CENTRAL SEROTONERGIC CONTROL OF CARDIOVASCULAR REFLEXES

A Thesis submitted for the Degree of
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by

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Abstract

Central serotonergic neurones control reflex parasympathetic outflow to the heart, airways and bladder in a number of species, and different 5-HT receptor subtypes are involved in this effect. 5-HT_{1A} and 5-HT_3 receptors in the brainstem facilitate these reflexes, whilst 5-$HT_{1B/1D}$, 5-HT_2 and 5-HT_4 receptors inhibit them. Recently, central 5-HT_7 receptors have been implicated in bladder reflexes.

Experiments on anaesthetised rats showed that the selective 5-HT_7 receptor antagonists SB-269970 and SB-656104, given intracisternally (i.c.), attenuated cardiopulmonary, baroreflex and chemoreflex bradycardias. Similarly, the selective 5-HT_{1A} receptor antagonist WAY-100635 attenuated cardiopulmonary and chemoreflex (but not baroreflex) bradycardia, whilst robalzotan and (-)-pindolol (antagonists at 5-HT_{1A} receptors) had no effect on cardiopulmonary and baroreflex bradycardias respectively.

Chemical stimulation of presumed serotonergic cell bodies in raphe magnus/pallidus evoked a bradycardia that could not be attenuated either by 5-HT receptor antagonists (given i.v.) or by prior 5-HT depletion. The latter did, however, significantly attenuate cardiovascular reflex sensitivity.

Activation of nucleus tractus solitarius (NTS) neurones by the vagus was inhibited by the iontophoretic AMPA receptor antagonist DNQX or by topical SB-269970. Subsequent histology suggested that 5-HT containing terminals do not make close appositions with these neurones.

Preliminary data demonstrate that SB-269970 (given i.c.) effectively attenuates the cardiopulmonary reflex in awake rats, but has variable effects on the chemoreflex.

The data suggest that 5-HT_7 receptors in the NTS are crucially involved in the central transmission of reflex bradycardias, at least in rats. The role of the 5-HT_{1A} receptor is less clear-cut than in the rabbit, and may reveal a species difference. The origin of 5-HT activating these receptors is unlikely to be the medullary raphe neurones, but may be primary afferents terminating in the NTS. Since recent ultrastructural evidence shows 5-HT terminals and NTS cardiovascular neurones are often separated by astroglial leaflets, astrocytes may be involved in serotonergic-glutamatergic signalling.

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Abbreviations

ANOVA	Analysis of variance
BP	Blood pressure
bpm	Beats per minute
cAMP	cyclic adenosine monophosphate
CNS	Central nervous system
CVLM	Caudal ventrolateral medulla
CVPN	Cardiac vagal preganglionic neurone
DVN	Dorsal vagal nucleus
EPSP	Excitatory postsynaptic potential
GABA	γ-aminobutyric acid
HR	Heart rate
HRP	Horseradish peroxidase
i.a.	Intra-atrial
i.c.	Intracisternal
i.c.v.	Intracerebroventricular
IML	Intermediolateral cell column (of the spinal cord)
i.p.	Intraperitoneal
IPNA	Integrated phrenic nerve activity
IPSP	Inhibitory postsynaptic potential
IRNA	Integrated renal nerve activity
i.t.	Intrathecal
i.v.	Intravenous
LSD test	Least significant difference test
MAP	Mean arterial pressure
min	Minutes
mRNA	Messenger ribonucleic acid
NTS	Nucleus tractus solitarius
PSTH	Peri-stimulus time histogram
PVN	Paraventricular nucleus of the hypothalamus
REM	Rapid eye movement
RVLM	Rostral ventrolateral medulla
s	Seconds

s.e.m.	Standard error of the mean
SERT	Serotonin transporter
SCN	Suprachiasmatic nucleus of the hypothalamus
SPN	Sympathetic preganglionic neurone
SSRI	Selective serotonin reuptake inhibitor

Abbreviated compounds (chemical names & functions)

(Compounds in bold are used in results chapters)

5,7-DHT (serotonergic neurotoxin) 5,7-dihydroxytryptamine

5-CT (5-$HT_{1/7}$ receptor agonist) 5-carboxamidotryptamine maleate

5-HT (serotonin) 5-hydroxytryptamine

8-OH-DPAT (5-$HT_{1/7}$ receptor agonist) (±)-8-hydroxy-2-dipropylaminotetralin hydrobromide

AMPA (AMPA receptor agonist) α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AP-5 (NMDA receptor antagonist) 2-amino-5-phosphonopentanoic acid

BIMU-8 (5-HT_4 receptor agonist) endo-*N*-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-isopropyl-2-oxo-1*H*-benzimidazol-1-carboxamide hydrochloride

BW-501C67 (peripheral 5-HT_2 receptor antagonist)

BW-723C86 (5-HT_{2B} receptor agonist) 1-[5-(2-thienylmethoxy)-1H-3-indolyl] propan-2-amine hydrochloride

CP-93129 (5-HT_{1B} receptor agonist) 1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrol[3,2-b]pyridin-5-one dihydrochloride

DLH (glutamate receptor agonist) DL-homocysteic acid

DMSO (organic solvent) dimethyl sulfoxide

DNQX (non-NMDA receptor antagonist) 6,7-dinitroquinoxaline-2,3-dione

DOI (5-HT_{2A} receptor agonist) 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane

DOB (5-HT_2 receptor agonist) 4-bromo-2,5-dimethoxyphenylisopropylamine

EMDT (5-HT_6 receptor agonist) 2-ethyl-5-methoxy-N, N-dimethyltryptamine

GR-113808 (5-HT_4 receptor antagonist) [1-[2-(methylsulfonylamino)ethyl]-4-piperidinyl]methyl 1-methyl-1H-indole-3-carboxylate

GR-125743 (5-$HT_{1B/1D}$ receptor antagonist) *n*-[4-methoxy-3-(4-methyl-1-piperizinyl)phenyl]-3methyl-4-(4-pyrinidinyl)benzamide

GR-127935 (5-$HT_{1B/1D}$ receptor antagonist) *N*-[4-Methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1'-biphenyl-4-carboxamide hydrochloride

GR-113808 (5-HT_4 receptor antagonist) [1,2[(methylsulphonyl)amino]ethyl]4-piperidinyl]methyl-1-methyl-1*H*-indole-3-carboxylate

GTI (5-$HT_{1B/1D}$ ligand) serotonin-O-carboxy-methyl-glycyl-tyrosinamide

ICS-205930 (5-HT_3 receptor antagonist) 3-tropanyl-indole-3-carboxylate

L-69247 (5-$HT_{1B/1D}$ receptor agonist) 2-[5-[3-(4-methylsulphonylamino)benzyl-1,2,4-oxadiazol-5-yl]-1*H*-indol-3-yl] ethanamine

LY-215840 (5-HT_7 receptor antagonist)

LSD (5-HT receptor partial agonist) lysergic acid diethylamide

m-CPP (5-$HT_{2B/2C}$ receptor agonist) 1-(3-chlorophenyl)piperazine hydrochloride

MDL-100907 (5-HT_{2A} receptor antagonist) ([R(+)-a-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol]

MDL-72222 (5-HT_3 receptor antagonist) 3-tropanyl-3.5-dichlorobenzoate

NAD-299 (robalzotan, 5-HT_{1A} receptor antagonist) (R)-3-N,N-dicyclobutylamino-8-fluoro-3,4-dihydro-2H-1-benzopyran-5-carboxamide hydrogen (2R,3R)-tartrate monohydrate

NAN-190 (5-HT_{1A} receptor antagonist) 1-(2-Methoxyphenyl)-4-(4-phthalimidobutyl)piperazine hydrobromide

NMDA (NMDA receptor agonist) *N*-methyl-D-aspartate

PEG (organic solvent) polyethylene glycol

PBG (5-HT_3 receptor agonist) 1-phenylbiguanide hydrochloride

***p*-CPA (5-HT synthesis inhibitor) para-chlorophenylalanine methyl ester hydrochloride**

Ro-650563 (5-HT_6 receptor antagonist) 4-amino-N-(2,6 bis-methylamino-pyridin-4-yl)-benzene sulphonamide

RS-67333 (5-HT_4 receptor agonist) 1-(4-amino-5-chloro-2-methoxyphenyl)-3-(1-n-butyl-4-piperidinyl)-1-propanone

RS-39604 (5-HT_4 receptor antagonist) 1-[4-Amino-5-chloro-2-(3,5-dimethoxyphenyl) methyloxy]-3-[1-[2-methylsulphonylamino]ethyl] piperidin-4-yl]propan-1-one

SB-204070 (5-HT_4 receptor antagonist) (8-amino-7-chloro-(N-butyl-4-piperidyl)methylbenzo-1,4-dioxan-5-carboxylate hydrochloride

SB-204741 (5-HT_{2B} receptor antagonist) *N*-(1-Methyl-*1H*-indolyl-5-yl)-*N*"-(3-methyl-5-isothiazolyl)urea

SB-216641 (5-HT_{1B} receptor antagonist) [1,1'-biphenyl]-4-carboxamide, N-[3-[2-(dimethylamino)ethoxy]-4-methoxyphenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)

SB-236057 (5-HT_{1B} antagonist) 1'-ethyl-5-(2'-methyl-4'-(5-methyl-1,3,4-oxadiazol-2-yl)biphenyl- 4-carbonyl)-2,3,6,7-tetrahydrospiro[furo[2,3-f]indole-3,4'-piperid ine]

SB-242084 (5-HT_{2C} receptor antagonist) 6-chloro-5-methyl-1-[6-(2-methylpiridin-3-yloxy)pyridin-3-yl carbamoyl] indoline

SB-258719 (5-HT_7 receptor antagonist) (R)-3,N-Dimethyl-N-[1-methyl-3-(4-methylpiperidin-1-yl)propyl]benzene sulfonamide

SB-258741 (5-HT_7 receptor antagonist) R-(+)-1-(toluene-3-sulfonyl)-2-[2-(4-methylpiperidin-1-yl)ethyl]-pyrrolidine

SB-269970 (5-HT_7 receptor antagonist) ((R)-1- [3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine

SB-357134 (5-HT_6 receptor antagonist) *N*-(2,5-dibromo-3-fluorophenyl)-4-methoxy-3-piperazin-1-ylbenzenesulfonamide

SB-656104 (5-HT_7 receptor antagonist) 6-((R)-2-(2-[4-(4-chloro-phenoxy)-piperidin-1-yl]-ethyl)-pyrrolidine-1-sulphonyl)-1H-indole hydrochloride

WAY-100135 (5-HT_{1A} receptor antagonist) (*S*)-*N*-tert-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide dihydrochloride

WAY-100635 (5-HT_{1A} receptor antagonist) *N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridinyl)cyclohexane carboxamide

Publications arising from this thesis

KELLETT DO, RAMAGE AG, & JORDAN D. (2003). Identification of medullary raphe sites involved in control of vagal drive to the heart in anaesthetised rats. *J Physiol* **547P**, PC83.

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KELLETT DO, RAMAGE AG, & JORDAN D. (2004). Evidence that vagal bradycardias evoked by baroreceptor and chemoreceptor afferents involve the activation of central 5-HT_7 receptors. *J Physiol* **555P**, C25.

RAMAGE AG, KELLETT DO & JORDAN D. (2004). Central 5-HT_7 receptors are involved in the reflex activation of vagal outflow to the heart. FASEB Journal **18**, 695.16

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KELLETT DO, JORDAN D & RAMAGE AG (2004). Effects of 5-HT depletion on cardiovascular reflex sensitivity in anaesthetised rats. *Br J Pharmacol* (pA_2 online) **2**(2), 18P

KELLETT DO, DAMASCO EL, BONAGAMBA LGH, MACHADO BH, JORDAN D & RAMAGE AG (2004). Involvement of central 5-HT_7 receptors in the autonomic responses to cardiopulmonary reflex activation in awake and anaesthetised rats. *Brazilian Biological Society (FESBE) Meeting* (in press)

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1. GENERAL INTRODUCTION

1.1. The autonomic nervous system

Langley's highly influential monograph entitled *The Autonomic Nervous System* (Langley, 1921) laid the foundation of our current understanding of the tandem sympathetic and parasympathetic systems controlling physiological processes, and was indebted to the prior works of Gaskell, Bayliss and Starling at the end of the 19th century. The autonomic nervous system is currently divided into three subdivisions: the sympathetic system and the parasympathetic system (both of which have neuronal cells bodies located in both the central (CNS) and peripheral nervous sytem), and the enteric system (whose neurones are located exclusively in the walls of the gut).

Both sympathetic and parasympathetic neurones provide a two-neurone motor output to various targets: a *preganglionic* neurone located in the CNS, and a *postganglionic* neurone, whose cell body is located in a ganglion, and whose axon innervates the target organ. Sympathetic ganglia are located in the paravertebral chains on either side of the spinal cord (levels T1 to L3), and are innervated by the preganglionic neurones in the intermediolateral (IML) cell columns of the spinal cord. In this system, the cell bodies of the postganglionic neurones are located close to those of the preganglionic neurones, and at a distance from the target organ. Conversely, parasympathetic ganglia are located on or close to the target organ, and are innervated by preganglionic neurones located in either the midbrain/brainstem (nuclei of cranial nerves III, VII, IX and X) or the sacral spinal cord (IML at levels S2 – S4).

All preganglionic neurones communicate with postganglionic neurones by releasing acetylcholine, which acts primarily on nicotinic receptors. Sympathetic postganglionic neurones release noradrenaline onto α and β adrenoceptors in the target organs (except for sweat glands, where the innervation is cholinergic *via* muscarinic receptors). Parasympathetic postganglionic neurones release acetylcholine onto muscarinic receptors in the target organ. Additionally, other

transmitters such as peptides and purines act as co-transmitters (see Burnstock, 1986; Lundberg & Hokfelt, 1986).

Sympathetic nerves innervate structures at many different levels, for example the heart and lungs *via* the superior cervical and stellate ganglia, the stomach *via* the coeliac ganglion, and the lower intestine *via* the inferior mesenteric ganglion. Similarly parasympathetic nerves innervate a range of structures: in the midbrain, the Edinger-Westphal nucleus innervates the ciliary ganglion of the eye *via* the IIIrd nerve. In the medulla, the salivatory nuclei innervate the lachrymal, nasal and parotid glands *via* the VIIth and IXth nerves, whereas the dorsal vagal nucleus (DVN) and nucleus ambiguus innervate the heart, lungs and gut *via* the Xth nerve. Finally the sacral IML innervates the colon, bladder and reproductive organs *via* the pelvic splanchnic nerve.

1.2. The cardiovascular system

The cardiovascular system is regulated by both peripheral and central neural structures, which are responsible for maintaining the internal environment of the body. Hence the central cardiovascular centres regulate the heart and vasculature, and work closely with the respiratory system. Together, these systems are under the control of various sets of sensory receptors, leading to a number of complex feedback loops known as cardiovascular and respiratory reflexes (see Spyer, 1990). Blood pressure (BP), cardiac output, blood volume, and blood gas tensions are regulated and constantly monitored by peripheral sensors, whose activation produces the appropriate neural or endocrine compensatory reponses. These receptors are broadly divided into mechanoreceptors and chemoreceptors. Mechanoreceptors detect mechanical force (usually stretch), such as arterial pressure (baroreceptors) in the aortic arch and carotid sinus; additionally, cardiac mechanoreceptors are located in the atria and ventricles and in the endings of great veins (see Spyer, 1990). Chemoreceptors are sensitive to the chemical environment, in particular to O_2 and CO_2 tensions and pH of arterial blood. These are located in the carotid body, and also on the ventral surface of the medulla, and together function to adjust ventilation and modulate cardiovascular variables accordingly (see Daly, 1997). These receptors relay information to the CNS where reflex responses are integrated. The

interactions between reflexes should not be underestimated: *in vivo,* different sets of afferents are often activated simultaneously. Furthermore, the reflex response to one afferent often activates a secondary reflex.

1.2.1. Parasympathetic control of the heart

Parasympathetic (vagal) drive has powerful negative chronotropic and inotropic actions on the heart, which in mammals is under a low to moderate level of tonic vagal influence. Hence withdrawal of vagal tone in itself causes a small tachycardia, whilst enhancement can cause a profound bradycardia up to and including atrioventricular block.

The vagus (X^{th}) nerve is the longest nerve in the body, exiting from the medulla, and taking a characteristic 'wandering' course through many parts of the thoracic and abdominal viscera. It is a mixed nerve consisting primarily of afferent fibres (80 % in the cat, with cell bodies in the nodose ganglia) as well as efferent fibres, which are the axons of parasympathetic (vagal) preganglionic neurones (see Daly, 1997).

Cardiac vagal preganglionic neurones

The location of vagal preganglionic neurones was first investigated by analysis of retrograde degeneration following vagotomy, which located vagal preganglionic neurones in the nucleus ambiguus (Bunzl-Federn, 1899) and DVN (Getz & Sirnes, 1949). Later the horseradish peroxidase (HRP) technique allowed retrograde labelling of specific neurones projecting to the heart (cardiac vagal preganglionic neurones; CVPNs) in rats (Nosaka *et al.*, 1979; Stuesse, 1982), cats (Kalia & Mesulam, 1980) and dogs (Bennett *et al.*, 1981), which were organised in two main cell groups, the DVN and nucleus ambiguus, but with a number of neurones scattered between these areas, as if not fully migrated to the nucleus ambiguus. CVPNs were found in more ventral parts of the nucleus ambiguus, but not in the cell columns innervating the striated muscles of the oesophagus, larynx and pharynx (Bieger & Hopkins, 1987). In the DVN, which also contains numerous gastrointestinal vagal preganglionic neurones, the CVPNs retrogradely labelled from the heart are mostly found in the lateral parts of the nucleus (Nosaka *et al.*, 1979; Bennett *et al.*, 1981), but in mammals this is thought to be a smaller population, the chief cardiac parasympathetic innervation arising from the nucleus ambiguus.

Projections to the nucleus ambiguus, identified by microinjection of HRP into parts of the nucleus that slowed the heart, are mainly from the ipsilateral medial nucleus of the tractus solitarius (NTS), but also from the parabrachial complex, the paraventricular nucleus of the hypothalamus (PVN), and the contralateral nucleus ambiguus (Stuesse & Fish, 1984), and ultrastructural analysis has shown the projection from the NTS to be monosynaptic (Deuchars & Izzo, 1991). Additionally, CVPNs receive synapses from terminals containing γ-aminobutyric acid (GABA) (Maqbool *et al.*, 1991), 5-hydroxytryptamine (5-HT, serotonin) (Izzo *et al.*, 1993), and substance P (Massari *et al.*, 1994).

Electrophysiological identification of cat CVPNs *via* antidromic stimulation of the cardiac branch of the vagus found that DVN neurones almost exclusively give rise to small diameter unmyelinated (C-fibre) efferents, whilst nucleus ambiguus neurones have small diameter myelinated (B-fibre) efferents (McAllen & Spyer, 1976). Further investigation found that nucleus ambiguus CVPNs are excited by baroreceptors, whereas nearby vagal bronchoconstrictor neurones are not (McAllen & Spyer, 1978). In the rabbit, some B-fibres also project from the DVN, but *only* B-fibres project from nucleus ambiguus (Jordan *et al.*, 1982). Similarly, in the rat C-fibres originate from DVN, whilst B-fibres originate from nucleus ambiguus (Nosaka *et al.*, 1982).

Rabbit and cat nucleus ambiguus CVPNs have a low spontaneous firing rate with a strong respiratory rhythm, firing during the post-inspiratory phase, and strongly inhibited during inspiration, which accounts for respiratory sinus arrthymia – the variability of heart rate (HR) during the respiratory cycle (McAllen & Spyer, 1976; McAllen & Spyer, 1978; Jordan *et al.*, 1982; Gilbey *et al.*, 1984). These studies also confirmed the powerful excitation of these neurones from baroreceptors, hence their ongoing activity strongly correlates with arterial pressure pulses. However, baroreceptors (and chemoreceptors) can only excite cardiac vagal outflow if they are timed during expiration (Haymet & McCloskey, 1975; Davidson *et al.*, 1976b), demonstrating that baroreceptor modulation of CVPNs is itself respiratory modulated. This inspiratory inhibition is attenuated by iontophoresis of atropine, whilst acetylcholine reduces firing (Gilbey *et al.*, 1984) suggesting a muscarinic receptor mechanism.

Figure 1.1 Central autonomic afferent and efferent systems

Schematic sagittal diagrams of rat brain (adapted from Saper, 2004) illustrating cell groups involved in central autonomic processing, and their main afferent and efferent connections.

Ascending: central cells groups receive afferent information either from the NTS (solid line) or indirectly *via* a relay in the parabrachial nucleus (dotted line)

Descending: Preganglionic cell groups (grey) receive direct innervation from various cells groups (solid line) and indirect inputs *via* premotor areas (dotted line).

A5	A5 noradrenaline group
Amyg	Central nucleus of the amygdala
AV	Anteroventral third ventricular area
BST	Bed nucleus of the stria terminalis
DVN	Dorsal vagal nucleus
ILC	Infralimbic cortex
IML	Intermediolateral cell column
Ins	Insular cortex
LH	Posterior lateral hypothalamus
nA	Nucleus ambiguus
NTS	Nucleus tractus solitarius
PB	Parabrachial nucleus
PVN	Paraventricular nucleus
Thal	Ventroposterior parvocellular nucleus of the thalamus
VLM	Ventrolateral medulla

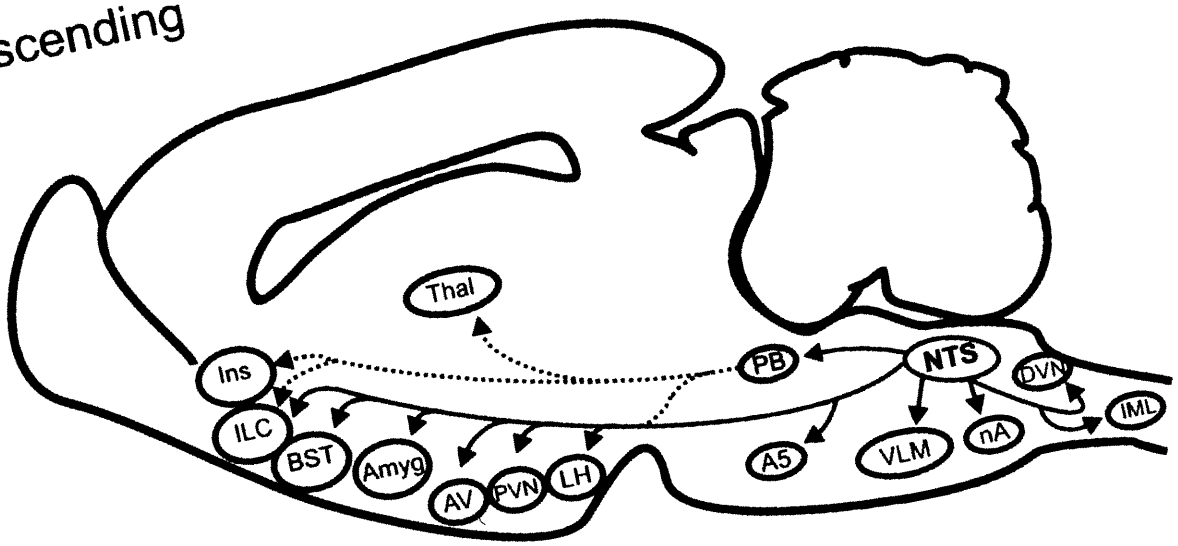
CVPNs in the DVN of rats and cats fire at a slow tonic rate that is not modulated by respiration or baroreceptors, but they are excited by cardiopulmonary afferents (Nosaka *et al.*, 1982; Nosaka, 1986; Jones *et al.*, 1998). A population of these neurones also receive a powerful and long lasting inhibition from cardiac afferents (Ford *et al.*, 1990).

The role of this dual B- and C-fibre innervation of the heart remains unresolved. It was assumed that B-fibres have negative chronotropic, whilst C-fibres have negative inotropic and coronary blood flow effects, although the latter is difficult to demonstrate as chronotropic changes will induce changes in the other variables. However, C-fibre efferents can be selectively stimulated using a triangular pulse anodal block technique, because they conduct slower than B-fibres. Selective C-fibre stimulation causes a slowing of the heart in rats, rabbits and cats (Woolley *et al.*, 1987; Jones *et al.*, 1995b), although this is smaller and slower in onset than the profound chronotropic effect due to B-fibre activation. It was later shown that C-fibre efferents also decrease atrioventricular conduction and contractility of the heart in rabbits (Garcia Perez & Jordan, 2001).

Cardiac vagal postganglionic neurones

Cardiac vagal *postganglionic* neurones have been extensively examined anatomically in the dog (Randall & Ardell, 1985; Randall *et al.*, 1986; Randall *et al.*, 1987), revealing a number of different ganglia usually embedded in fat pads around the vena cava, right pulmonary vein, interarterial groove and septum. Removing the fat pad at the junction of the inferior vena cava and left atrium abolished atrioventricular block due to vagal stimulation (Randall *et al.*, 1986), whereas removal of the fat pad over the right pulmonary vein attenuated vagal bradycardia without affecting atrioventricular block (Randall *et al.*, 1987), suggesting differential innervation of cardiac nodes. Vagal bradycardias are generally blocked by atropine, but the C-fibre efferent-evoked bradycardia is resistant to ganglionic blockade with hexamethonium (Ford & McWilliam, 1986) and chlorisondamine (Jones & Jordan, 1993), demonstrating that muscarinic as well as nicotinic mechanisms are present at the ganglia.

Ascending



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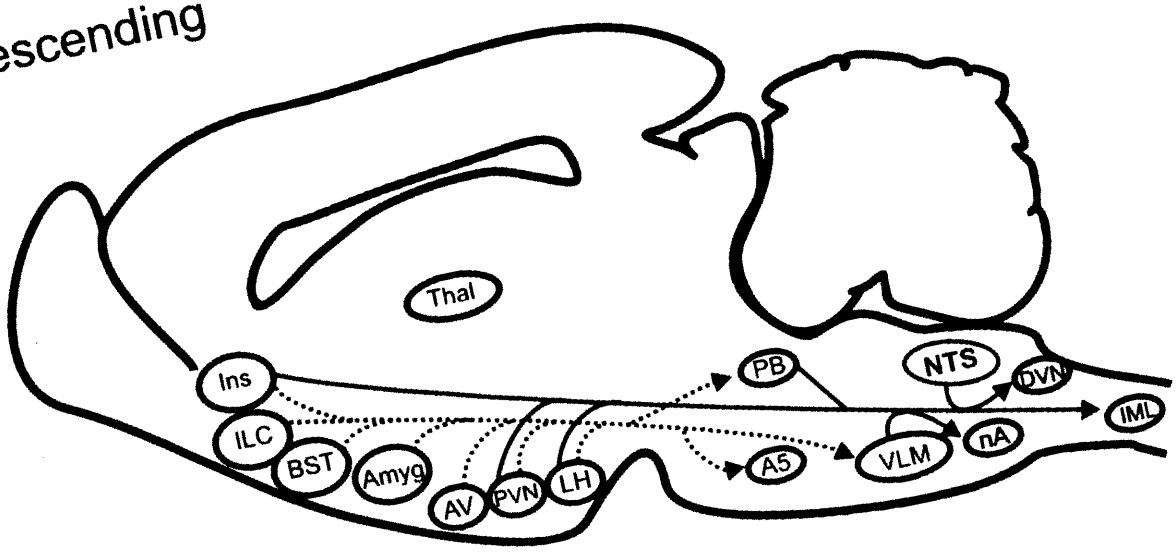


1.2.2. Sympathetic control of the vasculature

Sympathetic preganglionic neurones

Sympathetic preganglionic neurones (SPNs) are found predominantly in the IML of the spinal cord, where their cytoarchitecture was first described following their identification by rhizotomy (Petras & Cummings, 1972). SPNs are also found in the adjacent white matter of the lateral funiculus, in the intercalated cell columns between IML and the central canal, and dorsolateral to the central canal, also called the central autonomic nucleus (see Coote, 1988). SPNs tend to group together in clusters or 'nests' (Oldfield & McLachlan, 1981), with long dendrites extending mainly rostrocaudally (which may encourage entrainment of their rhythm with other spinal cord levels) but also laterally. The innervation of sympathetic ganglia was extensively studied using the retrograde fluorogold method, and found that each ganglion is innervated by several spinal cord segments, although one segment provides the principal innervation (Strack *et al.*, 1988) A separate investigation suggests that each SPN is specific to a particular ganglion (Appel & Elde, 1988). It is generally accepted that sympathetic outflows to different vascular beds are quite independent, and that each has its own functional properties (see Janig, 1988). This allows blood flow in different parts of the body to be separately adjusted without sympathetic outflow necessarily being globally modulated.

Sympathetic premotor neurones

A variety of neurones have been identified as providing control of SPNs from the brainstem or forebrain, and these are generally termed sympathetic premotor neurones. Anatomical tracing reveals that these neurones are found predominantly in the rostral ventrolateral medulla (RVLM) and medullary raphe nuclei (Amendt *et al.*, 1979; Loewy, 1981; Strack *et al.*, 1989), although the PVN and A5 noradrenaline cells also project to SPNs (Strack *et al.*, 1989). The role of raphe nuclei is described in 1.5.2 below.

The RVLM was first implicated in BP control when transections of the medulla or spinal cord at or below the RVLM were found to elicit a substantial fall in BP (Dittmar, 1873). Chemical inhibition of the RVLM *via* ventral surface applications

causes a similar fall (Guertzenstein & Silver, 1974; Feldberg & Guertzenstein, 1976), suggesting that the maintenance of BP by SPNs depends on a tonic excitation from RVLM neurones. Chemical stimulation of the RVLM causes a profound hypertension in anaesthetised (Dampney *et al.*, 1985; McAllen, 1986) and awake animals (Bachelard *et al.*, 1990). Neurones in the RVLM are organised topographically with respect to different vascular beds but not different body regions (McAllen & Dampney, 1990).

The RVLM is heavily innervated by the NTS (Loewy & Burton, 1978; Ross *et al.*, 1985), forming a major reflex pathway, and also by the area postrema (Shapiro & Miselis, 1985; Ross *et al.*, 1985) which may relay information on circulating hormones to the RVLM. There are also major inputs to the RVLM from the periaqueductal grey (Carrive *et al.*, 1988), the PVN and lateral hypothalamic area (Dampney *et al.*, 1987), which are thought to be involved in reflex modulation and hypertension.

Single unit recordings reveal that there are many barosensitive neurones in the RVLM, which have a high rate of discharge (10 – 20 Hz), and these can be divided into Type 1 and Type 2, sending slow-conducting and fast-conducting axons to the spinal cord respectively (Brown & Guyenet, 1985). Type 1 are inhibited by iontophoresis of noradrenaline and the α_2 adrenoceptor agonist clonidine, whilst Type 2 are insensitive to these agents. This suggested that a population of RVLM neurones can be inhibited by a noradrenergic pathway, probably from the A5 cell group (Sun & Guyenet, 1986). RVLM barosensitive neurones fire in strong correlation with BP pulses, firing only during the BP trough; the lowest probability of firing was calculated to be ~65 ms after the ECG R-wave, and ~25 ms after the discharge of a barosensitive NTS neurone, suggesting that this intramedullary pathway is very slow (~0.4 m s^{-1}) (Moore & Guyenet, 1983), as was reported for the cardiac limb of the baroreflex (McAllen & Spyer, 1978). Barosensitive RVLM neurones have an intrinsic pacemaker discharge, which appears independent of external inputs. Intracisternal (i.c.) administration of the glutamate receptor antagonist kynurenic acid abolishes reflex changes in their firing rate, but their intrinsic rhythm persists, and it is also seen *in vitro* in the rat perfused bulb preparation (Sun *et al.*, 1988).

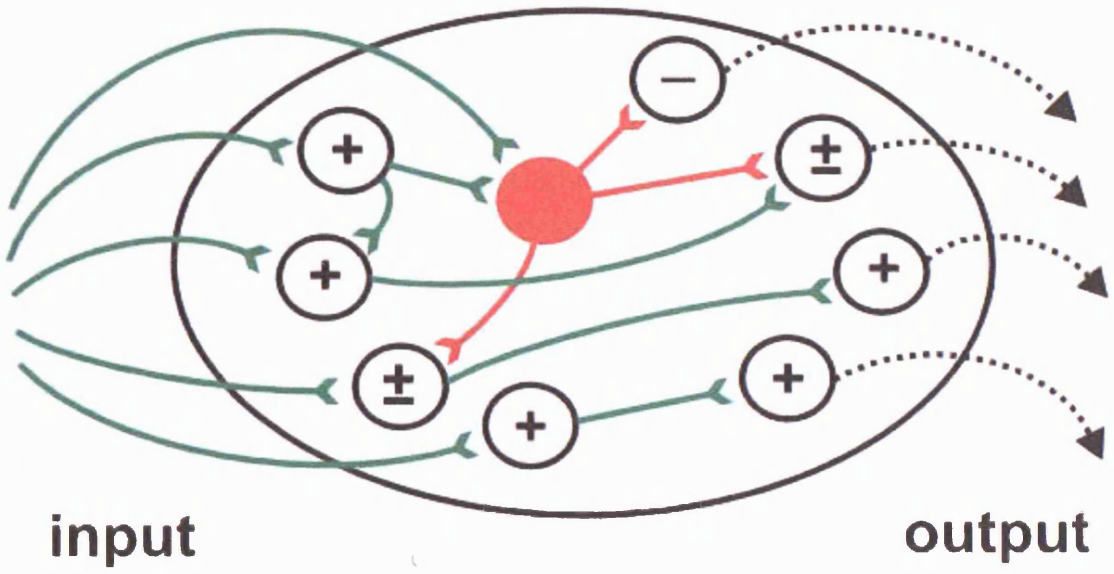


Figure 1.2 NTS microcircuitry

Schematic diagram of NTS (adapted from Spyer, 1994) based on intracellular recordings, illustrating some of the possible projections of primary afferents (input) to various NTS neurones (black circles and red circle which is an intrinsic GABAergic interneurone) and their possible connectivity. Green projections are excitatory, red are inhibitory.

The postsynaptic potentials evoked by afferent stimulation are shown in each cell:

+ excitatory postsynaptic potential (EPSP)

– inhibitory postsynaptic potential (IPSP)

± EPSP followed by IPSP

1.2.3. The nucleus tractus solitarius

The NTS is the principal site of termination of an array of visceral primary afferent fibres including arterial baroreceptors, chemoreceptors and lung stretch afferents (see Spyer, 1990). Together these provide moment to moment information on the pressure in arteries and great veins, and on the chemical composition of arterial blood. As such, the NTS is an essential integrator of cardiovascular reflexes, whose pathways are described in detail in 1.3 below.

The NTS is a sensory nucleus running longitudinally in the dorsomedial region of the medulla, and can be seen as the autonomic equivalent of the dorsal horn of the spinal cord. The NTS is centred around the level of the area postrema, and extends for a short distance caudally from the calamus scriptorius (caudal tip of the area postrema) and rostrally from the obex (caudal border of the IV^{th} ventricle). Hence it is divided into three rostrocaudal portions: rostral (above the obex), intermediate (at the level of the area postrema), and caudal (below the calamus scriptorius). The intermediate NTS is just ventral to the dorsal column nuclei, and just dorsal to the DVN and hypoglossal nuclei. Vagal afferents terminate primarily in the caudal two-thirds of the NTS, whilst glossopharyngeal afferents terminate primarily in the rostral two-thirds. Mediolaterally it can also be divided into medial, commisural, and lateral segments. Lung stretch afferents terminate mainly in the medial and commisural NTS (Donoghue *et al.*, 1982b). The termination of other afferents is discussed in 1.3 below.

Extracellular and intracellular recordings have been used to assess the degree of convergence of different afferents onto NTS neurones (Donoghue *et al.*, 1985). This study reported that the majority of NTS cells responding to aortic nerve, sinus nerve, or vagal stimulation, respond only to one input, although a low level of convergence was found, suggesting that generally a separate neuronal channel is used for each afferent type.

The NTS contains a variety of neurones involved in cardiovascular functions, as illustrated in Figure 1.2. Second order neurones are innervated by primary afferent fibres, and these project to higher order NTS neurones for further processing prior to

leaving the NTS. A number of interneurones may also be involved, but this is difficult to characterise. These pathways are generally excitatory, although a population of intrinsic GABAergic interneurones are found in the NTS (Izzo *et al.*, 1992), which receive primary afferents, as well as projecting to various second order neurones (see Spyer, 1994). Hence pathways through the NTS may be influenced by a number of excitatory and inhibitory inputs (quite apart from the wealth of neurochemical messengers discussed in 1.4 below), resulting in various electrophysiological phenotypes. Some neurones display excitatory postsynaptic potentials (EPSPs) when afferents are stimulated, some display inhibitory postsynaptic potentials (IPSPs), and some displaying an initial EPSP followed by an IPSP (Figure 1.2). A frequent problem with single unit recording studies is that the NTS neuronal type (second or higher order) is difficult to identify except by analysing the varibility of the latency, or by paired pulse activation. These techniques yield some information on whether a neurone is mono- or polysynaptically activated, but identification is not unequivocal. Therefore, microinjections are likely to activate all of these neurones, including inhibitory interneurones. There is much less information on higher order neurones in the NTS: there is thought to be some limited convergence *via* polysynaptic interactions (see Jordan & Spyer, 1986), which are under continuing investigation.

The NTS also contains two kinds of respiratory-related neurones: firstly P cells (Berger, 1977), which burst fire in phase with lung inflation, and are monosynaptically activated by slowly adapting lung stretch afferents (Berger & Dick, 1987). Secondly, Iβ cells are a subclass of dorsal respiratory group inspiratory neurones in the ventrolateral NTS. They are activated when the lungs are held inflated during expiration; the cells adapt and are excited by gasping, suggesting activation by rapidly adapting lung stretch afferents, but they are also innervated by slowly adapting lung stretch afferents (Averill *et al.*, 1984; Backman *et al.*, 1984).

Apart from primary afferents, the NTS receives inputs from all brain levels. These include, based on anatomical work, the periaqueductal grey (Bandler & Tork, 1987), PVN (van der Kooy *et al.*, 1984; Patel & Schmid, 1988), amygdala (Schwaber *et al.*, 1982), frontal cortex (Terreberry & Neafsey, 1983), and raphe (Thor & Helke, 1987; Schaffar *et al.*, 1988). Only some of these connections have been functionally

investigated. NTS neurones are excited by stimulation of the amygdala (Cox *et al.*, 1986). Stimulation of the parabrachial nucleus causes bradycardia *via* neurones in the commisural NTS (Hamilton *et al.*, 1981), and modulates carotid sinus nerve inputs to the NTS (Felder & Mifflin, 1988). Stimulation of the area postrema excites NTS neurones (Hay & Bishop, 1991), and stimulation of PVN excites 20 % of NTS neurones orthodromically, 2.5 % antidromically, and inhibits 6 % (Kannan & Yamashita, 1985). Conversely, stimulation of the hypothalamic defence area inhibits most NTS cells, and attenuates carotid sinus nerve inputs (Mifflin *et al.*, 1988). Stimulation of the RVLM also inhibits aortic nerve inputs to NTS neurones (Wang & Li, 1988) suggesting a reciprocal connection between these nuclei. Anatomical projections from the NTS to the parabrachial nucleus (Herbert *et al.*, 1990), the hypothalamus and amygdala (Ricardo & Koh, 1978; Riche *et al.*, 1990) have also been described, suggesting similar reciprocal connectivity.

1.2.4. The respiratory network

Normal ventilation consists of inspiration and expiration mediated by neuronal control of the diaphragm, thorax and abdominal wall, and these neuronal events fall into three respiratory phases: inspiration, post-inspiration, and expiration (see Richter *et al.*, 1992). The main generators of the respiratory rhythm are located in the brainstem, consisting of the ventral respiratory group in the ventrolateral medulla, and the dorsal respiratory group in the region of the NTS. The ventral respiratory group is considered to contain the premotor neurones that provide most of the efferent control of the respiratory muscles (Richter *et al.*, 1992). Neurones in the intermediate part of the ventral respiratory group retain respiratory-like activity even after blockade of excitatory transmission, suggesting they are pacemaker cells (Onimaru *et al.*, 1988). Further pacemaker cells are found in a respiratory related area known as the pre-Bötzinger complex (Smith *et al.*, 1991). In cats the dorsal respiratory group is thought to have a role in transmission of inspiratory drive to motor neurones *via* other groups of respiratory neurones, but in rats the existence of a dorsal respiratory group remains controversial, and NTS neurones are believed to make a weak contribution to respiratory drive (Bianchi *et al.*, 1995). In both species, however, inspiratory and post-inspiratory neurones form a complex network modulating cardiovascular variables (see Figure 1.4).

1.2.5. Other CNS structures

A variety of other CNS structures contribute to cardiovascular regulation (see Figure 1.1). A detailed description of these pathways is beyond the scope of this thesis, but they have been recently reviewed in depth (see Saper, 2004), as summarised below.

The *parabrachial nucleus* is a relay station between the NTS and higher areas, and in addition to autonomic afferents, it is involved in nociceptive processing. It is the only cardiovascular area to communicate with the thalamus. Various cardiovascular effects are evoked by stimulating this nucleus, depending on the area.

The *A5 noradrenaline cells* are the origin of the chief descending noradrenergic innervation of spinal cord SPNs. Stimulating the A5 area causes hypotension.

The *hypothalamus* has many autonomic and homeostatic roles. The anteroventral third ventricular region participates in functions such as thermoregulation and drinking. The PVN has diverse functions including secretion of releasing hormones but also has various projections to cardiovascular areas. Stimulating the PVN can increase or decrease BP. The lateral hypothalamic area has a sympathoexcitatory role, and stimulation causes hypertension and tachycardia.

The *central nucleus of the amygdala* contains neurones that are barosensitive *via* a parabrachial input. Various responses are elicited by amygdala stimulation, but it is clearly involved in the conditioned cardiovascular fear response.

Little is known of the cardiovascular functions of the *bed nucleus of the stria terminalis*, but there is some evidence that it contains neurones that are sensitive to certain cardiovascular stimuli. Stimulation increases gastric motility.

Stimulating the *insular cortex* can produce hypotension with bradycardia or hypertension with tachycardia depending on the area stimulated.

The *infralimbic cortex* may be a type of visceral motor cortex: stimulation affects gastric motility *via* a hypothalamic relay.

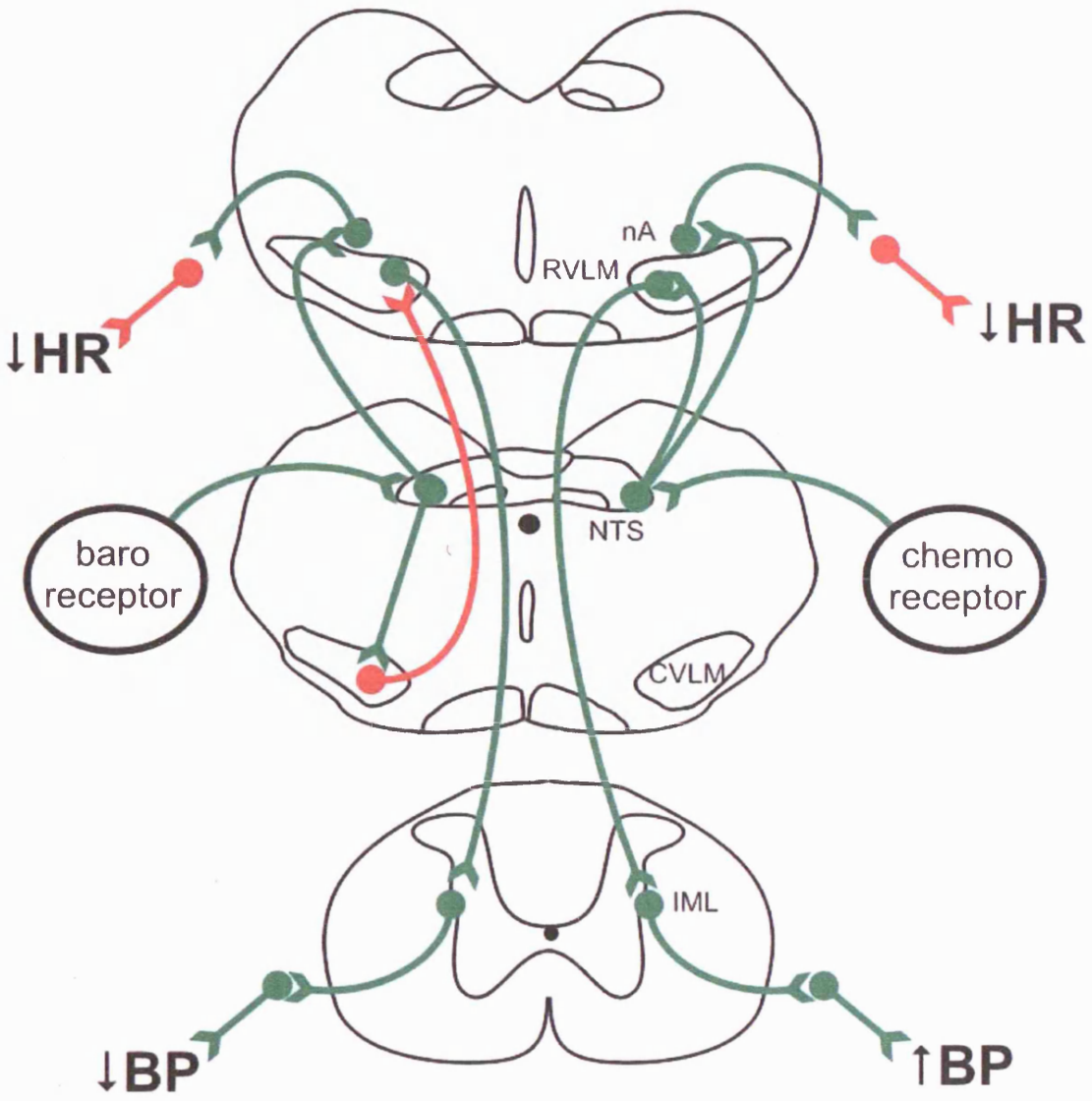


Figure 1.3 Cardiovascular reflex pathways

Schematic diagrams of rat medulla (at 2 levels) and thoracic spinal cord, illustrating main pathways of reflex responses to baroreceptor and chemoreceptor stimulation. Green are excitatory and red are inhibitory pathways. The output at the end organs (heart or vasculature) is indicated in terms or a rise (↑) or fall (↓) in heart rate (HR) or blood pressure (BP).

CVLM: caudal ventrolateral medulla, nA: nucleus ambiguus, NTS: nucleus tractus solitarius, IML: intermediolateral cell column, RVLM: rostral ventrolateral medulla.

1.3. Cardiovascular reflexes

1.3.1. The baroreflex

Signals from the arterial baroreceptors in the aortic arch and carotid sinuses reflexly modulate heart rate, sympathetic vasomotor activity and vasopressin release. As such, it is a crucial reflex responsible for maintaining appropriate perfusion of tissues by keeping arterial pressure within normal limits. Baroreflex dysfunction is thought to be one cause of hypertension, and therefore associated with considerable morbidity. At normal BP, baroreceptor afferents fire at a tonic, pulse-related rate, allowing reflex changes to occur if the afferent firing rate changes in either direction. Similarly, cardiac vagal efferents and sympathetic vasoconstrictor efferents fire at a tonic rate, allowing reflex increases or decreases to occur (see Spyer, 1990). When BP is elevated, baroreceptor afferents increase their firing rate through stretch activation, consequently cardiac vagal fibres increase their firing to lower HR, whereas vasoconstrictor fibres slow down. The consequent drop in cardiac output and total peripheral resistance helps return BP to baseline. Conversely, when arterial pressure drops (also called baroreceptor unloading) baroreceptor afferents slow down, vagal drive diminishes and sympathetic tone increases, causing tachycardia and vasoconstriction.

Experimentally, baroreflex responses can be elicited by artificially modulating BP. This is usually done with vasoactive substances, such as the vasoconstrictor phenylephrine, and the vasodilator sodium nitroprusside. Brief mechanical manipulations are also possible, such as occlusion of the abdominal aorta, which raises BP, or occlusion of the common carotid arteries, which unloads carotid sinus baroreceptors. The problem with these techniques is that changing BP can itself modulate respiration, alter the perfusion of the brain, and cause local cardiac reflexes. A more elegant technique is the electrical stimulation of afferent nerves, particularly the aortic nerve (Sapru *et al.*, 1981), which in the rat has mainly baroreceptor afferents, although recently some chemoreceptor afferents have been identified (Brophy *et al.*, 1999).

In the rabbit and cat, mapping of aortic nerve baroreceptor afferents using retrograde labelling from the nodose ganglion and antidromic mapping found that they project to the ipsilateral medial, lateral and ventrolateral NTS in the cat, and to the medial and lateral NTS in the rabbit (Donoghue *et al.*, 1982a). This study identified only myelinated fibres. Subsequent mapping of carotid sinus baroreceptor fibres with cell bodies in the petrosal ganglion reported that they project to the ipsilateral lateral NTS rostral to the obex *via* both myelinated and unmyelinated fibres (Donoghue *et al.*, 1984). Previously baroreceptor afferents had been characterised as both myelinated (A-fibres) and unmyelinated (C-fibres) (Coleridge & Coleridge, 1980), the latter (from the aortic wall) having a threshold of activation of 30 – 50 mmHg higher than A-fibres, a lower maximum discharge rate, and as such probably important for responses to sudden large pressure changes, whilst A-fibres regulate pressure under normal conditions (Thoren & Jones, 1977; Coleridge & Coleridge, 1977). Single carotid baroreceptor afferent fibres also consist of two types: Type I and Type II (Seagard *et al.*, 1993). Type I (A-fibres) have a high threshold for activation over a narrow range; Type II (C-fibres) have a low threshold, and operate over a wide range of pressures. Some myelinated Type II fibres also exist.

Baroreceptor *resetting* is a mechanism reported to occur in hypertension, characterised by an increase in the threshold and a reduction in the sensitivity and maximum frequency of impulses of baroreceptor fibres (Coleridge & Coleridge, 1980). More resetting was reported to occur in A-fibres than C-fibres (Jones & Thoren, 1977), and greater resetting occurs during chronic hypertension (Jones, 1977), highlighting the importance of early treatment. A reduced flexibility of arterial walls at least partly accounts for reduced baroreceptor responsiveness in chronic hypertension, although more complex transductional and indeed central mechanisms are also thought to contribute.

The central pathways of the baroreflex involve medullary and spinal structures (see Figure 1.3). Midline electrolytic lesions in the medulla had no effect on baroreflex function, nor did decerebration or lower pontine transection, whilst DVN and nucleus ambiguus lesions abolished the bradycardia (Lee *et al.*, 1972). This suggests that neither the medullary raphe nor more rostral brainstem or forebrain structures are involved in this reflex *per se,* although various supraspinal areas can modulate the

baroreflex. The cardiac component of the baroreflex may involve a monosynaptic pathway from NTS to nucleus ambiguus (Deuchars & Izzo, 1991). DVN neurones are not barosensitive, and therefore not involved in baroreflex bradycardia (Jones *et al.*, 1998). The symathetic component of the baroreflex involves an excitatory pathway from NTS to the caudal ventrolateral medulla (CVLM), followed by an inhibitory projection from CVLM to RVLM (Guyenet *et al.*, 1987; Terui *et al.*, 1990; Masuda *et al.*, 1991). Hence baroreflex sympathoinhibition involves switching off the tonic activity of RVLM, and consequently IML neurones, whereas baroreflex sympathoexcitation (during baroreceptor unloading) involves inhibition of the tonic brake imposed by the CVLM.

Within the NTS, glutamate is thought to be the primary neurotransmitter. Microinjection of glutamate (Talman *et al.*, 1980; Leone & Gordon, 1989) or NMDA (Kubo & Kihara, 1988b) into the NTS causes bradycardia and hypotension in anaesthetised rats, and bilateral microinjections of non-selective glutamate receptor antagonists strongly inhibit the baroreflex (Talman *et al.*, 1981; Guyenet *et al.*, 1987; Leone & Gordon, 1989). Interestingly, these researchers report hypertension after blockade of NTS glutamate receptors, illustrating the importance of the NTS in maintaining normal BP. In awake rats, both AMPA and NMDA receptors in the NTS (Machado, 2001) but not metabotropic glutamate receptors (Antunes & Machado, 2003) contribute to the baroreflex. Iontophoresis of selective antagonists onto NTS neurones excited by aortic nerve afferents found that AMPA receptors are located at the primary synapse between afferent and second order neurone, whilst NMDA receptors are only found on higher order neurones receiving polysynaptic inputs (Zhang & Mifflin, 1997; Zhang & Mifflin, 1998). As the NMDA receptor is involved in synaptic plasticity, this proposed a role in long-lasting changes in these higher order neurones, although this has not been confirmed. Indeed, recent evidence points to NMDA receptors on second order barosensitive NTS neurones (GA Jones & D Jordan, unpublished data). Another likely central mechanism for hypertension is increased inhibition of NTS barosensitive neurones *via* GABA receptors (see Mifflin, 2001).

There only appears to be a low degree of convergence of different afferents onto NTS neurones with respect to baroreceptor afferents (Donoghue *et al.*, 1985), but a

recent study demonstrated that lung stretch afferents tend to converge onto second order barosensitive NTS neurones, but not higher order neurones (Jones & Jordan, 2003).

Respiratory modulation in cardiovascular reflexes is the the process by which the reflex output is modulated by the activity of the respiratory network. The baroreflex bradycardia is subject to respiratory modulation (Haymet & McCloskey, 1975; Neil & Palmer, 1975; Davidson *et al.*, 1976a). Baroreflex changes in the activity of CVPNs (i.e. increases in vagal drive to the heart) cannot be elicited during inspiration. This is also seen in the chemoreflex (see 1.3.3), but not the cardiopulmonary reflex (see 1.3.2). The modulation seems to occur at the level of the nucleus ambiguus, *via* inhibition by inspiratory neurones (see Figure 1.4). Furthermore, stimulation of the hypothalamic defence area attenuates the baroreflex, but enhances the chemoreflex (Coote *et al.*, 1979), suggesting that under certain emergency situations it is desirable for the body to suppress the baroreflex.

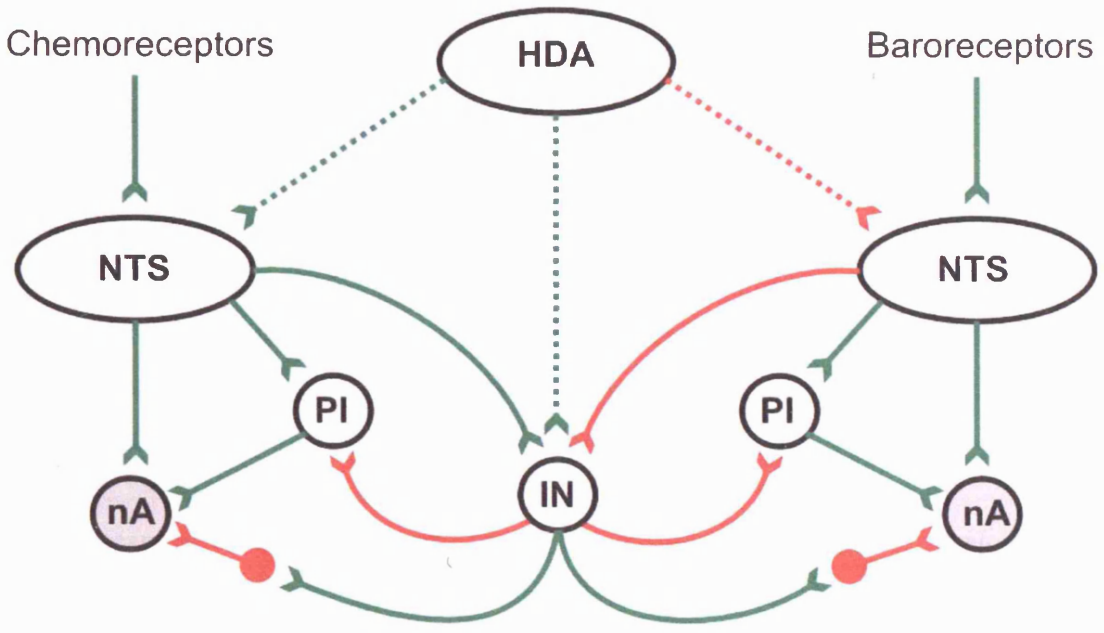


Figure 1.4 Reflex modulation

Schematic diagram (adapted from Spyer, 1994) illustrating synaptic influences of both the respiratory network (IN: inspiratory neurone, PI: post-inspiratory neurone) and higher centres (HDA: hypothalamic defence area) on the transmission of chemoreceptor (left) and baroreceptor (right) reflexes through the NTS and nucleus ambiguus (nA). Green denotes an excitatory and red an inhibitory pathway, neither of which is necessarily monosynaptic.

The chief mediators of respiratory modulation are the inspiratory neurones, which inhibit nucleus ambiguus neurones, probably *via* an inhibitory interneurone. Post-inspiratory neurones may provide an additional drive to nucleus ambiguus neurones during the post-inspiratory phase.

1.3.2. The cardiopulmonary reflex

First described by von Bezold and Hirt in 1867, and further characterised by Jarisch and Richter, the cardiopulmonary reflex has also been termed the von Bezold-Jarisch reflex or the pulmonary chemoreflex, although the von Bezold-Jarisch reflex refers specifically to the reflex evoked by C-fibre afferents in the coronary circulation (Dawes & Comroe, 1954). The cardiopulmonary reflex is physiologically stimulated when pulmonary capillary pressure rises, for example during pulmonary congestion (see Paintal, 1969).

The cardiopulmonary reflex can be elicited by injection of veratum alkaloids or other substances such as phenylbiguanide (PBG) or capsaicin into the cardiopulmonary circulation, although the latter compounds selectively activate C-fibre afferents in the heart and lungs, without activating mechanoreceptors (Coleridge & Coleridge, 1980). The action of PBG is mediated by 5-HT_3 receptors on the peripheral C-fibre terminals (Kay & Armstrong, 1990). The reflex response is a characteristic triad of bradycardia, hypotension and either apnoea or rapid shallow breathing (see Thoren, 1979), although additionally reflex bronchoconstriction and airway secretion can occur (see Coleridge & Coleridge, 1994).

Pulmonary C-fibres throughout the lungs were originally termed J-receptors, and reported to evoke the pulmonary depressor chemoreflex, whereas cardiac C-fibres located in the left ventricular wall, but also in the atria, evoked the cardiac chemoreflex (see Coleridge & Coleridge, 1979). The cardiac endings do not consistently respond to changes in BP, suggesting no role in the regulation of BP as such (Coleridge *et al.*, 1973), but they do respond to prostaglandins and bradykinin, which can be endogenously released from the myocardium, for example during hypoxia, ischaemia, and increased preload (Needleman, 1976). Indeed, the cardiopulmonary reflex is thought to be activated in myocardial ischaemia, and during the injection of contrast media for coronary angiography (Mark, 1983). During experimental stimulation *via* injections into the jugular vein or right atrium, reflex responses to pulmonary C-fibres can only be usefully distinguished from cardiac receptors in larger species (e.g. in the cat, where the pulmonary circulation

time is around 5 s) (Daly, 1991), so in small rodents it is more realistic to refer to the stimulated afferents as 'cardiopulmonary C-fibres.'

Unmyelinated cardiopulmonary afferents project to the NTS *via* the vagus nerve, and antidromic mapping in the cat has found that they terminate in the medial NTS rostral to the obex, and in the dorsal commisural NTS caudal to the obex (Kubin *et al.*, 1991) – a pattern that was confirmed in the rat and rabbit by c-fos expression following repetitive PBG injection (Gieroba *et al.*, 1995). Transmission within the NTS is thought to be glutamatergic, as microinjection of the non-selective glutamate receptor antagonist kynurenic acid into the NTS abolished the reflex (Verberne & Guyenet, 1992; Vayssettes-Courchay *et al.*, 1997). An iontophoretic study reported that NTS neurones are excited by cardiopulmonary afferents *via* AMPA receptors (Wilson *et al.*, 1996), although microinjections into the NTS also implicate NMDA receptors (Chianca & Machado, 1996) or both (Vardhan *et al.*, 1993). One possible explanation is that AMPA receptors are involved at the afferent synapse onto second order neurones, whereas NMDA receptors are located on higher order interneurones within the NTS (see Andresen *et al.*, 2004).

Whilst in the awake rat the depressor response to cardiopulmonary afferent stimulation is entirely atropine sensitive, hence secondary to the fall in cardiac output (Chianca & Machado, 1996), in the anaesthetised animal it is accompanied by a strong sympathoinhibition, which is thought to utilise the same pathway as the baroreflex. Microinjection of kynurenic acid into the CVLM, or of bicuculline into the RVLM attenuate the sympathoinhibition (Verberne & Guyenet, 1992) suggesting that the sympathetic component involves an excitatory glutamatergic projection from NTS to CVLM, followed by an inhibitory GABAergic projection from CVLM to RVLM (as for the baroreflex). The lateral tegmental field of the medulla has also been reported to contain sympathoexcitatory neurones projecting to the RVLM and sympathoinhibitory neurones projecting to the medullary raphe, which are activated by the cardiopulmonary reflex and may be involved in the integration of the sympathetic component, at least in the cat (Vayssettes-Courchay *et al.*, 1997). Earlier lesion experiments, however, suggested that only the NTS/DVN, ventrolateral medulla, and IML are involved in cardiopulmonary reflex function (Lee *et al.*, 1972).

In the cat, the cardiopulmonary reflex is *not* respiratory modulated (Daly & Kirkman, 1988), and this has also been demonstrated in the rabbit and rat (Jones, 1993). Stimulation of cardiopulmonary afferents activates CVPNs in the DVN (Jones *et al.*, 1998), and since these do not have respiratory rhythm, it was thought that the lack of respiratory modulation meant only DVN neurones are involved in the bradycardia. However, this cannot be the case, since selective stimulation of the cardiac C-fibre efferents of DVN neurones only causes a small bradycardia (Jones *et al.*, 1995b). Therefore the nucleus ambiguus CVPNs (with B-fibre axons) must contribute the larger part of the total bradycardia. Since these neurones have strong respiratory rhythm, the lack of respiratory modulation in this reflex could be explained by combined B-fibre and C-fibre outflow to the heart. Possibly the C-fibres uncouple the respiratory and sinoatrial node oscillators, suppressing the respiratory modulation caused by the B-fibres. Whether this is processed centrally or peripherally at cardiac ganglia remains somewhat mysterious. However, recordings from CVPNs in the nucleus ambiguus of the cat demonstrated that these neurones can be excited by cardiopulmonary afferents at various phases of the respiratory cycle (Wang *et al.*, 2000a).

1.3.3. The chemoreflex

The chemoreflex modifies ventilation according to the requirements of the body, and has additional complex cardiovascular effects. In the 1920s, JF and C Heymans reported first that the aortic and later that the carotid bodies were sensitive to asphyxia, hypoxia, hypercapnia and acidaemia, as part of a reflex control of breathing (see Daly, 1997). Chemical agents such as cyanide and nicotine also mimicked these effects. Later single fibre recordings from the carotid body or the sinus nerve were performed, and it is now established that a single fibre in this pathway can be sensitive to a decrease in arterial O_2 tension (PaO_2), and increase in $PaCO_2$, and an increase in plasma $[H^+]$. Furthermore, the relationship between fibre discharge and PaO_2 is exponential, whereas the relationship to $PaCO_2$ is linear (Biscoe *et al.*, 1970; Fitzgerald & Parks, 1971). When both stimuli (hypoxia and hypercapnia) are applied simultaneously, the respiratory response is greater than the sum of both stimuli (Lahiri *et al.*, 1981). Taken together, these findings impute a

complex system of transduction mechanisms which is under continuing investigation.

The greatest chemoreceptive response *in vivo* is generally accepted to arise from the carotid bodies (Lahiri *et al.*, 1981). Experimentally these can be stimulated by injection of small volumes of either a cyanide salt or CO_2-saturated saline to the vicinity of the carotid bifurcation, for example into the lingual artery (Hilton & Marshall, 1982). However, bolus doses of cyanide (NaCN or KCN) are frequently given systemically (into the femoral vein), producing chemoreflex effects that are abolished by sinus nerve transection (Franchini & Kreiger, 1993) or carotid body artery ligation (Barros *et al.*, 2002), suggesting selective stimulation of carotid bodies even through this route. Cyanide is thought to activate carotid body chemoreceptors by temporarily inhibiting tissue respiration and causing histotoxic hypoxia.

Early investigations using anaesthetised free-breathing dogs reported increased ventilation and bradycardia in response to intracarotid injection of chemical agents (Heymans & Bouckart, 1941). However, responses to chemoreceptor stimulation can vary between different animals, or in the same animal depending on experimental conditions, which caused confusing and conflicting responses. For example, the finding of Heymans & Bouckart (1941) conflicted with an earlier observation in an anaesthetised dog, in which the carotid bodies were vascularly isolated and perfused with CO_2-saturated Ringer's solution, causing hyperventilation and *tachycardia* (Heymans *et al.*, 1930). Variable changes were also seen by later researchers (Daly & Scott, 1958; Daly & Scott, 1963) who reported hyperventilation plus either an increase, a decrease, or no change in heart rate. Another study reported tachycardia unless respiration was held constant with a ventilator pump, in which case the response was bradycardia (Bernthal *et al.*, 1951). Daly & Scott (1958) showed that hypoxic blood delivered to the carotid bodies causes a sudden an intense hyperventilation and immediate tachycardia, which later slows down. Also, bradycardia tends to occur when the hyperventilatory response is smaller. In the cat, mainly bradycardic responses are reported (Macleod & Scott, 1964), whilst in the seal only bradycardia is seen (Elsner *et al.*, 1977). In free-breathing anaesthetised rats, a bradycardia followed by a tachycardia was seen, and under neuromuscular blockade this became a bradycardia only (Marshall, 1987).

The present consensus is that chemoreflex changes in respiration cause a subsequent change in the effectiveness of incoming chemoreceptor signals, as shown by the relationship between changes in ventilation and changes in heart rate (see Daly, 1997). Lung stretch afferents also interfere strongly with the bradycardic response to chemoreceptor stimulation (see Marshall, 1994), and for these reasons it is useful to clamp ventilation during chemoreflex experiments. A third influence on chemoreflex responses is anaesthesia: the profoundly depressant barbiturate anaesthetics tend to blunt reflexes (Marshall, 1987), especially vagal bradycardias, whereas α-chloralose is favoured for its milder effect on cardiovascular reflexes. Anaesthetics also influence the activity of the hypothalamic defence area: under light Saffan (alphaxalone/alphadalone) anaesthesia, rats exhibit a defence reaction pattern of responses to chemoreceptor stimulation, which is not seen in either deep Saffan or pentobarbitone anaesthesia (Marshall, 1987). In awake rats, a profound bradycardia and hypertension are observed, together with some behavioural alerting (Haibara *et al.*, 1995), and these latter responses may involve hypothalamic defence area participation.

Cell bodies of chemosensitive neurones in the petrosal ganglia were antidromically mapped and shown to project to the dorsal NTS rostrally, and to the medial and commisural NTS caudally, *via* unmyelinated fibres (Donoghue *et al.*, 1984). These projections were chiefly ipsilateral, although some contralateral collaterals were identified. Myelinated chemoreceptor afferents are also thought to exist (Fidone & Sato, 1969), but researchers have consistently failed to record from them. Further investigation found that the commisural NTS receives unmyelinated and probably also small myelinated carotid body chemoreceptor fibres (Izzo *et al.*, 1987). Intracellular recordings from NTS cells monosynaptically activated by chemoreceptor afferents showed that these cells do not have respiratory rhythm, nor is the input modulated by the respiratory cycle or inflation of the lungs (Mifflin, 1993a), suggesting that respiratory modulation does not occur at this early stage of the reflex pathway. However, simultaneous baroreceptor input does attenuate chemoreceptor input at the level of the NTS (Mifflin, 1993b). From the NTS, the bradycardic component of the chemoreflex is thought to project through the nucleus ambiguus as in the baroreflex.

Stimulating chemoreceptors also increases BP, which is preceded by excitation of RVLM vasomotor neurones, which are subsequently inhibited by the rise in BP (Sun & Spyer, 1991). RVLM neurones excited by chemoreceptors were most likely to fire during the postinspiratory phase, demonstrating respiratory modulation of the sympathetic response (Koshiya *et al.*, 1993). Microinjection of kynurenic acid into the RVLM inhibits the chemoreflex sympathoexcitation, but not the baroreflex sympathoinhibition, whilst microinjection into CVLM caused the reverse (Koshiya *et al.*, 1993), suggesting that the sympathetic components of these two reflexes take different pathways. Chemoreflex sympathoexcitation is probably integrated by a population of convergent NTS neurones that are excited by chemoreceptor afferents and the hypothalamic defence area, and inhibited by baroreceptor afferents (Silva-Carvalho *et al.*, 1995), but it is unlikely that a direct NTS-RVLM projection is the only pathway mediating this sympathoexcitation. In anaesthetised rats, microinjection of kynurenic acid into the NTS abolishes the chemoreflex pressor response (Zhang & Mifflin, 1993), but in awake rats kynurenic acid and DNQX into the NTS only reduce the pressor response, although abolishing the bradycardia (Haibara *et al.*, 1999). Similarly, blockade of NTS NMDA receptors inhibits the bradycardia but not the hypertension in awake rats (Haibara *et al.*, 1995), but the latter effect is attenuated by electrolytic lesions of the PVN (Olivan *et al.*, 2001). Furthermore, kynurenic acid microinjected into the RVLM did not alter the pressor response in awake rats (Mauad & Machado, 2001), but microinjection of lignocaine into the parabrachial nucleus did attenuate it (Haibara *et al.*, 2002). Together these data suggest that the established glutamatergic circuits through the NTS and RVLM are not the only pathways taken by the sympathetic chemoreflex: either different transmitters, or different nuclei are involved, and the data would suggest that both are likely.

The probable pathways of the bradycardic and sympathetic components of the chemoreflex are illustrated in Figure 1.3. Modulation of chemoreflex signal is also illustrated in Figure 1.4, showing that inputs from the hypothalamic defence area enhance reflex responses by stimulating the NTS, as well as enhancing respiratory modulation, which is thought to occur *via* rhythmical excitation of CVPNs in the nucleus ambiguus by post-insiratory neurones (see Spyer, 1994)

1.4. Neuropharmacology of the NTS

The NTS contains an extensive range of endogenous transmitters and their receptors, many of which are involved in cardiovascular control. Whilst a comprehensive description of their physiology and pharmacology is beyond the scope of this thesis, the basic effects of locally applied agonists and antagonists is outlined below, with particular reference to reflex control.

γ-Aminobutyric acid

The NTS is known to contain large amounts of GABA (Dietrich *et al.*, 1982). Bilateral microinjection of GABA into the NTS causes an immediate hypertension and baroreflex inhibition *via* $GABA_A$ receptors (Catelli *et al.*, 1987; Kubo & Kihara, 1988a), and selective activation of $GABA_B$ receptors also has this effect (Lalley, 1980; Catelli *et al.*, 1987). $GABA_A$ receptors tonically inhibit baroreflex transmission, as microinjection of bicuculline potentiates aortic nerve evoked responses (Kubo & Kihara, 1988a). The finding that spontaneously hypertensive rats have a higher density of $GABA_B$ receptors in the NTS suggests a role in hypertension (see Mifflin, 2001). The majority of GABAergic effects at this level are believed to be mediated by intrinsic interneurones.

Glycine

Microinjection of glycine into the NTS can produce hypertension (Kubo & Kihara, 1987), or hypotension and bradycardia (Talman & Robertson, 1989), but the antagonist strychnine had no effect on the baroreflex (Talman & Robertson, 1989) suggesting that glycine is not part of the reflex arc. However, the depressor effects of glycine were blocked by microinjection of atropine (Talman *et al.*, 1991), suggesting that the glycine receptor is modulating acetylcholine release.

Acetylcholine

Cholinesterase is found in a population of NTS neurones (Helke *et al.*, 1983), and acetylcholine, like glycine, elicits bradycardia and hypotension when microinjected into the NTS, which is inhibited by atropine (Criscione *et al.*, 1983). Interestingly, in this study atropine alone attenuated the baroreflex when bilaterally microinjected, so a muscarinic receptor mechanism may be involved in the reflex itself. Vagotomy

reduced M_1 receptor binding sites in the NTS, suggesting a presynaptic location (Reynolds *et al.*, 1994). Microinjection of nicotine also reduces BP and HR (Tseng *et al.*, 1993), proposing an additional cardiovascular role for nicotinic receptors.

Nitric oxide

Nitric oxide synthase is found in the NTS (Vincent & Kimura, 1992), and microinjection of nitrosocysteine, a nitric oxide donor, into the NTS produces immediate bradycardia and hypotension *via* soluble guanylate cyclase activation (Lewis *et al.*, 1991b). Nitric oxide may also aid the transduction of cardiopulmonary afferent information (Lewis *et al.*, 1991a), suggesting release from afferents. However, *in vivo* microdialysis suggests nitric oxide increases glutamate efflux in the NTS (Lawrence & Jarrott, 1993), hence is a modulator rather than transmitter. Nitric oxide also appears to interact with other transmitters, for example angiotensin II, which inhibits the baroreflex in the NTS *via* release of endothelial nitric oxide (Paton *et al.*, 2001). On the single neurone level, the predominant effect of nitric oxide is to inhibit NTS neuronal discharge (Ma *et al.*, 1995; Wang & Jordan, 1998).

Adenosine

The highest density of adenosine uptake sites in the brain is found in the NTS (Bisserbe *et al.*, 1985). Microinjection of adenosine causes bradycardia and hypotension. A selective A_1 receptor agonist causes the same responses, whilst a selective A_2 receptor agonist causes hypertension (Barraco & Phillis, 1991). Antagonists of adenosine receptors such as caffeine inhibit baroreflex bradycardia (Mosqueda-Garcia *et al.*, 1989), so the baroreflex may require adenosine as an endogenous transmitter. A_2 receptor activation has also been shown to potentiate the bradycardia and reduce the hypotension evoked by the cardiopulmonary and baroreflexes (Carey & Jordan, 1998; Carey & Jordan, 1999).

Noradrenaline

The NTS receives innervations from noradrenaline-containing neurones in the locus coeruleus (McBride & Sutin, 1976) and from the A2 cell group within the NTS (Levitt & Moore, 1979). Dysfunction of these noradrenergic neurones may account for age-related loss of cardiovascular control and hypertension (Itoh *et al.*, 1992). Whether noradrenaline has a reflex role, however, is not entirely clear. 6-

hydroxydopamine induced noradrenergic lesions did not affect the baroreflex (Itoh *et al.*, 1992; Healy *et al.*, 1981), but NTS microinjection of the α_2 adrenoceptor antagonists yohimbine and idozoxan caused hypertension and baroreflex inhibition (Sved *et al.*, 1992). Lowering BP with glyceryl trinitrate enhances noradrenaline efflux in the NTS (Yamazaki & Ninomiya, 1993) supporting a reflex role for noradrenaline, although the action of nitric oxide produced by glyceryl trinitrate cannot be ruled out.

Dopamine

A population of dopamine containing neurones are found in the A2 cell group of the NTS (Armstrong *et al.*, 1982), and microinjection of dopamine into the NTS causes bradycardia and hypotension (Zandberg *et al.*, 1979), although high doses cause tachycardia and hypertension (Granata & Woodruff, 1982). Central administration of the D_2 receptor antagonist sulpiride has no effect on cardiovascular reflexes (Bogle *et al.*, 1990; Dando, 1995). During severe hypoxia, however, dopamine efflux increases in the NTS, which is preventable by carotid sinus nerve transection (Goiny *et al.*, 1991). This does not occur in mild hypoxia, suggesting an emergency role.

5-hydroxytryptamine

The cardiovascular roles of 5-HT are discussed in detail in 1.6 with respect to the individual receptor subtypes involved.

Peptides

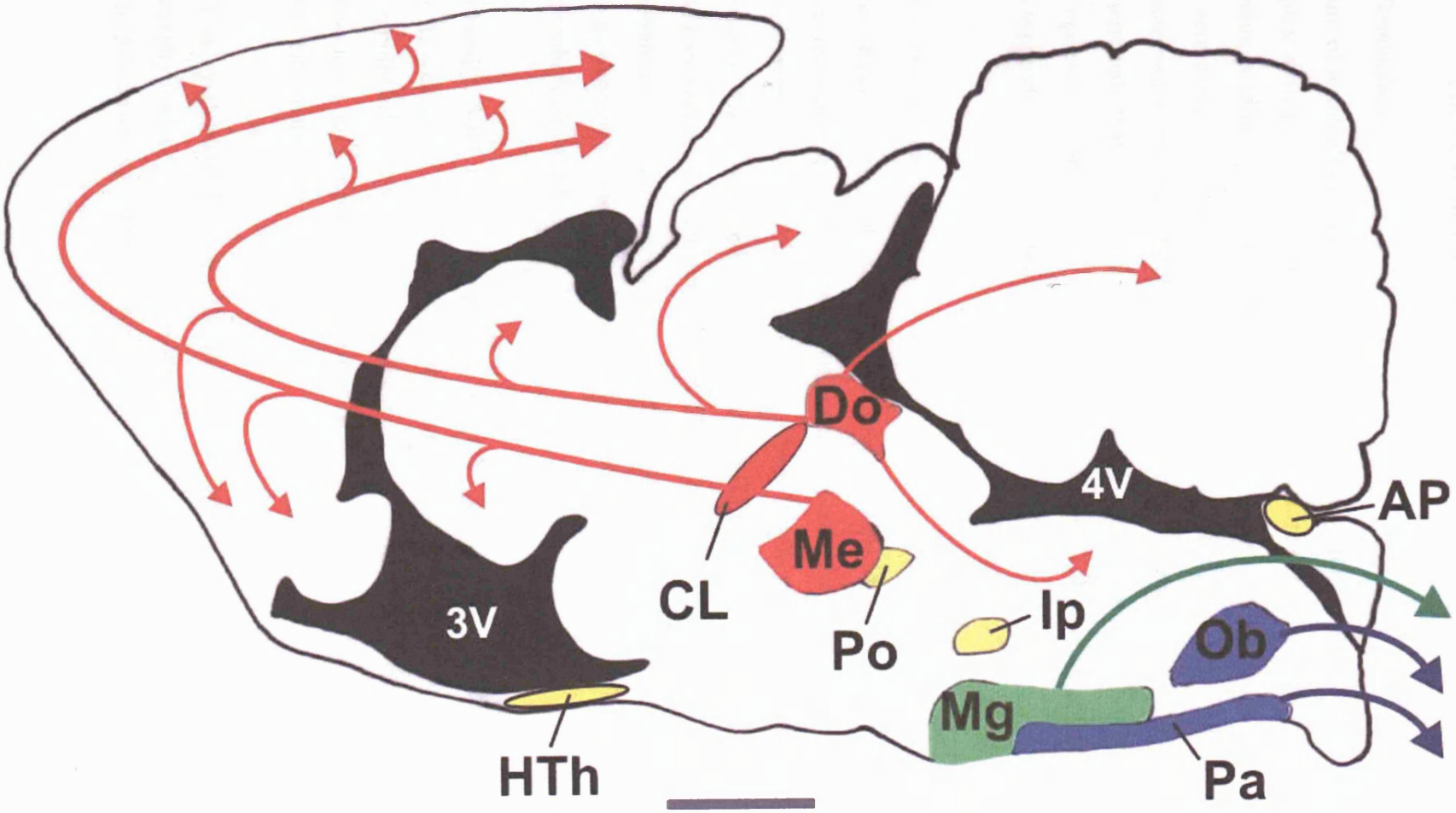
The NTS contains a variety of peptide transmitters, many of which cause cardiovascular changes when locally applied. These include substance P, neuropeptide Y, angiotensin II, vasopressin, cholecystokinin, bradykinin, somatostatin, opioid peptides, endothelin and neurotensin (see Lawrence & Jarrott, 1996).

Figure 1.5 Central 5-HT containing cell groups

Midline sagittal diagram of rat brain illustrating position of various 5-HT containing cell groups, and their major established projections. Colours are arbitrary, but yellow areas are the least understood.

Note: the size and shape of the cortex at this midline level has been expanded for illustration of projections, but the remainder is to scale (bar = 2 mm).

3V: Third ventricle
4V: Fourth ventricle
AP: Area postrema
CL: Caudal linear nucleus
Do: Dorsal raphe nucleus
HTh: Hypothalamic 5-HT containing neurones
Ip: Raphe interpositus nucleus
Me: Median raphe nucleus
Mg: Raphe magnus nucleus
Ob: Raphe obscurus nucleus
Pa: Raphe pallidus nucleus
Po: Raphe pontis nucleus


Do
4V
AP
Me
CL
3V
Ip
Po
Ob
Mg
HTh
Pa

1.5. The central serotonergic system

Terminology

A point of terminology should be clarified before attempting to describe this highly complex system. Neurotransmission mediated *via* release of 5-HT onto 5-HT receptors is termed 5-hydroxytryptaminergic, or *serotonergic* for simplicity. The term serotonergic will be used to describe neuronal pathways that are functionally characterised as involving 5-HT neurotransmission. Conversely, cell groups, fibres and terminals that are anatomically identified on the basis of 5-HT *content* (e.g. immunoreactivity or histofluorescence) will be termed *5-HT containing* when there is no supporting functional data.

5-hydroxytryptamine

The existence of a serum-borne factor affecting the tonus of blood vessels had been known for some time, hence the original name of *serotonin* (Rapport *et al.*, 1948). An unknown substance found in the gut, where it affected motility, was called *enteramine.* Later these were both identified as 5-HT (Amin *et al.*, 1954). 5-HT was located in varying amounts in different CNS structures, proposing its role as a neurotransmitter: an interesting proposition was that 5-HT and noradrenaline might serve as opposing central transmitters, much as acetylcholine and noradrenaline in the periphery (Brodie & Shore, 1957).

Peripherally, 5-HT is found in platelets and enterochromaffin cells throughout the body, as well as in the gastrointestinal tract, urinary system, kidneys, liver and heart. It is rapidly broken down by monoamine oxidase to produce 5-hydroxyindoleacetic acid, which in the periphery occurs primarily in the endothelial cells of the lungs and liver (Verbeuren, 1989).

5-HT is synthesised from the amino acid tryptophan, which is converted to 5-hydroxytryptophan by tryptophan hydroxylase (the rate limiting step), and then to 5-HT by 5-hydroxytryptophan decarboxylase.

1.5.2. 5-HT containing neurones

Anatomy

The location of 5-HT containing neurones was first demonstrated by the Falck-Hillarp histochemical fluorescence technique (Dahlstrom & Fuxe, 1961), revealing that the majority were in cell groups of the midline seam (*raphe*), first described in detail by Cajal in 1911 and characterised in detail thereafter (Cajal, 2000; Taber *et al.*, 1960). None of these authors had suspected that these neurones shared a common transmitter. Although 5-HT containing neurones comprise only a very small proportion of CNS neurones, in the rat it is estimated that there are about 6×10^6 5-HT containing varicosities per mm^3 of cortex (Audet *et al.*, 1989).

The atlas of Paxinos & Watson (1998) provides a general consensus on the anatomy of rat raphe nuclei, and the delineations are shown in Figure 1.5. The caudal linear nucleus is the most rostral group of 5-HT containing neurones, extending from the rostral tip of the dorsal raphe nucleus to the dorsal aspect of the interpeduncular nucleus. At its caudal end it is bordered ventrally by the decussation of the superior cerebellar peduncles. The caudal linear nucleus contains small to medium sized neurones, with the largest proportion of 5-HT containing neurones in the caudal portion (Steinbusch & Nieuwenhuys, 1983), although many dopamine-containing neurones belonging to the A10 cell group are also found in the caudal linear nucleus (Swanson, 1982).

The dorsal raphe is the largest raphe nucleus, and has received the most research interest. It extends from the level of the pons to the oculomotor nucleus, and the majority of its neurones are in the midbrain, dorsal to the median longitudinal fasciculi, but also ventrally between these fibre tracts. Laterally the dorsal raphe nucleus is difficult to differentiate from the periaqueductal grey. Various neuronal types have been characterised in dorsal raphe: small spherical, medium fusiform and large multipolar (Danner & Pfister, 1980), later arranged into Type 1, 2 and 3 neurones depending on their morphology – a classification that applied to other raphe areas too (Holzel & Pfister, 1981). Whilst dorsal raphe contains about half of all

central 5-HT containing neurones, it is estimated that only 40 – 50 % of dorsal raphe neurones contain 5-HT (Wiklund *et al.*, 1981; Descarries *et al.*, 1982). Noradrenaline containing neurones are found in the caudolateral part (Steinbusch, 1981), and dopamine containing neurones near the midline (Ochi & Shimizu, 1978). GABA-containing neurones are found throughout dorsal raphe, which can be divided into those sensitive to the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), and those that are insensitive, suggesting that one population is colocalised with 5-HT (Nanopoulos *et al.*, 1982; Belin *et al.*, 1983). A number of peptides are also found in dorsal raphe, such as substance P which coexists with 5-HT (Hokfelt *et al.*, 1978), and cholecystokinin in an independent neuronal population (Innis *et al.*, 1979).

The median raphe nucleus is found ventrally and caudally to dorsal raphe, and runs between the tectospinal tracts, with the raphe pontis nucleus at its caudal end. Only a minority of neurones contain 5-HT (Wiklund *et al.*, 1981). Three types of neurone with similar morphological characteristics to dorsal raphe are found in median raphe (Holzel & Pfister, 1981). Raphe pontis is a small nucleus continuing caudally from median raphe to the level of the raphe magnus nucleus, and its cells are not well differentiated from those of the underlying reticular tegmental nucleus.

Raphe magnus is a long and wide nucleus, forming a characteristic triangular lateral extension into the gigantocellular reticular nucleus and ventrolateral medulla, and contains a seemingly random scattering of additional 5-HT containing neurones, although the majority of raphe magnus 5-HT neurones are close to the midline. Raphe magnus extends from the facial nucleus rostrally, and joins into the raphe obscurus nucleus caudally, with the raphe pallidus nucleus running along most of its ventral surface, between the pyramids. Raphe magnus cytoarchitecture is different from the rostral raphe, as the cells are larger and more loosely distributed, with many neurones arranged at right angles to the midline, their dendrites extending far into the surrounding reticular formation. Approximately 50 % of raphe magnus neurones contain 5-HT, whilst 25 % are positive for substance P and thyrotropin releasing hormone; in some cases all three transmitters coexist in the same neurone (Johansson *et al.*, 1981). Other peptides located in raphe magnus include enkephalin (Hokfelt *et*

al., 1979), somatostatin (Finley *et al.*, 1981) and cholecystokinin (Kubota *et al.*, 1983).

Raphe obscurus continues from raphe magnus caudally in two paramedian columns remaining very close to the midline, and reaching the greatest dorsoventral height at the level of the obex. Raphe obscurus is flanked by reticular formation and by the inferior olive ventrally. The majority of neurones contain 5-HT, and are medium to large in size, with dendrites extending both along the midline and laterally (Steinbusch & Nieuwenhuys, 1983). Other transmitters such as substance P, thyrotropin releasing hormone and cholecystokinin are found here as is raphe magnus.

Raphe pallidus is a thin column of cells on the ventral surface of the medulla, flanked by the pyramids. It makes contact with raphe obscurus caudally and raphe magnus rostrally, and is sometimes interpreted as a continuation of these cell groups (Taber, 1961), although raphe pallidus neurones are very compactly arranged, consisting of small, medium and large cells. 5-HT containing neurones are amongst the medium and large type (Steinbusch & Nieuwenhuys, 1983), and again various peptides coexist.

Cell bodies in the hypothalamus capable of accumulating 5-HT that had been injected intracerebroventricularly (i.c.v.) were described some time ago (Fuxe & Ungerstedt, 1968). 5-HT immunoreactive neurones in the dorsomedial hypothalamic nucleus was subsequently confirmed (Frankfurt *et al.*, 1981), but only in rats pretreated with both L-tryptophan and the monoamine oxidase inhibitor pargyline. These neurones are small in size and in number, and their connections are unknown. They may be a group of small local neurones, and whilst they are difficult to reveal, their existence may explain why the 5-HT content of the hypothalamus is only reduced but not abolished by surgical lesioning of its brainstem afferents (Palkovits *et al.*, 1977).

A small scattering of 5-HT containing neurones is found lateral to raphe magnus, and also in the vicinity of the locus coeruleus, the dorsal tegmental nucleus, and the interpeduncular complex (Steinbusch, 1981). In the area postrema on the dorsal

surface of the medulla is a further population of very small 5-HT containing neurones, although little is known of their connections (Steinbusch, 1981). Finally, there is one report of a population of 5-HT immunoreactive neurones in the medial nucleus of the rat NTS (Calza *et al.*, 1985) but only in i.c. colchicine pretreated animals. Also, older (24 month old) animals showed greater numbers of these neurones. The implications are unclear, nor has the observation been confirmed elsewhere.

Connections

The major ascending projections from dorsal and median raphe have been clearly demonstrated to project to nearly all rostral brain areas ipsilaterally *via* the medial forebrain bundle, where they travel parallel to numerous noradrenaline containing fibres (Takagi *et al.*, 1980a). This bundle contains both myelinated and unmyelinated 5-HT immunoreactive fibres in the rat and monkey, with more myelination in the monkey (25 % *vs.* 1 % in the rat) (Azmitia & Gannon, 1983). The hippocampus receives afferents from dorsal and median raphe, the latter travelling in the cingulum bundle and fornix (Azmitia & Segal, 1978b). The hypothalamic medial preoptic area, suprachiasmatic nucleus and anterior area 5-HT content was reduced by dorsal but not median raphe lesions, whereas anterolateral and arcuate nucleus 5-HT content was also reduced by dorsal raphe lesion (Van De Kar & Lorens, 1979). Both dorsal and median raphe also project to various parts of the thalamus (Azmitia & Segal, 1978a), septum (Swanson & Cowan, 1979), and midbrain (Steinbusch, 1981) whereas the main projection to the caudate-putamen is from dorsal raphe (Jacobs *et al.*, 1978). Dorsal and median raphe also project to pial blood vessels, suggesting a role in the pathogenesis of migraine (Edvinsson *et al.*, 1983)

Early studies failed to find clear ultrastructural evidence of synapses between monoamine-containing fibres and cortical neurones, and described the mode of transmission as diffuse and non-specific (Descarries *et al.*, 1975; Beaudet & Descarries, 1976), fitting with an older concept of the brain as a neuroendocrine organ, where neurosecretion modulated general brain activity. Since then, however, specific synaptic connections have been well established (see Parnavelas & Papadopoulos, 1989), with axons selectively innervating certain types of cat cortical

neurones (Mulligan & Tork, 1988). In monkey visual cortex, 5-HT containing fibres preferentially innervate layer IV, whilst noradrenaline containing fibres are predominantly in layers V and VI (Morrison *et al.*, 1982).

In the hindbrain, the locus coeruleus receives afferents from many raphe areas including dorsal, median and raphe magnus (Cedarbaum & Aghajanian, 1978; Morgane & Jacobs, 1979). Brainstem motor nuclei, especially the facial and trigeminal motor nuclei, receive 5-HT containing synapses (Aghajanian & McCall, 1980; Schaffar *et al.*, 1984), although retrograde labelling failed to find a projection from any raphe areas (Travers & Norgren, 1983). The RVLM is also innervated by 5-HT containing neurones from dorsal raphe (Underwood *et al.*, 1999). The cerebellum receives a sparse innervation of 5-HT containing fibres (Takeuchi *et al.*, 1982).

The caudal or medullary raphe (magnus, obscurus, pallidus) have predominantly descending projections that are well-characterised. 5-HT immunoreactivity is found in the rat spinal cord (chiefly in substantia gelatinosa and IML); injection of HRP into the spinal cord retrogradely labels neurones in all medullary raphe areas, many of which contain 5-HT; lesion of the dorsolateral funiculus inhibits raphe labelling (Bowker *et al.*, 1982). Many 5-HT containing neurones in raphe magnus and pallidus projecting to the spinal cord also express enkephalin (Millhorn *et al.*, 1989), and in the guinea pig either somatostatin, substance P, enkephalin or thyrotropin releasing hormone (Chiba & Masuko, 1989). Anterograde transport of *phaesolus vulgaris* leucoagglutinin from rat raphe magnus/pallidus also labelled the C1 to C3, A1 and A2 catecholamine cell groups, with close appositions of 5-HT containing varicosities at neurones of the C1 group (Nicholas & Hancock, 1990), supporting a body of evidence for the interaction of monoamine systems.

The density of 5-HT containing fibres in the NTS has been widely reported (Steinbusch, 1981; Maley & Elde, 1982; Pickel *et al.*, 1984). Retrograde labelling reveals 5-HT containing neurones in dorsal raphe and raphe magnus (Schaffar *et al.*, 1988) as well as in raphe obscurus and pallidus that project to the NTS (Thor & Helke, 1987). Some of the 5-HT containing fibres in the NTS, however, are the

terminals of primary afferents from the nodose ganglia (Gaudin-Chazal *et al.*, 1982; Nosjean *et al.*, 1990; Sykes *et al.*, 1994).

5-HT and substance P immunoreactive fibres form a dense network around respiratory muscle motoneurones in the nucleus ambiguus, and the ventral respiratory group receives an innervation from the medullary raphe (Holtman, 1988; Holtman *et al.*, 1990a). Further examination found clear and frequent synaptic contacts between 5-HT containing terminals and phrenic motoneurones in the cat (Pilowsky *et al.*, 1990; Holtman *et al.*, 1990b).

Of the numerous other anatomical connections of the raphe, raphe obscurus projects to pudendal motoneurones (Hermann *et al.*, 1998), suggesting a role in pelvic floor autonomic and motor functions, and raphe magnus also projects to the septum, and together with raphe obscurus (but not pallidus) to the hypothalamus (Takagi *et al.*, 1980b). However others report that raphe pallidus has a reciprocal connection with the dorsal hypothalamus, which contains asymmetrical (excitatory) synapses from raphe pallidus (Hosoya, 1985; Hosoya *et al.*, 1989). In fact, the majority of afferents to the medullary raphe come from the dorsal hypothalamus and periaqueductal grey (Hermann *et al.*, 1997).

The area postrema sends a 5-HT containing projection to the parabrachial area in rats (Lanca & van der Kooy, 1985), as well as to the NTS, DVN and nucleus ambiguus (Shapiro & Miselis, 1985), suggesting it may modulate the processing of visceral afferent information.

Functions

The neuronal activity of dorsal raphe is believed to play a critical role in regulating mood, sleep, learning and memory (see Graeff *et al.*, 1996); some of the effects of manipulating rostral raphe neuronal activity on these functions is discussed in 1.6 below. On the autonomic side, electrical stimulation of dorsal or median raphe increases BP in anaesthetised rats, which was attenuated by pretreatment with the 5-HT synthesis inhibitor para-chlorophenylalanine (p-CPA) (Kuhn *et al.*, 1980). Electrical or chemical stimulation (with DL-homocysteic acid; DLH) of dorsal raphe reduces carotid blood flow in cats (Goadsby *et al.*, 1985). Furthermore, electrical

stimulation of rostral dorsal raphe reduces cerebral blood flow, whereas stimulation of the caudal part of the nucleus increases cerebral blood flow (Underwood *et al.*, 1992) Electrical stimulation of any raphe area must be interpreted with care, as it is possible to activate fibres of passage, many of which cross at the midline. Hence chemical stimulation is often used to confirm that cell bodies are activated. Chemical stimulation with kainate but not glutamate increased cerebral blood flow (Underwood *et al.*, 1995).

The medullary raphe have a number of established roles, especially the control of descending antinociception by raphe magnus (see Mason, 2001), although raphe obscurus may also be involved in this function (Dantas *et al.*, 1990). Raphe magnus and pallidus also control skin blood flow (Nalivaiko & Blessing, 2001), and the activity of raphe neurones is modulated by skin temperature (Dickenson, 1977; Rathner *et al.*, 2001). An autonomic role of the medullary raphe was first described in the 1970s (Coote & Macleod, 1974), and reported evoking a sympathoinhibition. Depletion of endogenous 5-HT, however, caused no change in BP or sympathetic nerve activity, suggesting that 5-HT is not crucially required (Coote *et al.*, 1978).

Since then, the autonomic roles of the medullary raphe have received much attention, although there is considerable disagreement. In cats, electrical stimulation causes a rise or fall in BP depending on which area is stimulated, although there was little clear pattern (Adair *et al.*, 1977). In rabbits there were similarly varied responses to electrical stimulation, with a pressor responses accompanied by tachycardia at dorsal sites, and a pressor/bradycardic response at ventral sites, the bradycardia being mainly reflexive (Haselton *et al.*, 1988). The concurrent bradycardia was also seen in cats (Lalley, 1986) and rats (from raphe obscurus) (Dreteler *et al.*, 1991). Chemical stimulation, however, can cause pressor (Dreteler *et al.*, 1991) or depressor (Bernard, 1998) responses without heart rate changes, or depressor with bradycardia (Coleman & Dampney, 1995) in rats. Effects on the sympathetic system are also varied: the raphe can mediate sympathoexcitation in anaesthetised rats (Nalivaiko & Blessing, 2001; Zhou & Gilbey, 1995), and rabbits (Blessing *et al.*, 1999), and sympathoinhibition in anaesthetised cats (Coote *et al.*, 1987; Gilbey *et al.*, 1981) and rats (Coleman & Dampney, 1995).

The caudal raphe also modulate central respiratory drive: raphe stimulation increases (Holtman *et al.*, 1986c; Haxhiu *et al.*, 1998) or decreases (Lalley *et al.*, 1997) phrenic nerve firing in cats, and increases it in rats (Bernard, 1998; Dreteler *et al.*, 1991). The raphe may also be involved in chemosensing in piglets (Dreshaj *et al.*, 1998) and rats (Bernard *et al.*, 1996).

Few studies have concentrated on parasympathetic raphe roles: medullary raphe stimulation reduces parasympathetic outflow to the airways in cats (Haxhiu *et al.*, 1998). Also electrical and chemical stimulation of raphe obscurus increases gastric motility in anaesthetised rats (McCann *et al.*, 1989), which is thought to occur *via* raphe activation of vagal preganglionic neurones in the DVN; another study reports that electrical stimulation of raphe obscurus augments distal bowel motility in anaesthetised rats *via* direct activation of sacral spinal parasympathetic neurones (Holmes *et al.*, 1997). As concerns cardiovascular reflexes, chemical stimulation of raphe pallidus can attenuate the cardiopulmonary reflex in anaesthetised rats (Edwards & Paton, 2000), whereas chemical stimulation of raphe obscurus attenuates chemoreflex bradycardia in conscious rats (BH Machado, unpublished data).

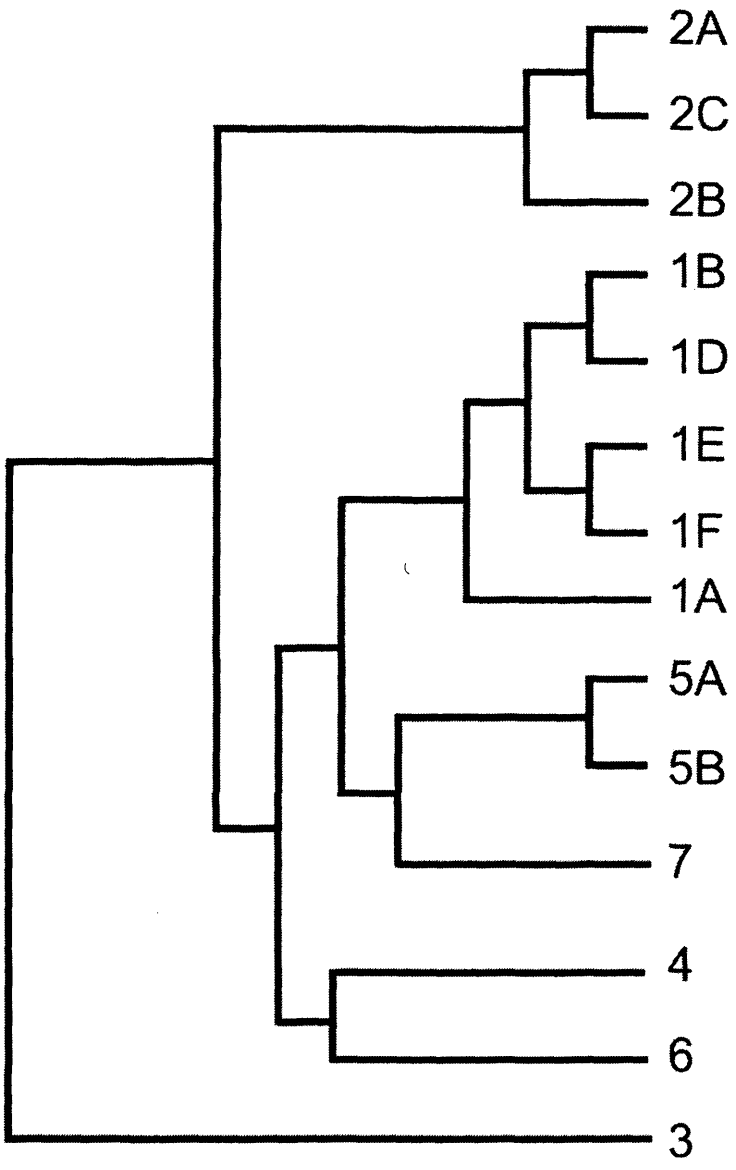


Figure 1.6 Evolution of 5-HT receptors

Dendrogram (adapted from Barnes & Sharp, 1999) illustrating evolutionary relationship between different 5-HT (1A – 7) receptor amino acid sequences (all from human except 5A and 5B, which are murine in origin).

1.6. 5-HT receptors

1.6.1. Classification

The effects of 5-HT are mediated by a variety of receptors, only one of which is a ligand-gated ion channel (5-HT_3), and the rest comprise a superfamily of at least 13 G protein-coupled receptors (see Figure 1.6). 5-HT receptors were first divided into M and D receptors by Gaddum & Picarelli (1957), and later into 5-HT_1 and 5-HT_2 receptors by Peroutka & Snyder (1979). In 1986, the M receptor was renamed 5-HT_3 by Bradley and colleagues. During the next few years the 5-HT_1 receptor began to be subdivided, whilst other putative 5-HT receptors were discovered and variously named, leading to the generally accepted reclassification by the International Union of Pharmacology in 1994 (Hoyer *et al.*, 1994). The molecular, functional, and pharmacological properties of 5-HT receptors continue to be regularly and extensively reviewed (see Barnes & Sharp, 1999; Hoyer *et al.*, 2002).

5-HT receptors are often classified as autoreceptors or heteroreceptors, referring to the neurochemical phenotype of the neurone expressing them. Autoreceptors are located on serotonergic neurones, and heteroreceptors on non-serotonergic neurones. Either type of receptor can be found either presynaptically, modulating transmitter release, or post-synaptically, modulating somatic membrane potential.

Whilst a comprehensive review of 5-HT receptors is beyond the scope of this thesis, the receptors targetted in later chapters are described in some detail, whilst other receptors are given an overview appropriate to their known contribution to autonomic functions.

1.6.2. Binding of 5-HT to its receptors

5-HT binds to its receptors with varying affinities. In human cloned receptors, 5-HT receptors can be grouped as follows. Receptors with relatively low affinity for 5-HT (pK_i 6 – 7) are 5-HT_{2A}, 5-HT_3, 5-HT_4, 5-ht_{5A} and 5-HT_6. Receptors with moderate affinity (pK_i 7 – 8) are 5-ht_{1E}, 5-HT_{1F}, 5-HT_{2B} and 5-HT_{2C}. Receptors with higher affinity (pK_i 8 – 9) are 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D} and 5-HT_7 (Roberts *et al.*, 2001b; Kilpatrick *et al.*, 1989). These variations suggest a hierarchy of different roles, with

some receptors active during normal and some only during high concentrations of 5-HT.

1.6.3. 5-HT_{1A} receptor

Molecular biology

Prior to its full molecular characterisation, the existence of the 5-HT_{1A} receptor was first inferred in a study describing two populations of 5-HT receptors in the rat brain, one with high affinity for spiperone in the cortex, and one with low affinity in the striatum (Pedigo *et al.*, 1981), later identified as 5-HT_{1A} and 5-HT_{1B} receptors respectively. Since then, the 5-HT_{1A} receptor has become the most intensively studied of all 5-HT receptors. An intronless gene with similarity to the β_2 adrenoceptor was first identified when the human genome was screened with a β_2 clone (Kobilka *et al.*, 1987), located on human chromosome 5 (q11.2 – 13), and later named as the 5-HT_{1A} receptor gene (Fargin *et al.*, 1988). Subsequently the receptor was cloned in the rat, as a 422 amino acid protein comprising 7 transmembrane spanning domains with sites for glycosylation and phosphorylation, having 89 % sequence homology with the human form (Albert *et al.*, 1990). Activation of the cloned receptor reduced cyclic adenosine monophosphate (cAMP) production. Also, 3 different messenger ribonucleic acid (mRNA) products of the gene were described in the rat (but not in the human), suggesting that different transcriptional or polyadenylation start sites might exist in the rat gene (Albert *et al.*, 1990).

The 5-HT_{1A} receptor is negatively coupled to adenlyate cyclase (Albert *et al.*, 1990; Pauwels *et al.*, 1993); agonist-induced inhibition of cAMP accumulation rapidly desensitises (van Huizen *et al.*, 1993). The receptor is also thought to couple directly to K^+ channels without a soluble intracellular messenger, at least in the hippocampus (Andrade *et al.*, 1986). The $G_{i\alpha}$ protein itself can directly activate inwardly rectifying K^+ channels independent of lowered cAMP (Codina *et al.*, 1987), so this direct coupling may be a significant *in vivo* signalling method.

Distribution

The 5-HT_{1A} receptor was first pharmacologically characterised as a binding site for the archetypal agonist 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT)

(Middlemiss & Fozard, 1983), and brain distribution of 5-HT_{1A} receptors was first described using [^{3}H]-8-OH-DPAT (Gozlan *et al.*, 1983), with the highest signal in the hippocampus. mRNA expression is highest in septum and hippocampus (Albert *et al.*, 1990). High mRNA is also described in subiculum, entorhinal cortex, raphe, dorsomedial and ventromedial hypothalamus, with lower signal in all layers of cerebral cortex, NTS, hypoglossal nucleus, spinal trigeminal nucleus, and the least signal in thalamus and cerebellum (Wright *et al.*, 1995). Combined immunocytochemistry, *in situ* hybridisation and radioligand binding describes all 3 signals in entorhinal cortex, dorsal raphe and hippocampus, with less intensity in the cortex (Miquel *et al.*, 1991). Many older papers describe 5-HT_1 binding sites, most of which correspond to known 5-HT_{1A} receptor sites: high density binding sites are found in the rat hippocampus, septum, dorsal raphe, with less in amygdala, substantia nigra, hypothalamus, and some in cortex, thalamus, basal ganglia, NTS, spinal trigeminal nucleus, nucleus ambiguus, and spinal cord. Virtually no binding is reported in cerebellum (Pazos & Palacios, 1985). Within the brainstem of the cat, 8-OH-DPAT binding sites have reported in NTS, DVN, nucleus ambiguus, and medullary raphe (Dashwood *et al.*, 1988).

In the human brain, highest densities are reported in layer II of cortex and area CA1 of hippocampus (Hoyer *et al.*, 1986). Autoradiography has also been performed using the more selective radioligand [^{3}H]WAY-100635 (therefore not necessitating masking compounds for non-selective bindings, as is required with [^{3}H]-8-OH-DPAT); in the human brain, [^{3}H]WAY-100635 has been useful in characterising cloned receptors (Khawaja *et al.*, 1997) and in confirming high receptor densities in hippocampus, raphe, neocortex, amygdala and septum, and low density in brainstem and cerebellum (Hall *et al.*, 1997). This distribution has been confirmed in the human brain *in vivo* using positon emission tomography (PET) with ^{11}[C]-WAY-100635 (Pike *et al.*, 1995).

5-HT_{1A} receptors in the dorsal raphe are thought to be somatodendritic autoreceptors with a high receptor reserve (Meller *et al.*, 1990), causing inhibition of raphe neuronal firing (Aghajanian *et al.*, 1968; Blier & de Montigny, 1987), whereas in the hippocampus the receptors are postsynaptic, with no such receptor reserve (Yocca *et al.*, 1992). This is supported by serotonergic lesions with 5,7-DHT abolishing

receptor expression in the dorsal raphe but not the hippocampus (Miquel *et al.*, 1992), indicating that raphe 5-HT_{1A} receptors are autoreceptors.

Pharmacology

5-HT binds to 5-HT_{1A} receptors with a pK_i of 8.8 in rat and human cortex (Hoyer *et al.*, 1985b; Hoyer *et al.*, 1986). Of the substituted tryptamines, 5-carboxamidotryptamine (5-CT) binds with a pK_i of 8 (Lovenberg *et al.*, 1993a) Several other classes of agonists for the 5-HT_{1A} receptor have been synthesised: the aminotetralins, including the archetypal agonist 8-OH-DPAT, with a pK_i of 8.5 in rat (Mellin *et al.*, 1991) and cloned human receptors (Millan *et al.*, 2000), although this also binds to 5-$HT_{1B/1D}$ and 5-HT_7 receptors (see 1.6.4 and 1.6.11 below). Another common full agonist is flesinoxan (pK_i 8.1) (Boess & Martin, 1994). The arylpiperazines are a class of partial agonists, of which the best known is buspirone, which has pK_i of 7.6 in rat cortex (Gozlan *et al.*, 1988) but also binds to dopamine D_2 and α_1 adrenoceptors with a pK_i of 7.4 and 6.2 respectively (see van Wijngaarden *et al.*, 1990). Similar drugs with comparable affinities are ipsapirone, tandospirone and zalospirone (Abou-Gharbia *et al.*, 1988; Hamik *et al.*, 1990).

Various non-selective antagonists have been used, including (with pK_i in rat brain) the antipsychotics spiperone (7.6) (Hoyer *et al.*, 1985b) and methiothepin (7.4) (Fozard *et al.*, 1987) and the β adrenoceptor antagonists (-)-pindolol (7.8) (Hoyer *et al.*, 1985b) and propranolol (6.6) (Gozlan *et al.*, 1988), although both spiperone and methiothepin display inverse agonist properties (McLoughlin & Strange, 2000). More recently, a number of selective arylpiperazine antagonists have been developed, including NAN-190 (pK_i 9.2; rat hippocampus) (Millan *et al.*, 1993), which also blocks dopamine D_2 and α_1 adrenoceptors, and NAD-299 (robalzotan; pK_i 9.2; rat hippocampus) with pK_i of only 6.6 at α_1 adrenoceptors (Johansson *et al.*, 1997). Perhaps the most widely used selective antagonist is WAY-100635 (Forster *et al.*, 1995), with a pK_i of 9.6 at rat cortical 5-HT_{1A} receptors, and of 7.3 and 7.1 at α_1 and D_2 respectively (Johansson *et al.*, 1997).

General physiology

In brain slices, activation of 5-HT_{1A} receptors causes hyperpolarisation of neuronal membranes in all areas studied, including hippocampus (Andrade & Nicoll, 1987;

Van den Hooff & Galvan, 1991), prefrontal cortex (Araneda & Andrade, 1991a), septum (Van den Hooff & Galvan, 1992) and dorsal raphe (Haj-Dahmane *et al.*, 1991). In anaesthetised rats, dorsal raphe neuronal firing was first reported to be inhibited by iontophoretic lysergic acid diethylamide (LSD) (Aghajanian *et al.*, 1972; Haigler & Aghajanian, 1974), and then by 8-OH-DPAT, which was blocked by (-)-propranolol (Sprouse & Aghajanian, 1986) and WAY-100635 (Wang *et al.*, 1995). Intravenous (i.v.) 8-OH-DPAT also inhibits dorsal raphe firing in awake cats, an effect which is blocked by spiperone, which by itself increases firing (Fornal *et al.*, 1994), suggesting that 5-HT_{1A} autoreceptors are tonically activated. This was also seen in anaesthetised rats, using WAY-100635 given iontophoretically (Wang *et al.*, 1995) or systemically (Gartside *et al.*, 1995), albeit to a lesser extent than in awake animals (Fornal *et al.*, 1996). Hence systemic 5-HT_{1A} receptor agonists would be expected to have a dual effect: firstly by inhibiting raphe firing and hence decreasing release at terminals; secondly by activating 5-HT_{1A} receptors on non-serotonergic neurones. Antagonists on the other hand should facilitate 5-HT release.

Systemic administration of non-selective 5-HT receptor agonists tends to produce a cluster of behavioural signs in rats (Jacobs, 1976) known as 5-HT syndrome or 5-HT related stereotyped behaviour. Typically this includes many of the following: lower-lip retraction, flat body posture, hindlimb abduction, forepaw treading, head weaving and wet dog shakes. Of these, lower lip retraction is the behaviour most closely attributed to activation of 5-HT_{1A} receptors, although a delicate interaction of other 5-HT receptor subtypes is also thought to be involved (Berendsen *et al.*, 1989). A further behaviour linked to 5-HT_{1A} receptor activation is feeding, since 8-OH-DPAT, at lower doses than those that cause head weaving and other behavioural disturbances, induces feeding (Dourish *et al.*, 1985a; Dourish *et al.*, 1985b). Likewise, microinjection of 8-OH-DPAT into dorsal raphe increased feeding (Bendotti & Samanin, 1986; Fletcher & Davies, 1990), again pointing to the autoreceptor as the mediator of this physiological role.

There has been considerable interest in 5-HT_{1A} receptor ligands in the treatment of depression and anxiety: learned helplessness – an animal model of depression – is reversed by 8-OH-DPAT and buspirone (Martin *et al.*, 1990) *via* activation of 5-HT_{1A} receptors in the septum, but not the dorsal raphe. Conversely, the anxiolytic

effects of buspirone relate to its actions on raphe neuronal firing, which is also inhibited by anxiolytic doses of benzodiazepines (Trulson *et al.*, 1982): microinjection of buspirone (Carli *et al.*, 1989) or 8-OH-DPAT (File *et al.*, 1996) into the median raphe has anxiolytic effects. However, in dorsal hippocampus 8-OH-DPAT has anxiogenic effects (File *et al.*, 1996), and the same results are reported elsewhere for microinjecting into *dorsal* raphe (Romaniuk *et al.*, 2001). Suffice it to say that anxiolysis appears autoreceptor-mediated, whereas anxiogenesis is heteroreceptor-mediated, and this may account for idiosyncratic anxiety caused by buspirone in some patients, at least initially.

A variety of antidepressant drugs (paroxetine, venlafaxine and clomipramine) inhibit dorsal raphe activity in anaesthetised rats, which is reversed by WAY-100635 (Gartside *et al.*, 1997). Since the efficacy of serotonergic antidepressants is thought to relate to their ability to increase extracellular concentrations of 5-HT, there has been some interest in adjunctive treatment with a 5-HT_{1A} receptor antagonist, to prevent the initial inhibition of raphe activity caused by antidepressants, and possibly reduce the latency of the therapeutic effect (see Kinney *et al.*, 2000). Clinical trials with an antidepressant plus pindolol have not, however, produced clear results (Isaac *et al.*, 2003; Perry *et al.*, 2004).

5-HT_{1A} receptors have various influences on neurotransmitter release. Firstly, using *in vivo* microdialysis, activation of 5-HT_{1A} receptors decreases efflux of 5-HT in the forebrain, probably *via* activation of raphe autoreceptors (Sharp & Hjorth, 1990; Hjorth *et al.*, 1995; Sharp *et al.*, 1996). Conversely, 5-HT_{1A} receptor antagonists have been demonstrated to facilitate the ability of 5-HT reuptake inhibitors (Invernizzi *et al.*, 1992; Hjorth & Sharp, 1993; Gartside *et al.*, 1995), monoamine oxidase inhibitors and tricyclic antidepressants (Romero *et al.*, 1996; Artigas *et al.*, 1996), to increase 5-HT efflux in the forebrain. Again, this is thought to reflect the antidepressant-evoked inhibition of raphe firing by increased synaptic concentrations of 5-HT in these nuclei, and the blockade by antagonists of these autoreceptors, removing the antidepressant-evoked inhibition.

Secondly, 8-OH-DPAT also increases the efflux of acetylcholine in the cortex of rats (Consolo *et al.*, 1996) and guinea pigs (Bianchi *et al.*, 1990; Wilkinson *et al.*, 1994).

5-HT_{1A} receptors are expressed in cholinergic cell bodies in the septum - an area that projects to the cortex. But given that these are inhibitory receptors, it is not yet understood how they increase release. Thirdly, 8-OH-DPAT also increases efflux of noradrenaline in hypothalamus (Suzuki *et al.*, 1995), ventral tegmental area (Chen & Reith, 1995), and hippocampus (Done & Sharp, 1994), and this effect is sensitive to WAY-100635 (Hajos-Korcsok & Sharp, 1996), but insensitive to 5,7-DHT lesions or 5-HT depletion with p-CPA (Suzuki *et al.*, 1995; Hajos-Korcsok *et al.*, 1999), suggesting that 5-HT_{1A} heteroreceptors mediate the modulation of noradrenergic transmission.

Autonomic functions

In respect of autonomic functions, systemic 8-OH-DPAT causes hypotension and vagal bradycardia with sympathoinhibition in anaesthetised cats (Fozard & Ramage, 1984; McCall *et al.*, 1987; Ramage & Fozard, 1987), but the antagonist WAY-100802 alone had no effect (Ramage & Mirtsou-Fidani, 1995), indicating that these 5-HT_{1A} receptors are not tonically activated. A similar bradycardia and hypotension is reported in normal and spontaneously hypertensive rats (Gradin *et al.*, 1985; Martin & Lis, 1985; Fozard *et al.*, 1987), rabbits (Hof & Fozard, 1989; Shepheard *et al.*, 1990) and conscious dogs (Di Francesco *et al.*, 1988). The hypotensive effects in awake animals are thought to be less profound due to behavioural alerting. Repeated doses of 8-OH-DPAT do not produce tolerance to the hypotensive effect (Kolbasa *et al.*, 1991). The agonist flesinoxan is also hypotensive, and the i.c. or intra-vertebral arterial route of administration produce greater hypotension than the i.v. route (Wouters *et al.*, 1988); furthermore i.c. administration is more hypotensive than i.c.v. (Mir & Fozard, 1987; Wouters *et al.*, 1988), and together these data point to a medullary location of sympathoinhibitory 5-HT_{1A} receptors. Also, 5,7-DHT lesions abolish the hypotensive effect, whereas depletion of 5-HT stores does not (Mir & Fozard, 1987), suggesting that intact serotonergic fibres, but not 5-HT stores, are required for this effect. Pretreatment with the antagonist idazoxan also confirmed that α_2 adrenoceptors are not involved in the hypotensive effect of 8-OH-DPAT (Mir & Fozard, 1987). Investigation of the site of action tested microinjection of 8-OH-DPAT and flesinoxan into raphe obscurus, which actually produced a pressor response, as did glutamate (Dreteler *et al.*, 1991). The preferred site of action is the RVLM, as supported by ventral surface application (Gillis *et al.*, 1989) and

microinjection (Laubie *et al.*, 1989) of 8-OH-DPAT. Pressor effects, however, are also seen when 5-HT_{1A} receptors are activated *via* the i.c.v. route, producing sympathoexcitation (Anderson *et al.*, 1992), possibly by activating raphe obscurus autoreceptors, although in this case a hypothalamic target such as the preoptic area (Szabo *et al.*, 1998) is more likely, as it is within quickest reach of i.c.v. injection.

The parasympathetic role of central 5-HT_{1A} receptors has also been widely investigated. Originally it was noted that LSD increases vagal drive in cats (Cervoni *et al.*, 1963). It is likely that this was due to 5-HT_{1A} receptor activation in the medulla, since 8-OH-DPAT administered into the IV^{th} ventricle causes a profound vagal bradycardia in cats (Shepheard *et al.*, 1994). The receptors are now thought to be located primarily in the vicinity of CVPNs in the nucleus ambiguus, as microinjection of 8-OH-DPAT (Izzo *et al.*, 1988) into this area produces a vagal bradycardia in the cat. However, they may also be located in the DVN, where microinjection of 8-OH-DPAT and flesinoxan produces a vagal bradycardia in the rat (Sporton *et al.*, 1991). A number of iontophoretic studies have compared 5-HT_{1A} receptors in the DVN and nucleus ambiguus: iontophoresis of 5-HT onto rat DVN neurones excited at low currents and inhibited at high currents. The excitation was blocked by WAY-100635 and pindolol. Iontophoretic 8-OH-DPAT inhibited the majority of neurones and excited some; only this excitation could be blocked by WAY-100635. By itself, WAY-100635 was inhibitory at most neurones, whilst exciting a few, and had an additive inhibitory effect with 8-OH-DPAT (Wang *et al.*, 1995). In cat CVPNs of the nucleus ambiguus, however, WAY-100635 by itself did not affect activity, posing the question of either a species difference or a pharmacological difference between DVN and nucleus ambiguus neurones. In agreement with DVN, however, 8-OH-DPAT inhibited at low currents and excited at high currents, and this excitation was WAY-100635 sensitive (Wang & Ramage, 2001).

The fact that these inhibitory receptors are activating neurones that cause bradycardia proposes an interesting mode of action, probably involving disinhibition. Indeed, microinjection of bicuculline into the nucleus ambiguus causes a vagal bradycardia (DiMicco *et al.*, 1979). Also, NTS-evoked inhibitory synaptic currents in DVN neurones *in vitro* were diminished by 5-HT_{1A} receptor activation, and this effect was

bicuculline sensitive (Browning & Travagli, 1999). Hence it is likely that 5-HT_{1A} receptors are located on GABAergic terminals presynaptic to CVPNs, which evokes excitation.

The triad of hypotension, bradycardia and sympathoinhibition caused by activating medullary 5-HT_{1A} receptors resembles the responses evoked by stimulating cardiopulmonary afferents, and this prompted the question of whether these receptors serve a reflex function. If they are not tonically involved in cardiovascular regulation, as shown by the lack of effect of antagonists on baseline variables, they may be activated in certain circumstances. This was first demonstrated in the anaesthetised rat, using the non-selective antagonists spiperone, methiothepin, and (±)-pindolol, which all attenuated cardiopulmonary reflex bradycardias when given i.c. (Bogle *et al.*, 1990). Additionally, buspirone caused an attenuation, whereas the 5-HT_2, α_1 adrenoceptor and D_2 receptor antagonists BW-501C67, alfuzosin and (-)-sulpiride were without effect, suggesting that 5-HT_{1A} receptors in the medulla are involved.

Later it was also found that reflex bronchoconstriction caused by inhaled capsaicin was attenuated by i.c. methiothepin, (-)-pindolol and WAY-100635, and potentiated by 8-OH-DPAT in the anaesthetised cat (Bootle *et al.*, 1996). Similarly, in the anaesthetised guinea pig, reflex bronchoconstriction was attenuated by i.c. (-)-pindolol and WAY-100635, and potentiated by buspirone, 8-OH-DPAT, and fluoxetine (Bootle *et al.*, 1998). In anaesthetised rabbits, the bradycardia evoked by stimulation of the upper airways with smoke was attenuated by i.c. (-)-pindolol and WAY-100635, and potentiated by buspirone (Dando *et al.*, 1998). In this study, however, 8-OH-DPAT failed to potentiate the bradycardia (indeed it attenuated it *via* 5-$HT_{1B/1D}$ receptor activation; see 1.6.4 below). This attenuation was previously observed (Futuro-Neto *et al.*, 1993), pointing to a possible species difference in the roles of these receptor subtypes. Further work in the anaesthetised rabbit confirmed the crucial role of the 5-HT_{1A} receptor in cardiovascular reflex transmission: cardiopulmonary, aortic nerve and chemoreceptor afferent-evoked bradycardias were all potentiated by i.c. buspirone and attenuated by WAY-100635, with the exception of the chemoreflex, which was potentiated by buspirone but not significantly attenuated by WAY-100635 (Skinner *et al.*, 2002). Here the sympathetic

components of these reflexes were also potentiated by buspirone and, in the case of the cardiopulmonary reflex, both the sympathetic and the respiratory components were attenuated by WAY-100635. This raised the possibility of the receptors being located in the NTS, where afferent inputs are distributed to the respective targets of the different components of the reflex, and receptor blockade would influence all components. Functional 5-HT_{1A} receptors have been located in the rat NTS (Wang *et al.*, 1997) using iontophoretic 8-OH-DPAT (but not confirmed with an antagonist); however, a reflex role has not been established within this nucleus. In the cat nucleus ambiguus, however, functional 5-HT_{1A} receptors are clearly demonstrated in the vicinity of CVPNs, and the excitation of these neurones by cardiopulmonary afferents is inhibited by iontophoretic WAY-100635 (Wang & Ramage, 2001). Whether this is specific to the cat remains to be seen, but the observation supports the direct facilitation of CVPNs by 5-HT_{1A} receptors in their vicinity, independent of NTS processes.

Additional to these cardiovascular reflex functions, 5-HT_{1A} receptors have been identified as a major player in bladder reflexes. In anaesthetised rats, the supraspinal micturition reflex was facilitated by 8-OH-DPAT given i.v., i.c.v, or intrathecally (i.t.; at the level of the sacral spinal cord), but not topically onto the bladder (Lecci *et al.*, 1992). Later studies found that WAY-100635 prevented reflex bladder contractions when given i.v. (Testa *et al.*, 1999; Conley *et al.*, 2001) or i.t. (Kakizaki *et al.*, 2001); additionally, WAY-100635 interferes with micturition when given either i.c.v. or i.t. in anaesthetised rats (Secker *et al.*, 2002). Hence the role of the 5-HT_{1A} in parasympathetic drive to the bladder is well documented, but the location is complex and poorly defined. The efficacy of i.c.v. as well as i.t. compounds suggests that functional receptors are located both supraspinally, possibly in the pontine micturition centre, and spinally. A spinal location in the vicinity of sacral parasympathetic preganglionic neurones would be comparable to the location in the nucleus ambiguus of the cat (Wang & Ramage, 2001), but this remains to be tested.

One further and no less complex parasympathetic role for 5-HT_{1A} receptors is in the outflow to the iris: in conscious mice (Prow *et al.*, 1996) and anaesthetised rats (Yu *et al.*, 2004), systemic 8-OH-DPAT produces pupillary dilatation, which is sensitive to 5-HT_{1A} and α_2 adrenoceptor antagonists, but unchanged by sympathetic nerve

section in the rat (Yu *et al.*, 2004), suggesting that 5-HT_{1A} receptors cause an inhibition of parasympathetic tone to the iris, *via* activation of noradrenergic neurones acting on α_2 adrenoceptors. Interestingly, this action seems to be specific to rodents, as buspirone causes pupillary constriction in humans *via* sympathetic nerves (Fanciullacci *et al.*, 1995) although parasympathetic tone may also be involved (Phillips *et al.*, 1999).

These observations support the fact that both multiplicity of effects and species differences are characteristics of the comparative physiology of the 5-HT_{1A} receptor, and as such any roles do not necessarily extend to other brain regions or other species.

1.6.4. 5-HT_{1B} and 5-HT_{1D} receptors

Molecular biology

The [^{3}H]-5-HT binding site in the rat striatum with low affinity for spiperone (Pedigo *et al.*, 1981) was later named the 5-HT_{1B} binding site, whereas another [^{3}H]-5-HT binding site in the bovine brain was termed the 5-HT_{1D} binding site on account of its different pharmacological profile (Heuring & Peroutka, 1987). Subsequent molecular discoveries led to considerable debate and revision of the nomenclature of these receptors, due to the existence of both intraspecies receptor subtypes and interspecies receptor homologues (see Hartig *et al.*, 1992; Hartig *et al.*, 1996).

The first clone of this receptor pair was of the 5-HT_{1D} receptor gene, then simply called RDC4 – an unassigned sequence from a canine thyroid cDNA library (Libert *et al.*, 1989). Although its homology to the 5-HT_{1A} receptor sequence was noted, it was only later identified as the canine 5-HT_{1D} receptor gene (Zgombick *et al.*, 1991). It was thought that rodent 5-HT_{1B} and non-rodent 5-HT_{1D} receptors were species homologues, based on their distribution and pharmacology (Hoyer & Middlemiss, 1989). This was confirmed by cloning of the rat 5-HT_{1B} receptor (Voigt *et al.*, 1991; Hartig *et al.*, 1992) with the pharmacological profile of the 5-HT_{1B} binding site. Very soon afterwards, however, an orthologous gene was isolated from the human genome, and this had a different pharmacological profile – namely that of the 5-HT_{1D}

receptor (Weinshank *et al.*, 1992; Levy *et al.*, 1992; Veldman & Bienkowski, 1992). The human 5-HT_{1D} receptor appeared to be a composite of 2 subtypes, encoded by different genes, which were then defined as 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ (Hartig *et al.*, 1992). The human 5-$HT_{1D\beta}$ has 96 % sequence homology to the rat 5-HT_{1B} receptor (Jin *et al.*, 1992), and their pharmacological difference has been attributed to a single amino acid substitution (Metcalf *et al.*, 1992). These differences are summarised in Figure 1.7, using the old nomenclature (5-$HT_{1D\alpha}$ etc.), which has now been revised to take into account these homologies: hence 5-$HT_{1D\beta}$ is now known simply as 5-HT_{1B}, and 5-$HT_{1D\alpha}$ has reverted to 5-HT_{1D}.

Both rat and human cloned 5-HT_{1B} receptors (Adham *et al.*, 1992; Levy *et al.*, 1992; Weinshank *et al.*, 1992) and 5-HT_{1D} receptors (Hamblin *et al.*, 1992; Weinshank *et al.*, 1992) inhibit forskolin-induced cAMP accumulation, suggesting negative coupling to adenylate cyclase.

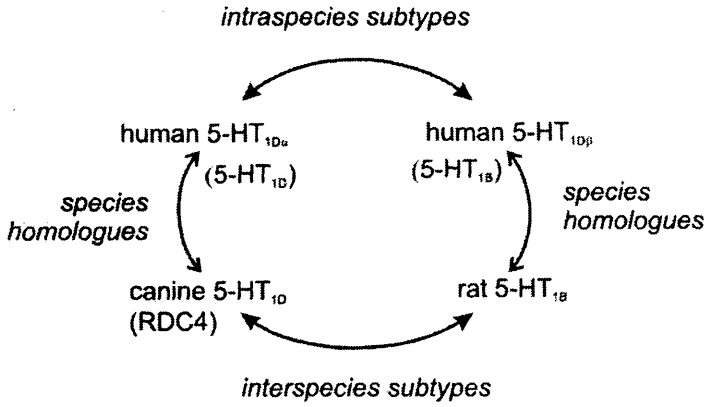


Figure 1.7 5-$HT_{1B/1D}$ receptors

Diagram illustrating relationships between species differences in 5-$HT_{1B/1D}$ receptors (adapted from Hartig, 1992)

Distribution

Mapping of differential tissue distributions of 5-HT_{1B} and 5-HT_{1D} receptors using radioligands poses some difficulties due to the lack of 5-HT_{1D} selective ligands. In the rat, the 5-$HT_{1B/1D}$ receptor radioligand [^{125}I]GTI, alone or in the presence of CP-93129 to mask 5-HT_{1B} receptors, reveals densest 5-HT_{1B} binding sites in the basal ganglia (globus pallidus, substantia nigra pars reticulata, nucleus accumbens and subthalamic nucleus), and 5-HT_{1D} binding sites in a very similar distribution, but with only a fraction of the intensity (Bruinvels *et al.*, 1993). The same researchers reported the highest 5-HT_{1B} receptor mRNA expression in the rat olfactory cortex, subthalamic nucleus, and hippocampal CA1, with lower signal in nucleus accumbens, caudate-putamen, and cerebellar Purkinje cells, and lowest expression in the amygdala, hypothalamus, thalamus, cortex and pons. Likewise, very weak 5-HT_{1D} receptor mRNA was present in olfactory cortex, nucleus accumbens, caudate-putamen, dorsal raphe and pons (Bruinvels *et al.*, 1994). Interestingly, they also found 5-HT_{1B} receptor mRNA in rat posterior communicating artery but not basilar artery.

Researchers focussing on the cellular location of 5-HT_{1B} receptors report mRNA in dorsal root ganglia, cerebral cortex layer IV, cerebellar Purkinje cells, and neurones of the dorsal and median raphe. These latter two signals are greatly reduced by 5,7-DHT lesion, suggesting 5-HT_{1B} autoreceptors in these raphe nuclei (Doucet *et al.*, 1995). Another *in situ* hybridisation study described highest 5-HT_{1B} receptor signal in caudate-putamen, olfactory tubercle, CA1 and cerebellar Purkinje cells, with less in cortex (layer IV), thalamus, dorsal raphe and spinal cord (Boschert *et al.*, 1994). However, additional autoradiography in this investigation showed that areas with high receptor mRNA have few binding sites and vice-versa, although the areas to which the mRNA-rich structures project are high in binding sites. Together with the previous lesion study, this suggests that 5-HT_{1B} receptors are located predominantly on axon terminals. Interestingly, inhibitory 5-HT_{1D} receptors are also located on raphe cell bodies (Bonaventure *et al.*, 1998), and these are colocalised with 5-HT_{1B} receptors, at least in the mouse (Evrard *et al.*, 1999).

With respect to the brainstem and spinal cord, binding sites were identified in human tissue using [^{3}H]-sumatriptan, plus ketanserin to differentiate between 5-HT_{1B} and 5-HT_{1D} (Castro *et al.*, 1997). Both binding sites were detected in (rank order of density) spinal trigeminal nucleus, substantia gelatinosa, NTS, and periaqueductal grey, although 5-HT_{1B} binding sites were between 3 and 20 fold denser in all these regions. The expression in these areas indicates a possible role in the processing and modulation of sensory and autonomic afferent information.

Pharmacology

5-$HT_{1B/1D}$ receptor binding is fraught with idiosyncrasies, and the following examples will illustrate such problems. Binding of common ligands at 5-HT_{1B} receptors in rat frontal cortex (Millan *et al.*, 2002) and guinea pig striatum (Audinot *et al.*, 1997) is as follows (pK_i rat *vs.* pK_i guinea pig). Agonists include 5-HT (9.1 *vs.* 8.6), 5-CT (9.5 *vs.* 9.1), L-694247 (10.4 *vs.* 10.1), CP-93129 (8.4 *vs.*< 6), and sumatriptan (7.4 *vs.* 6.8). Antagonists include GR-125743 (9.4 *vs.* 9), GR-127935 (8.2 *vs.* 8.4), (-)-pindolol (7.6 *vs.* 5.8), (-)-propranolol (7.4 *vs.* 5.6), methiothepin (7 *vs.* 8) and methysergide (6.5 *vs.* 6.7). These reveal that CP-93129 and the β-blockers are more selective for the rat receptor. Interestingly (-)-pindolol binds to rat cortical 5-HT_{1B} receptors with a pK_i of 7.1 (Hoyer *et al.*, 1985a), and to human cortical 5-HT_{1D} receptors with a pK_i of 5.1 (Peroutka *et al.*, 1989). Also, (-)-propranolol binds to cloned rat and cloned human 5-HT_{1B} receptors with a pK_i of 8.3 and 6.2 respectively (Hamblin *et al.*, 1992). Conversely, sumatriptan has pK_is of 7.3 and 8.7 respectively on cloned rat and human 5-HT_{1B} receptors (Hamblin *et al.*, 1992), and 8 and 8.4 respectively on cloned rat and human 5-HT_{1D} receptors (Hamblin & Metcalf, 1991; Hamblin *et al.*, 1992), whereas on cloned dog 5-HT_{1D} receptors it has a pK_i of 8.8 (Boess & Martin, 1994). This illustrates some of the variations shown in Figure 1.7 – human 5-$HT_{1B/1D}$ and dog 5-HT_{1D} receptors have similar affinities, whereas rat 5-HT_{1B} is pharmacologically distinct, although genetically related.

Compative binding (pK_i) to cloned human 5-HT_{1B} and 5-HT_{1D} (i.e. 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$) receptors (Weinshank *et al.*, 1992) using [^{3}H]-5-HT, was reported as follows: 5-HT (8.4 *vs.* 8.4), 5-CT (8.8 *vs.* 9.1), 8-OH-DPAT (6.6 *vs.* 6.9), sumatriptan (8.1 *vs.* 8.5), methysergide (7.6 *vs.* 8.4), spiperone (< 5 *vs.* 6). In a different study, again using [^{3}H]GR-125743 (Domenech *et al.*, 1997), the affinities (pIC_{50}) at human

cloned 5-HT_{1B} *vs.* 5-HT_{1D} receptors were: 5-HT (7.4 *vs.* 8.5), 5-CT (7.9 *vs.* 8.6), 8-OH-DPAT (6.1 *vs.* 7.3), sumatriptan (7.2 *vs.* 8.1), methiothepin (8.2 *vs.* 8.2), GR-127935 (9.4 *vs.* 8.7) and naratriptan (8 *vs.* 8.8). Therefore within the human species there tend to be less discrepancies between these subtypes than there are between the same receptor amongst different species. As such, the guinea pig is favoured over the rat as a species with comparable affinities to the human receptors.

For the most part, it remains difficult to distinguish between 5-HT_{1B} and 5-HT_{1D} receptors, especially in functional assays, so compounds are often referred to as acting on 5-$HT_{1B/1D}$ receptors, for the sake of simplicity. The most selective antagonists for 5-$HT_{1B/1D}$ receptors over other 5-HT receptors are GR-127935 (Skingle *et al.*, 1996) and GR-125743 (Millan *et al.*, 2002), although recently several highly potent and selective 5-HT_{1B} receptor antagonists have been developed, including SB-216641 (Price *et al.*, 1997) and SB-236057 (Roberts *et al.*, 2000).

General physiology

Inhibitory 5-HT_{1B} autoreceptors on serotonergic terminals are believed to fine tune 5-HT release at various CNS structures. For example, it has been demonstrated that these receptors limit the increased forebrain efflux of 5-HT caused by selective serotonin reuptake inhibitors (SSRIs) (Malagie *et al.*, 2001). On the other hand, 5-HT_{1B} receptors have been reported to facilitate dopamine efflux in the striatum (Galloway *et al.*, 1993), acetylcholine efflux in the hippocampus (Maura *et al.*, 1989), and to inhibit GABAergic transmission onto dopamine containing neurones (Morikawa *et al.*, 2000), so their effects on other transmitter systems are potentially very wide ranging. Functional roles *in vivo* have been described in the regulation of mood (Sipes & Geyer, 1996; Saudou *et al.*, 1994), reward (Fletcher & Korth, 1999; Belzung *et al.*, 2000), sleep (Boutrel *et al.*, 1999), locomotor activity (Chaouloff *et al.*, 1999), appetite (Lucas *et al.*, 1998), and cognition (Boulenguez *et al.*, 1998; Meneses, 1999). Due to the lack of selective ligands, 5-HT_{1B} receptor (-/-) knockout mice have been useful in differentiating 5-HT_{1B} from 5-HT_{1D} receptor mediated effects. There is no dramatic knockout phenotype, although they do display various behavioural differences, such as increased aggression (Saudou *et al.*, 1994) and increased paradoxical sleep (Boutrel *et al.*, 1999). Fenfluramine-induced anorexia is

abolished (Lucas *et al.*, 1998), and SSRI-induced 5-HT efflux is enhanced (Malagie *et al.*, 2001).

By far the most useful application of 5-$HT_{1B/1D}$ receptor agonists is in the treatment of migraine (see Goadsby, 1998; De Vries *et al.*, 1999; Tepper *et al.*, 2002). The effect of these agonists is thought to be spread across a number of targets: firstly, by constricting cerebral blood vessels *via* 5-HT_{1B} receptors (see Villalon *et al.*, 2002); secondly by inhibiting the release of inflammatory mediators; and thirdly, by reducing the excitability of sensory neurones (see Goadsby, 1998). This latter effect is demonstrated by iontophoresis of the agonists sumatriptan and zolmitriptan, which inhibit the excitation of second order trigeminal neurones by nociceptor afferents in anaesthetised cats (Storer & Goadsby, 1997).

Autonomic functions

Peripherally, 5-$HT_{1B/1D}$ receptor agonists cause internal carotid vasoconstriction in anaesthetised dogs, probably *via* smooth muscle 5-HT_{1B} receptors (Centurion *et al.*, 2001), although these receptors also mediate external carotid vasodilatation, possibly *via* a presynaptic sympathoinhibitory mechanism (Villalon *et al.*, 2001). In anaesthetised cats the inhibition of sympathetic ganglionic transmission by 5-HT and 5-CT was attributed to 5-$HT_{1B/1D}$ receptors (Jones *et al.*, 1995a).

Reflex activation of cardiac vagal and renal sympathetic outflow by stimulation of the upper airways with smoke in anaesthetised rabbits was attenuated by i.c. sumatriptan (Dando *et al.*, 1998), indicating that 5-$HT_{1B/1D}$ receptor activation has the same effect as 5-HT_{1A} receptor blockade, possibly reflecting inhibition of 5-HT release onto 5-HT_{1A} receptors. GR-127935 had no effect, so 5-$HT_{1B/1D}$ receptors in the medulla are not involved in reflex transmission *per se.* Similar results were found in the micturition reflex in anaesthetised rats, which was inhibited by sumatriptan and 5-CT, but not in the presence of GR-127935 (Read *et al.*, 2004). Within the NTS, iontophoresis of sumatriptan tended to inhibit ongoing neuronal firing, and this effect was inhibited by 5-HT_{1D} selective, and potentiated by 5-HT_{1B} selective antagonists. Also, the 5-HT_{1B} receptor selective agonist CP-39129 increased baseline, vagus-evoked, and cardiopulmonary afferent-evoked firing, whilst the latter

was reduced by sumatriptan (Jeggo *et al.*, 2000a; Jeggo, 2003). These data point to differential roles of these receptors within the NTS, with 5-HT_{1B} increasing, and 5-HT_{1D} decreasing neuronal excitability. Consequently, sumatripan is likely to attenuate autonomic reflexes *via* 5-HT_{1D} receptor activation. As such, the effects of non-selective 5-HT_1 receptor agonists must be treated with care.

1.6.5. 5-ht_{1E} and 5-HT_{1F} receptors

5-ht_{1E} receptor

Little is known about the 5-ht_{1E} receptor due to a lack of selective ligands, and it remains in lower case (5-ht) due to the lack of functional characterisation to date. The receptor was first characterised in human brain as having low affinity for 5-CT and ergotamine (Leonhardt *et al.*, 1989), distinguishing it from other 5-HT_1 receptors. The human receptor was cloned, with 64 % sequence homology to the human 5-$HT_{1B/1D}$ receptors, and shown to inhibit forskolin-induced cAMP accumulation (Zgombick *et al.*, 1992). *In situ* hybridisation signal was detected in human and monkey caudate nucleus and putamen, parietal, visual and entorhinal cortex, and medial hypothalamus (Bruinvels *et al.*, 1994).

Various non-selective ligands bind to the cloned human 5-ht_{1E} receptor, and their rank order of potencies is reported as: 5-HT > methysergide > ergotamine > 8-OH-DPAT > 5-CT > ketanserin (Zgombick *et al.*, 1992). Comparative binding of common ligands to human cloned 5-ht_{1E} (Zgombick *et al.*, 1992) and 5-HT_{1F} receptors (Adham *et al.*, 1993) (pK_i) was as follows: 5-HT (8 *vs.* 8), 5-CT (5.1 *vs.* 6.1), methiothepin (6.7 *vs.* 6.2), methysergide (6.6 *vs.* 7.5), and sumatriptan (5.6 *vs.* 7.6), suggesting that 5-ht_{1E} receptors tend to have lower affinities for known 5-HT_1 ligands, and especially that sumatripan binding is particularly selective for 5-HT_{1F} receptors.

5-HT_{1F} receptor

The 5-HT_{1F} receptor was the fifth human 5-HT_1 receptor to be cloned, also negatively coupled to adenylate cyclase (Adham *et al.*, 1993). *In situ* hybridisation revealed prominent receptor mRNA in guinea-pig frontal, olfactory, parietal,

entorhinal and cingulate cortices (layer V), and moderate signal in hippocampus, amygdala, pons, claustrum, and spinal trigeminal nucleus (Bruinvels *et al.*, 1994). In the human brainstem and spinal cord, [^{3}H]-sumatriptan in the presence of 5-CT revealed differential 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} receptor binding sites in the following rank order: NTS and spinal trigeminal nucleus (5-HT_{1B} > 5-HT_{1F} > 5-HT_{1D}), substantia gelatinosa (5-HT_{1F} > 5-HT_{1B} > 5-HT_{1D}) (Castro *et al.*, 1997).

Common 5-HT_1 receptor ligands including 5-CT, methysergide and sumatriptan have comparable affinities for the cloned human and rat 5-HT_{1F} receptors (Adham *et al.*, 1993; Lovenberg *et al.*, 1993b) suggesting that species variation is not an important factor as in the 5-$HT_{1B/1D}$ receptor. Sumatripan, zolmitriptan and naratriptan all have high and similar affinities (pK_i 7.6 – 9) for 5-$HT_{1B/1D/1F}$ receptors (see Barnes & Sharp, 1999) . A selective agonist, LY-334370, has also been developed, with a pK_i of 8.8 at the cloned 5-HT_{1F} receptor and 100-fold selectivity over 5-$HT_{1B/1D}$ receptors (Phebus *et al.*, 1997).

LY-334370 has been demonstrated to inhibit neurogenic dural inflammation in the guinea pig (Johnson *et al.*, 1997), and to inhibit the activation of second order trigeminal neurones by dural stimulation in the rat (Shepheard *et al.*, 1999). This suggests that the antimigraine effects of compounds such as sumatriptan may be mediated by 5-HT_{1F} in addition to 5-$HT_{1B/1D}$ receptors. Due to their distribution in areas such as hippocampus and cortex, there may be roles for the 5-HT_{1F} receptor in certain behavioural states, although systemic LY-334370 does not produce 5-HT stereotypical behaviour (see Barnes & Sharp, 1999).

1.6.6. 5-HT_2 receptors

The three 5-HT_2 receptor subtypes share a 46 – 50 % overall sequence identity, and couple preferentially to G_q leading to increased hydrolysis of phosphoinositide and a rise in intracellular Ca^{2+} concentration (see Hoyer *et al.*, 2002). The 5-HT_{2A} receptor was originally termed the D receptor (Gaddum & Picarelli , 1957), later renamed the 5-HT_2 receptor (Peroutka & Snyder, 1979). The 5-HT_{2B} receptor, firstly located in rat fundus, was previously known as the 5-HT_{2F} (fundus) receptor. The 5-HT_{2C} receptor

was previously known as the 5-HT_{1C} receptor, and reclassified by the International Union of Pharmacology in 1994 (see Hoyer *et al.*, 1994).

5-HT_{2A} receptor

The 5-HT_{2A} receptor was first cloned from a rat brain cDNA library, with high affinity for spiperone, ketanserin and mianserin, and low affnity for 8-OH-DPAT (Pritchett *et al.*, 1988). Cloning of the human counterpart showed an 87 % sequence homology with the rat receptor (Saltzman *et al.*, 1991). Receptor autoradiography using [^{125}I]-DOI or [^{3}H]-MDL-100907 combined with *in situ* hybridisation found receptor expression with good agreement in neocortex (layer V), caudate-putamen, and various brainstem nuclei (pontine, trigeminal motor and facial nuclei) (Mengod *et al.*, 1990b; Lopez-Gimenez *et al.*, 1997). This confirms an earlier *in situ* hybridisation study, which additionally found lower signal in various areas including CA3, entorhinal cortex, amygdala, substantia nigra, and lowest signal in various brainstem areas including NTS and raphe (Pompeiano *et al.*, 1994). Others have reported high levels of mRNA in rat DVN and nucleus ambiguus (Wright *et al.*, 1995). Peripherally, Northern blotting reveals highest levels of receptor mRNA in the liver and spleen (Bonhaus *et al.*, 1995).

5-HT_{2B} receptor

The receptor mediating 5-HT-evoked contraction of the rat stomach fundus was cloned from the mouse (Foguet *et al.*, 1992) and rat (Kursar *et al.*, 1992), and termed the 5-HT_{2B} receptor. Originally, it was thought to be only peripherally expressed. Northern blotting identified mRNA in rat kidney, pancreas and liver, but not in whole brain (Bonhaus *et al.*, 1995). Immunocytochemistry localised the receptor not only to longitudinal and circular smooth muscle of rat stomach fundus, but also to Purkinje cells of the cerebellum, the lateral septum, dorsal hypothalamus and medial amygdala, and to fibres but not cell bodies of the frontal cortex and spinal cord (Duxon *et al.*, 1997a). This distribution correlates well with mRNA expression in the rat brain (Flanigan *et al.*, 1995).

5-HT_{2C} receptor

The 5-HT_{2C} receptor was cloned in the rat (Julius *et al.*, 1988), mouse (Yu *et al.*, 1991) and human (Saltzman *et al.*, 1991), and is X-linked (human chromosome

Xq24). Although mRNA has been detected in rat heart, kidney and liver (Bonhaus *et al.*, 1995), this receptor it thought to be almost exclusive to the brain, where its mRNA is highly expressed in hippocampus, subiculum and subthalamic nucleus, moderately expressed in amygdala, lamina V of the spinal cord, and substantia nigra. Lower expression was found in NTS, nucleus ambiguus, raphe, and various hypothalamic and thalamic nuclei (Molineaux *et al.*, 1989; Wright *et al.*, 1995). A later study confirmed a similar distribution, including NTS, plus moderate levels in parts of cortex and septum, basal ganglia and locus coeruleus, but no signal in raphe (Pompeiano *et al.*, 1994). Additional to these sites, the choroid plexus is a very rich source of 5-HT_{2C} receptors (Mengod *et al.*, 1990a).

Pharmacology of 5-HT_2 receptors

A lot of pharmacological characterisation has been performed in cloned 5-HT_2 receptor subtypes. Importantly, there are no overt difference in the affinities of common ligands to human and rat cloned, and to human cloned and native 5-HT_2 receptors (Bonhaus *et al.*, 1995)

A wide variety of compounds non-selectively activate 5-HT_2 receptors, but there are no highly selective ($\geq$ 100-fold) agonists; pharmacological characterisation has relied on antagonists. 5-HT activates all subtypes with comparable potency (pEC_{50} of 7.7, 8.9 and 8 at human cloned 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors respectively); *m*-CPP is selective for 5-$HT_{2A/C}$ (pEC_{50} of 6.7, < 5 and 7), DOI is poorly selective for 5-HT_{2A} (pEC_{50} of 8.2, 7.5 and 7) (Jerman *et al.*, 2001), whereas α-methyl-5-HT is selective for 5-HT_{2B} (pEC_{50} of 7.4, 8.7, 7.9) and DOB and quipazine are non-selective (Porter *et al.*, 1999) LSD is a partial agonist with similar potency at all 3 subtypes, and lisuride a 5-HT_{2A} selective partial agonist (Porter *et al.*, 1999).

The antagonists methiothepin and mianserin have similar high affinities for all 5-HT_2 receptors, whereas ketanserin is 5-HT_{2A} selective (pK_B at cloned human receptors of 8.7, 6 and 7 respectively), SB-204741 is 5-HT_{2B} selective (pK_B of 5.8, 6.7 and 6.2) mesulergine is 5-HT_{2C} selective (pK_B of 7.4, 8.9 and 9.5) (Jerman *et al.*, 2001). With the exception of SB-204741, all these compounds have effects on other 5-HT and non-5-HT receptors. In this respect, cinanserin is a useful 5-HT_{2A} selective antagonist (pK_i of 8.3, 5.8, 6.7) with negligible α_1 adrenoceptor, histamine or

muscarinic receptor affinity (van Wijngaarden & Soudijn, 1997). More selective antagonists continue to be developed, including MDL-100907 for 5-HT_{2A} (Sorensen *et al.*, 1993), RS-127445 for 5-HT_{2B} (Bonhaus *et al.*, 1999), and SB-242084 for 5-HT_{2C} (Kennett *et al.*, 1997b).

General physiology

Activation of 5-HT_2 receptors causes depolarisation of neuronal membranes in a number of different brain areas, but whether the excitation (reduced K^+ conductance) depends upon phosphoinositide signalling is uncertain. In rat piriform cortical slices, 5-HT has excitatory effects *via* 5-HT_{2A} receptors on interneurones, and 5-HT_{2C} receptors on pyramidal cells (Sheldon & Aghajanian, 1991; Marek & Aghajanian, 1994). The effects of hallucinogenic and antipsychotic drugs (such as LSD and clozapine) which bind to these receptors are thought to be mediated by these cortical mechanisms. Excitatory 5-HT_2 receptors are also described in slices of nucleus accumbens (North & Uchimura, 1989), dentate gyrus (Piguet & Galvan, 1994) and prefrontal cortex (Araneda & Andrade, 1991b). *In vivo*, systemic 5-HT_2 receptor activation decreases noradrenaline efflux in the hippocampus (Done & Sharp, 1992; Done & Sharp, 1994), *via* indirect inhibition of locus coeruleus neurones, possibly involving other brainstem afferents (Gorea *et al.*, 1991).

Systemic administration of 5-HT_2 receptor agonists produces various behavioural signs such as head twitches, which are thought to be mediated by 5-HT_{2A} receptors. There is also a close correlation between the hallucinogenic properties of drugs in humans, and their affinity for 5-HT_{2A} receptors (Glennon, 1990), where they are thought to act as agonists or partial agonists. Additionally, atypical antipsychotics such as clozapine, olanzapine and risperidone have high affinity for 5-HT_{2A} receptors, suggesting a role in schizophrenia (Leysen *et al.*, 1993). Other effects of 5-HT_{2A} receptor activation include hyperthermia (Gudelsky *et al.*, 1986), and neuroendocrine secretion (Fuller, 1996). There is little evidence for a behavioural role for the 5-HT_{2B} receptor, which is poorly expressed in the CNS, with the exception of the amygdala. Indeed, microinjection of the selective 5-HT_{2B} receptor agonist BW-723C86 into the amygdala has an anxiolytic effect (Duxon *et al.*, 1997b), making it an interesting target in the treatment of anxiety. A number of behavioural responses are also attributed to 5-HT_{2C} receptor activation, including

hypolocomotion, hypophagia, anxiety, penile erections and hyperthermia (see Koek *et al.*, 1992), and the advent of new and highly selective ligands is allowing many formerly generalsed 5-HT_2 receptor mechanisms to be re-evaluated.

Autonomic functions

Systemic administration of DOI raises BP in rats *via* a combination of direct and angiontensin II-mediated vasoconstriction (Alper, 1990), as well as by a central mechanism: in cats i.v. DOI and quipazine also increases sympathetic nerve discharge (McCall *et al.*, 1987; Vayssettes-Courchay *et al.*, 1991) , which was prevented by pretreatment with ketanserin but not prazosin (McCall & Harris, 1988). Ketanserin itself decreased baseline sympathetic activity, but this was probably mediated by α_1 adrenoceptor blockade (Ramage, 1985). In rats, i.c.v. cinanserin does not affect baseline sympathetic activity (Knowles & Ramage, 1999), suggesting that these central receptors are not tonically activated. In the anaesthetised cat, however, i.v. cinanserin can cause a fall in blood pressure (Ramage, 1988).

Iontophoretically applied DOI had no effect on sympathetic preganglionic neurones that were excited by i.v. DOI (Clement & McCall, 1990), suggesting that the receptors are located supraspinally. Application of DOI to the ventral medulla of cats raised BP (without altering HR), suggesting the receptors are located in the RVLM (Mandal *et al.*, 1990). Additionally this study found that the pressor response to central DOI was reduced by i.v. propranolol or stellate ganglionectomy, suggesting that central sympathoexcitatory 5-HT_2 receptors have a cardiac inotropic but not chronotropic effect, i.e. that they are located on sympathetic premotor neurones controlling outflow to the ventricles but not nodal regions of the heart, hence increasing contractility and cardiac output, but not rate. This was supported by the observation that i.c.v. DOI increased cardiac nerve activity but not HR, and did not increase renal nerve activity (Anderson *et al.*, 1995). However, in the definitive experiment, where BP and HR were held constant in an anaesthetised cat, DOI onto the ventral medulla failed to increase cardiac contractility, although it did increase hindlimb resistance (Ramage & Daly, 1998), which nevertheless confirms that RVLM premotor neurones to the heart respond differently from those to other targets.

In rats, 5-HT_{2A} receptors regulate vasopressin release (Anderson *et al.*, 1992), and stimulation of 5-HT_{2A} receptors with i.c.v. agonists causes a tachycardia and rise in BP due in part to vasopressin release; additionally, this vasopressin release causes a secondary reduction in sympathetic nerve activity *via* 5-HT_{2B} receptors (Knowles & Ramage, 1999), although 5-HT_{2B} receptors can themselves excite sympathetic outflow to the kidney (Knowles & Ramage, 2000). These receptors are believed to be involved in central regulation of blood volume.

Vagally-activated NTS neurones are either excited or inhibited by iontophoretic DOI (Wang *et al.*, 1997). Further investigation found that second-order neurones in the NTS are more likely to be inhibited, whilst higher-order neurones tend to be excited by 5-HT_2 receptors (Sevoz-Couche *et al.*, 2000b), and these effects were probably mediated by 5-HT_{2C} (inhibition) and 5-$HT_{2A/B}$ (excitation) receptors respectively.

1.6.7. 5-HT_3 receptor

It was first demonstrated that 5-HT can cause neuronal depolarisation and the release of acetylcholine in the guinea pig ileum, which was attributed to the activation of the M receptor (Gaddum & Picarelli , 1957), thus termed because it interfered with the effects of morphine on gut contractions. Several decades later this was reclassified as the 5-HT_3 receptor (Bradley *et al.*, 1986).

Molecular biology

The 5-HT_3 receptor is a fast-conducting ligand-gated ion channel (Derkach *et al.*, 1989). Electron microscopic imaging of the 5-HT_3 receptor protein revealed a pentameric structure arranged around a central aperture which forms the ion channel, similar to the neuronal nicotinic receptor (Boess *et al.*, 1992). The 5-HT_{3A} subunit was first cloned from a neuroblastoma expression library (Maricq *et al.*, 1991), where it showed approximately equal sequence similarity to the neuronal nicotinic, $GABA_{Abl}$ and glycine receptors. An alternative murine splice variant with a 6 amino acid deletion in the cytoplasmic loop was also cloned (Hope *et al.*, 1993). The 5-HT_3 receptor causes fast depolarisation of neuronal membranes, and is selective for Na^+ and K^+ with near equal permeability. It is also prone to rapid desensitisation (see Peters *et al.*, 1992; Jackson & Yakel, 1995). *In vitro*, promiscuous co-

assemblies of 5-HT_3 and neuronal nicotinic subunits can be formed, which are Ca^{2+} permeable (van Hooft *et al.*, 1998), but whether these exist *in vivo* remains to be seen. Some 5-HT_3 receptor agonists have also been reported to increase phosphoinositide hydrolysis (Edwards *et al.*, 1991), so further versions of the receptor may be naturally occurring.

Distribution

Receptor autoradiography with [^{3}H]-GR-65630 reveals the highest density of 5-HT_3 binding sites in the cat NTS, DVN, nucleus ambiguus, and spinal trigeminal nucleus (Reynolds *et al.*, 1991), and a similar distribution is found in the rat and human brainstem using tritiated selective ligands (Barnes *et al.*, 1990; Waeber *et al.*, 1989). Outside of the brainstem, however, density is much lower, with highest binding in hippocampus and amygdala, moderate binding in several cortical and limbic regions, and virtually no binding in pons, cerebellum, or basal ganglia (Barnes *et al.*, 1990; Waeber *et al.*, 1989).

Another important location of 5-HT_3 receptors is on peripheral terminals of many sensory afferents. 5-HT applied to a blister base causes pain in humans, which is sensitive to 5-HT_3 receptor antagonists (Richardson *et al.*, 1985). 5-HT_3 receptors are thought to be responsible particularly for the peripheral transduction of chemically-induced pain (Giordano & Dyche, 1989). Centrally in the dorsal horn of the rat spinal cord, 5-HT_3 binding sites are reduced by capsaicin treatment (Hamon *et al.*, 1989) suggesting the receptors are located on C-fibre terminals. They have also been found electrophysiologically in the rat dorsal root ganglion (Todorovic & Anderson, 1990). High receptor densities are found in autonomic nerves and their ganglia, especially the nodose ganglia and along the length of the vagus in the cat (Hoyer *et al.*, 1989). Lesion of the cervical vagus in the mouse (Waeber *et al.*, 1988) or of the nodose ganglion in the rat (Pratt & Bowery, 1989) reduces binding sites in the NTS, suggesting a presynaptic location on primary afferent terminals, as in the spinal cord. Many of these afferents, however, may be abdominal, as subdiaphragmatic vagotomy abolishes 5-HT_3 binding sites, at least in the ferret (Leslie *et al.*, 1990).

Pharmacology

Pharmacological characterisation of 5-HT_3 receptors has been greatly facilitated by a wealth of selective ligands, especially when compared with other 5-HT receptors. 5-HT has a comparatively low affinity (pK_i 6.8) for rat 5-HT_3 receptors (Kilpatrick *et al.*, 1989; Barnes *et al.*, 1990). Full agonists include (with pK_i in rat brain) quipazine (9), 2-methyl-5-HT (6.9) (Kilpatrick *et al.*, 1989) and PBG (7.1) (Barnes *et al.*, 1990). Selective antagonists include zacopride (9.4), ICS-205930 (9.1), granisetron (8.7), ondansetron (8.6), MDL-72222 (7.9), and clozapine (7) (Barnes *et al.*, 1990). Binding affinities of granisetron at central (cortex) and peripheral (vagus) sites are quite similar (pK_i 9.2 *vs* 8.7) (Kilpatrick *et al.*, 1989), and also at rat and human brain receptors (8.6) (Barnes *et al.*, 1988a; Barnes *et al.*, 1988b). However, interspecies differences in pharmacology have been reported. For example, PBG and MDL-72222 have low affinities for the guinea pig receptor (Kilpatrick & Tyers, 1992; Lankiewicz *et al.*, 1998).

General physiology

The 5-HT_3 receptor has been researched within the framework of a number of different physiological functions and pathological conditions. The 5-HT_{3A} subunit knockout mouse displayed anxiolytic behaviour (Kelley *et al.*, 2003), and antagonists have a similar anxiolytic effect on the startle response (Nevins & Anthony, 1994). Furthermore, cocaine-induced behaviour (Herges & Taylor, 2000), dopamine efflux in the nucleus accumbens by drugs of abuse (Carboni *et al.*, 1989), and dopamine-induced locomotor behaviour (Costall *et al.*, 1990) are all sensitive to 5-HT_3 receptor antagonists. 5-HT_3 receptor activation caused dopamine efflux in the striatum *in vitro* (Blandina *et al.*, 1989) and in the nucleus accumbens *in vivo* (Chen *et al.*, 1991). Thus the 5-HT_3 receptor is implicated in anxiety, reinforcement, and possibly in aspects of schizophrenia.

5-HT_3 receptors mediate the vomiting reflex, probably *via* actions at the NTS and area postrema, and this is very efficiently inhibited by 5-HT_3 receptor antagonists (Miner & Sanger, 1986), which also inhibit radiation- and cytotoxic drug-induced emesis (Miner *et al.*, 1987; Bermudez & Sanger, 1994). Indeed, ondansetron and granisetron are widely used in the clinic for this reason. Additionally, the antagonist

tropisetron is clinically effective in the treatment of fibromyalgia (Farber *et al.*, 2001).

Autonomic functions

Central autonomic functions of 5-HT_3 receptors appear to relate to their ability to modulate glutamate release: DVN vagal preganglionic neurones are excited by iontophoretic PBG, and this excitation is sensitive to 5-HT_3, AMPA and NMDA receptor antagonists, and to inhibition of neurotransmitter release with Mg^{2+} (Wang *et al.*, 1996), suggesting that presynpatic 5-HT_3 receptors potentiate the release of glutamate onto these neurones. In NTS neurones activated by vagal afferents, a similar effect has been observed: both PBG- and cardiopulmonary afferent-evoked excitation are inhibited by granisetron, the NMDA receptor antagonist AP-5, and Mg^{2+} (Jeggo *et al.*, 2000b; Jeggo *et al.*, 2001). This further proposes a reflex function – that 5-HT_3 receptors in the NTS tonically contribute to reflex transmission, at least for the cardiopulmonary reflex – which is supported by the effects of i.c. or microinjected (into the NTS) granisetron, which inhibits both cardiopulmonary reflex bradycardia and hypotension in the anaesthetised rat (Pires *et al.*, 1998). Microdialysis in the NTS has shown increased glutamate efflux during local infusion of PBG (Ashworth-Preece *et al.*, 1995), supporting the gluatamergic mechanism of action. However, microinjection of 5-HT or 1-(*m*-chloro)-PBG into the NTS had also been shown to attenuate cardiopulmonary reflex bradycardia (Sevoz *et al.*, 1996), and chemoreflex bradycardia (Sevoz *et al.*, 1997), which were prevented by pretreatment (microinjection) with zacopride or ondansetron, and by bicuculline in the latter study, suggesting that 5-HT_3 receptor stimulation can also activate a GABAergic brake, which in these experiments overrides any glutamatergic effects.

1.6.8. 5-HT_4 receptor

Prior to its molecular characterisation, the 5-HT_4 receptor was identified in the 1980s (see Bockaert *et al.*, 1992). The receptor sequence was subsequently cloned in the rat (Gerald *et al.*, 1995), consisting of two splice variants, a short (5-HT_{4S}) and a long (5-HT_{4L}) version, later renamed 5-$HT_{4(a)}$ and 5-$HT_{4(b)}$ (Hoyer & Martin, 1997). The human gene was mapped to chromosome 5 (q.31 – q.33), sharing 94 % sequence

homology with the rat and mouse (Claeysen *et al.*, 1997). Further human splice variants (5-$HT_{4(c)}$ to 5-$HT_{4(h)}$) have since been described (see Hoyer *et al.*, 2002). The 5-HT_4 receptor couples positively to adenylate cyclase to increase cAMP production (Gerald *et al.*, 1995), and this causes neuronal depolarisation *via* phosophorylation and inactivation of the inwardly rectifying K^+ channel (Fagni *et al.*, 1992).

Reverse transcriptase polymerase chain reaction found 5-$HT_{4(a)}$ receptor signal in rat striatum, ileum, colon, and cardiac atria, and 5-$HT_{4(b)}$ receptor signal in striatum, olfactory bulb, hippocampus and brainstem, as well as in ileum and colon (Gerald *et al.*, 1995). Autoradiography with [^{3}H]-GR-113808 located 5-HT_4 binding sites in guinea pig brain, in the rank order striatum > olfactory tubercle > globus pallidus > hippocampus > substantia nigra, and in the rat brain in the rank order olfactory tubercle > globus pallidus > striatum > substantia nigra > superior colliculus > hippocampus (Grossman *et al.*, 1993). The same radioligand revealed binding sites in the human brain in the rank order caudate nucleus > globus pallidus > putamen > hippocampus (Reynolds *et al.*, 1995). Interestingly, the 5-$HT_{4(d)}$ isoform was detected only in the gut in humans, whereas 5-$HT_{4(a-c)}$ were also in brain and atrium (Blondel *et al.*, 1998).

5-HT binds to cloned rat 5-HT_4 receptors with a pK_i of 6.8 (Gerald *et al.*, 1995). Several compounds have comparable binding affinities in the guinea pig striatum and hippocampus, including (with pK_i at striatum) the full agonists GR-67333 (8.7) (Eglen, 1997) 5-HT (7.3) and 5-methoxytryptamine (6.5), the partial agonists BMU-8 (7.9), cisapride (7.5), zacopride (6.8) and metoclopramide (6.3), and the highly selective antagonists GR-113808 (9.5) (Grossman *et al.*, 1993), RS-39604 (9.1) (Hegde *et al.*, 1995), and SB-204070 (10.2) (van den Wyngaert *et al.*, 1997). 8-OH-DPAT, LSD, 5-CT and sumatriptan do not bind (pK_i < 5) (Gerald *et al.*, 1995).

A variety of neuromodulatory functions is ascribed to the 5-HT_4 receptor, especially acetylcholine release in the myenteric plexus of the gut (Tonini *et al.*, 1989; Craig & Clarke, 1990), which is the basis of cisapride treatment for constipation-predominant irritable bowel syndrome. Subsequently, 5-HT_4 receptor activation with i.c.v. BMU-8 was also shown to increase acetylcholine efflux in rat frontal cortex (Consolo *et al.*,

1994), possibly from cholinergic neurones in the septum – an area which contains some 5-HT_4 receptor mRNA (Ullmer *et al.*, 1996). This promoted interest in a cognitive role. Indeed, human post-mortem Alzheimer's disease brains were reported to have lower 5-HT_4 receptor densities in the hippocampus compared to age-matched controls (Reynolds *et al.*, 1995). In rats, the agonist GR-67333 reverses atropine-induced cognitive deficits (Fontana *et al.*, 1997), and similar memory improving effects are described elsewhere in rats (Letty *et al.*, 1997) and monkeys (Terry *et al.*, 1998).

Other roles of the 5-HT_4 receptor include facilitation of dopamine release in the rat striatum (Bonhomme *et al.*, 1995; Steward *et al.*, 1996), anxiolysis (caused by receptor antagonism) in the rat (Kennett *et al.*, 1997a), and tachycardia in the pig (Villalon *et al.*, 1990; Villalon *et al.*, 1991). This latter effect is attributed to 5-HT_4 receptors in the right atrium, which mediate 5-HT induced positive chronotropic effects, although positive inotropic effects are also reported, which are not thought to reflect the existence of ventricular 5-HT_4 receptors (Saxena *et al.*, 1992). There are notorious species differences in the 5-HT receptors mediating tachycardia (see Saxena & Villalon, 1991), so it is interesting to note that in human atrial tissue, 5-HT_4 receptors cause a positive chronotropic and inotropic effect (Blondel *et al.*, 1997). More recently, 5-HT_4 receptor stimulation has been shown to elicit atrial arrhythmias in man (see Kaumann, 1994). Cisapride, the only selective 5-HT_4 receptor (partial) agonist to be marketed, has now been withdrawn due to side effects of QT prolongation and ventricular arrhythmias (Wysowski *et al.*, 2001).

Although 5-HT_4 receptors are not involved in cardiovascular reflex transmission *per se*, a cAMP-dependent attenuation of the cardiopulmonary reflex was linked to 5-HT_4 receptor activation (Edwards & Paton, 1999), *i.e.* microinjection of 5-methoxytryptamine (a non-selective agonist) into the NTS of anaesthetised rats attenuated cardiopulmonary reflex bradycardia and tachypnoea, which was prevented by pretreatment (microinjection) with RS-39604, which by itself had no effect. Further investigation found that chemical stimulation of raphe pallidus attenuated the cardiopulmonary reflex unless RS-39604 was bilaterally microinjected into the NTS (Edwards & Paton, 2000), suggesting that serotonergic axons from raphe pallidus innervate 5-HT_4 receptors in the NTS, causing an attenuation of reflex bradycardia,

probably *via* activation of GABAergic inhibition, and with the possible function of blocking reflexes in an emergency situation.

A final interesting respiratory role was recently demonstrated in rats, where respiratory depression with the opioid receptor agonist fentanyl was reversed by 5-HT_4 receptor stimulation, without loss of analgesia (Manzke *et al.*, 2003), which suggests that 5-HT_4 receptors located in the pre-Bötzinger complex can counteract this major side effects of opioid therapy.

1.6.9. 5-ht_5 receptor

The 5-ht_5 receptor molecular characteristics are well established, but no function has yet been reported. Two subtypes, 5-ht_{5A} and 5-ht_{5B}, have been identified in the rat, sharing a 68 % amino acid homology (Erlander *et al.*, 1993). In humans, however, only the 5-ht_{5A} receptor gene is functional (Schanen *et al.*, 1996), whereas the 5-ht_{5B} receptor gene is interrupted by stop codons (Grailhe *et al.*, 2001), suggesting the receptor was lost during evolution. In transfected glioma cells, the 5-ht_{5A} receptor reduces cAMP accumulation (Carson *et al.*, 1996). It was later shown to couple to G_i/G_o (Francken *et al.*, 1998). Additionally, when expressed in *Xenopus* oocytes, the 5-ht_{5A} receptor can couple to and activate the inwardly rectifying K^+ channel (Grailhe *et al.*, 2001).

5-ht_{5A} receptor mRNA is found in rat hippocampal CA1, medial habenula, raphe, septum, and hypothalamus, whereas 5-ht_{5B} mRNA is found throughout the CNS (Erlander *et al.*, 1993; Grailhe *et al.*, 2001). No 5-ht_{5A} receptor mRNA is found in peripheral organs (Grailhe *et al.*, 2001), although both mRNA and immunoreactivity are reported in the carotid body, petrosal and superior cervical ganglia (Wang *et al.*, 2000b), suggesting a role in the chemoreceptor pathway. 5-ht_{5A} receptor mRNA is also found in rat astroctyes, at least during development (Carson *et al.*, 1996), proposing a possible role for serotonergic signalling in gliosis or other glial cell functions.

The human 5-ht_{5A} receptor has high affinities (pK_i) for LSD (9.7) and 5-CT (8) (Grailhe *et al.*, 2001), but no selective ligands have been identified. Several

commonly used 5-HT receptor ligands have comparable binding affinities at human and mouse cloned 5-ht_{5A} receptors, including (with pK_i at human receptor) methiothepin (8.5), ergotamine (8), 5-CT and ritanserin (7.6), 5-HT (6.7) and clozapine (6.5) (Grailhe *et al.*, 2001). The low affinity for 5-HT is noteworthy, and might suggest a function as a back-up receptor, activated only by high concentrations of endogenous transmitter, and hence a possible emergency control mechanism. Of further note is the potential overlap of 5-ht_{5A} receptor pharmacology with that of other 5-HT receptors. The overlap with 5-HT_{1A} is minimal, since 8-OH-DPAT has a pK_i of only 5.7 (Grailhe *et al.*, 2001), and WAY-100635 has a pK_i of < 5 (Thomas *et al.*, 2004) at the cloned human 5-ht_{5A} receptor. Significant overlap, however, exists with the 5-HT_7 receptor, not only in the binding of 5-CT and methiothepin, but of the selective antagonist SB-269970, which has a pK_i at the 5-ht_{5A} receptor of 7.2 (human), 6.9 (guinea pig) and 7.8 (rat) respectively (Thomas *et al.*, 2004).

Very little functional data is available. The 5-ht_{5A} (-/-) knockout mouse displays increased exploratory behaviour without accompanying anxiolytic behaviour, as well as, conversely, a reduction of LSD-evoked exploratory behaviour (Grailhe *et al.*, 1999), which supports a further functional binding site for LSD, but offers no real insight into receptor function. Based on deduction using a range of antagonists, 5-ht_{5A} receptor activation may also account for the cardiac sympathoinhibition caused by i.v. 5-HT in the pithed rat (Sanchez-Lopez *et al.*, 2003), but more selective ligands are required to characterise this and any other functions of this poorly understood receptor.

1.6.10. 5-HT_6 receptor

The rat 5-HT_6 receptor was identified by cloning, and found to increase cAMP production (Ruat *et al.*, 1993a). Cloning of the human receptor found it to be similar to rat in molecular, transductional and pharmacological properties (Kohen *et al.*, 1996).

In the rat and guinea pig, receptor mRNA was detected by Northern blotting and *in situ* hybridisation in the striatum, olfactory tubercle, nucleus accumbens and hippocampus (Ruat *et al.*, 1993a). Receptor immunoreactivity was demonstrated in

similar areas, plus frontal, entorhinal and piriform cortex, cerebellum, caudate-putamen, and some brainstem motor nuclei (Hamon *et al.*, 1999). Human receptor distribution is comparable to the rat and guinea pig, most noteably in the caudate nucleus (Kohen *et al.*, 1996). 5,7-DHT lesions did not affect 5-HT_6 receptor mRNA expression, suggesting it is not an autoreceptor (Gerard *et al.*, 1996).

Non-selective agonists at the human cloned 5-HT_6 receptor include (with pK_i) 5-methoxytryptamine (7.4), 5-HT (7.2), 2-methyl-5-HT (6.4) and 5-CT (6.1), and antagonists include methiothepin (9.4), mianserin (7.3) and methysergide (6.7) (Kohen *et al.*, 1996). Recently, highly selective antagonists have been developed, such as Ro-650563 (pK_i 7.9) (Sleight *et al.*, 1998) and SB-357134 (8.8) (Stean *et al.*, 2002). EMDT has been described as a moderately (10-fold) selective agonist (pK_i 7.8) (Glennon *et al.*, 2000). Several common antipsychotics also bind to 5-HT_6 receptors, including olanzapine (pK_i 8.6), clozapine (8.4) and pimozide (7.2) (Roth *et al.*, 1994).

Treatment with selective antagonists has produced a behavioural syndrome of stretching, yawning and chewing in rats (Sleight *et al.*, 1998), which was also found when i.c.v. antisense oligonucleotides were given (Bourson *et al.*, 1995), although a separate group instead reported an anxiogenic effect of i.c.v. antisense (Hamon *et al.*, 1999). The main current interests in this receptor are for the treatment of schizophrenia and cognitive decline (see Branchek & Blackburn, 2000). There are no reports or indications that the receptor may have any autonomic functions.

1.6.11. 5-HT_7 receptor

The 5-HT_7 receptor is the most recently identified 5-HT receptor, and has attracted a fair amount of pharmaceutical research interest, much of which has been recently reviewed (see Vanhoenacker *et al.*, 2000; Glennon, 2003; Thomas & Hagan, 2004; Hedlund & Sutcliffe, 2004). Prior to its classification in 1993, the 5-HT_7 was described as an as yet unidentified receptor different from other 5-HT receptors. 5-HT induced relaxation of the porcine vena cava was antagonised by methysergide (Trevethick *et al.*, 1984) suggesting a novel 5-HT receptor mediating vasodilatation. This receptor was further characterised as being positively coupled to adenylate

cyclase, and activated by 5-CT (Sumner *et al.*, 1989), making it different from any other 5-HT receptor known. A similar unidentified 5-HT receptor was found on the canine coronary artery (Cushing & Cohen, 1992) and in the vicinity of SPNs in the rat spinal cord (Lewis & Coote, 1990).

Molecular biology

In 1993 a novel nameless 5-HT receptor was cloned from a rat cDNA library (Meyerhof et al., 1993), and then also from a human library and described as the 5-HT_7 receptor (Bard *et al.*, 1993). Then in quick succession it was also cloned from mouse (Plassat *et al.*, 1993), rat (Lovenberg *et al.*, 1993a) and guinea pig (Tsou *et al.*, 1994), and later from pig (Bhalla *et al.*, 2002). The 5-HT_7 receptor gene has been localised to chromosome 10 of the human genome (q23.3 – q24.4) (Gelernter *et al.*, 1995). Alternative splicing has been described in rat and human, consisting of splice variants 5-$HT_{7(a)}$ and 5-$HT_{7(b)}$, which are homologous in both species, and a third splice variant termed 5-$HT_{7(c)}$ in rats and 5-$HT_{7(d)}$ in humans (see Vanhoenacker *et al.*, 2000). All 3 isoforms are functionally active: 5-$HT_{7(a)}$ is the most abundant in the brain, accounting for 55 % of 5-HT_7 mRNA in the human hippocampus, and 80 % in the rat (Heidmann *et al.*, 1997). The 5-HT_{7b} isoform is the second most abundant in both species, and the 5-HT_{7c} (rat) and 5-$HT_{7(d)}$ (human) isoforms account for less than 4 % of mRNA (Heidmann *et al.*, 1997). The splice variants display similar binding characteristics. In the human splice variants, however, different numbers of phosphorylation sites and different C terminus lengths have been found, which might lead to differences in desensitisation and trafficking properties, even if gross pharmacology is similar .

The 5-HT_7 receptor is positively coupled to adenylate cyclase (Bard *et al.*, 1993), presumably *via* G_s. The 5-$HT_{7(a)}$ isoform couples to the Ca^{2+}-calmodulin sensitive isoforms of adenylate cyclase (AC1 and AC8), which are neurone specific, leading to an increase in intracellular Ca^{2+}, both of which imply a role in neuronal excitability (Baker *et al.*, 1998). Indeed, activation of 5-HT_7 receptors increases neuronal excitability by suppressing the Ca^{2+}-activated K^+ current causing slow afterhyperpolarisation following a spike discharge, which has been demonstrated in brain slices containing hippocampal CA3 (Bacon & Beck, 2000) and midline thalamus (Goaillard & Vincent, 2002). This appears to be due to direct inhibition of

the Ca^{2+} activated K^+ channel, which also causes an increase in burst firing in CA3 (Gill et al., 2002). This 5-CT induced firing was absent in slices of 5-HT_7 receptor (-/-) knockout mice (HI Choudhury *et al.*, in press). *In vivo,* the increase in firing of CA1 neurones evoked by the SSRI fluvoxamine is abolished by 5-HT_7 receptor antagonists, which by themselves do not affect population spike activity (Matsumoto *et al.*, 2002) confirming a role for the 5-HT_7 receptor in neuronal activity *in vivo*, albeit not a tonic one.

Distribution

Receptor distribution has been reported in a wide range of CNS structures, mainly using autoradiography with [^{3}H]-5-CT or other compounds, *in situ* hybridisation, and rarely immunocytochemistry. Combined autoradiography and *in situ* hybridisation found overlapping binding and mRNA signals in rat superficial neocortex, lateral septum, hypothalamus, dorsal/midline thalamus, amygdala and hippocampus, as well as weaker signals in dorsal raphe, periaqueductal grey, pontine nuclei, NTS, and dorsal horn of the spinal cord (Gustafson *et al.*, 1996). The same technique in the guinea pig revealed overlapping signals in thalamus, hippocampus, hypothalamus, neocortex and amygdala, and weak binding in dorsal raphe, NTS and the reticular formation (To *et al.*, 1995). An antibody to the 5-HT_7 receptor was raised, binding and overlapping with mRNA expression in rat superficial neocortex, olfactory tubercle, septal nuclei, hippocampus, amygdala, thalamus and hypothalamus (Neumaier *et al.*, 2001). In this study, agonist induced (8-OH-DPAT in the presence of WAY-100635) c-fos expression was shown in neurones, demonstrating the effect of the 5-HT_7 receptor on neuronal excitability. Another study has also reported 5-HT_7 receptor immunoreactivity in the rat cerebellum, confined to Purkinje cells (Geurts *et al.*, 2002), and on hippocampal pyramidal cells (Bickmeyer *et al.*, 2002). More recently, the selective antagonist [^{3}H]-SB-269970 has been used to radiolabel 5-HT_7 receptors in the neocortex of the rat, pig, guinea pig, marmoset, and human (Thomas *et al.*, 2002). Hence it is clear that 5-HT_7 receptors are predominantly expressed in limbic areas of the brain, but also in the NTS of both the rat and guinea pig.

Pharmacology

The pharmacological profile of the 5-HT_7 receptor has redefined the classification of agonists and antagonists previously thought to be selective for other receptors. No selective agonists have been found to date, but the agonist with the highest affinity is 5-CT, with a pK_i of 9.1 at human 5-$HT_{7(a)}$ and of 8.8 at guinea pig cortex (Hagan *et al.*, 2000). 8-OH-DPAT is also an agonist with moderate affinity, having a pK_i of 6.6 at both human and guinea pig receptors (Hagan *et al.*, 2000), while 5-HT has a pK_i of 8.2 and 8 respectively (Hagan *et al.*, 2000). In the original cloned human receptors, the affinity of 5-CT (pK_i) was 9, 5-HT was 8.1, and 8-OH-DPAT was 6.3 (Bard *et al.*, 1993). Compared to 5-CT, 8-OH-DPAT has an efficacy of 0.73 or 0.87 depending on which G protein adapter type is transfected (Wood *et al.*, 2000). Both 5-CT and 8-OH-DPAT also have affinities for 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors. Reported affinities of 5-CT (pK_i) include 8 at 5-HT_{1A} (Lovenberg *et al.*, 1993a), 7.9 at 5-HT_{1B}, and 8.6 at 5-HT_{1D} (Domenech *et al.*, 1997), whilst affinities of 8-OH-DPAT include 7.6 at 5-HT_{1A} (Lovenberg *et al.*, 1993a), 6.2 at 5-HT_{1B} and 7.3 at 5-HT_{1D} (Domenech *et al.*, 1997) .

Many non-selective antagonists that were previously used to generate data on other receptor systems have affinities for the 5-HT_7 receptors including (with pK_i at guinea pig cortex): methiothepin (7.4), mesulergine (6.9) and clozapine (6.5) (Hagan *et al.*, 2000). Affinities of many antagonists were also described at the cloned human 5-HT_7 receptor (with pK_i): methiothepin (8.4), metergoline (8.2), mesulergine (7.7), ritanserin (7.3), methysergide (7.1) and spiperone (7) (Bard *et al.*, 1993). Ritanserin and mesulergine, for example, were previously thought to be selective for the 5-HT_2 receptor. Various other commonly used drugs are also reported to bind to 5-HT_7 receptors, including the antipsychotics pimozide (pK_i 9.3), risperidone (8.9), chlorpromazine (7.7), loxapine (7.4), olanzapine (7) and haloperidol (6.6) (Roth *et al.*, 1994); the antidepressants mianserin (7.2) and clomipramine (6.9), and the hallucinogen LSD (8) (Ruat *et al.*, 1993b).

The first antagonist with high (100-fold) selectivity for 5-HT_7 over other 5-HT receptors was the aryl sulphonamide SB-258719 (Thomas *et al.*, 1998a), although its affinity was only moderate. Variations on this structure led to the synthesis of

compounds with higher affinity, including SB-258741 and SB-269970 (Lovell *et al.*, 2000), the latter having a pK_i of 8.9 at cloned human 5-HT7$_{(a)}$ receptors and 8.3 at guinea pig cortex, and 100-fold selectivity over other 5-HT receptors except the 5-ht$_{5A}$ receptor (50-fold selectivity) (Hagan *et al.*, 2000). SB-269970 is CNS penetrant (with a brain:blood steady state of 0.83:1 in rats) but is rapidly cleared, with no compound detectable in the brain 1 hour after a 3 mg kg^{-1} intraperitoneal (i.p.) dose (Hagan *et al.*, 2000). Further structural modifications led to the development of SB-656104, with a pK_i of 8.7, which is orally active, and has a half-life of 1.4 hr following a 10 mg kg^{-1} i.p. injection, leading to a plasma concentration of ~1 μM (Thomas *et al.*, 2003). SB-656104 binds to other receptors moderately, with the following relative selectivity for the 5-HT$_7$ receptor: 5-HT$_{1D}$ (20-fold), dopamine D2 (70-fold), 5-HT$_{1A}$ (150-fold).

General physiology

The high level of receptor expression in the hypothalamus proposed a role for the 5-HT$_7$ receptor in temperature regulation (Gustafson *et al.*, 1996); indeed, the ability of new compounds to inhibit i.c.v. 5-CT induced hypothermia has become a reliable pharmacodynamic assay of 5-HT$_7$ receptor antagonism *in vivo*. This model has demonstrated the 5-HT$_7$ receptor antagonism of SB-269970 (Hagan *et al.*, 2000) and SB-656104 (Thomas *et al.*, 2003) in the guinea pig. In the mouse, 5-CT induced hypothermia was attenuated by the 5-HT$_7$ receptor antagonists SB-269970 and SB-258719, but not by the 5-HT$_{1A}$ or 5-HT$_{1B/1D}$ receptor antagonists WAY-100635 or GR-127935 (Guscott *et al.*, 2003), confirming the 5-HT$_7$ receptor mediated mechanism. 5-CT induced hypothermia is also absent in 5-HT$_7$ receptor (-/-) knockout mice (Hedlund *et al.*, 2003), although in this study oleamide still caused hypothermia. Oleamide is an endogenous lipid that is thought to be an allosteric modulator of 5-HT$_7$ (as well as 5-HT$_{1A}$ and 5-HT$_{2A/C}$) receptor function (Hedlund *et al.*, 1999). It induces sleep, and accumulates during sleep deprivation (Basile *et al.*, 1999; Thomas *et al.*, 1998b). Furthermore, neurones of the thalamus and hypothalamus that express c-fos in response to oleamide tend to express 5-HT$_7$ receptors (Thomas *et al.*, 1999b).

Within the hypothalamus, 5-HT$_7$ receptors are highly expressed in the suprachiasmatic nucleus (SCN) (Lovenberg *et al.*, 1993a) – a cell group involved in

circadian rhythms. *In vitro*, a 5-HT_7 receptor-induced phase shift of SCN neurones has been demonstrated, which is sensitive to transcription and translation inhibitors, suggesting that 5-HT_7 receptors contribute to circadian rhythms *via* protein synthesis (Jovanovska & Prosser, 2002). *In vivo,* both SB-269970 (Hagan *et al.*, 2000) and SB-656104 (Thomas *et al.*, 2003) modulate sleep architecture, the latter increasing the latency of rapid eye movement (REM) sleep and reducing the time spent in REM sleep. These effects have stimulated pharmaceutical interest for the treatment of depression. Disturbed sleep is a common symptom of unipolar depression: in humans, sleep cycles and circadian rhythms interact to influence mood (Boivin *et al.*, 1997), and unipolar depression commonly involves reduced sleep efficiency, time, and latency, combined with increased amount of REM sleep (Brunello *et al.*, 2000). Conversely, SSRIs modify sleep architecture in healthy and depressed patients (Schlosser *et al.*, 1998; Trivedi *et al.*, 1999). Hence the ability of 5-HT_7 receptor antagonists to mimic the sleep modulating profiles of antidepressants raised the possibility that this could be a good predictor of antidepressant efficacy. It is interesting to note that a number of antidepressants including fluoxetine and imipramine are reported to increase c-fos expression in SCN neurones *via* 5-HT_7 receptor activation, and also to downregulate these receptors with chronic treatment (Mullins *et al.*, 1999).

A role for 5-HT_7 receptors in anxiety is under preliminary investigation, based on the assumption that inhibition of ascending dorsal raphe neurones has an anxiolytic effect, as is seen with the 5-HT_{1A} receptor agonists (see Menard & Treit, 1999), which subsequently decrease 5-HT efflux in raphe terminal fields (Sharp *et al.*, 1993). In rat dorsal raphe slices, SB-269970 was originally reported as not affecting local 5-HT efflux (Roberts *et al.*, 2001a), but a subsequent adjustment of the protocol showed that SB-269970 does cause a bicuculline-sensitive decrease in 5-HT efflux, suggesting that 5-HT_7 receptors are located on GABAergic interneurones in this nucleus (Roberts *et al.*, 2004b). Whether these effects extend to other brain areas, or correlate to behaviour, remains to be established.

Additional to its role in the treatment of depression, there has been interest in the 5-HT_7 receptor's role in the treatment of psychotic illness, due to its affinity for clozapine (Plassat *et al.*, 1993). The antagonist SB-258719 was tested on 3 animal

models of the positive symptoms of schizophrenia, but its only effect was to normalise phencyclidine-disrupted prepulse inhibition, with no effect on the other models (Pouzet *et al.*, 2002). Amphetamine induced locomotion was reduced in this study, but so was basal locomotor activity, so the results are unclear. Suffice it to say that this antagonist does not display the typical profile of an antipsychotic drug.

There has also been some interest in the 5-HT_7 receptor's role in learning and memory. In contrast to the 5-HT_6 receptor, 5-HT_7 receptor *activation* may enhance memory, since the beneficial effects of 8-OH-DPAT on learning consolidation in rats was blocked by the non-selective 5-HT_7 receptor antagonists ritanserin and LY-215840 (Meneses & Terron, 2001). Although in this study the role of the 5-HT_7 receptor is not unequivocal, recent research with 5-HT_7 receptor (-/-) knockout mice describes an impaired contextual fear conditioning, together with reduced long-term potentiation in hippocampal CA1 (Roberts *et al.*, 2004a), but no other differences compared to (+/+) wild-type littermates on a battery of behavioural tests including locomotion, rotarod, light-dark transfer, tail flick analgesia, Barnes maze, and operant food conditioning. Further research, using selective antagonists, will be necessary for full characterisation of the behavioural functions of the 5-HT_7 receptor.

Autonomic functions

Very little has been reported on the autonomic roles of the 5-HT_7 receptor. Various peripheral functions have been described, including dilatation of canine coronary artery (Cushing & Cohen, 1992) and carotid artery (Centurion *et al.*, 2000; Villalon *et al.*, 2001). Additionally, 5-HT induced tachycardia is thought to be caused by cardiac 5-HT_7 receptors in the cat (Villalon *et al.*, 1997), although there is a marked species difference in this effect (see Saxena & Villalon, 1991).

Prior to the characterisation of the 5-HT_7 receptor, it was reported that 5-CT applied iontophoretically to rat sympathetic preganglionic neurones caused excitation that could not be blocked by selective antagonists, hence pointed to a novel (5-HT_1-like) receptor (Lewis & Coote, 1990). In retrospect, the 5-HT_7 receptor would be a strong candidate. Since then, this receptor has been implied in the transmission of spinal reflexes evoked by stimulation of the sural nerve in the decerebrate rabbit: in this

respect, reflex facilitation due to 8-OH-DPAT was inhibited by ritanserin (Ogilvie *et al.*, 1999), hence suggesting but not confirming 5-HT_7 receptor involvement.

Supraspinally, the 5-HT_7 receptor has been implicated in the control of micturition in anaesthetised rats: both SB-269970 and SB-656104 inhibit reflex-evoked bladder contractions when given i.c.v., but not when given i.t. (Read *et al.*, 2003). Also i.c.v. 5-CT, in the presence of GR-127935, causes spontaneous bladder contractions (Read *et al.*, 2004). Together these results suggest an important physiological role for supraspinal 5-HT_7 receptors in the control of bladder function, and raised the question of whether other parasympathetic functions also utilise this receptor.

1.6.12. 5-HT transporter

The 5-HT transporter, often referred to as SERT (serotonin transporter) is the principal mechanism of inactivation of released 5-HT, removing it from the extracellular space, back into neurones where it can be either repackaged into vesicles, or degraded by monoamine oxidase. SERT belongs to a large family of transporters, other members of which selectively sequester other monoamines (noradrenaline and dopamine), amino acids (glutamate, GABA, glycine), and other molecules (see Amara & Kuhar, 1993). The monoamine transporters constitute a distinct subfamily on the basis of their high amino acid homology, and their affinities for various antidepressants, amphetamine analogues, and cocaine.

SERT was cloned from the rat (Blakely *et al.*, 1991; Hoffman *et al.*, 1991), where it is a Na^+-dependent transporter (*i.e.* Na^+ is a co-factor co-transported with 5-HT), with 12 transmembrane spanning domains, highly expressed in raphe areas, and sensitive to a variety of antidepressants. The human cloned transporter shares 92 % of its sequence with the rat (Ramamoorthy *et al.*, 1993). Autoradiography with tritiated uptake inhibitors reveals dense binding in the raphe, and moderate binding throughout all their projection areas in humans (Cortes *et al.*, 1988) and rats (Hrdina *et al.*, 1990; Hensler *et al.*, 1994). A similar pattern of SERT immunoreactivity is found (Qian *et al.*, 1995), and the SERT antibody is a popular tool for staining 5-HT containing structures in general.

A variety of SSRIs and tricyclic antidepressants bind to cloned human SERT and rat cortical SERT with comparable affinities (Owens *et al.*, 1997). Of the SSRIs, the most potent is paroxetine, with a pK_i (human cloned SERT) of 10, compared with 7.1 at the human cloned noradrenaline transporter. The most selective with respect to this latter transporter is citalopram (pK_i 8.8 *vs.* 5.1), just ahead of fluoxetine (9 *vs.* 6.1) (Owens *et al.*, 1997). An example of a SERT-selective tricyclic is clomipramine (pK_i 9.8 *vs.* 7.3) (Millan *et al.*, 2001), whereas a non-selective tricyclic is amitriptyline (pK_i 8.6 *vs.* 7.7) (Owens *et al.*, 1997). Additionally, a number of compounds such as MDMA ('ecstasy') and fenfluramine require SERT to gain entry to neurones, (Hekmatpanah & Peroutka, 1990; Wichems *et al.*, 1995) causing non-vesicular release (retrotransport) of 5-HT, again *via* SERT.

SERT is regarded as the primary site of action of many antidepressants. Although their mode of action is complex, their initial effect is to increase synaptic concentrations of 5-HT. At first this would decrease raphe neuronal firing by autoreceptor activation, but chronic treatment is thought to desensitise autoreceptors, thus enhancing serotonergic transmission at the heteroreceptors (Blier *et al.*, 1990). This would account for the several week delay in therapeutic effect. The many roles of SERT and its ligands in the pathogenesis and treatment of affective and other disorders have been extensively reviewed (Lesch, 1997; Stanford, 1999).

Whilst older tricyclic antidepressants are renowned for cardiovascular side-effects (due to peripheral muscarinic and α_1 adrenoceptor antagonism, and Type 1A antiarrhythmic action), central autonomic effects of selective compounds are rarely reported. From the mode of action of SSRIs, they should be able to enhance certain serotonergic functions. Indeed, i.c. fluoxetine augments reflex bronchoconstriction in the anaesthetised guinea pig (Bootle *et al.*, 1998). Repeated (4 day) fluoxetine treatment also enhances baroreflex control of the sympathetic system in rats (Moffitt & Johnson, 2004), which may account for its success in the treatment of orthostatic intolerance. Similarly, human post traumatic stress disorder patients tend to display decreased heart rate variability, which is reversed with fluoxetine treatment (Cohen *et al.*, 2000). Other researchers report no effect of SSRIs on heart rate variablity in depressed patients (Sattler *et al.*, 2000) or in healthy volunteers (Siepmann *et al.*,

2003), although in this latter study the SSRI sertraline did lower baseline HR and skin conductance, possibly due to sympathoinhibition.

1.7. Aims of the thesis

The aims of this thesis are to characterise the serotonergic control of cardiovascular reflexes in rats with reference to the 5-HT receptor subtypes involved, the central pathways innervating these receptors, and the synaptic mechanisms responsible for interaction between serotonergic and glutamatergic reflex transmission in the medulla. These investigations are divided into the following parts:

1. To characterise which 5-HT receptors in the brainstem are involved in the transmission of the cardiopulmonary reflex, baroreflex and chemoreflex in anaesthetised rats, using new highly selective antagonists. The aim is to confirm previous findings on the role of the 5-HT_{1A} receptor in the rabbit, to spot any species differences, and also to investigate the possible role of the 5-HT_{7} receptor, which has recently been implicated in bladder reflex control. This study will also re-examine previous findings of the role of 5-HT_{1A} receptors in cardiopulmonary reflex control in anaesthetised rats, in which no sympathetic or respiratory variables were measured, and in which non-selective 5-HT receptor antagonists were used.

2. To investigate which central 5-HT containing cell groups may be responsible for the effects observed in 1. above. Initially this will involve electrically and chemically stimulating areas of the medullary raphe to locate any areas affecting cardiac vagal outflow. Subsequently, the role of endogenous 5-HT and 5-HT receptors in these responses will be evaluated. One of these techniques (5-HT depletion) will also allow the role of endogenous 5-HT in cardiovascular reflex function to be assessed. Additionally, an attempt will be made to reconcile the conflicting previous reports on sympathetic and respiratory roles of the raphe by careful topographical analysis of the roles of these nuclei.

3. To examine the site of action of intracisternally applied compounds characterised in 1. above, using extracellular single unit recordings from dorsal medullary nuclei. Additionally, the role of glutamatergic transmission in the NTS will be studied (using iontophoretically applied drugs) to assess the interactions between glutamatergic and serotonergic signalling in reflex pathways, with particular attention to higher order neurones in the NTS. Neurones will also be labelled by the juxtacellular technique, and subsequent 5-HT immunocytochemistry will be performed to assess the apposition of 5-HT containing terminals with these physiologically characterised neurones.

4. Having characterised the roles and mechanisms of 5-HT receptor contribution to cardiovascular reflexes in anaesthetised rats, a brief study will be carried out to confirm that these mechanisms are functioning in awake animals.

2. GENERAL METHODS

The methods outlined below describe the general methods used for the preparation of anaesthetised animals and the collection of data presented in this thesis. These have been divided as shown in Table 2.1. The specific protocols used will be outlined within the methods section of each chapter, for clarity. Additionally, the full methods for the preparation of the awake rats used in Chapter 6 are described in that chapter.

Table 2.1 Methods separated according to anaesthesia and techniques

Table illustrating different methods described in this chapter, depending on anaesthetic protocol (A or B), and how these are used in different chapters. The variables measured in each chapter are also shown (MAP: mean arterial pressure, ECG: electrocardiogram, IRNA integrated renal nerve activity, IPNA: integrated phrenic nerve activity, Cell: single unit extracellular potential, HR: heart rate).

	Anaesthesia	*Chapter*	*Techniques used*	*Variables*
A	α-chloralose	**3**	**Reflex pharmacology**	MAP
	α-bungarotoxin			ECG
	atenolol	**4**	**Raphe stimulation**	IRNA
			5-HT depletion	IPNA
B	pentobarbitone	**5**	**Single unit electrophysiology**	MAP
	gallamine		Iontophoresis	ECG
			Topical applications	IPNA
			juxtacellular labelling	Cell
C	None	**6**	**Reflex pharmacology**	MAP
			(awake rats)	HR
	See Ch 6			
	for methods			

2.1. Preparation of rats

Experiments were performed on adult male Sprague-Dawley rats (280 – 450 g) under a personal and project licence from the UK Home Office. At the end of experiments, animals were euthanased with pentobarbitone sodium (60 mg i.v.) All rats were obtained from a colony at the Comparative Biology Unit, Royal Free & University College Medical School.

Induction of anaesthesia

Animals were placed in a perpex induction chamber and anaesthetised with isoflurane (5 % in 100 % O_2), then placed supine on a homeothermic heating blanket (Harvard Apparatus), the isoflurane transferred to a face-mask (Fluovac), and the concentration reduced to 1.5 – 2.5 %. Core body temperature was maintained between 37 and 38°C. by means of a rectal temperature probe attached to the homeothermic system (Harvard Apparatus).

Cannulation of blood vessels

The left femoral vein was cannulated (Portex non-sterile tubing, ED 0.96 mm, ID 0.58 mm) and the cannula attached to a three-way valve for administration of drugs and fluids. The left femoral artery was cannulated (Portex non-sterile tubing, ED 0.96 mm, ID 0.58 mm); the tubing contained heparinised 0.9% saline (10 IU ml^{-1}), and was connected to a pressure transducer (Statham model P23XL) and amplifier (Grass Instruments 7PI). The cannula was used for continuous recording of arterial blood pressure and periodical collection of arterial blood samples. The skin incision was closed with stainless steel suture clips.

Maintenance of anaesthesia

Once intravenous access and blood pressure recordings were established, isoflurane was switched off, and anaesthesia was continued with α-chloralose (80 mg kg^{-1} i.v.) given over 1 min. Throughout the surgery the depth of anaesthesia was assessed by the absence of a withdrawal reflex in reponse to a noxious pinch, and supplementary doses of α-chloralose (10 mg kg^{-1} i.v.) were given if required.

2.1.2. Anaesthesia for single unit electrophysiology

For single unit electrophysiological experiments, animals were anaesthetised with pentobarbitone sodium (Sagatal®; 60 mg kg^{-1} i.p.). This provides greater stability of cardiovascular reflexes, and consequently greater stability of the brainstem and recording electrode. Once intravenous access was established, anaesthesia was maintained with supplementary doses of pentobarbitone sodium as required (approx. 20 mg kg^{-1} h^{-1} i.v.).

2.1.3. General surgical preparation

Cannulation of the trachea

A midline skin incision was made in the throat and the underlying salivary glands sprayed with Xylocaine® (lignocaine in 10 mg metered doses). The trachea was exposed by blunt dissection, and cannulated using a stainless steel tube (ED 1.8 mm) to enable connection to a ventilator (Harvard Apparatus model 683). A branch of the ventilator tubing was connected to a pressure transducer (Statham model P23XL) and amplifier (Grass Instruments 7PI) for measurement of tracheal pressure. Mechanical ventilation with oxygen-enriched room air was commenced once the animal was in the stereotactic frame, as described below.

Cannulation of the bladder

A 1 cm skin incision was made in the lower abdomen and sprayed with lignocaine. The abdomen was opened and the bladder was pulled to the surface, emptied by gentle digital pressure, and cannulated at the apex using a polythene tube (ED 1.6 mm, ID 1.0 mm). This allowed urine to drain freely, preventing any reflex effects from bladder distention. The abdomen and skin were closed with stainless steel suture clips.

Cannulation of the right atrium and cardiopulmonary reflex

The right external jugular vein was exposed following tracheal cannulation, and a polythene cannula (ED 0.8 mm, ID 0.4 mm) advanced 3 cm into the vein so that the tip lay within or close to the right atrium. This cannula was connected to a 100 µl Hamilton syringe and pre-filled with 0.05 mg ml^{-1} phenylbiguanide (PBG), which acts on 5-HT_3 receptors to activate cardiopulmonary afferents (Kay & Armstrong, 1990). The cardiopulmonary reflex was stimulated by injection of 1 – 5 µg PBG per

animal (20 – 100 µl) over 2 s. The dose of PBG was adjusted to evoked a submaximal bradycardia (40 – 100 bpm). At least 5 min was allowed to elapse between PBG injections to prevent tachyphylaxis. The skin incision was closed with stainless steel suture clips. In a few animals correct cannula placement was confirmed *post mortem.*

Intravenous injection of sodium cyanide

In experiments involving the arterial chemoreflex, a cannula (Portex non-sterile tubing, ED 0.96 mm, ID 0.58 mm) was inserted into the right femoral vein. This cannula was connected to a 100 µl Hamilton syringe and pre-filled with 0.5 mg ml^{-1} NaCN in 0.9 % saline. The chemoreflex was elicited by injection of 25 – 50 µg NaCN per animal (50 – 100 µl) over 2 s. The dose of NaCN was adjusted to evoke a submaximal bradycardia (30 – 100 bpm). To ensure a bradycardia rather than tachycardia in response to NaCN injection, arterial PaO_2 was maintained at 85 – 100 mmHg. At least 5 min was allowed to elapse between NaCN injections to prevent tachyphylaxis.

Intravenous injection of phenylephrine

In some experiment a cannula was inserted into the right femoral vein as described above, and pre-filled with the α-adrenoceptor agonist phenylephrine (0.1 mg ml^{-1} in 0.9 % saline). The baroreflex was elicited by injecting a bolus of 3 – 8 µg phenylephrine per animal (30 – 80 µl). The dose was adjusted to increase MAP by ~ 50 mmHg.

Positioning in stereotaxic frame

The animal's head was placed in a stereotaxic frame (Royal Free Medical Engineering) using ear and incisor bars, with the head ventroflexed at 20°. The body was placed on an adjustable stage, which was lowered so that the forelimbs were just clear of the stage. A midline skin incision was made over the back of the head and continued laterally over the right scapula. The nuchal muscles were dissected from the occipital bone and removed by cautery to reveal the atlanto-occipital membrane.

Cannulation of the cisterna magna and intracisternal injection

In experiments involving i.c. injection, a 23 gauge needle was used as a guide cannula. It was held in a Narashige micromanipulator at an angle of 20 - 40° from

the vertical and inserted through the atlanto-occipital membrane until its tip lay within the cisterna magna, as shown by the emergence of cerebrospinal fluid.

Test solutions for i.c. injection were drawn into a 25 µl glass syringe (Hamilton) attached via a short length of polythene tubing to a 25 gauge steel cannula. This was inserted into the cisterna magna *via* the guide needle. Test solutions were injected over 20 s, and the cannula left in place for 5 min to ensure diffusion.

Exposure of the phrenic nerve

A phrenic nerve was exposed by reflecting the right scapula laterally and removing the overlying connective tissue. The nerve was dissected and desheathed, crushed distally to block afferent traffic, and placed on a bipolar silver hook electrode. The nerve and electrode were covered in polyvinylsiloxane dental impression material (Super Dent®, Carlisle Laboratories). This prevented the nerve from drying out or of excess fluid short-circuiting the electrode. Whole nerve activity was amplified (Digitimer NL 104; gain 10 – 20 K), filtered (Digitimer NL 125; 500 – 5000 Hz), and displayed on an oscilloscope (Tektronix 5103N), and as audio output (Royal Free Medical Electronics). The signal was also passed through a solid state electronic integrator (Royal Free Medical Electronics), which quantified activity in 5 s bins above background noise. The resulting peaks are a measure of both frequency and size of burst firing.

Exposure of the aortic depressor nerve

In some experiments, the aortic depressor nerve was exposed following dissection of the phrenic nerve. The nerve was carefully dissected free from the cervical vagus and the sympathetic trunk, placed on a bipolar silver hook electrode, and insulated with polyvinylsiloxane dental impression material. The electrode was connected to a constant current stimulator box (Digitimer DS2) triggered by a digital programmer (Digitimer D4030). 5 s trains of 40 Hz pulses (0.1 ms pulse duration, 0.1 – 1 mA) were delivered and the current adjusted to evoke a submaximal bradycardia (40 – 100 bpm). The identity of the aortic depressor nerve was confirmed by the bradycardia, hypotension and renal nerve inhibition resulting from its stimulation.

Exposure of the renal nerve

In all but the single unit electrophysiolocial experiments, the animal's hindquarters were turned, with the legs both facing left. A lumbar skin incision was made just left of the midline, the lumbodorsal fascia cut and the retroperitoneum opened by dissecting through the underlying muscle. A retractor exposed the renal artery and part of the abdominal aorta. A renal sympathetic nerve was dissected from the surrounding tissue where it crosses the junction of aorta and renal artery, placed on a bipolar platinum hook electrode, and insulated with polyvinylsiloxane dental impression material. Whole nerve activity was amplified (Digitimer NL 104; gain 20 K), filtered (Digitimer NL 125; 100 – 500 Hz), and displayed on an oscilloscope (Tektronix 5103N) and as audio output above noise (Digitimer D130 Spike Processor).

Recording of electrocardiogram

ECG leads were attached to the front right and back left paw (ECG lead II) *via* needles inserted under the skin. The signal was amplified (Digitimer NL 104; gain 20 K), filtered (Digitimer NL115; 10 – 100 Hz) and displayed on an oscilloscope (Tektronix 5103N, Guernsey Ltd). The R wave was discriminated using a spike processor (Digitimer NL 201).

2.1.4. Additional preparation: raphe stimulation

Using an electric drill (RS Biotech Ltd) a rectangular occipital crainiotomy was performed from the atlanto-occipital membrane to the lambdoid suture. The crainiotomy was extended rostrally using microrongeurs, taking care not to damage the venous sinuses. The dura was cut and reflected laterally. For optimal exposure of the IV^{th} ventricle above the raphe, the overlying cerebellar lobules were removed by aspiration using a medical suction pump (MG Electronic Ltd) connected to a glass pipette. Any bleeding was controlled with haemostatic gauze (Surgicel®, Ethicon Sarl). The exposed brain was covered with a saline-moistened cotton swab.

2.1.5. Additional preparation: single unit electrophysiology

A small unilateral thoracotomy was performed to create a pneumothorax, and light traction was put on the base of the tail to straighten the spine. End-tidal tracheal pressure was held at 1 cmH_2O to prevent collapse of the lungs.

A small crainiotomy was performed to gain access to the medulla, and the atlanto-occipital membrane was removed. Just prior to the beginning of the experiment, the dura and arachnoid were cut and reflected laterally.

After isolation of the phrenic nerve, the cervical vagus nerve was also dissected away from the sympathetic trunk and aortic depressor nerve, and placed on a bipolar hook electrode, connected to a power supply and stimulus generator. A test stimulus of 50 Hz (1 ms pulse duration, 0.5 mA, 1 s train) producing an immediate maximal bradycardia confirmed a viable vagus. In some experiments the aortic depressor nerve was also identified and placed on a bipolar electrode. Nerves were insulated with polyvinylsiloxane.

2.2. Neuromuscular blockade and stabilisation

Once surgery was completed, animals were neuromuscularly blocked with α-bungarotoxin (75 μg per animal i.v.) to ensure mechanical stability and to control respiratory afferent activity. An intravenous infusion was commenced (6 ml kg^{-1} h^{-1}; Gilson Minipuls 2) consisting of 50 % Gelofusine plasma substitute and 50 % distilled water containing 100 mM $NaHCO_3$ and 10 mM glucose. This helped maintain blood volume and prevented metabolic acidosis. At regular intervals, arterial blood samples were collected in heparinised capillary tubes, and analysed using a pH/blood gas analyser (Ciba Corning 238).

Blood gases were maintained at PaO_2 90 – 120 mmHg, $PaCO_2$ 40 – 50 mmHg, and pH at 7.3 – 7.4, by adjusting the rate and/or stroke volume of the ventilator, or by giving 1 mmol $NaHCO_3$ by slow intravenous injection. A $PaCO_2$ range of slightly higher than physiological norms was chosen to ensure that phrenic nerve discharge was entrained to the ventilator cycle. The preparation was left to stabilise for at least 30 min. During neuromuscular blockade, depth of anaesthesia was continuously assessed by monitoring pupil diameter, the stability of blood pressure, heart rate and phrenic nerve activity, and the absence of cardiovascular response to noxious stimuli.

Neuromuscular blockade for single unit electrophysiology

In single unit electrophysiological experiments, neuromuscular blockade was commenced with gallamine triethiodide (initially 30 mg kg^{-1} i.v., followed by 6 mg kg^{-1} h^{-1} maintenance). This drug was selected because of its vagolytic properties, which prevent sudden changes in cardiac output due to reflex stimulation, thus reducing movement of the brainstem during these challenges. Accordingly, a dose of PBG that evoked a submaximal bradycardia was selected just prior to gallamine administration.

2.2.2. Cardiac sympathoadrenal blockade with atenolol

At the beginning of the protocol, animals were pretreated with the selective β_1 adrenoceptor antagonist atenolol (1 mg kg^{-1} i.v.) to block sympathoadrenal drive to the heart, so changes in heart rate could be assumed to reflect changes in cardiac vagal outflow. This drug has been chosen because it poorly penetrates the central nervous system (Street *et al.*, 1979) and has little or no affinity for 5-HT receptors (Middlemiss *et al.*, 1977). It also has a long duration of action, making it suitable for our protocol of approximately 1 hour. Atenolol was not given to animals where single unit electrophysiology was performed.

2.3. Raphe stimulation

2.3.1. Stereotaxic microinjections

3-barrel glass micropipettes were constructed from borosilicate glass (1.5 mm OD, 0.86 mm ID, Harvard Apparatus) bonded within brass collars (Royal Free Medical Engineering) using epoxy resin. These were pulled on a Narashige multibarrel electrode puller, and the tips broken back to 30 – 40 μm under microscopic guidance. The barrels were back-filled with 50 mM DL-homocysteic acid (DLH; in 0.9 % saline) for chemical stimulation, pontamine sky blue dye (2 % in 0.9 % saline) for marking of microinjection sites, and 0.9 % saline as a volume and pH control. All solutions were adjusted to pH 7.3 – 7.4. Each barrel was connected to a 3-way valve via a length of polythene tubing, and a 10 ml syringe used to apply positive pressure. A volume of 50 nl was injected by visualising the fluid meniscus *via* a binocular

operating microscope (Leica M651) fitted with an eyepiece graticule. In some experiments the saline barrel was filled instead with a 1:1 mixture of Wood's metal and indium for electrical stimulation. An indifferent electrode was also attached to the neck musculature, and electrical stimuli (0.2 ms pulses, 10 – 50 Hz, 50 – 100 μA, 5 – 15 s) were delivered via a stimulus generator (Digitimer D4030) attached to an isolated constant current stimulator (Digitimer DS2).

The micropipette was held vertically in a Narashige micromanipulator, and the tip zeroed at the calamus scriptorius (caudal tip of the area postrema). Distinct areas of the medullary midline were serially stimulated with reference to a stereotaxic atlas (Paxinos & Watson, 1998). The most ventral point on any micropipette penetration was marked by depositing 50 nl pontamine for subsequent histological verification.

2.4. Depletion of 5-HT stores with para-chlorophenylalanine

In a separate set of experiments, the effects of depleting stores of 5-HT were investigated using the brain-permeable tryptophan hydroxylase inhibitor para-chlorophenylalanine methyl ester (p-CPA), which depletes both the CNS as well as peripheral organs of 5-HT (Koe & Weissman, 1966)). p-CPA was dissolved as 100 mg ml^{-1} in sterile saline. Animals were given either p-CPA (350 mg kg^{-1} i.p.) or saline (3.5 ml kg^{-1} i.p.) on two consecutive days, and experiments performed 24 hours after the second dose. This dosing regimen has been reported to deplete 5-HT stores by 95 % in the hippocampus (Chaput *et al.*, 1990) and has previously been used to investigate whether an evoked response involved serotonergic transmission (Zhu & McNaughton, 1994). A single-blind design was adopted, in which the solutions were prepared by a technician, and their identity only revealed after data analysis and histology.

2.5. Single unit electrophysiology

2.5.1. Recording single unit activity

Single barrel recording electrodes were pulled from borosilicate glass and the tip broken against a glass rod to a diameter of ~1 μm. These were filled with 1M NaCl, resulting in an *in vitro* impedance of 5 – 15 MΩ. Electrodes were held in a headstage (AxoClamp HS-2A) connected to an amplifier (AxoClamp 2B). An indifferent

electrode was attached to the neck musculature. After dissection of the overlying pia mater, electrode penetrations were made along the edge of the area postrema, from 0.2 mm caudal to 0.8 mm rostral to the calamus scriptorius, and 0.1 to 0.6 mm lateral to the midline. Electrodes were lowered through the medulla in 1 μm steps, using a micromanipulator (Inchworm, Burleigh).

Neurones responding to stimulation of the ipsilateral vagus (1 Hz, 1 ms pulse, 0.1 – 1 mA) were recorded within the NTS at a depth of 350 – 900 μm, or in the DVN at a depth of 500 – 1100 μm. DVN (vagal efferent) neurones were distinguished from NTS neurones by the fact that they fire only a single evoked spike with no jitter (variability of latency), which is sensitive to the collision test, i.e. a vagal stimulus, triggered to fire at the same time as a spontanous spike, abolishes the evoked spike due to collision of the simultaneous afferent and efferent impulses.

Peri-stimulus time histograms (PSTHs; 1 ms bins) were plotted from 50 pulses (sweeps) of vagal stimulation at twice the latency of the evoked response. This characterised NTS neurones (according to the jitter of the evoked spikes) as receiving a monosynaptic or polysynaptic input.

Neurones were also functionally characterised with intra-atrial PBG, to test whether they receive cardiopulmonary afferent input (indicated by a burst of spikes within 2 s of PBG injection). In some experiments, neurones were also characterised as barosensitive or -insensitive depending on whether they fire in response to raising BP by 20 – 30 mmHg with i.v. phenylephrine (1 – 3 μg).

In some experiments, dorsal column (gracile nucleus) neurones were characterised as responding only to light touch (with a cotton bud) of the skin or fur of the ipsilateral hindquarters, and not responding to vagal stimulus. These neurones were found at a depth of 100 – 500 μm.

2.5.2. Pharmacology I: iontophoresis

In some experiments, compound electrodes were constructed, consisting of a single recording electrode bonded to a 5 barrelled iontophoresis electrode (tip diameter 5 –

10 μm; borosilicate glass, 1.5 mm OD, 0.86 mm ID bonded within brass collars with cyanoacrylate). The separation of the recording and iontophoresis tips was < 10 μm.

Iontophoresis barrels were filled with a selection of: PBG (10 mM, pH 10.5), AMPA HBr (20 mM, pH 8.5), NMDA (20 mM, pH 8.5), DNQX (2.5 mM, pH 8.5) and DNQX vehicle (2.5 % DMSO, pH 8). All drugs were dissolved in 0.9 % saline except DNQX which was dissolved in DMSO and diluted in saline. Each barrel was connected *via* a silver wire to an iontophoresis pump (Neurophore). Drugs were ejected using a negative current (5 – 300 nA). Between ejections, a positive retaining current of 15 nA was kept on the barrels to prevent leakage of drugs. To prevent artefacts due to current ejection, one barrel was filled with 2M NaCl and connected to the automatic current balancing module of the iontophoresis pump.

2.5.3. Pharmacology II: topical applications

In some experiments, the effect of test solutions, applied topically to the surface of the medulla, were tested on the baseline and evoked firing rate of NTS, DVN and dorsal column (gracile) neurones. In these experiments the medulla was conservatively exposed so that topical solution would remain over the obex region. Excess moisture was removed from the brain surface before each electrode penetration, but over the time taken to obtain a stable neuronal recording (10 – 20 min) a layer of CSF forms over the surface, so topical solution is added to and diluted by this CSF (akin to i.c. administration).

2.5.4. Juxtacellular labelling

In some experiments, the recording electrode was filled with 1M NaCl containing 2.5% (w/v) neurobiotin tracer for juxtacellular labelling (Pinault, 1996). Following electrophysiological characterisation of a neurone (with a spike amplitude of at least 400 μV at 10 MΩ impedance, suggesting juxtacellular placement of the electrode), positive current pulses (200 ms, 2.5 Hz) were applied to the electrode, starting at 0.5 nA. Neurobiotin is positively charged in 1M NaCl solution, and so is iontophoretically ejected by positive current pulses. Current was carefully increased in 0.1 nA steps until the neurone began to fire regular spikes in response to the pulse (entrainment). Current was adjusted where necessary to keep neuronal activity between 3 – 8 spikes per pulse. This entrainment was maintained for 2 – 10 min. A

maximum of 3 neurones were labelled on each side of the brain, separated by at least 300 μm in the rostrocaudal axis to prevent overlap.

2.6. Histological processing

2.6.1. Confirmation of microinjections

At the end of raphe microinjection experiments, the animals were killed with an overdose of pentobarbitone. The background noise of the nerve recordings were verified. Brains were removed and fixed in 4 % formaldehyde in 0.1 M phosphate buffered saline for at least 72 hours. 100 μm coronal sections were cut on either a freezing microtome or a vibrating microtome, mounted, stained with 1% neutral red, and coverslipped. The position of pontamine marks were reconstructed on coronal stereotactic diagrams (Paxinos & Watson, 1998).

2.6.2. Immunocytochemistry of 5-HT containing neurones

At the end of experiments involving pretreatment with p-CPA (or saline), animals were deeply anaesthetised with pentobarbitone (30 mg kg^{-1} i.v.) and transcardially perfused with 300 ml heparinised saline followed by 500 ml 4 % formaldehyde in 0.1 M phosphate buffered saline. The brains were removed and post-fixed for at least 72 hours, then cut at 100 μm on a vibrating microtome. Sections were washed (3 x 10 min) in tris-phosphate buffered saline (TPBS: 10 mM Tris, 0.9% NaCl, 0.05% thimerosal in 10 mM phosphate buffer, pH 7.4) containing 0.3 % Triton X-100, and incubated in 10 % normal horse serum (NHS; in TPBS-Triton) for 1 hour to block non-specific antibody binding sites, followed by primary antibody (rabbit anti-5-HT, Biogenesis, Poole; 1:4000 in 5 % NHS in TPBS-Triton) and left to incubate overnight. The following day sections were washed in TPBS (5 x 10 min) and incubated overnight with biotinylated secondary antibody (1:500 donkey anti-rabbit immunoglobulin; (Jackson ImmunoResearch Laboratories, West Grove PA). On the third day sections were washed in TPBS (3 x 10 min) and incubated overnight with ExtrAvidin® conjugated to horseradish peroxidase (1:1000 in TPBS-Triton). On the fourth day sections were washed in TPBS (3 x 10 min) and processed for the nickel-diaminobenzidine (Ni-DAB) reaction as follows: sections were incubated in Ni-DAB solution (20 ml consisting of 5 ml 0.4 M phosphate buffer, 13.8 ml distilled water, 0.2 ml 20 % glucose solution, 0.8 ml 1 % nickel ammonium sulphate solution, and

10 mg DAB in tablet form). After 10 min incubation period, glucose oxidase solution (final concentration 250 units ml^{-1}) was added to start the reaction. When optimum staining was observed, the reaction was stopped by washing with TBPS (2 x quickly and 2 x 10 min) and placing in distilled water. Sections were mounted on gelatinised slides, dehydrated, and coverslipped.

In some sections, the extent of background staining was confirmed by omitting the primary antibody from the protocol as a negative control.

2.6.3. Neurobiotin/5-HT double labelling

When juxtacellularly labelling was performed, at least 30 min was allowed to elapse after the last entrainment, to allow neurobiotin to diffuse throughout the neurone. Then animals were deeply anaesthetised, transcardially perfused, and the brains removed and post-fixed as described in 2.6.2 above.

50 μm coronal sections of medulla were cut on a vibrating microtome, and processed as follows, at room temperature and on a shaker. The sections were initially exposed to 1 % hydrogen peroxide (in distilled water) for 20 min to block endogenous perioxidase activity in red blood cells. Subsequently the sections were washed 3 × 10 min in TPBS-Triton as described above (see 4.2.3), and exposed to 10% normal horse serum (NHS) in TPBS-Triton for at least 30 min before incubation in primary antibody. Primary antibodies were diluted with 10% NHS in TPBS-Triton and secondary antibodies, with 1% NHS in TPBS-Triton. Sections were washed 3 × 10 min in TPBS after each exposure to an immunoreagent.

The sections were incubated for 2-3 days in 1:250 ExtrAvidin-horseradish peroxidase (Sigma) plus 1:7,500 rabbit anti-serotonin (Biogenesis). After washing, juxtacellularly labelled neurons were visualized *via* the imidazole-intensified diamidobenzidine (DAB-imidazole) reaction, peroxide being generated by glucose oxidase.

Sections were incubated in 20 ml DAB-imidazole solution, which was made up as follows: 10 ml of 100 mM Tris-HCl (pH 7.6), 200 μl of 1 M imidazole (w/v in distilled water), 200 μl of 0.4% NH_4Cl_2 and 200 μl of 20% D-glucose (in 0.05%

sodium azide). This was made up to 20 ml with distilled water, a 10 mg DAB tablet added and dissolved by stirring, filtered, and added to the sections.

After 10 min incubation, glucose oxidase solution (final concentration 250 units ml^{-1}) was added to start the reaction. When optimum staining was observed, the reaction was stopped by washing with TBPS (2 x quickly and 2 x 10 min).

The sections were then incubated overnight in 1:500 biotinylated donkey anti-rabbit immunoglobulin (Jackson ImmunoResearch Laboratories), followed by a 4 – 6 hr incubation in 1:1500 ExtrAvidin-HRP diluted with TPBS-Triton. Finally, 5-HT immunoreactive fibres were revealed with the nickel-DAB reaction, as described above (see 4.2.3.). This process visualises juxtacellularly labelled neurones in brown and 5-HT containing fibres in black. Processed sections were mounted on gelatinised slides, dehydrated and coverslipped.

2.7. Data Capture

All recorded variables were recorded onto computer hard disk using a CED 1401+ interface and Spike2 (version 4.1) software (Cambridge Electronic Design). In all experiments, BP, TP, raw phrenic nerve activity and ECG were captured as waveforms. HR was recorded as a digital input corresponding to R waves of the ECG (from the spike processor) and converted to instant frequency by Spike2 (bpm). BP and TP were displayed as mmHg and cmH_2O respectively, and the pressure transducers were calibrated regularly. A keyboard input was used to annotate the traces with text and stimulus marks. A digital output from the stimulus programmer was also displayed as a TTL pulse to record the timing of nerve stimuli.

2.7.1. Whole nerve electrophysiology

Phrenic and renal nerve activities were captured as raw signal (sampled at 1 and 2 kHz respectively), which was subsequently rectified and smoothed offline, using Spike2 functions. The time constants selected for the smoothing were 0.1 s for phrenic and 1 s for renal nerve, which. This form of processing is used for all illustrations of nerve activity, and is referred to as integrated phrenic and renal nerve

activity (IPNA and IRNA) in figures to reflect the fact that it has been rectified and smoothed.

Additionally, both raw nerve activities were passed through solid state integrators (Royal Free Medical Electronics), which were calibrated at the beginning of each experiment to quantify nerve activity above background noise. The time constant selected was 5 s, i.e. the integrator quantifies activity in 5 s bins (displayed as arbitrary units). In figures, this are referred to as IPNA units and IRNA units.

2.7.2. Single unit electrophysiology

Extracellular neuronal activity was recorded as a waveform (sampled at 5 kHz). Additionally, extracellular spikes were discriminated using a spike processor (Digitimer D130) and recorded as a Spike2 event channel. Vagus nerve stimuli were also recorded in this way. Spike activity was displayed as a continuous rate meter in 1 s bins (spikes s^{-1}, unless otherwise stated). If there was any question of the accuracy of the spike discrimination window, spikes were redescriminated offline using Spike2 spike sorting functions.

Each channel of the iontophoresis pump provided a waveform output to display the timing and amplitude (nA) of iontophoresis currents in Spike2.

2.8. Data analysis

2.8.1. Analysis of baselines and reflexes

Quantification

Annotated traces illustrating methods of measurement are shown in Figures 2.1 and 2.2. All baseline variables were calculated from the 30s period prior to the stimulation of a reflex. Control baselines were averaged from the 3 30s periods preceding the 3 control reflexes, and post-drug or vehicle baselines were measured from the 30s periods preceding each subsequent reflex.

Baseline mean arterial pressure (MAP; calculated as diastolic pressure+(pulse pressure÷3) was measured over the 30s before a reflex using best-fit horizontal cursors. Then MAP at the peak response following a reflex was measured, and absolute changes in MAP (peak response MAP-baseline MAP) were calculated for each reflex.

Baseline HR was measured in beats per min (bpm) over the 30s before a reflex using best-fit horizontal cursors. Then HR at the peak response following a reflex was measured. Since Spike2 only displays our discriminated R waves as bpm, HRs were converted to R-R interval (calculated as 60000÷HR; ms). This was done because R-R interval is linearly related to cardiac vagal outflow, therefore a more appropriate measure (see Daly, 1997, Appendix 1). From these calculations the absolute change in R-R interval (peak R-R interval-baseline R-R interval) was obtained. If during cardiopulmonary reflex stimulation there were 2 or 3 arrhythmic beats after PBG injection, these were discounted (as they can reflect movement of the cannula tip against the atrial wall) and the R-R interval immediately following them was measured.

Baseline IRNA (rectified and smoothed) was measured by bracketting the 30s prior to a reflex with vertical cursors, and averaging the waveform using Spike2 cursor functions. Then the peak change after a reflex was measured using a horizontal cursor, and calculated as the absolute fall for the cardiopulmonary reflex, and as the absolute rise within 10 s of NaCN injection for the chemoreflex.

Baseline IPNA (from the solid state integrator) was calculated by measuring the height of the 6 5s bins prior to a reflex, using a horizontal cursor, and taking the mean. For the chemoreflex, peak change was measured as the largest bin within 10 s of NaCN injection. For the cardiopulmonary reflex, phrenic changes were not consistent enough to be usefully quantified. For aortic depressor nerve stimulations, nerve activities were not measured due to frequent electrical interference.

Since the absolute values of both phrenic and renal integrated nerve activity vary substantially between animals, the baseline prior to each stimulation was normalised to 100 %, and changes expressed as % of control (calculated as (peak activity÷baseline activity×100). At the termination of each experiment with pentobarbitone (60 mg i.v.) the noise level of the neurograms was verified, and the position of zero adjusted accordingly in subsequent analysis (see Figures 2.1 and 2.2).

In a separate series of experiments the baroreflex was elicited by raising MAP with a bolus dose of the vasoconstrictor phenylephrine (3 – 15 μg kg^{-1} i.v.) to provide additional data on this reflex, namely reflex gain and reflex changes in integrated renal nerve activity (IRNA). Absolute changes in MAP and HR were measured as for the cardiopulmonary reflex. Changes in IRNA (rectified and smoothed) were measured as follows: baseline was averaged over the 30 s prior to phenylephrine, as for the cardiopulmonary reflex. Reflex-evoked change was taken as the mean of the 60 s after phenylephrine injection, this being a measure of both amplitude and duration of inhibition.

Reflex gain was quantified as described previously (Su *et al.*, 1992). An illustration of the method is shown in Figure 2.3. Using an in-house Spike2 script (see Appendix), the section of the experimental trace beginning with the phenylephrine-evoked rise in BP and ending with the trough of the reflex bradycardia was converted into beat-by-beat MAP values and their corresponding R-R intervals. These were displayed as an X-Y plot (SigmaPlot 8.0) and a regression line fitted through the points, measuring slope and correlation coefficient (r). In their raw form, the points have poor correlation because of the delay (~ 1 s) of the reflex bradycardia. In order to achieve the maximum correlation between the points ($r > 0.8$), the MAP values

were advanced (staggered) with respect to their corresponding R-R intervals, one beat at a time, and the correlation measured each time.. Typically MAPs would be staggered by about 10 beats to achieve the maximum correlation coefficient, and the slope would then be taken as the gain (in ms $mmHg^{-1}$) – a measure of reflex sensitivity.

Statistics

Raw data were collated and expressed as mean ± standard error of the mean (s.e.m.). Statistical analysis was performed using a 2-way analysis of variance (ANOVA) to compare a drug-treated experimental groups to its time-matched vehicle control group. Subsequent analysis was performed using the least significant difference (LSD) test, to calculate significant differences between means of drug and saline treated groups at specific time points.

In addition to the comparisons of drug and vehicle groups, the vehicle treated groups in themselves were analysed using 1-way ANOVA with repeated measures, followed by the Tukey test, or (non-parametrically) with 1-way ANOVA on ranks, followed by the Dunnett test. This compared the averaged control baselines and control reflexes with the baselines and reflexes at various time points after vehicle or drug injection.

For all statistical analysis, differences between groups were considered significant when $P < 0.05$.

Figure 2.1 Cardiopulmonary reflex measurements

Sample trace illustrating method of analysing baseline and cardiopulmonary afferent-evoked changes (Δ) in mean arterial pressure (MAP), heart rate (HR – converted to R-R interval), integrated renal (IRNA) and integrated phrenic (IPNA) nerve activity.

Baseline activity is averaged from the shaded area.
Reflex-evoked changes are maximum changes following afferent stimulation (PBG)

Termination of the experiment with pentobarbitone allows verification of IRNA and IPNA noise levels, and zero is set accordingly.

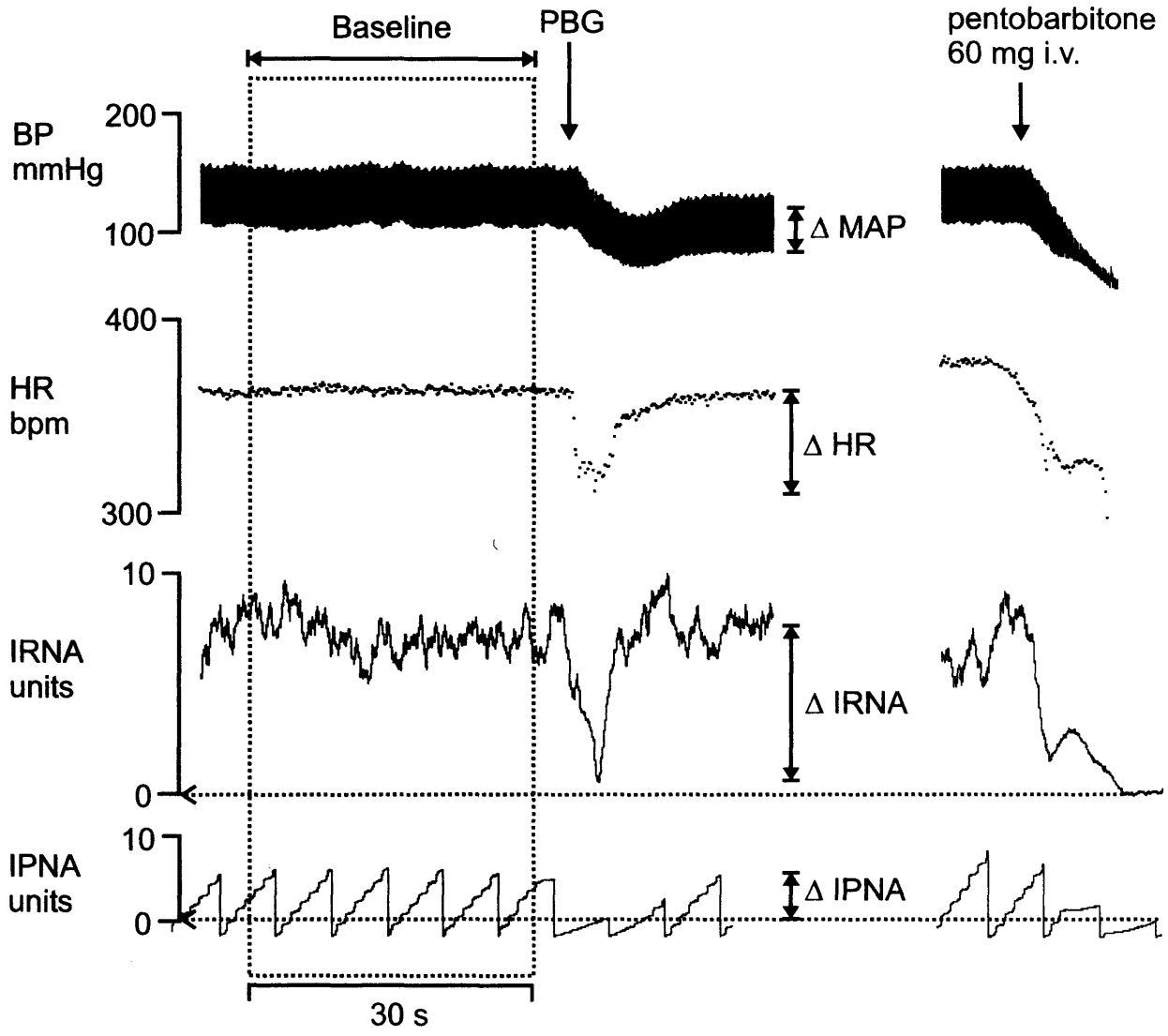

Baseline
PBG
pentobarbitone
60 mg i.v.
BP
mmHg
200
100
Δ MAP
400
HR
bpm
300
Δ HR
10
IRNA
units
0
Δ IRNA
10
IPNA
units
0
Δ IPNA
30 s

Figure 2.2 Chemoreflex measurements

Sample trace illustrating method of analysing baseline and chemoreflex-evoked changes (Δ) in mean arterial pressure (MAP), heart rate (HR – converted to R-R interval), integrated renal (IRNA) and integrated phrenic (IPNA) nerve activity.

Baseline activity is averaged from the shaded area.
Reflex-evoked changes are maximum changes following afferent stimulation with sodium cyanide (NaCN)

Termination of the experiment with pentobarbitone allows verification of IRNA and IPNA noise levels, and zero is set accordingly.

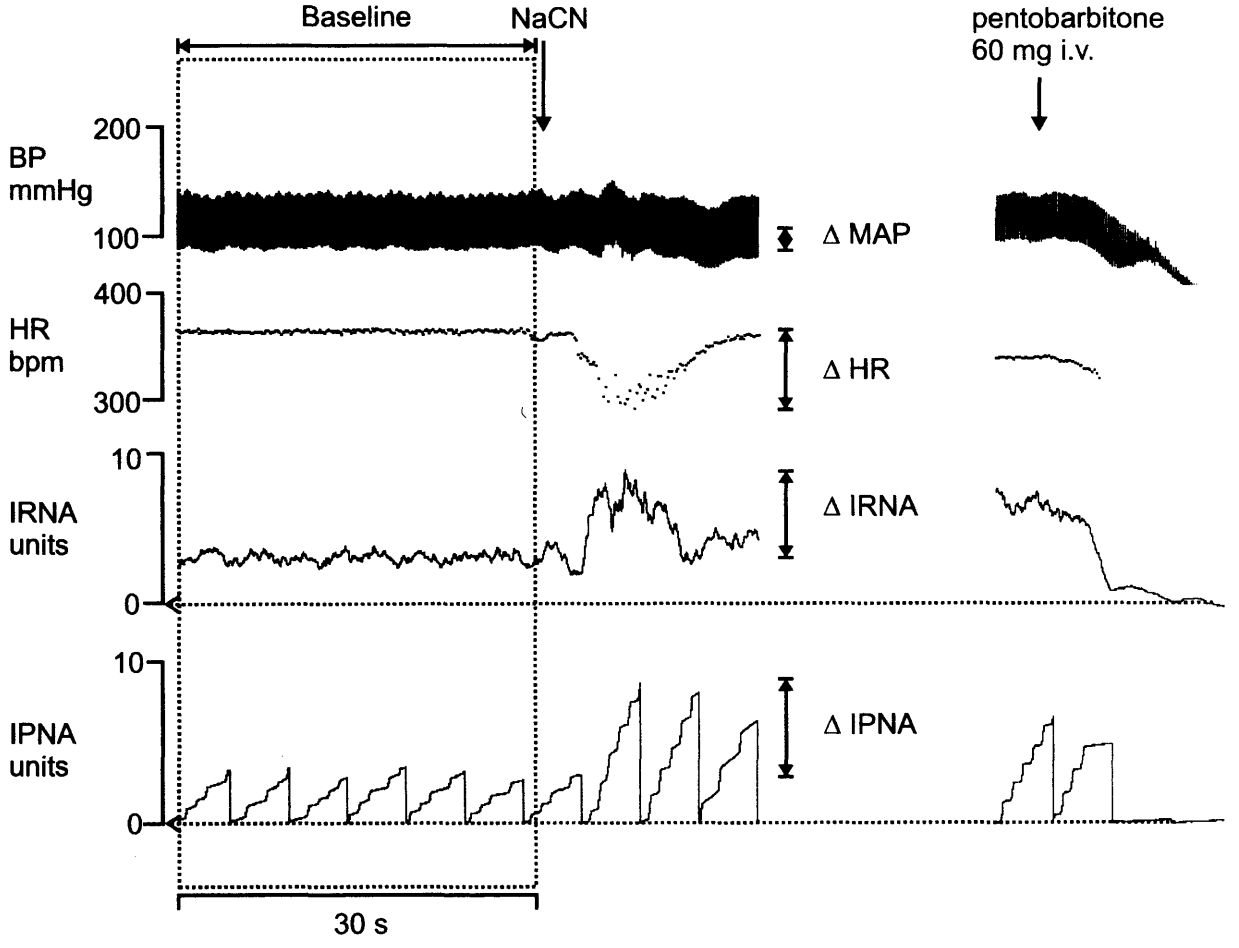
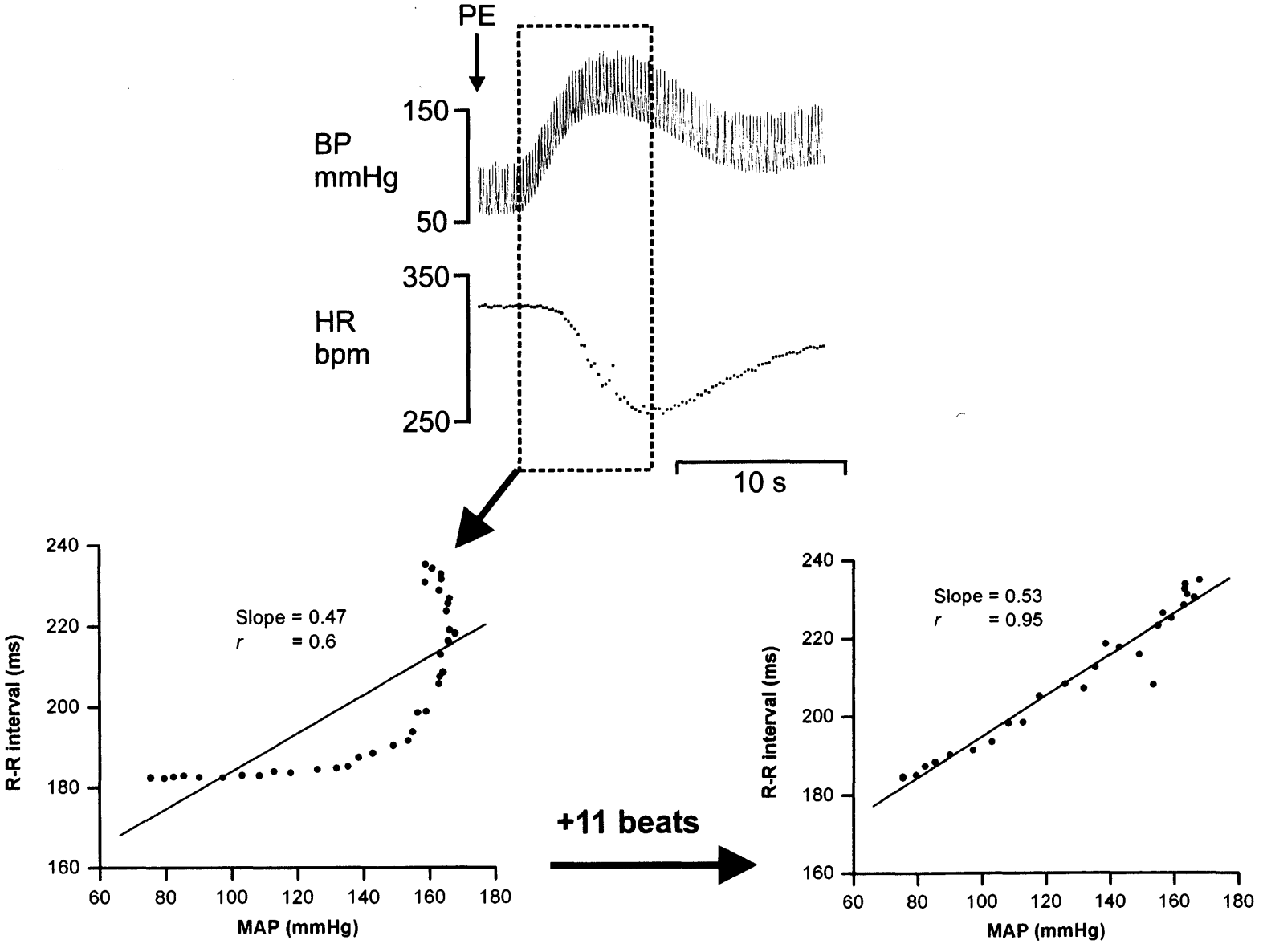

Baseline
NaCN
pentobarbitone
60 mg i.v.
200
BP
mmHg
100
Δ MAP
400
HR
bpm
300
Δ HR
10
IRNA
units
0
Δ IRNA
10
IPNA
units
0
Δ IPNA
30 s

Figure 2.3 Baroreflex gain calculation

Top: bolus injection of phenylephrine (PE) causes an elevation of blood pressure (BP) and a reflex drop in heart rate (HR). This area (shaded) is analysed by plotting each mean arterial pressure (MAP) point against the corresponding R-R interval and fitting a regression line *(bottom left)*.

In its raw form, the correlation coefficient (r) tends to be poor. Hence the MAP points are advanced (staggered) one beat at a time with respect to the R-R interval points, until the maximum correlation coefficient is found (*bottom right* – staggered by 11 beats).

The slope at this point (in ms $mmHg^{-1}$) is taken as the baroreflex gain.


PE
150
BP
mmHg
50
350
HR
bpm
250
10 s
240
220
200
180
160
R-R interval (ms)
Slope = 0.47
r = 0.6
60
80
100
120
140
160
180
MAP (mmHg)
+11 beats
240
220
200
180
160
R-R interval (ms)
Slope = 0.53
r = 0.95
60
80
100
120
140
160
180
MAP (mmHg)

2.8.2. Analysis of raphe stimulations

Quantification

All variables were measured as the baseline (from the 30s prior to a stimulation), and the peak change from baseline, as described in 2.8.1 above. MAP and HR were measured before a stimulus and at the peak response, and changes were calculated for each stimulus. As this was not a reflex study, HR was not converted to R-R interval. IRNA and IPNA were measured from the solid state integrators, and baselines were measured as the mean of the 6 bins immediately prior to stimulation. If there was a variation of over 20 %, no measurement could be made. After stimulation the bin showing the greatest change from baseline was measured. Since the absolute values of integrated nerve activity vary substantially between animals, the baseline prior to each stimulation was set at zero, and changes expressed as % change (calculated as (peak activity/baseline activity×100)-100). In addition, the peak latency of each evoked response was calculated by measuring the time (s) from the stimulus mark to the peak of the response for each variable.

Statistics

Statistical analysis of raphe stimulations was performed using a paired Student's t-test to compare baseline to peak BP and HR. Peak % change in IRNA and IPNA were analysed as a one-population t-test relative to 0.

For the analysis of receptor antagonist effects, evoked responses before and after the antagonist were compared to those before and after vehicle using 2-way ANOVA followed by the LSD test, to allow time-matched comparison of drug-induced changes with those of vehicle alone.

For comparison of the p-CPA and vehicle treated rats, peak changes were compared using an unpaired Student's t-test.

For all statistical tests, differences were considered significant when $P < 0.05$.

2.8.3. Analysis of single unit electrophysiological data

NTS and DVN neurones were characterised as shown in Figures 5.1, 5.2 and 5.3. The latency of the evoked spikes was measured by plotting a PSTH (in 1 ms bins) of 50 sweeps of 1 Hz vagal stimuli. The position of the largest bar was taken as the mean latency, and the number of bars on either side of this bar was taken as the jitter (variability) of the latency. Neurones were characterised according to the criteria described previously (Sevoz-Couche *et al.*, 2000b): neurones with < 3 ms jitter were Type 1 (second order neurones receiving monosynaptic input), those with jitter of 3 – 5 ms were Type 2 (intermediate), and those with > 5 ms jitter were Type 3 (higher-order neurones receiving polysynaptic input).

2.8.4. Analysis of iontophoretic data

Quantification

Iontophoretic data were analysed as outlined in Figure 2.4. Baseline neuronal activity was averaged (i.e to spikes s^{-1}) over a set period (equal to half the duration of the drug ejection) prior to the beginning of each drug ejection. Drug-evoked neuronal activity was averaged (spikes s^{-1}) over a period equal to the duration of the drug ejection, but beginning halfway through the current application and continuing beyond its termination. This allows for the delay between drug ejection and neuronal response (due to the separation of the drug and iontophoresis barrels). Iontophoretic agonist-evoked effects were characterised as excitatory, inhibitory, or no effect, depending on their ability to change baseline neuronal activity by > 20 %, as described previously (Wang *et al.*, 1995).

Vagus-evoked activity was measured in 20 s segments, as the total number of evoked spikes per 20 sweeps. Following DNQX, the 20 s segment corresponding to maximum inhibition was recorded.

Cardiopulmonary afferent-evoked activity was measured as the total number of spikes in the burst following PBG injection.

Statistics

The effects of agonist alone were compared to those of agonist in the presence of DNQX (or vehicle) using the paired Student's t-test (for parametric data) or the Mann-Whitney Rank Sum test (for non-parametric data).

Vagus-evoked effects during DNQX was compared to the mean of the 3 control 20 s segments using the Mann-Whitney Rank Sum test.

Cardiopulmonary afferent-evoked bursts during DNQX were compared to the mean of the control bursts using the paired Student's t-test.

For all statistical tests, $P < 0.05$ was considered significant.

2.8.5. Analysis of topical applications during single unit recording

Quantification

Neuronal activity evoked by vagal stimulation was measured (as illustrated in Figure 2.5) by plotting PSTHs for 50 sweeps (i.e. from 0 to 50 secs of each minute). Evoked spikes were counted from the PSTH, depending on the latency of the C-fibre evoked response (and its jitter) measured during initial neurone characterisation (i.e. for a neurone with latency of 30 ms and jitter of 2 ms, all evoked spikes of latency 29 – 31 ms are counted throughout the protocol). Data are presented as spikes 50 sweeps^{-1}, both before and after drug or saline. Hence 1 min after drug/saline refers to number of spikes in the opening 50 s of the 1st minute, etc.

Ongoing activity of DVN neurones was measured by calculating the mean spontaneous activity (spikes s^{-1}) for each min before and after drug or saline administration. Hence 1 min after drug/saline refers to mean firing in the 1st minute, etc.

Ongoing activity of neurones in the gracile nucleus was measured (as illustrated in Figure 2.6) by calculating the mean spontanous activity (spikes s^{-1}) during 30 s

preceding a stimulus. Touch-evoked activity was measured by counting the number of spikes starting at the tracheal pressure peak corresponding to the first stimulus, and ending at the tracheal pressure trough after the last stimulus (i.e. number of spikes 10 stimuli^{-1}). Data are presented as real values as well as being normalised (to % of control; control being the mean of the 3 pre-application values). This was to prevent variability within the samples from masking differences.

Statistics

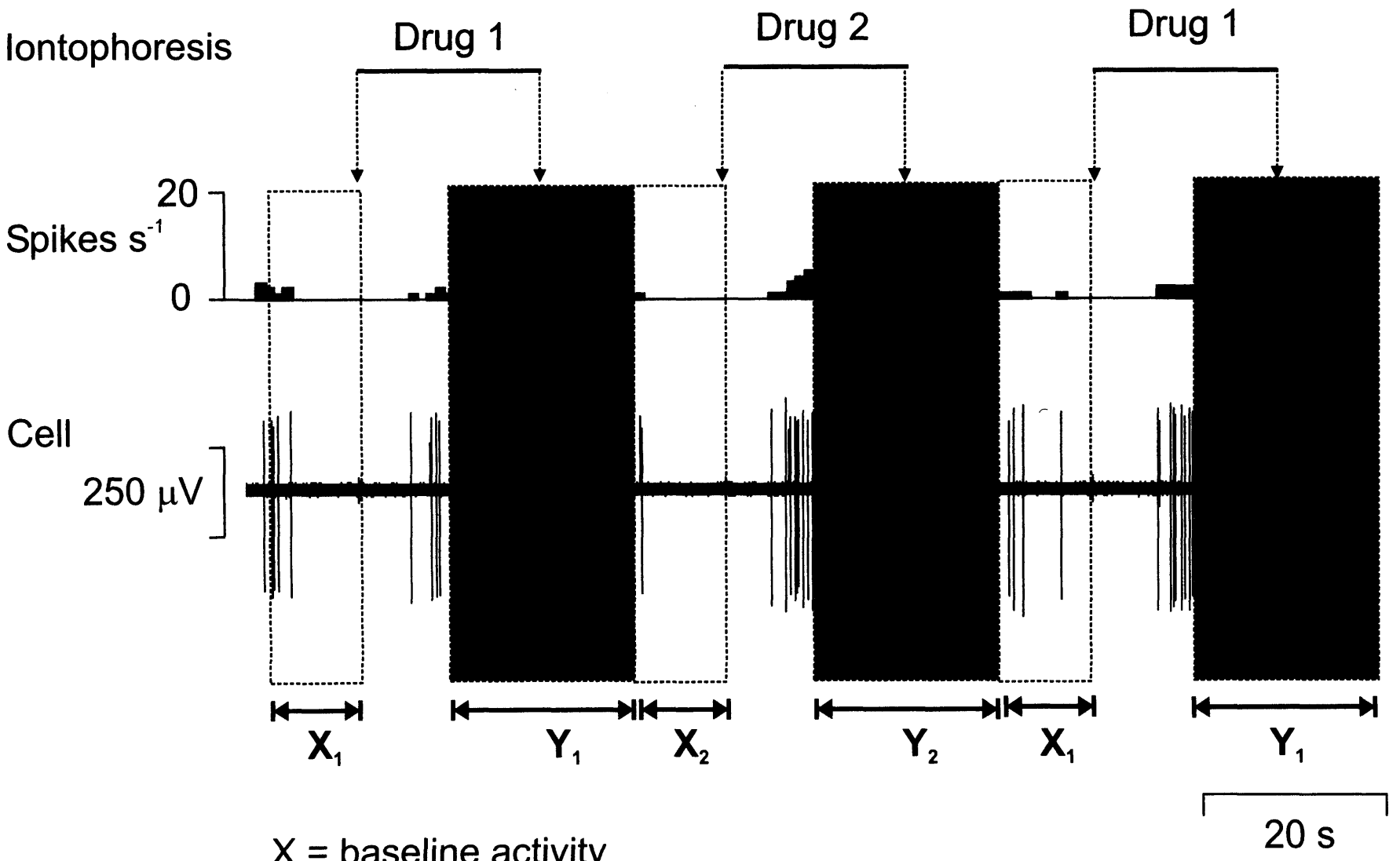
The effect of test solutions on NTS, DVN and gracile neuronal data at each time point is compared to that of their vehicle at the equivalent time, using 2-way ANOVA, followed by the LSD test. Additionally, the time course of the vehicle group is analysed using 1-way ANOVA. $P < 0.05$ is considered significant.

Figure 2.4 Analysis of iontophoretic data

Sample experimental trace illustrating method of analysing iontophoresis data. The trace shows extracellular neuronal activity (cell), the spike rate meter (s^{-1}; 1 s bins) and the timing of iontphoretic currents of Drug 1 and Drug 2.

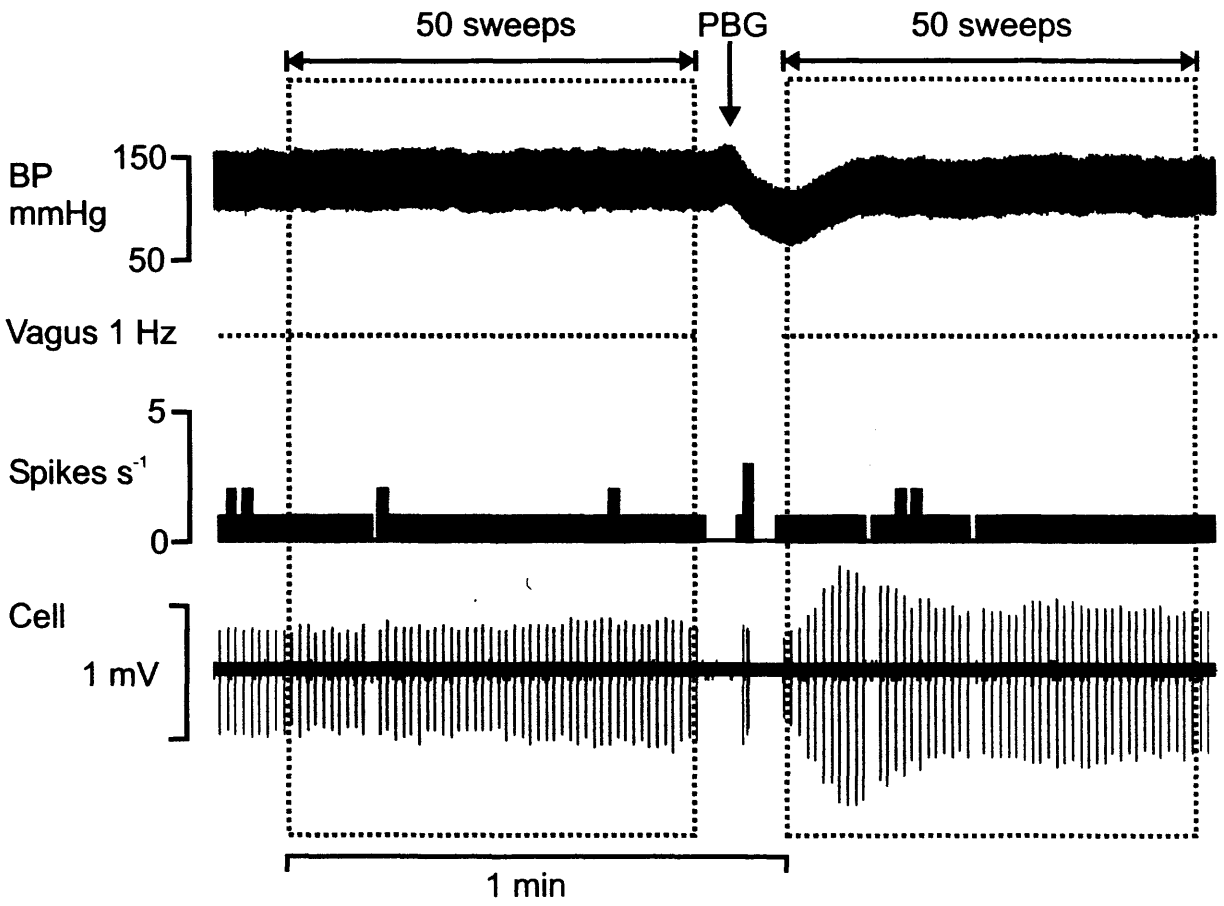
Baseline (X_1 for Drug 1 and X_2 for Drug 2) neuronal activity is averaged over 10 s prior to the beginning of the iontophoretic current of each drug. (10 s because this is half the duration of the iontophoretic current).

Drug-evoked neuronal activity is averaged over a period equal to the duration of the iontophoretic current (in this case 20 s) but beginning halfway through the current application. This allows for the delay in onset and termination of the drug effect due to the separation of the iontophoresis and recording barrels.



X = baseline activity

Y = drug-evoked activity


50 sweeps
PBG
50 sweeps
BP
mmHg
150
50
Vagus 1 Hz
5
Spikes s-1
0
Cell
1 mV
1 min

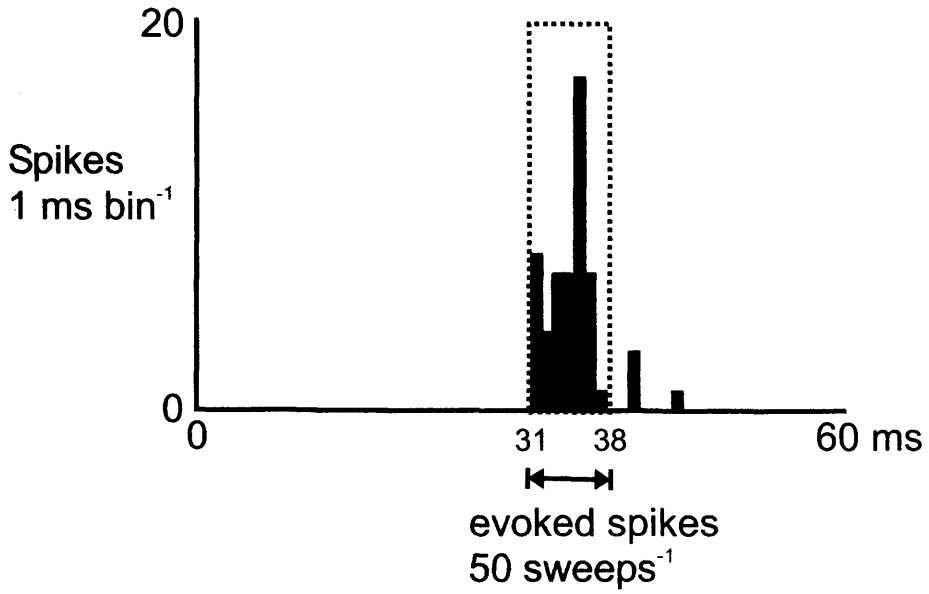

20
Spikes
1 ms bin-1
0
0
31
38
60 ms
evoked spikes
50 sweeps-1

Figure 2.5 Analysis of NTS topical applications

Sample experimental trace illustrating method of analysing vagally-evoked NTS activity following topical applications. The trace shows extracellular neuronal activity (cell), the spike rate meter (s^{-1}; 1 s bins) and the duration of 1 Hz vagal stimuli.

In each minute before and after a topical application, evoked activity is counted by plotting a PSTH (bottom) from the first 50 s of the minute (shaded areas; top). During the pre-application controls, the PSTH is analysed to set margins of the evoked response (shaded area; bottom). In this case the margins are from 31 to 38 ms post stimulus. The number of spikes within this region alone are counted throughout the protocol.

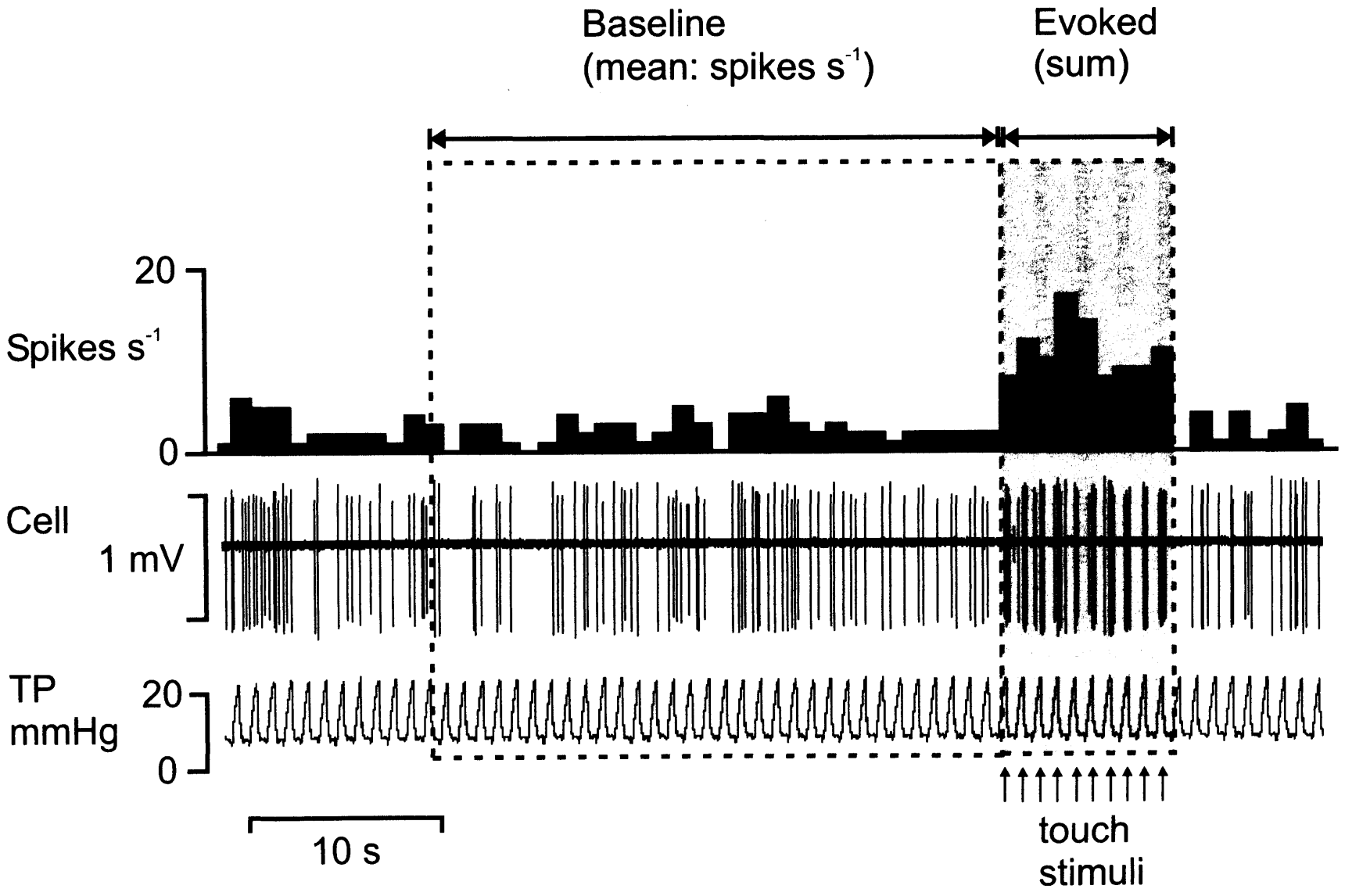
Counting the first 50 s only allows 10 s for the vagal stimulus to be switched off and PBG to be administered (top).

Figure 2.6 Analysis of gracile nucleus topical applications

Sample experimental trace illustrating method of analysing baseline and touch-evoked gracile neuronal activity following topical applications. The trace shows extracellular neuronal activity (cell), the spike rate meter (s^{-1}; 1 s bins) and tracheal pressure (TP) which is used to time the touch (cotton bud) stimuli.

Stimuli are presented 10 times (arrows), 1 ventilator cycle (~ 1 s) apart, at the beginning of each minute before and after a topical application. Evoked activity is measured by counting the total spikes within this time (dark shaded area), i.e. between the 1^{st} TP peak and the 10^{th} TP trough.

Baseline neuronal activity is measured as the mean firing rate (s^{-1}) over 30 s preceding each set of stimuli (light shaded area).


Baseline
(mean: spikes s-1)
Evoked
(sum)
20
Spikes s-1
0
Cell
1 mV
TP
mmHg
20
0
10 s
touch
stimuli

2.9. Drugs and solutions

Dissolved in 0.9 % saline:

5-HT creatinine sulphate (Sigma, USA)

8-OH-DPAT hydrobromide (Sigma, UK)

AMPA hydrobromide (Tocris, UK)

Atenolol (Sigma, UK)

Atropine methylnitrate (Sigma, UK)

α-bungarotoxin (Sigma, UK)

Cinanserin hydrochloride (a gift from Squibb, Princeton, USA)

DL-homocysteic acid (Sigma, UK)

DL-p-chlorophenylalanine methyl ester (Sigma, UK)

Evans blue (Sigma, USA)

Granisetron (a gift from GlaxoSmithKline, Harlow, UK)

Methiothepin mesylate (Sigma-RBI, UK)

NMDA (Tocris, UK)

Phenylbiguanide (Sigma, UK)

Phenylephrine (Sigma, UK)

Pontamine sky blue (BDH, UK)

SB-269970 (a gift from GlaxoSmithKline, Harlow, UK)

Sodium cyanide (BDH, UK)

Tribromoethanol (Sigma, USA)

WAY-100635, adjusted to pH. 6 with sodium bicarbonate (Sigma-RBI, UK)

Dissolved in 1M saline:

Neurobiotin tracer (biotin ethylenediamine hydrobromide; Vector Laboratories)

Dissolved in acidified saline and adjusted to pH 6 with sodium bicarbonate:

(-)-Pindolol (Sigma, UK)

Robalzotan (NAD-299; a gift from Pfizer, Sandwich, UK)

Dissolved in distilled water and salinated to 0.9 %:

SB-204070 (a gift from GlaxoSmithKline, Harlow, UK)

Dissolved in dimethyl sulfoxide

DNQX (Tocris, UK)

Dissolved in polyethylene glycol and dimethyl sulfoxide:

SB-656104 (a gift from GlaxoSmithKline, UK) was dissolved 0.6% (w/v) in a mixture of 50% polyethylene glycol and 50% dimethyl sulfoxide to form a stock solution. This was diluted with saline to yield 100 μg kg^{-1} in 10 μl (approx 55% stock and 45 % saline)

Dissolved in borax:

α-chloralose (Vickers Laboratories Ltd, UK) was dissolved (25 mg ml^{-1}) in 0.9 % saline containing 2.5 % (w/v) sodium tetraborate (Borax; Sigma, UK) to improve solubility, and heated to 40 – 60°C until dissolved.

Other drugs and reagents:

Biotinylated donkey anti-rabbit immunoglobulin (Jackson ImmunoResearch, USA)

D-glucose (BDH, UK)

Dimethyl sulfoxide (Sigma, UK)

ExtrAvidin® (Sigma, UK)

Gelofusine® (plasma substitute; Braun Medical Ltd, UK)

Glucose oxidase (Sigma, UK)

Isoflurane (Aerrane®; Baxter Healthcare Ltd, UK)

Lignocaine & noradrenaline (Lidostesin®; Probem, Brazil)

Lignocaine spray (Xylocaine®; AstraZeneca, UK).

Pentabiotico Veterinario (Fort Dodge Ltd, Campinas, Brazil)

Pentobarbitone sodium (Sagatal®; Rhône-Mérieux Ltd, UK)

Polyethylene glycol (Sigma, UK)

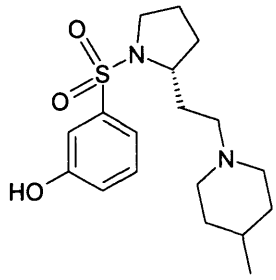
Rabbit serotonin antibody (Biogenesis, UK)

Sodium bicarbonate (BDH, UK)

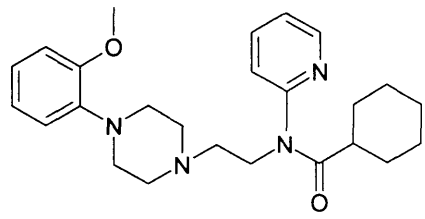
Thiopentone sodium (Thionembutal®; Abbott, São Paulo, Brazil)

Figure 2.7 Chemical structures of 5-HT receptor antagonists used

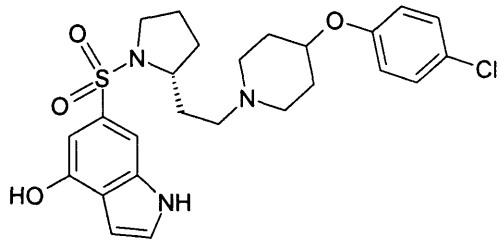
SB-269970



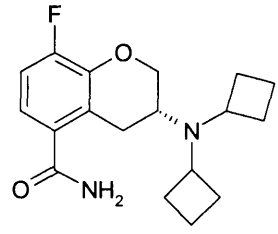
WAY-100635



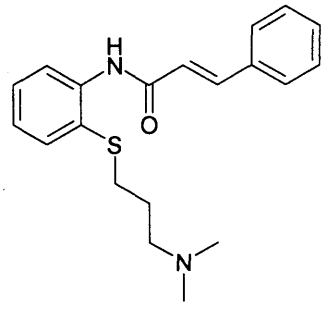
SB-656104



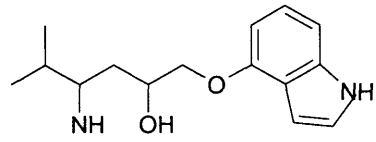
Robalzotan



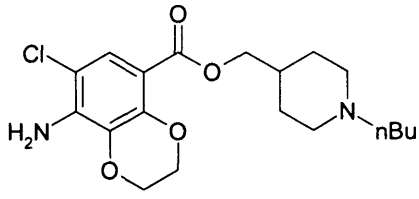
Cinanserin



Pindolol



SB-204070



3. THE ROLES OF CENTRAL 5-HT$_7$ AND 5-HT$_{1A}$ RECEPTORS IN THE REFLEX ACTIVATION OF CARDIAC VAGAL OUTFLOW

3.1. Introduction

3.1.1. Background

Central 5-HT$_{1A}$ receptors have been shown to contribute to the reflex activation of parasympathetic outflow in a number of species. Blockade of central 5-HT$_{1A}$ receptors attenuates reflex parasympathetic outflow to the heart in rats (Bogle *et al.*, 1990) and rabbits (Dando *et al.*, 1998; Skinner *et al.*, 2002), to the airways in guinea pigs (Bootle *et al.*, 1998), and to the bladder in rats (Conley *et al.*, 2001). More specifically, iontophoresis of the selective 5-HT$_{1A}$ receptor antagonist WAY-100635 onto cardiac vagal preganglionic neurones in the nucleus ambiguus of the cat attenuates their excitation by cardiopulmonary afferent stimulation (Wang & Ramage, 2001). The 5-HT$_{1A}$ receptor is negatively coupled to adenylate cyclase, suggesting that their facilitatory action in the nucleus ambiguus is due to disinhibition (see Ramage, 2000). They may be located presynaptically on the terminals of fibres containing GABA or another inhibitory transmitter, as has been shown in DVN slices (Browning & Travagli, 2001). Functional 5-HT$_{1A}$ receptors have been identified in the NTS (Wang *et al.*, 1997) and DVN (Wang *et al.*, 1995) in rats, but whether they are involved in reflex integration remains to be determined. Furthermore, central 5-HT$_{1A}$ receptors are not involved in all vagal reflexes: in rabbits, blockade of central 5-HT$_{1A}$ receptors attenuates reflex bradycardias evoked by stimulating cardiopulmonary and baroreceptor afferents, but not chemoreceptor afferents, although activation of 5-HT$_{1A}$ receptors potentiates chemoreflex bradycardia (Skinner *et al.*, 2002).

5-HT$_7$ receptors, as the most recently discovered sub-type, are now known to be pharmacologically similar to 5-HT$_{1A}$ receptors. The archetypal agonist 8-OH-DPAT, for example, has a moderate affinity for the 5-HT$_7$ receptor. Indeed it can have different effects on DVN cells when applied at low or high iontophoretic currents

(Wang *et al.*, 1995), possibly through activating these different receptors. Likewise the 5-$HT_{1/2}$ receptor antagonist methiothepin has a strong affinity for the 5-HT_7 receptor. Now that a number of highly selective 5-HT_7 receptor antagonists have become available, a re-examination of the complex area of 5-HT receptor control of parasympathetic outflow is possible.

Several observations of central importance to this study were made concerning parasympathetic outflow to the bladder: blockade of either supraspinal or sacral 5-HT_{1A} receptors strongly inhibits the micturition reflex in rats (Secker *et al.*, 2002). Additionally, blockade of supraspinal but not sacral 5-HT_7 receptors has the same effect (Read *et al.*, 2003), suggesting that both 5-HT_{1A} and 5-HT_7 receptor activation is necessary for successful reflex function. This posed the question of whether 5-HT_7 receptors contribute to other parasympathetic reflexes, and a report of the receptor mRNA being expressed in the NTS (Gustafson *et al.*, 1996) supported this possibility.

3.1.2. Aims of the study

The primary aim of this study was to investigate whether blockade of central 5-HT_7 receptors modulates the reflex activation of vagal outflow to the heart in anaesthetised rats, and whether there are any differences between cardiopulmonary, baroreceptor, and chemoreceptor evoked reflexes. The selective and structurally distinct 5-HT_7 receptor antagonists SB-269970 (Hagan *et al.*, 2000) and SB-656104 (Thomas *et al.*, 2003) were used. Secondly, the role of 5-HT_{1A} receptors was re-examined, to confirm previous findings in the rabbit using the selective antagonist WAY-100635 (Forster *et al.*, 1995) and to compare its role to that of the 5-HT_7 receptor. Additionally, the selective and structurally distinct 5-HT_{1A} receptor antagonist robalzotan (NAD-299) (Johansson *et al.*, 1997) and the non-selective antagonist (-)-pindolol were used. For further comparison, the role of some receptors that are not believed to be involved in vagal reflexes *per se* – the 5-HT_2 receptor family and the 5-HT_4 receptor – were examined, using the selective 5-HT_2 receptor antagonist cinanserin and the selective 5-HT_4 receptor antagonist SB-204070 (Wardle *et al.*, 1994).

3.2. Methods

3.2.1. Experimental protocols

After completion of surgery, animals were allowed to stabilise for at least 30 mins. Experiments were only performed in animals in which baseline variables were stable. Only animals in which stable control reflexes were obtained have been included in these studies.

At the start of each protocol, anaesthesia was supplemented by giving 15 mg kg^{-1} α-chloralose i.v. to ensure a stable level of anaesthesia during the protocol (which lasted a maximum of 80 min). Atenolol pretreatment (1 mg kg^{-1} i.v.) was also given at this time. 10 min were allowed to elapse before control reflexes were elicited. Schematic diagrams of the different protocols are shown in Figure 3.1.

Protocol 1

Protocol 1 was used to examine the effects of a selection of 5-HT receptor antagonists and their vehicles on the reflex responses evoked by stimulating cardiopulmonary afferents. 10 minutes after administering α-chloralose and atenolol, PBG was given intra-atrially every 10 mins until 3 stable control bradycardias had been obtained. A maximum of 4 control reflexes were obtained. 5 min after the final control, the test solution was administered i.c. PBG was then given 5, 15, 25, 35, and in some experiments 45 min after i.c. injection (i.e. until recovery was observed).

Protocol 1 was also used for the phenylephrine-evoked baroreflex, and phenylephrine was injected instead of PBG.

Protocol 2

Having identified compounds that significantly modulate the cardiopulmonary reflex in Protocol 1, Protocol 2 was used to compare the effect of these compounds on the reflex responses evoked by stimulating baroreceptor and chemoreceptor afferents. 10 min after administering α-chloralose and atenolol, baroreceptor afferents were activated by electrically stimulating the right aortic depressor nerve. 5 min later, chemoreceptor afferents were activated by injecting NaCN i.v. Challenges were alternated every 5 min until 3 stable control bradycardias had been obtained. A

maximum of 4 control reflexes were obtained. As soon as variables had returned to baseline after the final NaCN challenge (< 2 min), the test solution was administered i.c. Aortic nerve stimuli were given 5, 15, and 25 min after i.c. injection, and NaCN was given at 10, 20 and 30 min after drug or saline.

When using WAY-100635, which has a very short duration of action, chemoreceptor stimuli were delivered at 7, 17, and 27 min after test solution. In one supplementary experiment (illustrated in Figure 3.14) PBG was given instead of aortic nerve stimulation as a positive control.

Protocol 3

To reinvestigate the timescale of WAY-100635 at higher resolution, control reflexes (either NaCN injection or aortic nerve stimulation) were given every 10 min until 3 stable controls were obtained. 10 mins after the last control, the test solution (WAY-100635 or saline) was injected i.c., and the reflex stimulus delivered at 2 and 5 min *after the start of the injection* (which lasted 20 s).

Figure 3.1 Experimental protocols

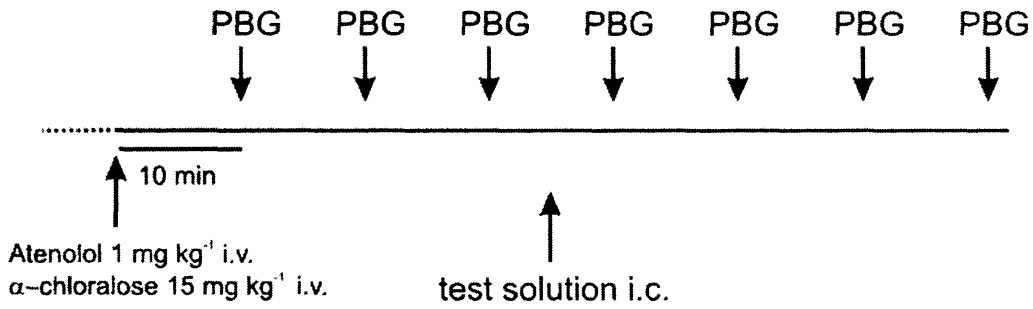
Schematic diagrams illustrating different experimental protocols for reflex pharmacology.

Protocol 1. To examine the effects of i.c. test solutions on phenylbiguanide (PBG) evoked cardiopulmonary reflex responses. (Also used for phenylephrine-evoked baroreflex).

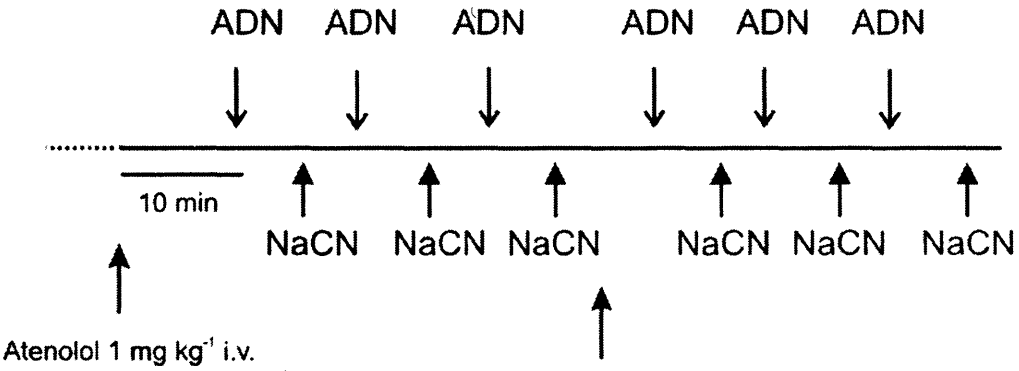
Protocol 2. To examine the effects of i.c. test solutions on aortic depressor nerve (ADN) stimulation evoked baroreflex, and sodium cyanide (NaCN) evoked chemoreflex responses. The two reflexes are alternated.
Note: when WAY-100635 is given as a test solution, the NaCN challenges are given at 7, 17 and 27 min after injection, instead of 10, 20 and 30 min.

Protocol 3. To re-examine the effects of test solutions on either sodium cyanide (NaCN) evoked chemoreflex, or aortic depressor nerve (ADN) evoked baroreflex, at 2 and 5 min after injection.

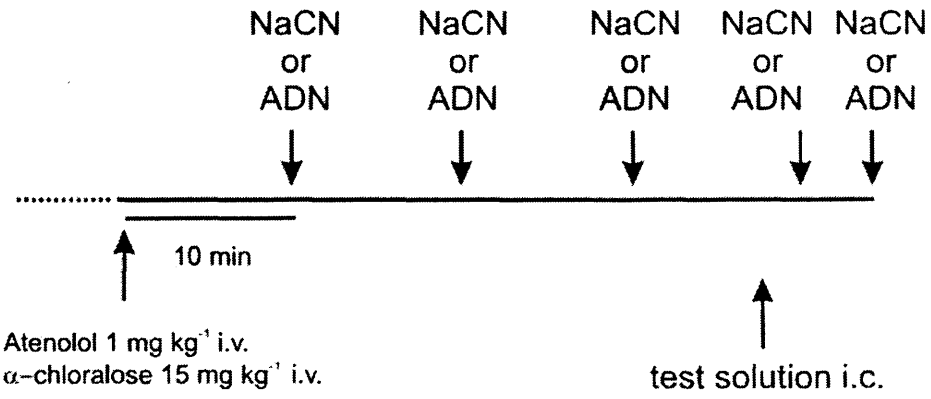
Protocol 1



Protocol 2



Protocol 3



3.3. Results

3.3.1. Effect of 5-HT receptor ligands on baseline variables

Effects of saline on baseline variables

Intracisternal administration of saline (10 μl i.c.; n = 5) at pH 5.8 (the average pH of SB-269970 in solution) had no significant effect on baseline MAP, IRNA or IPNA, but baseline R-R interval was significantly lower than control at all time points (Figure 3.2 and Table 9.1).

Effect of SB-269970 on baseline variables

Central administration of the selective 5-HT_7 receptor antagonist SB-269970 (30, 100 and 300 μg kg^{-1} i.c.; n = 5, all groups) had no significant effect on baseline MAP, R-R interval, or IRNA at any time point in the protocol, compared to i.c. saline (Figure 3.2, Tables 9.2, 9.3 and 9.4). However, baseline IPNA was significantly and dose-dependently attenuated at the middle and higher doses of SB-269970, with the greatest response 15 min after administration (88 ± 18 % of control after 100 μg kg^{-1} and 24 ± 12 % of control after 300 μg kg^{-1}). In some experiments, the high dose (300 μg kg^{-1}) caused a central apnoea. In a pilot study using this high dose in spontaneously breathing rats (n = 2), a central apnoea was also observed in both rats, requiring immediate mechanical ventilation.

Effects of intravenous SB-269970 on baseline variables

Intravenous administration of SB-269970 (100 μg kg^{-1} i.c.; n = 3) had no significant effect on baseline MAP, R-R interval or IRNA, 5 min after injection compared to pre-drug controls (Table 9.5).

Effect of vehicle for SB-656104 on baseline variables

Intracisternal administration of the vehicle for SB-656104 (45 % saline, 27.5 % DMSO, 27.5% PEG, 10 μl i.c.; n = 5) significantly raised baseline MAP at all time points after administration, but had no significant effect on baseline R-R interval, IRNA or IPNA (Figure 3.3, Table 9.6).

Effects of SB-656104 on baseline variables

Central administration of the selective 5-HT_7 receptor antagonist SB-656104 (100 μg kg^{-1} i.c.; n = 5) had no significant effect on baseline MAP, R-R interval, IRNA or IPNA at any time point in the protocol, compared to vehicle (Figure 3.3, Table 9.7).

Effects of WAY-100635 on baseline variables

Central administration of the selective 5-HT_{1A} receptor antagonist WAY-100635 (100 μg kg^{-1} i.c.; n = 5) had no significant effect on baseline MAP, R-R interval, IRNA, or IPNA at any time point in the protocol, compared to i.c. saline (Figure 3.4, Table 9.8).

Effects of robalzotan on baseline variables

Central administration of the selective 5-HT_{1A} receptor antagonist robalzotan (100 μg kg^{-1} i.c.; n = 5) had no significant effect on baseline MAP, R-R interval, IRNA, or IPNA at any time point in the protocol (Figure 3.4, Table 9.9).

Effects of (-)-pindolol on baseline variables

Central administration of the 5-HT_{1A} receptor antagonist (-)-pindolol (100 μg kg^{-1} i.c.; n = 5) had no significant effect on baseline MAP, IRNA or IPNA, but baseline R-R interval was significantly lower throughout the protocol (Figure 3.4, Table 9.10).

Effects of cinanserin on baseline variables

Central administration of the 5-HT_2 receptor antagonist cinanserin (100 μg kg^{-1} i.c.; n = 5) had no significant effect on baseline MAP or IPNA at any time point in the protocol, compared to i.c. saline (Figure 3.5, Table 9.11). However, it significantly increased baseline R-R interval 5 min after administration (194 ± 4 ms) and significantly increased IRNA 25 min after administration (131 ± 18 %).

Effects of SB-204070 on baseline variables

Central administration of the selective 5-HT_4 receptor antagonist cinanserin (100 μg kg^{-1} i.c.; n = 5) had no significant effect on baseline MAP, R-R interval, or IRNA at any time point in the protocol (Figure 3.5, Table 9.12). However, it significantly increased baseline IPNA 5 min after administration (211 ± 14 % of control), returning to control after 15 min.

Figure 3.2 Baseline graph: SB-269970

Graph showing the effects (mean ± s.e.m.) of 10 µl saline i.c. (□, n = 5), and SB-269970 30 µg kg^{-1} i.c. (●, n = 5), 100 µg kg^{-1} i.c. (▲, n = 5), and 300 µg kg^{-1} i.c. (▼, n = 5), on baseline mean arterial pressure (MAP), heart rate (HR), integrated renal nerve activity (IRNA) and integrated phrenic nerve activity (IPNA) in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

* $P < 0.05$, ** $P < 0.01$, 2-way ANOVA followed by LSD test.

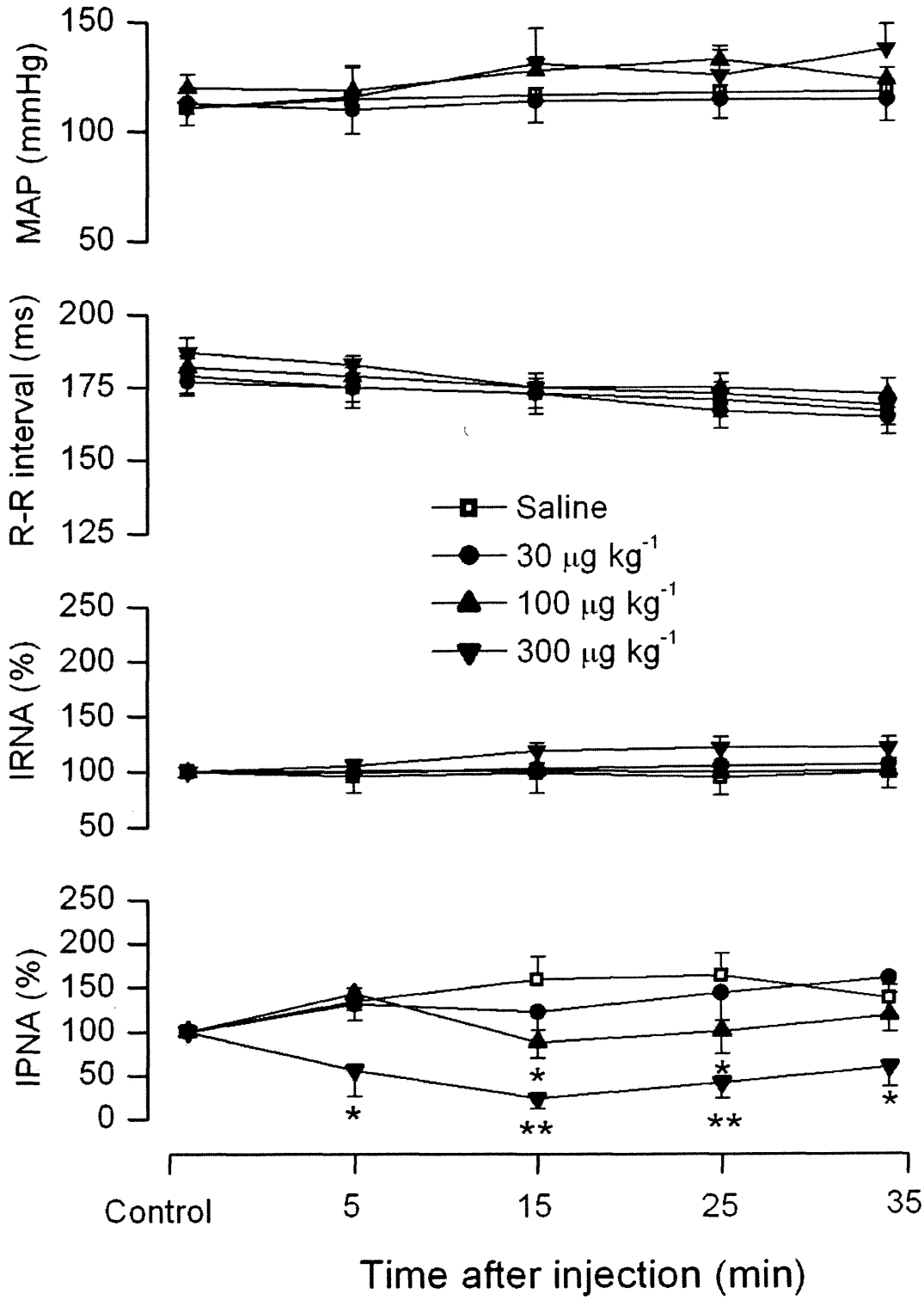

MAP (mmHg)
150
100
50
R-R interval (ms)
200
175
150
125
Saline
30 μg kg-1
100 μg kg-1
300 μg kg-1
IRNA (%)
250
200
150
100
50
IPNA (%)
250
200
150
100
50
0
*
**
**
*
Control
5
15
25
35
Time after injection (min)

Figure 3.3 Baseline graph: SB-656104

Graph showing the effects (mean ± s.e.m.) of 10 μl vehicle i.c. (□, n = 5), and SB-656104, 100 μg kg^{-1} i.c. (●, n = 5) on baseline mean arterial pressure (MAP), heart rate (HR), integrated renal nerve activity (IRNA) and integrated phrenic nerve activity (IPNA) in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

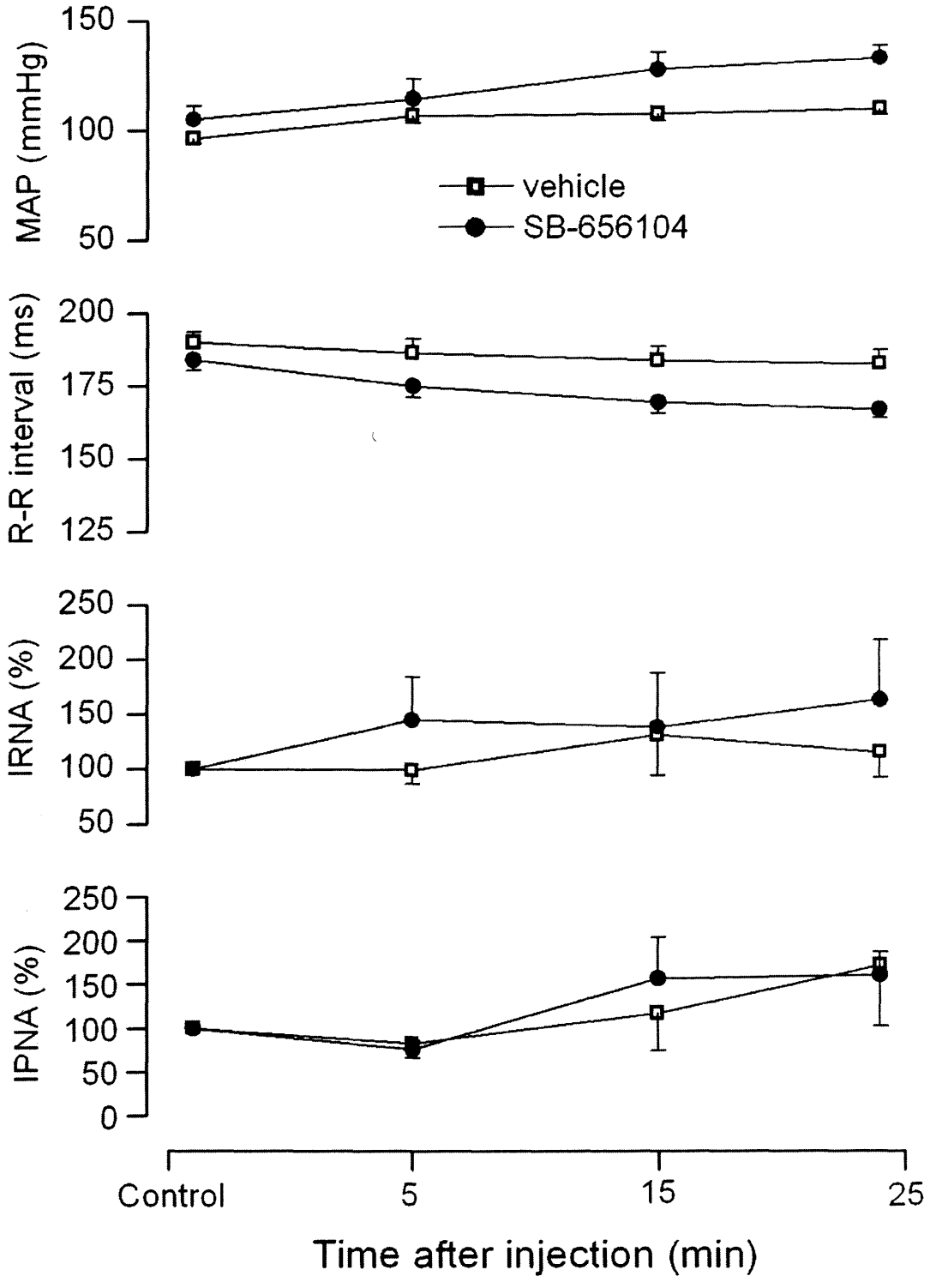

MAP (mmHg)
150
100
50
vehicle
SB-656104
R-R interval (ms)
200
175
150
125
IRNA (%)
250
200
150
100
50
IPNA (%)
250
200
150
100
50
0
Control
5
15
25
Time after injection (min)

Figure 3.4 Baseline graph: WAY-100635, robalzotan, (-)-pindolol

Graph showing the effects (mean ± s.e.m.) of 10 µl saline i.c. (□, n = 5), WAY-100635, 100 µg kg^{-1} i.c. (●, n = 5), robalzotan, 100 µg kg^{-1} i.c. (▲, n = 5), and (-)-pindolol, 100 µg kg^{-1} i.c. (▼, n = 5), on baseline mean arterial pressure (MAP), heart rate (HR), integrated renal nerve activity (IRNA) and integrated phrenic nerve activity (IPNA) in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

* $P < 0.05$, ** $P < 0.01$, 2-way ANOVA followed by LSD test.

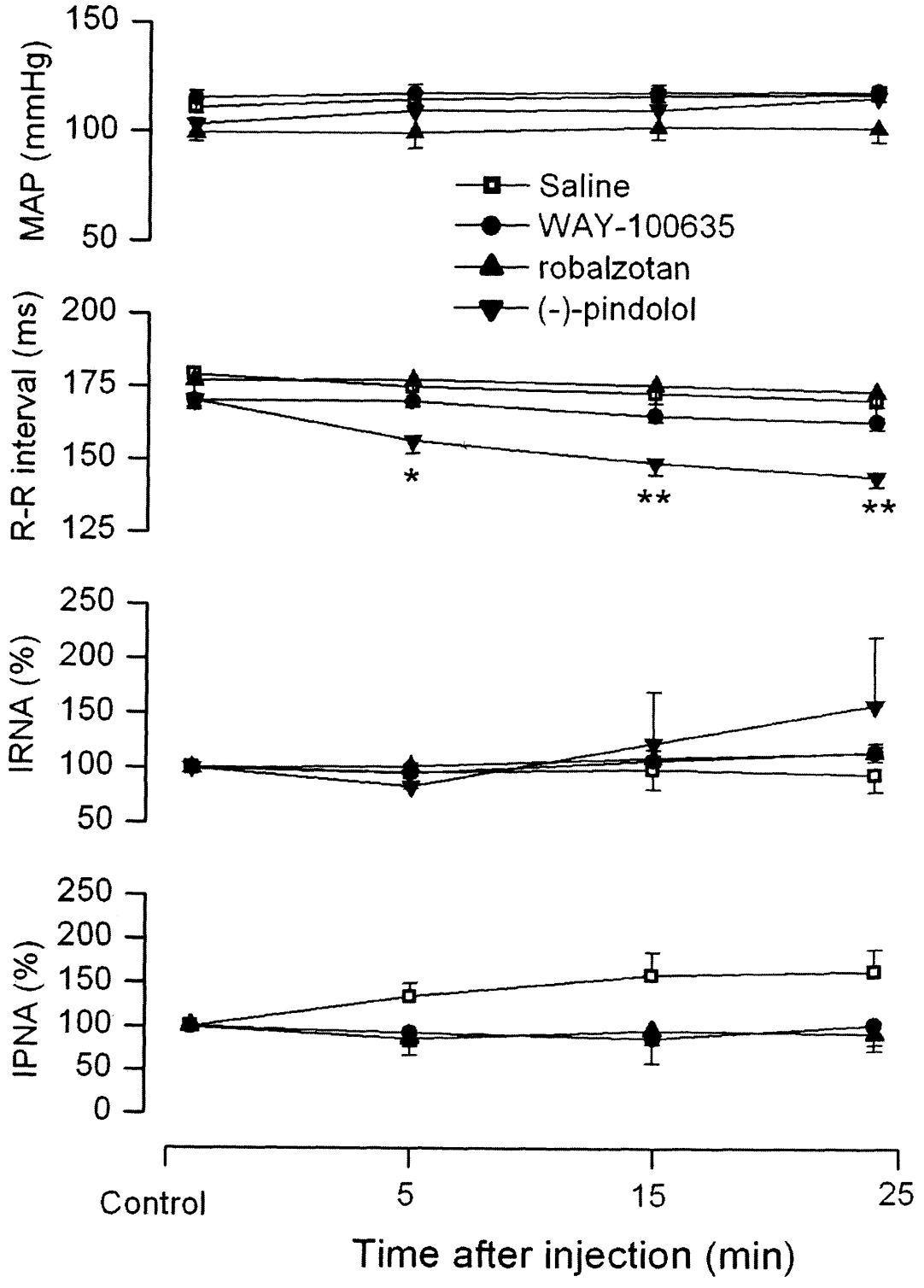
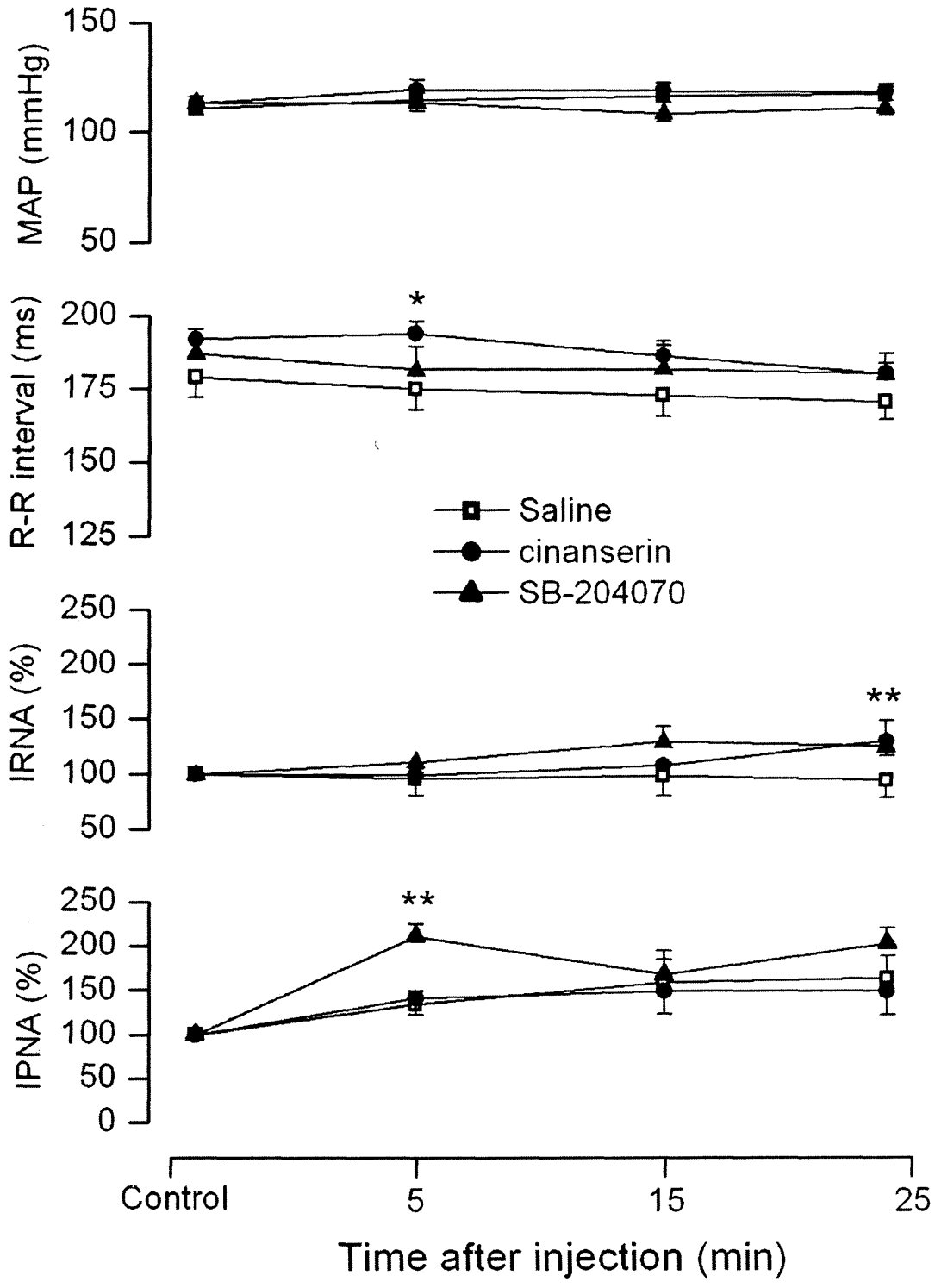

MAP (mmHg)
150
100
50
Saline
WAY-100635
robalzotan
(-)-pindolol
R-R interval (ms)
200
175
150
125
*
**
**
IRNA (%)
250
200
150
100
50
IPNA (%)
250
200
150
100
50
0
Control
5
15
25
Time after injection (min)

Figure 3.5 Baseline graph: cinanserin, SB-204070

Graph showing the effects (mean ± s.e.m.) of 10 µl saline i.c. (□, $n = 5$), cinanserin, 100 µg kg^{-1} i.c. (●, $n = 5$), and SB-204070, 100 µg kg^{-1} i.c. (▲, $n = 5$), on baseline mean arterial pressure (MAP), heart rate (HR), integrated renal nerve activity (IRNA) and integrated phrenic nerve activity (IPNA) in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

* $P < 0.05$, ** $P < 0.01$, 2-way ANOVA followed by LSD test.


MAP (mmHg)
150
100
50
R-R interval (ms)
200
175
150
125
*
Saline
cinanserin
SB-204070
IRNA (%)
250
200
150
100
50
**
IPNA (%)
250
200
150
100
50
0
**
Control
5
15
25
Time after injection (min)

3.3.2. Effect of 5-HT receptor ligands on cardiopulmonary reflex

Effects of right atrial phenylbiguanide and saline

Taking as a group all experiments in Protocol 1 (n = 53), right atrial bolus administration of phenylbiguanide (PBG, 1 – 5 μg) evoked an increase in R-R interval of 50 ± 3 ms from a baseline of 184 ± 2 ms, a fall in MAP of 35 ± 2 mmHg from a baseline of 112 ± 2 mmHg, and a fall in IRNA of 43 ± 3 %. The IPNA response in this mechanically ventilated preparation was inconsistent – either apnoea or tachypnoea was observed – and therefore has not been quantified. These reflex changes usually occurred within 2 s of PBG administration, and returned to baseline within 15 – 30 s (Figure 3.6).

Right atrial bolus administration of the same volume of saline (20 – 100 μl; n = 5) had no significant effect on MAP, R-R interval, IRNA or IPNA.

Effects of saline on cardiopulmonary reflex

Saline (10 μl, i.c., pH 5.8; n = 5) had no significant effect on the reflex MAP or R-R interval changes evoked by PBG at any time point in the protocol when compared to controls. The reflex IRNA response, however, was significantly potentiated at all time points after saline (Figure 3.7, Table 9.13).

Effects of SB-269970 on cardiopulmonary reflex

Central administration of SB-269970 (30, 100 and 300 μg kg^{-1}; n = 5 each group) significantly attenuated the response to stimulating cardiopulmonary afferents (Figures 3.6 and 3.7, Tables 9.14, 9.15 and 9.16). SB-269970 significantly and dose dependently attenuated the reflex increase in R-R interval compared to i.c. saline, with the greatest change after 15 min (24 ± 3 ms, 12 ± 3 ms, and 4 ± 2 ms respectively). Return to control reflex R-R interval (recovery) was observed after 25 and 35 min in the 30 and 100 μg kg^{-1} groups, respectively, whilst in the 300 μg kg^{-1} group, recovery took >45 min. SB-269970 had no significant effect on the reflex fall in MAP except at the high dose of 300 μg kg^{-1} (-1 ± 2 mmHg, 15 min after drug). Likewise the reflex renal sympathoinhibition was clearly attenuated only by 300 μg kg^{-1} SB-269970 (5 ± 2 % of control, 15 min after drug). However, the low and

medium doses did cause some significantly attenuation which was not dose-dependent (Figure 3.7).

Effects of intravenous SB-269970 on cardiopulmonary reflex

Intravenous administration of SB-269970 (100 μg kg^{-1}; n = 3) had no significant effect on changes in MAP, R-R interval or renal sympathoinhibition evoked by stimulating cardiopulmonary afferents, when compared to pre-drug controls (Table 9.17).

Effects of vehicle for SB-656104 on cardiopulmonary reflex

The vehicle for SB-656104 (45 % saline, 27.5 % DMSO, 27.5% PEG; 10 μl, i.c.; n = 5) had no significant effect on the reflex MAP, R-R interval orI RNA changes evoked by PBG at any time point in the protocol when compared to controls (Figure 3.8, Table 9.18).

Effects of SB-656104 on cardiopulmonary reflex

Central administration of SB-656104 (100 μg kg^{-1} i.c.; n = 5) significantly attenuated the reflex responses to stimulation of cardiopulmonary afferents (Figure 3.8, Table 9.19). It significantly attenuated the reflex increase in R-R interval 5 min after administration (9 ± 5 ms), with no recovery observable within the time frame of the protocol. It also significantly attenuated the reflex fall in MAP (15 ± 5 mmHg) at 5 min after drug administration, with recovery at 25 min. Reflex sympathoinhibition was not significantly affected.

Effects of WAY-100635 on cardiopulmonary reflex

Central administration of WAY-100635 (100 μg kg^{-1} i.c.; n = 5) significantly attenuated the reflex responses to stimulation of cardiopulmonary afferents (Figures 3.9 and 3.10, Table 9.20). WAY-100635 significantly attenuated the reflex increase in R-R interval only 5 min after administration (20 ± 3 ms), with no effect on the reflex fall in MAP or the reflex renal sympathoinhibition.

Effects of robalzotan on cardiopulmonary reflex

Central administration of robalzotan (100 μg kg^{-1} i.c.; n = 5) had no significant effect on the reflex increase in R-R interval, the reflex fall in MAP, or the reflex

sympathoinhibition evoked by stimulation of cardiopulmonary afferents, compared to i.c. saline (Figure 3.11, Table 9.21).

Effects of cinanserin on cardiopulmonary reflex

Central administration of cinanserin (100 μg kg^{-1} i.c.; n = 5) had no significant effect on the reflex increase in R-R interval, or the reflex fall in MAP evoked by stimulation of cardiopulmonary afferents, compared to i.c. saline (Figure 3.11, Table 9.22). However, cinanserin significantly attenuated the reflex sympathoinhibition 5 min after administration (90 ± 7 %), returning to control after 15 min.

Effects of SB-204070 on cardiopulmonary reflex

Central administration of SB-204070 (100 μg kg^{-1} i.c.; n = 5, and 300 μg kg^{-1} i.c.; n = 3) had no significant effect on the reflex increase in R-R interval, the reflex fall in MAP, or the reflex sympathoinhibition evoked by stimulation of cardiopulmonary afferents, compared to i.c. saline (see Figure 3.11, Table 9.23).

Figure 3.6 Cardiopulmonary reflex trace: SB-269970

Sample traces showing the effect of SB-269970 on heart rate (HR), blood pressure (BP), integrated renal (IRNA) and integrated phrenic (IPNA) nerve responses evoked by stimulation of cardiopulmonary afferents with phenylbiguanide (PBG; 2 μg) in an anaesthetised, neuromuscular blocked and atenolol pretreated rat.

Neurograms are rectified and smoothed (time constants: IRNA 1 s, IPNA 0.1 s)

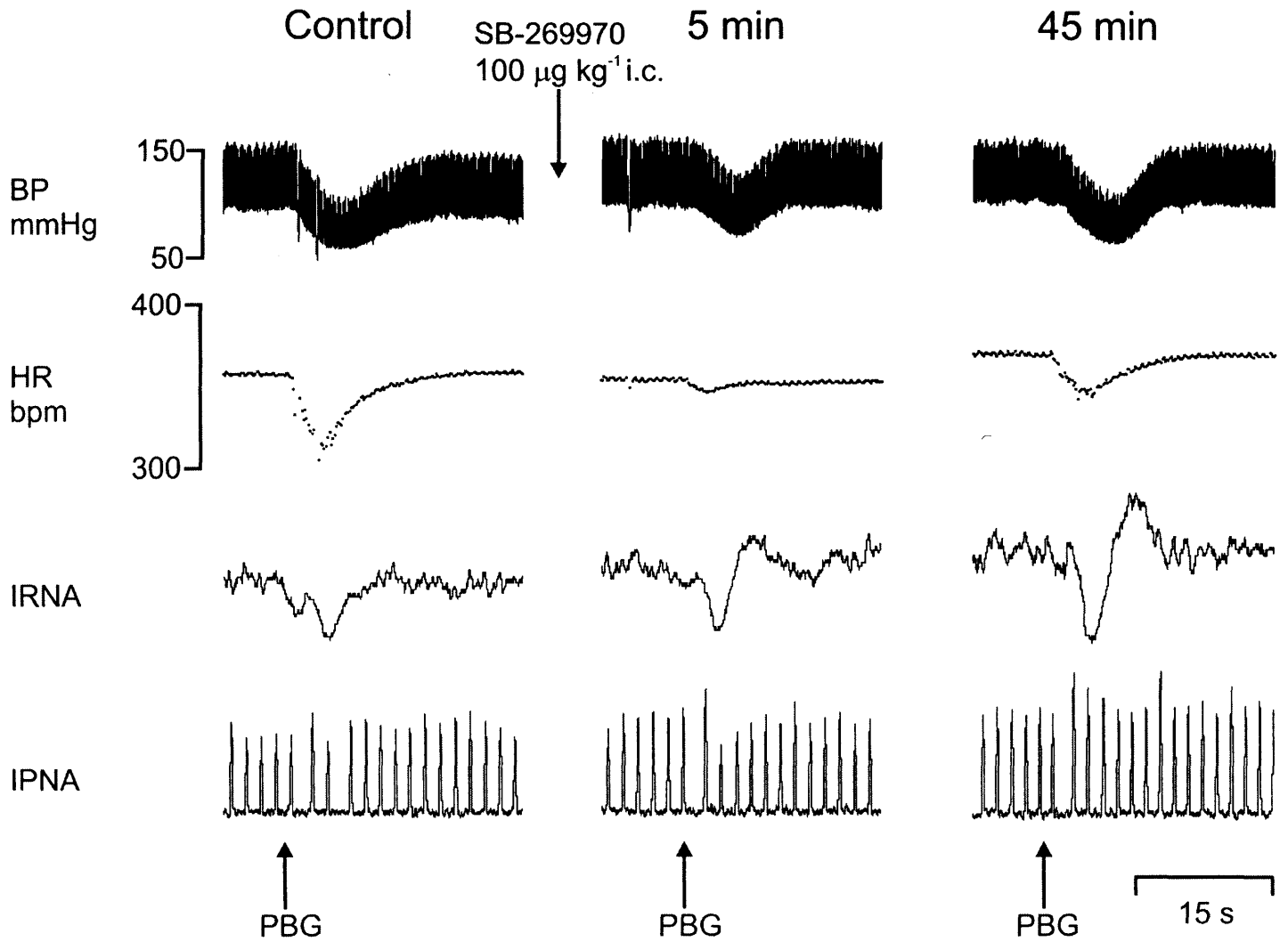

Control
SB-269970
100 μg kg-1 i.c.
5 min
45 min
150
50
BP
mmHg
400
300
HR
bpm
IRNA
IPNA
PBG
PBG
PBG
15 s

Figure 3.7 Cardiopulmonary reflex graph: SB-269970

Graph showing the effects (mean ± s.e.m.) of 10 µl saline i.c. (□, n = 5), and SB-269970 30 µg kg^{-1} i.c. (●, n = 5), 100 µg kg^{-1} i.c. (▲, n = 5), and 300 µg kg^{-1} i.c. (▼, n = 5), on changes (Δ) in mean arterial pressure (MAP), R-R interval, and integrated renal nerve activity (IRNA) evoked by cardiopulmonary reflex stimulation with phenylbiguanide in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

* $P < 0.05$, ** $P < 0.01$, 2-way ANOVA followed by LSD test (compared to time-matched saline control).

† $P < 0.05$, 1-way ANOVA followed by Tukey test (compared to pre-saline control).

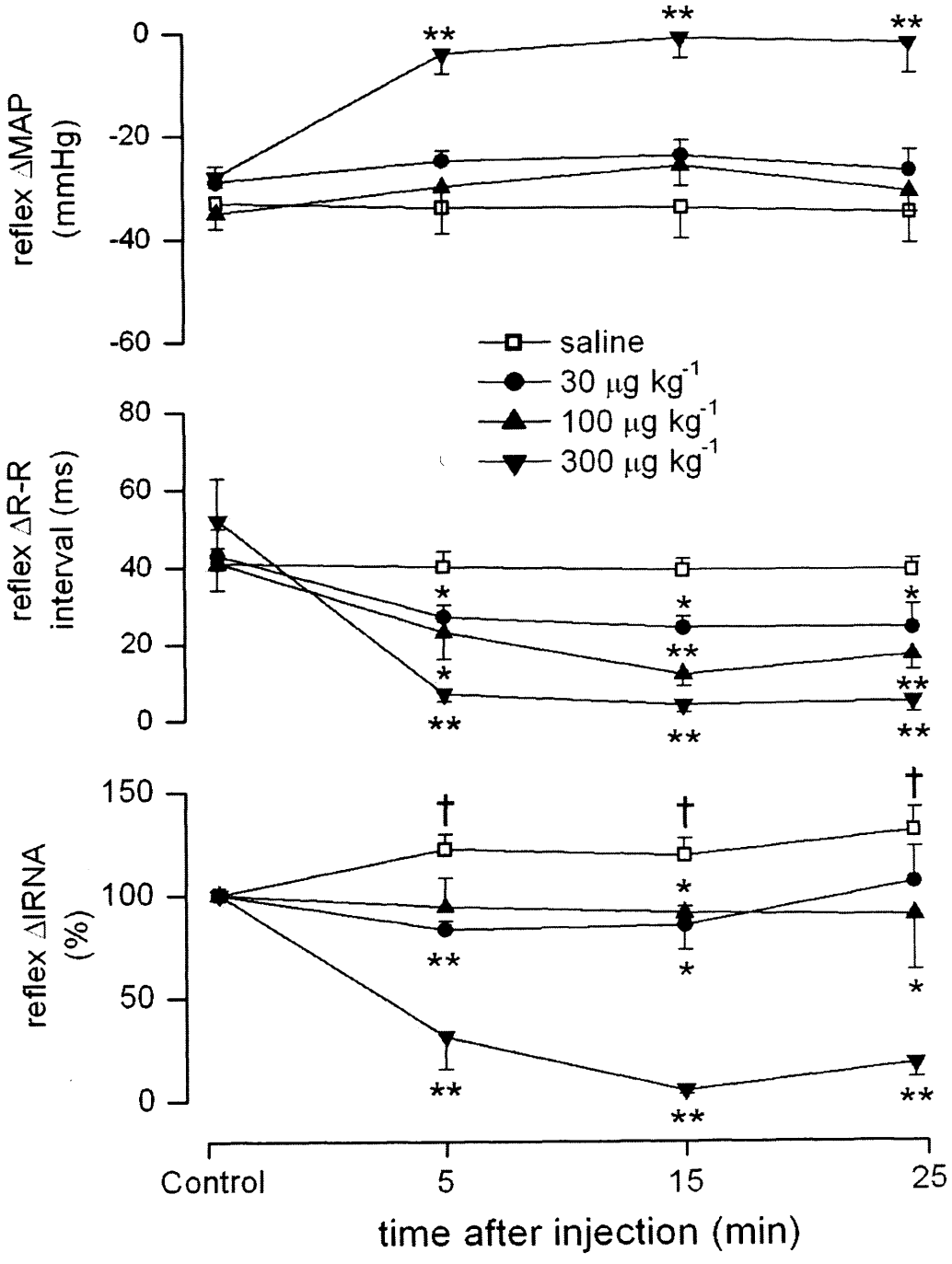

reflex ΔMAP (mmHg)
0
-20
-40
-60
saline
30 μg kg-1
100 μg kg-1
300 μg kg-1
reflex ΔR-R interval (ms)
80
60
40
20
0
reflex ΔIRNA (%)
150
100
50
0
Control
5
15
25
time after injection (min)

Figure 3.8 Cardiopulmonary reflex graph: SB-656104

Graph showing the effects (mean ± s.e.m.) of 10 μl vehicle i.c. (□, n = 5), and SB-656104, 100 μg kg^{-1} i.c. (●, n = 5) on changes (Δ) in mean arterial pressure (MAP), R-R interval, and integrated renal nerve activity (IRNA) evoked by cardiopulmonary reflex stimulation with phenylbiguanide in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

* $P < 0.05$, ** $P < 0.01$, 2-way ANOVA followed by LSD test.

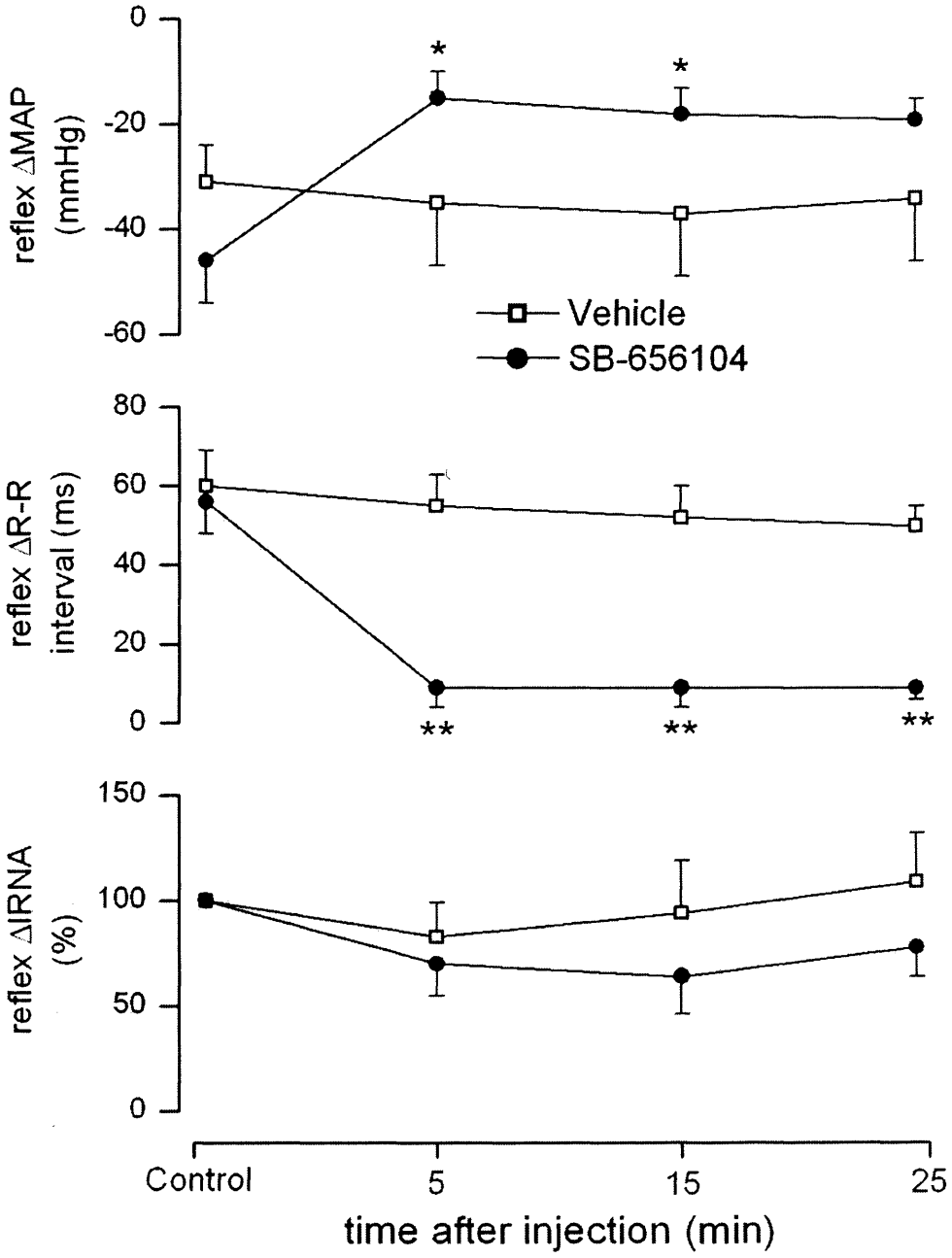

reflex ΔMAP (mmHg)
0
-20
-40
-60
*
*
Vehicle
SB-656104
reflex ΔR-R interval (ms)
80
60
40
20
0
**
**
**
reflex ΔIRNA (%)
150
100
50
0
Control
5
15
25
time after injection (min)

Figure 3.9 Cardiopulmonary reflex trace: WAY-100635

Sample traces showing effect of WAY-100635 on heart rate (HR), blood pressure (BP), integrated renal (INRA) and integrated phrenic (IPNA) nerve responses evoked by stimulation of cardiopulmonary afferents with phenylbiguanide (PBG; 2.5 μg) in an anaesthetised, neuromuscular blocked and atenolol pretreated rat.

Neurograms are rectified and smoothed (time constants: IRNA 1 s, IPNA 0.1 s)

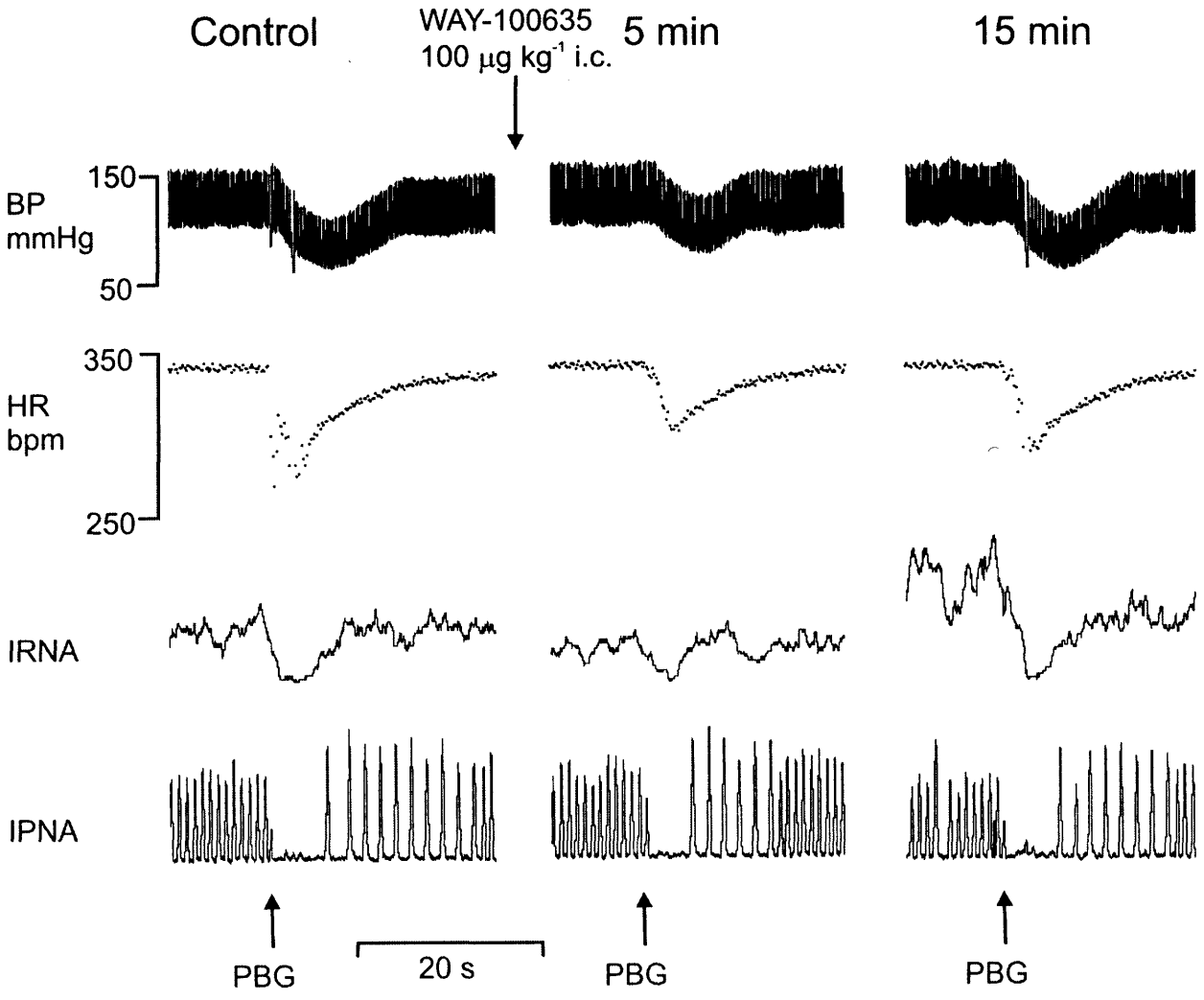

Control
WAY-100635
100 μg kg-1 i.c.
5 min
15 min
150
BP
mmHg
50
350
HR
bpm
250
IRNA
IPNA
PBG
20 s
PBG
PBG

Figure 3.10 Cardiopulmonary reflex graph: WAY-100635

Graph showing the effects (mean ± s.e.m.) of 10 µl saline i.c. (□, $n = 5$), and WAY-100635 100 µg kg^{-1} i.c. (●, $n = 5$), on changes (Δ) in mean arterial pressure (MAP), R-R interval, and integrated renal nerve activity (IRNA) evoked by cardiopulmonary reflex stimulation with phenylbiguanide in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

* $P < 0.05$, ** $P < 0.01$, 2-way ANOVA followed by LSD test.

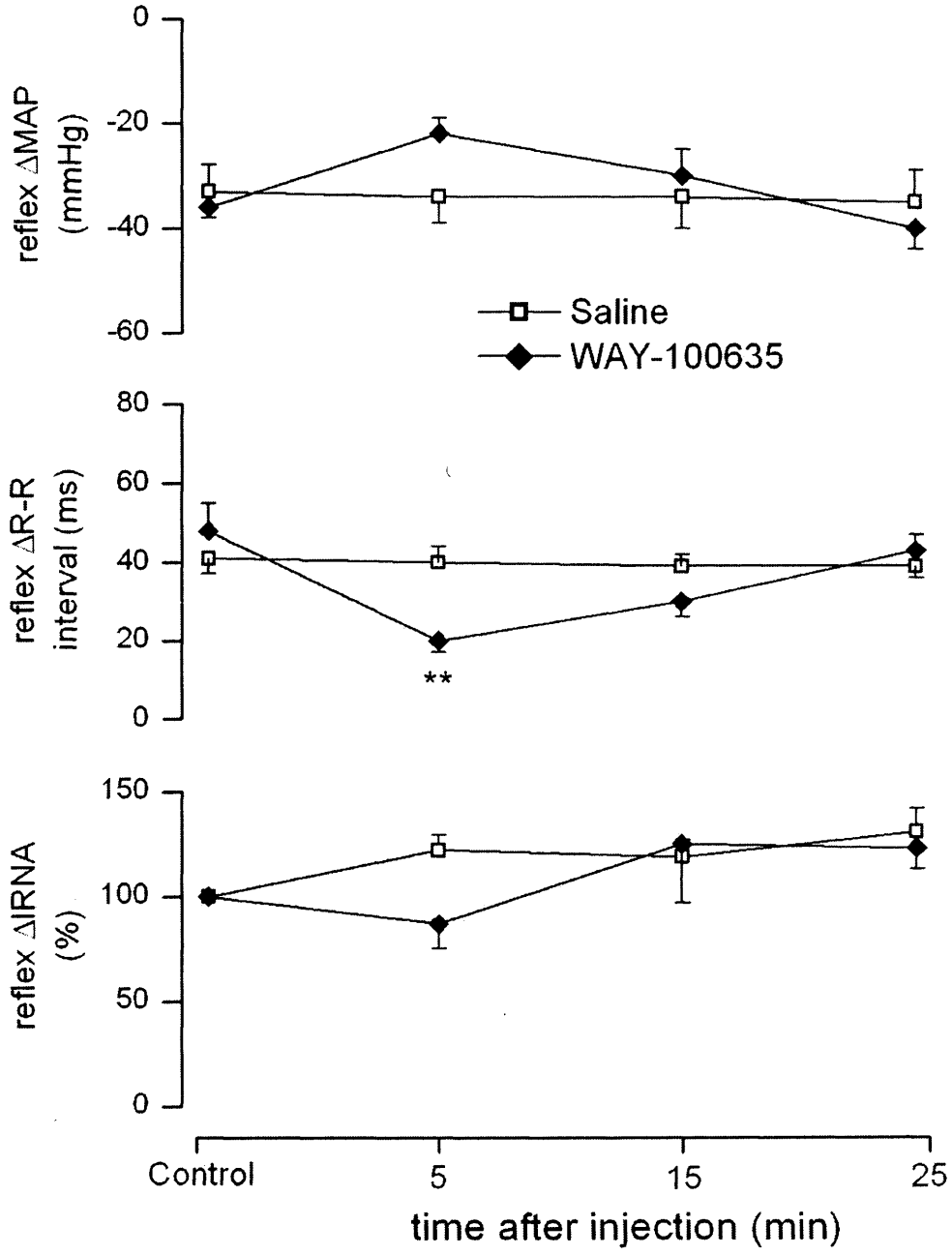
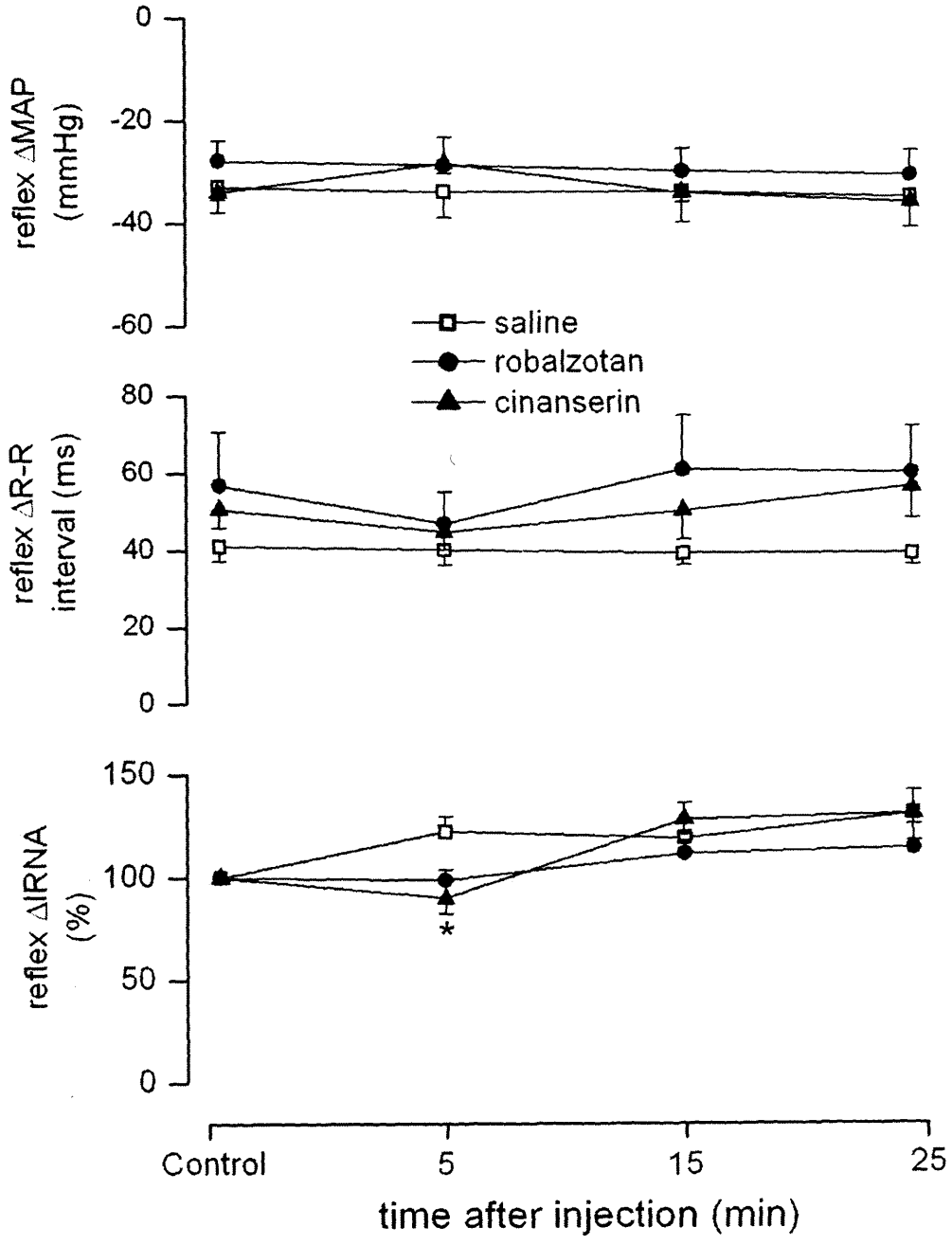

0
-20
-40
-60
reflex ΔMAP (mmHg)
Saline
WAY-100635
80
60
40
20
0
reflex ΔR-R interval (ms)
**
150
100
50
0
reflex ΔIRNA (%)
Control
5
15
25
time after injection (min)

Figure 3.11 Cardiopulmonary reflex graph: robalzotan & cinanserin

Graph showing the effects (mean ± s.e.m.) of 10 µl saline i.c. (□, n = 5), robalzotan 100 µg kg^{-1} i.c. (●, n = 5), and cinanserin 100 µg kg^{-1} i.c. (▲, n = 5) on changes (Δ) in mean arterial pressure (MAP), R-R interval, and integrated renal nerve activity (IRNA) evoked by cardiopulmonary reflex stimulation with phenylbiguanide in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

* $P < 0.05$, 2-way ANOVA followed by LSD test.


0
-20
-40
-60
reflex ΔMAP (mmHg)
saline
robalzotan
cinanserin
80
60
40
20
0
reflex ΔR-R interval (ms)
150
100
50
0
reflex ΔIRNA (%)
*
Control
5
15
25
time after injection (min)

3.3.3. Effects of 5-HT receptor ligands on chemoreflex

Effects of intravenous sodium cyanide and saline

Bolus injection of NaCN (25 – 50 μg kg^{-1} i.v.; n = 25) evoked an increase in R-R interval of 29 ± 2 ms from a baseline of 178 ± 3 ms , a fall in MAP of 31 ± 2 mmHg from a baseline of 110 ± 2 mm Hg, a rise in IRNA of 74 ± 11 %, and a rise in IPNA of 262 ± 45 % (Figure 3.12). These reflex responses began within 2 s of NaCN administration, peaked within 10 (except IRNA and IPNA, which sometimes continued to rise for another 10 s) and usually returned to baseline within 30 – 60 s (except IPNA which took up to 2 min to recover). Vehicle administration (0.1 ml saline i.v.) had no measurable effect. To control for possible direct cardiac effects of NaCN, atropine methonitrate (1 mg kg^{-1} i.v.; n = 2) administered at the end of a protocol, abolished the reflex increase in R-R interval resulting from NaCN injection.

Effects of saline on chemoreflex

Using Protocol 2, intracisternal administration of saline (10 μl, i.c., pH 5.8; n = 5) had no significant effect on the reflex MAP or IPNA changes evoked by NaCN at any time point in the protocol when compared to controls. The reflex R-R interval increase, however, was slightly potentiated at 10 and 20 min after saline administration, and the IRNA response was potentiated at 10 and 30 min (Figure 3.13, Table 9.24).

Using Protocol 3, saline (10 μl, i.c., pH 5.8; n = 5) had no significant effect on the reflex MAP, R-R interval, IRNA or IPNA changes evoked by NaCN at either 2 or 5 min after saline, when compared to controls (Figure 3.17, Table 9.27).

Effects of SB-269970 on chemoreflex

Central administration of SB-269970 (100 μg kg^{-1} i.c., n = 5) significantly attenuated components of the response to stimulating chemoreceptor afferents (Figure 3.12 and 3.13, Table 9.25). It significantly reduced the reflex increase in R-R interval, with the greatest response after 10 min (11 ± 2 ms). Control reflex R-R interval recovered 30 min after drug administration. SB-269970 had no significant effect on the fall in MAP, or on the reflex increase in IPNA. However, the reflex sympathoexcitation

was significantly attenuated 10 min after drug administration (46 ± 6 % of control), with recovery at 20 min.

Effects of WAY-100635 on chemoreflex

Central administration of WAY-100635 (100 μg kg^{-1}; n = 5) had no significant effect on the reflex increase in R-R interval, the reflex drop in MAP or the reflex increase in IPNA evoked by stimulating chemoreceptor afferents (Figure 3.14 and 3.15, Table 9.26). However, 7 min after drug administration WAY-100635 significantly attenuated the reflex sympathoexcitation (93 ± 9 % of control) with recovery 17 min after drug administration.

Further investigation using Protocol 3 to test NaCN challenges at 2 and 5 min after WAY-100635 found that the drug does indeed attenuate responses to chemoreceptor stimulation, but only at 2 min (Figure 3.16 and 3.17, Table 9.28). The reflex increase in R-R interval was significantly attenuated compared to i.c. saline (18 ± 7 *vs.* 36 ± 8 ms; n = 5), and the reflex renal sympathoexcitation was also significantly attenuated (101 ± 8 *vs.* 193 ± 40 % of control). There were no significant changes in reflex hypotension or phrenic excitation.

Figure 3.12 Chemoreflex trace: SB-269970

Sample traces showing the effect of SB-269970 on heart rate (HR), blood pressure (BP),integrated renal (IRNA) and integrated phrenic (IPNA) nerve responses evoked by stimulation of chemoreceptor afferents with sodium cyanide (NaCN; 50 μg) in an anaesthetised, neuromuscular blocked and atenolol pretreated rat.

Neurograms are rectified and smoothed (time constants: IRNA 1 s, IPNA 0.1 s)

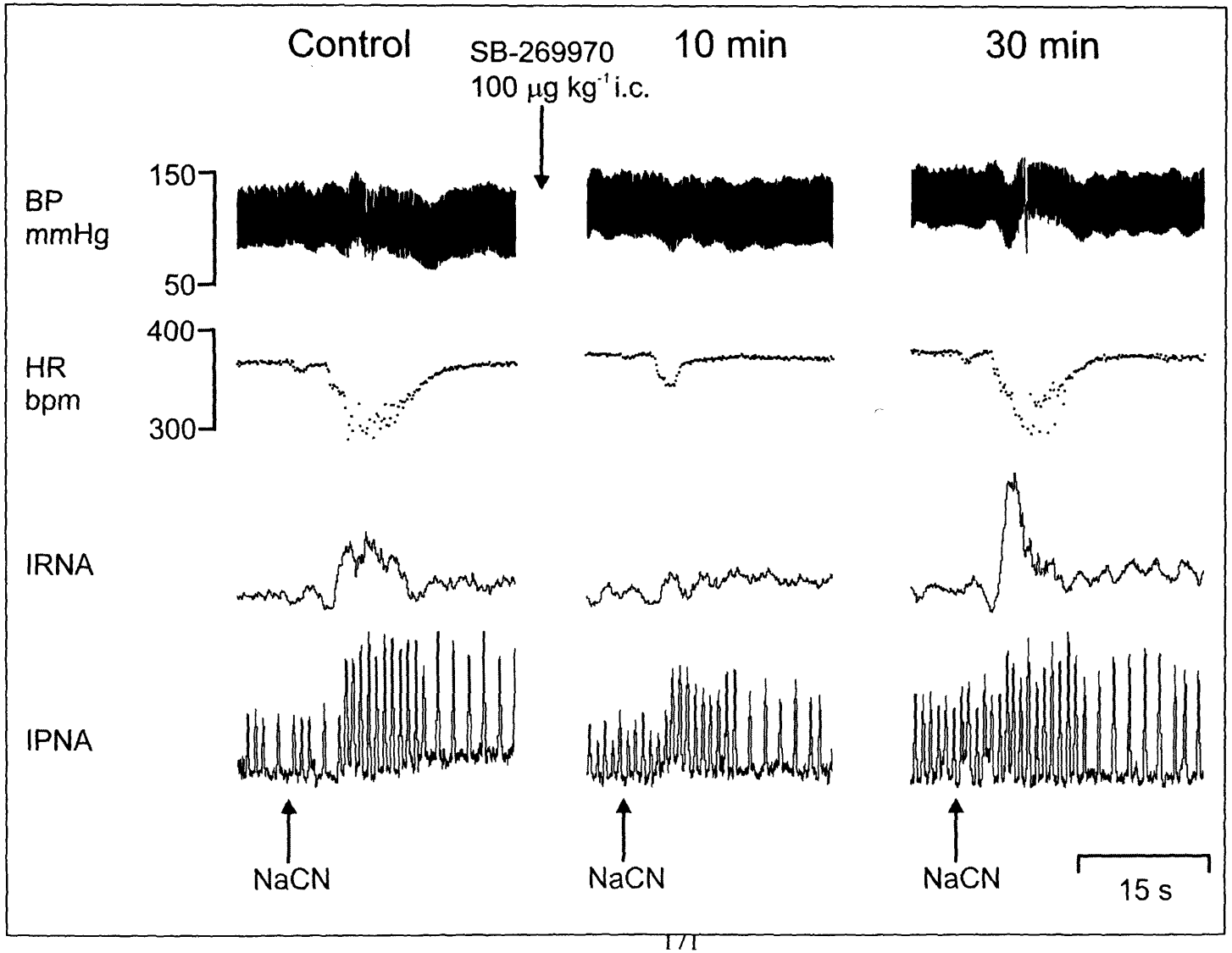

Control
SB-269970
100 μg kg-1 i.c.
10 min
30 min
BP
mmHg
150
50
HR
bpm
400
300
IRNA
IPNA
NaCN
NaCN
NaCN
15 s

Figure 3.13 Chemoreflex graph : SB-269970

Graph showing the effects (mean ± s.e.m.) of 10 µl saline i.c. (□, n = 5), and SB-269970, 100 µg kg^{-1} i.c. (●, n = 5), on changes (Δ) in mean arterial pressure (MAP), R-R interval, integrated renal nerve activity (IRNA), and integrated phrenic nerve activity (IPNA) evoked by chemoreflex stimulation with sodium cyanide in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

** $P < 0.01$, 2-way ANOVA followed by LSD test.

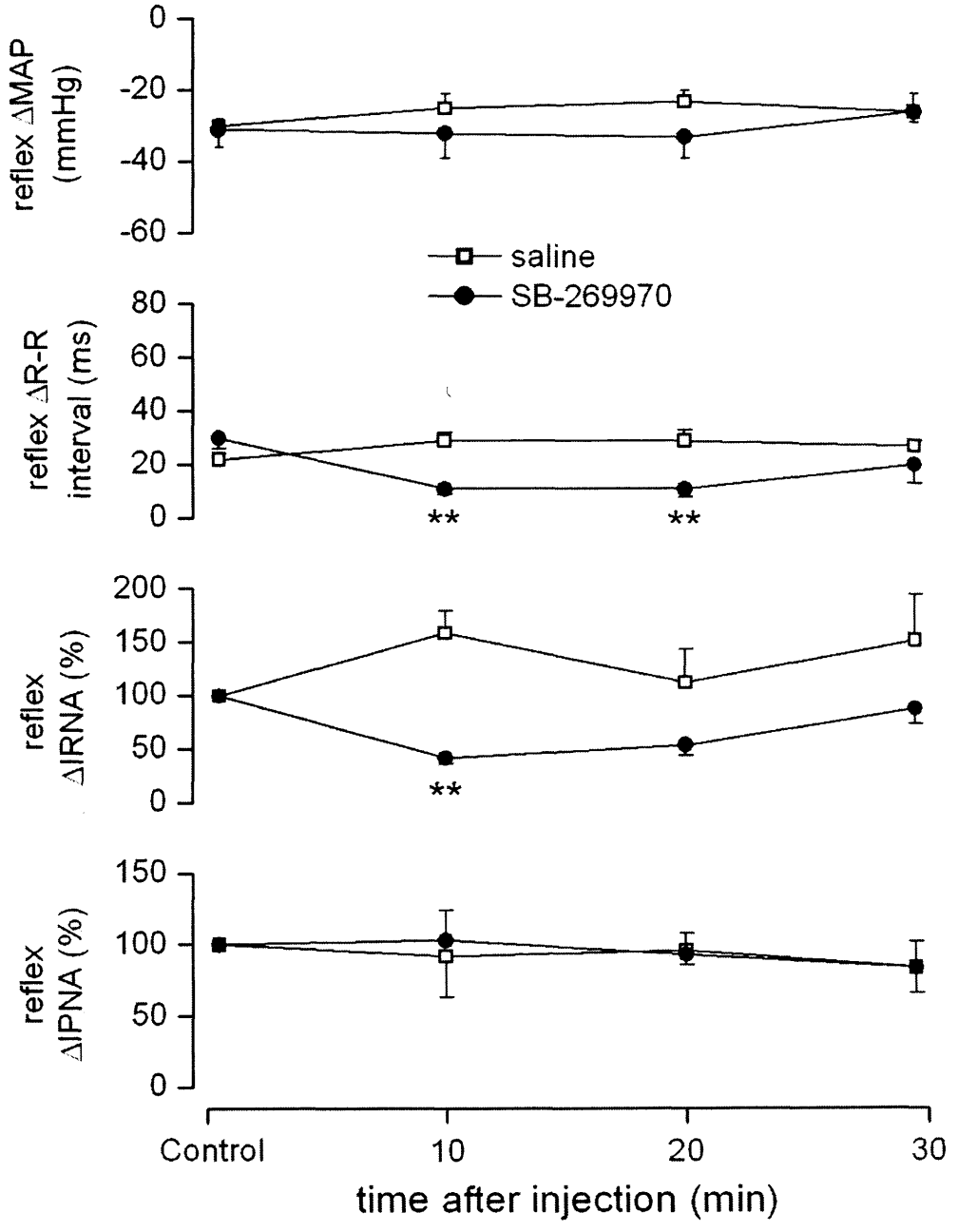

reflex ΔMAP (mmHg)
0
-20
-40
-60
saline
SB-269970
reflex ΔR-R interval (ms)
80
60
40
20
0
**
**
reflex ΔIRNA (%)
200
150
100
50
0
**
reflex ΔIPNA (%)
150
100
50
0
Control
10
20
30
time after injection (min)

Figure 3.14 Chemoreflex trace: WAY-100635

Sample traces comparing effect of WAY-100635 on heart rate (HR), blood pressure (BP), integrated renal (IRNA) and integrated phrenic (IPNA) nerve responses evoked by stimulation of cardiopulmonary afferents with phenybiguanide (PBG; 1.5 μg), and chemoreceptor afferents with sodium cyanide (NaCN; 50 μg) in an anaesthetised, neuromuscular blocked and atenolol pretreated rat.

Neurograms are rectified and smoothed (time constants: IRNA 1 s, IPNA 0.1 s)

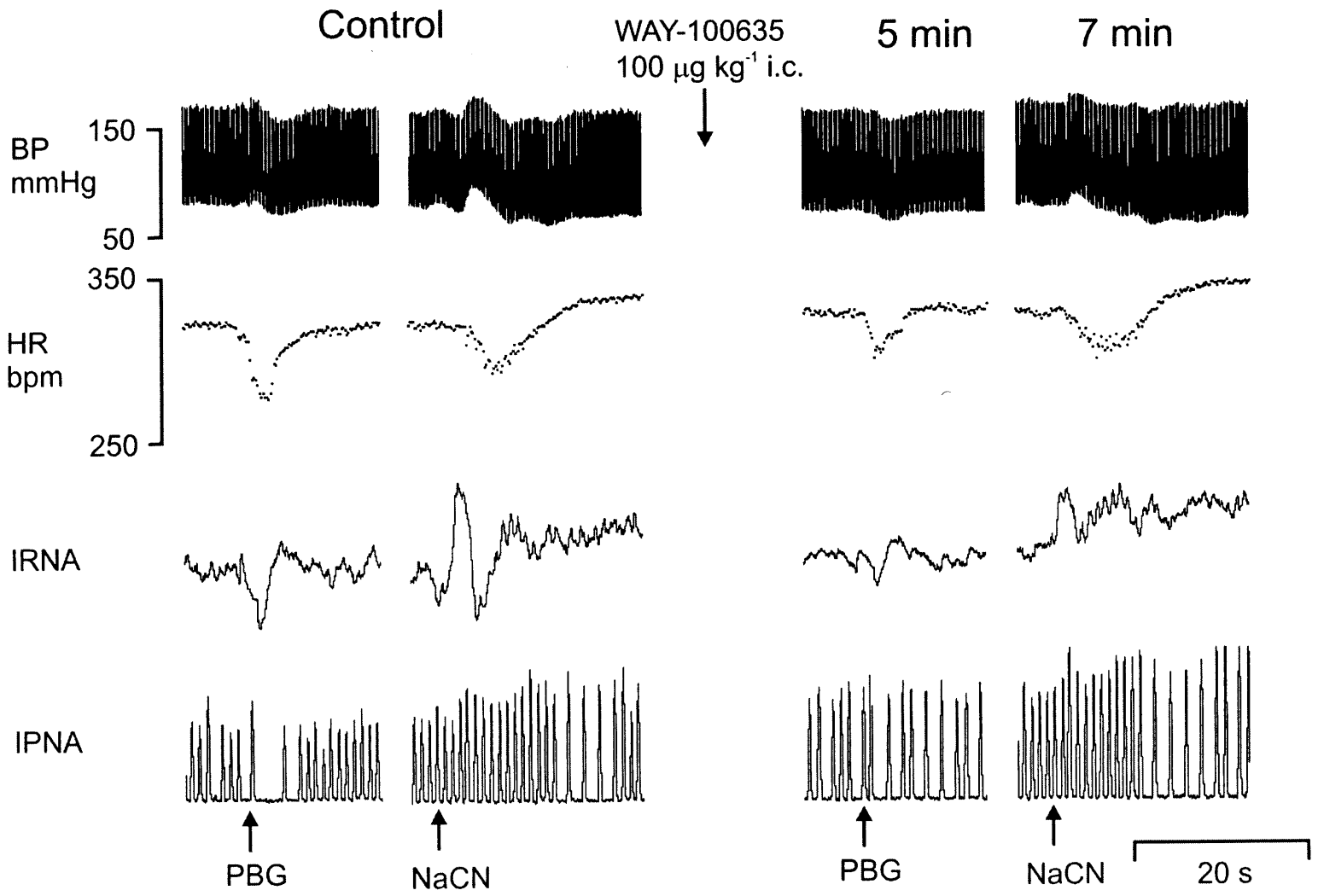

Control
WAY-100635
100 μg kg-1 i.c.
5 min
7 min
BP
mmHg
150
50
350
HR
bpm
250
IRNA
IPNA
PBG
NaCN
PBG
NaCN
20 s

Figure 3.15 Chemoreflex graph: WAY-100635

Graph showing the effects (mean ± s.e.m.) of 10 µl saline i.c. (□, n = 5), and WAY-100635, 100 µg kg^{-1} i.c. (●, n = 5), on changes (Δ) in mean arterial pressure (MAP), R-R interval, integrated renal nerve activity (IRNA), and integrated phrenic nerve activity (IPNA) evoked by chemoreflex stimulation with sodium cyanide in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

* $P < 0.05$, 2-way ANOVA followed by LSD test (comparing treated animals at 7, 17 and 27 min with saline conrols at 10, 20 and 30 min).

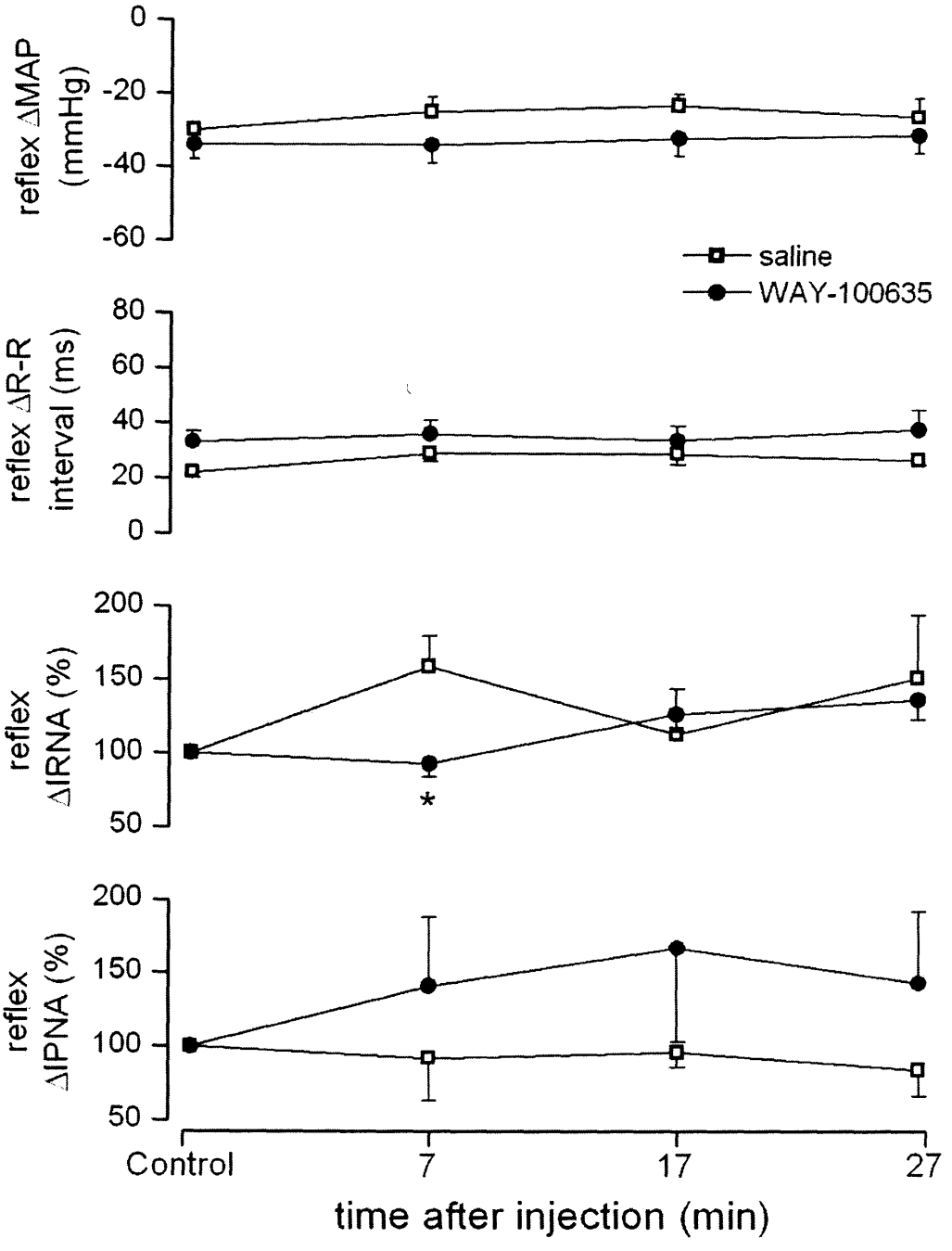

reflex ΔMAP (mmHg)
0
-20
-40
-60
saline
WAY-100635
reflex ΔR-R interval (ms)
80
60
40
20
0
reflex ΔIRNA (%)
200
150
100
50
*
reflex ΔIPNA (%)
200
150
100
50
Control
7
17
27
time after injection (min)

Figure 3.16 Chemoreflex trace (Protocol 3): WAY-100635

Sample trace illustrating effect of WAY-100635 at 2 and 5 min after injection on heart rate (HR), blood pressure (BP), integrated renal (IRNA) and phrenic (IPNA) nerve responses evoked by stimulation of chemoreceptor afferents with sodium cyanide (NaCN; 50 μg) in an anaesthetised, neuromuscular blocked and atenolol pretreated rat.

Neurograms are rectified and smoothed (time constants: IRNA 0.1 s, IPNA 0.1 s)

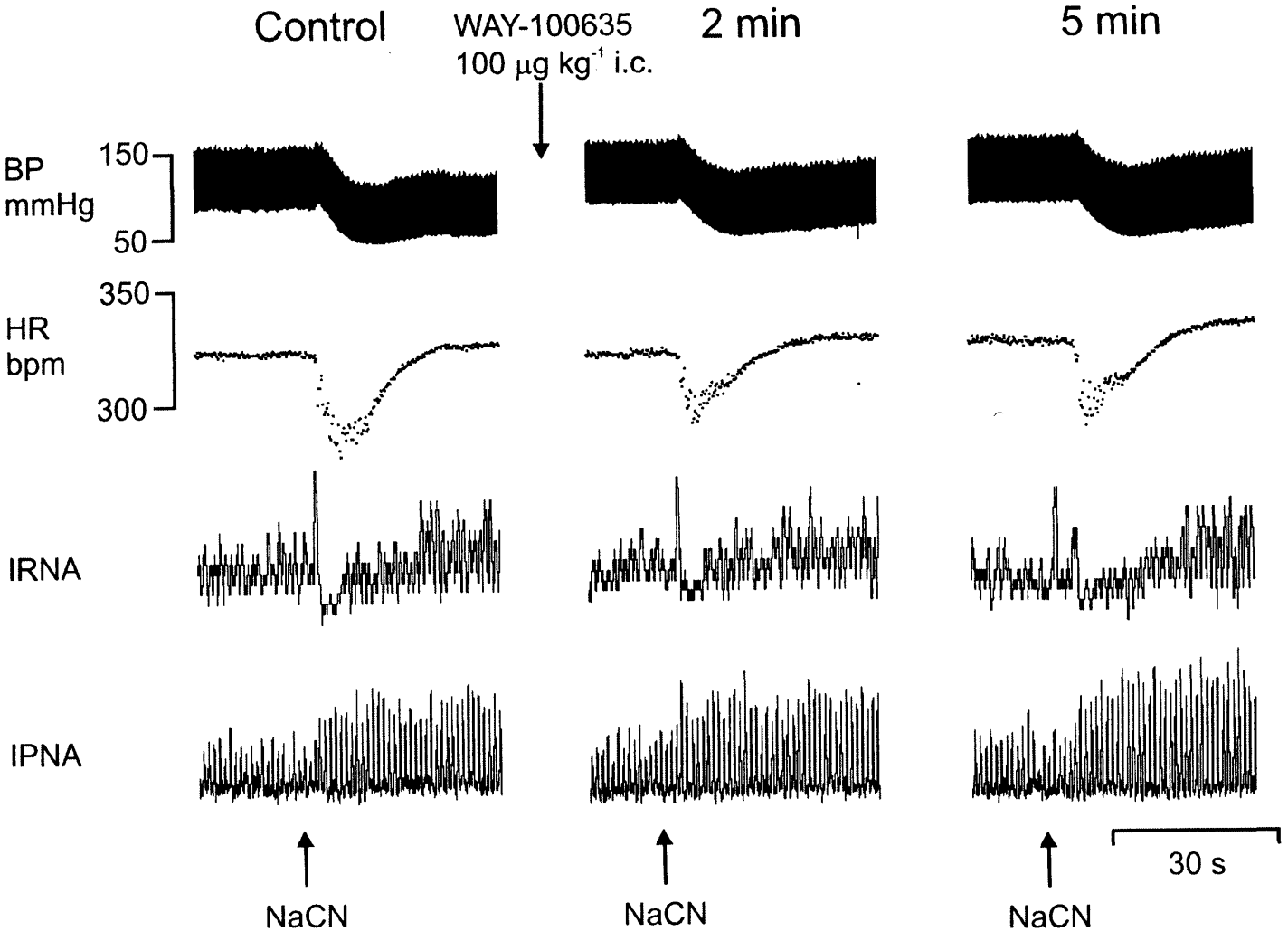
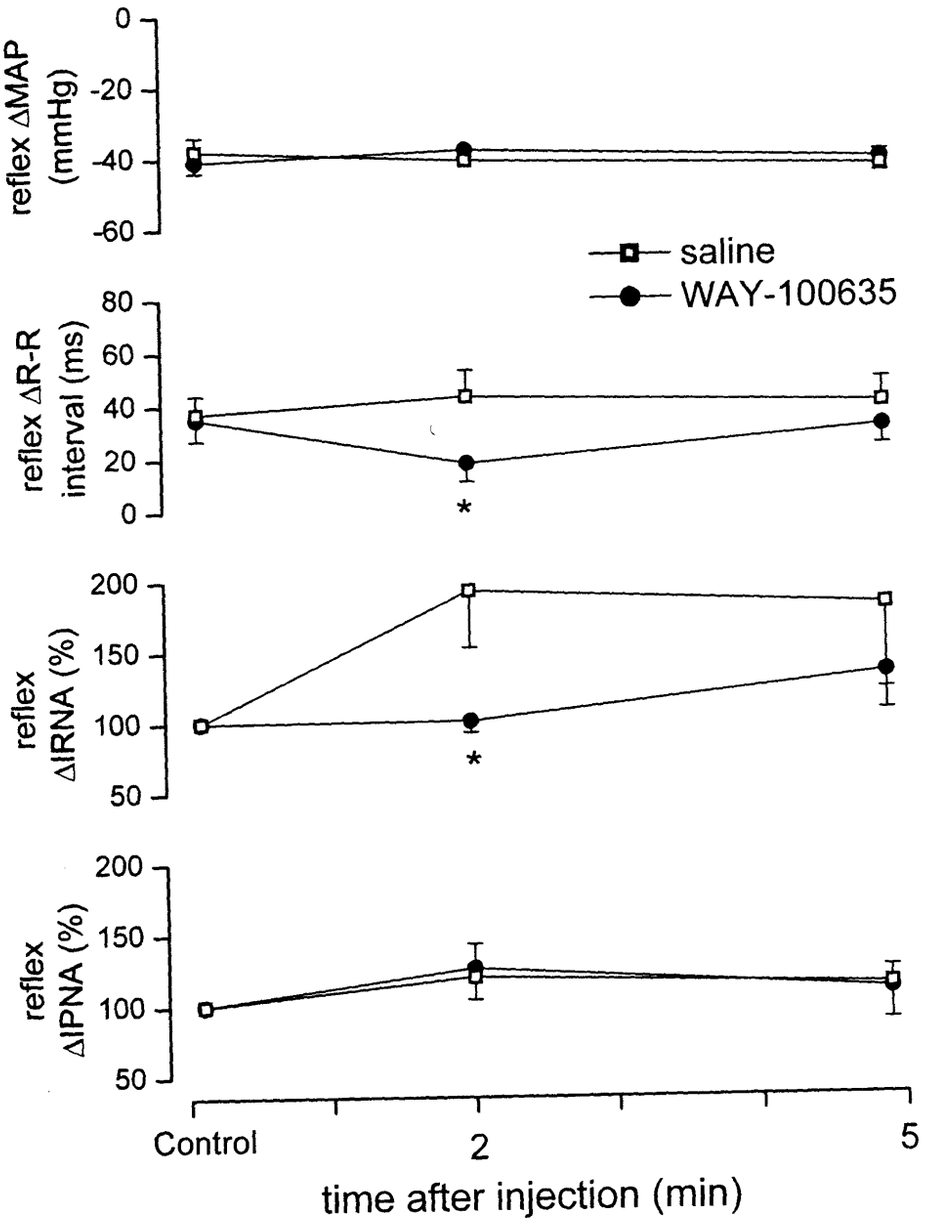

Control
WAY-100635
100 μg kg-1 i.c.
2 min
5 min
BP
mmHg
150
50
HR
bpm
350
300
IRNA
IPNA
NaCN
NaCN
NaCN
30 s

Figure 3.17 Chemoreflex graph (Protocol 3): WAY-100635

Graph showing the effects (mean ± s.e.m.) of 10 µl saline i.c. (□, n = 5), and WAY-100635, 100 µg kg^{-1} i.c. (●, n = 5), on changes (Δ) in mean arterial pressure (MAP), R-R interval, integrated renal nerve activity (IRNA), and integrated phrenic nerve activity (IPNA) evoked by chemoreflex stimulation with sodium cyanide in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

* $P < 0.05$, 2-way ANOVA followed by LSD test.


reflex ΔMAP (mmHg)
0
-20
-40
-60
saline
WAY-100635
reflex ΔR-R interval (ms)
80
60
40
20
0
*
reflex ΔIRNA (%)
200
150
100
50
*
reflex ΔIPNA (%)
200
150
100
50
Control
2
5
time after injection (min)

3.3.4. Effects of 5-HT receptor ligands on aortic nerve stimulation

Effects of aortic nerve stimulation

5 s trains of constant current stimulation (40 Hz, 0.1 ms pulse, 0.2 – 1 mA) of the right aortic depressor nerve (n = 26) evoked an increase in R-R interval of 58 ± 6 ms from a baseline of 175 ± 2 ms , and a fall in MAP of 29 ± 2 mmHg from a baseline of 106 ± 2 mmHg. These reflex responses began within 0.5 s of electrical stimulation, peaked within 5 s and usually returned to baseline within 20 s (Figure 3.18).

Effects of saline on aortic nerve stimulation

Saline (10 µl, i.c., pH 5.8; n = 5) had no significant effect on the reflex MAP or R-R interval changes evoked by aortic depressor nerve stimulation at any time point in the protocol when compared to controls (Figure 3.19, Table 9.29).

Effects of SB-269970 on aortic nerve stimulation

Central administration of SB-269970 (100 µg kg^{-1}; n = 5) significantly attenuated the response to stimulating the aortic depressor nerve (Figures 3.18 and 3.19, Table 9.30). SB-269970 significantly attenuated the reflex increase in R-R interval (compared to i.c. saline) 5 min after drug administration (24 ± 6 ms), with recovery at 15 min post drug. SB-269970 had no significant effect on the reflex fall in MAP.

Effects of WAY-100635 on aortic nerve stimulation

Central administration of WAY-100635 (100 µg kg^{-1} i.c.; n = 5) had no significant effect on the response to stimulation the aortic depressor nerve at 5, 15, or 25 min after drug administration (Figure 3.20, Table 9.31). On further investigation using Protocol 3, it was found that giving the aortic nerve stimulus at 2 min after drug delivery (100 µg kg^{-1}; n = 6) also had no significant effect (Figures 3.21 and 3.22, Table 9.32). However, administration of a second dose in the same animals (200 µg kg^{-1}; n = 6, 20 min after first dose) significantly attenuated the reflex increase in R-R interval at 2 min after drug administration (25 ± 8 ms, compared to control) with recovery at 3 min. Also the reflex hypotension was significantly attenuated at 2, 3

and 4 min after drug administration, with the greatest attenuation at 2 min (-16 ± 4 mmHg).

Effects of (-)-Pindolol on aortic nerve stimulation

Central administration of (-)-pindolol (100 μg kg^{-1} i.c.; n = 5) had no significant effect on the response to stimulation the aortic depressor nerve at 5, 15, or 25 min after drug administration (Figure 3.20, Table 9.33).

Figure 3.18 Aortic nerve stimulation trace: SB-269970

Sample trace illustrating the effect of SB-269970 on heart rate (HR), blood pressure (BP), integrated renal (IRNA) and integrated phrenic (IPNA) nerve responses evoked by electrical stimulation (40 Hz) of the right aortic depressor nerve in an anaesthetised, neuromuscular blocked and atenolol pretreated rat. For clarity, stimulus artefacts have been removed from the phrenic neurogram.

Neurograms are rectified and smoothed (time constants: IRNA 1 s, IPNA 0.1 s)

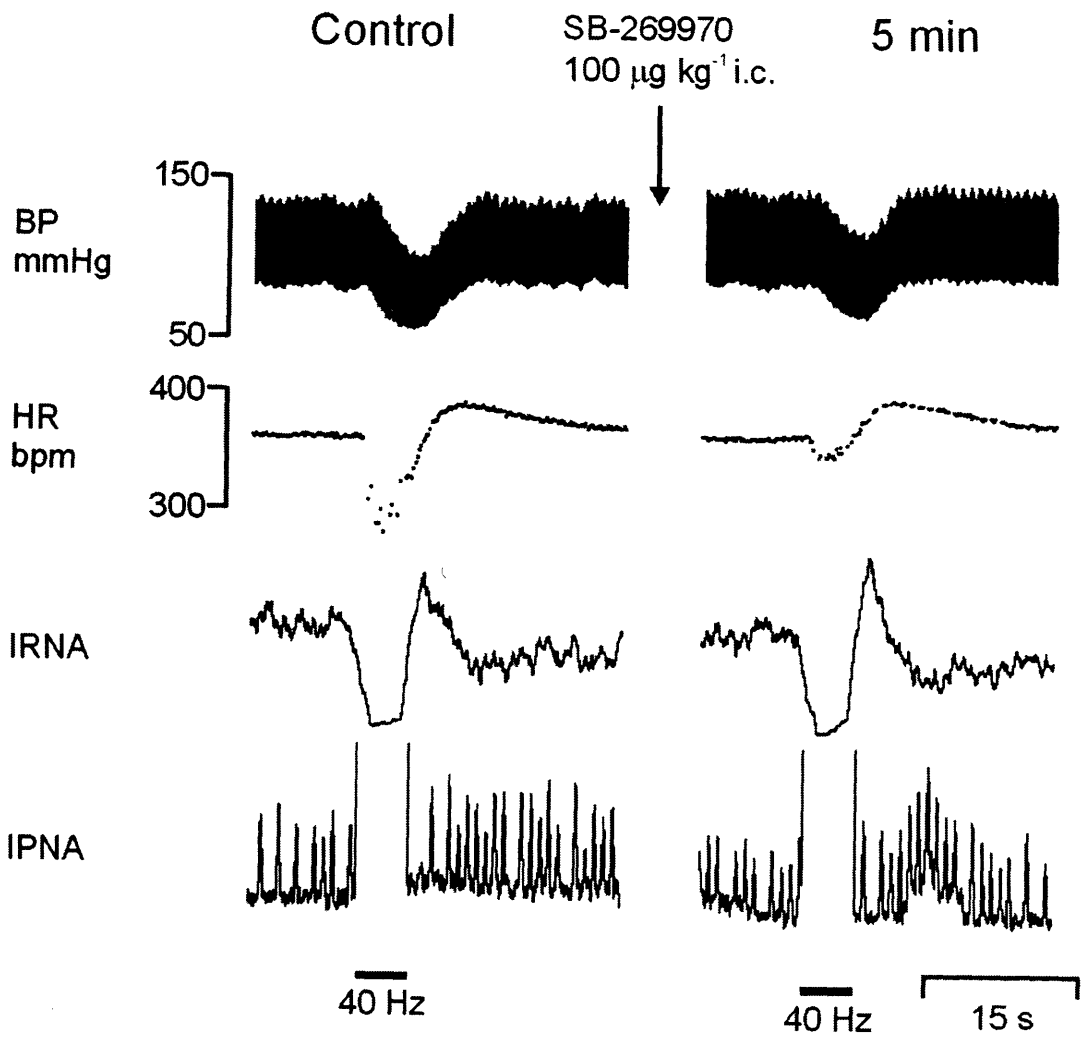

Control
SB-269970
100 μg kg-1 i.c.
5 min
150
BP
mmHg
50
400
HR
bpm
300
IRNA
IPNA
40 Hz
40 Hz
15 s

Figure 3.19 Aortic nerve stimulation graph: SB-269970

Graph showing the effects (mean ± s.e.m.) of 10 μl saline i.c. (□, n = 5), and SB-269970, 100 μg kg^{-1} i.c. (●, n = 5), on changes (Δ) in mean arterial pressure (MAP) and R-R interval evoked by electrical stimulation of the right aortic depressor nerve in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

* $P < 0.05$, 2-way ANOVA followed by LSD test.

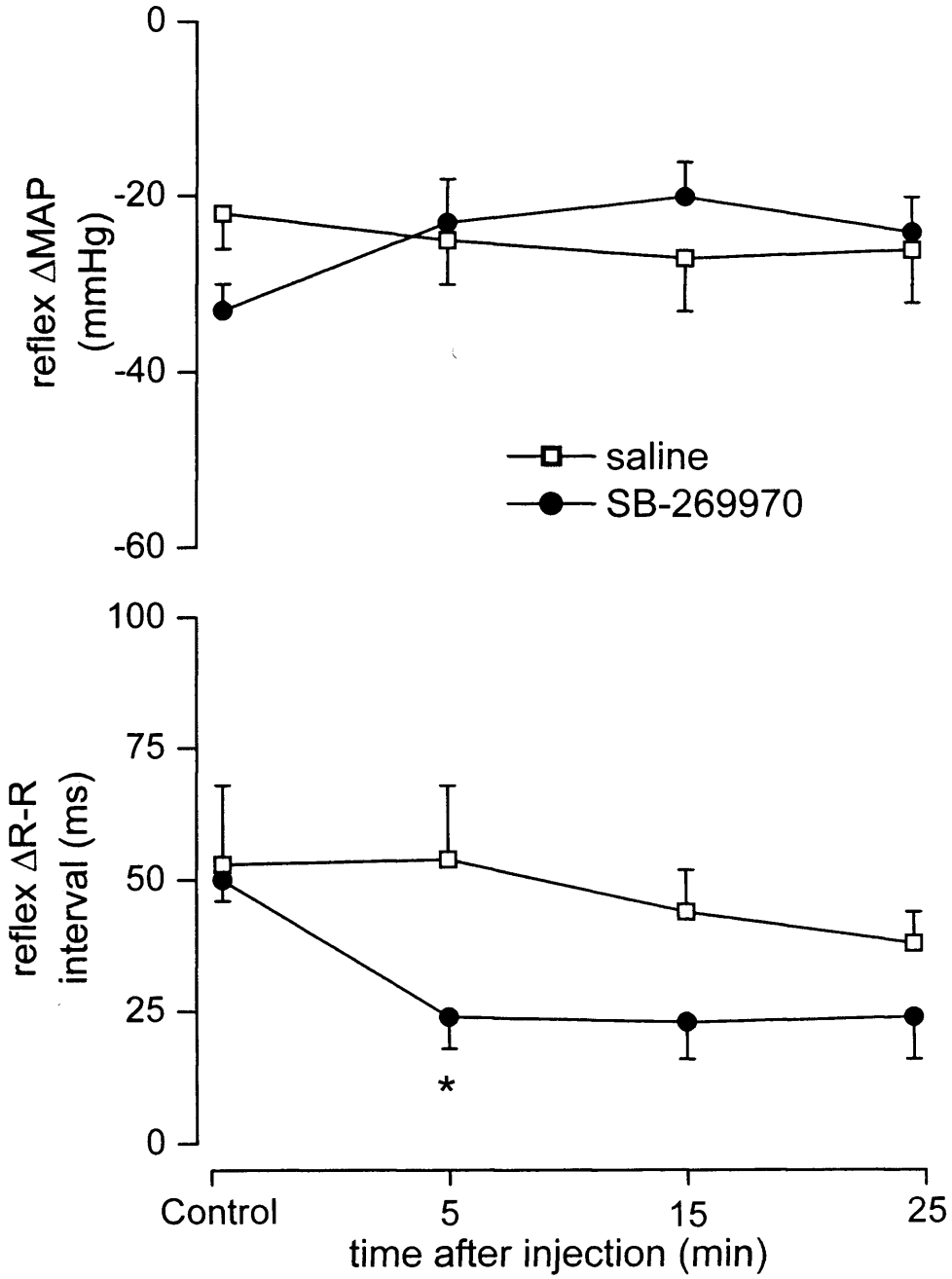
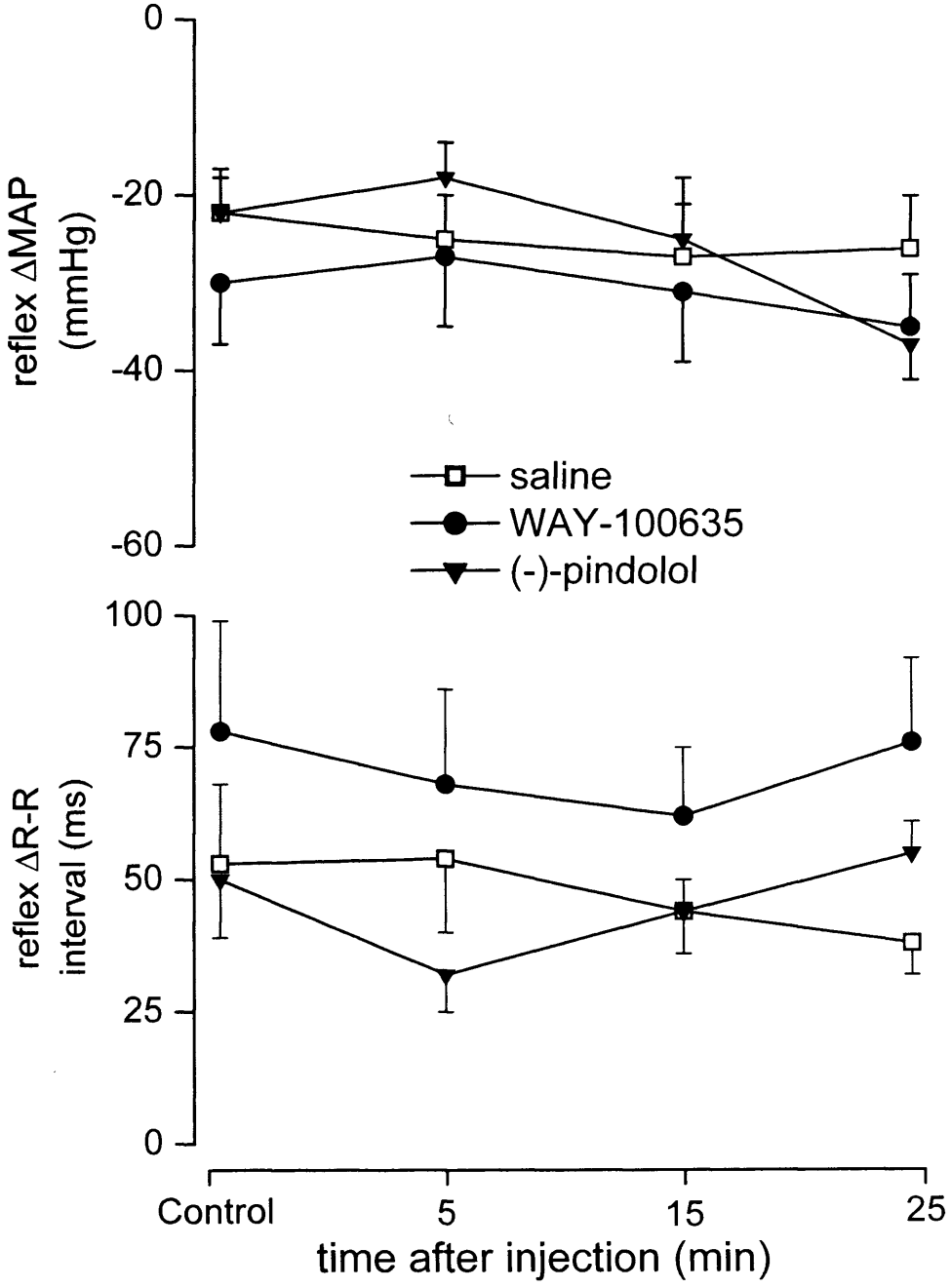

0
-20
-40
-60
reflex ΔMAP (mmHg)
saline
SB-269970
100
75
50
25
0
reflex ΔR-R interval (ms)
*
Control
5
15
25
time after injection (min)

Figure 3.20 Aortic nerve stimulation graph: WAY-100635 & pindolol

Graph showing the effects (mean ± s.e.m.) of 10 μl saline i.c. (□, n = 5), WAY-100635, 100 μg kg^{-1} i.c. (●, n = 5), and (-)-pindolol, 100 μg kg^{-1} i.c. (▼, n = 5) on changes (Δ) in mean arterial pressure (MAP) and R-R interval evoked by electrical stimulation of the right aortic depressor nerve in anaesthetised, neuromuscular blocked and atenolol pretreated rats.


0
-20
-40
-60
reflex ΔMAP (mmHg)
saline
WAY-100635
(-)-pindolol
100
75
50
25
0
reflex ΔR-R interval (ms)
Control
5
15
25
time after injection (min)

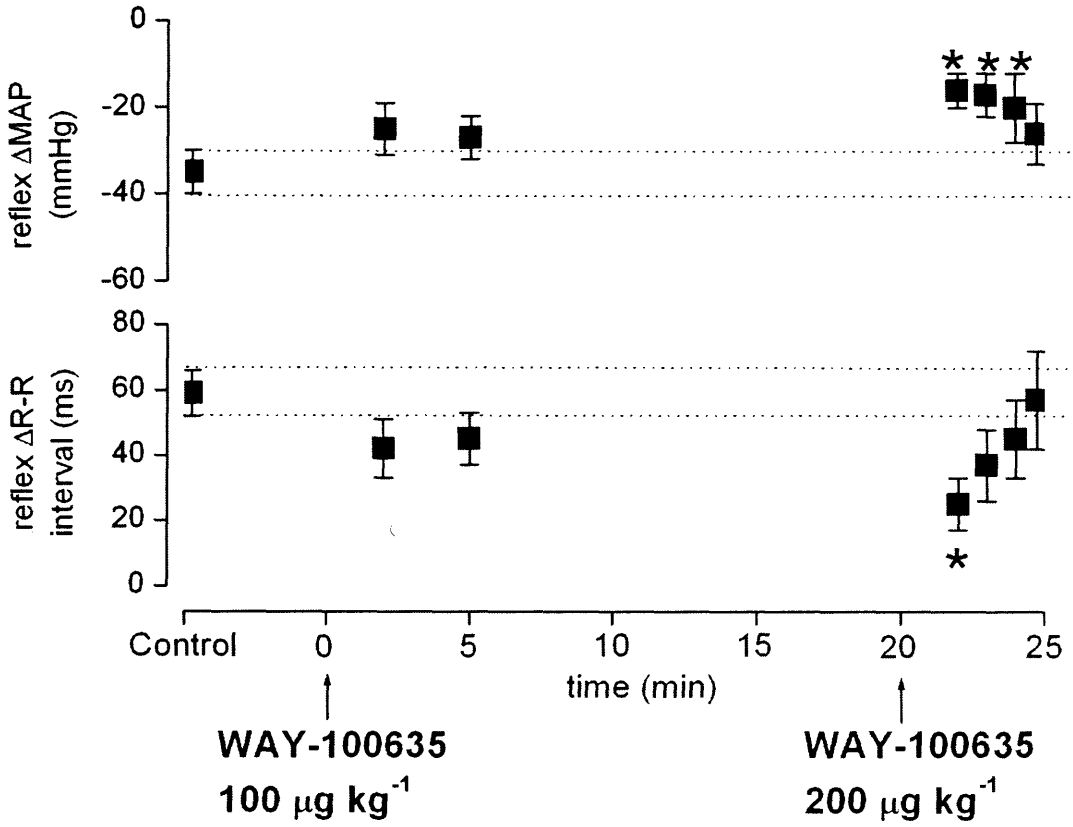


Figure 3.21 Aortic nerve stimulation graph (Protocol 3): WAY-100635

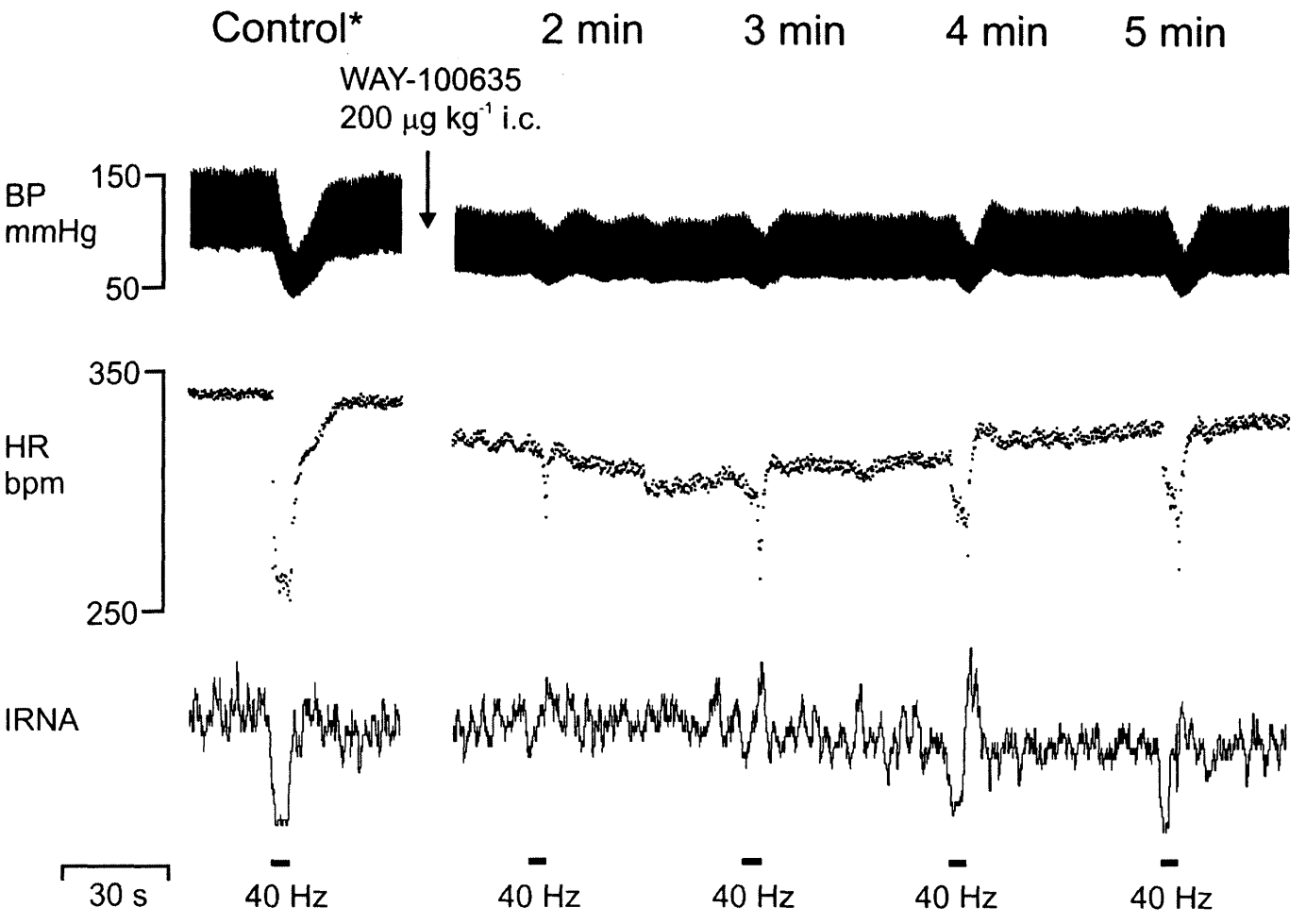
Graph showing the effects (mean ± s.e.m.) of WAY-100635 (100 and 200 μg kg^{-1} i.c.; $n = 6$) on changes (Δ) in mean arterial pressure (MAP) and R-R interval evoked by electrical stimulation of the right aortic depressor nerve (■) in anaesthetised, neuromuscular blocked and atenolol pretreated rats. Dotted line indicates s.e.m. of pre-drug control.

* $P < 0.05$, 1-way ANOVA followed by Tukey test.

Figure 3.22 Aortic nerve stimulation trace (Protocol 3): WAY-100635

Sample trace illustrating the effect of 200 μg kg^{-1} i.c. WAY-100635 (administered 20 min after the initial dose of 100 μg kg^{-1}, on heart rate (HR), blood pressure (BP), and integrated renal nerve (IRNA) responses evoked by electrical stimulation (40 Hz) of the right aortic depressor nerve in an anaesthetised, neuromuscular blocked and atenolol pretreated rat.

IRNA neurogram is rectified and smoothed (time constant 1 s)



* 20 min after WAY-100635 100 μg kg^{-1} i.c.

3.3.5. Effects of 5-HT receptor ligands on baroreflex

Effects of intravenous phenylephrine

Bolus injection of phenylephrine (3 – 15 μg kg^{-1}, n = 10) caused an increase in MAP of 77 ± 3 mmHg from a baseline of 98 ± 5 mmHg and a reflex increase in R-R interval of 22 ± 3 ms from a baseline of 184 ± 4 ms. The baroreflex gain calculated from plotting beat-by-beat changes in MAP and R-R interval was 0.24 ± 0.03 ms $mmHg^{-1}$. When MAP was raised IRNA was immediately switched off, but when averaged over 60 s following phenylephrine, IRNA reflexly dropped to 44 ± 6 % of control (Figure 3.23).

Effects of saline on baroreflex

Following saline (10 μl, i.c., pH 5.8; n = 5) the phenylephrine-evoked increase in MAP was significantly smaller at 25 min after saline, compared to control (64 ± 4 *vs.* 74 ± 4 mmHg), and the baroreflex gain was significantly greater at 5 min after saline (0.25 ± 0.04 *vs.* 0.34 ± 0.06 ms $mmHg^{-1}$). However, saline had no significant effects on the reflex increase in R-R interval or the reflex renal sympathoinhibition (Figures 3.24 and 3.25, Table 9.34).

Effects of SB-269970 on baroreflex

Central administration of SB-269970 (100 μg kg^{-1}; n = 5) significantly attenuated the reflex responses to raising MAP with phenylephrine (Figures 3.23, 3.24, 3.25 and Table 9.35). It significantly attenuated the reflex increase in R-R interval (compared to i.c. saline) with the greatest effect 15 min after drug administration (9 ± 1 ms) and recovery at 25 min. It also significantly attenuated baroreflex gain (to 0.1 ± 0.03 ms $mmHg^{-1}$, 15 min after drug administration). Reflex renal sympathoinhibition was unchanged until 25 min after drug administration, when it was significantly attenuated to 87 ± 10 % of control (compared to i.c. saline).

Figure 3.23 Baroreflex trace: SB-269970

Sample trace illustrating changes in blood pressure (BP), heart rate (HR), and integrated renal nerve (IRNA) evoked by phenylephrine (PE; 3 μg i.v.), both before (control) and after SB-269970 (100 μg kg^{-1} i.c.) in an anaesthetised, neuromuscular blocked and atenolol pretreated rat.

IRNA neurogram is rectified and smoothed (time constant 1 s)

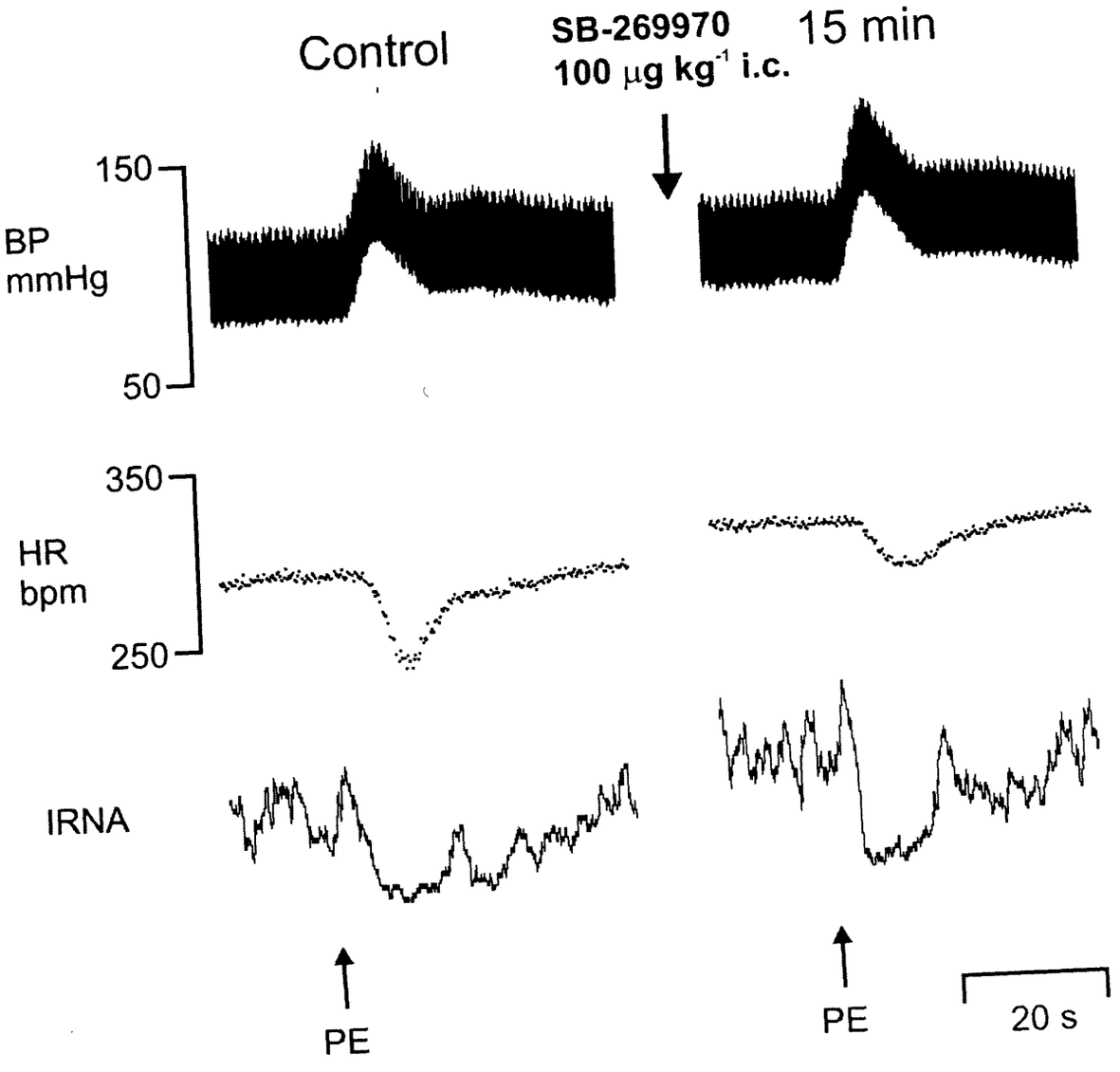

Control
SB-269970
100 μg kg-1 i.c.
15 min
150
BP
mmHg
50
350
HR
bpm
250
IRNA
PE
PE
20 s

Figure 3.24 Baroreflex gain: SB-269970

Sample plot illustrating phenylephrine-evoked baroreflex gain (regression line) both before (●, control) and 15 min after SB-269970 (▼, 100 μg kg^{-1} i.c.) in an anaesthetised, neuromuscular blocked and atenolol-pretreated rat.

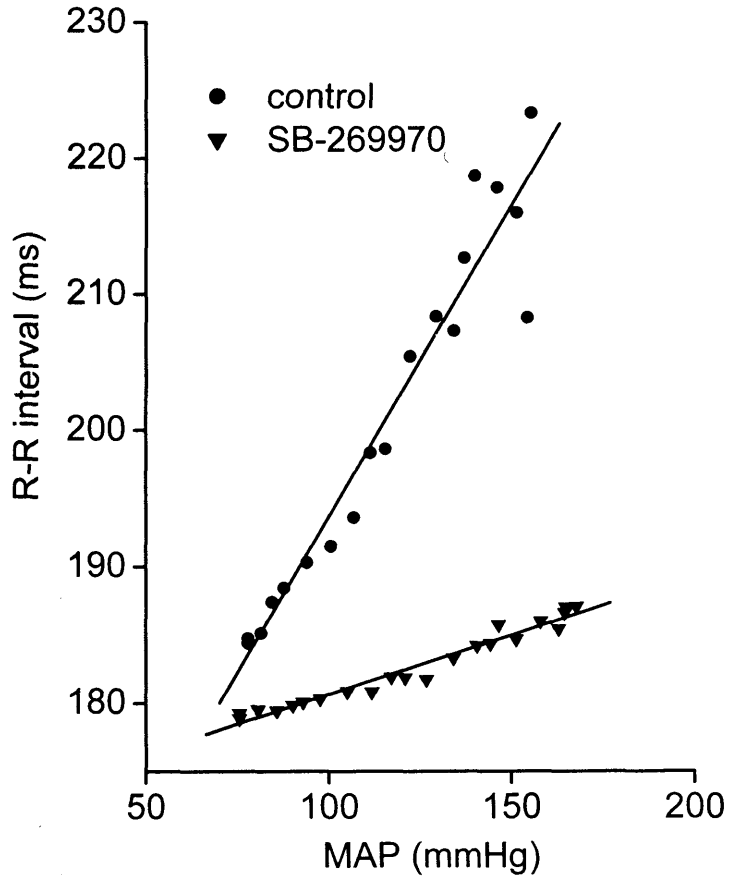
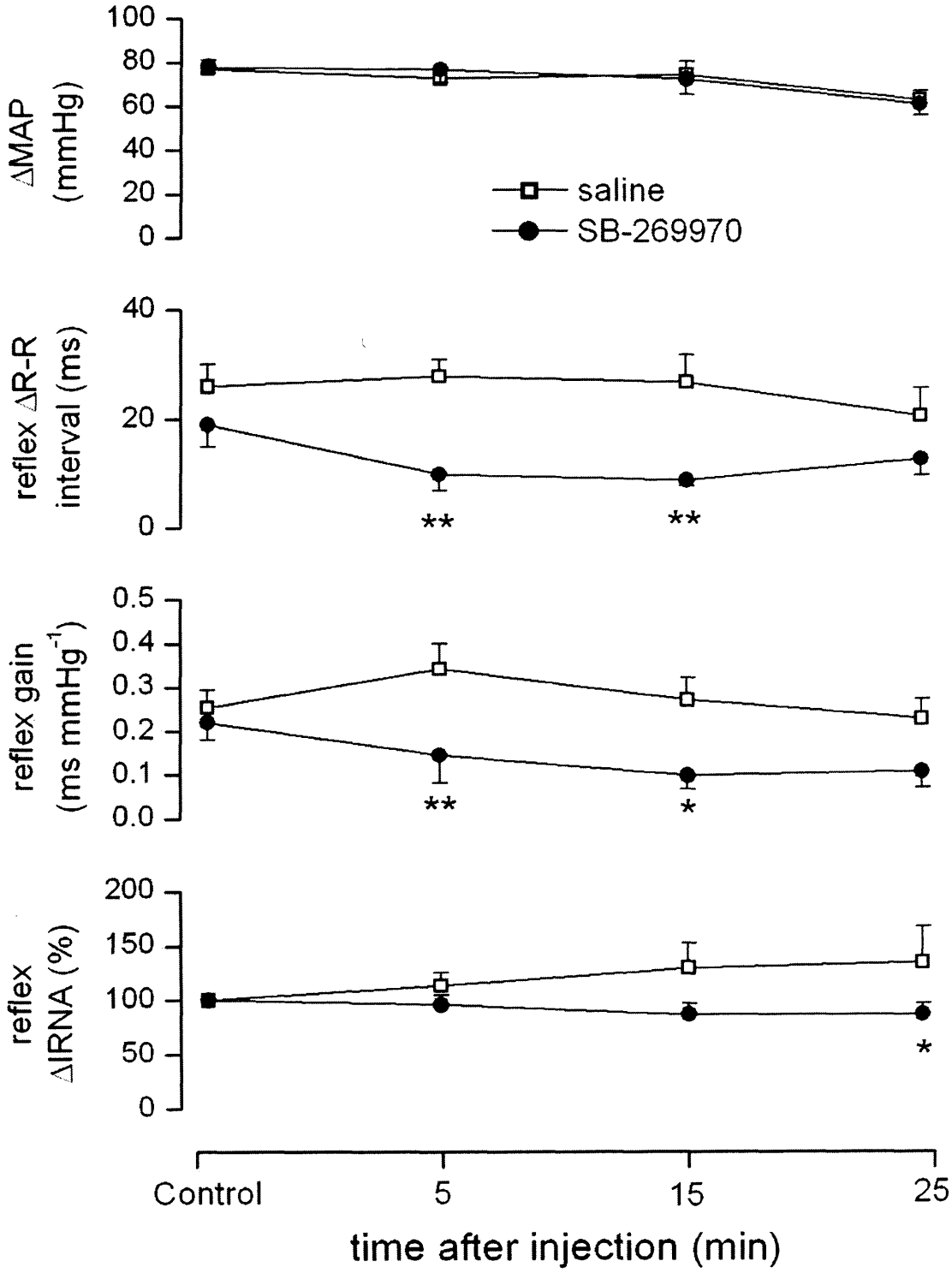


Figure 3.25 Baroreflex graph: SB-269970

Opposite: graph showing the effects (mean ± s.e.m.) of 10 µl saline i.c. (□, $n = 5$), and SB-269970, 100 µg kg^{-1} i.c. (●, $n = 5$), on changes (Δ) in mean arterial pressure (MAP) and reflex changes in R-R interval, baroreflex gain, and integrated renal nerve activity (IRNA), evoked by intravenous phenylephrine in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

* $P < 0.05$, ** $P < 0.01$, 2-way ANOVA followed by LSD test.


ΔMAP (mmHg)
100
80
60
40
20
0
saline
SB-269970
reflex ΔR-R interval (ms)
40
20
0
**
**
reflex gain (ms mmHg^{-1})
0.5
0.4
0.3
0.2
0.1
0.0
**
*
reflex ΔIRNA (%)
200
150
100
50
0
*
Control
5
15
25
time after injection (min)

3.3.6. Intracisternal injection of pontamine sky blue dye

To examine the potential diffusion and possible sites of action of intracisternally administered drugs, 10 µl pontamine sky blue dye (2.5 % in saline) was administered i.c. at the end of an experiment in several animals. After 15 min (the time at which the maximum response to SB-269970 was observed) the animals were deeply anaesthetised with pentobarbitone (30 mg kg^{-1} i.v.), transcardially perfused with 100 ml heparinised saline followed by 100 ml 4 % formal saline, and the brain removed and photographed (Figure 3.26).

The heaviest staining was seen on the dorsal surface of the medulla, near the tip of the intracisternal cannula, but considerable staining was also seen around the ventral surface of the medulla and pons. Light staining continued rostrally along the ventral surface as far as the optic chiasm, and caudally to approximately C2. No staining was seen in cerebellar or cortical areas, or in more caudal parts of the spinal cord.

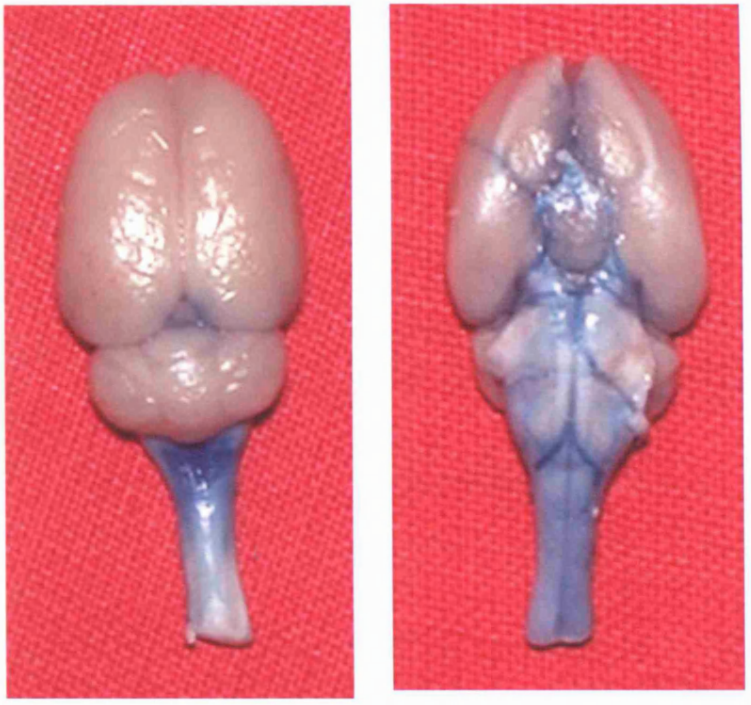


Figure 3.26 Intracisternal pontamine sky blue

Photographs illustrating staining of rat brain 15 min after intracisternal injection of pontamine sky blue dye (10 µl).

Left: dorsal view

Right: ventral view

Table 3.1 Summary of drug effects

Effects of different 5-HT receptor antagonists on bradycardic (R-R) and sympathetic (IRNA) components of reflexes. ↓ attenuation, o no effect († at 100 μg kg^{-1} dose).

Drug	*Cardiopulmonary*	*Chemoreflex*	*Baroreflex*
SB-269970	↓R-R ?↓IRNA	↓R-R ↓IRNA	↓R-R oIRNA
SB-656104	↓R-R oIRNA		
WAY-100635	↓R-R oIRNA	↓R-R ↓IRNA	oR-R†
Robalzotan	oR-R oIRNA		
(-)-Pindolol			oR-R
Cinanserin	oR-R ↓IRNA		
SB-204070	oR-R oIRNA		

Table 3.2 Maximum % inhibition

Comparison of the maximum % inhibition of reflex-evoked increases in R-R interval caused by different drugs, and different reflexes (PBG: cardiopulmonary, NaCN: chemoreflex, ADN: aortic depressor nerve stimulation. Changes are relative to pre-injection controls. Asterisks indicate that the true values are significantly different from vehicle (* $P < 0.05$, ** $P < 0.01$).

Time of maximum inhibition (min) in parentheses. All drug doses are 100 μg kg^{-1}.

Drug	*PBG*	*NaCN*	*ADN*
SB-269970	70 ± 6 (15)**	72 ± 5 (10)**	59 ± 11 (5)*
SB-656104	87 ± 6 (15)**		
WAY-100635	58 ± 2 (5)**	49 ± 10 (2)*	26 ± 16 (2)
Robalzotan	-6 ± 20 (5)		
(-)-Pindolol			27 ± 17 (15)
Cinanserin	11 ± 7 (5)		
SB-204070	37 ± 5 (5)		
Saline	7 ± 6 (5)	-24 ± 18 (10)	12 ± 16 (25)

3.4. Discussion

3.4.1. Main findings

These experiments demonstrate, for the first time, that central 5-HT_7 receptors are involved in the reflex activation of parasympathetic outflow to the heart in anaesthetised rats. Intracisternal injection of the selective 5-HT_7 receptor antagonist SB-269970 (Hagan *et al.*, 2000) dose-dependently attenuated the vagally mediated cardiac slowing evoked by cardiopulmonary afferent fibres, arterial baroreceptor and chemoreceptor afferents. This effect is most likely due to blockade of 5-HT_7 receptors and not compound specific, as SB-656104, another selective 5-HT_7 receptor antagonist (Thomas *et al.*, 2003), also strongly attenuated the bradycardic component of the cardiopulmonary reflex. SB-656104, although from the same chemical series, is sufficiently distinct in terms of structure and selectivity profile as to rule out this latter possibility. These effects are most likely due to actions on 5-HT_7 receptors located within the brainstem, since the same dose of SB-269970 given i.v. was without effect.

The role of brainstem 5-HT_{1A} receptors is more ambiguous. Although the selective 5-HT_{1A} antagonist WAY-100635 attenuated the bradycardic component of the cardiopulmonary reflex, the structurally different selective antagonist robalzotan failed to have any effect on this reflex. WAY-100635 also failed to attenuate the bradycardic component of the chemoreflex, which confirms previous experiments performed on rabbits (Skinner *et al.*, 2002). On further investigation, however, it was found that WAY-100635 does attenuate the bradycardic component of the chemoreflex, but only at 2 min after drug administration. Finally, the bradycardic response to aortic nerve stimulation was not attenuated by either WAY-100635 (including at the 2 min time point) or by the 5-HT_{1A} receptor antagonist (-)-pindolol.

These data suggest that the role of 5-HT_{1A} receptors in these reflexes is far less clear that the role of the 5-HT_7 receptor, at least in the rat. Possibly a compound-specific mechanism is revealed, with its most important role in the cardiopulmonary reflex, a lesser role in the chemoreflex, and no role in the aortic nerve-mediated baroreflex.

Blockade of brainstem 5-HT_2 and 5-HT_4 receptors with the selective antagonists cinanserin and SB-204070 had no effect on the bradycardic component of the cardiopulmonary reflex. Finally, the antagonists used generally had no effect on baseline variables, although SB-269970 dose-dependently diminished central respiratory drive.

3.4.2. Selectivity of antagonists

The chief compounds used, SB-269970, SB-656104, and WAY-100635, are thought to be highly selective for the 5-HT_7 and 5-HT_{1A} receptor respectively, based on binding data principally from human cloned receptors, as summarised in Table 3.3. These human cloned data, however, may not necessarily be the same at rat receptors. Various species differences in 5-HT receptor binding are reported, as discussed in 1.6 above. SB-269970, however, has a similar binding affinity at 5-HT_7 receptors in a variety of species (Thomas *et al.*, 2002).

In general, both SB-269970 and SB-656104 are very selective for the 5-HT_7 receptor, although SB-656104 has 5-HT_{1D} receptor affinity, which SB-269970 has not. Both drugs have negligible (SB-269970) or low affinity (SB-656104) for the 5-HT_{1A} receptor. The only possible non-5-HT_7 receptor affinity that these drugs have in common is at the 5-ht_{5A} receptor. WAY-100635 is highly selective for the 5-HT_{1A} receptor, but also has moderate affinity for the 5-HT_7 receptor.

Further comments on the selectivity of the antagonists used will be made in reference to the reflexes and protocols on which they were used.

	Binding affinity (pK_i)		
Receptor	**SB-269970**	**SB-656104**	**WAY-100635**
5-HT_{1A}	**<5.0**	**6.3**	**9.0**
5-HT_{1B}	6.0	6.2	<5.5
5-HT_{1D}	5.8	7.6	6.7
5-HT_{1E}	<5.2	<5.3	<5.3
5-HT_{1F}	<5.5	<5.7	<5.3
5-HT_{2A}	<5.5	7.2	6.2
5-HT_{2B}	5.0	7.0	7.3
5-HT_{2C}	<5.0	6.6	6.2
5-HT_4	5.9	5.7	<4.3
5-ht_{5A}	7.2	6.7	<5.0
5-HT_6	5.2	6.1	<5.1
5-HT_7	**8.9**	**8.7**	**6.9**
α_{1B}	<5.0	6.7	7.4*
D2	6.5	7.0	7.1
D3	5.6	6.5	7.2

Table 3.3 Binding affinities at human cloned receptors

Binding affinities at human cloned 5-HT receptors, α_1 adrenoceptors and dopamine receptors. Data for SB-269970 are taken from Lovell *et al.* (2000), data for SB-656104 from Thomas *et al.* (2003), and data for WAY-100635 from Roberts *et al.* (2001b) and Johansson *et al.* (1997). * Data from rat cortical α_1 adrenoceptors.

3.4.3. Sites of action

Administration of pontamine suggests that a 10 µl i.c. injection has the greatest effects at the dorsal surface of the medulla at the level of the obex, but also significantly diffuses to the ventral surface. Over time, further diffusion is expected both rostrally and caudally, so effects seen late in the protocol are cautiously interpreted. The most likely site of action for the antagonists here used is the NTS, for the following reasons. Firstly it is the most accessible cardiovascular structure for an i.c. injection, secondly it is the main integrative nucleus for cardiovascular reflexes, with a high level of neurochemical complexity (Lawrence & Jarrott, 1996), thirdly it is also the only medullary structure where 5-HT_7 receptor distribution has been reported (To *et al.*, 1995; Gustafson *et al.*, 1996), as well as containing 5-HT_{1A} receptors (Wright *et al.*, 1995; Dashwood *et al.*, 1988). However, 5-HT_{1A} receptors are also located in the nucleus ambiguus, at least in the cat (Dashwood *et al.*, 1988), where they are involved in cardiopulmonary reflex bradycardia (Wang & Ramage, 2001), so it is possible that i.c. drugs are also diffusing into the nucleus ambiguus at sufficient concentrations. Experiments to investigate the site of action of SB-269970 are described in Chapter 5.

3.4.4. Baseline variables

The general lack of effect of 5-HT receptor antagonists on baselines confirms that 5-HT *per se* has no clear tonic role in cardiovascular regulation in the anaesthetised rat. It should be noted, however, that i.c. injection of saline produced a small but significant lowering of baseline R-R interval (corresponding to an increase in HR of 13 ± 4 bpm after 15 min), which might seem surprising. Since there were no significant differences between saline and drug-treated groups, however, these changes could be caused either by the volume of the injection, or by its pH, or by time. Without a separate control in which no solution was injected, it cannot be unequivocally concluded that time alone was the factor causing the change. However, if either volume or pH caused the changes, other phenomena would be expected. For example, sufficiently acidic solutions would be expected to increase central respiratory drive *via* activation of medullary chemoreceptors, but as the saline solution used here was not sufficiently acidic (pH 5.8) to cause such changes, the R-R interval changes are unlikely to be due to this. Also, the volume of 10 µl is

unlikely to cause significant pressure changes; after injection the guide cannula was unobstructed, allowing cerebrospinal fluid pressure to equilibrate with atmospheric pressure. The likely cause of the decreased R-R interval is time, which could be due to atenolol wearing off. This is unlikely, since the protocol has been extensively used before (Bogle *et al.*, 1990; Dando *et al.*, 1998; Skinner *et al.*, 2002), and this dose of atenolol shown to be sufficient for the duration of the protocols. In the present experiments, a further dose of atenolol at the end of the protocol did not further decrease HR, suggesting adequate lasting β_1 adrenoceptor blockade. Therefore the cause of the decreased R-R interval is likely to be a reduction in vagal drive to the heart, possibly as a consequence of the level of anaesthesia slowly changing. For this reason, the same supplementary dose of anaesthetic was given at the beginning of each protocol, and the comparison with time-matched vehicle controls can take such changes into account.

The vehicle for SB-656104 itself caused an increase in MAP which was no different from that caused by SB-656104 alone, and this effect was not seen in the saline group. This suggests that the vehicle alone causes this pressor response, which is not surprising considering it consists of about 25 % DMSO and 25 % PEG. SB-656104 is not commercially available and was supplied pre-dissolved in DMSO and PEG for these experiments, hence there was no choice but to use this rather severe concentration. DMSO in particular is thought to increase the permeability of neurones and thus increase their excitability (as is shown in Chapter 5, Figure 5.6). A possible mechanism, therefore, is that the vehicle is centrally raising MAP by, for example, activating sympathoexcitatory neurones in the RVLM. The fact that no decrease in baseline R-R interval is seen in this vehicle group may be because the pressor response is causing a reflex increase in baseline R-R interval which is counteracting the presumed time-related decrease in R-R interval.

Neither SB-269970 nor SB-656104 significantly increase baseline MAP. This is an important observation, supporting a modulatory role for central 5-HT_7 receptors involved in cardiovascular reflexes. Blockade of glutamate receptors in the NTS, for example, raises MAP (Talman *et al.*, 1981; Guyenet *et al.*, 1987) as does blockade of α_2 adrenoceptors in the NTS (Sved *et al.*, 1992). Both these effects were associated with a baroreflex inhibition. In the present experiments, baroreflex inhibition is

associated with normal resting MAP, suggesting that when central 5-HT_7 receptors are blocked, there is no loss of control of baseline MAP. Possibly the continuing activity of other neurochemical systems, such as the major glutamatergic pathways, is responsible for this.

The effect of SB-269970 on central respiratory drive was also surprising. At the highest dose, the drug immediately (in < 5 min) reduced IPNA, but at the medium dose it took 15 min, and the low dose was without effect. The failure of SB-656104 to affect IPNA raises doubts over the role of the 5-HT_7 receptor in this effect. Furthermore, the fact that only the higher doses have an effect would support the notion that a different receptor is involved. The affinity of SB-269970 for the dopamine D_2 receptor (pK_i 6.5) (Lovell *et al.*, 2000) is unlikely to be the cause, as the D_2 receptor antagonist sulpiride had no effect on central respiratory drive when given i.c. to anaesthetised rabbits (Dando, 1995). Based on its affinity, a possible mechanisms is *via* the 5-ht_{5A} receptor, but this is purely speculative as no information on its respiratory functions is available.

The lack of effect of WAY-100635 and robalzotan on baseline variables confirms earlier findings (Dando *et al.*, 1998; Skinner *et al.*, 1997; Secker, 2004), illustrating the lack of tonic effect of 5-HT_{1A} receptors in the medulla on cardiovascular regulation. The ability of (-)-pindolol to cause a significant reduction in baseline R-R interval also confirms previous findings (Bogle *et al.*, 1990; Dando *et al.*, 1998), and is probably due to its partial agonist activity at the β adrenoceptor (Hicks *et al.*, 1987). It is unclear whether the central actions involve a cardiac sympathoexcitation or a vagal inhibition. The atenolol pretreatment would be expected to prevent the former, although if it were sufficiently strong it could override the blockade.

The initial increase in IPNA caused by SB-204070 was surprising. Previous research found that *activation* of the 5-HT_4 receptor reverses opioid-induced respiratory depression (Manzke *et al.*, 2003), suggesting that the 5-HT_4 receptor stimulates respiratory drive (although the effect of the agonist BIMU8 alone was not reported). The present experiments show that 5-HT_4 receptor blockade augments respiratory drive, suggesting the 5-HT_4 receptor is tonically dampening respiration. The effect is also very short-lived (only seen at 5 min). Hence, unless compensatory measures are

activated to bring central respiratory drive back down (which is unlikely as the animals are ventilated) it is more likely that this is a non-selective effect of the drug.

The general lack of effects of cinanserin on baseline variables confirms the effects of i.c.v. cinanserin reported previously (Knowles & Ramage, 1999). However, at 5 min after cinanserin, R-R interval was slightly higher than saline, which was surprising. The control value for the cinanserin group, however, was slightly higher than the corresponding saline control value, so this may be an artefact caused by this difference. Furthermore, baseline IRNA was significantly higher at 25 min in the cinanserin group. There is no previous research to suggest that blockade of 5-HT_2 receptors should cause sympathoexcitation, and the long latency of this effect would probably exclude 5-HT_2 receptors in the medulla, so this may reflect a change in stability of anaesthetic level at this later time in this group.

In general, it should be noted that this experimental design is not ideally suited to the study of baseline effects: the stimulation of cardiovascular reflexes by definition alters baselines, and these repeated challenges could have effects that have not been controlled for in these experiments. For this reason the baseline effects here reported are those from the groups in which only the cardiopulmonary reflex was elicited (or, in the case of (-)-pindolol, the aortic nerve mediated baroreflex). In these groups there was minimal perturbation of baselines, as the cardiopulmonary reflex changes are very short-lived.

3.4.5. The cardiopulmonary reflex

Intra-atrial PBG is a reliable tool for the study of the cardiopulmonary reflex, as it acts on the 5-HT_3 receptors located on the peripheral terminals of both cardiac and pulmonary vagal C-fibres (Kay & Armstrong, 1990). The latency of the bradycardia thus elicited is very rapid (< 2 s) and thus clearly within the circulation time of right atrium to left ventricle. Therefore there is no time for downstream (e.g. gastrointestinal) afferents to contribute to these effects. In the rabbit and cat the circulation time is sufficiently long (approximately 5 s) for the pulmonary afferent-evoked effect to be distinguished from the cardiac (Bezold-Jarisch) component, but in the rat these cannot be reliably distinguished. In these experiments the dose of PBG has been titrated to evoke submaximal bradycardias, to allow either attenuation

or potentiation to be observed. The strength of the stimulation is therefore probably not great enough to cause consistent reflex apnoeas, hence the reflex changes in IPNA were variable. The pretreatment with atenolol means that changes in R-R interval will directly reflect changes in the activity of CVPNs.

5-HT$_7$ receptors

SB-269970 dose-dependently and reversibly attenuated the bradycardic component of the cardiopulmonary reflex. The dose of 100 μg kg^{-1} i.c. was subsequently chosen as the most appropriate to use in other protocols, since it caused a moderate attenuation of the bradycardia. The same dose of SB-656104 also strongly attenuated the cardiopulmonary reflex bradycardia with a longer time-course, consistent with its longer plasma half-life (Thomas *et al.*, 2003). Both drugs are selective for the 5-HT$_7$ receptor, and their selectivity profiles are sufficiently different to conclude that their reflex effects reflect 5-HT$_7$ receptor blockade (see Table 3.3). Both drugs have been used to attenuate the micturition reflex in anaesthetised rats (Read *et al.*, 2003). In that investigation, they were given by the i.c.v. route, and at a slightly lower dose (10 – 100 μg kg^{-1} and 30 μg kg^{-1} respectively) and smaller volume (5 μl). However, since the target structures involved in the micturition reflex (probably the pontine micturition centre) are different, a comparison is meaningless. To date there have been no other studies using these compounds centrally *in vivo* (i.e. by the i.c or i.c.v. route), nor has the 5-HT$_7$ receptor previously been implicated in cardiovascular reflexes. However, the original report of the role of the 5-HT$_{1A}$ receptor in the cardiopulmonary reflex (Bogle *et al.*, 1990) used two non-selective antagonists that also have affinities for the 5-HT$_7$ receptor, namely spiperone and methiothepin (Bard *et al.*, 1993). At the time this was not known, but it may be significant to these findings.

In brain slices, SB-269970 is effective at nanomolar concentrations (Roberts *et al.*, 2004b), raising the question of whether the present dose of 100 μg kg^{-1} (26 nmol kg^{-1}) is too high to have a selective effect. In the present experiments, the end concentration at the target neurones is nigh impossible to estimate, but the selectivity profiles of the drugs may give clues: SB-269970 has higher affinity at the 5-ht$_{5A}$ receptor than SB-656104 (see Table 3.3). Therefore it is unlikely that both drugs are attenuating the bradycardia *via* 5-ht$_{5A}$ receptor blockade. The 5-HT$_{1D}$ and 5-HT$_{2A}$

blocking properties of SB-656104 are also not expected to have an effect on the reflex (Jeggo, 2003; Bogle *et al.*, 1990). SB-269970 has negligible affinity for the 5-HT_{1A} receptor, whereas SB-656104 has low affinity (p*K*i 6.25). The latter may contribute to the very strong attenuation of the bradycardia achieved with this drug, but the primary effect appears to be 5-HT_7 receptor mediated.

The effect of SB-269970 on cardiopulmonary reflex sympathoinhibition is more ambiguous. It should be noted that in the saline-treated group, the size of the sympathoinhibition grows larger over time. This is likely to be due to time rather than the saline injection. Whilst every care was taken to verify IRNA noise levels at the end of the experiment, these may also vary during the protocol. Although no changes in baseline IRNA were observed, various factors might cause a gradual increase in the reflex effect. Possibly a central mechanism is involved, by which successive reflex stimuli cause an increasingly efficient response. A possible candidate receptor for such an effect would be the NMDA receptor, which is known to be involved in cardiopulmonary reflex transmission in the NTS (Chianca & Machado, 1996). As such, the effect of SB-269970 (at the low and medium doses) is not to attenuate reflex sympathoinhibition, but to prevent its potentiation. This may be due to an interplay of glutamatergic and serotonergic mechanisms in the NTS. Only the high dose of 300 $\mu g\ kg^{-1}$ clearly attenuated the reflex sympathoinhibition (and with it the reflex hypotension). Furthermore, there is no clear dose-depedency in this response.

If the 5-HT_7 receptor involved in this reflex are located in the NTS, where afferent information is integrated and distributed to the different limbs of the reflex, it is surprising that these components are not equally attenuated. Three possibilities should be considered: firstly, that the reflex sympathoinhibition has a different sensitivity from the reflex bradycardia; secondly, that it involves a different neuronal pathway, and thirdly that a different 5-HT receptor is involved. For the baroreflex, there is emerging evidence that the bradycardic and sympathetic components are physiologically quite different. For example, baroreflex sympathoinhibition is more sensitive and has a shorter latency than baroreflex bradycardia (Simms *et al.*, 2004). This may be due to the different properties of sympathetic and parasympathetic preganglionic neurones, but another hypothesis is that different afferents are

involved. When the aortic nerves are cut in the rat, the baroreflex bradycardia is almost entirely abolished, but the sympathoinhibition remains fairly robust (Pickering *et al.*, 2004), suggesting that the bradycardia preferentially uses the aortic nerve afferents, whilst the sympathoinhibition is maintained by the carotid sinus nerve afferents. As such, the present effects of SB-269970 on the cardiopulmonary reflex resemble the effects of cutting the aortic nerves on the baroreflex. Although the cardiopulmonary afferents all travel in the vagus nerve, as opposed to the baroreceptor afferents, which are both vagal (aortic nerve) and glossopharyngeal (carotid sinus nerve), there may be different types of afferent mediating the different components of reflexes. Like afferents terminating in the dorsal horn of the spinal cord (to which the NTS is often compared) these may terminate in very different and discrete parts of the NTS. These possibilities remain hypothetical, but raise the second question of a different 5-HT receptor being involved. The reflex sympathetic effects of SB-269970 (albeit unclear) are not confirmed by SB-656104. If the 5-HT_7 receptor is indeed involved, this is unlikely to be a question of insufficient dose, as SB-656104 attenuates the bradycardia almost as strongly as the high dose of SB-269970 (which itself strongly attenuates the sympathoinhibition). Therefore it is possible that the effect of SB-269970 on reflex sympathoinhibition is non-selective, possibly *via* the 5-ht_{5A} receptor. The lack of effect of SB-656104 on reflex sympathoinhibtion, but its strong effect on reflex hypotension (comparable to that of the high dose of SB-269970) suggests that a large part of the reflex hypotension is vagally mediated. In awake rats it has been shown that the cardiopulmonary reflex hypotension is entirely vagal (Chianca & Machado, 1996) but in these anaesthetised rats some reflex hypotension remains after administration of atropine.

The major problem with interpreting 5-HT_7 receptor mechanisms is the lack of selective agonists to confirm the effect. The non-selective agonist 5-CT has been extensively used, but has its limitations. 5-CT induces hypothermia in guinea pigs, which is inhibited by SB-269970 (Hagan *et al.*, 2000) or SB-656104 (Thomas *et al.*, 2003). Also 5-CT can evoke micturition in anaesthetised rats only when 5-$HT_{1B/1D}$ receptors are masked with GR-127935 (Read *et al.*, 2004). This latter approach could be used in the present experiments to confirm whether 5-CT can potentiate the reflex, although this would still not exclude possible effects at the 5-ht_{5A} receptor, for which 5-CT has a high affinity.

A final consideration is that SB-269970 is dose-dependently reducing central respiratory drive, which is known to modulate vagal bradycardias. Hence when IPNA is reduced, one would expect there to be less inhibition of CVPNs, causing an increase in reflex vagal outflow. In these cardiopulmonary reflex experiments, however, this is not an issue since the reflex bradycardia has been shown not to be respiratory modulated (Wang *et al.*, 2000a).

5-HT_{1A} receptors

The attenuating effect of WAY-100635 on cardiopulmonary reflex bradycardia confirms the role of the 5-HT_{1A} receptor in this effect, as previously reported in the rat (Bogle *et al.*, 1990), rabbit (Skinner *et al.*, 2002) and cat (Wang & Ramage, 2001). The initial study in the rat did not use selective antagonists, nor look at reflex sympathoinhibition. The subsequent study in the rabbit, using the present dose of WAY-100635 given i.c., reported that *all* components of the cardiopulmonary reflex were attenuated (including sympathetic and respiratory components), suggesting that the receptors are located in the NTS, which mediates all components of the reflex. The study in the cat, however, found that reflex activation of CVPNs in the nucleus ambiguus was attenuated by iontophoretic WAY-100635, suggesting that the receptors are located here. Possibly the receptors are located in both nuclei. A further possibility is that WAY-100635 is acting at medullary raphe autoreceptors to increase firing, causing a release of 5-HT in the NTS, where it could inhibit reflexes. A possible pathway has been described from raphe pallidus to the NTS, which attenuates cardiopulmonary reflex bradycardia *via* 5-HT_4 receptors (Edwards & Paton, 2000). Raphe pallidus neurones on the ventral surface of the medulla could be rapidly reached by an i.c. injection. 5-HT_{1D} receptors in the NTS also attenuate activation of NTS neurones by cardiopulmonary afferents (Jeggo, 2003). Whether these receptors are innervated by a functional pathway from the raphe is not known, but illustrates that there are at least two 5-HT receptors that might inhibit reflexes if raphe neuronal firing increased.

WAY-100635 is considered a highly selective and potent 5-HT_{1A} receptor antagonist, with almost 100-fold selectivity over the nearest 5-HT receptor (5-HT_{1D}). However, recently it has also been shown to have a moderate affinity for 5-HT_7

receptors (see Table 3.3). The selectivity of the present dose (100 μg kg^{-1} i.c.) has not been verified: in the rabbit, this dose inhibited the potentiation of cardiovascular reflexes by i.c. buspirone, but it was only given *intravenously* (the dose itself having no effect on the reflex) (Skinner *et al.*, 2002). The fact that buspirone potentiated the reflex but i.v. WAY-100635 had no effect can be explained by the relative difficulty of antagonising synaptic events with a systemic antagonist. However, these data do not exclude the possibility that this dose of WAY-100635, given i.c., has non-selective effects.

Robalzotan (NAD-299), on the other hand, is a highly potent and selective 5-HT_{1A} receptor antagonist with a 5-HT_{1A} binding affinity comparable to that of WAY-100635, but negligible affinity for the 5-HT_7 receptor (Johansson *et al.*, 1997). Robalzotan i.c. had no effects on the cardiopulmonary reflex in these experiments, raising doubts over whether the effects of WAY-100635 are 5-HT_{1A} receptor mediated. A set of experiments is described in Chapter 5, which investigate whether robalzotan can inhibit 8-OH-DPAT induced bradycardia (see 5.3.2). These found that an *intravenous* dose of 100 μg kg^{-1} of robalzotan was able to inhibit the bradycardia elicited by i.v. 8-OH-DPAT, suggesting adequate receptor occupancy when given systemically. Those data are presented in Chapter 5 to avoid confusing different anaesthetic protocols, and in those experiments robalzotan was not given i.c. because subsequent single-unit recordings were performed, which could be influenced by this pretreatment. Therefore robalzotan was given i.v. to ensure its rapid clearance from the body. This minor control is by no means unequivocal evidence, but it seems unlikely that the dose of robalzotan used i.c. in the present Chapter was insufficient to occupy medullary 5-HT_{1A} receptors to a similar extent to WAY-100635. Similar doses of robalzotan and WAY-100635 increase bladder capacity in rats (Pehrson *et al.*, 2002), suggesting similar potency when given centrally *in vivo*.

5-HT_2 and 5-HT_4 receptors

The lack of effect of cinanserin and SB-204070 confirms that there is no tonic role of 5-HT_2 and 5-HT_4 receptors in the cardiopulmonary reflex. 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors are found in the NTS, with 5-HT_{2C} receptors causing neuronal inhibition and 5-$HT_{2A/B}$ causing excitation (Sevoz-Couche *et al.*, 2000b). Activation of 5-HT_4 receptors in the NTS also attenuates the cardiopulmonary reflex (Edwards

& Paton, 1999). The present experiments are consistent with previous findings that the 5-HT_2 receptor antagonist BW-501C67 given i.c. (Bogle *et al.*, 1990) has no effect on the cardiopulmonary reflex. The present dose of cinanserin is marginally higher than the i.c.v. dose previously reported to block completely the effects of i.c.v. quipazine in rats (Knowles & Ramage, 1999), suggesting that it is a sufficient dose to occupy 5-HT_2 receptors in the medulla. Cinanserin did, however, attenuate the reflex sympathoinhibition only at 5 min. This short-lasting effect is not consistent with the long duration of this antagonist when given i.c.v. (Knowles & Ramage, 1999), and therefore seems unlikely to be due to 5-HT_2 receptor blockade. Although other components of the reflex in this group were stable, this could represent a change in the condition of nerve recording in a few animals, which has skewed the mean of this small group.

It has been previously shown that activation of 5-HT_4 receptors in the NTS attenuates the cardiopulmonary reflex (Edwards & Paton, 1999), but the 5-HT_4 receptor antagonist RS-39604 alone had no effect. These present data demonstrate the lack of effect of another more potent 5-HT_4 receptor antagonist, SB-204070, with a pK_i of 10.2 (van den Wyngaert *et al.*, 1997). A 1 mg kg^{-1} i.p. dose of SB-204070 is sufficient to occupy striatal 5-HT_4 receptors effectively (Porras *et al.*, 2002), suggesting that the current dose of 100 μg kg^{-1} given i.c. is sufficient.

3.4.6. The chemoreflex

Bolus doses of i.v. cyanide were used to activate peripheral chemoreceptors in the carotid bodies. This mechanism has been previously verified: cutting of the carotid sinus nerves completely abolished the effect of i.v. cyanide in awake rats (Franchini & Kreiger, 1993). In the present experiments it was also found that atropine at the end of the experiment abolished cyanide-evoked bradycardias, indicating that the doses of cyanide used did not have direct cardioinhibitory effects. The chief effect on MAP in these experiments was a depressor response. Although a small initial increase in MAP was sometimes observed, the decrease in MAP was the dominant effect, and this was recorded. In awake animals the normal effect of chemoreceptor stimulation with cyanide is a marked pressor response, which turns into a depressor response when the animal is anaesthetised (Franchini & Kreiger, 1993), which may reflect the greater excitability of neurones in the unanesthetised state, and include

some behavioural alerting. Also in the anaesthetised rabbit a pressor response is achieved when small doses of cyanide are applied locally to the carotid bodies via the lingual artery (Skinner *et al.*, 2002), probably because with this method the doses of cyanide are not sufficient to cause peripheral vasodilatation by direct effects on the vasculature, which is probably what is causing the hypotension in the present experiments. The disadvantage in the lingual artery route is that only one carotid body is stimulated; in the present experiments the stimulation is bilateral. Another possibility is that the sympathoexcitation observed is either not of sufficient strength and duration to raise MAP, or is only restricted to certain targets (such as the kidney).

In the i.c. saline group, the reflex bradycardia was slightly potentiated with time, which was not seen in the cardiopulmonary reflex bradycardia, but was seen in the cardiopulmonary sympathoinhibition. As before, this may involve central mechanisms of increased synaptic efficacy, but is most likely due to changing anaesthesia level. However, it aids the interpretation of drug-evoked attenuation, as the normal tendency is for repeated stimuli to increase in size.

5-HT_7 receptors

The medium dose of SB-269970 used in the cardiopulmonary reflex also caused a comparable attenuation of chemoreflex bradycardias. This illustrates for the first time the role of 5-HT receptors in chemoreflex transmission, as it was previously shown that 5-HT_{1A} receptors are involved in cardiopulmonary but not chemoreflex bradycardia in rabbits (Skinner *et al.*, 2002). From NTS neuronal recordings it was proposed that different reflexes use different neuronal channels in the NTS as well as different afferents (Donoghue *et al.*, 1985), which would indicate an influence of 5-HT_7 receptors on multiple channels, if in fact they are functioning within the NTS. The similar sizes of the cardiopulmonary and chemoreflex bradycardic attenuations (~ 70 %) suggest that the receptor has a similar role and importance in both reflexes.

The effect of SB-269970 on chemoreflex sympathoexcitation is a clear attenuation, not merely the prevention of potentiation seen in the cardiopulmonary reflex. Furthermore the effect follows the same time course as for the bradycardic attenuation, suggesting a similar site of action. This implies that in the chemoreflex,

5-HT_7 receptors facilitate sympathoexcitation. The receptors could be located on or in the vicinity of NTS neurones projecting to the RVLM. The ability of the drug to attenuate this response, where it failed to have such a clear effect on cardiopulmonary reflex sympathoinhibition, could reflect a lower excitability of neurones mediating the effect. The control sympathoexcitation was an increase of 74 ± 11 %, which is comparatively small, and may reflect the depressant action of the anaesthetic. Hence it is not surprising that this effect is attenuated, whereas the huge reflex respiratory response (an increase of 262 ± 45 %) was unaffected. The 5-HT_7 receptors facilitating the sympathoexcitation could also be located on RVLM premotor neurones; the nature of the connection between NTS and RVLM in the chemoreflex is still under debate, and appears to be more complex than a simple glutamatergic projection (Mauad & Machado, 2001), possibly involving other transmitters. Hence 5-HT is another possible candidate, but there is no evidence of expression of 5-HT_7 receptors in the RVLM.

The lack of effect on reflex increase in IPNA is interesting, and suggests that the neuronal channel mediating the respiratory response to chemoreceptor activation through the NTS is not facilitated by 5-HT_7 receptors. Again this illustrates the differential role of the receptor in reflex integration. A similar effect was seen in the micturition reflex in anaesthetised rats, where SB-269970 reduced reflex bladder contractions, but did not affect reflex urethral dilatation (Read *et al.*, 2003). The respiratory response to chemoreceptor stimulation (by simulated hypoxia in this case) is certainly the most important component of the reflex in terms of survival of the organism, so one would expect it to be more resistant to modulation.

Chemoreflex bradycardia is known to be respiratory modulated (Davidson *et al.*, 1976), so the lack of change in the reflex ventilatory response is advantageous to the interpretation of the bradycardic attenuation: the latter is unlikely to be affected, as the former has not changed. The *baseline* IPNA, however, is lowered by SB-269970, suggesting that cardiac vagal outflow is generally more excitable after the drug, due to reduced inspiratory drive to inhibit it. The fact that evoked bradycardias are still strongly attenuated even when IPNA is lowered confirms that the reduced bradycardia is not an artefact of altered respiratory modulation.

5-HT_{1A} receptors

The same dose of WAY-100635 that attenuated cardiopulmonary reflex bradycardia 5 min after injection had no effect on chemoreflex bradycardia at 7 min. This confirms previous findings in the rabbit (Skinner *et al.*, 2002), where the same dose of WAY-100635 had no effect (at 5 min), although buspirone potentiated the bradycardia. This study, however, reported no attenuation of the sympathoexcitation at 5 min. This is at odds with the present data, which show that the sympathoexcitation is not diminished as such, but fails to be potentiated. In these experiments, the first chemoreceptor stimulus was given at 7 min after i.c. WAY-100635, as opposed to at 10 min after SB-269970 (and saline). This was because the inhibitory effect of WAY-100635 on the cardipulmonary reflex was gone by 15 min, and previous experiments with WAY-100635 suggested a very short duration of action when given i.c. (Skinner *et al.*, 2002). Subsequently it was considered that even 7 min might be too long, so a new protocol was designed, with stimuli given at 2 and 5 min after WAY-100635 or saline. Here effects are compared with truly time-matched controls, rather than reflexes at 7 min being compared to those at 10 min in the saline group.

At 2 min after WAY-100635 the chemoreflex bradycardia was indeed attenuated, and the sympathoexcitation was again prevented from potentiating, as seen in the original protocol at 7 min. Both of these effects were gone at 5 min. This raises the question of whether the previously reported lack of effect in rabbits (Skinner *et al.*, 2002) was also due to the short duration of action rather than the lack of 5-HT_{1A} receptor involvement. Such a short duration of action was very surprising, as WAY-100635 has a plasma half-life of ~15 min (Pfizer Ltd., personal communication). When given centrally, it may redistribute very rapidly, losing effective concentration in a much shorter time. Another possibility is a non-selective action. The selectivity of WAY-100635 for the 5-HT_{1A} over the 5-HT_7 receptor has been reported to be between 75-fold (see Forster *et al.*, 1995, discussion) and 100-fold (Roberts *et al.*, 2001b). WAY-100635 appears to diffuse into the brain very rapidly following an i.c. injection, as it virtually abolished aortic nerve-mediated bradycardia in rabbits just 90 seconds after administration (Skinner *et al.*, 2002). The rat medulla is smaller, so the time to reach the target could be even less. It is conceivable that at this early time of 2 min after injection, the concentration of WAY-100635 at a relatively superficial

structure such as the NTS could be high enough to occupy 5-HT_7 receptors. This could therefore be responsible for the very short-lived attenuation of chemoreflex bradycardia and sympathoexcitation. WAY-100635 also binds to α_1 adrenoceptors, but there is no evidence for their involvement in the chemoreflex. Indeed α_1 adrenoceptor blockade has no effect on cardiopulmonary reflex bradycardia (Bogle *et al.*, 1990).

3.4.7. The baroreflex

Electrical stimulation is an accepted and elegant way to activate the baroreflex (Sapru *et al.*, 1981), as it directly stimulates the afferents without the need to change BP artificially. Therefore reflex hypotension as well as bradycardia can be observed. The disadvantage is that all the afferents are stimulated, and at the same time, rather than incrementally as would be the case with increasing BP. However, the aortic nerves in the rat have been shown to contain very few chemoreceptor afferents, and are the primary mediators of baroreflex bradycardia (Pickering *et al.*, 2004). In the present experiments, stimulating of the right aortic nerve produced consistent bradycardias and hypotensions, though these were slightly less stable and reproducible than other reflexes. The use of constant current rather than constant voltage stimulation should overcome small changes in the impedance of the electrode-nerve preparation. However, as can happen with repeated electrical stimulation of neural tissue, there is some wind-down of the response. This may account for the slight downward trend in the saline group. Consistent sympathoinhibition was difficult to record in these experiments. In several animals there were stimulus artefacts in the renal nerve signal, possibly due to insufficient grounding. For this reason the baroreflex investigation was continued using the traditional method of raising MAP with phenylephrine. With this method, the real sensitivity of the baroreflex can be quantified. The method of plotting the phenylephrine-evoked MAP slope beat-by-beat against the corresponding R-R intervals, and staggering the two until the maximum correlation coefficient was reached (Su *et al.*, 1992), was chosen because it takes into account the delay in the onset of the bradycardia, and shows the true linear relationship between MAP and R-R interval.

5-HT$_7$ receptors

SB-269970 again caused a significant attenuation of the aortic nerve-evoked bradycardia, with no effect on the evoked hypotension, which suggests that central 5-HT$_7$ receptors facilitate the cardioinhibitory but not the sympathoinhibitory component of the baroreflex. The size of the attenuation was not quite as great as that of the cardiopulmonary and chemoreflex bradycardias, nor did it reach the same level of significance with respect to saline control, which may reflect the tendency of the saline group to wind-down over time. For an initial investigation, however, the results are clear. In previous experiments, a variety of stimulus frequencies (10 – 80 Hz) were delivered in the presence of buspirone, which has a long duration of action when given i.c. (Skinner *et al.*, 2002). However, in the presence of WAY-100635 this was problematic due to its short duration of action, and the fact that at least 1.5 min needed to elapse between each train of stimuli. If the drug is rapidly wearing off during a set of successive stimuli, the results become difficult to interpret. Therefore a single frequency of 40 Hz was chosen in this study.

SB-269970 also attenuated the absolute bradycardia evoked by phenylephrine injection, and flattened the slope, indicating a reduced gain or sensitivity of the reflex. This validates the aortic nerve experiments, and confirms that central 5-HT$_7$ receptors facilitate bradycardias evoked by baroreceptors, as well as chemoreceptors and cardiopulmonary afferents. As suspected from the aortic nerve experiments, there was no effect on baroreflex sympathoinhibition until 25 min post drug, which was not attenuated *per se,* but presented from potentiating. As for the cardiopulmonary reflex sympathoinhibition, the effect on this component is inconsistent with the effect on the cardioinhibitory limb, and raises several alternate possibilities, including a distant site of action, a different receptor, or a change in the stability of the preparation at this late stage in the protocol.

5-HT$_{1A}$ receptors

The role of the 5-HT$_{1A}$ receptor in the baroreflex is even more ambiguous than its role in the chemoreflex. Surprisingly, neither WAY-100635 nor (-)-pindolol significantly affected the aortic nerve-evoked responses. In the rabbit, WAY-100635 did inhibit baroreflex but not chemoreflex bradycardia (Skinner *et al.*, 2002) – an effect which at the time was attributed possibly to the different afferents activated

(aortic nerve afferents in the vagus and chemoreceptor afferents in the glossopharyngeal nerve), which might have different central circuitry and pharmacology. The present experiments suggest that if this is the case, there is a species difference from the rat, in which two different 5-HT_{1A} receptor antagonists are inactive at the baroreflex.

The pindolol group appears to be slightly attenuated: of 5 animals 1 had a strongly attenuated bradycardia at 5 min only, but the remaining 4 were unaffected. The lack of effect was particularly surprising as the same dose of (±)-pindolol has been reported to attenuate cardiopulmonary reflex bradycardia in anaesthetised rats (Bogle *et al.*, 1990), and the same dose of (-)-pindolol has been reported to attenuate smoke-induced bradycardia in anaesthetised rabbits (Dando *et al.*, 1998). In the study of Bogle *et al.* (1990), (±)-pindolol was the only antagonist used that did not bind to 5-HT_7 receptors, and in retrospect this drug provided the confirmation that the 5-HT_{1A} was involved. Subsequent to these present experiments, a brief investigation was made to reproduce the conditions in the study of Bogle *et al.* (1990), stimulating the cardiopulmonary reflex in spontaneously breathing anaesthetised rats. However, neither 100 μg kg^{-1} i.c. (±)-pindolol (n = 2), nor the same dose of (-)-pindolol (n = 2) had much effect on cardiopulmonary reflex bradycardias.

After the findings that chemoreflex bradycardias were attenuated by WAY-100635 only at 2 min, this protocol was tried on the aortic nerve-evoked baroreflex, but the attenuation was not as large as in the chemoreflex group, and not significant. Addition of a further 200 μg kg^{-1} i.c. WAY-100635 (20 min after the first dose) then did attenuate the bradycardia between 2 and 3 min after drug, and the hypotension between 2 and 5 min. At such high doses one cannot expect a selective response, therefore these data were treated as minor observations and not supported with a vehicle control. From these experiments, however, it is clear that the role of the 5-HT_{1A} receptor in cardiovascular reflexes is not at all clear in rats, and that WAY-100635 has different potencies at different reflexes, the aortic nerve-evoked baroreflex being the most resistant to attenuation by this drug.

3.4.8. Comparative receptor mechanisms

Species differences are regularly encountered in the serotonergic system. For example, in the mouse and rat 8-OH-DPAT induced hypothermia is WAY-100635 sensitive, therefore was originally thought to be 5-HT_{1A} receptor mediated (Forster *et al.*, 1995). On the other hand, in the guinea pig 5-CT induced hypothermia is sensitive to SB-269970 (Hagan *et al.*, 2000) and SB-656104 (Thomas *et al.*, 2003), suggesting that in this species the effect is 5-HT_7 receptor mediated. However, studies with 5-HT_7 receptor knockout mice have shown that 8-OH-DPAT acts on 5-HT_7 receptors at low doses, and at 5-HT_{1A} receptors at higher doses, both producing hypothermia (Hedlund *et al.*, 2004). In the guinea pig, activation of 5-HT_{1B} receptors can also cause hypothermia (Hagan *et al.*, 1997). Therefore what was previously thought to be a clear species difference of 5-HT_{1A} receptors in rats and 5-HT_7 receptors in guinea pigs, now appears to be a more complex picture of multiple receptor mechanisms, possibly with different emphasis in different species. With respect to the present experiments, there was an early speculation that in the rabbit, reflex bradycardias might be 5-HT_{1A} dependent, whilst in the rat the 5-HT_7 receptor is dominant. This will remain purely hypothetical until 5-HT_7 receptor effects are examined in other species, namely the rabbit and guinea pig. However, in the micturition reflex in the rat, the 5-HT_{1A} receptor is clearly a major player (Testa *et al.*, 1999; Conley *et al.*, 2001; Pehrson *et al.*, 2002), and this reflex is exquisitely sensitive to antagonists. WAY-100635 at doses as low as 1 $\mu g\ kg^{-1}$ i.c.v. inhibits micturition (Secker, 2004). This would suggest that 5-HT_{1A} receptors do have a major parasympathetic reflex role in rats. The pharmacology of reflex micturition and reflex bradycardia are comparable, as 5-HT_{1A} and 5-HT_7 receptors facilitate whereas 5-$HT_{1B/1D}$ receptors inhibit (Dando *et al.*, 1998; Read, 2004). In the rat, however, it would appear that the 5-HT_{1A} receptor is closely involved with micturition, but only a minor player in cardiovascular reflexes. One confounding factor in these experiments is that the micturition experiments in the rat (and indeed the cardiovascular reflex studies in the rabbit) were performed under urethane anaesthesia, whilst the rat cardiovascular experiments used α-chloralose. Therefore the different 5-HT_{1A} effects could be anaesthesia-related.

Both 5-HT_{1A} and 5-HT_7 mechanisms co-operate in micturition (Read *et al.*, 2003; Pehrson *et al.*, 2002) and hypothermia (Hedlund *et al.*, 2004). In the physiological context of micturition, blockade of either receptor inhibits the reflex. This suggests that they are located in series within the reflex arc, raising the question of how receptors with opposite effects on neuronal excitability (inhibition *vs.* excitation) can have the same effect. Some clues have come from hippocampal membranes, where 5-HT_7 receptors stimulate cAMP production, and 5-HT_{1A} receptors further fine tune (i.e. augment) this effect (Thomas *et al.*, 1999a). This raised the possibility of a population of 5-HT_{1A} receptors positively coupled to adenylate cyclase, but from that study using homogenised tissue it is uncertain whether the two receptors are located on the same neurone. In the cat nucleus ambiguus, 5-HT_{1A} receptors are believed to inhibit a tonic GABAergic inhibition of CVPNs (Wang & Ramage, 2001), causing their excitation indirectly. An important experiment would be to test whether functional 5-HT_7 receptors are located in the cat NTS, as this would illustrate the hypothesis that 5-HT_7 and 5-HT_{1A} receptors are in series (one in the sensory and one in the premotor nucleus). One must bear in mind that in the rat, 5-HT_{1A} receptors involved in micturition are located both supraspinally and in the sacral spinal cord (Secker, 2004), which is a comparable sensory and premotor distribution. It is likely that 5-HT_{1A} receptors are found in the nucleus ambiguus of all mammals, as systemic agonists produce bradycardia in a variety of species (see Ramage, 2001). Therefore, if species differences exist in the 5-HT receptors involved in cardiovascular reflexes, the NTS may be the site involved.

3.4.9. Technical limitations

The use of anaesthesia and neuromuscular blockade allows a high level of control over confounding variables such as animal movement, changes in ventilation and associated afferent information, and blood gases. Whilst every care was taken to keep animals as stable and physiologically normal as possible, anaesthesia is a major limitation, but this is addressed in the awake experiments of Chapter 6.

A serious potential limitation of these studies is the small group size. Groups of 5 are often used in these types of experiments, and pose no problem when an effect is clear cut. Indeed in such a case small groups should be encouraged to reduce the number of animals required. However, in more variable responses (such as reflex effects on

nerve activity) and more ambiguous effects (such as the 5-HT_{1A} receptor role), a higher number (e.g. 8 per group) would give a clearer result. Within the limited time frame of this project, larger group sizes would have meant reducing the scope of the study.

Another possible confounding factor is the use of multiple reflexes in Protocol 2. This has been done previously in this laboratory in the rabbit (Skinner *et al.*, 2002), and has the advantage of reducing the number of animals required for a study, as well as providing an internal control in the case of one drug having different actions on different reflexes. The problem is that the stimulation of one reflex might have an effect on the other, requiring further controls. In these experiments, the effect of WAY-100635 on aortic nerve and chemoreflex evoked changes (Protocol 2) was subsequently re-tested using Protocol 3, to confirm that the lack of effect on the chemoreflex was due to time, not due to the aortic nerve stimuli. Similarly, the effects of aortic nerve stimulation were similar in the presence and in the absence of chemoreflex stimluli. The effects of SB-269970 on the chemoreflex, however, were not confirmed in the absence of aortic nerve stimulation. The effects on the phenylephrine-evoked baroreflex were comparable to the effects on aortic nerve stimulation, suggesting that this latter effect was not influenced by the chemoreflex in the same protocol. Lastly, the effect on pindolol was tested on aortic nerve stimulation in the absence of chemoreflex stimulation, and as such is not entirely comparable to the effects of WAY-100635 (which was tested on both reflexes in the same protocol). As such, the mixing of different reflexes has been counterproductive in terms of reduction of animals, and would best be avoided in future.

3.4.10. Conclusion

In anaesthetised rats, the central 5-HT_7 receptor makes a major contribution to the activation of cardiac vagal outflow in response to a variety of peripheral stimuli, and these receptors are likely to be located in the medulla, possibly the NTS. Conversely, central 5-HT_{1A} receptors have a questionable role: the antagonist WAY-100635 has an effect on reflex bradycardias in the rank order of cardiopulmonary reflex > chemoreflex > baroreflex, but this is not supported by other antagonists, so the possibility of a non-selective (e.g. 5-HT_7 receptor mediated) or compound-specific effect cannot be excluded. In terms of sympathetic components, 5-HT_7 receptors are

clearly involved in chemoreflex sympathoexcitation, but sympathoinhibitions in response to other reflexes are ambiguous.

There are various clinical implications, as a number of commonly used drugs bind to these receptors. In particular, antipsychotic medications such as clozapine bind to 5-HT_7 receptors, and whether the cardiovascular side-effects of such compounds relate to actions on reflexes is an interesting question. Changes in the efficacy of serotonergic neurotransmission may be involved in depressive disorders, and most commonly used antidepressants aim to restore this hypothetical functional deficit. In either case, reuptake inhibitors potentiate the action of endogenous 5-HT, so possible changes in cardiovascular reflex efficacy might be expected either in depression, or during antidepressant administration, or both. An interesting finding is that patients with depression have lower heart rate variability (Tulen *et al.,* 1996; Stein *et al.*, 2000) and increased mortality after cardiac surgery than non-depressed patients (Baker *et al.*, 2001; Blumenthal *et al.*, 2003).

A major remaining question is which neurones are releasing 5-HT onto 5-HT_7 and 5-HT_{1A} receptors during these reflexes. This will be addressed during the following 2 chapters.

3.4.11. Future experiments

The most important follow-up confirmation required for these experiments is of the mixed effects of 5-HT_{1A} receptor antagonists. This would best be approached using an old and a new antagonist (e.g. pindolol and robalzotan), given in several doses, and tested only against highly stable vehicle controls. The effects of 5-HT_7 receptor antagonists on reflex sympathetic activity should also be further evaluated, including the effect on sympathoexcitation evoked by stimulation of the upper airways with smoke, or by baroreceptor unloading with sodium nitroprusside. In such a study, a more detailed and elegant method of analysing reflex changes in sympathetic outflow could be used (Scheuer & Mifflin, 2001). A vital observation would also be the effect of these new 5-HT_7 antagonists on cardiovascular reflexes in other species, particularly the guinea pig and the rabbit.

4. THE CONTRIBUTION OF 5-HT CONTAINING CELL GROUPS TO AUTONOMIC BASELINES AND REFLEXES

4.1. Introduction

4.1.1. Background

Many brainstem cell groups involved in cardiovascular regulation are innervated by 5-HT containing fibres. The NTS in particular receives a dense innervation. In the cat this consists of unmyelinated fibres with both varicosities and specialised synapses (Maley & Elde, 1982). The origin of these fibres is unclear, although some are likely to be 5-HT containing vagal afferents (Sykes *et al.*, 1994) from the nodose ganglion (Gaudin-Chazal *et al.*, 1982; Nosjean *et al.*, 1990) or other peripheral sources. From retrograde tracing it would appear that a further source is neurones of raphe magnus and dorsal raphe (Schaffar *et al.*, 1988). Thirdly it has been suggested that interneurones within the NTS contain 5-HT (Calza *et al.*, 1985). Whether or not these fibres serve a role in cardiovascular regulation is also not clear, but the growing body of evidence identifying roles for various 5-HT receptor subtypes in the NTS in cardiovascular control suggests that these are the fibres releasing 5-HT onto the relevant NTS neurones. Other autonomic cell groups receive a similar innervation: retrograde viral tracing discloses a projection from the caudal raphe to the DVN and nucleus ambiguus (Hadziefendic & Haxhiu, 1999). Such anatomical connections are not necessarily functional. The medullary raphe, however, are the cell groups of interest to this study, since they are reported to have predominantly descending projections, and a variety of functions which have been discussed in 1.5.1 above.

4.1.2. Aims of the study

The previous chapter suggests that 5-HT is released during cardiovascular reflexes. The aim of this study is to investigate the origin of this 5-HT. This took the form of an initial mapping study of the medullary raphe, paying particular attention to cardiac vagal outflow, but also monitoring central respiratory drive and sympathetic tone. Chemical and electrical stimulation were used to identify areas that mediate changes in baselines resembling autonomic reflexes. Particular areas of interest were then

further characterised by attempting to block the responses using 5-HT receptor antagonists. Furthermore some animals were depleted of 5-HT to confirm firstly the findings of the antagonist study, and secondly that endogenous 5-HT is required for successful reflex function.

4.2. Methods

4.2.1. Protocols

Raphe mapping study

Distinct areas of the medullary midline were serially stimulated with reference to the calamus scriptorius (rostrocaudal axis) and the floor of the IV^{th} ventricle (dorsoventral axis), using the stereotaxic deliniations of Paxinos and Watson (1998). In each animal up to four electrode penetrations were performed on the midline, between 0.5 and 4 mm rostral, and between 1 and 3 mm ventral. Individual stimulations were separated by 0.5 – 1 mm in the dorsoventral axis, and by 1 mm in the rostrocaudal axis. 10 min was allowed to elapse between chemical stimulations, and 5 min between electrical stimulations, to prevent tachyphylaxis, depolarisation block, or excitotoxic damage. In some experiments stimulations were made 1 mm lateral to the midline to control for the effect of drug diffusion or current spread. The most ventral point on any penetration was marked by depositing 50 nl pontamine sky blue dye for subsequent histological verification.

Pharmacology

When an area of interest had been characterised in the mapping study, the effects of 5-HT receptor antagonists on the responses to stimulating this area with DLH were investigated. The following antagonists were used: methiothepin (broad spectrum 5-HT receptor antagonist; (Monachon *et al.*, 1972) 1 & 3 mg kg^{-1}), granisetron (selective 5-HT_3 receptor antagonist; (Bermudez *et al.*, 1988) 0.3 mg kg^{-1}) and SB-204070 (selective 5-HT_4 receptor antagonist; (Wardle *et al.*, 1994) 3 mg kg^{-1}). Since the location of 5-HT release caused by raphe stimulation was not known, antagonists were given i.v., and comparisons were made between drug and saline pretreated groups.

Only one raphe area was stimulated in each experiment. After micropipette placement, atenolol (1 mg kg^{-1} i.v.) and α-chloralose (15 mg kg^{-1} i.v.) were given. 10 min were allowed to elapse, then control microinjections were commenced (2.5 nmol DLH) every 10 min. 5 min after the third control, the test solution was given i.v. in a volume of 1 ml kg^{-1}, and a further microinjection performed at 5 min after injection.

Depletion of 5-HT stores

In a further set of experiments, the effect of depleting 5-HT stores with p-CPA were investigated on raphe-evoked responses and cardiovascular reflexes. A detailed protocol is shown in Figure 4.1. Serial microinjections were made at 6 distinct locations in the raphe, separated by 10 min. At the beginning and the end of the protocol, baroreflex sensitivity was tested by giving 10 μg phenylephrine i.v., and cardiopulmonary reflex sensitivity was tested by giving 2.5 μg PBG into the right atrium.

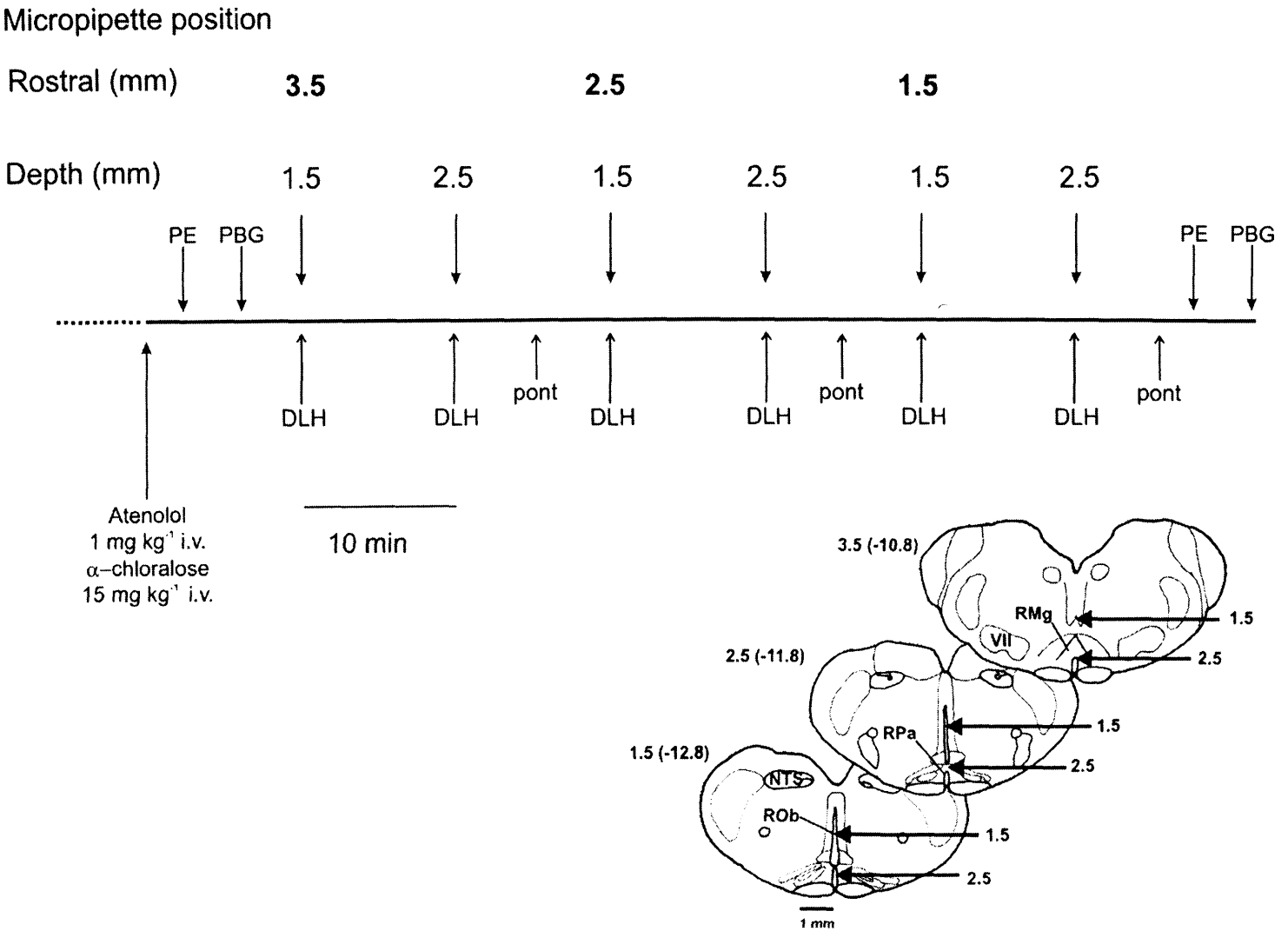
Figure 4.1 Microinjection protocol for depletion study

Diagram of protocol for stimulating areas of medullary raphe at various positions relative to calamus scriptorius (rostral) and floor of the IV^{th} ventricle (depth) with DLH (2.5 nmol) in p-CPA or saline treated rats.

PE: phenylephrine 10 μg i.v.
PBG: phenylbiguanide 2.5 μg intra-atrial
pont: pontamine sky blue (50 nl).

Inset: coronal diagrams of medulla illustrating sites of microinjection (relative to bregma in parentheses).

NTS: nucleus tractus solitarius
ROb: raphe obscurus
RPa: raphe pallidus
RMg: raphe magnus
VII: facial nucleus.


Micropipette position
Rostral (mm)
3.5
2.5
1.5
Depth (mm)
1.5
2.5
1.5
2.5
1.5
2.5
PE
PBG
PE
PBG
DLH
DLH
pont
DLH
DLH
pont
DLH
DLH
pont
Atenolol
1 mg kg-1 i.v.
α–chloralose
15 mg kg-1 i.v.
10 min
3.5 (-10.8)
RMg
VII
1.5
2.5
2.5 (-11.8)
RPa
1.5
2.5
1.5 (-12.8)
NTS
ROb
1.5
2.5
1 mm

4.3. Results

4.3.1. Chemical stimulation of medullary raphe

Baseline variables

Microinjections were performed in anaesthetised, neuromuscular blocked and atenolol-pretreated rats (n = 12). Baselines at the beginning of the protocol were a MAP of 118 ± 2 mmHg and a HR of 362 ± 10 bpm.

1.5 mm rostral (Bregma A -12.8, L 0, D 10.1-10.6)

Microinjection of DLH (2.5 nmol in 50 nl) into parts of raphe obscurus and pallidus 1.5 mm rostral to calamus scriptorius evoked the following changes: at 2 mm depth (n = 6) DLH significantly reduced baseline IRNA (-45 ± 8 %) and increased baseline IPNA (+116 ± 21 %), with no effect on baseline MAP or HR (Figures 4.2 and 4.3, Table 9.36).

At 2.5 mm depth (n = 5) DLH significantly reduced baseline IRNA (-40 ± 8 %) and increased baseline IPNA (+123 ± 46 %), with no effect on baseline MAP or HR.

Microinjection of saline at depth 2.5 mm (50 nl, pH 7.4; n = 3) had no observable effect on any variable (Table 9.36).

Lateral control microinjections of DLH (into gigantocellular reticular nucleus, Figure 4.3; n = 3) had no significant effect on any variable (Table 9.36).

2.5 mm rostral (Bregma A -11.8, M 0, V 9.3-10.3)

Microinjection of DLH (2.5 nmol in 50 nl) into parts of raphe obscurus and pallidus 2.5 mm rostral to calamus scriptorius evoked the following changes: it significantly reduced baseline IRNA at all 3 depths tested: -35 ± 3 % at 1.5 mm depth (n = 9), -29 ± 8 % at 2 mm depth (n = 7), and -20 ± 9 % at 2.5 mm depth (n = 8). It had no significant effects on baseline MAP, HR, or IPNA at any depth (Figures 4.4 and 4.5, Table 9.37).

Lateral control microinjections of DLH (into gigantocellular reticular nucleus, Figure 4.5, Table 9.37; n = 4) significantly raised baseline MAP by 22 ± 3 mmHg (126 ± 5

to 149 ± 8 mmHg), and baseline IRNA (+36 ± 3 %), with no significant effect on baseline HR or IPNA.

3.5 mm rostral (Bregma A -10.8, L 0, V 10.0-10.5)

Microinjection of DLH (2.5 nmol in 50 nl) into parts of raphe magnus and pallidus 2.5 mm rostral to calamus scriptorius evoked the following changes: at 2 mm depth (n = 6) it significantly reduced baseline HR by 30 ± 7 bpm (367 ± 16 to 340 ± 15 bpm), increased baseline IRNA (+31 ± 6 %) and reduced baseline IPNA (-53 ± 7 %), with no significant effects on baseline MAP (Figures 4.6 and 4.7, Table 9.38).

At 2.5 mm depth (n = 7) DLH significantly reduced baseline HR by 42 ± 6 bpm (378 ± 10 to 338 ± 8 bpm), increased baseline IRNA (+46 ± 6 %), and decreased baseline IPNA (-60 ± 6 %), with no significant effects on baseline MAP. Stimulation of the corresponding area 1mm further rostral (4.5 mm rostral to calamus scriptorius) consistently had no effect on any variable.

Microinjection of pontamine sky blue (50 nl, pH 7.4; n = 7) at depth 2.5 mm had no significant effect on any variable. The histological appearance of such a pontamine deposit is shown in Figure 4.9.

Lateral control microinjections of DLH (into gigantocellular reticular nucleus, Figure 4.7; n = 5) significantly increased baseline MAP by 13 ± 1 mmHg (127 ± 5 to 139 ± 6 mmHg), increased baseline IRNA (+33 ± 10 %), and decreased baseline IPNA (-47 ± 8 %), with no significant effect on baseline HR.

4.3.2. Electrical stimulation of medullary raphe

Electrical stimulation of the medullary raphe *via* a Wood's metal/indium filled micropipette was attempted using various protocols (0.1 ms pulses at 10 – 50 Hz, 50 – 100 μA, 3 – 15 s). The results of these stimulations were highly inconsistent and have not been quantified. In general, stimulation of raphe obscurus produced dramatic pressor or depressor responses with a high degree of variability between experiments. These effects were frequency dependent, with the greatest response at 50 Hz, and no effect at 10 Hz. Occasionally a response was seen that resembled that evoked by chemical stimulation, as illustrated in Figure 4.8.

Figure 4.2 Raphe obscurus stimulation trace: 1.5 mm rostral

Experimental trace illustrating effect of microinjecting DLH into a part of raphe obscurus (1.5 mm rostral, 1.5 mm depth) on baseline BP, HR, integrated renal (IRNA) and phrenic (IPNA) nerve activity in an anaesthetised, neuromuscular blocked and atenolol pretreated rat.

Nerve activities are presented as rectified and smoothed neurograms (time constants: IRNA 1 s, IPNA 0.1 s) as well as in 5 s bins (arbitrary units).

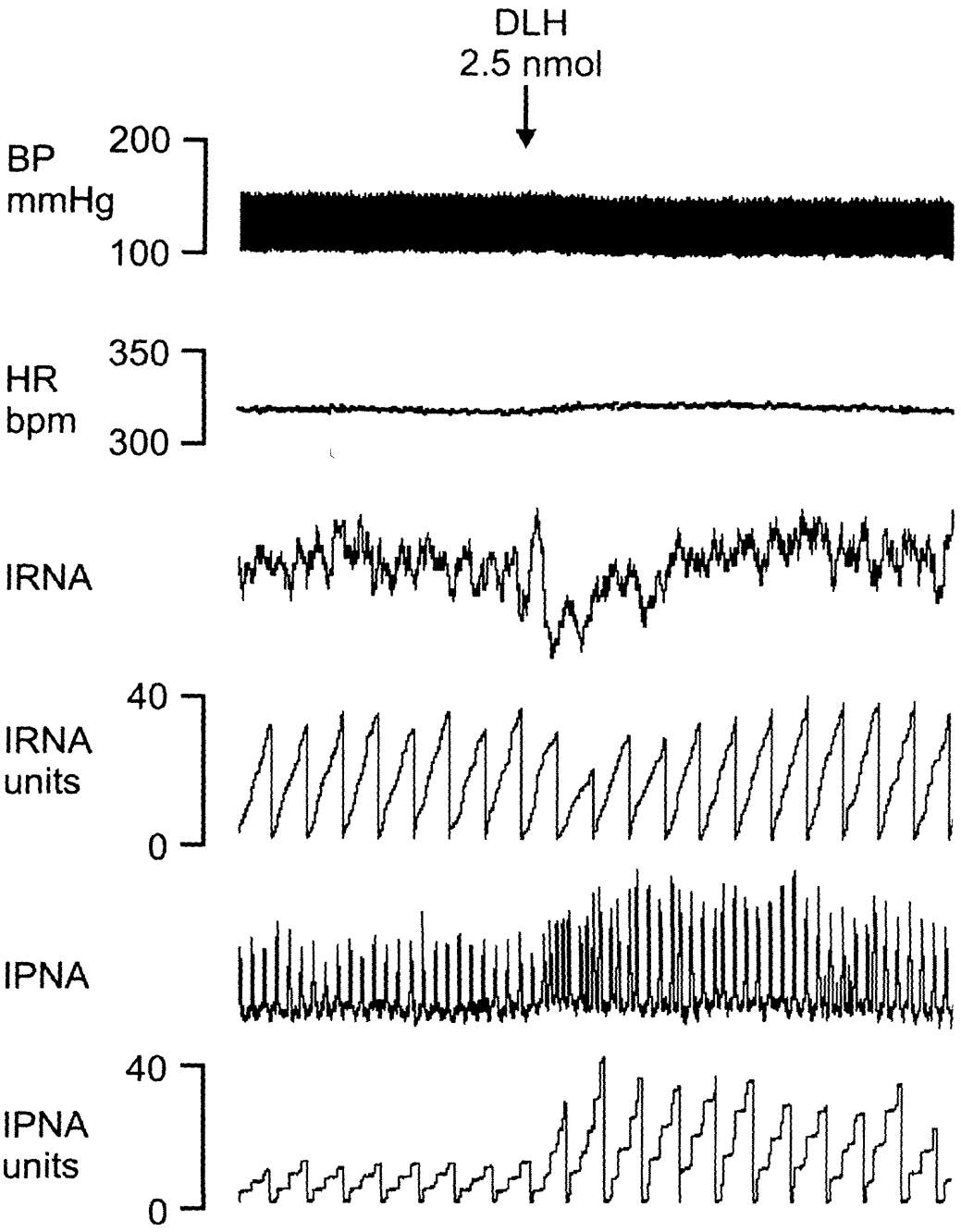
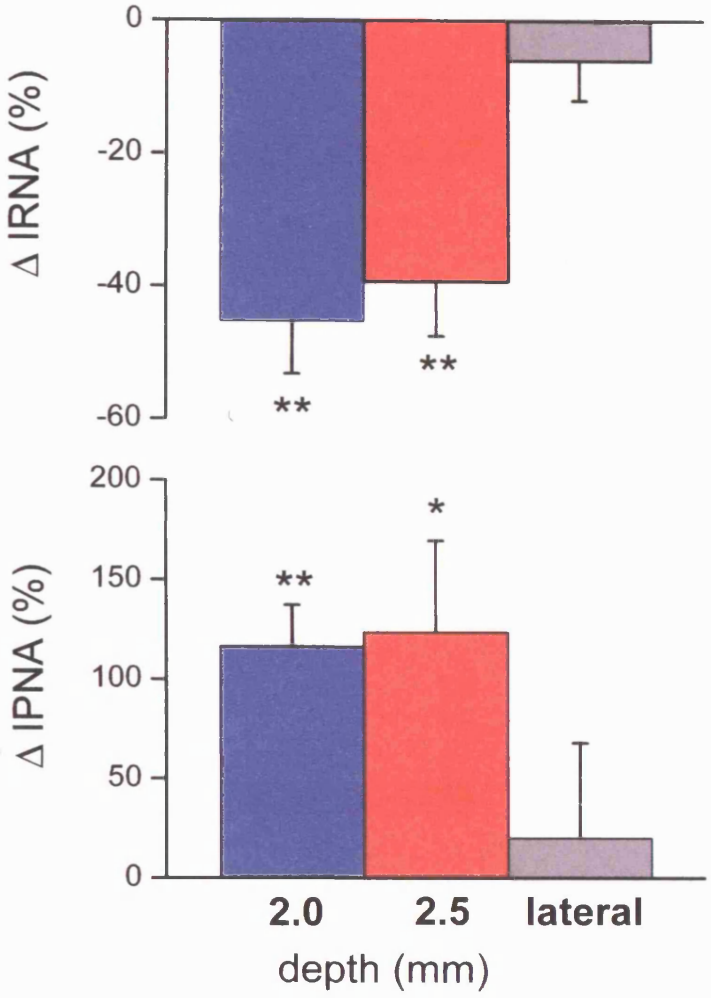

DLH
2.5 nmol
BP
mmHg
200
100
HR
bpm
350
300
IRNA
IRNA
units
40
0
IPNA
IPNA
units
40
0

Figure 4.3 Raphe stimulation histogram: 1.5 mm rostral

Top: Histogram of mean (± s.e.m.) changes in baseline integrated renal nerve (IRNA) and phrenic nerve (IPNA) activity evoked by microinjection of DLH into raphe 1.5 mm rostral and at 1.5 and 2.5 mm depth (n = 6, 5 respectively), and lateral control microinjection (n = 3) in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

Bottom: Coronal diagram of rat medulla illustrating position of microinjections reconstructed from pontamine sky blue deposits


0
-20
-40
-60
Δ IRNA (%)
**
**
200
150
100
50
0
Δ IPNA (%)
**
*
2.0
2.5
lateral
depth (mm)

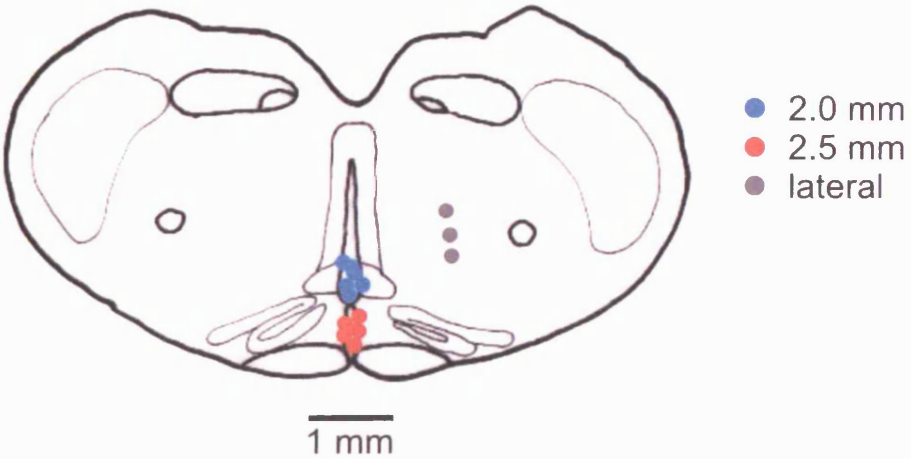

2.0 mm
2.5 mm
lateral
1 mm

Figure 4.4 Raphe pallidus stimulation trace: 2.5 mm rostral

Experimental trace illustrating effect of microinjecting DLH into a part of raphe pallidus (2.5 mm rostral, 2.5 mm depth) on baseline BP, HR, integrated renal (IRNA) and phrenic (IPNA) nerve activity in an anaesthetised, neuromuscular blocked and atenolol pretreated rat.

Nerve activities are presented as rectified and smoothed neurograms (time constants: IRNA 1 s, IPNA 0.1 s) as well as in 5 s bins (arbitrary units).

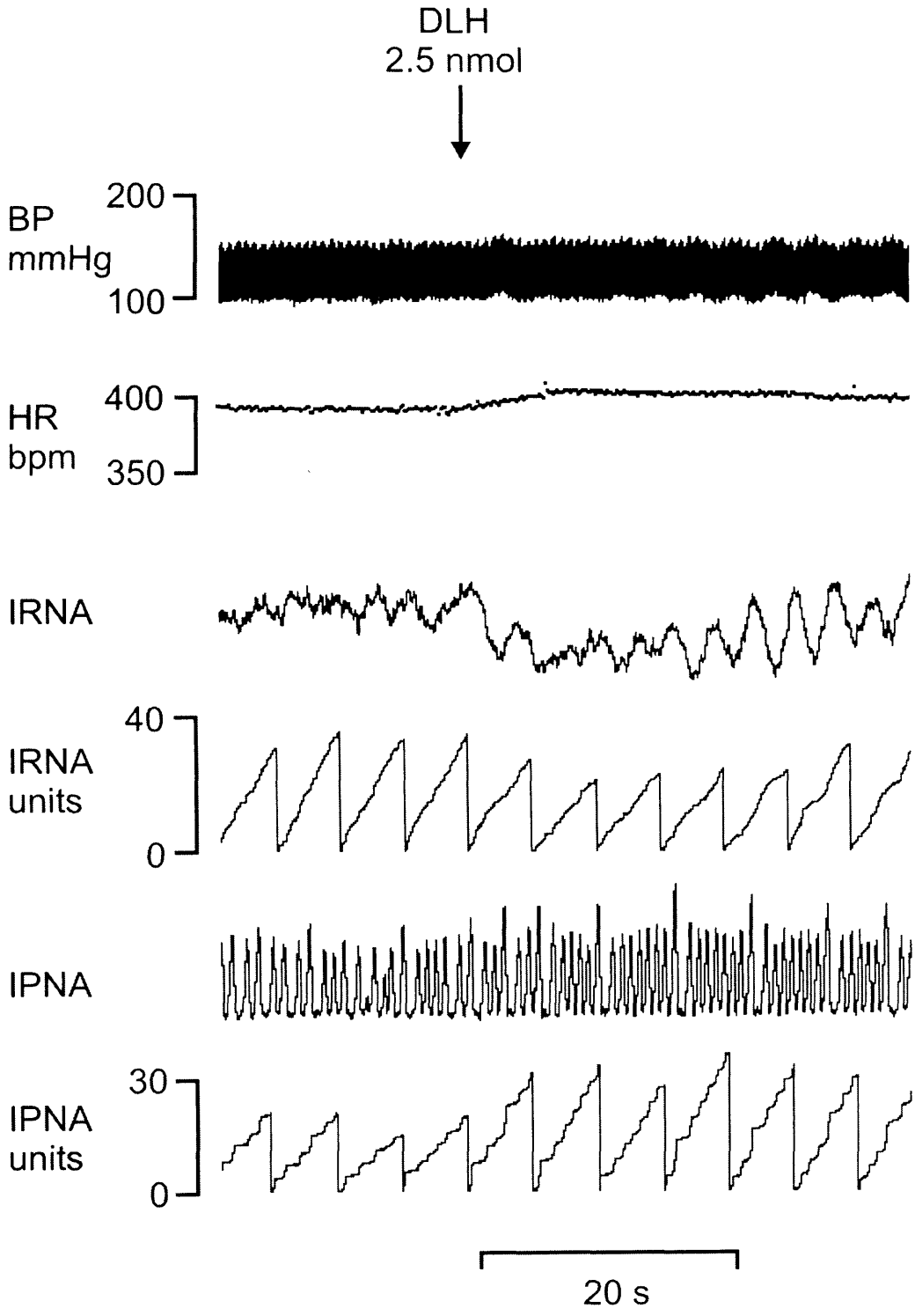
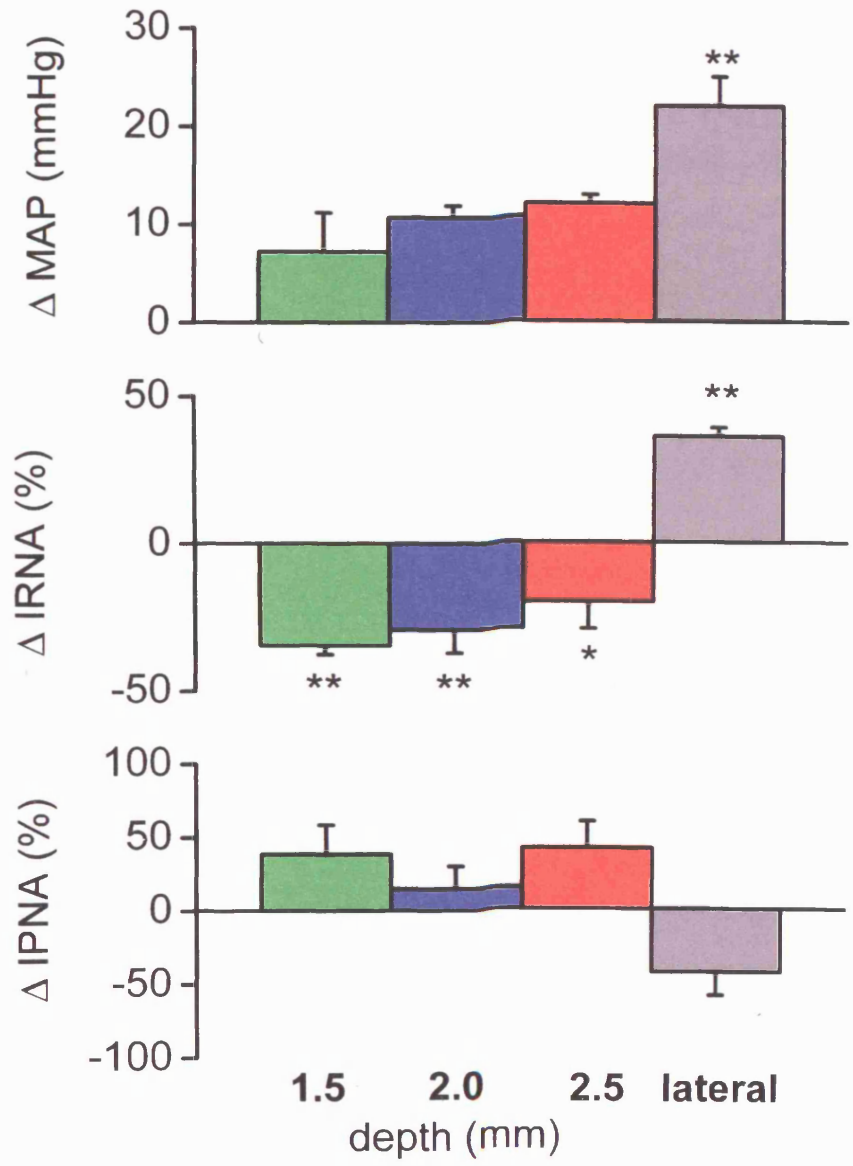

DLH
2.5 nmol
BP
mmHg
200
100
HR
bpm
400
350
IRNA
IRNA
units
40
0
IPNA
IPNA
units
30
0
20 s

Figure 4.5 Raphe stimulation histogram: 2.5 mm rostral

Top: Histogram of mean (± s.e.m.) changes in baseline IRNA and IPNA evoked by microinjection of DLH into raphe 2.5 mm rostral and at 1.5, 2.0 and 2.5 mm depth (n = 9, 7, 8 respectively), and lateral control microinjection (n = 4) in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

Bottom: Coronal diagram of rat medulla illustrating position of microinjections reconstructed from pontamine sky blue deposits.


Δ MAP (mmHg)
30
20
10
0
**
Δ IRNA (%)
50
0
-50
**
**
*
**
Δ IPNA (%)
100
50
0
-50
-100
1.5
2.0
2.5
lateral
depth (mm)

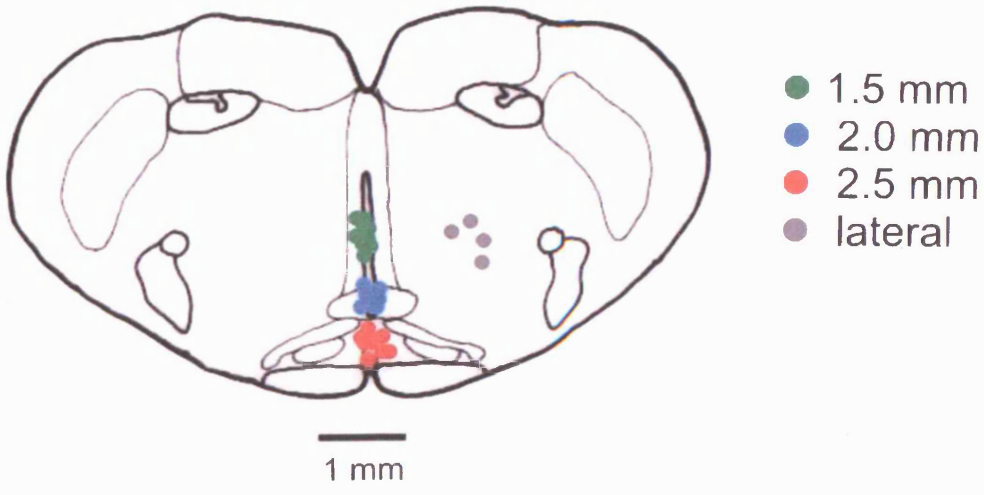

1.5 mm
2.0 mm
2.5 mm
lateral
1 mm

Figure 4.6 Raphe magnus stimulation trace: 3.5 mm rostral

Experimental trace illustrating effect of microinjecting DLH into a part of raphe magnus/pallidus (3.5 mm rostral, 2.5 mm depth) on baseline BP, HR, integrated renal (IRNA) and phrenic (IPNA) nerve activity in an anaesthetised, neuromuscular blocked and atenolol pretreated rat.

Nerve activities are presented as rectified and smoothed neurograms (time constants: IRNA 1 s, IPNA 0.1 s) as well as in 5 s bins (arbitrary units).

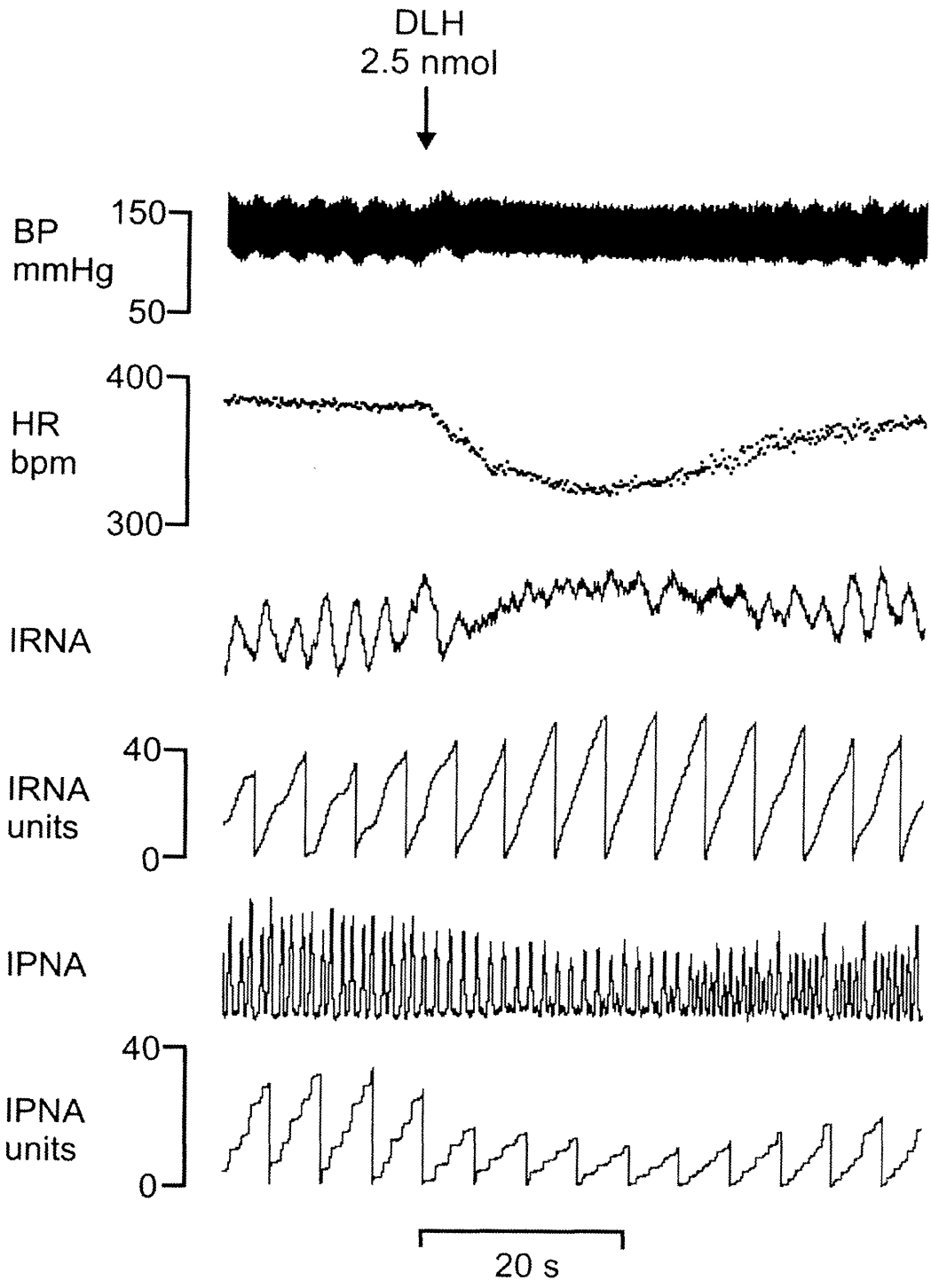
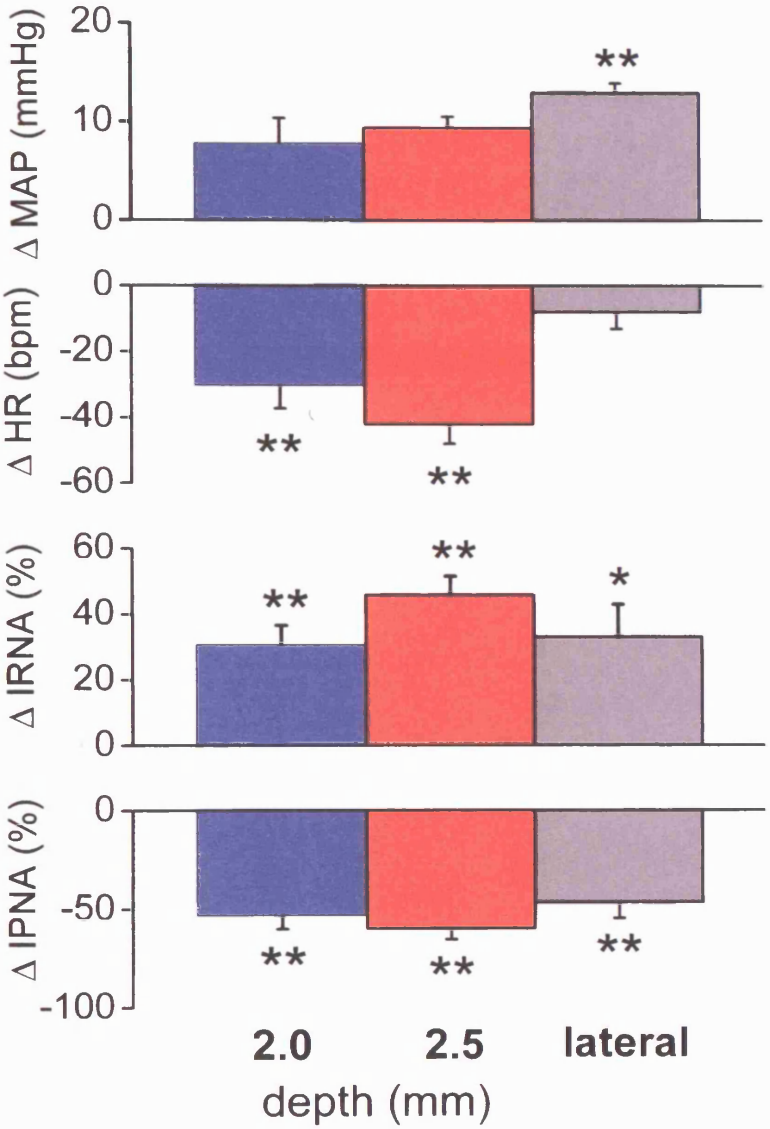

DLH
2.5 nmol
BP
mmHg
150
50
HR
bpm
400
300
IRNA
IRNA
units
40
0
IPNA
IPNA
units
40
0
20 s

Figure 4.7 Raphe stimulation histogram: 3.5 mm rostral

Top: Histogram of mean (± s.e.m.) changes in baseline IRNA and IPNA evoked by microinjection of DLH into raphe 3.5 mm rostral and at 2.0 and 2.5 mm depth (n = 6, 7 respectively), and lateral control microinjection (n = 5) in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

Bottom: Coronal diagram of rat medulla illustrating position of microinjections reconstructed from pontamine sky blue deposits, including areas where DLH had no effect on any variable.


Δ MAP (mmHg)
20
10
0
**
Δ HR (bpm)
0
-20
-40
-60
**
**
Δ IRNA (%)
60
40
20
0
**
**
*
Δ IPNA (%)
0
-50
-100
**
**
**
2.0
2.5
lateral
depth (mm)

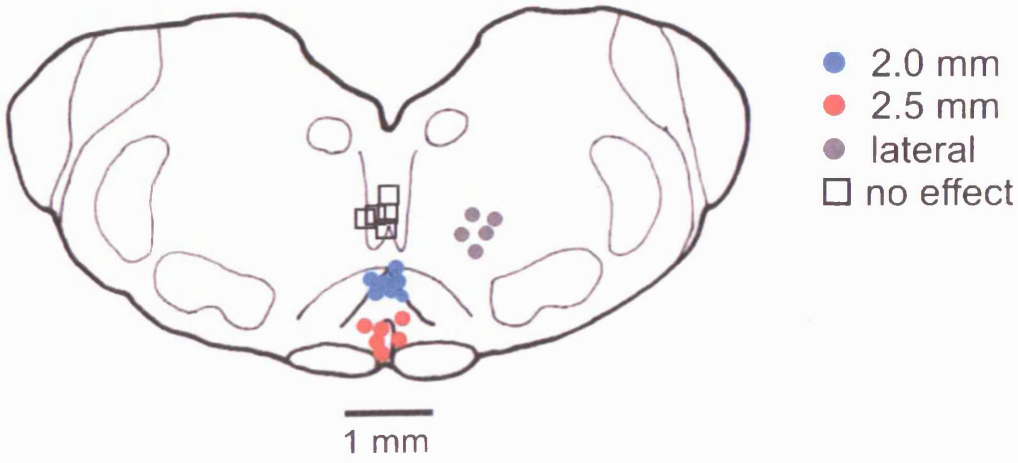

2.0 mm
2.5 mm
lateral
no effect
1 mm

Figure 4.8 Electrical stimulation of raphe magnus

Experimental trace illustrating effect of electrical stimulation of raphe magnus/pallidus (3.5 mm rostral, 2.5 mm depth) at 10 – 30 Hz (0.1 ms pulses, 50 μA, 5 s train) as well as chemical stimulation (DLH) on baseline HR, BP and IRNA in an anaesthetised, neuromuscular blocked and atenolol-pretreated rat.

IRNA is rectified and smoothed (1 s time constant).

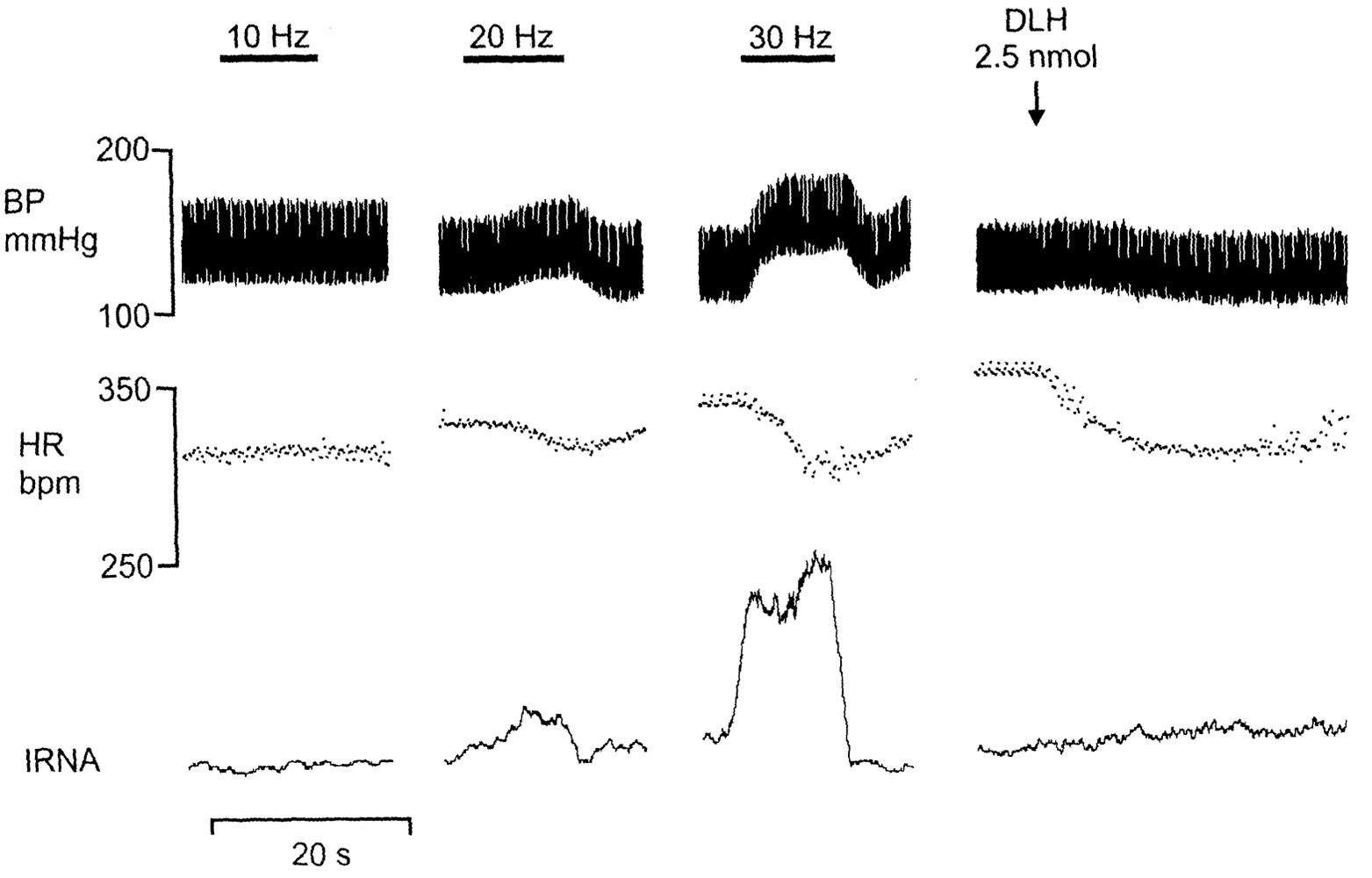

10 Hz
20 Hz
30 Hz
DLH
2.5 nmol
200
BP
mmHg
100
350
HR
bpm
250
IRNA
20 s

Figure 4.9 Histological verification of microinjection

Micrographs of coronal sections of rat medulla (3.5 mm rostral to calamus scriptorius) counterstained with neutral red, illustrating location of a 50 nl deposit of pontamine sky blue after chemical stimulation with DLH.

Left: Gross appearance (scale bar = 1 mm)

RMg: raphe magnus

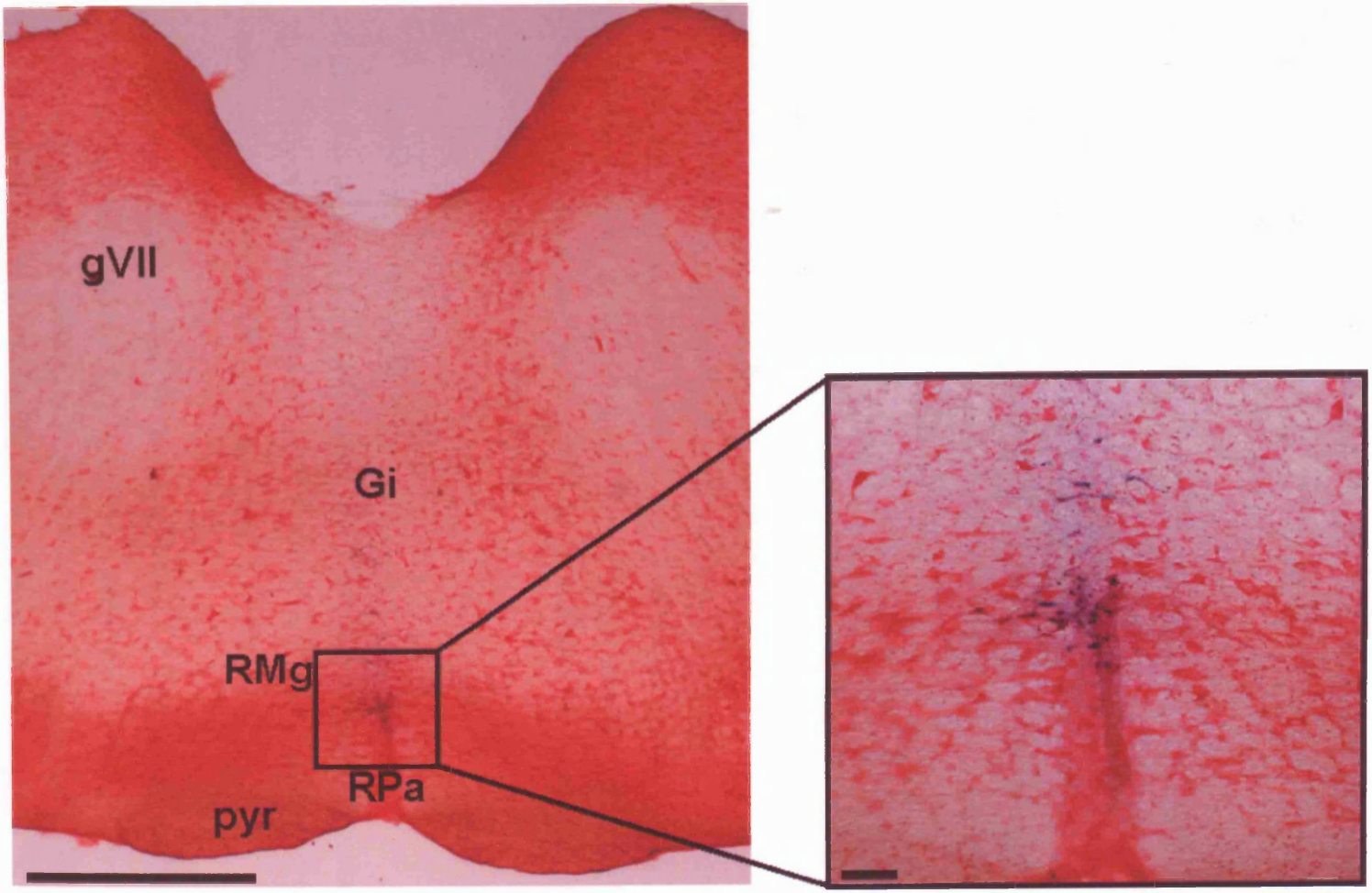
RPa: raphe pallidus

pyr: pyramid

Gi: gigantocellular reticular nucleus

gVII: genu of the VIIth nerve

Right: Magnification of boxed area (scale bar = 100 μm)


gVII
Gi
RMg
RPa
pyr

4.3.3. Effects of 5-HT receptor antagonists on baselines

Intravenous injection of the 5-HT receptor antagonists methiothepin (1 and 3 mg kg^{-1}; n = 5, 4), granisetron (0.3 mg kg^{-1}; n = 2) and SB-204070 (3 mg kg^{-1}; n = 2) had no effect on baseline MAP, HR, IRNA or IPNA when compared to i.v. saline, with one exception: HR in animals given 3 mg kg^{-1} methiothepin was significantly lower than in those given saline, although the pre-drug control HR was also significantly lower than the pre-saline controls (Table 9.39).

4.3.4. Effects of 5-HT receptor antagonists on raphe-evoked changes

To investigate the role of 5-HT receptors in the triad of responses (bradycardia, renal sympathoexcitation, phrenic inhibition) to raphe magnus/pallidus stimulation (Bregma A -10.8, L 0, V 10.5), various 5-HT receptor antagonists were given i.v. and compared to time-matched i.v. saline controls.

Saline (1 ml kg^{-1} i.v.)

Microinjection of DLH (2.5 nmol in 50 nl) into raphe magnus/pallidus evoked an increase in MAP of 5 ± 4 mmHg from a baseline of 122 ± 5 mmHg, a bradycardia of 29 ± 5 bpm from a baseline of 345 ± 4 bpm , a renal sympathoinhibition of +101 ± 29 %, and a phrenic inhibition of -81 ± 5 %. Intravenous administration of saline (1 ml kg^{-1}; n = 5) had no significant effects on DLH-evoked responses 5 min after administration (Figure 4.11, Table 9.40).

Methiothepin (1 & 3 mg kg^{-1} i.v.)

Intravenous injection of the broad-spectrum 5-HT receptor antagonist methiothepin (1 mg kg^{-1}; n = 5) had no significant effect on DLH-evoked responses 5 min after administration, compared to i.v. saline (Figure 4.11. Table 9.40).

Intravenous injection of 3 mg kg^{-1} methiothepin (n = 4) significantly potentiated the renal sympathoexcitation evoked by DLH microinjection (+355 ± 100 % *vs.* +80 ± 29 %, 5 min after administration, Figures 4.10 and 4.11, Table 9.40). Methiothepin had no significant effects on other DLH-evoked responses.

Granisetron (0.3 mg kg^{-1} i.v.)

Intravenous injection of the selective 5-HT_3 receptor antagonist granisetron (0.3 mg kg^{-1}; $n = 2$) had no effect on DLH-evoked responses (Table 9.40).

SB-204070 (3 mg kg^{-1} i.v.)

Intravenous injection of the selective 5-HT_4 receptor antagonist SB-204070 (3 mg kg^{-1}; $n = 2$) had no effect on DLH-evoked responses (Table 9.40).

Figure 4.10 Raphe magnus trace: effects of methiothepin

Experimental trace illustrating effects of microinjecting DLH into raphe magnus/pallidus (3.5 mm rostral and 2.5 mm depth) on baseline HR, BP, IRNA and IPNA before (control) and 5 min after methiothepin (3 mg kg^{-1} i.v.) in an anaesthetised, neuromuscular blocked and atenolol pretreated rat.

Neurograms are rectified and smoothed (time constants: IRNA 1 s, IPNA 0.1 s)

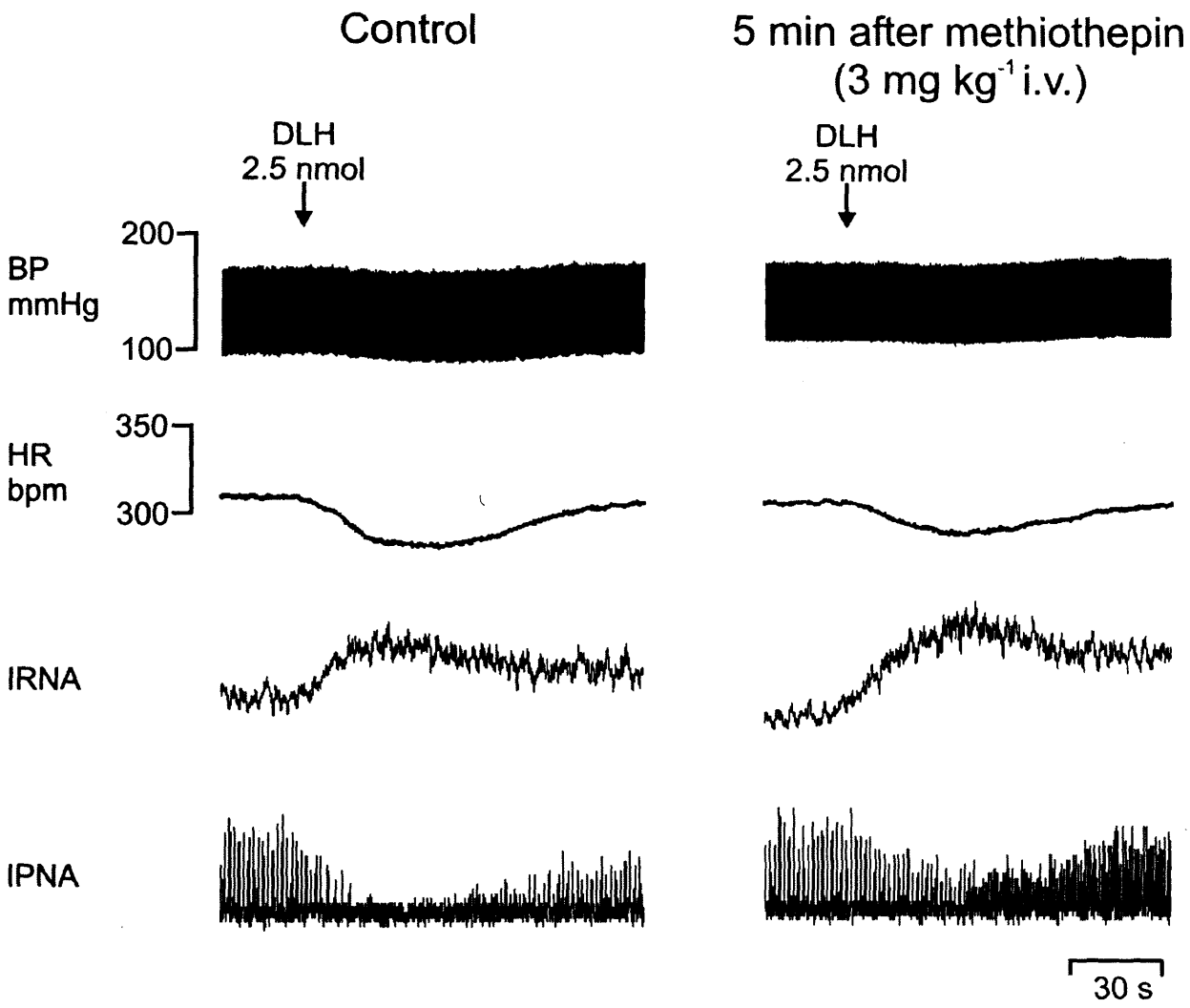
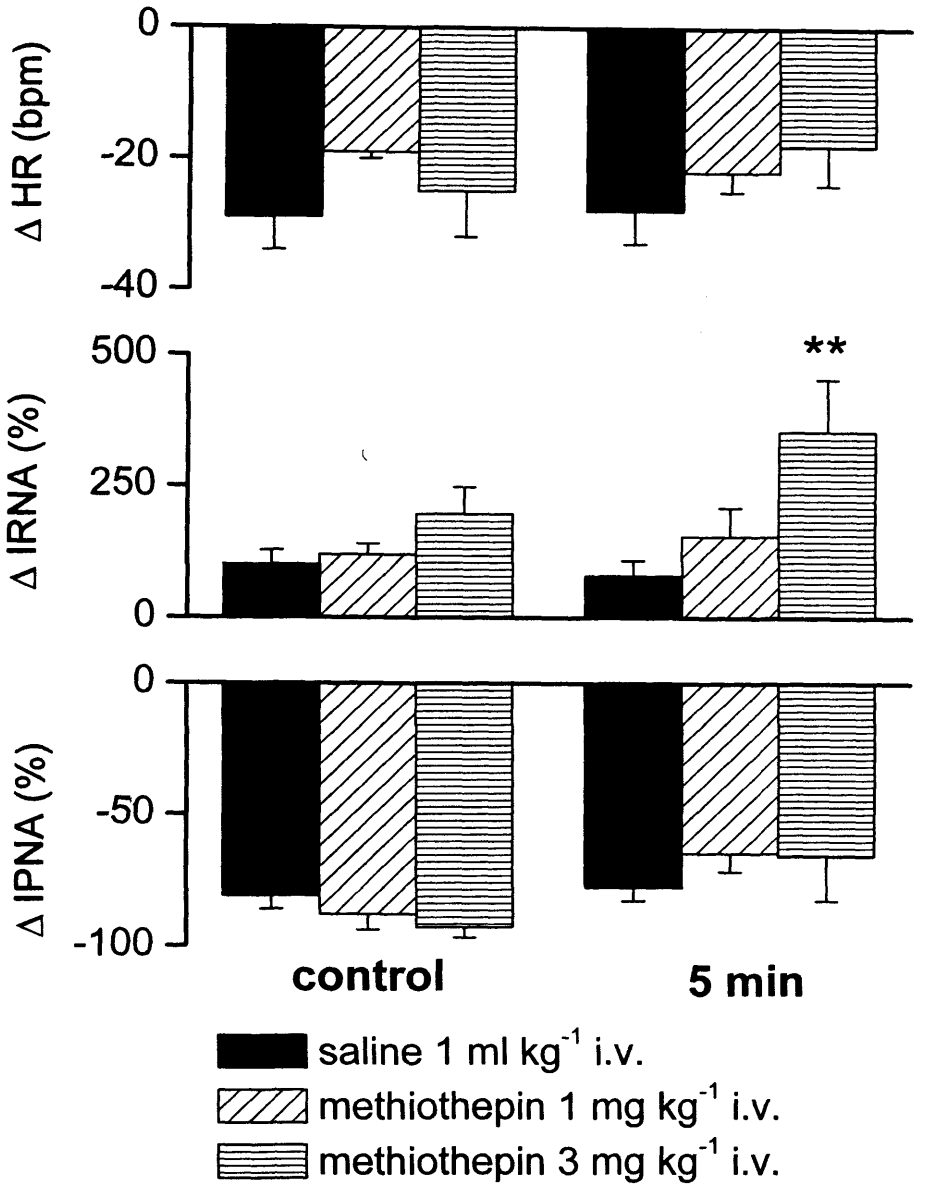

Control
5 min after methiothepin
(3 mg kg-1 i.v.)
DLH
2.5 nmol
DLH
2.5 nmol
200
BP
mmHg
100
350
HR
bpm
300
IRNA
IPNA
30 s

Figure 4.11 Raphe magnus histogram: effects of methiothepin

Histogram of mean (± s.e.m.) changes (Δ) in baseline HR, IRNA and IPNA evoked by microinjection of DLH into raphe magnus/pallidus before (control) and 5 min after i.v. saline (n = 5), 1 mg kg^{-1} methiothepin (n = 5) and 3 mg kg^{-1} methiothepin (n = 4) in anaesthetised, neuromuscular blocked and atenolol pretreated rats.

** $P < 0.01$ (relative to saline, 2-way ANOVA followed by LSD test).


0
-20
-40
Δ HR (bpm)
500
250
0
Δ IRNA (%)
**
0
-50
-100
Δ IPNA (%)
control
5 min
saline 1 ml kg-1 i.v.
methiothepin 1 mg kg-1 i.v.
methiothepin 3 mg kg-1 i.v.

4.3.5. Effects of 5-HT depletion

Baseline variables

Rats pretreated with p-CPA (350 mg kg^{-1} i.p on 2 consecutive days; n = 7) had a significantly lower baseline MAP (86 ± 7 *vs.* 107 ± 5 mmHg) than rats pretreated with saline (3.5 ml kg^{-1} i.p.; n = 7). Baseline HR was unchanged (321 ± 6 *vs.* 311 ± 5 bpm; Table 9.42) . p-CPA pretreated animals did not show a marked behavioural difference from controls, although a few were more resistant to handling.

Cardiopulmonary reflex

Intra-atrial injection of a bolus dose of PBG (2.5 μg in 50 μl) evoked a significantly smaller increase in R-R interval in p-CPA pretreated rats compared to controls (38 ± 7 *vs.* 130 ± 31 ms; n = 7, Figure 4.12, Table 9.42). The PBG-evoked hypotensions, however, were not significantly different (-27 ± 4 *vs.* -31 ± 6 mmHg).

Baroreflex gain

Intravenous administration of a bolus dose of phenylephrine (10 μg) evoked an increase in MAP of 77 ± 7 mmHg (from a baseline of 86 ± 8 mmHg; n = 5) in p-CPA treated animals, compared to an increase of 72 ± 6 mmHg (from a baseline of 113 ± 2 mmHg; n = 5) in saline treated animals (Table 9.42). This hypertension was associated with an increase in R-R interval of 55 ± 12 ms in p-CPA treated animals and 82 ± 12 ms in saline treated animals. Neither the hypertensions nor the changes in R-R interval were statistically different. Analysis of the baroreflex gain, however, revealed that the baroreflex slope was significantly attenuated in the p-CPA group (0.19 ± 0.05 *vs.* 0.66 ± 0.07 ms $mmHg^{-1}$; Figure 4.14). The correlation coefficients of MAP to R-R interval, and the rates of change of MAP, were not significantly different in p-CPA and saline groups (Table 9.42).

Raphe stimulation

3 micropipette penetrations were made in the medulla, at 1.5, 2.5 and 3.5 mm rostral to calamus scriptorius (-12.8, -11.8 and -10.8 mm from bregma). DLH (2.5 nmol in 50 nl) was microinjected at 1.5 and 2.5 mm depth in each track (Figure 4.15, Table

9.41). In the p-CPA pretreated group the DLH-evoked responses were not significantly different from those of the saline pretreated group at any location, with the following exceptions: the DLH-evoked changes in MAP were significantly different at 2 locations in raphe obscurus: firstly at 1.5 mm rostral and 1.5 mm depth (-10 ± 3 mmHg (p-CPA) *vs.* 0 ± 2 mmHg (saline); $n = 5$), and also at 2.5 mm rostral and 1.5 mm depth (-3 ± 4 mmHg (p-CPA) *vs.* 11 ± 2 mmHg (saline); $n = 5$). Furthermore, in raphe pallidus at 2.5 mm rostral and 2.5 mm depth, the MAP increase was significantly larger in the depleted group (18 ± 1 *vs.* 7 ± 3 mmHg; $n = 5$) and the renal sympathoinhibition was likewise significantly larger (-54 ± 5 *vs.* -8 ± 11 %; $n = 5$).

Immunocytochemistry

Immunocytochemical staining of rat brainstem sections, with a rabbit antibody for 5-HT followed by a donkey anti-rabbit secondary antibody, clearly revealed neuronal cell bodies belonging to the raphe cell groups. No staining of cell bodies was seen in cell groups outside of the raphe structures previously described (Törk, 1985). Additionally, the staining revealed a dense network of fibres in various cell groups, most notably the DVN and the NTS, which contained a marginally lower density of fibres than the DVN. Moderate density fibres were observed in structures including RVLM, nucleus ambiguus, gracile nucleus, and the spinal trigeminal nucleus. Sections treated with secondary antibody alone did not show any staining of cell bodies or fibres (Figure 4.16, *F*).

Medullary sections from rats pretreated with saline ($n = 7$) were indistiguishable from controls (no pretreatment – data not shown), whereas medullary sections from rats pretreated with p-CPA ($n = 7$) displayed a dramatic reduction in the density of fibre staining, compared to saline-pretreated rats. The depletion was particularly clear in the NTS and DVN (Figure 4.16, *A* and *B*). Staining of cell bodies in the medullary raphe, however, was unchanged (Figure 4.16, *C* and *D*).

Figure 4.12 Cardiopulmonary reflex trace: effects of p-CPA

Experimental trace illustrating cardiopulmonary reflex elicited by 2.5 μg intra-atrial phenylbiguanide (PBG) in 2 anaesthetised, neuromuscular blocked and atenolol pretreated rats, pretreated with saline (3.5 ml kg^{-1} i.p.) and p-CPA (350 mg kg^{-1} i.p.) respectively, for 2 days.

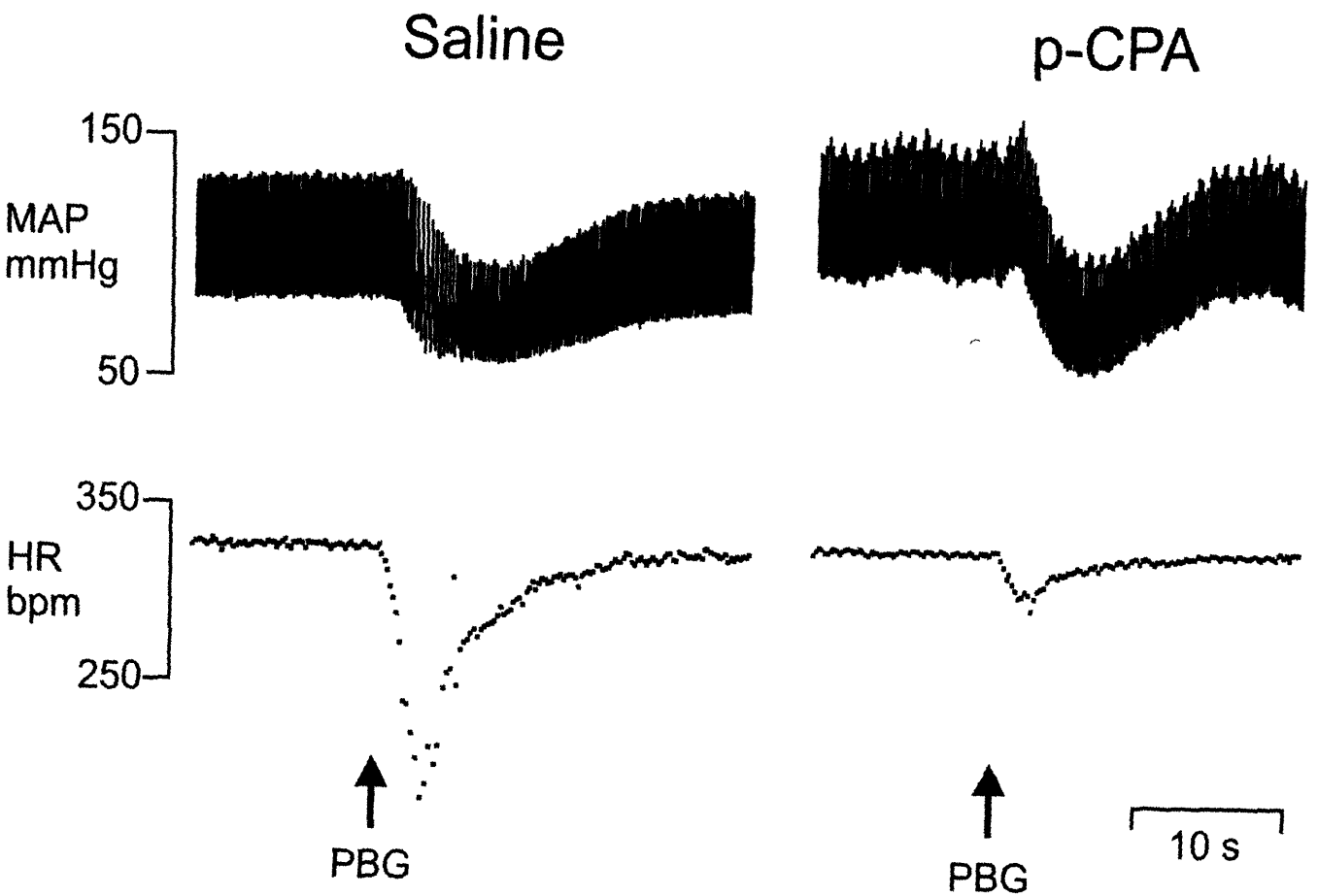

Saline
p-CPA
150
MAP
mmHg
50
350
HR
bpm
250
PBG
PBG
10 s

Figure 4.13 Cardiopulmonary reflex histogram: effects of p-CPA

Mean (± s.e.m.) changes (Δ) in MAP and R-R interval evoked by stimulation of cardiopulmonary afferents with PBG in anaesthetised, neuromuscular blocked and atenolol pretreated rats pretreated with either saline (n = 7) or p-CPA (n = 7).

** $P < 0.01$ relative to saline, Student's unpaired t-test.

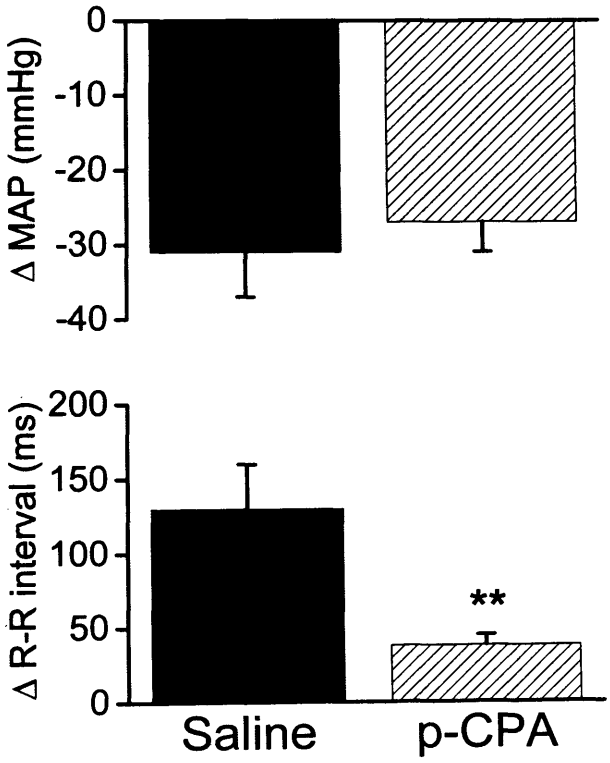
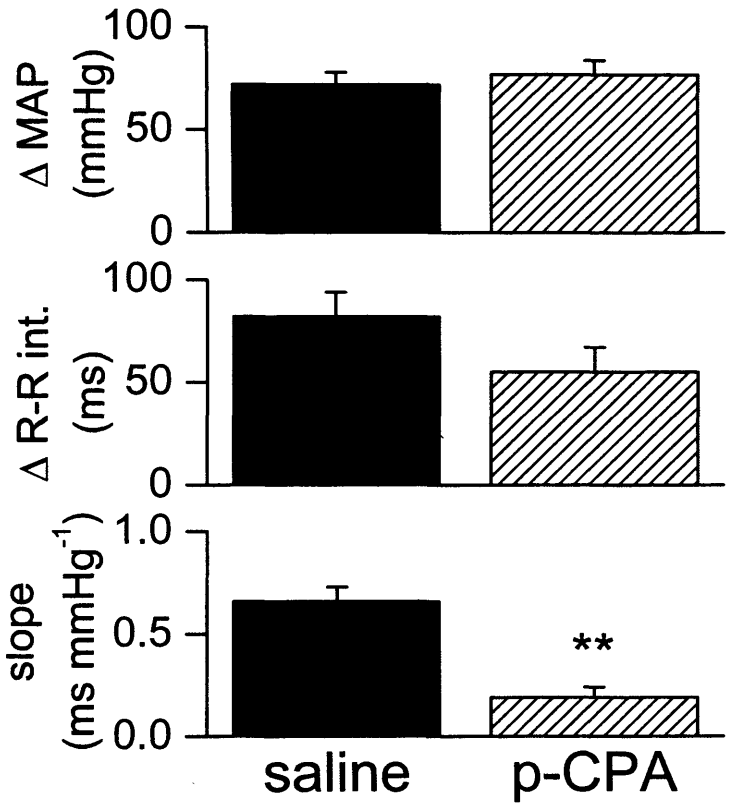


Figure 4.14 Baroreflex graphs: effects of p-CPA

Top: Mean (± s.e.m.) changes (Δ) in MAP, R-R interval, and baroreflex slope evoked by phenylephrine in anaesthetised, neuromuscular blocked and atenolol pretreated rats treated with either saline (n = 5) or p-CPA (n = 5).

** $P < 0.001$ relative to saline, Student's unpaired t-test.

Bottom: Phenylephrine (10 μg i.v.) evoked baroreflex slope analysis in 2 anaesthetised, neuromuscular blocked and atenolol pretreated rats, pretreated with either saline (3.5 ml kg^{-1} i.p.) or p-CPA (350 mg kg^{-1} i.p.) for 2 days.


Δ MAP (mmHg)
100
50
0
Δ R-R int. (ms)
100
50
0
slope (ms mmHg^{-1})
1.0
0.5
0.0
**
saline
p-CPA

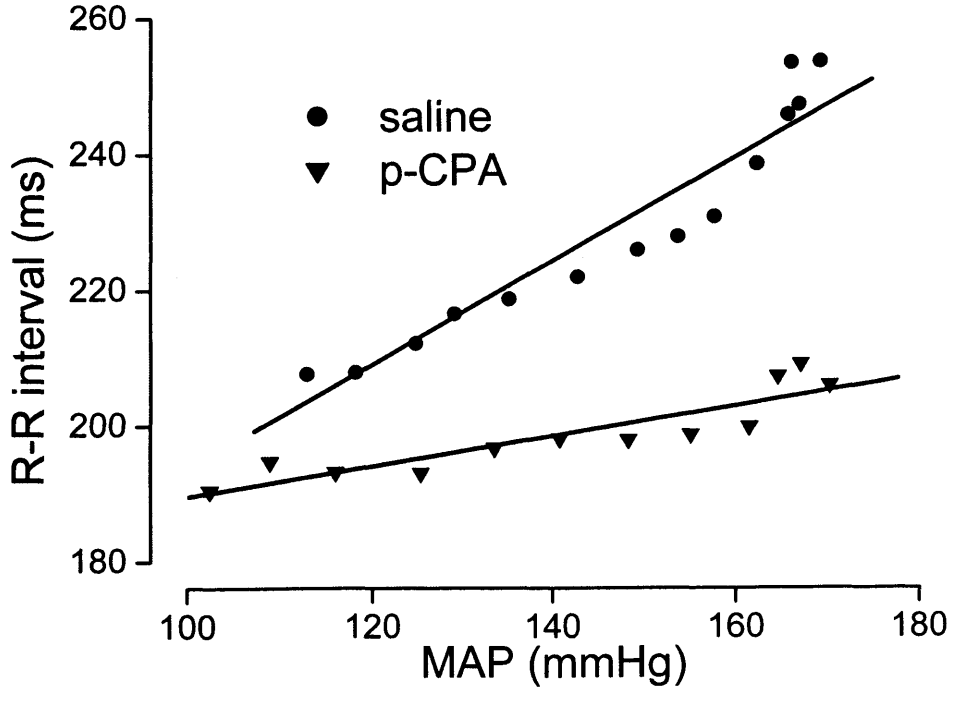

R-R interval (ms)
260
240
220
200
180
saline
p-CPA
100
120
140
160
180
MAP (mmHg)

Figure 4.15 Raphe stimulation histogram: effects of p-CPA

Histogram of mean (± s.e.m.) changes in baseline MAP, HR, IRNA and IPNA evoked by microinjection of DLH into various raphe areas of anaesthetised, neuromuscular blocked and atenolol pretreated rats, pretreated with either saline (3.5 ml kg^{-1} i.p.; $n = 5$) or p-CPA (350 mg kg^{-1} i.p.; $n = 5$) for 2 days.

Location of the microinjection (reconstructed from pontamine deposits) is shown in mm relative to calamus scriptorius (rostral) and brain surface (depth).

* $P < 0.05$, ** $P < 0.01$ (unpaired Student's t-test)

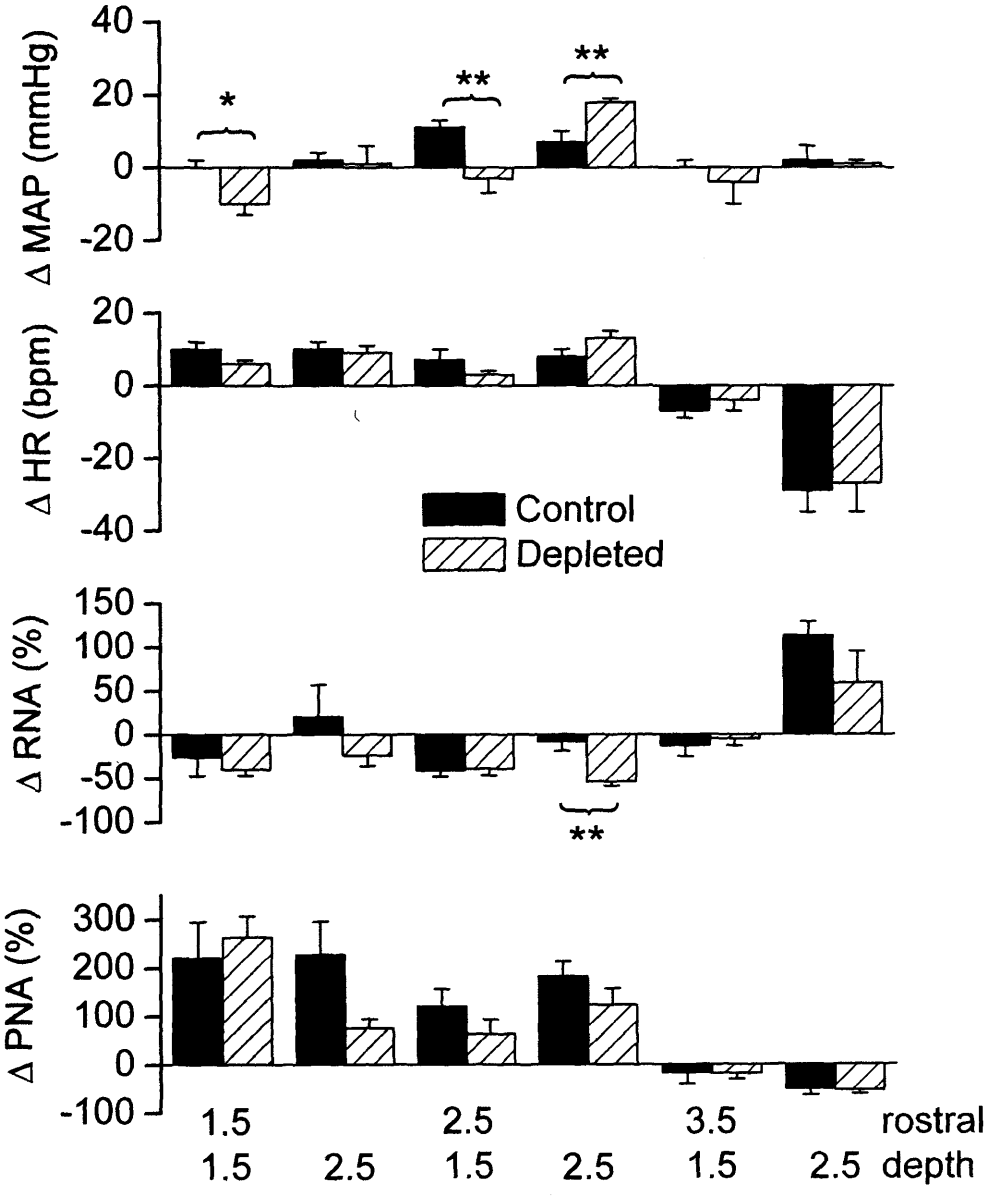

Δ MAP (mmHg)
40
20
0
-20
*
**
**
Δ HR (bpm)
20
0
-20
-40
Control
Depleted
Δ RNA (%)
150
100
50
0
-50
-100
**
Δ PNA (%)
300
200
100
0
-100
1.5
2.5
3.5
rostral
1.5
2.5
1.5
2.5
1.5
2.5
depth

Figure 4.16 5-HT immunocytochemistry: effects of p-CPA

Photomicrographs of coronal sections of rat medulla immunostained for 5-HT (except negative control in *F*), illustrating effects of pretreatment with saline or p-CPA.

A. 5-HT containing fibres in the caudal NTS/DVN of a saline pretreated rat

B. Caudal NTS/DVN in a p-CPA pretreated rat

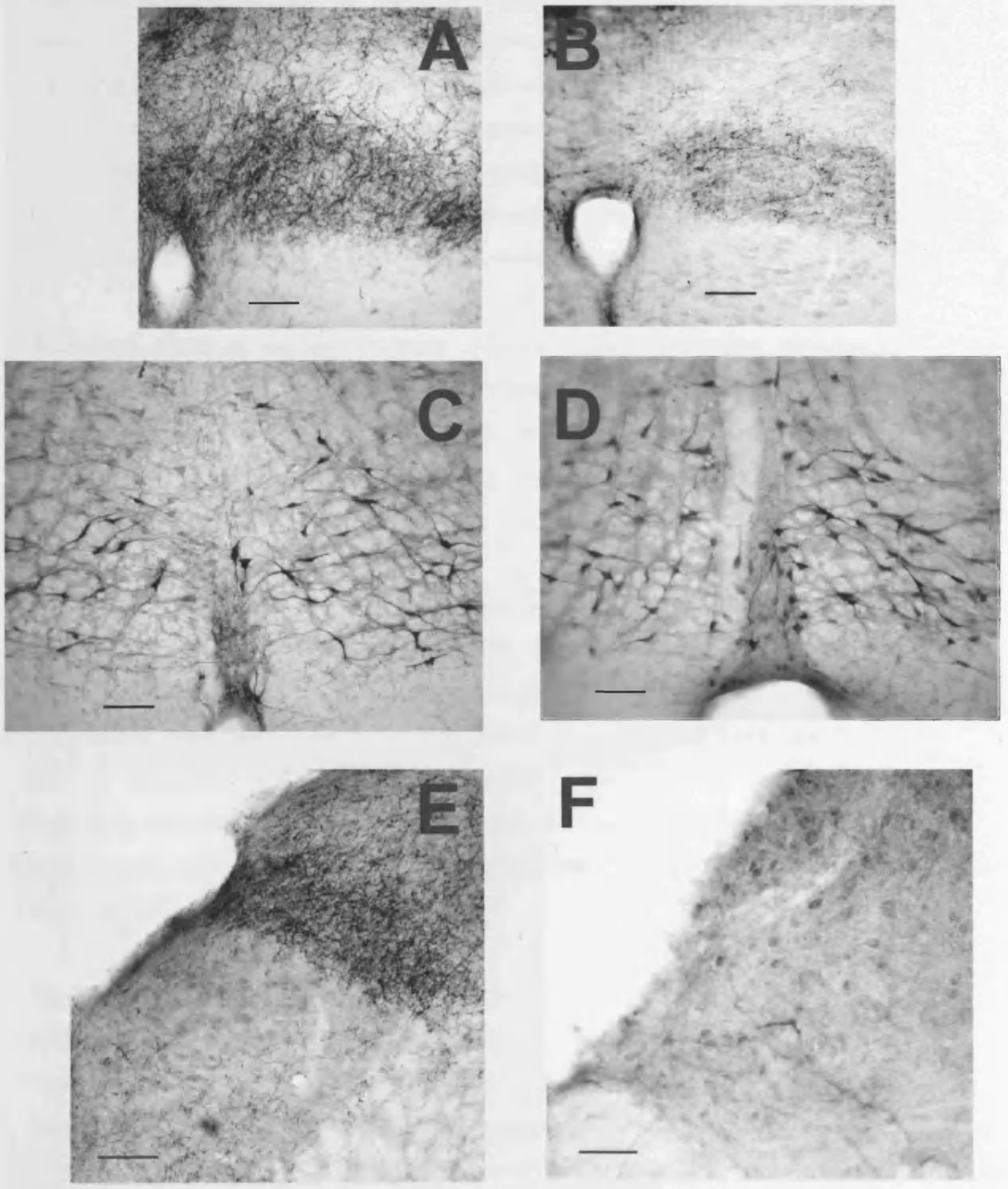
C. 5-HT containing neurones in the raphe magnus of a saline pretreated rat

D. Raphe magnus in a p-CPA pretreated rat

E. 5-HT containing fibres in the rostral NTS of a saline pretreated rat

F. Negative control (using only secondary antibody) from rostral NTS in a saline pretreated rat.

Scale bars: 200 μm


A
B
C
D
E
F

4.4. Discussion

4.4.1. Main Findings

The data demonstrate that the medullary raphe can have both an excitatory or inhibitory influence on various cardiovascular and respiratory variables, depending on which area is stimulated. The most caudal areas of raphe obscurus and pallidus caused sympathoinhibition and increased respiratory drive; the more rostral areas of raphe obscurus and pallidus caused similar sympathoinhibition and either raised or lowered respiratory drive; finally the most rostral area (raphe magnus/pallidus) caused bradycardia, sympathoexcitation, and respiratory depression.

Effects of electrical stimulation rarely matched those of chemical stimulation, indicating the unsuitability of passing current through these midline structures. They do, after all, contain many axons either crossing the midline or travelling longitudinally; if any of these fibres have a cardiovascular or respiratory role, the results would be uninterpretable.

The rostral area of raphe magnus/pallidus became the focus of further investigation because its influence on HR imputed a possible role in vagal reflexes. However, the bradycardia could not be attenuated by methiothepin (which is an antagonist at 5-HT_1, 5-HT_2, 5-ht_5, 5-HT_6 and 5-HT_7 receptors). In addition, the 5-HT_3 and 5-HT_4 receptor antagonists granisetron and SB-204070 respectively were without effect, suggesting that the bradycardia does not involve the activation of 5-HT receptors. Methiothepin did, however, augment the sympathoexcitation evoked by raphe magnus/pallidus stimulation.

To investigate further the role of 5-HT in the transmission of the effects of raphe stimulation, rats were depleted of 5-HT using p-CPA. This had no effect on raphe-evoked reponses with the exception of augmenting renal sympathoinhibition in one caudal area. This confirms that the raphe magnus/pallidus evoked bradycardia is probably not 5-HT related. It also suggests that the other responses do not involve 5-HT, or that other transmitters are able to compensate in the absence of 5-HT.

Depletion of 5-HT lowered baseline MAP with no corresponding elevation in baseline R-R interval. Cardiopulmonary reflex bradycardia was much smaller in depleted animals, as was the sensitivity of the baroreflex, demonstrating for the first time that endogenous 5-HT is required for the successful function of these reflexes.

In general these data propose that whilst vagal reflexes involve the release of 5-HT from fibres within the NTS or nucleus ambiguus, the neurones from which these fibres originate are unlikely to be located in the medullary raphe.

4.4.2. Raphe stimulation

The glutamate receptor agonist DLH was used in preference to glutamate because the distribution of 5-HT containing neurones in the medullary raphe is relatively sparse; DLH is not rapidly removed by a reuptake mechanism like glutamate, so is likely to activate a greater number of neurones. Unfortunately DLH is also likely to spread further and activate other neurones. Pontamine injections suggest that a 50 nl volume does not spread further laterally than 0.5 mm, however pontamine diffusion does not necessarily reflect DLH diffusion. Further control microinjections were performed at 1 mm lateral to the raphe, and at the caudal areas these were different from the midline stimulations, suggesting that the renal sympathoinhibitory and phrenic excitatory responses are indeed mediated by raphe obscurus and pallidus. At the rostral locus, however, lateral injections elicited a smaller but still noticeable triad of responses (bradycardia, renal sympathoexcitation, phrenic inhibition). This would suggest that the responses involve neurones spread over some distance – a distribution that is characteristic of this area of raphe. The other possibility is that the reticular area stimulated laterally happens to have a similar functional influence on the autonomic system although the neurones activated are different. One cannot exclude the possibility that DLH is activating neurones whose cell bodies are not in the vicinity of the micropipette, but whose dendrites extend a long distance.

The problem with many past raphe stimulation studies is that they offer no comprehensive functional topography of these nuclei with respect to their multiple cardiovascular, respiratory, and sympathetic roles. Therefore these experiments have

measured central sympathetic, parasympathetic, and respiratory drive simultaneously.

Blood pressure responses

Stimulation of the medullary raphe had no significant effect on baseline MAP, therefore other variables (particularly central respiratory drive) are free from the confounding factor of MAP change. Previous research found that chemical stimulation of the medullary raphe (particularly raphe obscurus) in anaesthetised animals causes a small to moderate (10 – 30 mmHg) pressor response (Haselton *et al.*, 1988; D'Amico *et al.*, 1996; Dreteler *et al.*, 1991). Numerous electrical stimulation experiments (e.g. Adair *et al.*, 1977) report more pronounced pressor responses not unlike those found in the present experiments. However, since the present results of chemical and electrical stimulation rarely correspond, there is no reason for further analysis of the electrical stimulations.

Heart rate responses

Bradycardic responses to stimulation of raphe magnus/pallidus have not been previously described. Bradycardia with concurrent hypotension has been demonstrated in a more caudal raphe area (Coleman & Dampney, 1995), but not in an atenolol-treated animal. Considering the strong sympathoinhibition reported in that study, the small bradycardia may well be due to cardiac sympathoinhibition. An atropine-sensitive hypotension resulting from microinjection of 5-HT_{1A} receptor agonists into dorsal raphe (which would inhibit neuronal activity) has been reported (Connor & Higgins, 1990), suggesting that this area tonically inhibits vagal drive. Other raphe stimulation studies have occasionally reported bradycardias, which are secondary to an evoked pressor response (Haselton *et al.*, 1988; Dreteler *et al.*, 1991).

The bradycardia in these present experiments was of interest because it might represent the serotonergic component of vagal reflexes. The triad of bradycardia, sympathoinhibition and apnoea resembled the diving response previously shown to involve 5-HT_{1A} receptors in rabbits (Futuro-Neto *et al.*, 1993; Dando *et al.*, 1998). Indeed, there is a projection from raphe magnus to NTS, and many of these neurones also contain 5-HT (Schaffar *et al.*, 1988). It is unlikely that this is the case, however,

for the following reasons: firstly, the bradycardia is too slow to participate in a fast reflex such as the cardiopulmonary reflex (it took up to 15 s to reach a maximum in these experiments, whilst the cardiopulmonary reflex bradycardia is almost instantaneous); secondly, it is not sensitive to a variety of 5-HT receptor antagonists; thirdly, it is not affected by 5-HT depletion. The lack of a role of the raphe in reflex bradycardias would agree with the midine lesion studies of Lee *et al.* (1972). Further investigation using reversible inhibition of the raphe (by microinjection of GABA, cobalt chloride or similar) would be needed to conclude unequivocally that there is no reflex role in these areas.

A possibility is that the bradycardia is either caused or potentiated by the phrenic inhibition, resulting from respiratory modulation of CVPNs. This is unlikely for the following reasons: firstly, bradycardia was observed in several animals in the absence of any phrenic changes; secondly, the onset of bradycardia often preceded the onset of phrenic inhibition; thirdly, bradycardia size was unchanged when animals were put into central apnoea by hyperventilation. Respiratory modulation of HR is more likely to be seen during the strong increases of respiratory drive evoked by raphe obscurus stimulation. This was usually accompanied by a tachycardia of 3 – 10 bpm, which one would expect to be due to respiratory inhibition of CVPNs.

It is unlikely that the raphe magnus-evoked bradycardia demonstrates the anatomical connection between raphe magnus and NTS (Schaffar *et al.*, 1988). There is no evidence that the responses to raphe stimulation are due to a direct pathway: several other cell groups may well be involved, which might account for the slow responses. A further anatomical connection from the medullary raphe to nucleus ambiguus has been reported (Haxhiu *et al.*, 1993), but these present data do not demonstrate such a functional connection. Single unit electrophysiology would be the most reliable way of demonstrating a functional connection between raphe and either NTS or CVPNs. Preliminary experiments in the cat were unable to find such a connection (Wang & Ramage, unpublished observations). Another possible connection could be between raphe and CVPNs in the DVN with C-fibre efferents. Selective stimulation of these efferents causes a relatively small and slow bradycardia (Jones *et al.*, 1995b). The long time taken for this raphe-evoked bradycardia to reach a maximum was surprising at first. It was thought that this could be due to slowly reverberating

activity in a network, but it could also be explained by its similarity to the C-fibre efferent-evoked bradycardia.

Sympathetic responses

The sympathetic changes evoked by raphe stimulation are the most widely reported. Some studies report sympathoexcitation and some sympathoinhibition. Sympathoexcitatory responses are reported due to both myelinated (Zhou & Gilbey, 1995) and unmyelinated (Huangfu *et al.*, 1994; Morrison, 1993) direct projections from raphe obscurus and pallidus to spinal cord. As such these disagree with the present data. Increased sympathetic drive, as evidenced by reduced blood flow, has been reported in the rat tail after stimulation of raphe magnus/pallidus (Blessing & Nalivaiko, 2001), and whilst these effects were shown not to reflect global sympathoexcitation, they agree with the present renal sympathoexcitation evoked from the same area. Care must be taken in interpreting the effects on different sympathetic targets, as these are known to behave differently. Hence a report of decreased tail blood flow in one report does not necessarily support the increased renal nerve activity in another, or, for that matter, contradict a reduced mesenteric blood flow in another.

Many researchers have reported sympathoinhibition due to raphe stimulation, including in raphe of the cat (Gilbey *et al.*, 1981), and raphe obscurus/pallidus of the rabbit (Coleman & Dampney, 1995; Coleman & Dampney, 1998) and raphe obscurus of rat (Verberne *et al.*, 1999). In the rabbit and the rat, these responses have been shown to be due to raphe-mediated inhibition of RVLM (Coleman & Dampney, 1998; Verberne *et al.*, 1999). In the cat, the sympathoinhibition was blocked by spinal LSD, suggesting a direct connection with spinal cord SPNs. Other earlier reports also suggest that the direct raphe-spinal pathway is sympathoinhibitory (Coote & Macleod, 1975) However, the majority of spinal cord SPNs are excited by iontophoretic 5-HT (Coote *et al.*, 1981), so the role of 5-HT receptors in this effect is still unclear.

The present data demonstrate, for the first time, that the sympathoinhibitory response is limited to the caudal portion of the medullary raphe, and becomes a sympathoexcitation in the corresponding dorsoventral portion of raphe

magnus/pallidus. This suggests different populations of raphe neurones with projections to sympathetic or presympathetic areas. These could be mediated by different receptors within the same structure (e.g the spinal cord, as suggested by the iontophoretic study of Lewis & Coote, 1990), or by different structures (the sympathoinhibition being indirect *via* RVLM inhibition, and the sympathoexcitation being a direct raphe-spinal pathway). This supposition warrants further investigation.

Respiratory responses

A variety of studies report increases or decreases in central respiratory drive when stimulating the raphe. Raphe obscurus stimulation in the cat caused an increase in respiratory drive (Holtman *et al.*, 1986a; Holtman *et al.*, 1987) which was reduced by the 5-HT receptor antagonist methysergide (Holtman *et al.*, 1987). A separate group, however, reported decreased respiratory drive evoked from the same area (Lalley, 1986; Lalley *et al.*, 1997) which was prevented by the 5-HT_{1A} receptor antagonist NAN-190 (Lalley *et al.*, 1997). One clear difference in these studies is that the first group used α-chloralose/urethane anaesthesia, and the second pentobarbitone. This could explain the different results. In the rat, chemical stimulation of raphe obscurus has been shown to increase respiratory drive (Dreteler *et al.*, 1991; Bernard, 1998), and stimulation of raphe pallidus or magnus had a smaller effect (Bernard, 1998). In brain slices, raphe neurones increase their firing rate when $PaCO_2$ in the bath is lowered (Richerson, 1995), suggesting a role in chemosensing. *In vivo* it was found that some raphe sites respond to local acidosis by increasing repiratory drive (Bernard *et al.*, 1996).

As seen in the sympathetic responses, the present data demonstrate for the first time that the phrenic nerve increases are limited to the caudal medullary raphe, especially raphe obscurus. Stimulation of the rostral part (magnus/pallidus) caused a strong inhibition of phrenic activity, often including apnoea. Whether these two functional populations have different projections remains to be determined. The excitatory population could project directly to phrenic motoneurones, which have been shown to receive 5-HT containing synaptic terminals (Holtman *et al.*, 1990b; Pilowsky *et al.*, 1990), but whether these are the synapses involved in this raphe-evoked response is disputed by the following evidence: firstly, the effects of electrical raphe stimulation are too rapid to be caused by slow-conducting raphe-spinal fibres

(Holtman *et al.*, 1986b; Lalley, 1986), secondly iontophoresis of 5-HT onto phrenic motoneurones had no effect on their activity (Lalley, 1986). Therefore the serotonergic innervation of phrenic premotor neurones or other components of the network may be responsible for this raphe-evoked effect. In the present data, raphe stimulation affected both amplitude and rate of phrenic nerve activity (see Figures 4.2 and 4.6). However, discharge of the phrenic nerve was locked to the rhythm of the ventilator in a ratio of either 1:1 or 1:2, therefore changes in the rate of phrenic discharge typically presented as either doubling or halving of frequency. From these observations it would appear that the raphe-respiratory influence is not merely at the level of the phrenic motoneurones.

4.4.3. Pharmacology

Raphe-evoked responses are notoriously difficult to interpret because of the variety of neurotransmitters found within the somata (and therefore expected to be found in axon terminals), of which 5-HT generally makes up only about 50 % (see 1.5). Whilst 5-HT containing neurones can be distinguished electrophysiologically from their steady discharge rate, there is no technique to stimulate that population selectively. Therefore pharmacological tools were used to investigate the role of 5-HT in the raphe-evoked responses.

Methiothepin is an antagonist with a broad spectrum of action, especially at 5-HT_1 and 5-HT_2 receptors. Additionally it has a high affinity at 5-HT_7 receptors, and also at 5-ht_5 and 5-HT_6 receptors, dopamine and α adrenoceptors (see 1.6). Hence many monoaminergic effects should be sensitive to methiothepin. The doses used in this study are high, and should be able to antagonise even synaptic effects (Chaput *et al.*, 1986; Terron, 1997), which are less antagonist-sensitive than exogenous agonist effects. In the present experiments, 1 mg kg^{-1} methiothepin (i.v.) completely blocked the pressor effects of 5 μg phenylephrine (i.v.) for over 20 minutes, demonstrating that the α adrenoceptor antagonist properties of methiothepin are potent and long-lasting.

Administration of i.v. saline, methiothepin (1 and 3 mg kg^{-1}), granisetron and SB-204070 caused no significant changes in baseline variables, which is to be expected if 5-HT is not involved in resting cardiovascular variables. Methiothepin did cause an initial depressor response, probably due to peripheral α_1 adrenoceptor blockade, but this rapidly recovered. Additionally, the 3 mg kg^{-1} methiothepin group has a lower control HR than the saline group, and following methiothepin this is reduced by approximately 5 bpm from control, which appears highly significant compared to the time-matched saline control, but is probably an artefact caused by that group having a lower starting HR.

Stimulation of raphe magnus/pallidus with DLH evoked changes comparable to those in the initial mapping study, and i.v. saline did not significantly affect the size of these responses. The tendency was for evoked responses to get smaller, as might be expected with repeated microinjections due to factors such as depolarisation block, excitotoxicity and mechanical damage. Hence in example traces it can appear that methiothepin is having an effect (e.g. Figure 4.10). Compared to the time-matched control, however, methiothepin had no effect on bradycardia or phrenic inhibition. This lack of effect of two high doses of methiothepin suggest that 5-HT receptors are not involved in the transmission of these responses. It cannot be ruled out, however, that this is a failure of the drug: it may be that antagonising multiple 5-HT receptors with opposing effects fails to dissect out the receptor of interest, or that the route of administration fails to provide a sufficient synaptic concentration of the drug, even at these high doses (McCall & Aghajanian, 1980). To exclude the possibility that the effects are mediated by 5-HT_3 or 5-HT_4 receptors, which methiothepin does not antagonise, granisetron and SB-204070 were used at doses greater than those previously shown to be peripherally effective (Bingham *et al.*, 1995). The dose of SB-204070 here used was particularly high. From preliminary experiments with two animals in each group, it seemed unlikely that these receptors were involved.

One clear results of methiothepin, however, was to *potentiate* the sympathoexcitation evoked from raphe magnus/pallidus. This suggests that the evoked sympathoexcitation is the net effect of an excitation with a concurrent inhibitory component *via* a 5-HT receptor. Therefore when this receptor is blocked, the full

excitation is unmasked. As sympathoinhibitions are evoked from the more caudal raphe areas investigated, it is not unreasonable to consider that this area may have a mixed population of neurones. Considering the small group size, however, this was treated with caution, and further investigated using 5-HT depletion.

Within the raphe there are not only separate populations of neurones containing transmitters other than 5-HT (such as GABA and dopamine) but also peptides existing as co-transmitters with 5-HT, such as substance P, cholecystokinin, thyrotropin releasing hormone, enkephalin and somatostatin (see Törk, 1985). The relative contributions of these transmitters has not been established. It may be that blockade of the serotonergic component alone is not sufficient to abolish the effect of the co-transmitters.

In the past, various responses to raphe stimulation have been blocked or attenuated with 5-HT receptor antagonists. Phrenic inhibition is prevented by the 5-HT_{1A} receptor antagonist NAN-190, at a dose of 24 $\mu g\ kg^{-1}$ i.v. (Lalley *et al.*, 1997). Likewise raphe-evoked bronchodilatation was abolished by brainstem application of the broad-spectrum 5-HT receptor antagonist methysergide (Haxhiu *et al.*, 1998), and sympathoinhibition was reduced by the partial agonist LSD, given i.v. or spinally (Gilbey *et al.*, 1981).

4.4.4. 5-HT depletion

In order to verify the data obtained using 5-HT receptor antagonists, further experiments were performed on rats depleted of 5-HT using the synthesis inhibitor p-CPA. This technique was first described by Koe & Weissman (1966), who suggested that the drug inhibits tryptophan hydroxylase, causing a rapid depletion of 5-HT stores throughout the body. Neurochemical analysis showed that doses of up to 316 $mg\ kg^{-1}$ i.p. had no effect on brain noradrenaline or dopamine concentrations. Administration of 100 mg p-CPA per rat for 3 days similarly caused no change in brain noradrenaline, but a depletion of brain 5-HT of over 90 % (Ito & Schanberg, 1975). Similar results have been confirmed (Chaput *et al.*, 1990; Datla & Curzon, 1996; Geranton *et al.*, 2004), so in the present experiments no neurochemical assay was performed. Depletion was verified with immunocytochemistry.

Immunocytochemistry

The 5-HT antibody used is highly selective for 5-HT, with minimal cross-reactivity with other catecholamines, 5-HT precursors, or metabolites (Wallace *et al.*, 1982). Medullary sections showed dense staining in the areas expected to contain serotonergic fibres, such as the NTS, DVN, RVLM, facial nucleus and trigeminal complex. The density in the NTS in particular is well known (Steinbusch, 1981; Maley & Elde, 1982; Pickel *et al.*, 1984). However, the present histology did not reveal 5-HT containing cell bodies in the NTS, meaning that this observation of Calza *et al.* (1985) remains unconfirmed. The only cell bodies stained by the 5-HT antibody were those in raphe obscurus, pallidus, and magnus in agreement with the descriptions of Törk (1985). This included a more diffuse scattering of 5-HT containing neurones lateral to the classical boundaries of raphe magnus, including some within the pyramids. Hence it is unlikely that a 50 nl midline DLH injection will activate all raphe neurones at this level, or at least not to the same degree.

After 2-day treatment with p-CPA, the staining of 5-HT containing fibres was almost entirely absent. This is thought to be simply due to absence of the transmitter; there is no evidence that p-CPA destroys fibres. Co-transmitters, therefore, are expected to remain. The pretreatment did not affect the staining of raphe cell bodies, which probably represents a non-releasable pool of 5-HT within the cell body that is unaffected by synthesis inhibition. A rough estimate by eye would suggest a depletion of approximately 90 % in fibres, in agreement with earlier neurochemical measurements (e.g. Ito & Schanberg, 1975). In one extra experiment, administration of p-CPA for 3 days instead of 2 did not further decrease staining, suggesting that the animals were maximally depleted.

Baseline variables

The finding that 5-HT depletion with p-CPA significantly lowered baseline MAP was surprising as 5-HT is not thought to be involved in the maintenance of MAP. In this thesis, a variety of 5-HT receptor antagonists given i.c. or i.v. have consistently failed to affect baseline MAP. It was considered that the lower MAP was due to some change in the condition of the depleted animals. The first possibility is that 5-

HT depletion is potentiating the effect of the anaesthetic, as deeper anaesthesia typically presents as lowered MAP. Pretreatment with methiothepin potentiates the effect of general anaesthetics in rats (Dringenberg, 2000), suggesting a tonic role for 5-HT at some level of consciousness. However, the effect of anaesthetics on consciousness is probably due to the exquisite sensitivity of the neuronal *networks* generating this phenomenon, whereas the effects of anaesthetics on the brainstem cardiovascular system is due to depressant actions on the *neurones* involved, which occurs at much higher doses of anaesthetic. As such, rate of loss of consciousness and rate of loss of cardiovascular control cannot be compared.

To shed some light on the matter, a previous report found a comparable fall in baseline MAP in conscious rats after a single 400 mg kg^{-1} i.p. p-CPA injection (Buckingham *et al.*, 1976) and this hypotension lasted 8 days. This suggests that the present results are not an artefact of anaesthetic. Similar lowered MAP is reported in the rabbit (Wing & Chalmers, 1974). Other studies have found no baseline MAP response to 100 mg kg^{-1} i.p. p-CPA for 3 days in pentobarbitone-anaesthetised rats (Smits *et al.*, 1978) although that protocol did attenuate median raphe-evoked hypertension. A further study reports a rise in MAP due to 100 mg per rat for 3 days (Ito & Schanberg, 1975), but in α-chloralose/urethane anaesthetised rats, which were also vagotomised. As such the results are not directly comparable to the present data.

Cardiopulmonary reflex

Comparison of the sensitivity of the cardiopulmonary reflex in different animals is difficult because of the inherent variability. From the reflex studies of Chapter 3 it was found that PBG doses need to be adjusted in each animal, at least to produce a submaximal bradycardia. From these experiments, a dose of 2.5 μg per animal was selected as the most appropriate. To ensure that the stimulation of cardiopulmonary afferents was similar in all animals, PBG was delivered at exactly the same rate (25 μl s^{-1}), and the intra-atrial cannula was always in the same place (3 cm into the jugular vein from the same point of insertion). The present data demonstrate, for the first time, that cardiopulmonary reflex bradycardia is smaller in p-CPA pretreated animals than in controls, but that cardiopulmonary reflex hypotension is unchanged. This is in direct agreement with the findings of Chapter 3, where central 5-HT_7 receptor blockade produced a similar effect (*cf.* Figures 3.6 and 4.12), illustrating

that either blockade of the receptor involved, or removal of the endogenous transmitter, selectively inhibits the reflex bradycardia.

The reduced bradycardia is unlikely to be a haemodynamic consequence of the lowered MAP. As this hypotension is not associated with a significant tachycardia, cardiac output should be lower in the depleted animals. Therefore intra-atial injection will be slower passing through lungs, which would cause, if anything, greater binding to receptors on cardiopulmonary afferents. Furthermore, the reduced reflex bradycardia does not always relate to reduced MAP. For example, in Figure 4.12 the baseline MAP in the saline-pretreated animal is actually lower than in the p-CPA pretreated animal, but the latter's bradycardia is much smaller.

Baroreflex gain

10 μg phenylephrine caused a rise in MAP, evoking a clear baroreflex bradycardia in all animals. The hypertension is larger than ideal (75 ± 4 mmHg), and it is thought that vasoconstrictor-evoked increases in MAP of over 50 mmHg can cause direct cardiac reflexes. These, however, typically present as arrhythmic beats and profound bradycardias, which were not seen in the present experiments. In the depleted animals, the reflex bradycardia was smaller but not significantly different from controls, which is what was previously found in awake rats with serotonergic lesions (using 5,7-DHT) of the nodose ganglia or NTS (Orer *et al.*, 1991). However, when the reflex gain was calculated with the more sensitive method of Su *et al.* (1992), it was found to be much smaller in the depleted group. This demonstrates for the first time that p-CPA pretreatment lowers the sensitivity of the phenylephrine-evoked baroreflex, and again confirms the findings of Chapter 3, albeit by removing the transmitter rather than blocking its receptor. A similar regimen of p-CPA pretreatment has previously been shown to inhibit the carotid sinus reflex in anaesthetised, vagotomised rats (Ito & Schanberg, 1975), i.e. to reduce the pressor response to carotid baroreceptor unloading (hence suggesting that 5-HT facilitates reflex sympathoexcitation). As the data of Chapter 3 demonstrate that 5-HT (*via* 5-HT_7 receptors) facilitates chemoreflex sympathoexcitation, but did not test its role in baroreflex sympathoexcitation, this warrants further investigation.

Again the possibility that the lowered resting MAP is a confounding factor must be considered. Baroreceptors are sensitive to change as well as rate of change, and the present data demonstrate no significant difference between either absolute change or rate of change in different groups (see Table 9.42). Furthermore, in the p-CPA group the correlation between MAP and R-R interval is no different from the saline group.

Raphe-evoked changes

The data demonstrate that this protocol of p-CPA administration is sufficient to cause histologically and functionally identifiable changes. It does not, however, cause any clear changes in raphe-evoked responses. The lack of effect of 5-HT receptor antagonists on the important raphe-evoked bradycardia is confirmed, showing that 5-HT is not involved in this effect. Depletion with p-CPA has previously been shown to be effective in attenuating raphe-evoked responses both on cardiovascular variables (Smits *et al.*, 1978) and on serotonergic transmission in other brain areas (Chaput *et al.*, 1990; Zhu & McNaughton, 1994). In the present experiments a very tight protocol was used to stimulate raphe areas sequentially, beginning rostrally. The control results obtained are comparable to those from the initial mapping study (which used sequential stimulation, beginning caudally), suggesting that the order in which the areas are stimulated (and any accompanying tissue damage) is not a confounding factor.

The maintenance of raphe-evoked effects despite severe 5-HT depletion may involve other transmitters, such as peptides. Thyrotropin-releasing hormone and substance P are examples of peptides found in the raphe (Chiba & Masuko, 1989). In the rat spinal cord, thyrotropin releasing hormone exerts a postsynaptic effect similar to that of 5-HT (Barbeau & Bedard, 1981; Hansen *et al.*, 1983), whilst substance P enhances 5-HT release, probably *via* terminal 5-HT autoreceptor blockade (Mitchell & Fleetwood-Walker, 1981). It is possible that an analogous situation is involved here to maintain serotonergic neurotransmission despite depletion. Another explanation could be receptor supersensitivity due to p-CPA, but this usually occurs after a longer period than the 48 hours of this protocol, and is more likely after destruction of serotonergic fibres with 5,7-DHT, as described previously (Ashby *et al.*, 1994; Sawynok & Reid, 1994).

Some effects of raphe stimulation, however, were significantly different in the p-CPA group, but these are difficult to interpret. Stimulation of caudal raphe obscurus in depleted rats caused a hypotension that was not seen in control rats, but the sympathoinhibition was of a similar size. At a more rostral site, stimulation of raphe obscurus caused a small rise in MAP that was abolished in the depleted group, whilst stimulation of raphe pallidus caused a rise in MAP that was potentiated in the depleted group. The latter effect was associated with a potentiated sympathoinhibition. As seen in the methiothepin experiments, 5-HT depletion may be uncovering a previously eclipsed mechanism. If this is the case, it is most likely to cause the rise in MAP, the sympathoinhibition being reflexive, hence proportional to the MAP change. The lack of concurrent reflex bradycardia would be explained by the large increase in phrenic activity, which could easily swamp such an effect by respiratory modulation of CVPNs.

Surprisingly none of the main raphe-evoked effects were significantly changed by depletion. However, some effects are smaller in the depleted groups, for example the phrenic excitation at parts of raphe obscurus and pallidus, and the sympathoexcitation at raphe magnus/pallidus. Possibly the group sizes (n = 5) are too small to detect such differences, although more importantly the numbers seem sufficient to demonstrate the lack of effect on the bradycardia evoked by raphe magnus/pallidus.

4.4.5. Technical limitations

Microinjections have their limitations, even when not performed in cell groups as complex as the raphe. These include drug spread, limited reproducibility, and activation of a variety of neurones (including interneurones). Similarly the additional methods used here (systemic drug pretreatment and global 5-HT depletion) have inherent problems due to the wide range of possible effects. Many of these problems have already been discussed, but suffice it to say that this study was intended to be merely a preliminary investigation of a possible mechanisms, and as such the techniques are appropriate.

4.4.6. Conclusion

This study has not addressed the reflex role of the medullary raphe directly; previous researchers have found that extensive lesioning of the midline (in fact of any brainstem region other than NTS/DVN and RVLM) does not affect the cardiopulmonary or baroreflexes (Lee *et al.*, 1972). Instead, these experiments tried to find functional evidence to support the anatomical connections between the medullary raphe and the NTS, DVN and nucleus ambiguus. The data very clearly demonstrate that a specific area of raphe magnus/pallidus exerts an excitatory influence on CVPNs. However, this does not seem to involve serotonergic transmission, as 5-HT receptor antagonists as well as depletion of 5-HT had no effect on this response. 5-HT depletion did, however, decrease the sensitivity of the bradycardic response to cardiopulmonary and baroreceptor stimulation, demonstrating that endogenous 5-HT is a necessary part of successful cardiopulmonary and baroreflex function.

4.4.7. Future experiments

The autonomic functions of the medullary raphe have been extensively mapped since the 1970s, however the effects of chemical stimulation of dorsal and medial raphe have not been characterised, although many reports of electrical stimulation exist. To conclude the present study, this needs to be done. The effect of chemically inactivating the raphe (with GABA or muscimol) is not expected to have much effect under α-chloralose anaesthesia, since raphe neurones usually have no spontaneous activity except under chloral hydrate or saffan anaesthesia. This could be tested to investigate any tonic roles. Furthermore, this would show unequivocally that the raphe are not involved in reflex bradycardias.

A more sensitive measure is neuronal recording from raphe, and testing the effects of selective 5-HT_7 receptor antagonists on these cells *in vivo* is an important experiment. Neurochemical assays *in vitro* suggest that SB-269970 reduces dorsal raphe neuronal activity (Roberts *et al.*, 2004b), and this needs to be confirmed.

The p-CPA findings of the present experiments require further investigation. This would best be done by comparing the effects firstly of different doses of p-CPA on baselines and reflexes; secondly of different doses of reflex stimulants (e.g. PBG, NaCN, phenylephrine) in the same animal; thirdly, and most importantly, the effects need to be observed in awake rats to exclude the effects of anaesthesia.

5. SEROTONERGIC AND GLUTAMATERGIC MECHANISMS IN THE NUCLEUS TRACTUS SOLITARIUS

5.1. Introduction

5.1.1. Background

The NTS is the site of termination of cardiopulmonary, baroreceptor and chemoreceptor afferents (see Jordan & Spyer, 1986). Integration of cardiovascular reflexes occurs within the complex microcircuitry of this nucleus, which distributes incoming stimuli to sympathetic, parasympathetic and respiratory neurones for generation of a motor response. Previous single-unit electrophysiological investigations have used iontophoretic application of drugs to characterise the role of glutamate receptors in reflex transmission (Wilson *et al.*, 1996; Jeggo *et al.*, 2001). Primary afferents release glutamate onto NTS second order neurones, which originally was thought to act *via* only AMPA receptors at this stage (see Andresen *et al.*, 2004), although NMDA receptors are now also implicated (Jeggo, 2003; Jones & Jordan, unpublished data). Higher order neurones in the NTS (receiving polysynaptic inputs) also receive glutamatergic inputs *via* both AMPA and NMDA receptors (see Andresen *et al.*, 2004). Additionally, 5-HT_3 receptors are thought to be located presynaptically on glutamatergic terminals in the NTS, where they cause excitation *via* NMDA receptors (Jeggo *et al.*, 2001), although the role of AMPA receptors in this effect was not confirmed.

Other 5-HT receptors involved in reflex transmission have not been identified in the NTS. 5-HT_{1A} receptors participating in the cardiopulmonary reflex are found in the nucleus ambiguus in the cat (Wang & Ramage, 2001), but a previous study failed to find a clear role for 5-HT_{1A} receptors in the NTS of rats (Ramage & Mifflin, 1998). The reflex role of the 5-HT_7 receptor was characterised in Chapter 3 above, but the location of these receptors has not been elucidated. Theoretically they are likely to be either in the NTS or the nucleus ambiguus.

The NTS is known to be richly innervated by 5-HT containing fibres (e.g. Steinbusch, 1981). In the cat these make synaptic contact with NTS neurones (Maley

& Elde, 1982), but whether this is the case in identified cardiovascular neurones of the rat NTS is not known.

5.1.2. Aims of the study

The aims of the present experiments are as follows: Firstly to confirm the role of AMPA/kainate receptors in the transmission of vagal afferent-evoked signals (and in particular the cardiopulmonary reflex) within the NTS, using the selective antagonist DNQX, applied iontophoretically, as previously described (Wang *et al.*, 1998). Secondly, to confirm that the excitation of vagally-evoked NTS neurones by the 5-HT_3 receptor agonist PBG, applied iontophoretically, is mediated *via* glutamate release onto AMPA as well as NMDA receptors. Thirdly, to test whether the 5-HT_7 receptor antagonist SB-269970 (at a dose known to attenuate cardiovascular reflexes) has any effect on vagally-evoked NTS neurones, DVN neurones, or gracile nucleus (touch-sensitive) neurones, when applied topically. Fourthly, to examine whether NTS neurones labelled by the juxtacellular method (Pinault, 1996) receive close appositions from 5-HT containing terminals. Lastly, in some of these experiments the effect of i.v. robalzotan was tested on the i.v. 8-OH-DPAT evoked bradycardia, as additional supporting data for Chapter 3 (see 3.4.5)

5.2. Methods

5.2.1. Protocols

Characterisation

The firing of all neurones was tested for at least 1 min in the presence of 1 Hz vagal (or aortic nerve) stimulation (for construction of PSTHs and analysis of latency and jitter) and for at least 1 min in the absence of any stimuli to note ongoing activity. During vagal stimulation the threshold (lowest current required to evoke a spike) was noted. Subsequent vagal stimuli were presented at just above (approximately 1.5 times) threshold. The effect of cardiopulmonary afferent activation with PBG (2 – 4 μg) on neuronal firing was also recorded.

Iontophoresis

Before the start of any iontophoretic protocol, anaesthesia was supplemented to prevent changes in neuronal activity due to anaesthetic wearing off during the protocol. Then the effect of various currents of AMPA, NMDA and PBG were tested until a currents were determined that caused a clear increase in neuronal activity within 10 s. Depending on the times taken for the neurone to begin firing and stop firing due to each drug, a time was selected for application of drugs and a time for recovery. Typically this consisted of 20 s on followed by 20 s off. The iontophoresis pumps were set to cycle automatically between drug ejection and drug retention, and between different drugs. Following at least 2 stable control applications, a continous current of DNQX was ejected, and agonist cycles continued in the presence of DNQX. After a maximum of 3 cycles, DNQX was switched off and agonist cycles continued until full recovery was observed. In some experiments several currents of DNQX were applied to determine its selectivity for the AMPA-evoked response

Once an AMPA-selective current was determined, the effects of DNQX were tested on vagally-evoked neuronal activity. Vagal stimulation was commenced at 1 Hz, and at least 3 min allowed to elapse as a control period. DNQX was switched on continuously until regular failures of the evoked spikes were observed. The time taken for this effect was recorded. Then DNQX was switched off and the neurone allowed to recover. The time to recovery was also noted.

Once the time for DNQX to affect vagus-evoked activity was estimated, the effect of DNQX on cardiopulmonary afferent-evoked neuronal activity was tested in some experiments. Control intra-atrial PBG injections were given every 5 min until at least 2 stable controls were obtained. Several minutes were allowed to elapse, then DNQX was switched on continuously. After several minutes (typically 3 min) the reflex was retested, then DNQX switched off and the reflex tested 5 min later.

Topical applications

NTS neuronal activity was evoked by continuous stimulation (1 Hz) of the vagus. Periodically the stimulus was switched off for the last 10 s of a min to allow cardiopulmonary afferent stimulation. After at least 3 min stable control stimulation,

either SB-269970 (100 μg kg^{-1}) or saline (10 μl) were applied topically, and stimulation continued up to a maximum of 25 min.

The ongoing activity of DVN neurones were recorded in the absence of any stimuli. After at least 3 min stable control activity, either SB-269970 (100 μg kg^{-1}) or saline (10 μl) were applied topically, and neuronal activity followed for a maximum of 25 min.

In neurones of the gracile nucleus, the receptive field was first mapped using gentle touch with the tip of a cotton bud, to establish the region of skin evoking the greatest burst firing of the neurone. Then, when spontanous activity had stabilised, stimuli were presented every 1 min, consisting of 10 touches of the receptive field. These touches were presented approximately once a second, and synchronised with the click of the ventilator pump (rate 60 – 70 min^{-1}), so each stimulus corresponded to the peak of tracheal pressure (for subsequent analysis). After at least 3 stable control responses, either SB-269970 (100 μg kg^{-1}) or saline (10 μl) was applied topically, and stimuli presented every minute thereafter (to a maximum of 15 mins).

When pharmacology was performed on more than one neurone in a given animal, at least 30 min were allowed to elapse between successive protocols. Drug and vehicle applications were alternated, and a maximum of 3 applications were made in each animal. Previous research on the micturition reflex in anaesthetised rats has demonstrated that SB-269970 can be given repeatedly, with no desensitisation of its inhibitory effect (Read, 2004).

Intravenous robalzotan

To provide some information on the functional efficacy of systemic robalzotan in support of Chapter 3 data, in some experiments (n = 10) 8-OH-DPAT (65 μg kg^{-1} i.v.) was given either alone or 2 min after robalzotan (100 μg kg^{-1} i.v.). This was done shortly after induction of anaesthesia (approx 90 min before any single unit electrophysiology), allowing plenty of time for elimination of the drugs.

5.3. Results

5.3.1. Glutamatergic study

Characterisation

A total of 22 neurones were recorded, all of which responded to electrical stimulation of the vagus, with a spike latency of between 22 and 35 ms, indicating activation by C-fibre afferents. 3 of 22 neurones were activated by a predominantly monosynaptic (Type 1) pathway, 8 by an intermediate (Type 2), and 11 by a polysynaptic (Type 3) pathway (Figures 5.1 and 5.2). Evoked spikes could not be cancelled by triggering the vagal stimulus from a spontanous spike (collision test) as can be done for DVN neurones (Figure 5.3) suggesting that they were orthodromically activated NTS neurones. They were recorded at a depth of 500 – 980 μm from the surface of the medulla, and between 100 μm caudal and 800 μm rostral to the calamus scriptorius.

Iontophoresis

Iontophoretic application of AMPA (20 – 120 nA) significantly increased the firing rate of all 13 neurones tested (0.3 ± 0.3 to 8.2 ± 0.8 spikes s^{-1}, Table 9.43). NMDA (40 – 180 nA) also increased the firing rate of all 13 neurones tested (0.5 ± 0.2 to 6.4 ± 0.9 spikes s^{-1}, Table 9.43). PBG (80 – 300 nA; $n = 9$) increased the firing rate of 7 of 9 neurones (2.7 ± 1.4 to 4.2 ± 1.6 spikes s^{-1}; Table 9.43), and had no effect on 2.

Iontophoretic application of DNQX (10 – 80 nA) significantly inhibited the AMPA-evoked firing in all 13 neurones tested (8.2 ± 0.8 to 2.6 ± 0.7 spikes s^{-1}, Figures 5.4 and 5.6, Table 9.44). It also produced a small, non-significant inhibition of the NMDA-evoked firing in 6 out of 13 neurones (6.6 ± 0.9 to 4.4 ± 1.2 spikes s^{-1}), and a small, non-significant potentiation in the response in 3 neurones (5.3 ± 4.6 to 7 ± 2.2 spikes s^{-1}) and had no effect on 4 neurones (Figures 5.4 and 5.7, Table 9.45). Taken as a group, however, the NMDA-evoked response was not significantly inhibited by DNQX (6.4 ± 0.9 *vs.* 5.9 ± 1.1 spikes s^{-1}, $n = 13$). Lastly, DNQX inhibited the PBG-evoked firing in 5 of 7 neurones (3.5 ± 2.1 to 2.2 ± 1.7 spikes s^{-1}, Figures 5.5 and 5.6, Table 9.44) and had no effect in 2 further neurones.

Iontophoresis of a similar current of DNQX (30 – 100 nA) significantly inhibited neuronal discharge evoked by electrical stimulation of the vagus (1 Hz) in all 12 neurones tested (35.8 ± 7.5 spikes 20 s^{-1} before DNQX *vs.* 15.3 ± 1.9 spikes 20 s^{-1} after DNQX; Figures 5.9 and 5.10, Table 9.47). Of these 12 neurones, 6 were Type 2 (intermediate) and 6 were Type 3 (polysynaptic). The mean time taken for DNQX to cause the maximum inhibition was 3.5 ± 0.4 min, and the mean time to recovery of control values was 3.7 ± 0.3 min.

DNQX (30 – 80 nA) also attenuated the cardiopulmonary afferent-evoked firing in 5 of 8 neurones (57 ± 25 to 33 ± 20 spikes, Figures 5.8 and 5.10, Table 9.46). Of these 5 neurones, 2 were Type 2 (intermediate) and 3 were Type 3 (polysynaptic). DNQX had no effect in 2 neurones, and potentiated the firing in 1.

Iontophoresis of the vehicle of DNQX (40 – 80 nA) potentiated the AMPA-evoked firing of all 4 neurones tested (7.3 ± 1.3 to 10.1 ± 1.9 spikes s^{-1}, Figures 5.6).

Figure 5.1 NTS neuronal characterisation (traces)

Sample experimental traces recorded extracellularly from NTS neurones in anaesthetised and neuromuscular blocked rats.

* vagal stimulus artefact

Type 1 (monosynaptic). Example of a neurone with very little variability of latency (jitter) over 5 sweeps. This is activated by a monosynaptic input.

Type 2 (intermediate). Example of a neurone with moderate jitter over 5 sweeps. This is probably activated by a mixture of mono- and polysynaptic inputs.

Type 3 (polysynaptic). Example of a neurone with pronounced jitter over 5 sweeps. This is activated by a presumed polysynaptic input.

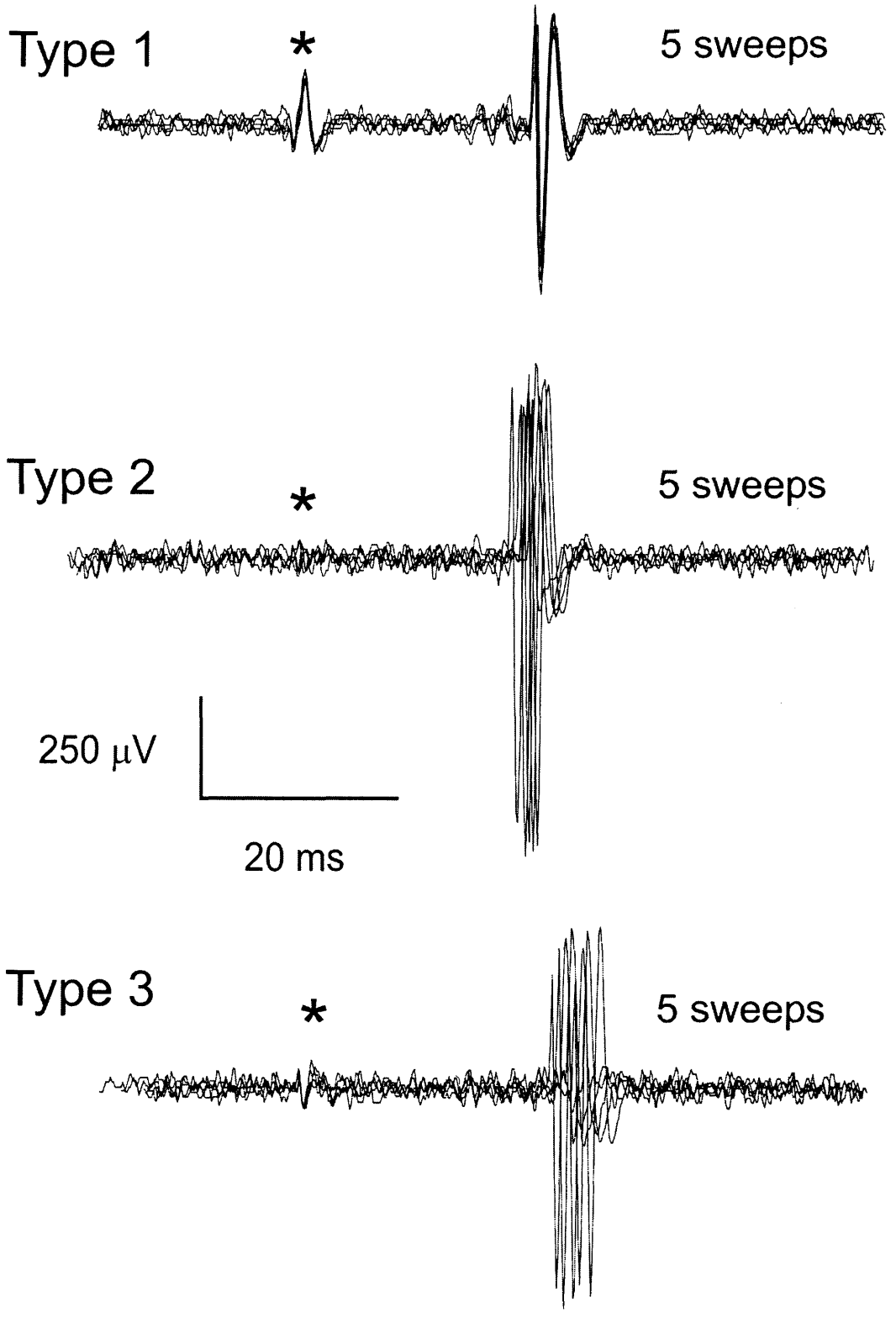

Type 1
*
5 sweeps
Type 2
*
5 sweeps
250 μV
20 ms
Type 3
*
5 sweeps

Figure 5.2 NTS neuronal characterisation (PSTHs)

Peri stimulus-time histograms (PSTHs) illustrating variability (jitter) of latency of the evoked spikes of NTS neurones over 50 sweeps of vagal stimulus (1 Hz). Number of spikes per bin (1 ms bins) is plotted against time after vagal stimulus.

Asterisks denote the bin reflecting the mean latency (i.e. mode).

A. Example of a Type 1 neurone with very low latency (monosynaptic).

B. Example of a Type 2 neurone with 3 ms jitter (intermediate).

C. Example of a Type 2 neurone firing a spike doublet. The first spike has a 2 ms jitter.

D. Example of a Type 3 neurone with 10 ms jitter (polysynaptic).

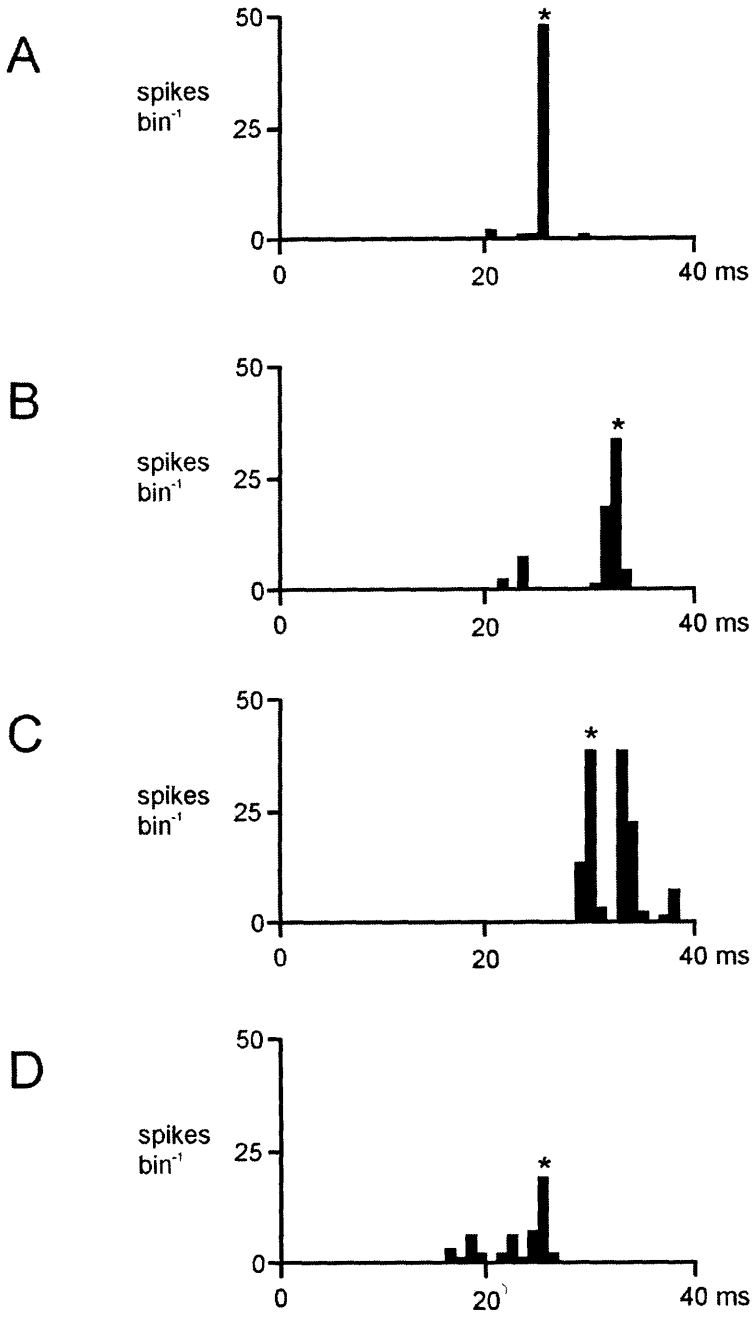

A
spikes bin-1
50
25
0
0
20
40 ms
*
B
spikes bin-1
50
25
0
0
20
40 ms
*
C
spikes bin-1
50
25
0
0
20
40 ms
*
D
spikes bin-1
50
25
0
0
20
40 ms
*

Figure 5.3 DVN neuronal characterisation

Sample experimental traces recorded extracellularly from a DVN neurone in an anaesthetised and neuromuscular blocked rat.

This neurone is antidromically activated, as shown by the following:

Top. The evoked spike has no jitter. (* vagal stimulus artefact)

Middle. The spontanous spikes occurring around the time of the vagal stimulus collide with the antidromic impulse, causing a cancellation of the evoked spike (‡).

Bottom. PSTH of 50 sweeps of 1 Hz vagal stimulus. Number of spikes per bin (1 ms bins) is plotted against time after vagal stimulus.

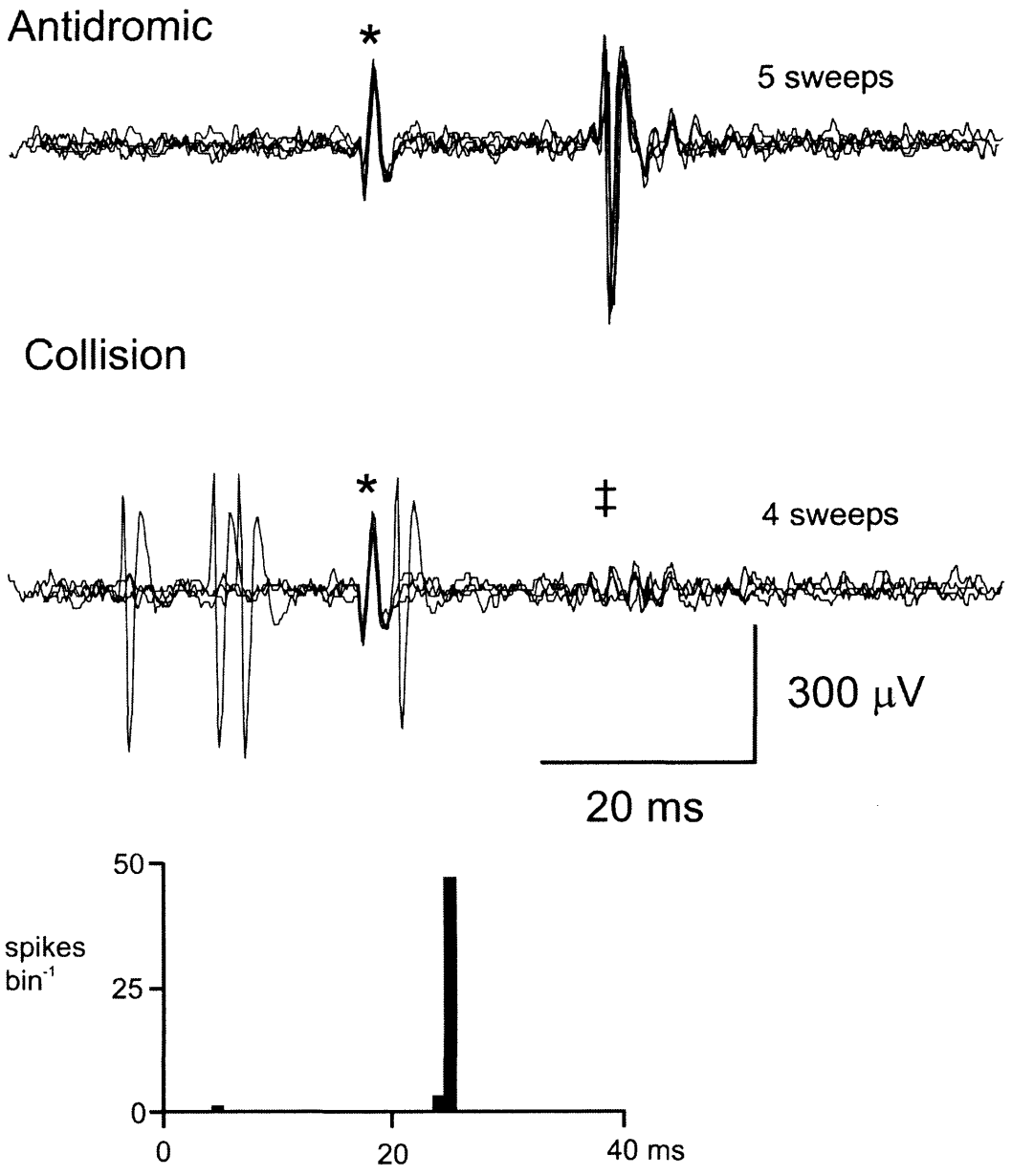

Antidromic
*
5 sweeps
Collision
*
‡
4 sweeps
300 μV
20 ms
50
spikes
bin-1
25
0
0
20
40 ms

Figure 5.4 Iontophoresis trace: selectivity of DNQX

Experimental trace of an NTS extracellular recording with iontophoresis in an anaesthetised, neuromuscular blocked rat, illustrating selectivity of iontophoretically applied DNQX (80 nA) for the AMPA-evoked neuronal response, over the NMDA-evoked neuronal response.

Bars indicate duration and amplitude of iontophoretic currents.

Cell: raw extracellular signal.
Spikes s^{-1}: rate meter (1 s bins)
BP: blood pressure.

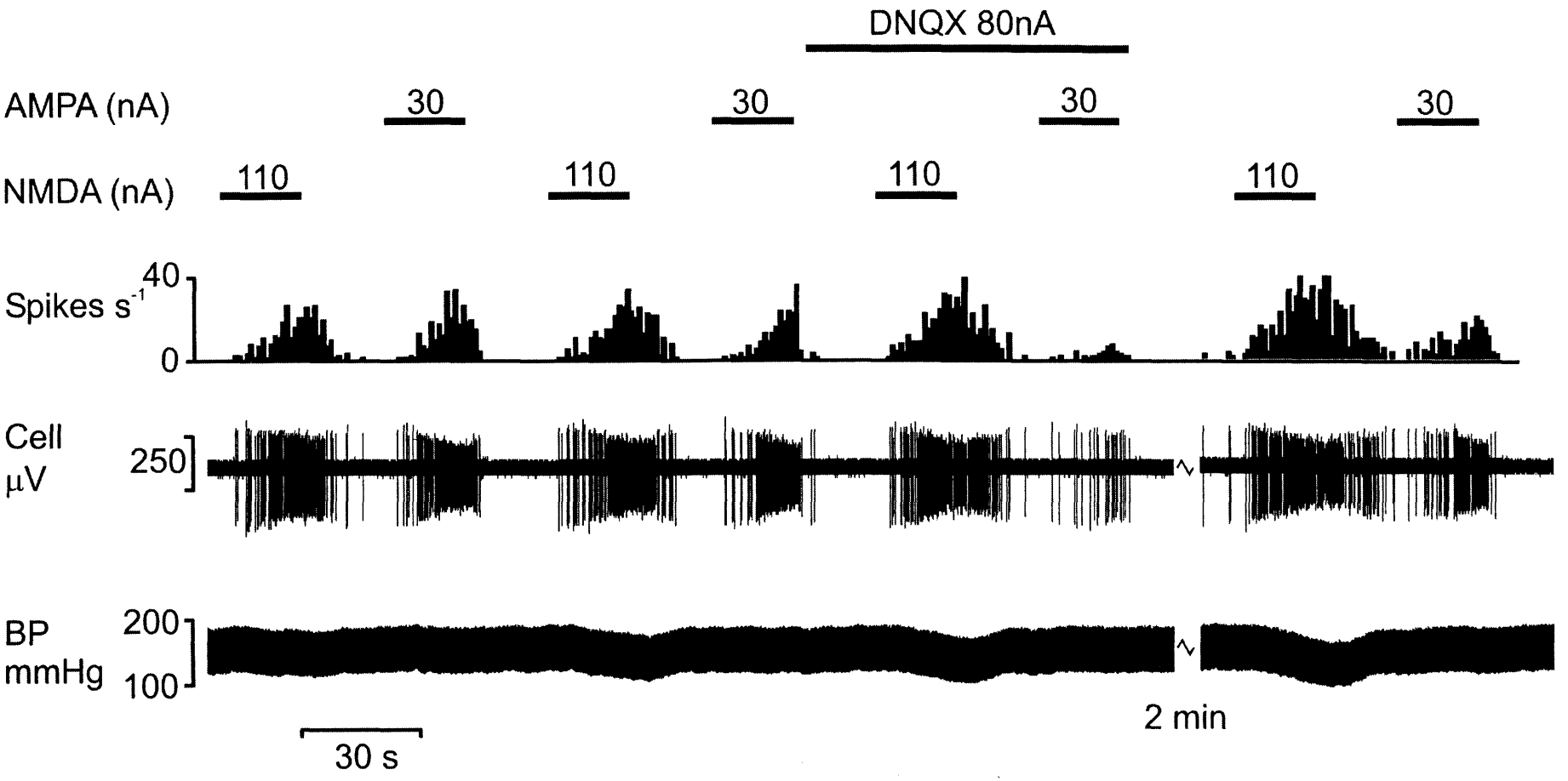

DNQX 80nA
AMPA (nA)
30
30
30
30
NMDA (nA)
110
110
110
110
Spikes s-1
40
0
Cell
μV
250
BP
mmHg
200
100
30 s
2 min

Figure 5.5 Iontophoresis trace: effects of DNQX on AMPA and PBG

Experimental trace of an NTS extracellular recording with iontophoresis in an anaesthetised, neuromuscular blocked rat, illustrating inhibitory effect of iontophoretically applied DNQX (10 nA) on both the PBG-evoked and AMPA-evoked neuronal responses, but not the NMDA-evoked neuronal response.

Bars indicate duration and amplitude of iontophoretic currents.
Cell: raw extracellular signal.
Spikes s^{-1}: rate meter (1 s bins)

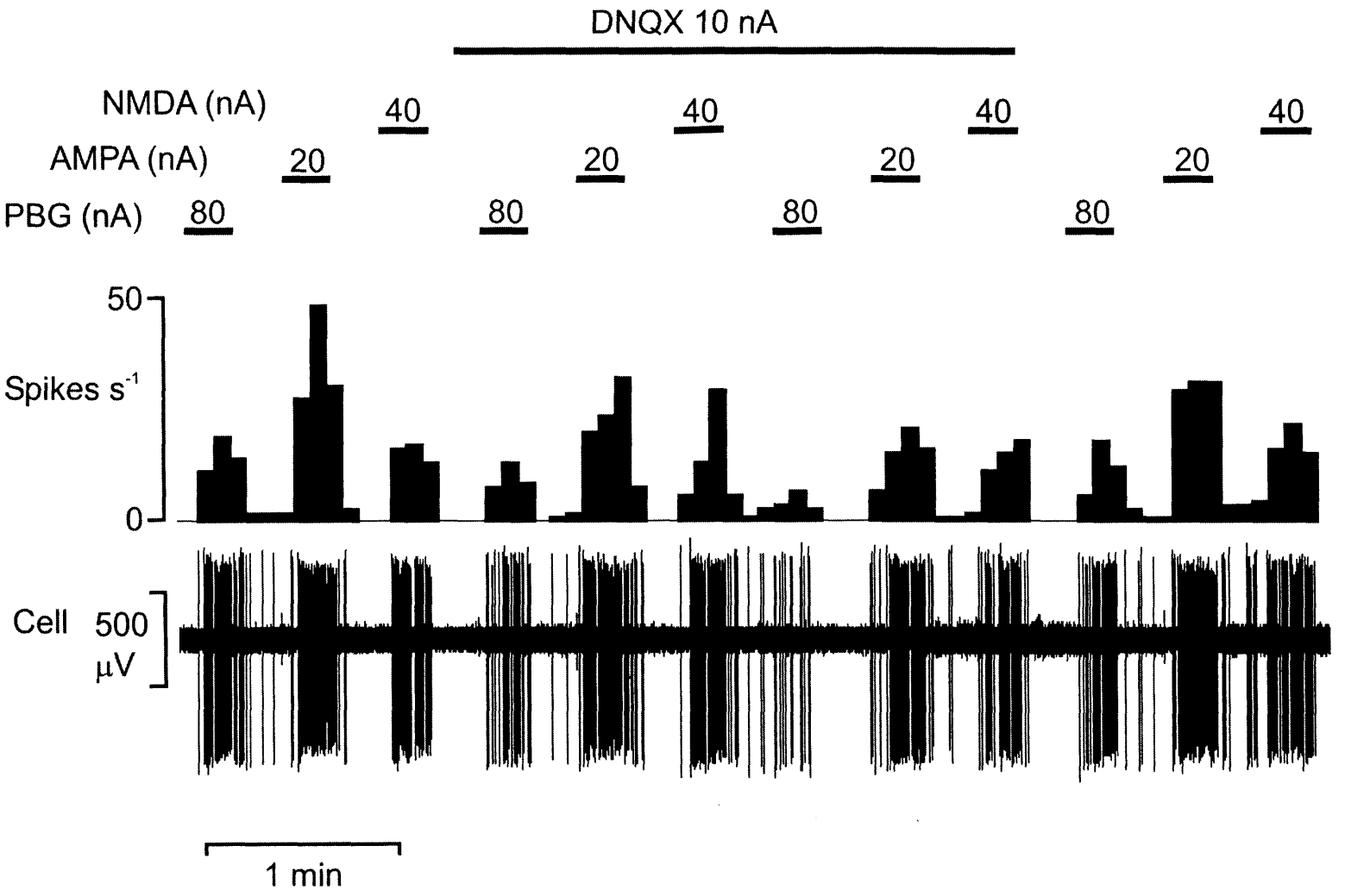

DNQX 10 nA
NMDA (nA)
AMPA (nA)
PBG (nA)
40
20
80
50
Spikes s-1
0
Cell
500
μV
1 min

Figure 5.6 Histograms: effects of DNQX on AMPA and PBG

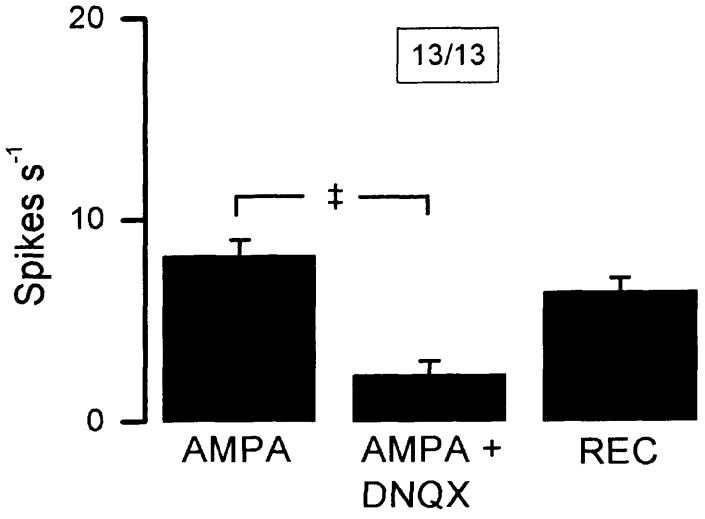
A. Histogram of mean (± s.e.m.) NTS neuronal activity evoked by iontophoretic application of AMPA (n = 13), AMPA in the presence of DNQX (n = 13), and recovery (REC; n = 11).
‡ $P < 0.01$ (Mann-Whitney Rank Sum test).

B. Histogram of mean (± s.e.m.) NTS neuronal activity evoked by iontophoretic application of AMPA (n = 4), AMPA in the presence of the vehicle for DNQX (n = 4), and recovery (REC; n = 4).
NS: non-significant (paired Student's t-test).

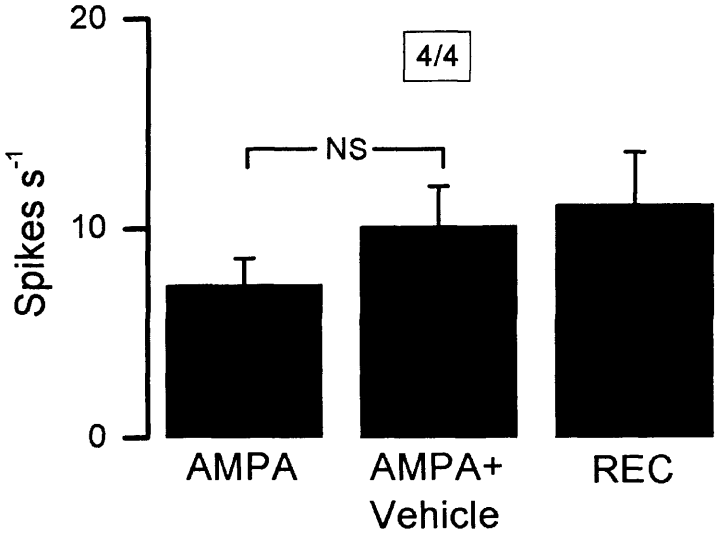
C. Histogram of mean (± s.e.m.) NTS neuronal activity evoked by iontophoretic application of PBG (n = 5), PBG in the presence of DNQX (n = 5), and recovery (REC; n = 4).
NS: non-significant (Mann-Whitney Rank Sum test).

Boxed numbers show proportion of neurones in which the effect was observed.

A


20
10
0
Spikes s-1
13/13
‡
AMPA
AMPA +
DNQX
REC

B


20
10
0
Spikes s-1
4/4
NS
AMPA
AMPA+
Vehicle
REC

C

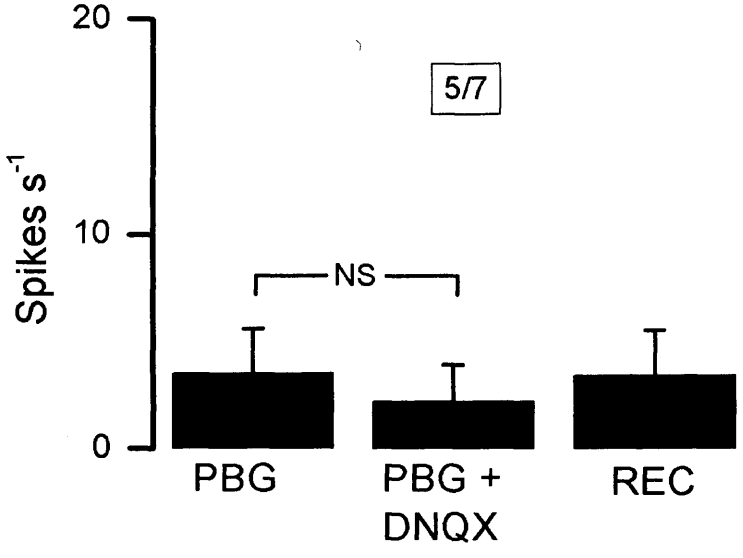

20
10
0
Spikes s-1
5/7
NS
PBG
PBG +
DNQX
REC

Figure 5.7 Histograms: effects of DNQX on NMDA

Histograms illustrating the effects of DNQX on NMDA-evoked NTS neuronal activity, depending on whether firing was inhibited (by > 20 %), attenuated (by > 20 %) or unaffected (< 20 % change).

A: Inhibition. Histogram of mean (± s.e.m.) NTS neuronal activity evoked by iontophoretic application of NMDA (n = 6), AMPA in the presence of DNQX (n = 6), and recovery (REC; n = 6).
NS: non-significant (Mann-Whitney Rank Sum test).

B: No change. Histogram of mean (± s.e.m.) NTS neuronal activity evoked by iontophoretic application of NMDA (n = 4), AMPA in the presence of DNQX (n = 4), and recovery (REC; n = 4).
NS: non-significant (Mann-Whitney Rank Sum test).

C: Potentiation. Histogram of mean (± s.e.m.) NTS neuronal activity evoked by iontophoretic application of NMDA (n = 3), AMPA in the presence of DNQX (n = 3), and recovery (REC; n = 2).

Boxed numbers show proportion of neurones in which the effect was observed.

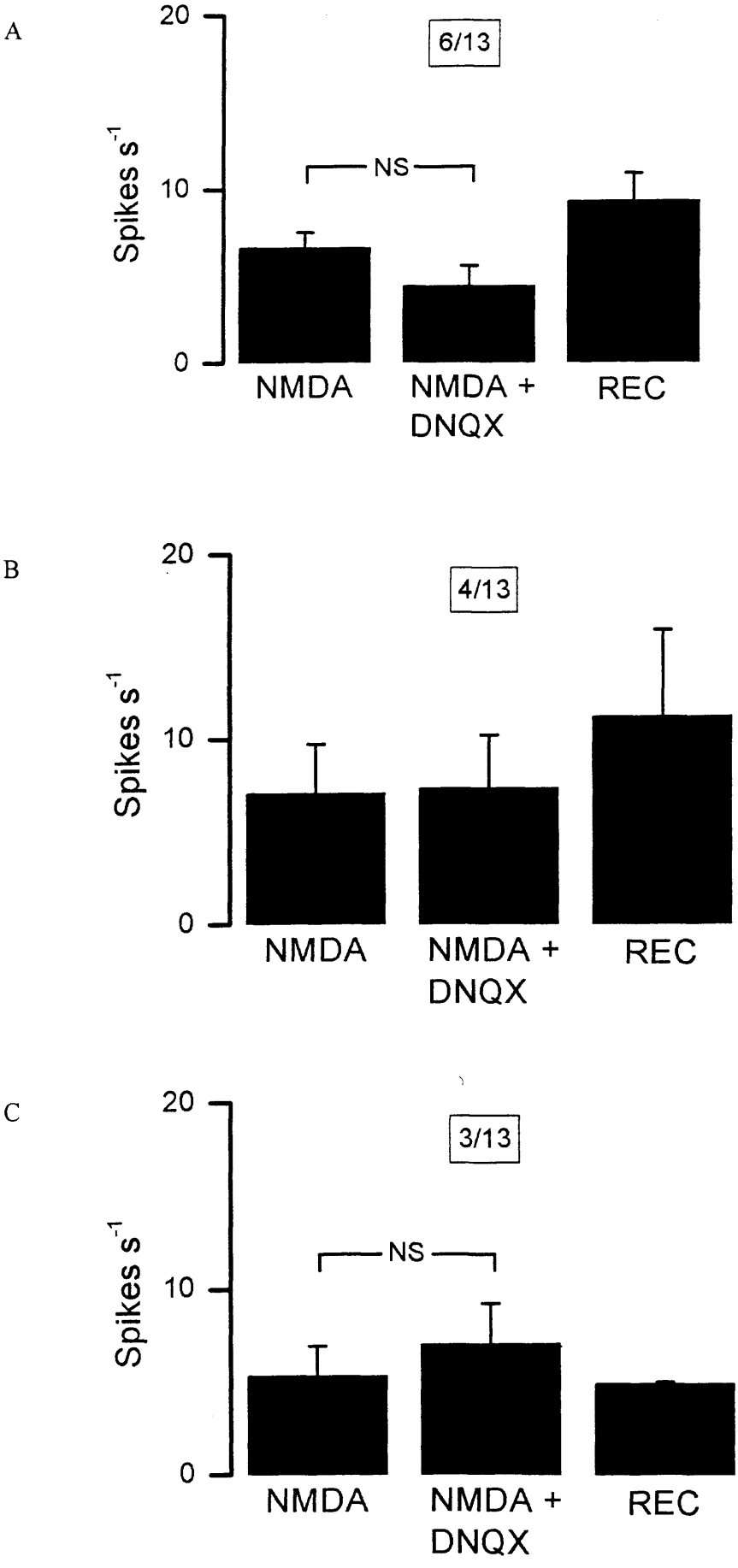

A
20
10
0
Spikes s-1
6/13
NS
NMDA
NMDA + DNQX
REC
B
20
10
0
Spikes s-1
4/13
NMDA
NMDA + DNQX
REC
C
20
10
0
Spikes s-1
3/13
NS
NMDA
NMDA + DNQX
REC

Figure 5.8 Trace of effects of DNQX on cardiopulmonary reflex

Experimental trace from an anaesthetised rat illustrating effect of activating cardiopulmonary afferents with intra-atrial PBG (4 μg) on blood pressure (BP) and NTS neuronal discharge, before (control), 2 min after commencement of iontophoretically applied DNQX (80 nA), and 5 min after termination of the DNQX current (recovery). Heart rate is not shown because the animal is pretreated with the vagolytic neuromuscular blocker gallamine.

Spikes s^{-1}: rate meter (1 s bins)

Numbers in circles indicate number of spikes per burst.

Insets: details of extracellular neuronal activity during reflex-evoked discharge before and during DNQX.

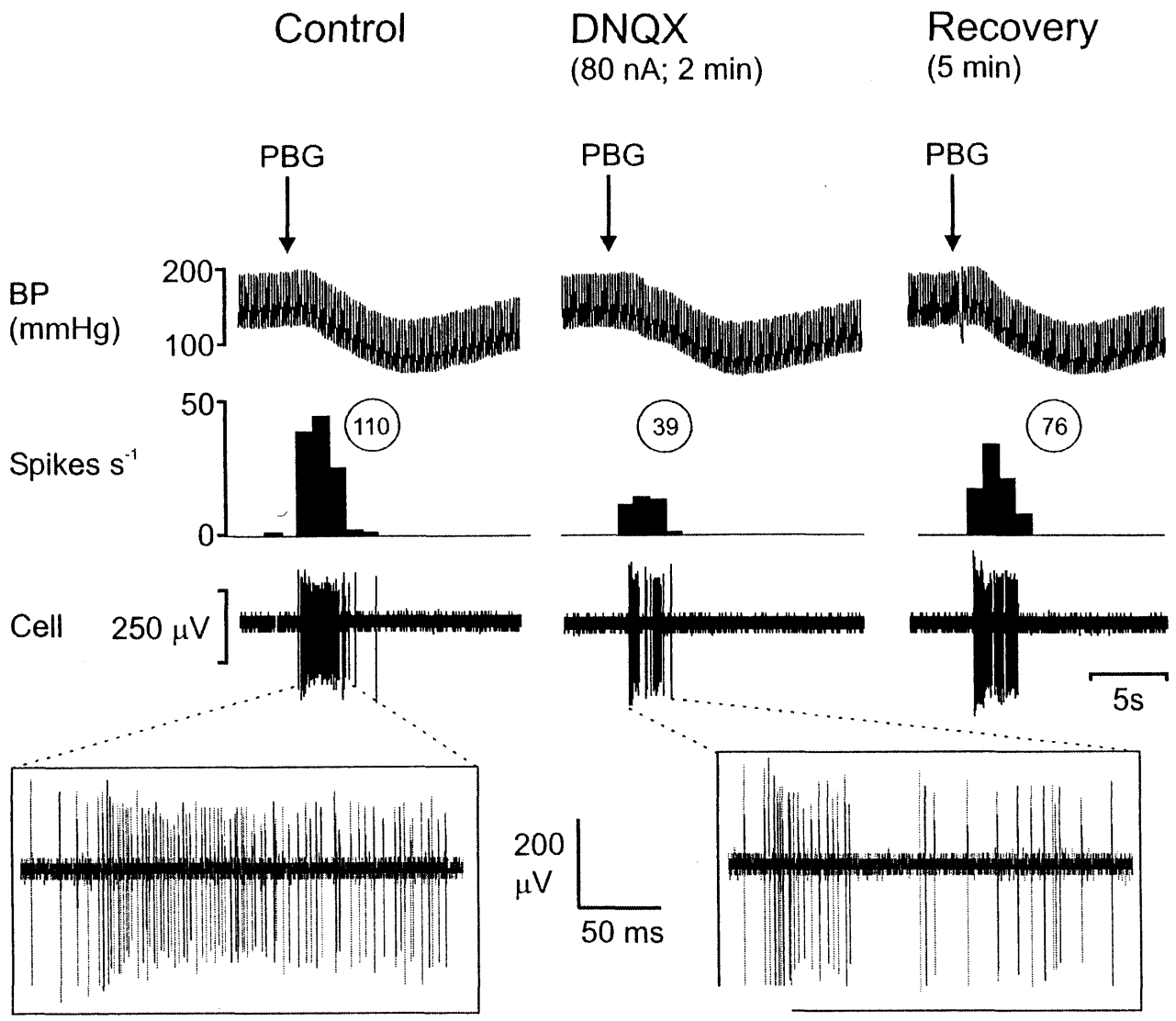

Control
DNQX
(80 nA; 2 min)
Recovery
(5 min)
PBG
PBG
PBG
BP
(mmHg)
200
100
50
0
Spikes s-1
110
39
76
Cell
250 μV
5s
200
μV
50 ms

Figure 5.9 Trace of effects of DNQX on vagus-evoked activity

Experimental trace from an anaesthetised rat illustrating effect of iontophoretically applied DNQX (20 and 40 nA) on vagally-evoked discharge of NTS neurones. The ipsilateral vagus nerve is being continuously stimulated (1 Hz, 1 ms pulse, 150 μA).

Spikes s^{-1}: rate meter (1 s bins)

Insets: 5 superimposed sweeps of evoked extracellular neuronal activity before (left) and during (right) DNQX, illustrating failure of evoked spikes during antagonist application.

* stimulus artefact.

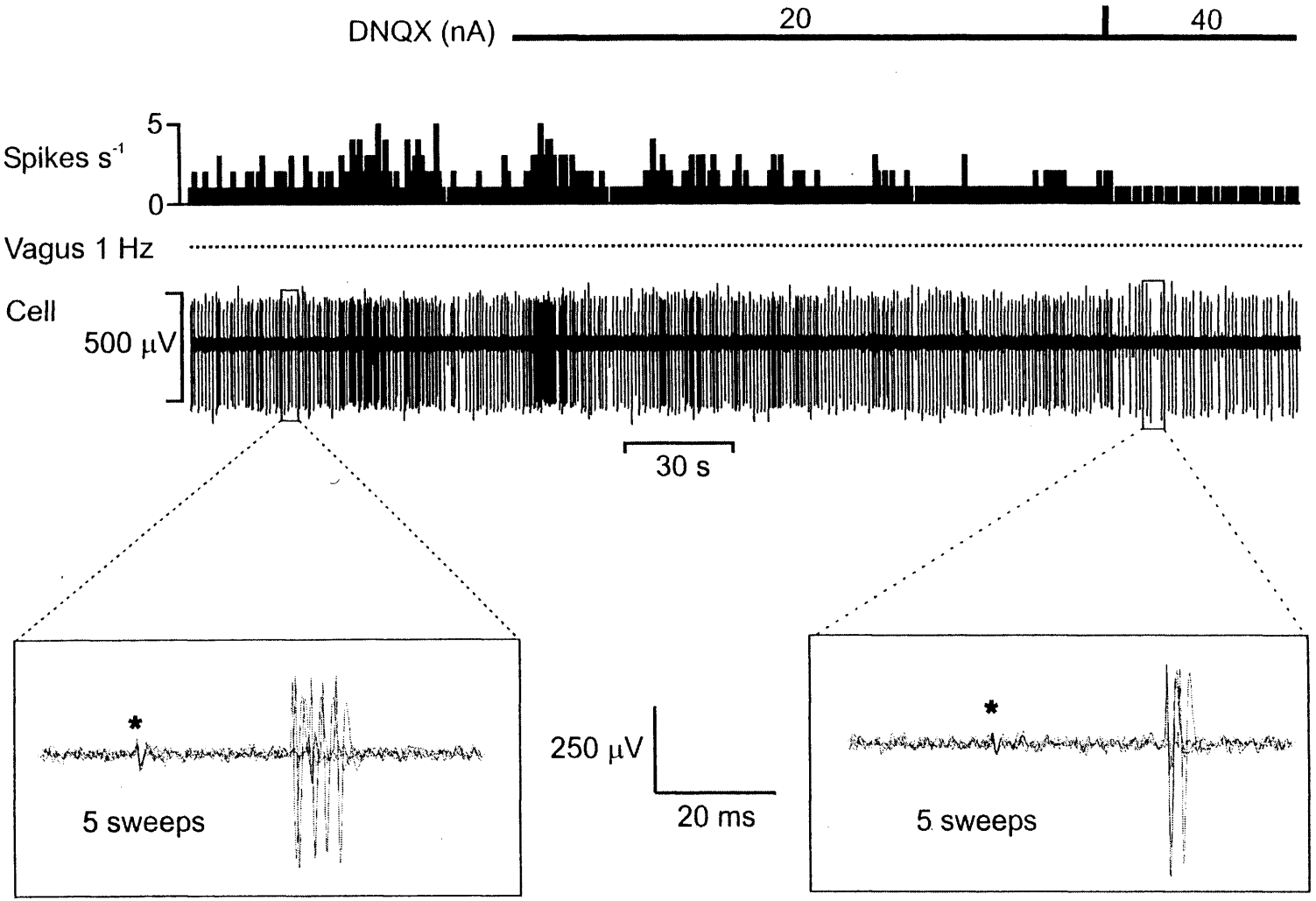

DNQX (nA)
20
40
Spikes s-1
5
0
Vagus 1 Hz
Cell
500 µV
30 s
*
5 sweeps
250 µV
20 ms
*
5 sweeps

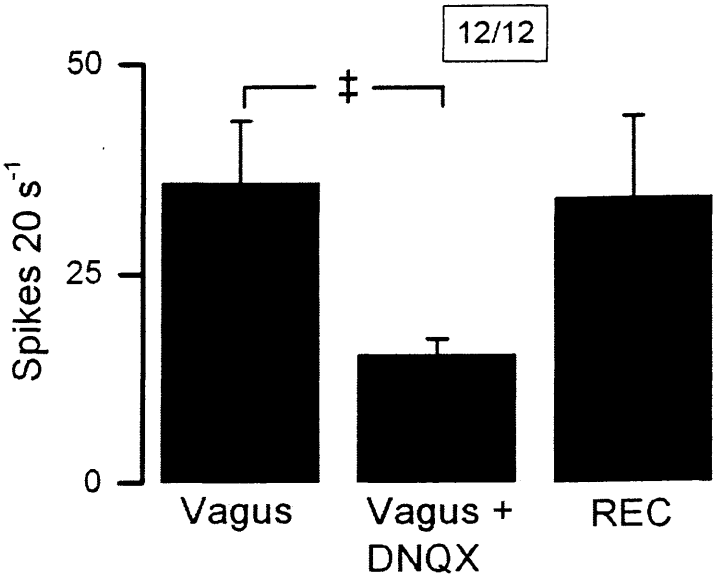
Figure 5.10 Histograms of effects of DNQX on evoked activity

A. Histogram of mean (± s.e.m.) NTS neuronal activity evoked by electrical stimulation (1 Hz) of the vagus (number of evoked spikes per 20 sweeps), both before (n = 12) and during iontophoretic application of DNQX (n = 12), and recovery (REC; n = 11).
‡ $P < 0.01$ (Mann-Whitney Rank Sum test).

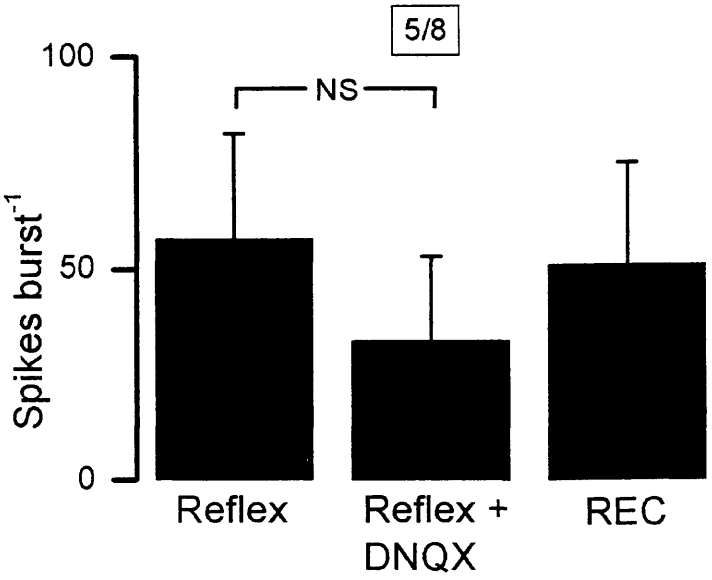
B. Histogram of mean (± s.e.m.) NTS neuronal activity evoked by activation of cardiopulmonary afferents with intra-atrial PBG (Reflex), both before (n = 5) and during iontophoretic application of DNQX (n = 5), and recovery (REC; n = 4). NS: non-significant (paired Student's t-test).

Boxed numbers show proportion of neurones in which the effect was observed.

A


12/12
‡
50
25
0
Spikes 20 s-1
Vagus
Vagus +
DNQX
REC

B


5/8
NS
100
50
0
Spikes burst-1
Reflex
Reflex +
DNQX
REC

5.3.2. Serotonergic study

Effects of intravenous 8-OH-DPAT in the presence of intravenous robalzotan

Intravenous administration of 8-OH-DPAT (65 μg kg^{-1}; n = 5) caused a fall in MAP of 18 ± 7 mmHg (from a baseline of 115 ± 9 mmHg) and a fall in HR of 48 ± 6 bpm (from a baseline of 370 ± 23 bpm). In a separate group, pretreatment with robalzotan (100 μg kg^{-1} i.v.; n = 5) caused a rise in BP of 4 ± 4 mmHg (from a baseline of 117 ± 2 mmHg) and a rise in HR of 34 ± 5 bpm (from a baseline of 384 ±20 bpm). 2 min after robalzotan pretreatment, 8-OH-DPAT (65 μg kg^{-1} i.v.) caused a fall in MAP of 5 ± 1 mmHg, and a fall in HR of 10 ± 2 bpm. This latter effect was significantly inhibited with respect to the non-pretreated group ($P < 0.001$, Student's unpaired t-test).

Effect of SB-269970 on NTS neurones

A total of 10 NTS neurones were recorded from 4 rats at a depth of 595 – 775 μm from the brain surface. All neurones responded to vagal stimulation with a latency of 16 – 38 ms, suggesting activation by C-fibre afferents. Of these, 1 was Type 1 (monosynaptic), 3 were Type 2 (intermediate) and the remaining 6 were Type 3 (polysynaptic). 3 were excited by intra-atrial PBG, 2 were inhibited, and the remaining 5 unaffected. 6 neurones had no spontaneous activity, and 4 had a firing rate of 0.6 – 4.2 Hz.

Topically applied saline (10 μl, pH 5.8; n =5) had no significant effect on vagally-evoked activity (Figure 5.12, Table 9.48).

Topically applied SB-269970 (100 μg kg^{-1}; n = 5) significantly inhibited vagally-evoked activity in the 5th minute after application (Figures 5.11 and 5.12, Table 9.48), with the greatest effect in the 7th minute, compared to saline (23.6 ± 8 *vs.* 47 ± 2 spikes 50 $sweeps^{-1}$). Recovery was observed in the 25th minute.

Effect of SB-269970 on DVN neurones

A total of 10 DVN neurones were recorded from 4 rats at a depth of 690 – 840 μm from the brain surface. All responded to antidromic stimulation of the vagus (as

shown by the collision test; see methods) with a latency of 15 to 29 ms, indicating they had C-fibre axons. Also, 6 of 10 neurones received an additional inhibitory vagal input, as shown by the post-stimulus inhibition of baseline activity. 5 neurones were excited by intra-atrial PBG, 4 were inhibited, and 1 was unaffected. All neurones had spontaneous ongoing activity (mean 4.1 ± 0.5 Hz).

Neither topically applied saline (10 µl, pH 5.8; $n = 4$) nor SB-269970 (100 µg kg^{-1}; $n = 6$) had any significant effect on baseline neuronal activity (Figures 5.13 and 5.14, Table 9.49).

Effect of SB-269970 on gracile neurones

A total of 14 gracile nucleus neurones were recorded in 4 rats, at a depth of 220 – 575 µm. All responded to light touch of discrete parts of the hindquarters or flank; none responded to vagal stimulation. 2 neurones had no spontaneous activity, and the remaining 8 had a mean baseline firing rate of 3.7 ± 1 Hz.

Topically applied saline (10 µl, pH 5.8) had no significant effect on either baseline activity (real or normalised; $n = 5$), nor on touch-evoked activity (real or normalised; $n = 7$; Figures 5.16 and 5.17, Tables 9.50, 9.51, 9.52 and 9.53).

Topically applied SB-269970 (100 µg kg^{-1}; $n = 7$) had no significant effect on either real or normalised baseline activity, or on real evoked activity (Figures 5.15, 5.16 and 5.17, Tables 9.50, 9.52 and 9.53). However, when the evoked activity was normalised, evoked activity was significantly lower in the drug-treated group, at 1, 3, 4, 7, 8, 9 and 10 min post drug (Figure 5.17, Table 9.51).

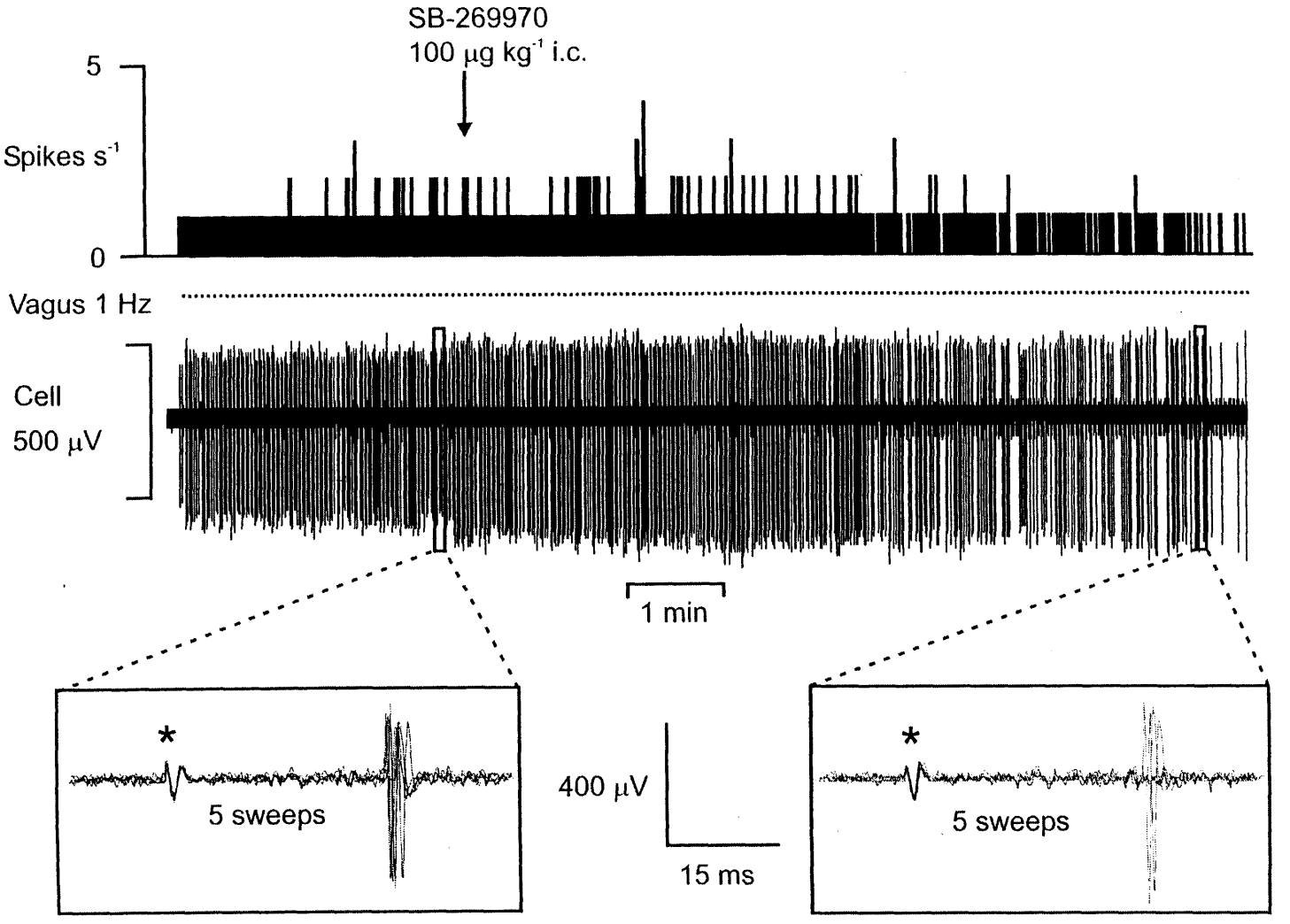
Figure 5.11 Trace of effects of SB-269970 on vagus-evoked activity

Experimental trace from an anaesthetised rat illustrating effect of topically (i.c.) applied SB-269970 (100 μg kg^{-1}) on the vagally-evoked discharge of an NTS neurone. The ipsilateral vagus nerve is being continuously stimulated (1 Hz, 1 ms pulse, 100 μA).

Spikes s^{-1}: rate meter (1 s bins)

Insets: 5 superimposed sweeps of evoked extracellular neuronal activity before (left) and after (right) SB-269970, illustrating failure of evoked spikes during antagonist application.

* stimulus artefact.


SB-269970
100 μg kg-1 i.c.
5
Spikes s-1
0
Vagus 1 Hz
Cell
500 μV
1 min
*
5 sweeps
400 μV
15 ms
*
5 sweeps

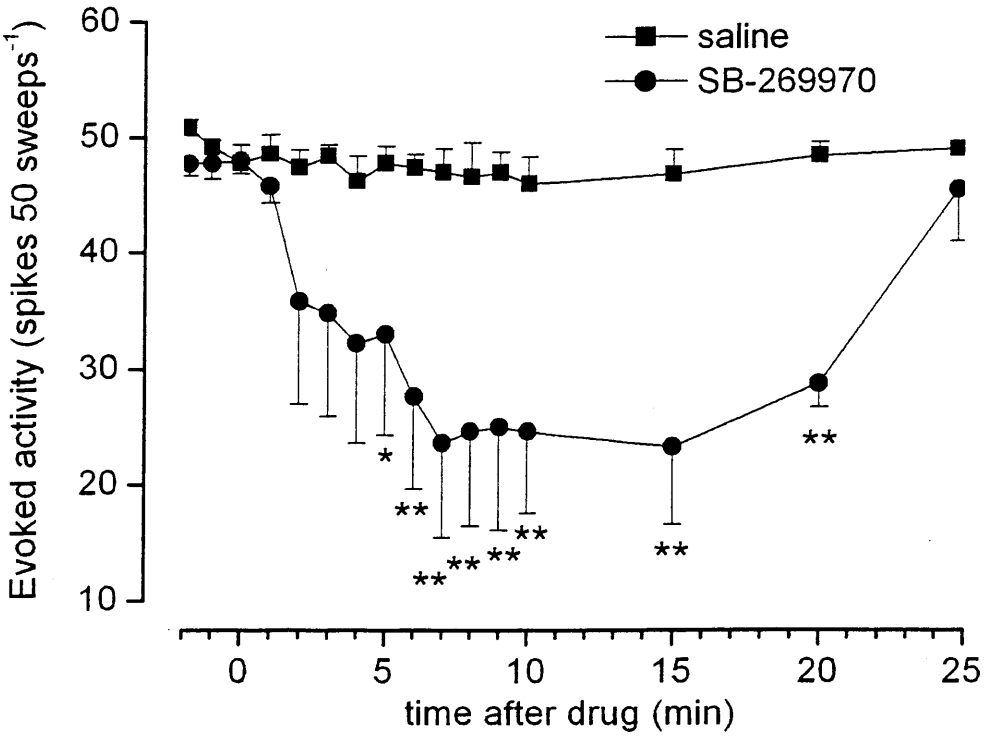


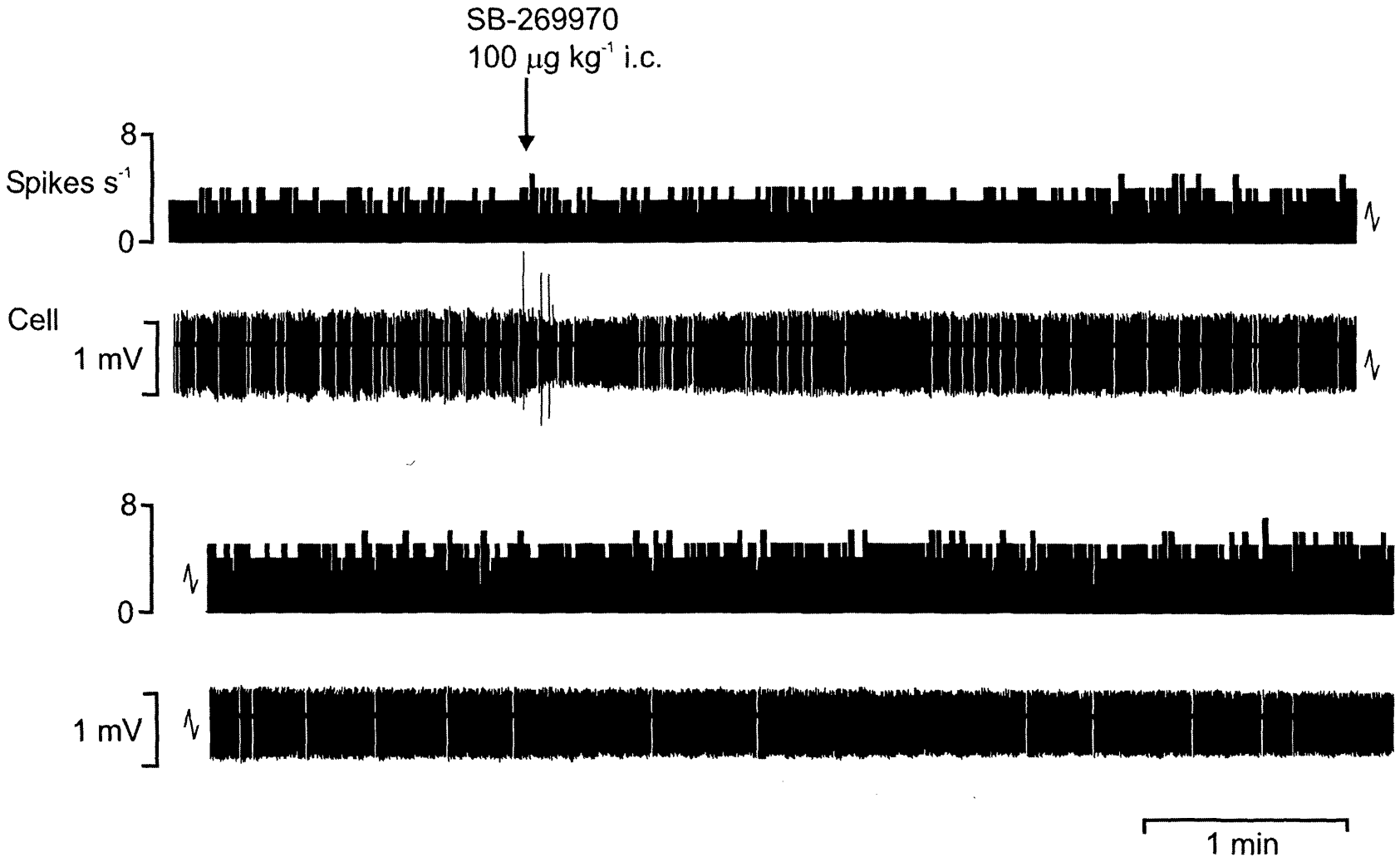
Figure 5.12 Graph of effects of SB-269970 on vagus-evoked activity

Graph illustrating mean (± s.e.m.) effects of topical saline (10 µl; n = 5) and SB-269970 (100 µg kg^{-1}; n = 5) on vagally-evoked NTS neuronal activity in anaesthetised and neuromuscularly blocked rats. * $P < 0.05$, ** $P < 0.01$ (2-way ANOVA & least significant difference test).

Figure 5.13 Trace of effects of SB-269970 on DVN activity

Experimental trace from an anaesthetised and neuromuscular blocked rat illustrating effect of topically (i.c.) applied SB-269970 (100 μg kg^{-1}) on the ongoing discharge of a DVN neurone.

Spikes s^{-1}: rate meter (1 s bins)


SB-269970
100 μg kg-1 i.c.
8
Spikes s-1
0
Cell
1 mV
8
0
1 mV
1 min

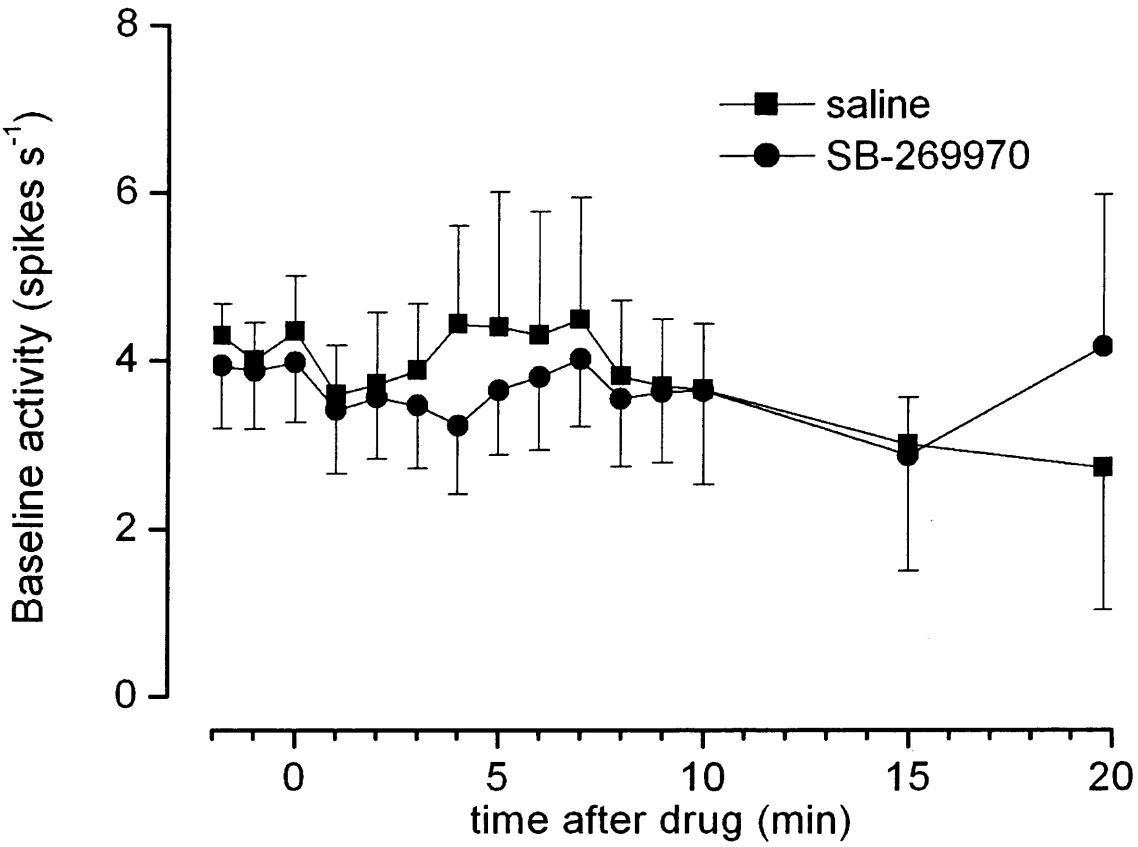


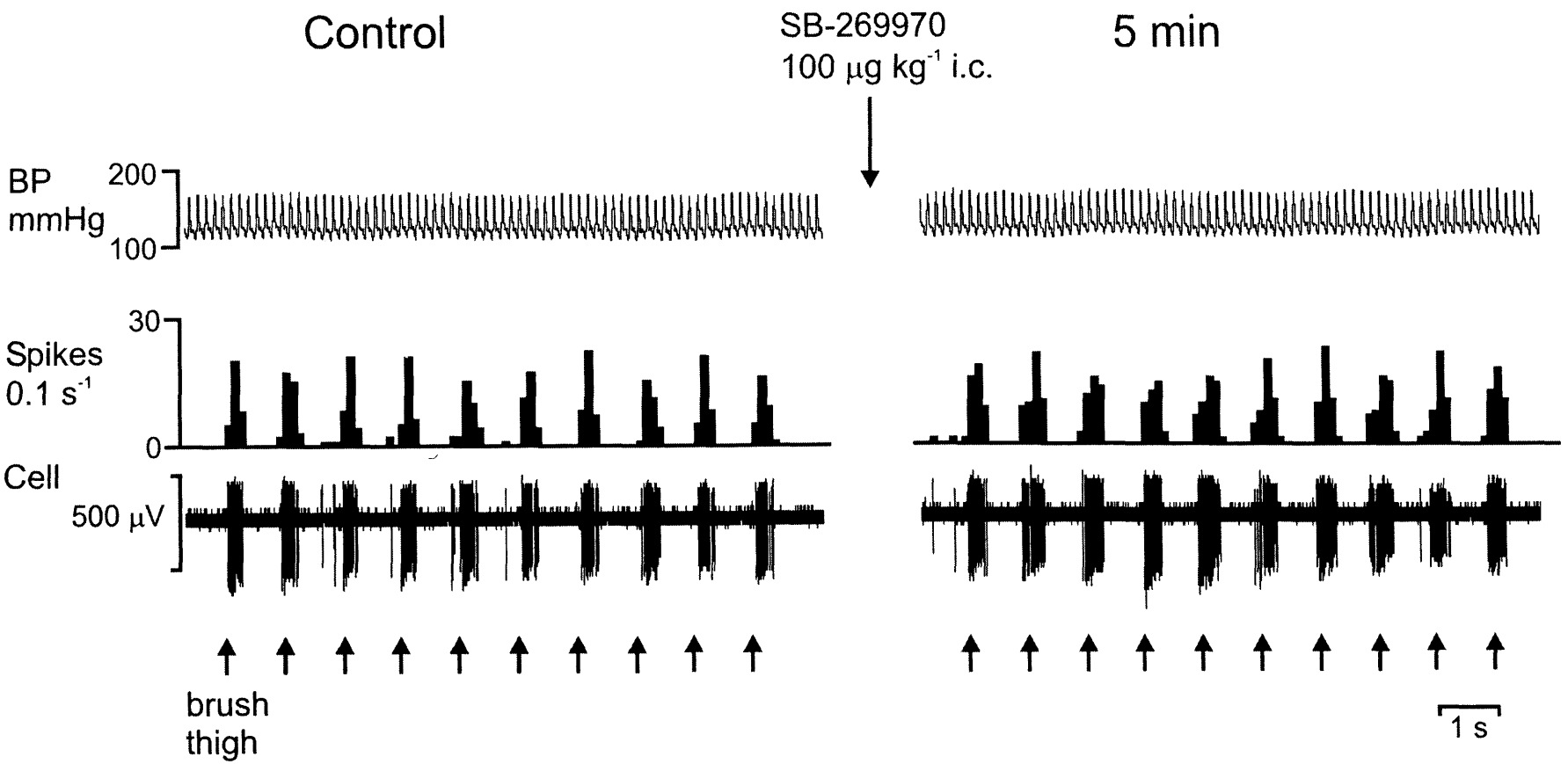
Figure 5.14 Graph of effects of SB-269970 on DVN activity

Graph illustrating mean (± s.e.m.) effects of topical saline (10 µl; n = 4) and SB-269970 (100 µg kg^{-1}; n = 6) on baseline DVN neuronal activity in anaesthetised and neuromuscularly blocked rats.

Figure 5.15 Trace of effects of SB-269970 on evoked gracile activity

Experimental trace from an anaesthetised neuromuscular blocked rat illustrating effect of topically (i.c.) applied SB-269970 (100 μg kg^{-1}) on the discharge of a gracile neurone evoked by lightly brushing the ipsilateral thigh with a cotton bud (arrows).

Spikes 0.1 s^{-1}: rate meter (0.1 s bins)


Control
SB-269970
100 μg kg-1 i.c.
5 min
BP
mmHg
200
100
Spikes
0.1 s-1
30
0
Cell
500 μV
brush
thigh
1 s

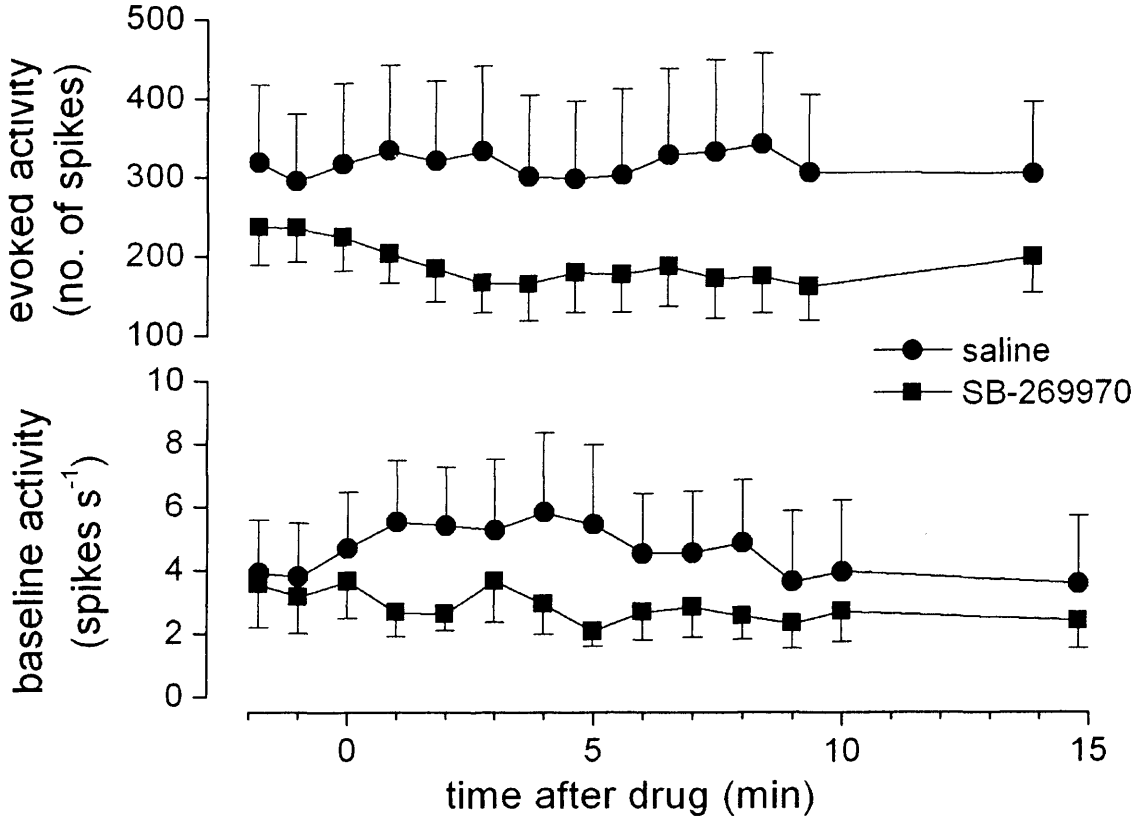


Figure 5.16 Graph of effects of SB-269970 on gracile activity

Graph illustrating mean (± s.e.m.) effects of topical saline (10 μl) and SB-269970 (100 μg kg^{-1}) on touch-evoked (n = 7) and baseline (n = 5) gracile neuronal activity in anaesthetised and neuromuscularly blocked rats.

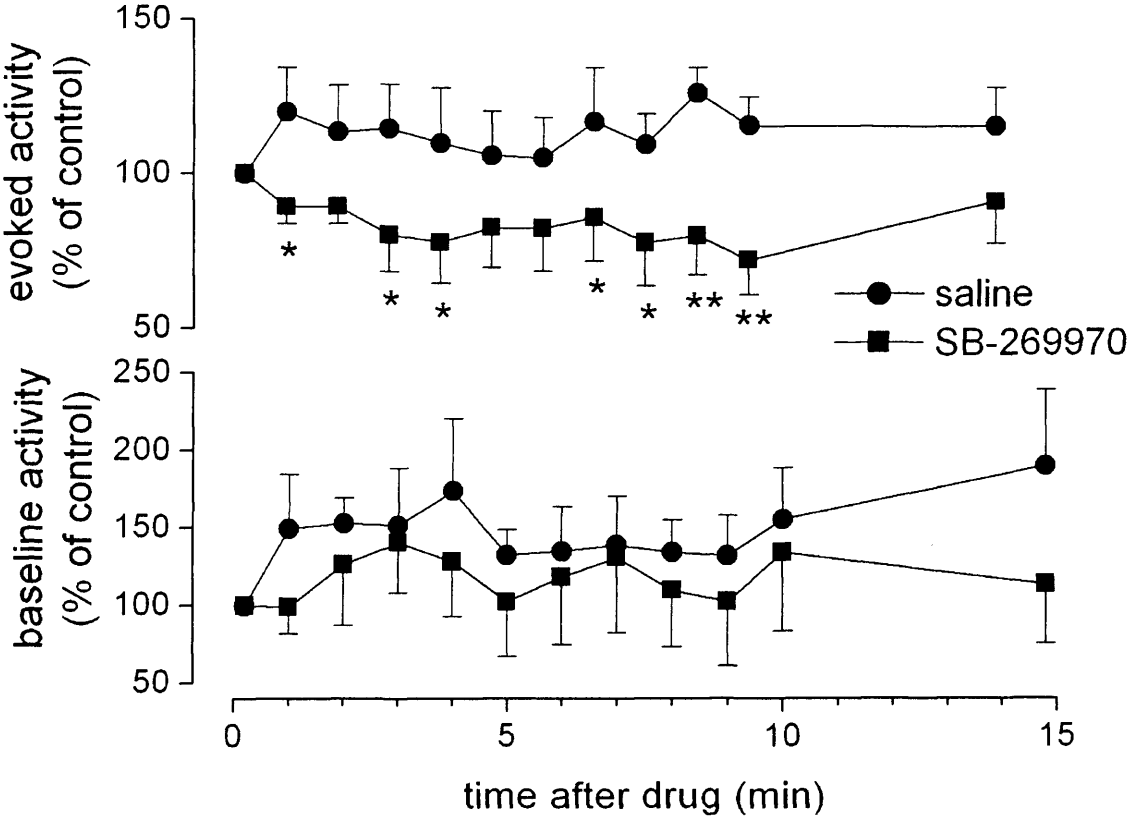


Figure 5.17 Normalised effects of SB-269970 on gracile activity

Graph illustrating normalised (% of control) mean (± s.e.m.) effects of topical saline (10 µl) and SB-269970 (100 µg kg^{-1}) on touch-evoked (n = 7) and baseline (n = 5) gracile neuronal activity in anaesthetised and neuromuscularly blocked rats.

* $P < 0.05$, ** $P < 0.01$ (2-way ANOVA & least significant difference test).

5.3.3. Juxtacellular labelling

5 juxtacellularly labelled neurones were histologically identified (4 or which are illustrated in Figure 5.19, *A – D*). All were found within the NTS, at a depth of 440 – 725 μm. 3 neurones (*A, B, C*) responded to electrical stimulation of the aortic nerve at a C-fibre latency, with a jitter suggesting Type 3 (polysynaptic). These neurones did not respond to vagal stimulation, but one of them responded to i.v. phenylephrine, confirming barosensitivity. The remaining 2 neurones (e.g. *D*) responded to electrical stimulation of the vagus, also at C-fibre (Type 3) latency, but had no spontaneous activity. Aortic nerve stimulation was not tested on those cells.

Light microscopic analysis showed that these neurones are surrounded by a dense network of 5-HT containing axons, but on closer inspection these rarely form close appositions. In Figure 5.19, the varicose terminals at *A* (asterisks) and *E* are clearly not closely apposed, whereas those at *F* and *G* may be forming proper synapses, but not clearly. The only convincing close appositions were at *A* (arrowheads).

Figure 5.18 Juxtacellular labelling trace

Sample trace of a juxtacellular recording from an NTS neurone in an anaesthetised, neuromuscular blocked rat.

2 nA current pulses are being applied *via* the recording electrode, causing entrainment of neuronal firing to the current pulse (beginning at the arrow).

* current ejection stimulus artefacts (truncated for clarity).

Inset below: 5 superimposed sweeps of vagally-evoked NTS spikes taken immediately before, and immediately after a 5 min period of entrainment, indicating that the same neurone is being recorded (spike amplitude has increased with time).

* vagal stimulus artefact.

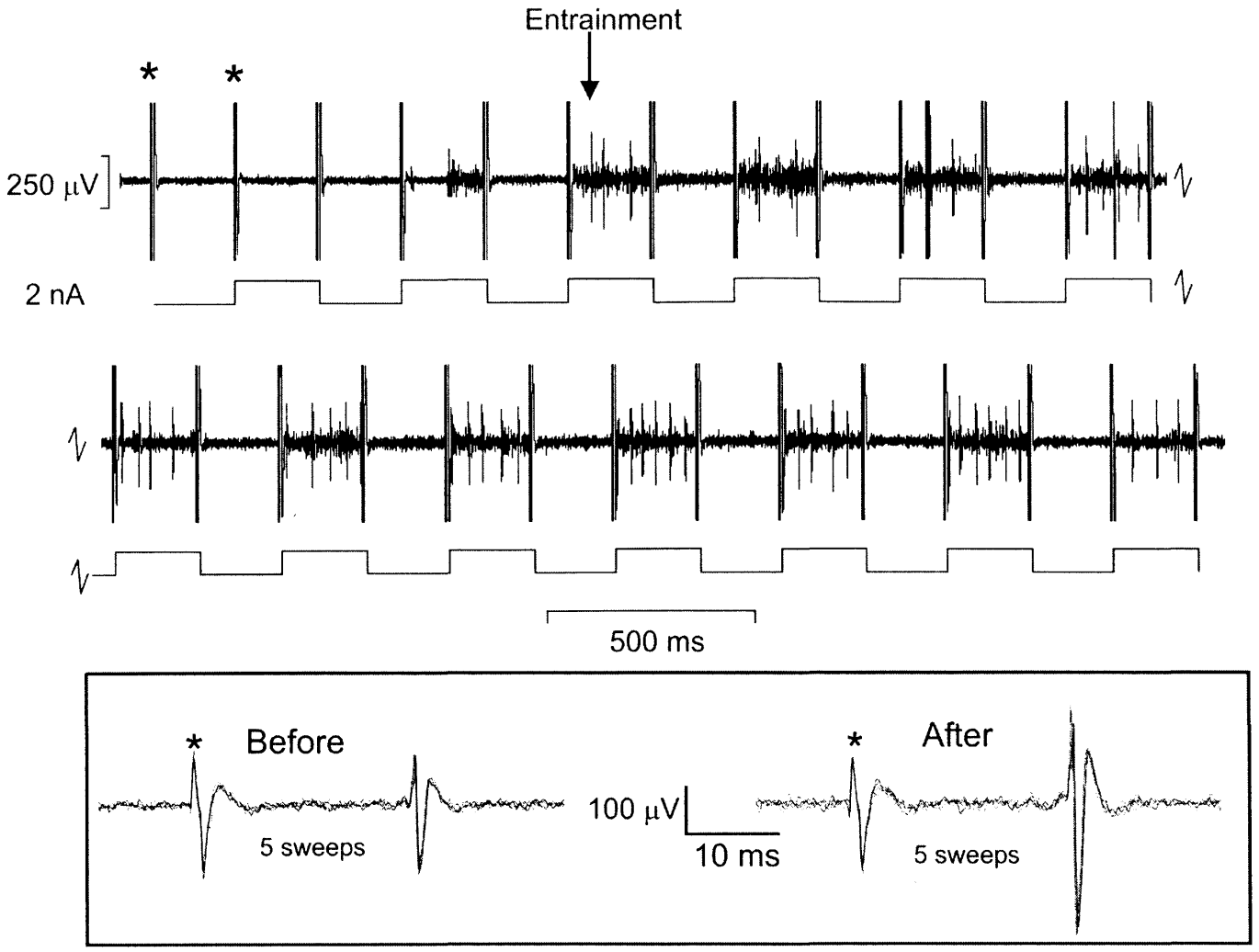

Entrainment
*
*
250 μV
2 nA
500 ms
*
Before
5 sweeps
100 μV
10 ms
*
After
5 sweeps

Figure 5.19 Micrographs of double-labelled neurones

Light micrographs of NTS coronal sections double labelled to show NTS neurones (juxtacellularly labelled with neurobiotin and visualised with DAB-imidazole) in brown, and 5-HT immunoreactive fibres (visualised with nickel-DAB) in black.

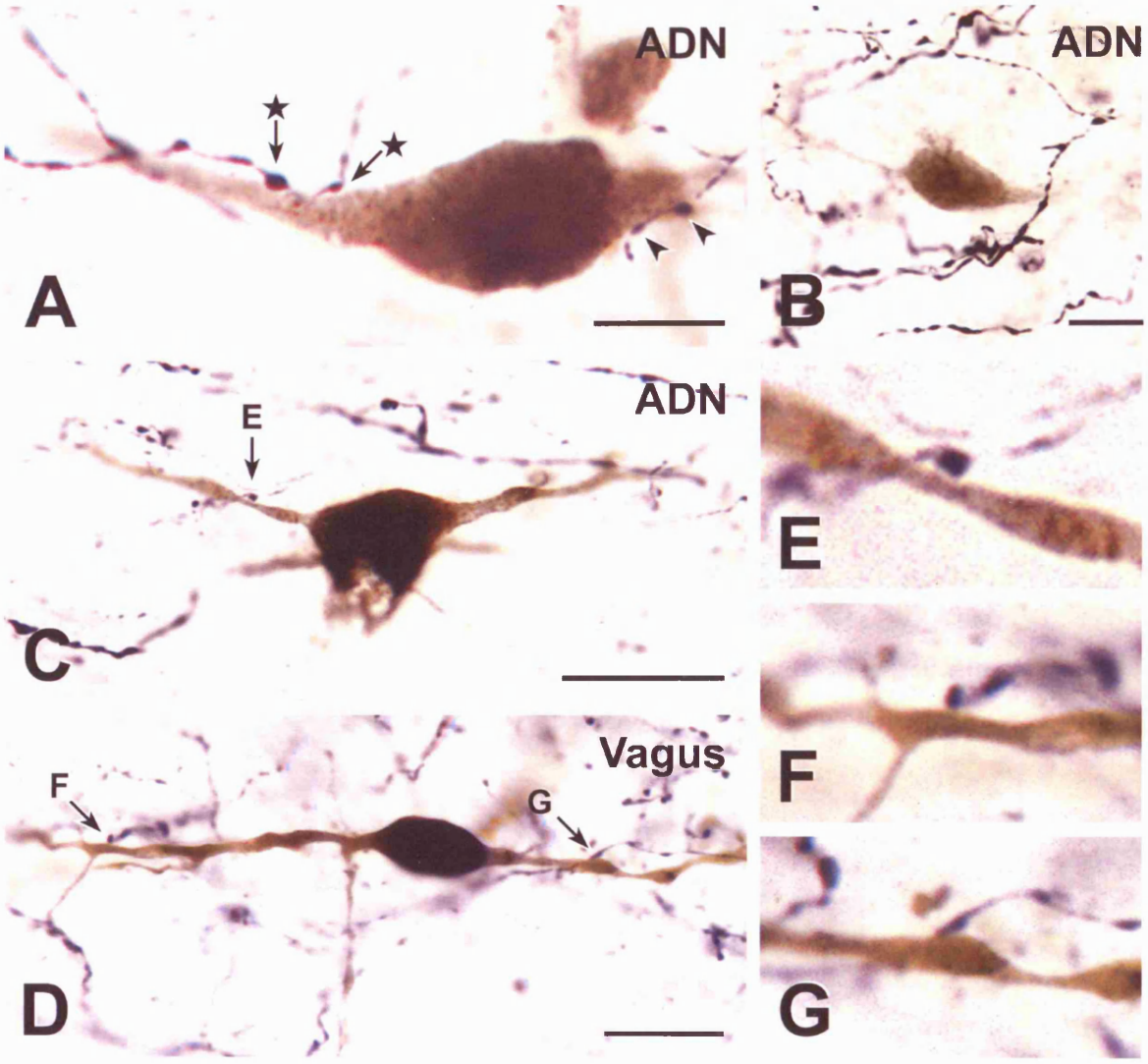
A, B, C: NTS neurones activated by electrical stimulation of the ipsilateral aortic depressor nerve (ADN).

Arrowheads (*A*, ▲) indicate possible close appositions of 5-HT immunoreactive varicosities.
Arrows (↓) indicate probable lack of close apposition (asterisks in *A*, letters in *C*, shown at higher magnification in *E*).

D An NTS neurone activated by electrical stimulation of the ipsilateral vagus nerve.

Arrows (↓) indicate probable lack of close apposition of 5-HT immunoreactive terminals (shown at higher magnification in *F* and *G*).

Scale bars: 20 μm


ADN
A
ADN
B
ADN
C
E
E
F
F
Vagus
G
G
D

5.4. Discussion

5.4.1. Main findings

The above data demonstrate that both AMPA (i.e AMPA/kainate) receptors, and 5-HT_7 receptors, are involved in the activation of NTS neurones by vagal afferents. Firstly, blockade of AMPA receptors with iontophoretically applied DNQX (at currents selective for the AMPA-evoked response) inhibited the activation of NTS neurones by electrical stimulation of the vagus, as well as inhibiting the cardiopulmonary afferent-evoked response in the majority of neurones tested, suggesting that glutamate, acting *via* AMPA receptors is a neurotransmitter at a number of sites within the cardiopulmonary reflex pathway in the NTS. Secondly, iontophoretic PBG excited NTS neurones, and this effect was also attenuated by iontophoretic DNQX, suggesting that 5-HT_3 receptors facilitate glutamate release onto AMPA receptors *via* a presynaptic action. These data complete previous results from this laboratory, which observed the same effects using the NMDA receptor antagonist AP-5 (Jeggo *et al.*, 2001), demonstrating that both AMPA and NMDA receptors are recruited within these pathways. Thirdly, these data show that the 5-HT_7 receptor antagonist SB-269970, given topically at the dose used to attenuate reflex bradycardias, inhibited the excitation of NTS neurones by vagal afferents, suggesting that the blockade of 5-HT_7 receptors by topical or intracisternal SB-269970 is occurring at the level of the NTS. The time course of SB-269970 in this respect was very similar to that observed in the reflex pharmacology of Chapter 3. Furthermore, SB-269970 did not affect the ongoing activity of DVN or gracile neurones, and did not have any clear effect on the ongoing or afferent-evoked excitation of gracile neurones, suggesting that the drug does not interfere with neuronal firing *per se*, and does not have other (e.g. local anaesthetic) actions. With respect to the systems tested, 5-HT_7 receptor activation would appear to be specific to neurones activated by vagal afferents, rather than neurones activated by discriminative touch (skin) afferents. Lastly, juxtacellularly labelled NTS neurones receiving vagal and aortic nerve afferent input were surrounded by a dense network of 5-HT immunoreactive fibres, but closely apposed terminals were very rare, whilst non-apposed terminals were more frequent. This suggests that there is no direct serotonergic innervation of these neurones.

5.4.2. Single unit electrophysiology

Single unit extracellular recordings were make from NTS, DVN and gracile neurones based on their depth from the brain surface and their physiological characteristics. NTS neurones were identified as previously described in this laboratory (Wang *et al.*, 1997), based on their orthodromic activation by vagal afferents. The majority of these neurones had no spontaneous ongoing activity, which is what previous studies using pentobarbitone-anaesthetised rats have reported (Wang *et al.*, 1997; Sevoz-Couche *et al.*, 2000a). Their synaptic position within the NTS (i.e. whether they are second order or higher order neurones) was assessed on the basis of the variability of their evoked latencies (also called synaptic jitter), which is considered the most reliable way of discriminating monosynaptically activated from polysynaptically activated NTS neurones (Doyle & Andresen, 2001). Recorded neurones were separated into 3 groups (Type 1, 2 and 3) based on the criteria of Sevoz-Couche *et al.* (2000a), who found that the intermediate (Type 2) neurones were pharmacologically distinct. For the purpose of this study, however, they can be seen as monosynaptic (Type 1) and polysynaptic (Types 2 and 3). All the NTS neurones recorded in this study had latencies in the C-fibre range (20 – 35 ms), suggesting activation by unmyelinated primary afferents. They tended to be found in the medial and commisural parts of the NTS, where unmyelinated pulmonary, bronchial and chemoreceptors terminated (see Loewy & Burton, 1978; Kalia & Mesulam, 1980; Jordan & Spyer, 1986).

DVN neurones were identified by the collision test, and by their regular ongoing discharge (Wang *et al.*, 1995). All DVN neurones recorded had latencies suggesting unmyelinated efferents. Gracile neurones, which are part of a completely different system from NTS and DVN (the dorsal columns, mediating the sense of discriminative touch) were identified by their superficial position within the brain, their biophysical property of tending to fire in spike doublets, and their response to touch of the skin (i.e. fur) but not to stimulation of the vagus.

Therefore the present experiments have investigated 3 different types of neurone: firstly the autonomic sensory neurones of the NTS activated by primary afferents; secondly the parasympathetic preganglionic neurones of the DVN controlled by

integrative autonomic centres such as the NTS; thirdly (as a non-autonomic control) the sensory neurones in the gracile nucleus, activated by skin afferents *via* the dorsal horn of the spinal cord (Tomasulo & Emmers, 1972).

5.4.3. Role of glutamate receptors

As would be expected, iontophoresis of AMPA and NMDA excited all NTS neurones tested. The AMPA/kainate receptor antagonist DNQX very effectively inhibited the excitation of NTS neurones by AMPA, and was reasonably selective for the AMPA-evoked response at currents of up to 80 nA. Although it inhibited the NMDA-evoked response in some neurones, taken as a group there were no significant effects, suggesting that for the purpose of this study the currents of DNQX used were selective for AMPA receptors. DNQX has a pK_i of 6.3 at AMPA receptors, and of 4.4 at NMDA receptors, but this NMDA receptor antagonism at higher doses is non-competitive, *via* the glycine site at that receptor (see Watkins *et al.*, 1990).

The DNQX solution used in the present experiments contained a small amount of DMSO to aid solubility, as used previously in this laboratory (Jones *et al.*, 2002). DMSO has direct neuronal effects even at these concentrations, as shown by the fact that the vehicle potentiated the AMPA-evoked excitation. This confirms, however, that the inhibitory effects of DNQX are drug-related, not vehicle-related.

AMPA-selective currents of DNQX inhibited the activation of NTS neurones by vagal afferents. All the neurones tested were Type 2 or 3 (presumed higher order). The evoked spikes began to fail within 2 min of current application, and the greatest response was typically at around 3.5 min. This may seem a long time, but is similar to previous iontophoretic studies in the NTS (Jeggo *et al.*, 2000b). The location of the synapse(s) in question may be at some distance from the iontophoresis barrel, so it may take some time for enough DNQX to accumulate at sufficient synaptic concentrations. Since a retaining current is placed on the DNQX barrel between ejections, the charged moiety of the drug may not be at the tip of the barrel at the time that ejection current commences. This can cause further delays.

DNQX also attenuated the cardiopulmonary afferent-evoked burst firing of the majority of NTS neurones tested. Whilst the group data was not statistically significant, the attenuation in the majority was > 20 %, which is a conservative criterion previously established to show change in these experiments, as NTS neuronal firing is relatively stable during pentobarbitone anaesthesia (Wang *et al.*, 1996).

Together these data suggest that glutamate acting at AMPA receptors (as well as at NMDA receptors, as previously shown by Jeggo *et al.*, 2001) is a major neurotransmitter within the NTS. A wealth of information implicates glutamate as the chief transmitter of primary vagal afferents (see Lawrence & Jarrott, 1996). Iontophoresis has previously shown that cardiopulmonary afferents activate second order NTS neurones *via* AMPA (but not NMDA) receptors (Wilson *et al.*, 1996), and subsequent work *in vivo* and *in vitro* suggested that NMDA receptors are found on higher order but not second order NTS neurones (see Andresen *et al.*, 2004). Whilst the present experiments support the view that glutamate is a transmitter within the NTS (i.e. between second and higher order neurones), recent work in this laboratory also suggests that NMDA receptors are found at second order as well as higher order neurones (Jeggo, 2003; Jones & Jordan, unpublished data). In the present experiments it cannot be ruled out that DNQX is diffusing as far as the synapse of primary afferents with second order neurones, but it is unlikely to have effects here for the following reason: the effect of DNQX on the AMPA-evoked response was one of inhibition not total blockade, indicating only partial receptor occupancy at this relatively proximal site. Therefore at a site as distant as several synapses away, the concentration of DNQX would probably be too low to antagonise synaptic events.

5.4.4. Role of 5-HT_3 receptors

Iontophoretic PBG excited the majority of neurones tested, which confirms previous data (Jeggo *et al.*, 2001), although in the present study the excitation was not as great. This may be because the recording barrel was positioned further from the iontophoresis barrel in the compound electrode construction, so PBG has to diffuse further. Iontophoretic PBG-evoked excitation has previously been shown to be inhibited both by 5-HT_3 and NMDA receptor antagonists in the NTS (Jeggo *et al.*,

2001) and by 5-HT_3, NMDA and AMPA receptor antagonists in the DVN (Wang *et al.*, 1998), suggesting that in both areas 5-HT_3 receptors are facilitating glutamate release by a presynaptic mechanism. This facilitation had already been reported in the NTS using microdialysis (Ashworth-Preece *et al.*, 1995). The glutamatergic fibres involved may be primary afferents, or the axons connecting second order and higher order neurones within the NTS. In the both these and the present experiments, a mixture of presumed second and higher order neurones were tested, and pharmacological differences were not identified.

An opposing role for 5-HT_3 receptors in the NTS has also been proposed by studies using microinjection. NMDA-induced bradycardia in awake rats was prevented by prior microinjection of a 5-HT_3 receptor agonist (Bonagamba *et al.*, 2000), suggesting that 5-HT_3 receptors can inhibit glutamate release, presumably by activating an inhibitory pathway. Indeed in earlier studies, microinjection of 5-HT_3 agonists into the NTS attenuated cardiopulmonary and chemoreflex bradycardias, the latter shown to be mediated by $GABA_A$ receptors (Sevoz *et al.* 1996, 1997). Although the larger-scale nature of these experiments (i.e. microinjection) is difficult to compare to the single unit iontophoretic studies of this laboratory, this disagreement might also imply two functional populations of 5-HT_3 receptors in the NTS, but this remains to be determined. Indeed, Jeggo (2003) found that less than 1 % of vagally-activated NTS neurones were inhibited by iontophoretic PBG, at least in this caudal/commisural part of the NTS.

5.4.5. Role of 5-HT_7 receptors

The intracisternal experiments of Chapter 3 have clearly defined the effective doses and time course over which the 5-HT_7 receptor antagonist SB-269970 attenuates reflex bradycardias. The NTS seemed the most likely location of the 5-HT_7 receptors involved, for the following reasons: firstly, it is within easiest reach of i.c. injections; secondly, it is the site of the greatest complexity in terms of neurochemical integration of cardiovascular reflexes; thirdly, it is very rich in 5-HT containing fibres. SB-269970 was administered topically because the effective dose was known. Possible negative iontophoretic data would be more difficult to interpret. Another speculation was that the 5-HT_7 receptors mediating bradycardia are located in the

nucleus ambiguus, as are 5-HT_{1A} receptors in the cat (Wang & Ramage, 2001). If this were the case, topical SB-269970 would have no effect on NTS neurones.

For the first few minutes, topical SB-269970 had little effect on vagus-evoked NTS activity. At around 5 min the evoked spikes began to fail, as they did during iontophoretic DNQX. In one experiment evoked activity was virtually abolished for several minutes. Such an effect suggests that a major neurotransmitter pathway is blocked, and would support the view that 5-HT is released from primary afferents. Some afferents with cell bodies in the nodose ganglia (i.e. cardiopulmonary and aortic baroreceptor fibres) contain 5-HT (Gaudin-Chazal *et al.*, 1982; Nosjean *et al.*,1990; Sykes *et al.*, 1994), but little is known of their function. Ablation of 5-HT containing fibres with 5,7-DHT microinjected into the nodose ganglia, or into the NTS, did not noticeably affect the baroreflex (Orer *et al.*, 1991, but see also 4.4.3 for discussion). Otherwise there is no further functional data on these putative fibres. However, the strength of the effect of SB-269970 in the present experiments would suggest more than a neuromodulatory role for 5-HT in the NTS.

In the drug-treated group, only 1 neurone was presumed second order, and this was inhibited in a similar way to the remaining presumed higher order neurones. Topical application will certainly reach all neurones in the NTS, even more so than microinjections. Hence this technique cannot unequivocally identify the synaptic localisation of the 5-HT_7 receptors. At the very least many more second order neurones need to be tested, but at this stage it is unclear whether the receptor is located on second order or higher order neurones, or both.

It was considered that topical SB-269970 might be interfering directly with neuronal activity. As the NTS neurones recorded had little if any ongoing activity, the drug was tested on DVN neurones. These have a regular ongoing discharge, which was unaffected by the drug, suggesting it has no serious non-serotonergic effects (e.g. blockade of Na^+ or activation of K^+ channels). A possiblity for the lack of effect, however, is that concentrations of the drug are insufficient once it has diffused as far as the DVN. To eliminate this possiblity, and to examine the effect on a non-autonomic system, SB-269970 was tested on gracile neurones.

Gracile neurones typically have ongoing discharge, though not as regular as DVN neurones, and are highly excited by light touch of their receptive field (usually the hindlimbs and surrounding areas). Touch with a cotton bud or paint brush is a reproducible non-noxious stimulus frequently used in the study of Aβ *vs.* Aδ and C-fibre inputs to the spinal cord (Dickenson & Sullivan, 1986), and in the present experiments produced consistent responses. SB-269970 had no effect on baseline neuronal activity, confirming the lack of effect on DVN neurones. On the evoked response there was no significant difference, but the considerable variability of the saline group led to the results being normalised in case this revealed any differences. There were indeed differences at some time points (1, 3, 4, 7, 8, 9 and 10 min post drug), which is difficult to interpret. Taken individually, of the 7 neurones in the drug-treated group, 6 were not affected by more than 20 %, whereas 1 was strongly and irreversibly inhibited. Possibly this neurone represents a separate population, but it is more likely that a change in responsiveness took place (perhaps at the level of the receptive field, as its spontaneous activity was unchanged). It is therefore unlikely that 5-HT_7 receptors have any role in the transmission of touch, at least at this level of the brain. Therefore SB-269970 seems to be specific in reducing the excitability of NTS neurones in response to vagal stimuli. This demonstrates, for the first time, the effects of SB-269970 on neuronal activity *in vivo.*

5.4.6. Juxtacellular labelling

The method of Pinault (1996) is a useful way of revealing the soma, dendritic field and even axonal projections of an extracellularly recorded neurone, and has previously been used in this laboratory to characterise NTS neurones physiologically, pharmacologically, and immunocytochemically (Jones *et al.*, 2002). In the present experiments no pharmacological characterisation was performed, and only basic physiology. All neurones were activated by either the vagus or aortic nerve, at C-fibre latency. Immunocytochemistry for 5-HT showed a dense network of 5-HT containing fibres surrounding the somata and dendrites of these NTS neurones. In some instances there appeared to be close appositions between varicosities and NTS neurones, but closer inspection found that these tended not to touch. This brings to mind the original *reticularist* view of Golgi in the late 19th century, that axon terminals are fused to dendrites (based on light microscopic appearance), whereas

Ramon y Cajal's *Neurone Doctrine* insisted they were *touching, not fused.* The examples in Figure 5.19 certainly look neither touching, nor fused, suggesting that they are not conventional synapses.

The lack of specialised synapses would support the original idea of monoaminergic transmission being diffuse and non-specific (e.g. Descarries *et al.*, 1975) – that monoamines are required to diffuse to nearby targets, like a neural aerosol. This idea was based more on endocrinological ideas than on hard neurophysiology, and was discredited by the finding of conventional specialised synapses (see Parnavelas & Papadopoulos, 1989). Electron microscopic analysis of the feline NTS also found 5-HT containing terminals making synaptic contacts with NTS neurones (Maley & Elde, 1982). However, the function of these NTS neurones was not known, so whether this extends to cardiovascular neurones was uncertain. A recent study, however, examined the ultrastructure of barosensitive NTS neurones (expressing c-fos in response to elevated MAP), and reported that out of 53 synaptic inputs to c-fos positive neuronal cell bodies, not a single one contained 5-HT (Llewellyn-Smith *et al.*, 2004). Even c-fos negative NTS neurones received very few (~2 %) 5-HT containing synapses, compared to non-5-HT ones. Staining with an antibody for SERT revealed similar results. This suggests that on barosensitive NTS neurones, serotonergic innervation is sparse and, if present, targetted mainly to distal dendrites. However, the researchers noticed that thin astroglial leaflets usually separated NTS neurones from 5-HT containing terminals. This could account for the lack of close appositions seen with light microscopy, and could represent an entirely new dimension of monoaminergic transmission.

Astrocytes were originally believed to have a supportive, relatively passive role. More recent observations, almost exclusively *in vitro*, show that astrocytes also behave like additional targets of neuronal signals (see Fellin & Carmignoto, 2004). One role of astrocytes was to sequester glutamate, and they can also release glutamate. The crucial observation was that elevating intracellular calcium ($[Ca^{2+}]_i$) in cultured astrocytes causes release of glutamate, which activates ionotropic glutamate receptors on neighbouring neurones, increasing their $[Ca^{2+}]_i$ (Parpura *et al.*, 1994). Similar effects have been recently reported in the cortex of the anaesthetised rat (Hirase *et al.*, 2004). Hence the emerging theory of

gliotransmission proposes that astrocytes can release neurotransmitter onto neurones to transmit information in the CNS. This release is likely to be vesicular (Calegari *et al.*, 1999; Araque *et al.*, 2000), at least in the hippocampus. Astrocytes can respond to various neurotransmitters including glutamate, GABA, noradrenaline and acetylcholine (see Fellin & Carmignoto, 2004), and recent research proposes that astrocyte processes alongside a synapse respond to the transmitters within it, and then release their own transmitters as part of a feedback or feed-forward mechanism. The speculation from the present experiments and the findings of Llewellyn-Smith *et al.* (2004) is that 5-HT released from a serotonergic terminal onto an astrocyte process causes release of glutamate onto an NTS neurone. This could help explain the interaction between serotonergic and glutamatergic mechanisms in cardiovascular reflexes. Such an arrangement of terminal-astrocyte-dendrite (i.e. lacking any synaptic specialisation) has not previously been described, and remains hypothetical. Astrocytes do express a variety of receptors, however, and 5-ht_{5A} receptor mRNA is particularly prominent in rat glia (Carson *et al.*, 1996) at least during development.

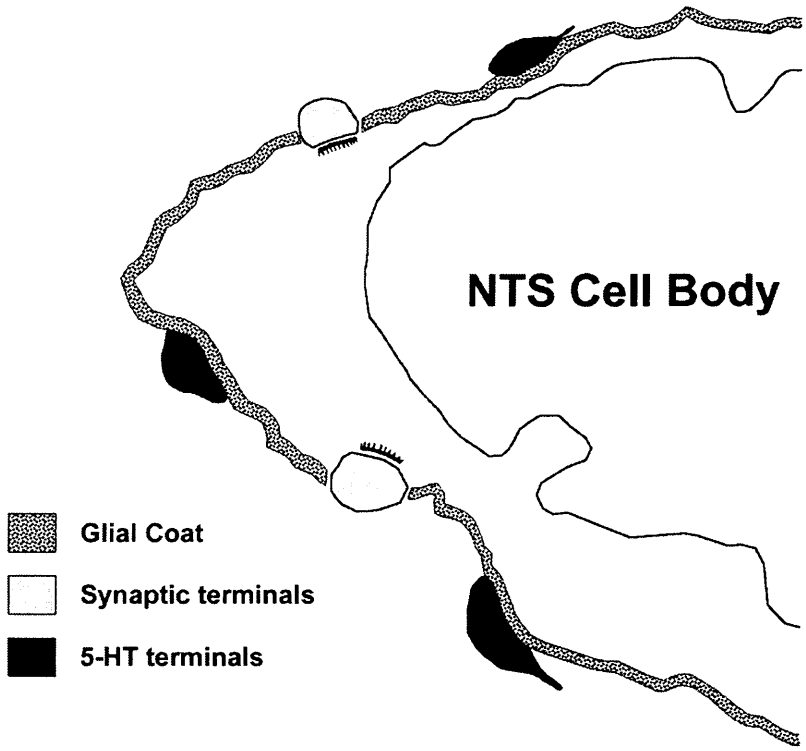


Figure 5.20 Diagram of NTS ultrastructure

Diagram of a barosensitive NTS neurone (adapted from Llewellyn-Smith *et al.*, 2004) as seen by electron microscopy, illustrating non-5-HT axon terminals (light grey) making conventional synaptic contact, whereas 5-HT containing axons terminals (black) are separated from the neurone by a glial coat (grey).

5.4.7. Limitations

Iontophoresis

In this study, care was taken to demonstrate that the currents of DNQX used were indeed selective for AMPA receptors, and this was done by showing inhibition of the effects of iontophoretic AMPA, but not of NMDA. This poses the following problem: iontophoretic agonists will act at receptors closest to the multibarrel aperture, and thus selectivity is true for the concentration of antagonist found at this site. However, the effects of the antagonist on synaptic actions (e.g. vagus-evoked activity) are likely to be at some distance from the iontophoresis barrels. It cannot be known how far away on a dendrite the synapse or synapses in question are located. The concentration of antagonist needed to inhibit synaptic actions also tends to be higher than that needed to inhibit exogenous agonists. Therefore this method of showing selectivity can be a problem, particularly when there is negative data (i.e. if the drug is not selective for agonists, it does not mean it will not be selective at synapses; conversely if it *is* selective for agonists, it may not be sufficiently concentrated at synapses). Here, however, DNQX is selective for AMPA, *and* inhibits synaptic events, therefore there should not be a problem.

In this study no controls were performed to monitor the effect of iontophoretic current ejection on neuronal activity (i.e. the effect of passing a negative iontophoretic current through saline at pH 8.5 – 10.5). However, the Neurophore system used in this study does use automatic current balancing. Previous studies in this laboratory indicated that iontophoresis of saline at this pH has no effect on neuronal activity (Wang *et al.*, 1998; Jeggo, 2003).

Topical application

As comparison is being made between these results and those of Chapter 3, the topical drug was not applied to the dry surface of the medulla, but to the layer of CSF covering it. As such it was as close to an i.c. injection as possible. However, it is possible that the final concentration of the solution on the surface is higher than that in Chapter 3, and that the diffusion characteristics are altered by the exposure and

partial drainage of the cisterna magna. Therefore some selectivity problems with the drug cannot be ruled out.

Juxtacellular labelling

The juxtacellular technique is becoming an increasingly popular way of identifying recorded neurones. It is technically easier than directly filling a neurone with HRP, which requires stable intracellular recording. However, unlike intracellular filling, there is less certainty that the recorded neurone has been filled with the juxtacellular technique. There is always the possibility that between recording and filling (or during the filling process) some movement of the electrode has occurred. However, the shape and amplitude of spikes are always compared during the entire recording-filling protocol to minimise this possibility. For example, in Figure 5.18 the shape of the spike is identical before and after filling, although the amplitude has increased (presumably due to increased impedance of the electrode), suggesting it is the same neurone. Using the technique of juxtacellular labelling, only the neurone that is entrained to fire by current pulses is labelled. If the current pulses are comparatively small (< 10 nA, as in the present experiments) the chance of entraining multiple neurones is very low.

5.4.8. Conclusion

The data support the view that glutamate is the major transmitter between vagal afferents and NTS neurones, and between second order and higher order NTS neurones. Furthermore, this release of glutamate is aided by 5-HT_3 receptors located presynpatically on glutamatergic terminals. However, 5-HT_7 receptors must now be included as major players in the NTS. A variety of reflex bradycardias have been shown to involve 5-HT_7 receptor activation, and the present experiments have provided compelling preliminary evidence that they are located in the NTS. Indeed, the inhibitory effect of SB-269970 on vagus-activated NTS neurones was so great that the possibility must be considered that serotonergic and glutamatergic primary afferents are working in tandem. From histological examination, however, the synaptic arrangment of 5-HT containing fibres in the NTS has been given an added complexity. Either the dated but long-held dogma of serotonergic transmission as diffuse (like an aerosol) is resurfacing in the present data, or another mechanism is

emerging. This latter possibility might be the increasingly popular theory that astrocytes participate in neurotransmission.

5.4.9. Future experiments

The effects of SB-269970 and of another antagonist (i.e. SB-656104) need to be confirmed topically using a lower dose, and also iontophoretically. It is vital to elucidate whether the effects are occuring at second order or higher order neurones. Whether WAY-100635 (and other 5-HT_{1A} receptor antagonists) have similar effects at the NTS must also be addressed, as this would imply a dual location of the receptors at NTS and nucleus ambiguus (comparable to the supraspinal and spinal location of micturition-controlling 5-HT_{1A} receptors).

The idea of glial roles in the NTS is certainly worth following. A simple double stain of glial cells and 5-HT containing terminals would yield clues to how these might interact. Further juxtacellular labelling experiments are currently underway to look at the nature of 5-HT appositions in the NTS with the DVN, and compare these with substance P and other peptide-containing fibres.

Finally the role of serotonergic vagal afferents needs to be addressed, and this might be done by application of 5,7-DHT to the nodose ganglia. If the 5-HT_7 receptor mediated effect is transmitted from primary afferents, one would expect topical antagonists to have no effect in such animals.

6. ROLE OF CENTRAL 5-HT$_7$ RECEPTORS IN CARDIOVASCULAR REFLEX INTEGRATION IN AWAKE RATS

6.1. Introduction

6.1.1. Background

The physiology and pharmacology of cardiovascular reflexes has been most widely studied in anaesthetised animals, where a wider range of variables can be monitored and controlled with moderate technical ease. However, cardiovascular reflexes serve to maintain the physiological functions of the awake animal, and it is in this state that they function normally, since anaesthetics can have a number of modulatory effects principally through their depressant mechanisms. Fewer studies have concentrated on reflexes in awake animals due to technical difficulty and limitations. The technique developed by Michelini & Bonagamba (1988) allows microinjections to be made into the NTS of awake, freely moving and unrestrained rats, whilst simultaneously stimulating cardiovascular reflexes. Using this technique, it was found that microinjection of glutamate into the NTS evoked a pressor response (Machado & Bonagamba, 1992), whilst previous studies in anaesthetised rats reported a depressor response (Talman *et al.*, 1980), mimicking baroreceptor activation. When these awake rats were anaesthetised with urethane, however, the hypertension evoked by glutamate reversed to a hypotension (Machado & Bonagamba, 1992). Furthermore, when rats were anaesthetised with α-chloralose, the depressor response was even greater than in the urethane group. This demonstrates the potential influence of anaesthesia on NTS cardiovascular mechanisms. There are several possible explanations: firstly, under anaesthesia glutamate receptors in the NTS may not be under the influence of other neuromodulatory mechanisms; secondly, the CVLM may be more active in the anaesthetised animal, where glutamate causes hypertension when muscimol is microinjected into CVLM (Urbanski & Sapru, 1988); thirdly, that the chemoreflex pathway is more important and easily activated in awake animals, leading to a chemoreflex hypertension. Consequently it is possible that reflex mechanisms (such as a

pharmacological effect) observed under anaesthesia may be quite different in the absence of this physiological and pharmacological complication.

6.1.2. Aims of the study

To date there are no reports of a serotonergic mechanism contributing to cardiovascular reflex transmission in awake animals. The present experiments were performed to re-examine the modulatory effects of the 5-HT_7 receptor antagonist SB-269970 on the cardiopulmonary and chemoreflex, as described in Chapter 3. The same dose (100 μg kg^{-1}) and route (i.c.) were used. Furthermore the effect of the antagonist on baseline MAP and HR, as well as any gross observable behavioural effects, were monitored.

6.2. Methods

This study was carried out at the Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil, following approval by the Ethical Committee of the University of São Paulo. All experiments were performed on adult male Wistar rats (280 – 330 g) obtained from a colony at the Faculty of Medicine of Ribeirão Preto. Animals were housed in groups of 2 – 4 at ambient temperature and humidity, with food and water *ad libitum*. After vascular cannulation, animals were housed singly (to prevent interference with the cannulae) and kept overnight in the room where experiments would be performed the next day, to avoid moving and disturbing the animals on the day of the experiment.

6.2.1. Implantation of intracisternal cannulae

Four days before the experiment, rats were anaesthetised with tribromoethanol (250 mg kg^{-1} i.p.) and placed in a stereotaxic frame (David Kopf) using atraumatic ear bars. Supplementary doses of anaesthetic (25 mg i.p.) were given if required during surgery. The scalp and nuchal muscles were injected with 0.2 ml Lidostesin (3 % lignocaine and 1:50 000 noradrenaline). The scalp was incised and the underlying connective tissue and

periosteum removed. The bregmoid and lamdoid sutures were located, and the incisor bar adjusted to achieve a flat skull position. The nuchal muscles were retracted from the occipital bone by blunt dissection. To allow placement of a vertical guide cannula into the cisterna magna, a small craniotomy was performed from the occipital notch to within 1 mm of the atlanto-occipital membrane using a dental drill. Also, 2 burr holes were made in the parietal bones several millimeters lateral to the midline for the placement of watch screws.

A guide cannula (22 gauge, 15 mm) was placed in the cisterna magna using stereotaxic coordinates (from Paxinos & Watson, 1998) relative to bregma (AP –14.5, L 0.0, DV – 7.9), and bonded to the skull and watch screws using dental acrylic. Postoperatively, rats were treated with a compound antibiotic preparation (Pentabiotico Veterinario; 0.2 ml intramuscular).

6.2.2. Cannulation of femoral artery and vein

On the day before experiment, rats were again anaesthetised with tribromoethanol (250 mg kg^{-1} i.p. followed by 25 mg i.p. when required) and a 0.5 cm incision made to isolate the femoral artery and vein. The femoral artery and vein was tied distally. This is thought to aid the opening of collateral circulation to the leg to prevent ischaemia. Care was taken not to damage the femoral nerve. Polyethylene cannulae were constructed using 18 cm of PE50 tubing (Clay Adams, USA, ID 0.58 mm, OD 0.965 mm) attached to 2.5 cm (for vein) or 5 cm (for artery) PE10 tubing (ID 0.28 mm, OD 0.61 mm). The cannulae were pre-filled with saline, sealed distally, and inserted into the femoral vein and artery so the tips lay within the inferior vena cava and abdominal aorta, respectively. The distal ends of the cannulae were tunneled subcutaneously, exteriorised through the back of the neck, and sutured to the skin. The incision was closed with a single suture.

6.2.3. Recording of arterial pressure and heart rate

On the day of the experiment, the cannulae were flushed with saline and the arterial cannula connected to a pressure transducer *via* a length of PE50 tubing under conscious, freely moving conditions. Pulsatile arterial pressure and mean arterial pressure were measured by a transducer (Model CDX III, Cobe Labs, USA) connected to a physiological recorder (Narcotrace 80, Narco Bio-Systems, USA). Heart rate was derived from the pulsatile arterial pressure with a biotachometer coupler (Narco, Model 7302). Variables were displayed on a chart recorder, and calibrated at the beginning of each experiment. The venous cannula was connected to a length of saline-filled PE50 tubing for intravenous injection under freely moving conditions.

6.2.4. Stimulation of cardiovascular reflexes

Once baseline variables had stabilized, intravenous injections were given to stimulate a variety of afferents. Chemoreceptor afferents were stimulated by injecting potassium cyanide (KCN; 10 – 80 μg per animal in a volume of 100 μl; see Franchini & Kreiger, 1993). The dose was adjusted to produce a bradycardia of 100 – 200 bpm. Cardiopulmonary afferents were stimulated by injecting 5-HT (0.1 %; 1 – 3 μg per animal). The dose was adjusted to produce a bradycardia of 200 – 300 bpm. Afer drug injection, the cannula was flushed with 0.3 ml saline and the time marked with an event marker on the chart recorder. 5 min was allowed to elapse between reflex stimulations to prevent tachyphylaxis.

6.2.5. Intracisternal injection

Drug or vehicle was injected once at least 2 stable control bradycardias had been recorded. When variables returned to baseline and the animal was calm, an injector constructed from a 30 gauge gingival needle (length 15.8 mm), and attached *via* a length of PE10 tubing to a 10 μl syringe (Hamilton, USA), was carefully inserted into the guide cannula. This was done without restraining or cornering the animal in any way. Drug or vehicle (saline) was injected in a volume of 5 μl over 20 s, and the injector left in place

for 1 min to ensure diffusion. Reflexes were elicited at 5, 10, 15, 20, 25 and 30 min post drug.

At the end of the experiments, 5 μl of dye (2 % Evans blue) was injected i.c. for verification of drug delivery. The animal was then immediately anaesthetised with thiopentone sodium (50 mg kg^{-1} i.v.) and perfused transcardially with saline followed by 10 % buffered formaldehyde. The brain was removed and examined for staining. Data was only used from animals that showed good staining on all medullary surfaces.

6.2.6. Data analysis

Mean arterial pressure (MAP) and heart rate (HR; derived from the pulsatile arterial pressure wave) were measured directly from the polygraph printout, using the calibration points inserted at the beginning of each experiment. Baseline MAP and HR were calculated by taking the best-fit average from the 30 s prior to each reflex stimulation. Readings were only taken if the baseline was stable (± 5 mmHg for MAP, ± 10 bpm for HR). Reflex-evoked changes were calculated as the absolute changes in baseline variables following an intravenous injection.

Baseline and reflex-evoked changes in drug-treated animals were compared with time-matched saline controls using 2-way ANOVA followed by the LSD test, as in Chapter 3. Similarly, data in saline-treated animals were themselves compared to averaged pre-saline controls using 1-way ANOVA followed by the Tukey test (and this test was also used to analyse the effect of SB-269970 on the chemoreflex, where no saline controls were available). Values of $P < 0.05$ were considered significant.

6.2.7. Drugs and solutions

See 2.9 above.

6.3. Results

6.3.1. Effect of SB-269970 on baseline variables

Intracisternal injection of SB-269970 (100 μg kg^{-1}, n = 5) had no significant effect on baseline MAP or HR when compared to i.c. saline (Figure 6.1, Table 9.56). Intracisternal saline (5 μl, n = 5) also had no effect on baselines (Figure 6.1, Table 9.54). SB-269970 had no noticeable effects on locomotor or exploratory activity.

6.3.2. Effect of SB-269970 on cardiopulmonary reflex

Injection of 5-HT (1 – 3 μg i.v., n = 10) caused an immediate bradycardia of -278 ± 14 bpm (from a baseline of 376 ± 17 bpm) and a hypotension of -56 ± 6 mmHg (from a baseline of 104 ± 4 mmHg). Intracisternal injection of SB-269970 (100 μg kg^{-1}, n = 5) significantly attenuated both the bradycardia (-142 ± 54 bpm) and the hypotension (-22 ± 7 mmHg) 5 min after injection, compared to i.c. saline. At 15 min the difference was no longer significant, and at 30 min full recovery was observed (Figures 6.2 and 6.3, Table 9.57). Saline (5 μl i.c.; n = 5) had no significant effect on reflex-evoked changes (Figure 6.3, Table 9.55).

6.3.3. Effect of SB-269970 on chemoreflex

Injection of KCN (20 – 40 μg i.v., n = 5) caused an immediate bradycardia of -188 ± 23 bpm (from a baseline of 343 ± 11 bpm) and a hypertension of 41 ± 4 mmHg (from a baseline of 103 ± 4 mmHg). Intracisternal injection of SB-269970 (100 μg kg^{-1}, n = 5) did not significantly affect the chemoreflex (Figure 6.5, Table 9.58), although in 3 out of 5 animals the bradycardia was strongly attenuated, and in 2 out of 5 the hypertension was attenuated (Figure 6.4). No vehicle controls were performed.

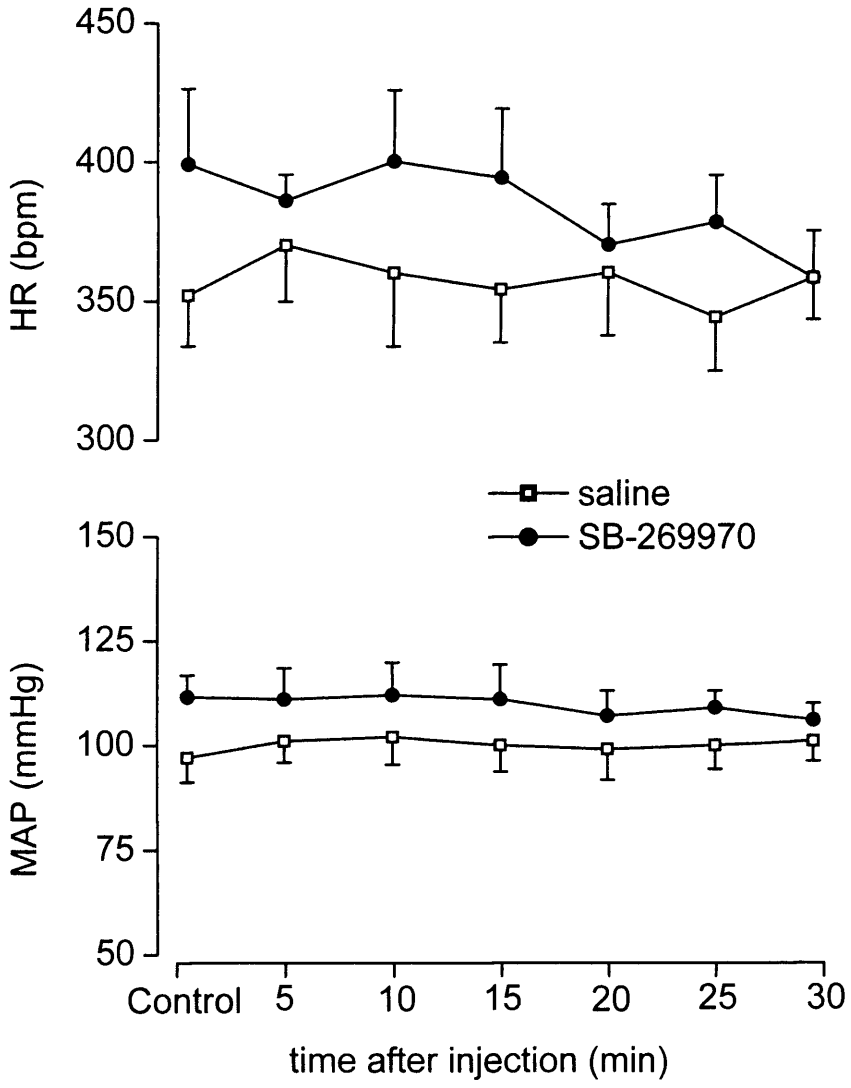
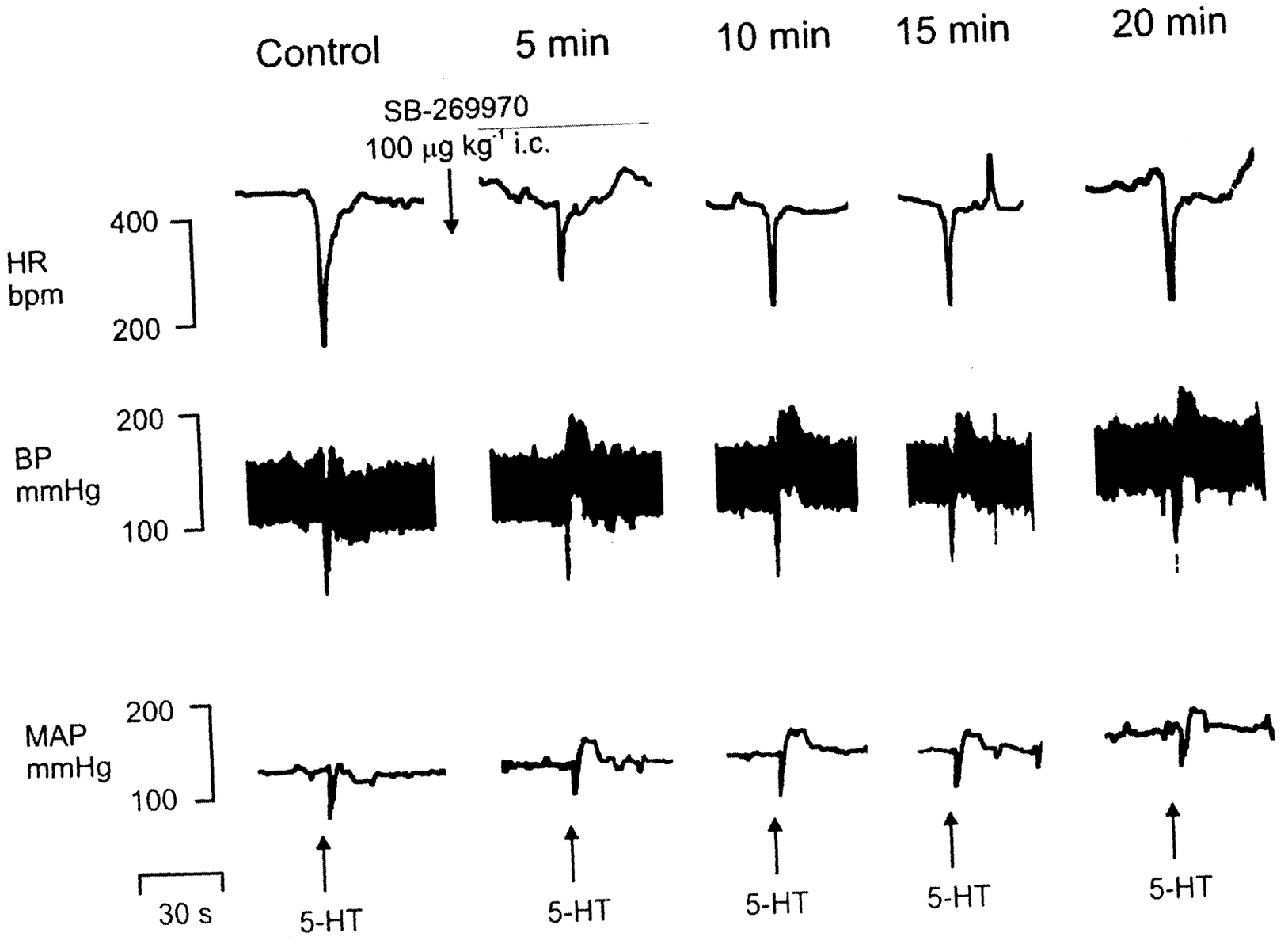


Figure 6.1 Baseline graph: SB-269970

Graph showing the effects (mean ± s.e.m.) of 5 µl saline i.c. (□, n = 5) and SB-269970, 100 µg kg^{-1} i.c. (●, n = 5) on baseline mean heart rate (HR) and mean arterial pressure (MAP) in awake rats.

Figure 6.2 Cardiopulmonary reflex trace: SB-269970

Sample trace showing effect of SB-269970 (100 μg kg^{-1} i.c.) on heart rate (HR), pulsatile blood pressure (BP), and mean arterial pressure (MAP) responses evoked by stimulation of cardiopulmonary afferents with 5-HT (3 μg i.v.) in an awake rat.


Control
5 min
10 min
15 min
20 min
SB-269970
100 µg kg⁻¹ i.c.
400
HR
bpm
200
200
BP
mmHg
100
200
MAP
mmHg
100
30 s
5-HT
5-HT
5-HT
5-HT
5-HT

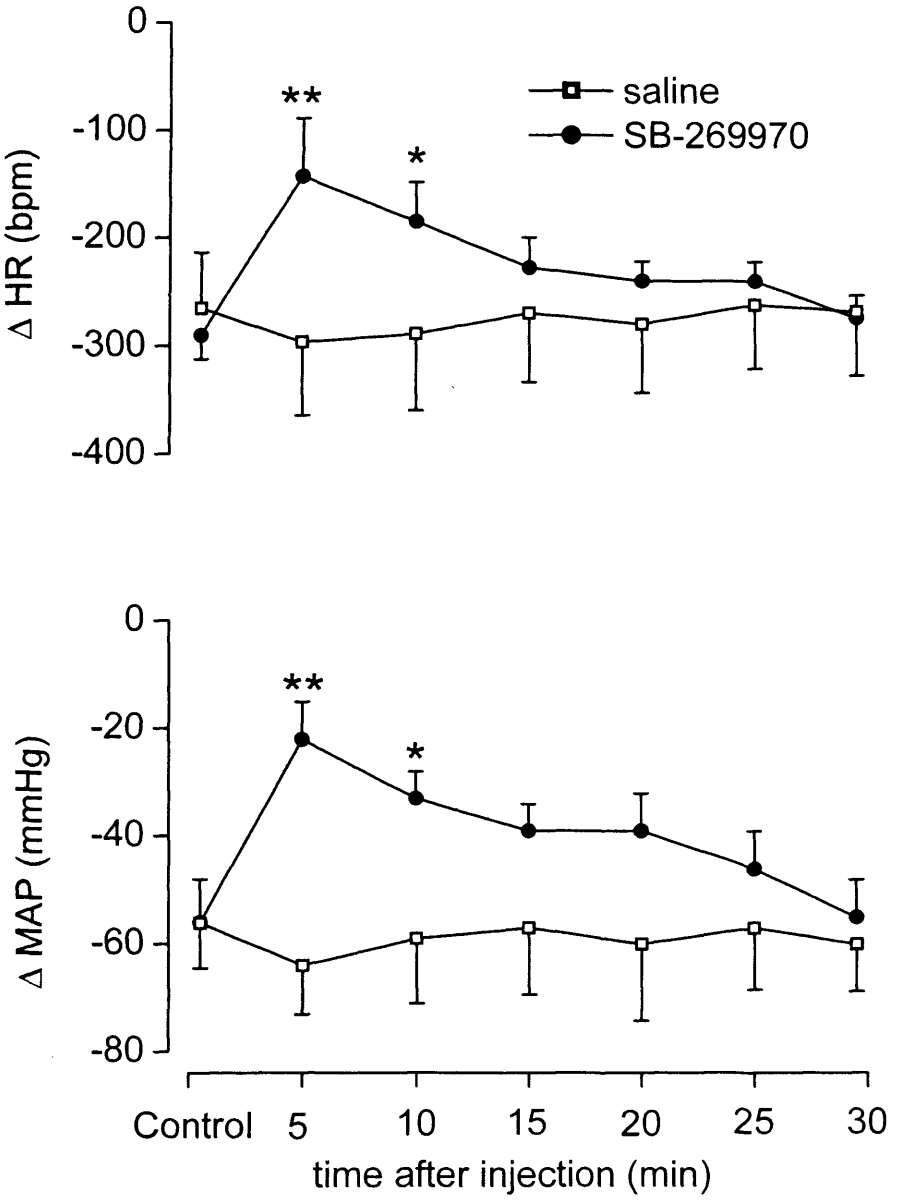


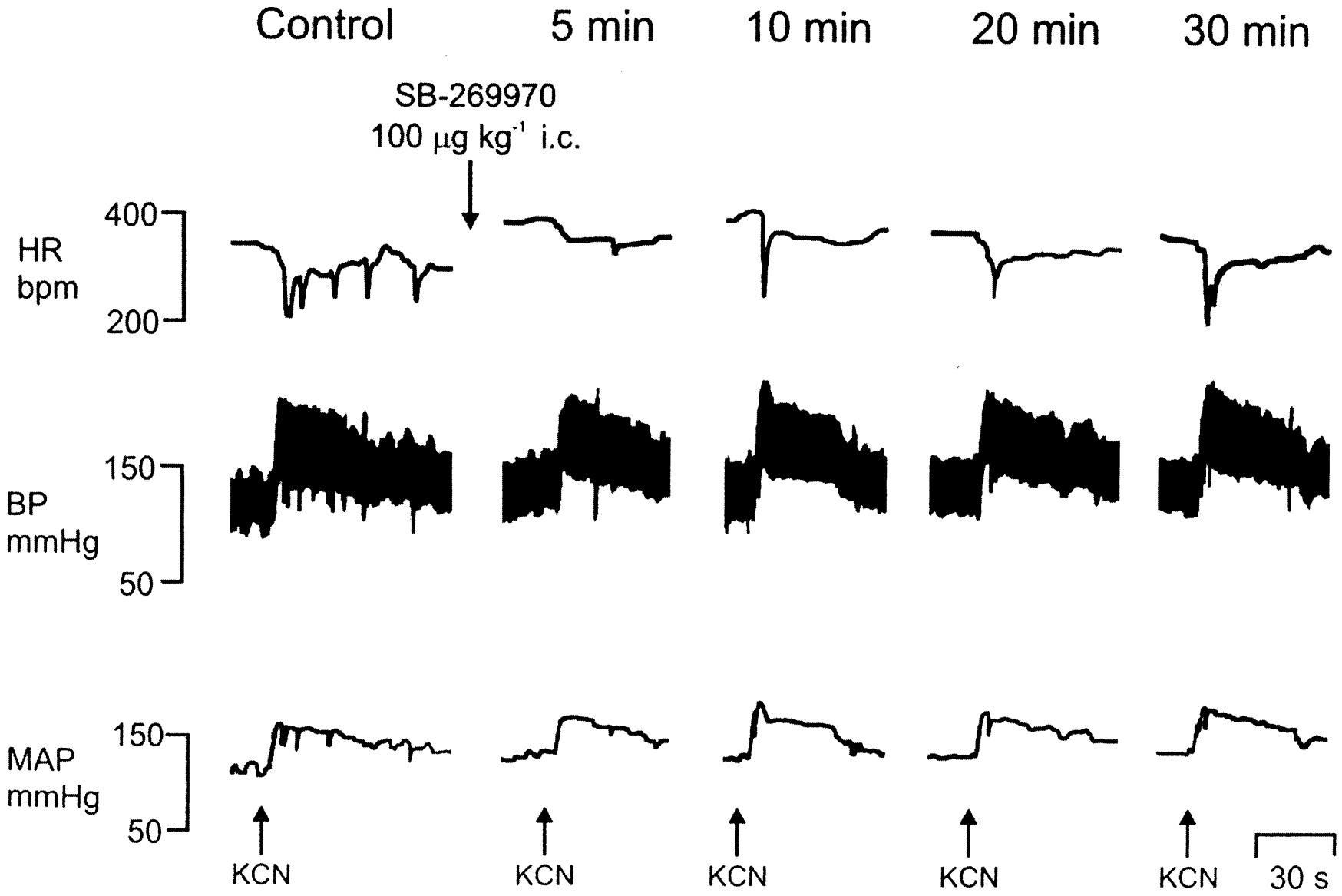
Figure 6.3 Cardiopulmonary reflex graph: SB-269970

Graph showing the effects (mean ± s.e.m.) of 5 µl saline i.c. (□, n = 5) and SB-269970, 100 µg kg^{-1} i.c. (●, n = 5) on changes (Δ) in heart rate (HR) and mean arterial pressure (MAP) evoked by cardiopulmonary afferent stimulation with 5-HT in awake rats.

* $P < 0.05$, ** $P < 0.01$, 2-way ANOVA followed by LSD test.

Figure 6.4 Chemoreflex trace: SB-269970

Sample trace showing effect of SB-269970 (100 μg kg^{-1} i.c.) on heart rate (HR), pulsatile blood pressure (BP), and mean arterial pressure (MAP) responses evoked by stimulation of chemoreceptor afferents with potassium cyanide (KCN; 20 μg i.v.) in an awake rat.


Control
5 min
10 min
20 min
30 min
SB-269970
100 μg kg-1 i.c.
HR
bpm
400
200
BP
mmHg
150
50
MAP
mmHg
150
50
KCN
KCN
KCN
KCN
KCN
30 s

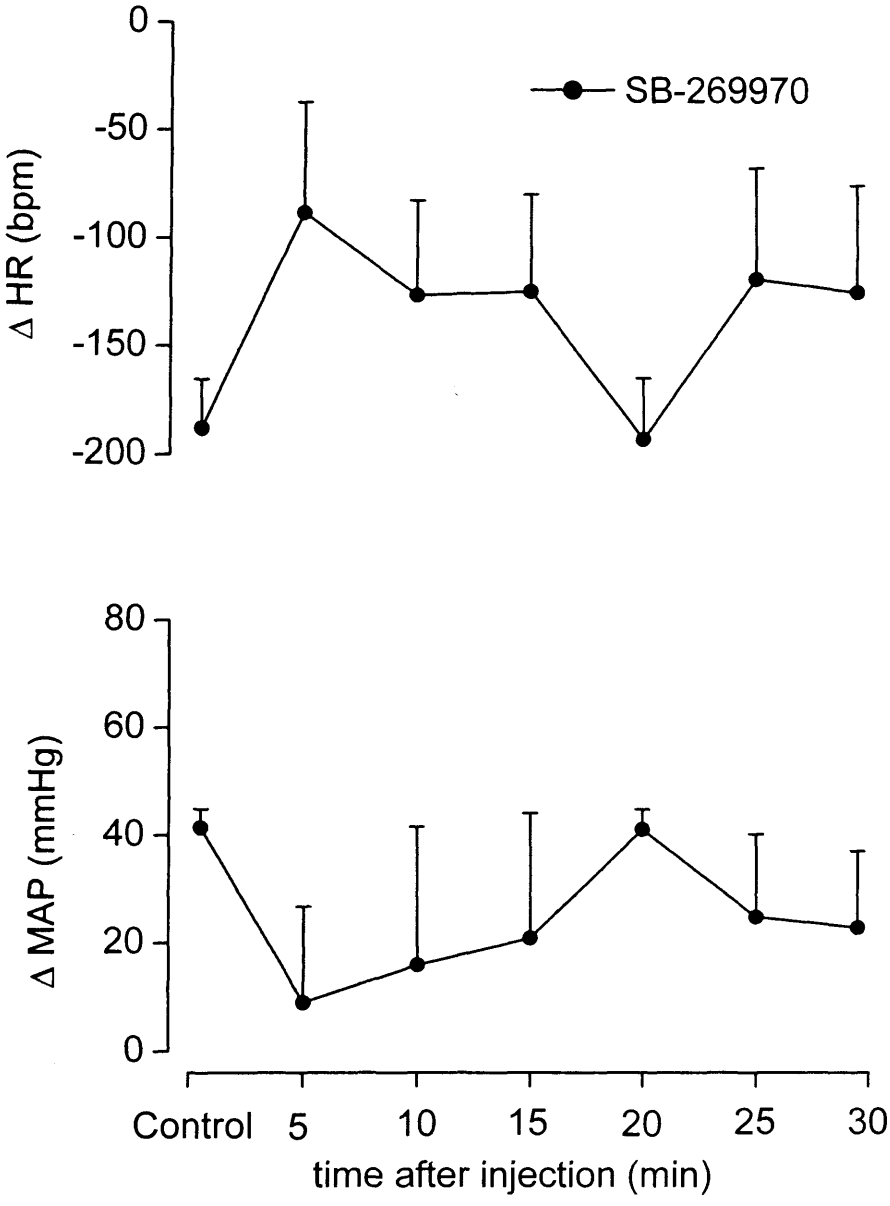


Figure 6.5 Chemoreflex graph: SB-269970

Graph showing the effects (mean ± s.e.m.) of SB-269970, 100 μg kg^{-1} i.c. (●, n = 5) on changes (Δ) in heart rate (HR) and mean arterial pressure (MAP) evoked by chemoreceptor afferent stimulation with potassium cyanide (KCN) in awake rats.

6.4. Discussion

6.4.1. General findings

The data demonstrate that blockade of 5-HT_7 receptors located in the brainstem significantly attenuates cardiopulmonary reflex bradycardia and hypotension in awake rats. This is in agreement with the findings of Chapter 3, and confirms that the cardiac vagal component of the cardiopulmonary reflex involves activation of central 5-HT_7 receptors, both in the anaesthetised and awake animal. It should be noted that in the awake rat, the cardiopulmonary reflex hypotension is atropine-sensitive (Chianca & Machado, 1996) and does not reflect a withdrawal of sympathetic tone, as is seen in the anaesthetised animal. In the present experiments no atenolol pretreatment was given, demonstrating that similar responses can be observed in the absence of cardiac sympathoadrenal blockade.

The present data also confirm that the antagonist has no effect on baseline HR and BP, which is also in agreement with the previous data from the anaesthetised animal. However, these preliminary experiments failed to demonstrate a significant effect of SB-269970 on the chemoreflex.

6.4.2. Technical considerations

In this study the antagonist was given in a lower volume than in the anaesthetised study (5 µl instead of 10 µl) to minimise subsequent pressure changes in the cisterna magna which could either have an effect on medullary activity, or facilitate the escape of drug through the guide cannula. Consequently the concentration of the antagonist is higher than in the anaesthetised study, but this is unlikely to be a problem since it rapidly disperses into the cerebrospinal fluid.

The standard method of activating cardiopulmonary afferents is intra-atrial PBG (Kay & Armstrong, 1990) which acts on 5-HT_3 receptors located on vagal sensory endings. In this awake rat preparation (Michelini & Bonagamba, 1988) it is not possible to inject directly into the right atrium, hence 5-HT was selected for injection into the femoral vein. 5-HT is rapidly cleared by pulmonary monoamine oxidase, so

it is unlikely to activate receptors downstream of the cardiopulmonary circulation. It may, however, have direct tachycardic effects on the heart, but since the results are similar to those using PBG in anaesthetised rats, this is unlikely to be an important complication.

The bradycardias evoked in this study are much larger than in the anaesthetised series (200 – 300 bpm compared with 40 – 100 bpm). However, seeing that these bradycardias are attenuated in a way comparable to the anaesthetised experiments (55 ± 17 % attenuation compared to 63 ± 7 % in anaesthetised rats) it would appear that 5-HT_7 receptors contribute to a wide range of reflex vagal tones to the heart, and are not just involved in submaximal bradycardias.

6.4.3. Limitations

The main difficulty in working with awake animals is the greater variability of data, which can eclipse subtle changes. Even in the very controlled system of the anaesthetised animal, both baselines and reflexes have a certain natural variability. In an awake animal that may be resting, exploring or grooming at different points in the experiment, and which may have a behavioural response to a reflex challenge, there are many factors influencing both baselines and reflexes, and the general noise in the system is thus amplified.

The present chemoreflex data are very variable and not significant in this small group size, although the antagonist was having some effect in the majority of animals. This is likely to be clarified by a few more experiments, and by a separate vehicle control group. What is apparent, however, is that the chemoreflex is more complex in its central integration and circuitry than the cardiopulmonary reflex, and as such is subject to more perturbations and variations. This is especially true in the conscious animal, where the reflex is accompanied by a marked behavioral response. The chemoreflex is augmented by the hypothalamic defence area, whereas the baroreflex is attenuated (Coote *et al.*, 1979). The hypothalamic defence area is quite sensitive to anaesthetics, so in an anaesthetised animal it is not expected to have an active role in these reflexes. In the awake animal, however, behavioural alerting will have the effect of increasing the size of the chemoreflex, which may mask any changes caused

by the 5-HT_7 receptor antagonist. Behavioural alerting is caused by the chemoreflex itself, and also by anxiety. Suffice it to say that the chemoreflex is technically more difficult and more variable, hence negative data based on a sample size of 5 should be interpreted cautiously.

6.4.4. Conclusion

From the close similarities between the present data, and the findings in Chapter 3, it is clear that the samed presumed 5-HT_7 receptor mechanism is involved in the cardiopulmonary reflex arc both in anaesthetised and awake rats.

6.4.5. Future experiments

As this was only a preliminary set of experiments, a full investigation of the role of 5-HT_7 as well as 5-HT_{1A} receptors would be useful in confirming both the very clear-cut and more surprising results discussed in Chapter 3 (particularly with respect to 5-HT_{1A} receptors). Any findings could then be retested further using bilateral microinjection of the antagonist into various NTS regions.

This model would also be a very useful way of confirming the effects of 5-HT depletion with p-CPA on cardiovascular reflex sensitivity and baseline MAP, as described in Chapter 4.

7. GENERAL DISCUSSION

7.1. Conclusions

The present experiments have investigated the neuropharmacology of three cardiovascular reflexes in anaesthetised rats (and began to re-examine two of these in awake rats). Furthermore, the roles of some of the cell groups associated with the serotonergic system were examined, and the physiology, pharmacology, and anatomy of these reflex pathways was taken to the single cell level in the NTS. Based on these data, the following conclusions can be made:

1. Central 5-HT receptors facilitate neurotransmission of reflex bradycardias. The 5-HT_7 receptor is involved in all reflex bradycardias here tested. This suggests that the 5-HT_7 receptor is the chief mediator of the serotonergic contribution to reflex bradycardias, at least in the rat. Preliminary evidence suggests that this role extends to the awake as well as the anaesthetised rat.

2. The 5-HT_{1A} receptor also facilitates reflex bradycardias, but the effect is not as clear in the rat as in the rabbit. The effect depends on the antagonist used, as well as which reflex is stimulated. Using WAY-100635, the cardiopulmonary reflex bradycardia is the most sensitive, the chemoreflex second and the baroreflex bradycardia least sensitive.

3. At least in the anaesthetised rat, central and peripheral 5-HT receptors are not tonically involved in controlling cardiovascular variables, although depletion of endogenous 5-HT lowers blood pressure. 5-HT depletion also demonstrates that endogenous 5-HT is required for successful reflex bradycardias

4. Chemical stimulation of various parts of the medullary raphe revealed that the rostral and caudal parts of these nuclei have opposing influences on sympathetic and respiratory drive. The cardiac vagal effects of raphe stimulation do not involve serotonergic mechanisms, and there is no evidence that these nuclei tonically contribute to reflex bradycardias.

5. Within the NTS, glutamate is a major neurotransmitter both at second and higher order neurones, acting at AMPA and NMDA receptors, and presynaptic 5-HT_3 receptors facilitate its release. 5-HT_7 receptors at this level also facilitate transmission of vagal afferent signals, raising the possibility that 5-HT is released from primary afferent fibres.

6. The cell bodies and proximal dendrites of NTS neurones activated by vagal afferents are rarely contacted by serotonergic terminals, therefore this interaction is either diffuse and non-specific, or another mechanism is involved. Astrocytes could be the intermediary, activated by 5-HT and releasing glutamate onto NTS neurones.

Figure 7.1 **Central serotonergic control of cardiovascular reflexes**

Schematic diagram illustrating some possible synaptic arrangements of 5-HT (and amino acid) receptors involved in cardiovascular reflex control in mammals, based on present and previous data. Two NTS neurones (one second order and one higher order) and one cardiac vagal preganglionic neurone (CVPN) are shown. Additionally an NTS astrocyte is shown (grey).

Dotted lines represent serotonergic fibres either from identified (e.g. raphe pallidus: RPa) or unknown sources (?). Unbroken lines are glutamatergic (or GABAergic) axons. Axon terminals are shown in terms of excitatory (●) or inhibitory (○) synaptic inputs.

References

① Wang & Ramage (2001)

② Wang *et al.* (1996)

③ Sevoz-Couche *et al.* (2000a)

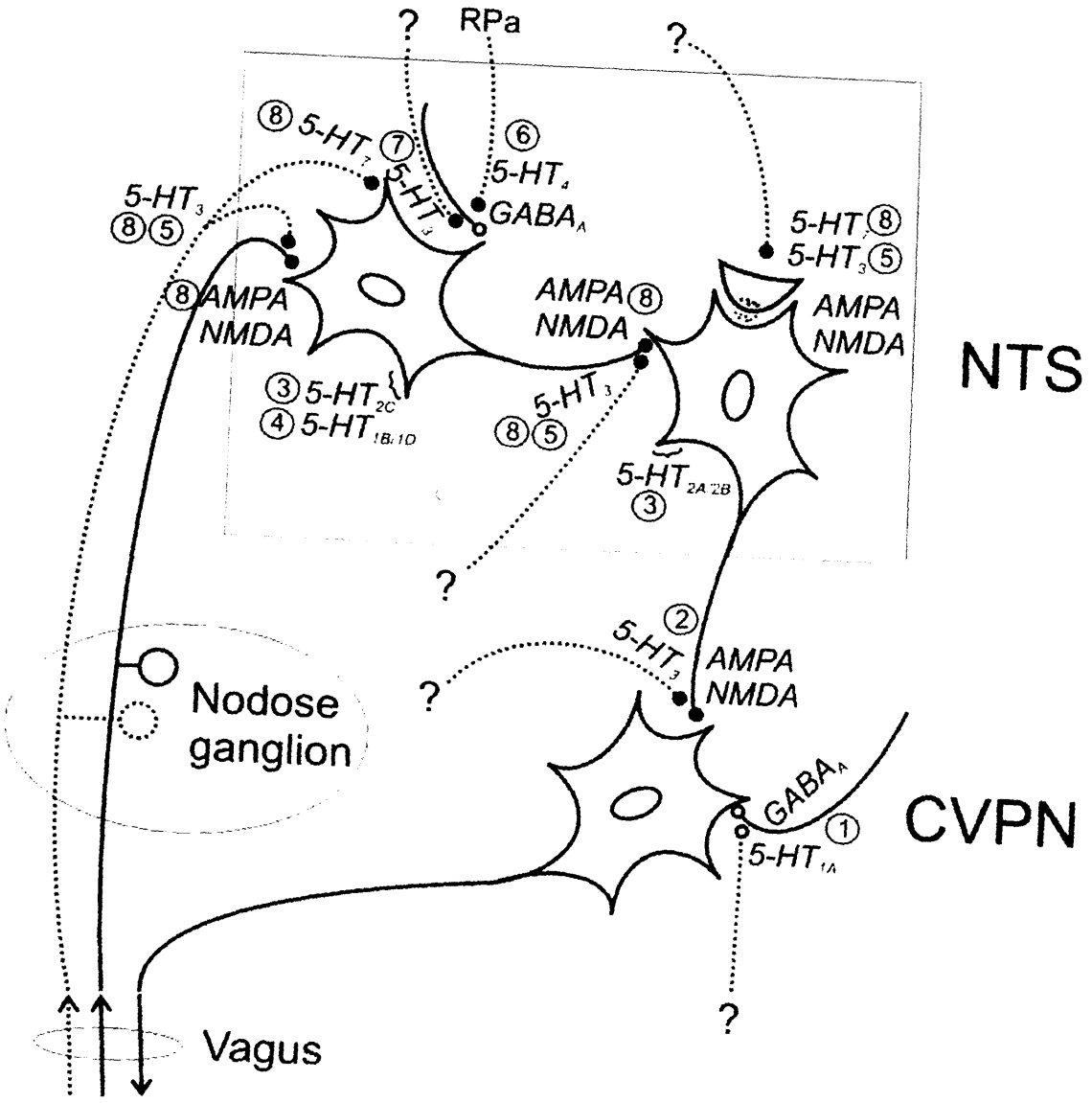
④ Jeggo *et al.* (2000a)

⑤ Jeggo *et al.* (2000b, 2001)

⑥ Edwards & Paton (1999, 2000)

⑦ Sevoz *et al.* (1996, 1997)

⑧ Present experiments


RPa
?
?
⑧ 5-HT1
⑦ 5-HT1
⑥ 5-HT4
GABAA
5-HT3 ⑧⑤
⑧ AMPA NMDA
③ 5-HT2C
④ 5-HT1B/1D
AMPA ⑧ NMDA
5-HT7 ⑧
5-HT3 ⑤
AMPA NMDA
NTS
5-HT3 ⑧⑤
5-HT2A/2B ③
?
② 5-HT3
AMPA NMDA
?
Nodose ganglion
GABAA
① 5-HT1A
CVPN
?
Vagus

7.2. Roles of the serotonergic system

From an overview of the literature as well as the present experiments, the role of the serotonergic system in cardiovascular reflexes is a particularly complex picture, due to numerous different receptors being involved, as well as the receptors being located in different brain areas. The precise synaptic arrangment of 5-HT receptors (and the axons innervating them) remains quite poorly understood. There are many possibilities (as summarised in Figure 7.1), some based on established and compelling evidence, and some on relatively preliminary data.

Central 5-HT_{1A} receptors, whose activation causes bradycardia, are located in the nucleus ambiguus. Since these are inhibitory receptors causing increased activity in CVPNs, they are thought to be presynaptic on an inhibitory terminal, probably inhibiting a tonic GABAergic brake. In terms of the cardiopulmonary reflex, this has been shown in the cat, and may well be the target of 5-HT_{1A} receptor antagonists in the rabbit. In the DVN, 5-HT_3 receptors potentiate glutamate release, therefore are probably on glutamatergic terminals. Hence it is possible that such 5-HT_3 receptors are also located in the nucleus ambiguus. The origin of the 5-HT containing fibres, however, is uknown.

In the NTS, 5-HT_{1A} receptors involved in cardiovascular reflexes have not been demonstrated, but again 5-HT_3 receptors are thought to be presynaptic on glutamatergic terminals contacting both second and higher order neurones. The innervation may come from serotonergic vagal afferents, or from elsewhere. The glutamatergic transmission is now thought to be *via* AMPA and NMDA receptors at both second and higher order neurones. Furthermore, 5-HT_7 receptors contributing to reflex bradycardias are probably in the NTS, though whether second or higher order neurones express them remains to be seen. 5-HT_7 receptors could be located on NTS neurones receiving serotonergic primary afferents, or fibres from elsewhere. They could also be found on astrocytes, which are then releasing glutamate onto NTS neurones. Alternatively, the 5-HT_3 receptor could be located on astrocytes instead of, or in addition to, glutamatergic terminals.

Several 5-HT receptors are also found in the NTS without being tonically involved in reflexes. The 5-HT_4 receptor inhibits reflexes, and is thought to be innervated by raphe pallidus neurones. Since it is an excitatory receptor, it is likely to involve activation of a GABAergic brake. Likewise 5-HT_3 receptors can also inhibit reflexes *via* GABAergic mechanisms in the NTS. Whether this is part of a physiological pathway is not known. Similarly, the 5-HT_{1B} receptor increases NTS neuronal and reflex-evoked activity, whilst the 5-HT_{1D} receptor decreases it. Also the 5-HT_{2C} receptor inhibits second order NTS neurones, and the 5-HT_{2A} and 5-HT_{2B} receptors excite higher order NTS neurones. None of these receptors, however, is involved in tonic reflex pathways.

One possibility that has received little attention is that blocking 5-HT_{1A} and 5-HT_7 receptors simply increases raphe neuronal firing, and activates inhibitory pathways (for example the 5-HT_4 receptor pathway described above). In this case, the serotonergic system would not be involved in reflexes at all, except when its activity were modulated by blockade of somatodendritic autoreceptors. Whilst this effect has been demonstrated using 5-HT_{1A} receptor antagonists (e.g. Wang *et al.,* 1995), the effect of 5-HT_7 receptor antagonists on raphe neuronal firing is unclear, but an inhibition rather than excitation has been suggested (Roberts *et al.,* 2004b). The present experiments have demonstrated that rats depleted of 5-HT have poor reflex bradycardias. This supports the role of 5-HT *per se* in reflexes, rather than the effects being secondary to modulation of serotonergic neurones.

7.3. Clinical implications

These findings have a variety of clinical implications. The serotonergic system has a variety of important influences, most noteably in higher brain functions such as mood. The autonomic system is critically associated with mood and affective states (see Jordan, 1990), and the most widespread use of drugs acting on serotonergic neurones is in the treatment of affective disorders, such as unipolar depression. Whether depression is caused by a functional deficit of serotonergic transmission remains contentious, but the efficacy of 5-HT reuptake inhibitors in treating depression is clear. Additionally, drugs acting at 5-HT receptors are used for various clinical conditions including migraine, anxiety, nausea, and schizophrenia.

Antidepressants have various cardiovascular side-effects. This is most prominent in the older tricyclics, which are especially dangerous in overdose, typically causing arrhythmias and heart block due to various non-selective actions (Glassman *et al.*, 1987). In addition to antagonising muscarinic and α adrenoceptors, tricyclic antidepressants are also classified as Type 1A antiarrhythmic drugs, hence their particular danger after myocardial infarction (Roose & Glassman, 1994). However, the use of imipramine as an antiarrhythmic yielded an interesting result: that non-depressed patients tend not to develop orthostatic hypotension, which is a common intolerable side effect in depressed patients treated with imipramine or similar (Glassman *et al.*, 1987). The obvious cause of such orthostatic hypotension would be baroreflex dysfunction in the depressed state, aggravated by the peripheral α_1 adrenoceptor blockade caused by the drug, leading to a failure of the system to adapt sufficiently to postural changes in blood pressure. This remains to be confirmed.

Unipolar depression has not been associated with cardiovascular risk factors such as hypertension, although bipolar affective disorder has been (Yates & Wallace, 1987). However, there is a growing body of evidence that unipolar depression carries a greater risk of mortality after coronary artery bypass surgery (Baker *et al.*, 2001; Blumenthal *et al.*, 2003). The depression in these cases was considered independent of the heart disease. Depressed patients with coronary heart disease have markedly lower heart rate variability than those without depression (Stein *et al.*, 2000). Indeed this is also seen in otherwise healthy depressed patients (Tulen *et al.*, 1996). In both cases this was observed in the absence of antidepressant medication, and suggests depression (rather than its treatment) alters cardiac autonomic modulation. Patients with cardiovascular disease frequently have at least one psychiatric diagnosis, such as depression (~30 %) or anxiety (24 %) (Bankier *et al.*, 2004).

Whether 5-HT is involved is far from clear. Many other factors and neurochemical phenomena are involved with depression, including inflammatory mediators (Joynt *et al.*, 2003). Treatment with SSRIs can protect against myocardial infarction (Sauer *et al.*, 2001), but whether this is due to amelioration of depression or other effects is uncertain. The SSRI sertraline can prolong bleeding time (Calhoun & Calhoun, 1996), so the protective effect might be to inhibit 5-HT mediated platelet activation.

In themselves, SSRIs have few cardiovascular side effects. Fluoxetine has been shown to reverse the decreased heart rate variability in post traumatic stress disorder patients (Cohen *et al.*, 2000). As this is a comparatively selective drug, it suggests that the altered heart rate variability (seen also in depression) is due to a serotonergic mechanism. Short-term fluoxetine treatment enhances baroreflex control of sympathetic nerves in rats (Moffitt & Johnson, 2004), possibly by temporarily reducing raphe neuronal firing, which takes a few weeks of SSRI treatment to recover (de Montigny *et al.*, 1990). This illustrates again how 5-HT receptors and serotonergic neurones can positively or negatively influence aspects of the cardiovascular system. As such, the influence of serotonergic drugs, and the relationship between serotonergic higher brain and autonomic functions, remain very complex questions, with many mechanisms and roles remaining to be elucidated.

7.4. Future studies

The present experiments have begun to investigate the origin of the endogenous 5-HT that facilitates cardiovascular reflexes, but this needs to be conclusively identified. The possibilities that vagal afferents release 5-HT, or that the *antagonists* are modulating raphe firing and thus causing the effect, can be investigated by various comparatively simple techniques, such as single unit recording (raphe) and microdialysis from the NTS (vagal afferents).

The exact mechanisms of the interactions between glutamate and 5-HT in the NTS need to be verified, as these could possibly extend to other brain areas. A combination of *in vivo* and *in vitro* electrophysiology would be useful in detecting synaptic mechanisms. Combined with further light and electron microsopic analysis, the possible and exciting role of astrocytes might be confirmed.

The role of 5-HT_7 receptors in other species needs to be confirmed, and in this respect similar experiments in the guinea pig and rabbit are important. This would verify any species differences, and begin to address whether these effects apply to mammals in general, and to man in particular. Further information on the 5-HT_7

receptor is also needed to characterise its role in other systems, especially mood and depression, in which it has been implicated.

Further scientific and clinical studies are also required to investigate links between depression and cardiovascular disease. These are both enormous health problems, and the identification of a common neuropharmacological factor is of great potential benefit.

8. REFERENCES

ABOU-GHARBIA M, PATEL UR, WEBB MB, MOYER JA, ANDREE TH & MUTH EA (1988). Polycyclic aryl- and heteroarylpiperazinyl imides as 5-HT1A receptor ligands and potential anxiolytic agents: synthesis and structure-activity relationship studies. *J Med Chem* **31**, 1382-1392.

ADAIR JR, HAMILTON BL, SCAPPATICCI KA, HELKE CJ & GILLIS RA (1977). Cardiovascular responses to electrical stimulation of the medullary raphe area of the cat. *Brain Res* **128**, 141-145.

ADHAM N, KAO HT, SCHECTER LE, BARD J, OLSEN M, URQUHART D *et al.* (1993). Cloning of another human serotonin receptor (5-HT1F): a fifth 5-HT1 receptor subtype coupled to the inhibition of adenylate cyclase. *Proc Natl Acad Sci U S A* **90**, 408-412.

ADHAM N, ROMANIENKO P, HARTIG P, WEINSHANK RL & BRANCHEK T (1992). The rat 5-hydroxytryptamine1B receptor is the species homologue of the human 5-hydroxytryptamine1D beta receptor. *Mol Pharmacol* **41**, 1-7.

AGHAJANIAN GK, FOOTE WE & SHEARD MH (1968). Lysergic acid diethylamide: sensitive neuronal units in the midbrain raphe. *Science* **161**, 706-708.

AGHAJANIAN GK, HAIGLER HJ & BLOOM FE (1972). Lysergic acid diethylamide and serotonin: direct actions on serotonin-containing neurons in rat brain. *Life Sci I* **11**, 615-622.

AGHAJANIAN GK & MCCALL RB (1980). Serotonergic synaptic input to facial motoneurons: localization by electron-microscopic autoradiography. *Neuroscience* **5**, 2155-2162.

ALBERT PR, ZHOU QY, VAN TOL HH, BUNZOW JR & CIVELLI O (1990). Cloning, functional expression, and mRNA tissue distribution of the rat 5-hydroxytryptamine1A receptor gene. *J Biol Chem* **265**, 5825-5832.

ALPER RH (1990). Hemodynamic and renin responses to (+-)-DOI, a selective 5-HT2 receptor agonist, in conscious rats. *Eur J Pharmacol* **175**, 323-332.

AMARA SG & KUHAR MJ (1993). Neurotransmitter transporters: recent progress. *Annu Rev Neurosci* **16**, 73-93.

AMENDT K, CZACHURSKI J, DEMBOWSKY K & SELLER H (1979). Bulbospinal projections to the intermediolateral cell column: a neuroanatomical study. *J Auton Nerv Syst* **1**, 103-107.

AMIN AH, CRAWFORD TB & GADDUM JH (1954). The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. *J Physiol* **126**, 596-618.

ANDERSON IK, MARTIN GR & RAMAGE AG (1992). Central administration of 5-HT activates 5-HT1A receptors to cause sympathoexcitation and 5-HT2/5-HT1C receptors to release vasopressin in anaesthetized rats. *Br J Pharmacol* **107**, 1020-1028.

ANDERSON IK, MARTIN GR & RAMAGE AG (1995). Evidence that activation of 5-HT2 receptors in the forebrain of anaesthetized cats causes sympathoexcitation. *Br J Pharmacol* **116**, 1751-1756.

ANDRADE R, MALENKA RC & NICOLL RA (1986). A G protein couples serotonin and GABAB receptors to the same channels in hippocampus. *Science* **234**, 1261-1265.

ANDRADE R & NICOLL RA (1987). Pharmacologically distinct actions of serotonin on single pyramidal neurones of the rat hippocampus recorded in vitro. *J Physiol* **394**, 99-124.

ANDRESEN MC, DOYLE MW, BAILEY TW & JIN YH (2004). Differentiation of autonomic reflex control begins with cellular mechanisms at the first synapse within the nucleus tractus solitarius. *Braz J Med Biol Res* **37**, 549-558.

ANTUNES VR & MACHADO BH (2003). Antagonism of glutamatergic metabotropic receptors in the NTS of awake rats does not affect the gain of the baroreflex. *Auton Neurosci* **103**, 65-71.

APPEL NM & ELDE RP (1988). The intermediolateral cell column of the thoracic spinal cord is comprised of target-specific subnuclei: evidence from retrograde transport studies and immunohistochemistry. *J Neurosci* **8**, 1767-1775.

ARANEDA R & ANDRADE R (1991a). 5-Hydroxytryptamine2 and 5-hydroxytryptamine 1A receptors mediate opposing responses on membrane excitability in rat association cortex. *Neuroscience* **40**, 399-412.

ARANEDA R & ANDRADE R (1991b). 5-Hydroxytryptamine2 and 5-hydroxytryptamine 1A receptors mediate opposing responses on membrane excitability in rat association cortex. *Neuroscience* **40**, 399-412.

ARAQUE A, LI N, DOYLE RT & HAYDON PG (2000). SNARE protein-dependent glutamate release from astrocytes. *J Neurosci* **20**, 666-673.

ARMSTRONG DM, ROSS CA, PICKEL VM, JOH TH & REIS DJ (1982). Distribution of dopamine-, noradrenaline-, and adrenaline-containing cell bodies in the rat medulla oblongata: demonstrated by the immunocytochemical localization of catecholamine biosynthetic enzymes. *J Comp Neurol* **212**, 173-187.

ARTIGAS F, ROMERO L, DE MONTIGNY C & BLIER P (1996). Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT1A antagonists. *Trends Neurosci* **19**, 378-383.

ASHBY CR, ZHANG JY, EDWARDS E & WANG RY (1994). The induction of serotonin3-like receptor supersensitivity and dopamine receptor subsensitivity in the rat medial prefrontal cortex after the intraventricular administration of the neurotoxin 5,7-dihydroxytryptamine: a microiontophoretic study. *Neuroscience* **60**, 453-462.

ASHWORTH-PREECE MA, JARROTT B & LAWRENCE AJ (1995). 5-Hydroxytryptamine3 receptor modulation of excitatory amino acid release in the rat nucleus tractus solitarius. *Neurosci Lett* **191**, 75-78.

AUDET MA, DESCARRIES L & DOUCET G (1989). Quantified regional and laminar distribution of the serotonin innervation in the anterior half of adult rat cerebral cortex. *J Chem Neuroanat* **2**, 29-44.

AUDINOT V, LOCHON S, NEWMAN-TANCREDI A, LAVIELLE G & MILLAN MJ (1997). Binding profile of the novel 5-HT1B/1D receptor antagonist, [3H]GR 125,743, in guinea-pig brain: a comparison with [3H]5-carboxamidotryptamine. *Eur J Pharmacol* **327**, 247-256.

AVERILL DB, CAMERON WE & BERGER AJ (1984). Monosynaptic excitation of dorsal medullary respiratory neurons by slowly adapting pulmonary stretch receptors. *J Neurophysiol* **52**, 771-785.

AZMITIA E & GANNON P (1983). The ultrastructural localization of serotonin immunoreactivity in myelinated and unmyelinated axons within the medial forebrain bundle of rat and monkey. *J Neurosci* **3**, 2083-2090.

AZMITIA EC & SEGAL M (1978a). An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J Comp Neurol* **179**, 641-667.

AZMITIA EC & SEGAL M (1978b). An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J Comp Neurol* **179**, 641-667.

BACHELARD H, GARDINER SM & BENNETT T (1990). Cardiovascular responses elicited by chemical stimulation of the rostral ventrolateral medulla in conscious, unrestrained rats. *J Auton Nerv Syst* **31**, 185-190.

BACKMAN SB, ANDERS C, BALLANTYNE D, ROHRIG N, CAMERER H, MIFFLIN S *et al.* (1984). Evidence for a monosynaptic connection between slowly adapting pulmonary stretch receptor afferents and inspiratory beta neurones. *Pflugers Arch* **402**, 129-136.

BACON WL & BECK SG (2000). 5-Hydroxytryptamine(7) receptor activation decreases slow afterhyperpolarization amplitude in CA3 hippocampal pyramidal cells. *J Pharmacol Exp Ther* **294**, 672-679.

BAKER LP, NIELSEN MD, IMPEY S, METCALF MA, POSER SW, CHAN G *et al.* (1998). Stimulation of type 1 and type 8 Ca2+/calmodulin-sensitive adenylyl cyclases by the Gs-coupled 5-hydroxytryptamine subtype 5-HT7A receptor. *J Biol Chem* **273**, 17469-17476.

BAKER RA, ANDREW MJ, SCHRADER G & KNIGHT JL (2001). Preoperative depression and mortality in coronary artery bypass surgery: preliminary findings. *ANZ J Surg* **71**, 139-142.

BANDLER R & TORK I (1987). Midbrain periaqueductal grey region in the cat has afferent and efferent connections with solitary tract nuclei. *Neurosci Lett* **74**, 1-6.

BANKIER B, JANUZZI JL & LITTMAN AB (2004). The high prevalence of multiple psychiatric disorders in stable outpatients with coronary heart disease. *Psychosom Med* **66**, 645-650.

BARBEAU H & BEDARD P (1981). Similar motor effects of 5-HT and TRH in rats following chronic spinal transection and 5.7-dihydroxytryptamine injection. *Neuropharmacology* **20**, 477-481.

BARD JA, ZGOMBICK J, ADHAM N, VAYSEE P, BRANCHEK TA & WEINSHANK RL (1993). Cloning of a novel human serotonin receptor (5-HT7)

positively linked to adenylate cyclase. *Journal of Biological Chemistry* **268**, 23422-23426.

BARNES JM, BARNES NM, CHAMPANERIA S, COSTALL B & NAYLOR RJ (1990). Characterisation and autoradiographic localisation of 5-HT3 receptor recognition sites identified with [3H]-(S)-zacopride in the forebrain of the rat. *Neuropharmacology* **29**, 1037-1045.

BARNES NM, COSTALL B, IRONSIDE JW & NAYLOR RJ (1988a). Identification of 5-HT3 recognition sites in human brain tissue using [3H]zacopride. *J Pharm Pharmacol* **40**, 668.

BARNES NM, COSTALL B & NAYLOR RJ (1988b). [3H]zacopride: ligand for the identification of 5-HT3 recognition sites. *J Pharm Pharmacol* **40**, 548-551.

BARNES NM & SHARP T (1999). A review of central 5-HT receptors and their function. *Neuropharmacology* **38**, 1083-1152.

BARRACO RA & PHILLIS JW (1991). Subtypes of adenosine receptors in the brainstem mediate opposite blood pressure responses. *Neuropharmacology* **30**, 403-407.

BARROS RC, BONAGAMBA LG, OKAMOTO-CANESIN R, DE OLIVEIRA M, BRANCO LG & MACHADO BH (2002). Cardiovascular responses to chemoreflex activation with potassium cyanide or hypoxic hypoxia in awake rats. *Auton Neurosci* **97**, 110-115.

BASILE AS, HANUS L & MENDELSON WB (1999). Characterization of the hypnotic properties of oleamide. *Neuroreport* **10**, 947-951.

BEAUDET A & DESCARRIES L (1976). Quantitative data on serotonin nerve terminals in adult rat neocortex. *Brain Res* **111**, 301-309.

BELIN MF, NANOPOULOS D, DIDIER M, AGUERA M, STEINBUSCH H, VERHOFSTAD A *et al.* (1983). Immunohistochemical evidence for the presence of gamma-aminobutyric acid and serotonin in one nerve cell. A study on the raphe nuclei of the rat using antibodies to glutamate decarboxylase and serotonin. *Brain Res* **275**, 329-339.

BELZUNG C, SCEARCE-LEVIE K, BARREAU S & HEN R (2000). Absence of cocaine-induced place conditioning in serotonin 1B receptor knock-out mice. *Pharmacol Biochem Behav* **66**, 221-225.

BENDOTTI C & SAMANIN R (1986). 8-Hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) elicits eating in free-feeding rats by acting on central serotonin neurons. *Eur J Pharmacol* **121**, 147-150.

BENNETT JA, KIDD C, LATIF AB & MCWILLIAM PN (1981). A horseradish peroxidase study of vagal motoneurones with axons in cardiac and pulmonary branches of the cat and dog. *Q J Exp Physiol* **66**, 145-154.

BERENDSEN HH, JENCK F & BROEKKAMP CL (1989). Selective activation of 5HT1A receptors induces lower lip retraction in the rat. *Pharmacol Biochem Behav* **33**, 821-827.

BERGER AJ (1977). Dorsal respiratory group neurons in the medulla of cat: spinal projections, responses to lung inflation and superior laryngeal nerve stimulation. *Brain Res* **135**, 231-254.

BERGER AJ & DICK TE (1987). Connectivity of slowly adapting pulmonary stretch receptors with dorsal medullary respiratory neurons. *J Neurophysiol* **58**, 1259-1274.

BERMUDEZ J, BOYLE EA, MINER WD & SANGER GJ (1988). The anti-emetic potential of the 5-hydroxytryptamine3 receptor antagonist BRL 43694. *Br J Cancer* **58**, 644-650.

BERMUDEZ J & SANGER GJ (1994). Prolonged anti-emetic activity and 5-HT3-receptor antagonism by BRL 46470 in conscious ferrets. *J Pharm Pharmacol* **46**, 520-521.

BERNARD DG (1998). Cardiorespiratory responses to glutamate microinjected into the medullary raphe. *Respir Physiol* **113**, 11-21.

BERNARD DG, LI A & NATTIE EE (1996). Evidence for central chemoreception in the midline raphe. *J Appl Physiol* **80**, 108-115.

BERNTHAL T, GREEN W & REVZIN AM (1951). Role of carotid chemoreceptors in hypoxic cardiac acceleration. *Proceedings of the Society of Experimental Biology and Medicine, New York* **76**, 121-124.

BHALLA P, SAXENA PR & SHARMA HS (2002). Molecular cloning and tissue distribution of mRNA encoding porcine 5-HT7 receptor and its comparison with the structure of other species. *Mol Cell Biochem* **238**, 81-88.

BIANCHI AL, DENAVIT-SAUBIE M & CHAMPAGNAT J (1995). Central control of breathing in mammals: neuronal circuitry, membrane properties, and neurotransmitters. *Physiol Rev* **75**, 1-45.

BIANCHI C, SINISCALCHI A & BEANI L (1990). 5-HT1A agonists increase and 5-HT3 agonists decrease acetylcholine efflux from the cerebral cortex of freely-moving guinea-pigs. *Br J Pharmacol* **101**, 448-452.

BICKMEYER U, HEINE M, MANZKE T & RICHTER DW (2002). Differential modulation of I(h) by 5-HT receptors in mouse CA1 hippocampal neurons. *Eur J Neurosci* **16**, 209-218.

BIEGER D & HOPKINS DA (1987). Viscerotopic representation of the upper alimentary tract in the medulla oblongata in the rat: the nucleus ambiguus. *J Comp Neurol* **262**, 546-562.

BINGHAM S, KING BF, RUSHANT B, SMITH MI, GASTER L & SANGER GJ (1995). Antagonism by SB 204070 of 5-HT-evoked contractions in the dog stomach: an in-vivo model of 5-HT4 receptor function. *J Pharm Pharmacol* **47**, 219-222.

BISCOE TJ, PURVES MJ & SAMPSON SR (1970). The frequency of nerve impulses in single carotid body chemoreceptor afferent fibres recorded in vivo with intact circulation. *J Physiol* **208**, 121-131.

BISSERBE JC, PATEL J & MARANGOS PJ (1985). Autoradiographic localization of adenosine uptake sites in rat brain using [3H]nitrobenzylthioinosine. *J Neurosci* **5**, 544-550.

BLAKELY RD, BERSON HE, FREMEAU RT, JR., CARON MG, PEEK MM, PRINCE HK *et al.* (1991). Cloning and expression of a functional serotonin transporter from rat brain. *Nature* **354**, 66-70.

BLANDINA P, GOLDFARB J, CRADDOCK-ROYAL B & GREEN JP (1989). Release of endogenous dopamine by stimulation of 5-hydroxytryptamine3 receptors in rat striatum. *J Pharmacol Exp Ther* **251**, 803-809.

BLESSING WW & NALIVAIKO E (2001). Raphe magnus/pallidus neurons regulate tail but not mesenteric arterial blood flow in rats. *Neuroscience* **105**, 923-929.

BLESSING WW, YU YH & NALIVAIKO E (1999). Raphe pallidus and parapyramidal neurons regulate ear pinna vascular conductance in the rabbit. *Neurosci Lett* **270**, 33-36.

BLIER P & DE MONTIGNY C (1987). Modification of 5-HT neuron properties by sustained administration of the 5-HT1A agonist gepirone: electrophysiological studies in the rat brain. *Synapse* **1**, 470-480.

BLIER P, DE MONTIGNY C & CHAPUT Y (1990). A role for the serotonin system in the mechanism of action of antidepressant treatments: preclinical evidence. *J Clin Psychiatry* **51**, S14-20.

BLONDEL O, GASTINEAU M, DAHMOUNE Y, LANGLOIS M & FISCHMEISTER R (1998). Cloning, expression, and pharmacology of four human 5-hydroxytryptamine 4 receptor isoforms produced by alternative splicing in the carboxyl terminus. *J Neurochem* **70**, 2252-2261.

BLONDEL O, VANDECASTEELE G, GASTINEAU M, LECLERC S, DAHMOUNE Y, LANGLOIS M *et al.* (1997). Molecular and functional characterization of a 5-HT4 receptor cloned from human atrium. *FEBS Lett* **412**, 465-474.

BLUMENTHAL JA, LETT HS, BABYAK MA, WHITE W, SMITH PK, MARK DB *et al.* (2003). Depression as a risk factor for mortality after coronary artery bypass surgery. *Lancet* **362**, 604-609.

BOCKAERT J, FOZARD JR, DUMUIS A & CLARKE DE (1992). The 5-HT4 receptor: a place in the sun. *Trends Pharmacol Sci* **13**, 141-145.

BOESS FG, LUMMIS SC & MARTIN IL (1992). Molecular properties of 5-hydroxytryptamine3 receptor-type binding sites purified from NG108-15 cells. *J Neurochem* **59**, 1692-1701.

BOESS FG & MARTIN IL (1994). Molecular biology of 5-HT receptors. *Neuropharmacology* **33**, 275-317.

BOGLE RG, PIRES JG & RAMAGE AG (1990). Evidence that central 5-HT1A-receptors play a role in the von Bezold- Jarisch reflex in the rat. *Br J Pharmacol* **100**, 757-760.

BOIVIN DB, CZEISLER CA, DIJK DJ, DUFFY JF, FOLKARD S, MINORS DS *et al.* (1997). Complex interaction of the sleep-wake cycle and circadian phase modulates mood in healthy subjects. *Arch Gen Psychiatry* **54**, 145-152.

BONAGAMBA LG, SEVOZ-COUCHE C, N'DIAYE A, UYGUN-LOUVET K, CALLERA J, MACHADO BH *et al.* (2000). Bradycardic responses to microinjection of N-methyl-D-aspartate into the nucleus tractus solitarius are inhibited by local activation of 5-HT(3) receptors. *Neuropharmacology* **39**, 2336-2345.

BONAVENTURE P, VOORN P, LUYTEN WH, JURZAK M, SCHOTTE A & LEYSEN JE (1998). Detailed mapping of serotonin 5-HT1B and 5-HT1D receptor messenger RNA and ligand binding sites in guinea-pig brain and trigeminal ganglion: clues for function. *Neuroscience* **82**, 469-484.

BONHAUS DW, BACH C, DESOUZA A, SALAZAR FH, MATSUOKA BD, ZUPPAN P *et al.* (1995). The pharmacology and distribution of human 5-hydroxytryptamine2B (5-HT2B) receptor gene products: comparison with 5-HT2A and 5-HT2C receptors. *Br J Pharmacol* **115**, 622-628.

BONHAUS DW, FLIPPIN LA, GREENHOUSE RJ, JAIME S, ROCHA C, DAWSON M *et al.* (1999). RS-127445: a selective, high affinity, orally bioavailable 5-HT2B receptor antagonist. *Br J Pharmacol* **127**, 1075-1082.

BONHOMME N, DE DEURWAERDERE P, LE MOAL M & SPAMPINATO U (1995). Evidence for 5-HT4 receptor subtype involvement in the enhancement of striatal dopamine release induced by serotonin: a microdialysis study in the halothane-anesthetized rat. *Neuropharmacology* **34**, 269-279.

BOOTLE DJ, ADCOCK JJ & RAMAGE AG (1996). Involvement of central 5-HT1A receptors in the reflex activation of pulmonary vagal motoneurones by inhaled capsaicin in anaesthetized cats. *Br J Pharmacol* **117**, 724-728.

BOOTLE DJ, ADCOCK JJ & RAMAGE AG (1998). The role of central 5-HT receptors in the bronchoconstriction evoked by inhaled capsaicin in anaesthetised guinea-pigs. *Neuropharmacology* **37**, 243-250.

BOSCHERT U, AMARA DA, SEGU L & HEN R (1994). The mouse 5-hydroxytryptamine1B receptor is localized predominantly on axon terminals. *Neuroscience* **58**, 167-182.

BOULENGUEZ P, PETERS SL, MITCHELL SN, CHAUVEAU J, GRAY JA & JOSEPH MH (1998). Dopamine release in the nucleus accumbens and latent inhibition in the rat following microinjections of a 5-HT1B agonist into the dorsal subiculum: implications for schizophrenia. *J Psychopharmacol* **12**, 258-267.

BOURSON A, BORRONI E, AUSTIN RH, MONSMA FJ, JR. & SLEIGHT AJ (1995). Determination of the role of the 5-ht6 receptor in the rat brain: a study using antisense oligonucleotides. *J Pharmacol Exp Ther* **274**, 173-180.

BOUTREL B, FRANC B, HEN R, HAMON M & ADRIEN J (1999). Key role of 5-HT1B receptors in the regulation of paradoxical sleep as evidenced in 5-HT1B knock-out mice. *J Neurosci* **19**, 3204-3212.

BOWKER RM, WESTLUND KN, SULLIVAN MC & COULTER JD (1982). Organization of descending serotonergic projections to the spinal cord. *Prog Brain Res* **57**, 239-265.

BRADLEY PB, ENGEL G, FENIUK W, FOZARD JR, HUMPHREY PP, MIDDLEMISS DN *et al.* (1986). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacology* **25**, 563-576.

BRANCHEK TA & BLACKBURN TP (2000). 5-ht6 receptors as emerging targets for drug discovery. *Annu Rev Pharmacol Toxicol* **40**, 319-334.

BRODIE BB & SHORE PA (1957). A concept for a role of serotonin and norepinephrine as chemical mediators in the brain. *Ann N Y Acad Sci* **66**, 631-642.

BROPHY S, FORD TW, CAREY M & JONES JF (1999). Activity of aortic chemoreceptors in the anaesthetized rat. *J Physiol* **514 (Pt 3)**, 821-828.

BROWN DL & GUYENET PG (1985). Electrophysiological study of cardiovascular neurons in the rostral ventrolateral medulla in rats. *Circ Res* **56**, 359-369.

BROWNING KN & TRAVAGLI RA (1999). Characterization of the in vitro effects of 5-hydroxytryptamine (5-HT) on identified neurones of the rat dorsal motor nucleus of the vagus (DMV). *Br J Pharmacol* **128**, 1307-1315.

BROWNING KN & TRAVAGLI RA (2001). The peptide TRH uncovers the presence of presynaptic 5-HT1A receptors via activation of a second messenger pathway in the rat dorsal vagal complex. *J Physiol* **531**, 425-435.

BRUINVELS AT, LANDWEHRMEYER B, GUSTAFSON EL, DURKIN MM, MENGOD G, BRANCHEK TA *et al.* (1994). Localization of 5-HT1B, 5-HT1D alpha, 5-HT1E and 5-HT1F receptor messenger RNA in rodent and primate brain. *Neuropharmacology* **33**, 367-386.

BRUINVELS AT, PALACIOS JM & HOYER D (1993). Autoradiographic characterisation and localisation of 5-HT1D compared to 5-HT1B binding sites in rat brain. *Naunyn Schmiedebergs Arch Pharmacol* **347**, 569-582.

BRUNELLO N, ARMITAGE R, FEINBERG I, HOLSBOER-TRACHSLER E, LEGER D, LINKOWSKI P *et al.* (2000). Depression and sleep disorders: clinical relevance, economic burden and pharmacological treatment. *Neuropsychobiology* **42**, 107-119.

BUCKINGHAM RE, HAMILTON TC & MOORE RA (1976). Prolonged effects of p-chlorophenylalanine on the blood pressure of conscious normotensive and DOCA/saline hypertensive rats. *Br J Pharmacol* **56**, 69-75.

BUNZL-FEDERN E (1899). Der zentrale Urpsrung des Nervus Vagus. *Psychiatria Neurologia* **5**, 1-22.

BURNSTOCK G (1986). Purines and cotransmitters in adrenergic and cholinergic neurones. *Prog Brain Res* **68**, 193-203.

CAJAL SR (2000). *Texture of the Nervous System of Man and the Vertebrates* Springer, Vienna.

CALEGARI F, COCO S, TAVERNA E, BASSETTI M, VERDERIO C, CORRADI N *et al.* (1999). A regulated secretory pathway in cultured hippocampal astrocytes. *J Biol Chem* **274**, 22539-22547.

CALHOUN JW & CALHOUN DD (1996). Prolonged bleeding time in a patient treated with sertraline. *Am J Psychiatry* **153**, 443.

CALZA L, GIARDINO L, GRIMALDI R, RIGOLI M, STENBUSCH HWM & TIENGO M (1985). Presence of 5-HT-positive neurons in the medial nuclei of the solitary tract. *Brain Research* **347**, 135-139.

CARBONI E, ACQUAS E, FRAU R & DI CHIARA G (1989). Differential inhibitory effects of a 5-HT3 antagonist on drug-induced stimulation of dopamine release. *Eur J Pharmacol* **164**, 515-519.

CAREY PA & JORDAN D (1998). Effects of central A2 receptors on aortic baroreceptor and cardiopulmonary receptor reflexes in anaesthetized rabbits. *J Physiol* **513**, 79P.

CAREY PA & JORDAN D (1999). A2 receptor-mediated modulation of the aortic baroreceptor and cardiopulmonary receptor reflexes in rabbits is antagonized by 3,7-dimethyl-1-propargylxanthine (DMPX). *J Physiol* **518**, 28-29P.

CARLI M, PRONTERA C & SAMANIN R (1989). Evidence that central 5-hydroxytryptaminergic neurones are involved in the anxiolytic activity of buspirone. *Br J Pharmacol* **96**, 829-836.

CARRIVE P, BANDLER R & DAMPNEY RA (1988). Anatomical evidence that hypertension associated with the defence reaction in the cat is mediated by a direct projection from a restricted portion of the midbrain periaqueductal grey to the subretrofacial nucleus of the medulla. *Brain Res* **460**, 339-345.

CARSON MJ, THOMAS EA, DANIELSON PE & SUTCLIFFE JG (1996). The 5HT5A serotonin receptor is expressed predominantly by astrocytes in which it inhibits cAMP accumulation: a mechanism for neuronal suppression of reactive astrocytes. *Glia* **17**, 317-326.

CASTRO ME, PASCUAL J, ROMON T, DEL ARCO C, DEL OLMO E & PAZOS A (1997). Differential distribution of [3H]sumatriptan binding sites (5-HT1B, 5-HT1D and 5-HT1F receptors) in human brain: focus on brainstem and spinal cord. *Neuropharmacology* **36**, 535-542.

CATELLI JM, GIAKAS WJ & SVED AF (1987). GABAergic mechanisms in nucleus tractus solitarius alter blood pressure and vasopressin release. *Brain Res* **403**, 279-289.

CEDARBAUM JM & AGHAJANIAN GK (1978). Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique. *J Comp Neurol* **178**, 1-16.

CENTURION D, SANCHEZ-LOPEZ A, DE VRIES P, SAXENA PR & VILLALON CM (2001). The GR127935-sensitive 5-HT(1) receptors mediating canine internal carotid vasoconstriction: resemblance to the 5-HT(1B), but not to the 5- HT(1D) or 5-ht(1F), receptor subtype. *Br J Pharmacol* **132**, 991-998.

CENTURION D, SANCHEZ-LOPEZ A, ORTIZ MI, DE VRIES P, SAXENA PR & VILLALON CM (2000). Mediation of 5-HT-induced internal carotid vasodilatation in GR127935- and ritanserin-pretreated dogs by 5-HT7 receptors. *Naunyn Schmiedebergs Arch Pharmacol* **362**, 169-176.

CERVONI P, BERTINO JR & GEIGER LE (1963). Medullary vagal effects of D-lysergic acid diethylamide in the decerebrate cat. *Nature* **199**, 700-701.

CHAOULOFF F, COURVOISIER H, MOISAN MP & MORMEDE P (1999). GR 127935 reduces basal locomotor activity and prevents RU 24969-, but not D-amphetamine-induced hyperlocomotion, in the Wistar-Kyoto hyperactive (WKHA) rat. *Psychopharmacology (Berl)* **141**, 326-331.

CHAPUT Y, BLIER P & DE MONTIGNY C (1986). In vivo electrophysiological evidence for the regulatory role of autoreceptors on serotonergic terminals. *J Neurosci* **6**, 2796-2801.

CHAPUT Y, LESIEUR P & DE MONTIGNY C (1990). Effects of short-term serotonin depletion on the efficacy of serotonin neurotransmission: electrophysiological studies in the rat central nervous system. *Synapse* **6**, 328-337.

CHEN JP, VAN PRAAG HM & GARDNER EL (1991). Activation of 5-HT3 receptor by 1-phenylbiguanide increases dopamine release in the rat nucleus accumbens. *Brain Res* **543**, 354-357.

CHEN NH & REITH ME (1995). Monoamine interactions measured by microdialysis in the ventral tegmental area of rats treated systemically with (+/-)-8-hydroxy-2-(di-n-propylamino)tetralin. *J Neurochem* **64**, 1585-1597.

CHIANCA DA & MACHADO BH (1996). Microinjection of NMDA antagonist into the NTS of conscious rats blocks the Bezold-Jarisch reflex. *Brain Res* **718**, 185-188.

CHIBA T & MASUKO S (1989). Coexistence of varying combinations of neuropeptides with 5- hydroxytryptamine in neurons of the raphe pallidus et obscurus projecting to the spinal cord. *Neurosci Res* **7**, 13-23.

CLAEYSEN S, FAYE P, SEBBEN M, LEMAIRE S, BOCKAERT J & DUMUIS A (1997). Cloning and expression of human 5-HT4S receptors. Effect of receptor density on their coupling to adenylyl cyclase. *Neuroreport* **8**, 3189-3196.

CLEMENT ME & MCCALL RB (1990). Studies on the site and mechanism of the sympathoexcitatory action of 5-HT2 agonists. *Brain Res* **515**, 299-302.

CODINA J, YATANI A, GRENET D, BROWN AM & BIRNBAUMER L (1987). The alpha subunit of the GTP binding protein Gk opens atrial potassium channels. *Science* **236**, 442-445.

COHEN H, KOTLER M, MATAR M & KAPLAN Z (2000). Normalization of heart rate variability in post-traumatic stress disorder patients following fluoxetine treatment: preliminary results. *Isr Med Assoc J* **2**, 296-301.

COLEMAN MJ & DAMPNEY RA (1995). Powerful depressor and sympathoinhibitory effects evoked from neurons in the caudal raphe pallidus and obscurus. *Am J Physiol* **268**, R1295-R1302.

COLEMAN MJ & DAMPNEY RA (1998). Sympathoinhibition evoked from caudal midline medulla is mediated by GABA receptors in rostral VLM. *Am J Physiol* **274**, R318-R323.

COLERIDGE HM & COLERIDGE JC (1979). Chemoreflex regulation of the heart. In *Handbook of Physiology, Sect 2 Vol I: Circulation.*, ed. Berne RM, pp. 653-676. American Physiological Society, Washington D.C.

COLERIDGE HM & COLERIDGE JC (1980). Cardiovascular afferents involved in regulation of peripheral vessels. *Annu Rev Physiol* **42**, 413-427.

COLERIDGE HM & COLERIDGE JC (1994). Pulmonary reflexes: neural mechanisms of pulmonary defense. *Annu Rev Physiol* **56**, 69-91.

COLERIDGE HM, COLERIDGE JC, DANGEL A, KIDD C, LUCK JC & SLEIGHT P (1973). Impulses in slowly conducting vagal fibers from afferent endings in the veins, atria, and arteries of dogs and cats. *Circ Res* **33**, 87-97.

COLERIDGE JC & COLERIDGE HM (1977). Afferent C-fibers and cardiorespiratory chemoreflexes. *Am Rev Respir Dis* **115**, 251-260.

CONLEY RK, WILLIAMS TJ, FORD AP & RAMAGE AG (2001). The role of alpha(1)-adrenoceptors and 5-HT(1A) receptors in the control of the micturition reflex in male anaesthetized rats. *Br J Pharmacol* **133**, 61-72.

CONNOR HE & HIGGINS GA (1990). Cardiovascular effects of 5-HT1A receptor agonists injected into the dorsal raphe nucleus of conscious rats. *Eur J Pharmacol* **182**, 63-72.

CONSOLO S, ARNABOLDI S, GIORGI S, RUSSI G & LADINSKY H (1994). 5-HT4 receptor stimulation facilitates acetylcholine release in rat frontal cortex. *Neuroreport* **5**, 1230-1232.

CONSOLO S, RAMPONI S, LADINSKY H & BALDI G (1996). A critical role for D1 receptors in the 5-HT1A-mediated facilitation of in vivo acetylcholine release in rat frontal cortex. *Brain Res* **707**, 320-323.

COOTE JH (1988). The organisation of cardiovascular neurons in the spinal cord. *Rev Physiol Biochem Pharmacol* **110**, 147-285.

COOTE JH, DALTON DW, FENUIK W & HUMPHREY PP (1987). The central site of the sympatho-inhibitory action of 5- hydroxytryptamine in the cat. *Neuropharmacology* **26**, 147-154.

COOTE JH, HILTON SM & PEREZ-GONZALEZ JF (1979). Inhibition of the baroreceptor reflex on stimulation in the brain stem defence centre. *J Physiol* **288**, 549-560.

COOTE JH & MACLEOD VH (1974). The influence of bulbospinal monoaminergic pathways on sympathetic nerve activity. *J Physiol* **241**, 453-475.

COOTE JH & MACLEOD VH (1975). The spinal route of sympatho-inhibitory pathways descending from the medulla oblongata. *Pflugers Arch* **359**, 335-347.

COOTE JH, MACLEOD VH, FLEETWOOD-WALKER S & GILBEY MP (1981). The response of individual sympathetic preganglionic neurones to microelectrophoretically applied endogenous monoamines. *Brain Res* **215**, 135-145.

COOTE JH, MACLEOD VH & MARTIN IL (1978). Bulbospinal tryptaminergic neurones. A search for the role of bulbospinal tryptaminergic neurones in the control of sympathetic activity. *Pflugers Arch* **377**, 109-116.

CORTES R, SORIANO E, PAZOS A, PROBST A & PALACIOS JM (1988). Autoradiography of antidepressant binding sites in the human brain: localization using [3H]imipramine and [3H]paroxetine. *Neuroscience* **27**, 473-496.

COSTALL B, DOMENEY AM & NAYLOR RJ (1990). 5-HT3 receptor antagonists attenuate dopamine-induced hyperactivity in the rat. *Neuroreport* **1**, 77-80.

COX GE, JORDAN D, MORUZZI P, SCHWABER JS, SPYER KM & TURNER SA (1986). Amygdaloid influences on brain-stem neurones in the rabbit. *J Physiol* **381**, 135-148.

CRAIG DA & CLARKE DE (1990). Pharmacological characterization of a neuronal receptor for 5-hydroxytryptamine in guinea pig ileum with properties similar to the 5-hydroxytryptamine receptor. *J Pharmacol Exp Ther* **252**, 1378-1386.

CRISCIONE L, REIS DJ & TALMAN WT (1983). Cholinergic mechanisms in the nucleus tractus solitarii and cardiovascular regulation in the rat. *Eur J Pharmacol* **88**, 47-55.

CUSHING DJ & COHEN ML (1992). Serotonin-induced relaxation in canine coronary artery smooth muscle. *J Pharmacol Exp Ther* **263**, 123-129.

D'AMICO M, BERRINO L, PIZZIRUSSO A, DE N, V & ROSSI F (1996). Opposing effects on blood pressure following the activation of metabotropic and ionotropic glutamate receptors in raphe obscurus in the anaesthetized rat. *Naunyn Schmiedebergs Arch Pharmacol* **353**, 302-305.

DAHLSTROM A & FUXE K (1961). Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in cell bodies of brain stem neurons. *Acta Physiol Scand Suppl* **232**, 1-55.

DALY MB (1991). Some reflex cardioinhibitory responses in the cat and their modulation by central inspiratory neuronal activity. *J Physiol* **439**, 559-577.

DALY MB (1997). *Peripheral Arterial Chemoreceptors and Respiratory-Cardiovascular Integration* Clarendon Press, Oxford.

DALY MB & SCOTT MJ (1958). The effects of stimulation of the carotid body chemoreceptors on heart rate in the dog. *J Physiol* **144**, 148-166.

DALY MB & SCOTT MJ (1963). The cardiovascular responses to stimulation of the carotid body chemoreceptors in the dog. *J Physiol* **165**, 179-197.

DALY MD & KIRKMAN E (1988). Cardiovascular responses to stimulation of pulmonary C fibres in the cat: their modulation by changes in respiration. *J Physiol* **402**, 43-63.

DAMPNEY RA, CZACHURSKI J, DEMBOWSKY K, GOODCHILD AK & SELLER H (1987). Afferent connections and spinal projections of the pressor region in the rostral ventrolateral medulla of the cat. *J Auton Nerv Syst* **20**, 73-86.

DAMPNEY RA, GOODCHILD AK & TAN E (1985). Vasopressor neurons in the rostral ventrolateral medulla of the rabbit. *J Auton Nerv Syst* **14**, 239-254.

DANDO SB (1995). *The role of 5-hydroxytryptamine receptors in the reflex activation of cardiac vagal motoneurones in the anaesthetised rabbit and rat.* Ph.D Thesis, University of London.

DANDO SB, SKINNER MR, JORDAN D & RAMAGE AG (1998). Modulation of the vagal bradycardia evoked by stimulation of upper airway receptors by central 5-HT1 receptors in anaesthetized rabbits. *Br J Pharmacol* **125**, 409-417.

DANNER H & PFISTER C (1980). Untersuchungen zur Zytoarchitektonik des Nucleus raphe dorsalis der Ratte. *J Hirnforsch* **21**, 655-664.

DANTAS MA, CO W & FUTURO-NETO HA (1990). Responses of neurons of the nucleus raphe obscurus to noxious stimuli. *Braz J Med Biol Res* **23**, 923-926.

DASHWOOD MR, GILBEY MP, JORDAN D & RAMAGE AG (1988). Autoradiographic localisation of 5-HT1A binding sites in the brainstem of the cat. *Br J Pharmacol* **386**, 94P.

DATLA KP & CURZON G (1996). Effect of p-chlorophenylalanine at moderate dosage on 5-HT and 5-HIAA concentrations in brain regions of control and p-chloroamphetamine treated rats. *Neuropharmacology* **35**, 315-320.

DAVIDSON NS, GOLDNER S & MCCLOSKEY DI (1976). Respiratory modulation of barareceptor and chemoreceptor reflexes affecting heart rate and cardiac vagal efferent nerve activity. *J Physiol* **259**, 523-530.

DAWES GS & COMROE JH (1954). Chemoreflexes from the heart and lungs. *Physiol Rev* **34**, 167-201.

DE MONTIGNY C, CHAPUT Y & BLIER P (1990). Modification of serotonergic neuron properties by long-term treatment with serotonin reuptake blockers. *J Clin Psychiatry* **51 Suppl B**, 4-8.

DE VRIES P, VILLALON CM & SAXENA PR (1999). Pharmacological aspects of experimental headache models in relation to acute antimigraine therapy. *Eur J Pharmacol* **375**, 61-74.

DERKACH V, SURPRENANT A & NORTH RA (1989). 5-HT3 receptors are membrane ion channels. *Nature* **339**, 706-709.

DESCARRIES L, BEAUDET A & WATKINS KC (1975). Serotonin nerve terminals in adult rat neocortex. *Brain Res* **100**, 563-588.

DESCARRIES L, WATKINS KC, GARCIA S & BEAUDET A (1982). The serotonin neurons in nucleus raphe dorsalis of adult rat: a light and electron microscope radioautographic study. *J Comp Neurol* **207**, 239-254.

DEUCHARS J & IZZO PN (1991). Demonstration of a monosynaptic pathway from the nucleus tractus soliatrius to regions of the ventral lateral medulla with specific reference to vagal motoneurones in the nucleus ambiguus of the anaesthetised cat. *J Physiol* **438**, 80P.

DI FRANCESCO GF, PETTY MA & FOZARD JR (1988). Antihypertensive effects of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) in conscious dogs. *Eur J Pharmacol* **147**, 287-290.

DICKENSON AH (1977). Specific responses of rat raphe neurones to skin temperature. *J Physiol* **273**, 277-293.

DICKENSON AH & SULLIVAN AF (1986). Electrophysiological studies on the effects of intrathecal morphine on nociceptive neurones in the rat dorsal horn. *Pain* **24**, 211-222.

DIETRICH WD, LOWRY OH & LOEWY AD (1982). The distribution of glutamate, GABA and aspartate in the nucleus tractus solitarius of the cat. *Brain Res* **237**, 254-260.

DIMICCO JA, GALE K, HAMILTON B & GILLIS RA (1979). GABA receptor control of parasympathetic outflow to heart: characterization and brainstem localization. *Science* **204**, 1106-1109.

DITTMAR C (1873). Über die Lage des sogenannten Gefässcentrums in der Medulla oblongata. *Bericht über die Verhandlungen der Sachischen* **25**, 449-469.

DOMENECH T, BELETA J & PALACIOS JM (1997). Characterization of human serotonin 1D and 1B receptors using [3H]-GR-125743, a novel radiolabelled serotonin 5HT1D/1B receptor antagonist. *Naunyn Schmiedebergs Arch Pharmacol* **356**, 328-334.

DONE CJ & SHARP T (1992). Evidence that 5-HT2 receptor activation decreases noradrenaline release in rat hippocampus in vivo. *Br J Pharmacol* **107**, 240-245.

DONE CJ & SHARP T (1994). Biochemical evidence for the regulation of central noradrenergic activity by 5-HT1A and 5-HT2 receptors: microdialysis studies in the awake and anaesthetized rat. *Neuropharmacology* **33**, 411-421.

DONOGHUE S, FELDER RB, GILBEY MP, JORDAN D & SPYER KM (1985). Post-synaptic activity evoked in the nucleus tractus solitarius by carotid sinus and aortic nerve afferents in the cat. *J Physiol* **360**, 261-273.

DONOGHUE S, FELDER RB, JORDAN D & SPYER KM (1984). The central projections of carotid baroreceptors and chemoreceptors in the cat: a neurophysiological study. *J Physiol* **347**, 397-409.

DONOGHUE S, GARCIA M, JORDAN D & SPYER KM (1982a). Identification and brain-stem projections of aortic baroreceptor afferent neurones in nodose ganglia of cats and rabbits. *J Physiol* **322**, 337-352.

DONOGHUE S, GARCIA M, JORDAN D & SPYER KM (1982b). The brain-stem projections of pulmonary stretch afferent neurones in cats and rabbits. *J Physiol* **322**, 353-363.

DOUCET E, POHL M, FATTACCINI CM, ADRIEN J, MESTIKAWY SE & HAMON M (1995). In situ hybridization evidence for the synthesis of 5-HT1B receptor in serotoninergic neurons of anterior raphe nuclei in the rat brain. *Synapse* **19**, 18-28.

DOURISH CT, HUTSON PH & CURZON G (1985a). Characteristics of feeding induced by the serotonin agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT). *Brain Res Bull* **15**, 377-384.

DOURISH CT, HUTSON PH & CURZON G (1985b). Low doses of the putative serotonin agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) elicit feeding in the rat. *Psychopharmacology* **86**, 197-204.

DOYLE MW & ANDRESEN MC (2001). Reliability of monosynaptic sensory transmission in brain stem neurons in vitro. *J Neurophysiol* **85**, 2213-2223.

DRESHAJ IA, HAXHIU MA & MARTIN RJ (1998). Role of the medullary raphe nuclei in the respiratory response to CO2. *Respir Physiol* **111**, 15-23.

DRETELER GH, WOUTERS W, SAXENA PR & RAMAGE AG (1991). Pressor effects following microinjection of 5-HT1A receptor agonists into the raphe obscurus of the anaesthetized rat. *Br J Pharmacol* **102**, 317-322.

DRINGENBERG HC (2000). Serotonergic receptor antagonists alter responses to general anaesthetics in rats. *Br J Anaesth* **85**, 904-906.

DUXON MS, FLANIGAN TP, REAVLEY AC, BAXTER GS, BLACKBURN TP & FONE KC (1997a). Evidence for expression of the 5-hydroxytryptamine-2B receptor protein in the rat central nervous system. *Neuroscience* **76**, 323-329.

DUXON MS, KENNETT GA, LIGHTOWLER S, BLACKBURN TP & FONE KC (1997b). Activation of 5-HT2B receptors in the medial amygdala causes anxiolysis in the social interaction test in the rat. *Neuropharmacology* **36**, 601-608.

EDVINSSON L, DEGUEURCE A, DUVERGER D, MACKENZIE ET & SCATTON B (1983). Central serotonergic nerves project to the pial vessels of the brain. *Nature* **306**, 55-57.

EDWARDS E, HARKINS K, ASHBY CR, JR. & WANG RY (1991). Effect of 5-hydroxytryptamine3 receptor agonists on phosphoinositides hydrolysis in the rat fronto-cingulate and entorhinal cortices. *J Pharmacol Exp Ther* **256**, 1025-1032.

EDWARDS E & PATON JF (1999). 5-HT(4) receptors in nucleus tractus solitarii attenuate cardiopulmonary reflex in anesthetized rats. *Am J Physiol* **277**, H1914-H1923.

EDWARDS E & PATON JF (2000). Glutamate stimulation of raphe pallidus attenuates the cardiopulmonary reflex in anaesthetised rats. *Auton Neurosci* **82**, 87-96.

EGLEN RM (1997). 5-Hydroxytryptamine (5-HT)4 receptors and central nervous system function: an update. *Prog Drug Res* **49**, 9-24.

ELSNER R, ANGELL-JAMES JE & DE BURGH DM (1977). Carotid body chemoreceptor reflexes and their interactions in the seal. *Am J Physiol* **232**, H517-H525.

ERLANDER MG, LOVENBERG TW, BARON BM, DE LECEA L, DANIELSON PE, RACKE M *et al.* (1993). Two members of a distinct subfamily of 5-hydroxytryptamine receptors differentially expressed in rat brain. *Proc Natl Acad Sci U S A* **90**, 3452-3456.

EVRARD A, LAPORTE AM, CHASTANET M, HEN R, HAMON M & ADRIEN J (1999). 5-HT1A and 5-HT1B receptors control the firing of serotoninergic

neurons in the dorsal raphe nucleus of the mouse: studies in 5-HT1B knock-out mice. *Eur J Neurosci* **11**, 3823-3831.

FAGNI L, DUMUIS A, SEBBEN M & BOCKAERT J (1992). The 5-HT4 receptor subtype inhibits K+ current in colliculi neurones via activation of a cyclic AMP-dependent protein kinase. *Br J Pharmacol* **105**, 973-979.

FANCIULLACCI M, SICUTERI R, ALESSANDRI M & GEPPETTI P (1995). Buspirone, but not sumatriptan, induces miosis in humans: relevance for a serotoninergic pupil control. *Clin Pharmacol Ther* **57**, 349-355.

FARBER L, STRATZ TH, BRUCKLE W, SPATH M, PONGRATZ D, LAUTENSCHLAGER J *et al.* (2001). Short-term treatment of primary fibromyalgia with the 5-HT3-receptor antagonist tropisetron. Results of a randomized, double-blind, placebo-controlled multicenter trial in 418 patients. *Int J Clin Pharmacol Res* **21**, 1-13.

FARGIN A, RAYMOND JR, LOHSE MJ, KOBILKA BK, CARON MG & LEFKOWITZ RJ (1988). The genomic clone G-21 which resembles a beta-adrenergic receptor sequence encodes the 5-HT1A receptor. *Nature* **335**, 358-360.

FELDBERG W & GUERTZENSTEIN PG (1976). Vasodepressor effects obtained by drugs acting on the ventral surface of the brain stem. *J Physiol* **258**, 337-355.

FELDER RB & MIFFLIN SW (1988). Modulation of carotid sinus afferent input to nucleus tractus solitarius by parabrachial nucleus stimulation. *Circ Res* **63**, 35-49.

FELLIN T & CARMIGNOTO G (2004). Neurone-to-astrocyte signalling in the brain represents a distinct multifunctional unit. *J Physiol* **559**, 3-15.

FIDONE SJ & SATO A (1969). A study of chemoreceptor and baroreceptor A and C-fibres in the cat carotid nerve. *J Physiol* **205**, 527-548.

FILE SE, GONZALEZ LE & ANDREWS N (1996). Comparative study of pre- and postsynaptic 5-HT1A receptor modulation of anxiety in two ethological animal tests. *J Neurosci* **16**, 4810-4815.

FINLEY JC, MADERDRUT JL, ROGER LJ & PETRUSZ P (1981). The immunocytochemical localization of somatostatin-containing neurons in the rat central nervous system. *Neuroscience* **6**, 2173-2192.

FITZGERALD RS & PARKS DC (1971). Effect of hypoxia on carotid chemoreceptor response to carbon dioxide in cats. *Respir Physiol* **12**, 218-229.

FLANIGAN TP, REAVLEY AC & CAREY JE (1995). Evidence for expression of the 5-HT1B receptor mRNA in rat brain. *Br J Pharmacol* **114**, 369P.

FLETCHER PJ & DAVIES M (1990). Dorsal raphe microinjection of 5-HT and indirect 5-HT agonists induces feeding in rats. *Eur J Pharmacol* **184**, 265-271.

FLETCHER PJ & KORTH KM (1999). Activation of 5-HT1B receptors in the nucleus accumbens reduces amphetamine-induced enhancement of responding for conditioned reward. *Psychopharmacology (Berl)* **142**, 165-174.

FOGUET M, HOYER D, PARDO LA, PAREKH A, KLUXEN FW, KALKMAN HO *et al.* (1992). Cloning and functional characterization of the rat stomach fundus serotonin receptor. *EMBO J* **11**, 3481-3487.

FONTANA DJ, DANIELS SE, WONG EH, CLARK RD & EGLEN RM (1997). The effects of novel, selective 5-hydroxytryptamine (5-HT)4 receptor ligands in rat spatial navigation. *Neuropharmacology* **36**, 689-696.

FORD TW, BENNETT JA, KIDD C & MCWILLIAM PN (1990). Neurones in the dorsal motor vagal nucleus of the cat with non-myelinated axons projecting to the heart and lungs. *Exp Physiol* **75**, 459-473.

FORD TW & MCWILLIAM PN (1986). The effects of electrical stimulation of myelinated and non-myelinated vagal fibres on heart rate in the rabbit. *J Physiol* **380**, 341-347.

FORNAL CA, LITTO WJ, METZLER CW, MARROSU F, TADA K & JACOBS BL (1994). Single-unit responses of serotonergic dorsal raphe neurons to 5-HT1A agonist and antagonist drug administration in behaving cats. *J Pharmacol Exp Ther* **270**, 1345-1358.

FORNAL CA, METZLER CW, GALLEGOS RA, VEASEY SC, MCCREARY AC & JACOBS BL (1996). WAY-100635, a potent and selective 5-hydroxytryptamine1A antagonist, increases serotonergic neuronal activity in behaving cats: comparison with (S)-WAY-100135. *J Pharmacol Exp Ther* **278**, 752-762.

FORSTER EA, CLIFFE IA, BILL DJ, DOVER GM, JONES D, REILLY Y *et al.* (1995). A pharmacological profile of the selective silent 5-HT_{1A} receptor antagonist, WAY-100635. *Eur J Pharmacol* **281**, 81-88.

FOZARD JR, MIR AK & MIDDLEMISS DN (1987). Cardiovascular response to 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) in the rat: site of action and pharmacological analysis. *J Cardiovasc Pharmacol* **9**, 328-347.

FOZARD JR & RAMAGE AG (1984). The effects of 8-hydroxy-2-(di-n-propylamino) tetralin on the cardiovascular system of the cat: comparison with clonidine. *Br J Pharmacol* **83**, 391P.

FRANCHINI KG & KREIGER EM (1993). Cardiovascular responses of conscious rats to carotid body chemoreceptor stimulation by intravenous KCN. *J Auton Nerv Syst* **42**, 63-70.

FRANCKEN BJ, JURZAK M, VANHAUWE JF, LUYTEN WH & LEYSEN JE (1998). The human 5-ht5A receptor couples to Gi/Go proteins and inhibits adenylate cyclase in HEK 293 cells. *Eur J Pharmacol* **361**, 299-309.

FRANKFURT M, LAUDER JM & AZMITIA EC (1981). The immunocytochemical localization of serotonergic neurons in the rat hypothalamus. *Neurosci Lett* **24**, 227-232.

FULLER RW (1996). Serotonin receptors involved in regulation of pituitary-adrenocortical function in rats. *Behav Brain Res* **73**, 215-219.

FUTURO-NETO HA, PIRES JG, GILBEY MP & RAMAGE AG (1993). Evidence for the ability of central 5-HT1A receptors to modulate the vagal bradycardia induced by stimulating the upper airways of anesthetized rabbits with smoke. *Brain Res* **629**, 349-354.

FUXE K & UNGERSTEDT U (1968). Histochemical studies on the distribution of catecholamines and 5-hydroxytryptamine after intraventricular injections. *Histochemie* **13**, 16-28.

GADDUM JH & PICARELLI ZP (1957). Two kinds of tryptamine receptor. *Br J Pharmacol* **12**, 323-328.

GALLOWAY MP, SUCHOWSKI CS, KEEGAN MJ & HJORTH S (1993). Local infusion of the selective 5HT-1b agonist CP-93,129 facilitates striatal dopamine release in vivo. *Synapse* **15**, 90-92.

GARCIA PEREZ M & JORDAN D (2001). Effect of stimulating non-myelinated vagal axons on atrio-ventricular conduction and left ventricular function in anaesthetized rabbits. *Auton Neurosci* **86**, 183-191.

GARTSIDE SE, UMBERS V, HAJOS M & SHARP T (1995). Interaction between a selective 5-HT1A receptor antagonist and an SSRI in vivo: effects on 5-HT cell firing and extracellular 5-HT. *Br J Pharmacol* **115**, 1064-1070.

GARTSIDE SE, UMBERS V & SHARP T (1997). Inhibition of 5-HT cell firing in the DRN by non-selective 5-HT reuptake inhibitors: studies on the role of 5-HT1A autoreceptors and noradrenergic mechanisms. *Psychopharmacology (Berl)* **130**, 261-268.

GAUDIN-CHAZAL G, PORTALIER P, BARRIT MC & PUIZILLOUT JJ (1982). Serotonin-like immunoreactivity in paraffin-sections of the nodose ganglia of the cat. *Neurosci Lett* **33**, 169-172.

GELERNTER J, RAO PA, PAULS DL, HAMBLIN MW, SIBLEY DR & KIDD KK (1995). Assignment of the 5HT7 receptor gene (HTR7) to chromosome 10q and exclusion of genetic linkage with Tourette syndrome. *Genomics* **26**, 207-209.

GERALD C, ADHAM N, KAO HT, OLSEN MA, LAZ TM, SCHECHTER LE *et al.* (1995). The 5-HT4 receptor: molecular cloning and pharmacological characterization of two splice variants. *EMBO J* **14**, 2806-2815.

GERANTON SM, HEAL DJ & STANFORD SC (2004). 5-HT has contrasting effects in the frontal cortex, but not the hypothalamus, on changes in noradrenaline efflux induced by the monoamine releasing-agent, d-amphetamine, and the reuptake inhibitor, BTS 54 354. *Neuropharmacology* **46**, 511-518.

GERARD C, EL MESTIKAWY S, LEBRAND C, ADRIEN J, RUAT M, TRAIFFORT E *et al.* (1996). Quantitative RT-PCR distribution of serotonin 5-HT6 receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. *Synapse* **23**, 164-173.

GETZ B & SIRNES T (1949). The localisation within the dorsal motor vagal nucleus. *J Comp Neurol* **90**, 95-110.

GEURTS FJ, DE SCHUTTER E & TIMMERMANS JP (2002). Localization of 5-HT2A, 5-HT3, 5-HT5A and 5-HT7 receptor-like immunoreactivity in the rat cerebellum. *J Chem Neuroanat* **24**, 65-74.

GIEROBA ZJ, MACKENZIE L, WILLOUGHBY JO & BLESSING WW (1995). Fos-determined distribution of neurons activated during the Bezold- Jarisch reflex in the medulla oblongata in conscious rabbits and rats. *Brain Res* **683**, 43-50.

GILBEY MP, COOTE JH, MACLEOD VH & PETERSON DF (1981). Inhibition of sympathetic activity by stimulating in the raphe nuclei and the role of 5-hydroxytryptamine in this effect. *Brain Res* **226**, 131-142.

GILBEY MP, JORDAN D, RICHTER DW & SPYER KM (1984). Synaptic mechanisms involved in the inspiratory modulation of vagal cardio-inhibitory neurones in the cat. *J Physiol* **356**, 65-78.

GILL CH, SOFFIN EM, HAGAN JJ & DAVIES CH (2002). 5-HT7 receptors modulate synchronized network activity in rat hippocampus. *Neuropharmacology* **42**, 82-92.

GILLIS RA, HILL KJ, KIRBY JS, QUEST JA, HAMOSH P, NORMAN WP *et al.* (1989). Effect of activation of central nervous system serotonin 1A receptors on cardiorespiratory function. *J Pharmacol Exp Ther* **248**, 851-857.

GIORDANO J & DYCHE J (1989). Differential analgesic actions of serotonin 5-HT3 receptor antagonists in the mouse. *Neuropharmacology* **28**, 423-427.

GLASSMAN AH et al. (1987). Cardiovascular effects of tricyclic antidepressants. In *Psychopharmacology: The Third Generation of Progress*, ed. Meltzer HY, pp. 1437-1442. Raven Press, New York.

GLENNON RA (2003). Higher-end serotonin receptors: 5-H5, 5-HT6 and 5-HT7. *J Med Chem* **46**, 2795-2812.

GLENNON RA (1990). Do classical hallucinogens act as 5-HT2 agonists or antagonists? *Neuropsychopharmacology* **3**, 509-517.

GLENNON RA, LEE M, RANGISETTY JB, DUKAT M, ROTH BL, SAVAGE JE *et al.* (2000). 2-Substituted tryptamines: agents with selectivity for 5-HT(6) serotonin receptors. *J Med Chem* **43**, 1011-1018.

GOADSBY PJ (1998). Serotonin receptors and the acute attack of migraine. *Clin Neurosci* **5**, 18-23.

GOADSBY PJ, PIPER RD, LAMBERT GA & LANCE JW (1985). Effect of stimulation of nucleus raphe dorsalis on carotid blood flow. II. The cat. *Am J Physiol* **248**, R263-R269.

GOAILLARD JM & VINCENT P (2002). Serotonin suppresses the slow afterhyperpolarization in rat intralaminar and midline thalamic neurones by activating 5-HT(7) receptors. *J Physiol* **541**, 453-465.

GOINY M, LAGERCRANTZ H, SRINIVASAN M, UNGERSTEDT U & YAMAMOTO Y (1991). Hypoxia-mediated in vivo release of dopamine in nucleus tractus solitarii of rabbits. *J Appl Physiol* **70**, 2395-2400.

GOREA E, DAVENNE D, LANFUMEY L, CHASTANET M & ADRIEN J (1991). Regulation of noradrenergic coerulean neuronal firing mediated by 5-HT2 receptors: involvement of the prepositus hypoglossal nucleus. *Neuropharmacology* **30**, 1309-1318.

GOZLAN H, EL MESTIKAWY S, PICHAT L, GLOWINSKI J & HAMON M (1983). Identification of presynaptic serotonin autoreceptors using a new ligand: 3H-PAT. *Nature* **305**, 140-142.

GOZLAN H, PONCHANT M, DAVAL G, VERGE D, MENARD F, VANHOVE A *et al.* (1988). 125I-Bolton-Hunter-8-methoxy-2-[N-propyl-N-propylamino]tetralin as a new selective radioligand of 5-HT1A sites in the rat brain. In vitro binding and autoradiographic studies. *J Pharmacol Exp Ther* **244**, 751-759.

GRADIN K, PETTERSSON A, HJORTH S, HEDNER T, ARVIDSSON LE & PERSSON B (1985). Cardiovascular effects in the Sprague-Dawley rat of 8-hydroxy-2(di-N-propylamino) tetralin, a selective 5-hydroxytryptamine receptor agonist. *J Pharm Pharmacol* **37**, 263-265.

GRAEFF FG, GUIMARAES FS, DE ANDRADE TG & DEAKIN JF (1996). Role of 5-HT in stress, anxiety, and depression. *Pharmacol Biochem Behav* **54**, 129-141.

GRAILHE R, GRABTREE GW & HEN R (2001). Human 5-HT(5) receptors: the 5-HT(5A) receptor is functional but the 5-HT(5B) receptor was lost during mammalian evolution. *Eur J Pharmacol* **418**, 157-167.

GRAILHE R, WAEBER C, DULAWA SC, HORNUNG JP, ZHUANG X, BRUNNER D *et al.* (1999). Increased exploratory activity and altered response to LSD in mice lacking the 5-HT(5A) receptor. *Neuron* **22**, 581-591.

GRANATA AR & WOODRUFF GN (1982). Dopaminergic mechanisms in the nucleus tractus solitarius and effects on blood pressure. *Brain Res Bull* **8**, 483-488.

GROSSMAN CJ, KILPATRICK GJ & BUNCE KT (1993). Development of a radioligand binding assay for 5-HT4 receptors in guinea-pig and rat brain. *Br J Pharmacol* **109**, 618-624.

GUDELSKY GA, KOENIG JI & MELTZER HY (1986). Thermoregulatory responses to serotonin (5-HT) receptor stimulation in the rat. Evidence for opposing roles of 5-HT2 and 5-HT1A receptors. *Neuropharmacology* **25**, 1307-1313.

GUERTZENSTEIN PG & SILVER A (1974). Fall in blood pressure produced from discrete regions of the ventral surface of the medulla by glycine and lesions. *J Physiol* **242**, 489-503.

GUSCOTT MR, EGAN E, COOK GP, STANTON JA, BEER MS, ROSAHL TW *et al.* (2003). The hypothermic effect of 5-CT in mice is mediated through the 5-HT7 receptor. *Neuropharmacology* **44**, 1031-1037.

GUSTAFSON EL, DURKIN MM, BARD JA, ZGOMBICK J & BRANCHEK TA (1996). A receptor autoradiographic and *in situ* hybridization analysis of the distribution of the 5-ht_7 receptor in rat brain. *Br J Pharmacol* **117**, 657-666.

GUYENET PG, FILTZ TM & DONALDSON SR (1987). Role of excitatory amino acids in rat vagal and sympathetic baroreflexes. *Brain Res* **407**, 272-284.

HADZIEFENDIC S & HAXHIU MA (1999). CNS innervation of vagal preganglionic neurons controlling peripheral airways: a transneuronal labeling study using pseudorabies virus. *J Auton Nerv Syst* **76**, 135-145.

HAGAN JJ, PRICE GW, JEFFREY P, DEEKS NJ, STEAN T, PIPER D *et al.* (2000). Characterization of SB-269970-A, a selective 5-HT(7) receptor antagonist. *Br J Pharmacol* **130**, 539-548.

HAGAN JJ, SLADE PD, GASTER L, JEFFREY P, HATCHER JP & MIDDLEMISS DN (1997). Stimulation of 5-HT1B receptors causes hypothermia in the guinea pig. *Eur J Pharmacol* **331**, 169-174.

HAIBARA AS, BONAGAMBA LG & MACHADO BH (1999). Sympathoexcitatory neurotransmission of the chemoreflex in the NTS of awake rats. *Am J Physiol* **276**, R69-R80.

HAIBARA AS, COLOMBARI E, CHIANCA DA, JR., BONAGAMBA LG & MACHADO BH (1995). NMDA receptors in NTS are involved in bradycardic but not in pressor response of chemoreflex. *Am J Physiol* **269**, H1421-H1427.

HAIBARA AS, TAMASHIRO E, OLIVAN MV, BONAGAMBA LG & MACHADO BH (2002). Involvement of the parabrachial nucleus in the pressor response to chemoreflex activation in awake rats. *Auton Neurosci* **101**, 60-67.

HAIGLER HJ & AGHAJANIAN GK (1974). Lysergic acid diethylamide and serotonin: a comparison of effects on serotonergic neurons and neurons receiving a serotonergic input. *J Pharmacol Exp Ther* **188**, 688-699.

HAJ-DAHMANE S, HAMON M & LANFUMEY L (1991). K+ channel and 5-hydroxytryptamine1A autoreceptor interactions in the rat dorsal raphe nucleus: an in vitro electrophysiological study. *Neuroscience* **41**, 495-505.

HAJOS-KORCSOK E, MCQUADE R & SHARP T (1999). Influence of 5-HT1A receptors on central noradrenergic activity: microdialysis studies using (+/-)-MDL 73005EF and its enantiomers. *Neuropharmacology* **38**, 299-306.

HAJOS-KORCSOK E & SHARP T (1996). 8-OH-DPAT-induced release of hippocampal noradrenaline in vivo: evidence for a role of both 5-HT1A and dopamine D1 receptors. *Eur J Pharmacol* **314**, 285-291.

HALL H, LUNDKVIST C, HALLDIN C, FARDE L, PIKE VW, MCCARRON JA *et al.* (1997). Autoradiographic localization of 5-HT1A receptors in the post-mortem human brain using [3H]WAY-100635 and [11C]way-100635. *Brain Res* **745**, 96-108.

HAMBLIN MW & METCALF MA (1991). Primary structure and functional characterization of a human 5-HT1D-type serotonin receptor. *Mol Pharmacol* **40**, 143-148.

HAMBLIN MW, METCALF MA, MCGUFFIN RW & KARPELLS S (1992). Molecular cloning and functional characterization of a human 5-HT1B serotonin receptor: a homologue of the rat 5-HT1B receptor with 5-HT1D-like pharmacological specificity. *Biochem Biophys Res Commun* **184**, 752-759.

HAMIK A, OKSENBERG D, FISCHETTE C & PEROUTKA SJ (1990). Analysis of tandospirone (SM-3997) interactions with neurotransmitter receptor binding sites. *Biol Psychiatry* **28**, 99-109.

HAMILTON RB, ELLENBERGER H, LISKOWSKY D & SCHNEIDERMAN N (1981). Parabrachial area as mediator of bradycardia in rabbits. *J Auton Nerv Syst* **4**, 261-281.

HAMON M, DOUCET E, LEFEVRE K, MIQUEL MC, LANFUMEY L, INSAUSTI R *et al.* (1999). Antibodies and antisense oligonucleotide for probing the distribution and putative functions of central 5-HT6 receptors. *Neuropsychopharmacology* **21**, 68S-76S.

HAMON M, GALLISSOT MC, MENARD F, GOZLAN H, BOURGOIN S & VERGE D (1989). 5-HT3 receptor binding sites are on capsaicin-sensitive fibres in the rat spinal cord. *Eur J Pharmacol* **164**, 315-322.

HANSEN S, SVENSSON L, HOKFELT T & EVERITT BJ (1983). 5-Hydroxytryptamine-thyrotropin releasing hormone interactions in the spinal cord: effects on parameters of sexual behaviour in the male rat. *Neurosci Lett* **42**, 299-304.

HARTIG PR, BRANCHEK TA & WEINSHANK RL (1992). A subfamily of 5-HT1D receptor genes. *Trends Pharmacol Sci* **13**, 152-159.

HARTIG PR, HOYER D, HUMPHREY PP & MARTIN GR (1996). Alignment of receptor nomenclature with the human genome: classification of 5-HT1B and 5-HT1D receptor subtypes. *Trends Pharmacol Sci* **17**, 103-105.

HASELTON JR, WINTERS RW, LISKOWSKY DR, HASELTON CL, MCCABE PM & SCHNEIDERMAN N (1988). Cardiovascular responses elicited by electrical and chemical stimulation of the rostral medullary raphe of the rabbit. *Brain Res* **453**, 167-175.

HAXHIU MA, EROKWU B, BHARDWAJ V & DRESHAJ IA (1998). The role of the medullary raphe nuclei in regulation of cholinergic outflow to the airways. *J Auton Nerv Syst* **69**, 64-71.

HAXHIU MA, JANSEN AS, CHERNIACK NS & LOEWY AD (1993). CNS innervation of airway-related parasympathetic preganglionic neurons: a transneuronal labeling study using pseudorabies virus. *Brain Res* **618**, 115-134.

HAY M & BISHOP VS (1991). Effects of area postrema stimulation on neurons of the nucleus of the solitary tract. *Am J Physiol* **260**, H1359-H1364.

HAYMET BT & MCCLOSKEY DI (1975). Baroreceptor and chemoreceptor influences on heart rate during the respiratory cycle in the dog. *J Physiol* **245**, 699-712.

HEALY DP, JEW JY, BLACK AC, JR. & WILLIAMS TH (1981). Bradycardia following injection of 6-hydroxydopamine into the intermediate portion of nucleus tractus solitarius medialis. *Brain Res* **206**, 415-420.

HEDLUND PB, CARSON MJ, SUTCLIFFE JG & THOMAS EA (1999). Allosteric regulation by oleamide of the binding properties of 5-hydroxytryptamine7 receptors. *Biochem Pharmacol* **58**, 1807-1813.

HEDLUND PB, DANIELSON PE, THOMAS EA, SLANINA K, CARSON MJ & SUTCLIFFE JG (2003). No hypothermic response to serotonin in 5-HT7 receptor knockout mice. *Proc Natl Acad Sci U S A* **100**, 1375-1380.

HEDLUND PB, KELLY L, MAZUR C, LOVENBERG T, SUTCLIFFE JG & BONAVENTURE P (2004). 8-OH-DPAT acts on both 5-HT1A and 5-HT7 receptors to induce hypothermia in rodents. *Eur J Pharmacol* **487**, 125-132.

HEDLUND PB & SUTCLIFFE JG (2004). Funcitonal, molecular and pharmacological advances in 5-HT7 receptor research. *Trends Pharmacol Sci* **25**, 481-486.

HEGDE SS, BONHAUS DW, JOHNSON LG, LEUNG E, CLARK RD & EGLEN RM (1995). RS 39604: a potent, selective and orally active 5-HT4 receptor antagonist. *Br J Pharmacol* **115**, 1087-1095.

HEIDMANN DE, METCALF MA, KOHEN R & HAMBLIN MW (1997). Four 5-hydroxytryptamine7 (5-HT7) receptor isoforms in human and rat produced by alternative splicing: species differences due to altered intron-exon organization. *J Neurochem* **68**, 1372-1381.

HEKMATPANAH CR & PEROUTKA SJ (1990). 5-hydroxytryptamine uptake blockers attenuate the 5-hydroxytryptamine-releasing effect of 3,4-methylenedioxymethamphetamine and related agents. *Eur J Pharmacol* **177**, 95-98.

HELKE CJ, HANDELMANN GE & JACOBOWITZ DM (1983). Choline acetyltransferase activity in the nucleus tractus solitarius: regulation by the afferent vagus nerve. *Brain Res Bull* **10**, 433-436.

HENSLER JG, FERRY RC, LABOW DM, KOVACHICH GB & FRAZER A (1994). Quantitative autoradiography of the serotonin transporter to assess the distribution of serotonergic projections from the dorsal raphe nucleus. *Synapse* **17**, 1-15.

HERBERT H, MOGA MM & SAPER CB (1990). Connections of the parabrachial nucleus with the nucleus of the solitary tract and the medullary reticular formation in the rat. *J Comp Neurol* **293**, 540-580.

HERGES S & TAYLOR DA (2000). Involvement of 5-HT(3) receptors in the nucleus accumbens in the potentiation of cocaine-induced behaviours in the rat. *Br J Pharmacol* **131**, 1294-1302.

HERMANN DM, LUPPI PH, PEYRON C, HINCKEL P & JOUVET M (1997). Afferent projections to the rat nuclei raphe magnus, raphe pallidus and reticularis gigantocellularis pars alpha demonstrated by iontophoretic application of choleratoxin (subunit b). *J Chem Neuroanat* **13**, 1-21.

HERMANN GE, BRESNAHAN JC, HOLMES GM, ROGERS RC & BEATTIE MS (1998). Descending projections from the nucleus raphe obscurus to pudendal motoneurons in the male rat. *J Comp Neurol* **397**, 458-474.

HEURING RE & PEROUTKA SJ (1987). Characterization of a novel 3H-5-hydroxytryptamine binding site subtype in bovine brain membranes. *J Neurosci* **7**, 894-903.

HEYMANS C & BOUCKART JJ (1941). Au sujet des influences de l'alpha-nicotine et de la bêta-nicotine sur la respiration, la fréquence cardiaque et la pression artérielle. *Archives Internationales de Pharmacodynamie et de Thérapie* **65**, 196-205.

HEYMANS C, BOUCKART JJ & DAUTREBANDE L (1930). Sinus carotidien et réflexes respiratoires. II. Influences respiratoires réflexes de l'acidose, de l'alcalose, de l'anhydride carbonique, de l'ion hydrogène et de l'anoxémie. *Archives Internationales de Pharmacodynamie et de Thérapie* **39**, 400-448.

HICKS PE, CAVERO I, MANOURY P, LEFEVRE-BORG F & LANGER SZ (1987). Comparative analysis of beta-1 adrenoceptor agonist and antagonist potency and selectivity of cicloprolol, xamoterol and pindolol. *J Pharmacol Exp Ther* **242**, 1025-1034.

HILTON SM & MARSHALL JM (1982). The pattern of cardiovascular response to carotid chemoreceptor stimulation in the cat. *J Physiol* **326**, 495-513.

HIRASE H, QIAN L, BARTHO P & BUZSAKI G (2004). Calcium dynamics of cortical astrocytic networks in vivo. *PLoS Biol* **2**, E96.

HJORTH S, BENGTSSON HJ, MILANO S, LUNDBERG JF & SHARP T (1995). Studies on the role of 5-HT1A autoreceptors and alpha 1-adrenoceptors in the inhibition of 5-HT release--I. BMY7378 and prazosin. *Neuropharmacology* **34**, 615-620.

HJORTH S & SHARP T (1993). In vivo microdialysis evidence for central serotonin1A and serotonin1B autoreceptor blocking properties of the beta adrenoceptor antagonist (-)penbutolol. *J Pharmacol Exp Ther* **265**, 707-712.

HOF RP & FOZARD JR (1989). 8-OH-DPAT, flesinoxan and guanfacine: systemic and regional haemodynamic effects of centrally acting antihypertensive agents in anaesthetized rabbits. *Br J Pharmacol* **96**, 864-871.

HOFFMAN BJ, MEZEY E & BROWNSTEIN MJ (1991). Cloning of a serotonin transporter affected by antidepressants. *Science* **254**, 579-580.

HOKFELT T, LJUNGDAHL A, STEINBUSCH H, VERHOFSTAD A, NILSSON G, BRODIN E *et al.* (1978). Immunohistochemical evidence of substance P-like immunoreactivity in some 5-hydroxytryptamine-containing neurons in the rat central nervous system. *Neuroscience* **3**, 517-538.

HOKFELT T, TERENIUS L, KUYPERS HG & DANN O (1979). Evidence for enkephalin immunoreactive neurons in the medulla oblongata projecting to the spinal cord. *Neurosci Lett* **14**, 55-60.

HOLMES GM, MARTAU JM, HERMANN GE, ROGERS RC, BRESNAHAN JC & BEATTIE MS (1997). Nucleus raphe obscurus (nRO) regulation of anorectal motility in rats. *Brain Res* **759**, 197-204.

HOLTMAN JR (1988). Immunohistochemical localization of serotonin- and substance P-containing fibers around respiratory muscle motoneurons in the nucleus ambiguus of the cat. *Neuroscience* **26**, 169-178.

HOLTMAN JR, ANASTASI NC, NORMAN WP & DRETCHEN KL (1986a). Effect of electrical and chemical stimulation of the raphe obscurus on phrenic nerve activity in the cat. *Brain Res* **362**, 214-220.

HOLTMAN JR, BULLER AL, HAMOSH P & GILLIS RA (1986b). Central respiratory stimulation produced by thyrotropin-releasing hormone in the cat. *Peptides* **7**, 207-212.

HOLTMAN JR, DICK TE & BERGER AJ (1986c). Involvement of serotonin in the excitation of phrenic motoneurons evoked by stimulation of the raphe obscurus. *J Neurosci* **6**, 1185-1193.

HOLTMAN JR, DICK TE & BERGER AJ (1987). Serotonin-mediated excitation of recurrent laryngeal and phrenic motoneurons evoked by stimulation of the raphe obscurus. *Brain Res* **417**, 12-20.

HOLTMAN JR, MARION LJ & SPECK DF (1990a). Origin of serotonin-containing projections to the ventral respiratory group in the rat. *Neuroscience* **37**, 541-552.

HOLTMAN JR, VASCIK DS & MALEY BE (1990b). Ultrastructural evidence for serotonin-immunoreactive terminals contacting phrenic motoneurons in the cat. *Exp Neurol* **109**, 269-272.

HOLZEL B & PFISTER C (1981). Untersuchungen zur Topographie und Zytoarchitektonik der Raphe-Kerne der Ratte. *J Hirnforsch* **22**, 697-708.

HOPE AG, DOWNIE DL, SUTHERLAND L, LAMBERT JJ, PETERS JA & BURCHELL B (1993). Cloning and functional expression of an apparent splice variant of the murine 5-HT3 receptor A subunit. *Eur J Pharmacol* **245**, 187-192.

HOSOYA Y (1985). Hypothalamic projections to the ventral medulla oblongata in the rat, with special reference to the nucleus raphe pallidus: a study using autoradiographic and HRP techniques. *Brain Res* **344**, 338-350.

HOSOYA Y, SUGIURA Y, ZHANG FZ, ITO R & KOHNO K (1989). Direct projection from the dorsal hypothalamic area to the nucleus raphe pallidus: a study using anterograde transport with Phaseolus vulgaris leucoagglutinin in the rat. *Exp Brain Res* **75**, 40-46.

HOYER D, CLARKE DE, FOZARD JR, HARTIG PR, MARTIN GR, MYLECHARANE EJ *et al.* (1994). International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol Rev* **46**, 157-203.

HOYER D, ENGEL G & KALKMAN HO (1985a). Characterization of the 5-HT1B recognition site in rat brain: binding studies with (-)[125I]iodocyanopindolol. *Eur J Pharmacol* **118**, 1-12.

HOYER D, ENGEL G & KALKMAN HO (1985b). Molecular pharmacology of 5-HT1 and 5-HT2 recognition sites in rat and pig brain membranes: radioligand

binding studies with [3H]5-HT, [3H]8-OH-DPAT, (-)[125I]iodocyanopindolol, [3H]mesulergine and [3H]ketanserin. *Eur J Pharmacol* **118**, 13-23.

HOYER D, HANNON JP & MARTIN GR (2002). Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav* **71**, 533-554.

HOYER D & MARTIN G (1997). 5-HT receptor classification and nomenclature: towards a harmonization with the human genome. *Neuropharmacology* **36**, 419-428.

HOYER D & MIDDLEMISS DN (1989). Species differences in the pharmacology of terminal 5-HT autoreceptors in mammalian brain. *Trends Pharmacol Sci* **10**, 130-132.

HOYER D, PAZOS A, PROBST A & PALACIOS JM (1986). Serotonin receptors in the human brain. I. Characterization and autoradiographic localization of 5-HT1A recognition sites. Apparent absence of 5-HT1B recognition sites. *Brain Res* **376**, 85-96.

HOYER D, WAEBER C, KARPF A, NEIJT H & PALACIOS JM (1989). [3H]ICS 205-930 labels 5-HT3 recognition sites in membranes of cat and rabbit vagus nerve and superior cervical ganglion. *Naunyn Schmiedebergs Arch Pharmacol* **340**, 396-402.

HRDINA PD, FOY B, HEPNER A & SUMMERS RJ (1990). Antidepressant binding sites in brain: autoradiographic comparison of [3H]paroxetine and [3H]imipramine localization and relationship to serotonin transporter. *J Pharmacol Exp Ther* **252**, 410-418.

HUANGFU D, HWANG LJ, RILEY TA & GUYENET PG (1994). Role of serotonin and catecholamines in sympathetic responses evoked by stimulation of rostral medulla. *Am J Physiol* **266**, R338-R352.

INNIS RB, CORREA FM, UHL GR, SCHNEIDER B & SNYDER SH (1979). Cholecystokinin octapeptide-like immunoreactivity: histochemical localization in rat brain. *Proc Natl Acad Sci U S A* **76**, 521-525.

INVERNIZZI R, BELLI S & SAMANIN R (1992). Citalopram's ability to increase the extracellular concentrations of serotonin in the dorsal raphe prevents the drug's effect in the frontal cortex. *Brain Res* **584**, 322-324.

ISAAC MT, ISAAC MB, GALLO F & TOURNOUX A (2003). Milnacipran and pindolol: a randomized trial of reduction of antidepressant latency. *Hum Psychopharmacol* **18**, 595-601.

ITO A & SCHANBERG SM (1975). Effect of serotonin depletion on the central regulation of the carotid sinus reflex in rats. *Japanese Heart Journal* **16**, 148-155.

ITOH H, ALPER RH & BUNAG RD (1992). Baroreflex changes produced by serotonergic or catecholaminergic lesions in the rat nucleus tractus solitarius. *J Pharmacol Exp Ther* **261**, 225-233.

IZZO PN, DEUCHARS J & SPYER KM (1993). Localization of cardiac vagal preganglionic motoneurones in the rat: immunocytochemical evidence of synaptic inputs containing 5- hydroxytryptamine. *J Comp Neurol* **327**, 572-583.

IZZO PN, JORDAN D & RAMAGE AG (1988). Anatomical and pharmacological evidence supporting the involvement of serotonin in the central control of cardiac vagal motoneurones in the anaesthetized cat. *J Physiol* **406**, 19P.

IZZO PN, LIN RJ, RICHTER DW & SPYER KM (1987). Physiological and morphological identification of neurones receiving arterial chemoreceptive afferent input in the nucleus tractus solitarius of the cat. *J Physiol* **399**, 31P.

IZZO PN, SYKES RM & SPYER KM (1992). gamma-Aminobutyric acid immunoreactive structures in the nucleus tractus solitarius: a light and electron microscopic study. *Brain Res* **591**, 69-78.

JACKSON MB & YAKEL JL (1995). The 5-HT3 receptor channel. *Annu Rev Physiol* **57**, 447-468.

JACOBS BL (1976). An animal behavior model for studying central serotonergic synapses. *Life Sci* **19**, 777-785.

JACOBS BL, FOOTE SL & BLOOM FE (1978). Differential projections of neurons within the dorsal raphe nucleus of the rat: a horseradish peroxidase (HRP) study. *Brain Res* **147**, 149-153.

JANIG W (1988). Pre- and postganglionic vasoconstrictor neurons: differentiation, types, and discharge properties. *Annu Rev Physiol* **50**, 525-539.

JEGGO RD (2003). *5-HT receptors and brainstem cardiorespiratory neurones* Ph.D thesis, University of London.

JEGGO RD, WANG Y, JORDAN D & RAMAGE AG (2000a). The effects of 5-HT1B/1D/1F receptor ligands on the activity of nucleus tractus solitarius (NTS) neurones in anaesthetized rats: an in vivo ionophoretic study. *Br J Pharmacol* **129**, 63P.

JEGGO RD, WANG Y, JORDAN D & RAMAGE AG (2000b). The role of 5-HT3 receptors in the cardiopulmonary afferent-evoked response of NTS neurones in the anaesthetized rat. *J Physiol* **523**, 258P-259P.

JEGGO RD, WANG Y, JORDAN D & RAMAGE AG (2001). Evidence to suggest that pre-synaptic 5-HT3 receptors are involved in the excitation of NTS neurones in anaesthetised rats. *J Physiol* **533**, 92-93P.

JERMAN JC, BROUGH SJ, GAGER T, WOOD M, COLDWELL MC, SMART D *et al.* (2001). Pharmacological characterisation of human 5-HT2 receptor subtypes. *Eur J Pharmacol* **414**, 23-40.

JIN H, OKSENBERG D, ASHKENAZI A, PEROUTKA SJ, DUNCAN AM, ROZMAHEL R *et al.* (1992). Characterization of the human 5-hydroxytryptamine1B receptor. *J Biol Chem* **267**, 5735-5738.

JOHANSSON L, SOHN D, THORBERG SO, JACKSON DM, KELDER D, LARSSON LG *et al.* (1997). The pharmacological characterization of a novel selective 5-hydroxytryptamine1A receptor antagonist, NAD-299. *J Pharmacol Exp Ther* **283**, 216-225.

JOHANSSON O, HOKFELT T, PERNOW B, JEFFCOATE SL, WHITE N, STEINBUSCH HW *et al.* (1981). Immunohistochemical support for three putative transmitters in one neuron: coexistence of 5-hydroxytryptamine, substance P- and thyrotropin releasing hormone-like immunoreactivity in medullary neurons projecting to the spinal cord. *Neuroscience* **6**, 1857-1881.

JOHNSON KW, SCHAUS JM, DURKIN MM, AUDIA JE, KALDOR SW, FLAUGH ME *et al.* (1997). 5-HT1F receptor agonists inhibit neurogenic dural inflammation in guinea pigs. *Neuroreport* **8**, 2237-2240.

JONES GA & JORDAN D (2003). Convergence of cardiorespiratory inputs on nucleus tractus solitarii (NTS) neurones at different stages in the baroreceptor reflex pathway in anaesthetized rats. *J Physiol* **547P**, C11.

JONES GA, LLEWELLYN-SMITH IJ & JORDAN D (2002). Physiological, pharmacological, and immunohistochemical characterisation of juxtacellularly labelled neurones in rat nucleus tractus solitarius. *Auton Neurosci* **98**, 12-16.

JONES JF & JORDAN D (1993). Evidence for a chronotropic response to cardiac vagal motor C-fibre stimulation in anaesthetized cats, rats and rabbits. *J Physiol* **483**, 89P.

JONES JF, MARTIN GR & RAMAGE AG (1995a). Evidence that 5-HT1D receptors mediate inhibition of sympathetic ganglionic transmission in anaesthetized cats. *Br J Pharmacol* **116**, 1715-1717.

JONES JF, WANG Y & JORDAN D (1995b). Heart rate responses to selective stimulation of cardiac vagal C fibres in anaesthetized cats, rats and rabbits. *J Physiol* **489**, 203-214.

JONES JF, WANG Y & JORDAN D (1998). Activity of C fibre cardiac vagal efferents in anaesthetized cats and rats. *J Physiol* **507**, 869-880.

JONES JFX (1993). *The central control of the pulmonary chemoreflex* University of London, PhD Thesis.

JONES JV (1977). Time course and extent of carotid sinus baroreceptor threshold resetting in rats with renovascular hypertension. *Acta Physiol Scand* **99**, 173-182.

JONES JV & THOREN PN (1977). Characteristics of aortic baroreceptors with non-medullated afferents arising from the aortic arch of rabbits with chronic renovascular hypertension. *Acta Physiol Scand* **101**, 286-293.

JORDAN D (1990). Autonomic changes in affective behavior. In *Central Regulation of Autonomic Functions*, eds. Loewy AD & Spyer KM, pp. 349-366. Oxford University Press, New York.

JORDAN D, KHALID MEM, SCHNEIDERMAN N & SPYER KM (1982). The location and properties of preganglionic vagal cardiomotor neurones in the rabbit. *Pflugers Arch* **395**, 244-250.

JORDAN D & SPYER KM (1986). Brainstem integration of cardiovascular and pulmonary afferent activity. *Prog Brain Res* **67**, 295-314.

JOVANOVSKA A & PROSSER RA (2002). Translational and transcriptional inhibitors block serotonergic phase advances of the suprachiasmatic nucleus circadian pacemaker in vitro. *J Biol Rhythms* **17**, 137-146.

JOYNT KE, WHELLAN DJ & O'CONNOR CM (2003). Depression and cardiovascular disease: mechanisms of interaction. *Biol Psychiatry* **54**, 248-261.

JULIUS D, MACDERMOTT AB, AXEL R & JESSELL TM (1988). Molecular characterization of a functional cDNA encoding the serotonin 1c receptor. *Science* **241**, 558-564.

KAKIZAKI H, YOSHIYAMA M, KOYANAGI T & DE GROAT WC (2001). Effects of WAY100635, a selective 5-HT(1A)-receptor antagonist on the micturition-reflex pathway in the rat. *Am J Physiol Regul Integr Comp Physiol* **280**, R1407-R1413.

KALIA M & MESULAM MM (1980). Brain stem projections of sensory and motor components of the vagus complex in the cat: II. Laryngeal, tracheobronchial, pulmonary, cardiac, and gastrointestinal branches. *J Comp Neurol* **193**, 467-508.

KANNAN H & YAMASHITA H (1985). Connections of neurons in the region of the nucleus tractus solitarius with the hypothalamic paraventricular nucleus: their possible involvement in neural control of the cardiovascular system in rats. *Brain Res* **329**, 205-212.

KAUMANN AJ (1994). Do human atrial 5-HT4 receptors mediate arrhythmias? *Trends Pharmacol Sci* **15**, 451-455.

KAY IS & ARMSTRONG DJ (1990). Phenylbiguanide not phenyldiguanide is used to evoke the pulmonary chemoreflex in anaesthetized rabbits. *Experimental Physiology* **75**, 383-389.

KELLEY SP, BRATT AM & HODGE CW (2003). Targeted gene deletion of the 5-HT3A receptor subunit produces an anxiolytic phenotype in mice. *Eur J Pharmacol* **461**, 19-25.

KENNETT GA, BRIGHT F, TRAIL B, BLACKBURN TP & SANGER GJ (1997a). Anxiolytic-like actions of the selective 5-HT4 receptor antagonists SB 204070A and SB 207266A in rats. *Neuropharmacology* **36**, 707-712.

KENNETT GA, WOOD MD, BRIGHT F, TRAIL B, RILEY G, HOLLAND V *et al.* (1997b). SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist. *Neuropharmacology* **36**, 609-620.

KHAWAJA X, ENNIS C & MINCHIN MC (1997). Pharmacological characterization of recombinant human 5-hydroxytryptamine1A receptors using a novel antagonist radioligand, [3H]WAY-100635. *Life Sci* **60**, 653-665.

KILPATRICK GJ, JONES BJ & TYERS MB (1989). Binding of the 5-HT3 ligand, [3H]GR65630, to rat area postrema, vagus nerve and the brains of several species. *Eur J Pharmacol* **159**, 157-164.

KILPATRICK GJ & TYERS MB (1992). Inter-species variants of the 5-HT3 receptor. *Biochem Soc Trans* **20**, 118-121.

KINNEY GG, TABER MT & GRIBKOFF VK (2000). The augmentation hypothesis for improvement of antidepressant therapy: is pindolol a suitable candidate for testing the ability of 5HT1A receptor antagonists to enhance SSRI efficacy and onset latency? *Mol Neurobiol* **21**, 137-152.

KNOWLES ID & RAMAGE AG (1999). Evidence for a role for central 5-HT2B as well as 5-HT2A receptors in cardiovascular regulation in anaesthetized rats. *Br J Pharmacol* **128**, 530-542.

KNOWLES ID & RAMAGE AG (2000). Evidence that activation of central 5-HT(2B) receptors causes renal sympathoexcitation in anaesthetized rats. *Br J Pharmacol* **129**, 177-183.

KOBILKA BK, FRIELLE T, COLLINS S, YANG-FENG T, KOBILKA TS, FRANCKE U *et al.* (1987). An intronless gene encoding a potential member of the family of receptors coupled to guanine nucleotide regulatory proteins. *Nature* **329**, 75-79.

KOE BK & WEISSMAN A (1966). p-Chlorophenylalanine: a specific depletor of brain serotonin. *J Pharmacol Exp Ther* **154**, 499-516.

KOEK W, JACKSON A & COLPAERT FC (1992). Behavioral pharmacology of antagonists at 5-HT2/5-HT1C receptors. *Neurosci Biobehav Rev* **16**, 95-105.

KOHEN R, METCALF MA, KHAN N, DRUCK T, HUEBNER K, LACHOWICZ JE *et al.* (1996). Cloning, characterization, and chromosomal localization of a human 5-HT6 serotonin receptor. *J Neurochem* **66**, 47-56.

KOLBASA KP, MCCALL RB & LUDENS JH (1991). Effect of chronic treatment on the cardiovascular and behavioral responses of 8-OH-DPAT in conscious normotensive rats. *Eur J Pharmacol* **193**, 275-281.

KOSHIYA N, HUANGFU D & GUYENET PG (1993). Ventrolateral medulla and sympathetic chemoreflex in the rat. *Brain Res* **609**, 174-184.

KUBIN L, KIMURA H & DAVIES RO (1991). The medullary projections of afferent bronchopulmonary C fibres in the cat as shown by antidromic mapping. *J Physiol* **435**, 207-228.

KUBO T & KIHARA M (1987). Evidence for the presence of GABAergic and glycine-like systems responsible for cardiovascular control in the nucleus tractus solitarii of the rat. *Neurosci Lett* **74**, 331-336.

KUBO T & KIHARA M (1988a). Evidence for gamma-aminobutyric acid receptor-mediated modulation of the aortic baroreceptor reflex in the nucleus tractus solitarii of the rat. *Neurosci Lett* **89**, 156-160.

KUBO T & KIHARA M (1988b). Evidence of N-methyl-D-aspartate receptor-mediated modulation of the aortic baroreceptor reflex in the rat nucleus tractus solitarii. *Neurosci Lett* **87**, 69-74.

KUBOTA Y, INAGAKI S, SHIOSAKA S, CHO HJ, TATEISHI K, HASHIMURA E *et al.* (1983). The distribution of cholecystokinin octapeptide-like structures in the lower brain stem of the rat: an immunohistochemical analysis. *Neuroscience* **9**, 587-604.

KUHN DM, WOLF WA & LOVENBERG W (1980). Pressor effects of electrical stimulation of the dorsal and median raphe nuclei in anesthetized rats. *J Pharmacol Exp Ther* **214**, 403-409.

KURSAR JD, NELSON DL, WAINSCOTT DB, COHEN ML & BAEZ M (1992). Molecular cloning, functional expression, and pharmacological characterization of a novel serotonin receptor (5-hydroxytryptamine2F) from rat stomach fundus. *Mol Pharmacol* **42**, 549-557.

LAHIRI S, MOKASHI A, MULLIGAN E & NISHINO T (1981). Comparison of aortic and carotid chemoreceptor responses to hypercapnia and hypoxia. *J Appl Physiol* **51**, 55-61.

LALLEY PM (1980). Inhibition of depressor cardiovascular reflexes by a derivative of gamma-aminobutyric acid (GABA) and by general anesthetics with suspected GABA-mimetic effects. *J Pharmacol Exp Ther* **215**, 418-425.

LALLEY PM (1986). Serotoninergic and non-serotoninergic responses of phrenic motoneurones to raphe stimulation in the cat. *J Physiol* **380**, 373-385.

LALLEY PM, BENACKA R, BISCHOFF AM & RICHTER DW (1997). Nucleus raphe obscurus evokes 5-HT-1A receptor-mediated modulation of respiratory neurons. *Brain Res* **747**, 156-159.

LANCA AJ & VAN DER KOOY D (1985). A serotonin-containing pathway from the area postrema to the parabrachial nucleus in the rat. *Neuroscience* **14**, 1117-1126.

LANGLEY JN (1921). *The Autonomic Nervous System* Heffer & Sons, Cambridge.

LANKIEWICZ S, LOBITZ N, WETZEL CH, RUPPRECHT R, GISSELMANN G & HATT H (1998). Molecular cloning, functional expression, and pharmacological characterization of 5-hydroxytryptamine3 receptor cDNA and its splice variants from guinea pig. *Mol Pharmacol* **53**, 202-212.

LAUBIE M, DROUILLAT M, DABIRE H, CHERQUI C & SCHMITT H (1989). Ventrolateral medullary pressor area: site of hypotensive and sympatho-inhibitory effects of (+/-)8-OH-DPAT in anaesthetized dogs. *Eur J Pharmacol* **160**, 385-394.

LAWRENCE AJ & JARROTT B (1993). Nitric oxide increases interstitial excitatory amino acid release in the rat dorsomedial medulla oblongata. *Neurosci Lett* **151**, 126-129.

LAWRENCE AJ & JARROTT B (1996). Neurochemical modulation of cardiovascular control in the nucleus tractus solitarius. *Prog Neurobiol* **48**, 21-53.

LECCI A, GIULIANI S, SANTICIOLI P & MAGGI CA (1992). Involvement of 5-hydroxytryptamine1A receptors in the modulation of micturition reflexes in the anesthetized rat. *J Pharmacol Exp Ther* **262**, 181-189.

LEE TM, KUO JS & CHAI CY (1972). Central integrating mechanism of the Bezold-Jarisch and baroceptor reflexes. *Am J Physiol* **222**, 713-720.

LEONE C & GORDON FJ (1989). Is L-glutamate a neurotransmitter of baroreceptor information in the nucleus of the tractus solitarius? *J Pharmacol Exp Ther* **250**, 953-962.

LEONHARDT S, HERRICK-DAVIS K & TITELER M (1989). Detection of a novel serotonin receptor subtype (5-HT1E) in human brain: interaction with a GTP-binding protein. *J Neurochem* **53**, 465-471.

LESCH K-P (1997). Molecular biology, pharmacology, and genetics of the serotonin transporter: psychobiological and clinical implications. In *Serotoninergic neurons and 5-HT receptors in the CNS*, eds. Baumgarten HG & Gothert M, Springer, Berlin.

LESLIE RA, REYNOLDS DJ, ANDREWS PL, GRAHAME-SMITH DG, DAVIS CJ & HARVEY JM (1990). Evidence for presynaptic 5-hydroxytryptamine3 recognition sites on vagal afferent terminals in the brainstem of the ferret. *Neuroscience* **38**, 667-673.

LETTY S, CHILD R, DUMUIS A, PANTALONI A, BOCKAERT J & RONDOUIN G (1997). 5-HT4 receptors improve social olfactory memory in the rat. *Neuropharmacology* **36**, 681-687.

LEVITT P & MOORE RY (1979). Origin and organization of brainstem catecholamine innervation in the rat. *J Comp Neurol* **186**, 505-528.

LEVY FO, GUDERMANN T, PEREZ-REYES E, BIRNBAUMER M, KAUMANN AJ & BIRNBAUMER L (1992). Molecular cloning of a human serotonin receptor (S12) with a pharmacological profile resembling that of the 5-HT1D subtype. *J Biol Chem* **267**, 7553-7562.

LEWIS DI & COOTE JH (1990). The influence of 5-hydroxytryptamine agonists and antagonists on identified sympathetic preganglionic neurones in the rat, in vivo. *Br J Pharmacol* **99**, 667-672.

LEWIS SJ, MACHADO BH, OHTA H & TALMAN WT (1991a). Processing of cardiopulmonary afferent input within the nucleus tractus solitarii involves activation of soluble guanylate cyclase. *Eur J Pharmacol* **203**, 327-328.

LEWIS SJ, OHTA H, MACHADO B, BATES JN & TALMAN WT (1991b). Microinjection of S-nitrosocysteine into the nucleus tractus solitarii decreases arterial pressure and heart rate via activation of soluble guanylate cyclase. *Eur J Pharmacol* **202**, 135-136.

LEYSEN JE, JANSSEN PM, SCHOTTE A, LUYTEN WH & MEGENS AA (1993). Interaction of antipsychotic drugs with neurotransmitter receptor sites in vitro and in vivo in relation to pharmacological and clinical effects: role of 5HT2 receptors. *Psychopharmacology (Berl)* **112**, S40-S54.

LIBERT F, PARMENTIER M, LEFORT A, DINSART C, VAN SANDE J, MAENHAUT C *et al.* (1989). Selective amplification and cloning of four new members of the G protein-coupled receptor family. *Science* **244**, 569-572.

LLEWELLYN-SMITH I, KELLETT DO, JONES GA & JORDAN D (2004). Do serotonergic axons directly innervate cardiovascular neurons in rat nucleus tractus solitarius (NTS)? *J Physiol* **557P**, C99.

LOEWY AD (1981). Raphe pallidus and raphe obscurus projections to the intermediolateral cell column in the rat. *Brain Res* **222**, 129-133.

LOEWY AD & BURTON H (1978). Nuclei of the solitary tract: efferent projections to the lower brain stem and spinal cord of the cat. *J Comp Neurol* **181**, 421-449.

LOPEZ-GIMENEZ JF, MENGOD G, PALACIOS JM & VILARO MT (1997). Selective visualization of rat brain 5-HT2A receptors by autoradiography with [3H]MDL 100,907. *Naunyn Schmiedebergs Arch Pharmacol* **356**, 446-454.

LOVELL PJ, BROMIDGE SM, DABBS S, DUCKWORTH DM, FORBES IT, JENNINGS AJ *et al.* (2000). A novel, potent, and selective 5-HT(7) antagonist: (R)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulfonyl) phen ol (SB-269970). *J Med Chem* **43**, 342-345.

LOVENBERG TW, BARON BM, DE LECEA L, MILLER JD, PROSSER RA, REA MA *et al.* (1993a). A novel adenylyl cyclase-activating serotonin receptor (5-HT7) implicated in the regulation of mammalian circadian rhythms. *Neuron* **11**, 449-458.

LOVENBERG TW, ERLANDER MG, BARON BM, RACKE M, SLONE AL, SIEGEL BW *et al.* (1993b). Molecular cloning and functional expression of 5-HT1E-like rat and human 5-hydroxytryptamine receptor genes. *Proc Natl Acad Sci U S A* **90**, 2184-2188.

LUCAS JJ, YAMAMOTO A, SCEARCE-LEVIE K, SAUDOU F & HEN R (1998). Absence of fenfluramine-induced anorexia and reduced c-Fos induction in the hypothalamus and central amygdaloid complex of serotonin 1B receptor knock-out mice. *J Neurosci* **18**, 5537-5544.

LUNDBERG JM & HOKFELT T (1986). Multiple co-existence of peptides and classical transmitters in peripheral autonomic and sensory neurons--functional and pharmacological implications. *Prog Brain Res* **68**, 241-262.

MA S, ABBOUD FM & FELDER RB (1995). Effects of L-arginine-derived nitric oxide synthesis on neuronal activity in nucleus tractus solitarius. *Am J Physiol* **268**, R487-R491.

MACHADO BH (2001). Neurotransmission of the cardiovascular reflexes in the nucleus tractus solitarii of awake rats. *Ann N Y Acad Sci* **940**, 179-196.

MACHADO BH & BONAGAMBA LG (1992). Microinjection of L-glutamate into the nucleus tractus solitarii increases arterial pressure in conscious rats. *Brain Res* **576**, 131-138.

MACLEOD RD & SCOTT MJ (1964). The heart rate responses to carotid body chemoreceptor stimulation in the cat. *J Physiol* **175**, 193-202.

MALAGIE I, TRILLAT AC, BOURIN M, JACQUOT C, HEN R & GARDIER AM (2001). 5-HT1B Autoreceptors limit the effects of selective serotonin re-uptake inhibitors in mouse hippocampus and frontal cortex. *J Neurochem* **76**, 865-871.

MALEY B & ELDE R (1982). The ultrastructural localization of serotonin immunoreactivity within the nucleus of the solitary tract of the cat. *J Neurosci* **2**, 1499-1506.

MANDAL AK, KELLAR KJ, NORMAN WP & GILLIS RA (1990). Stimulation of serotonin2 receptors in the ventrolateral medulla of the cat results in nonuniform increases in sympathetic outflow. *Circ Res* **67**, 1267-1280.

MANZKE T, GUENTHER U, PONIMASKIN EG, HALLER M, DUTSCHMANN M, SCHWARZACHER S *et al.* (2003). 5-HT4(a) receptors avert opioid-induced breathing depression without loss of analgesia. *Science* **301**, 226-229.

MAQBOOL A, BATTEN TF & MCWILLIAM PN (1991). Ultrastructural Relationships Between GABAergic Terminals and Cardiac Vagal Preganglionic Motoneurons and Vagal Afferents in the Cat: A Combined HRP Tracing and Immunogold Labelling Study. *Eur J Neurosci* **3**, 501-513.

MAREK GJ & AGHAJANIAN GK (1994). Excitation of interneurons in piriform cortex by 5-hydroxytryptamine: blockade by MDL 100,907, a highly selective 5-HT2A receptor antagonist. *Eur J Pharmacol* **259**, 137-141.

MARICQ AV, PETERSON AS, BRAKE AJ, MYERS RM & JULIUS D (1991). Primary structure and functional expression of the 5HT3 receptor, a serotonin-gated ion channel. *Science* **254**, 432-437.

MARK AL (1983). The Bezold-Jarisch reflex revisited: clinical implications of inhibitory reflexes originating in the heart. *J Am Coll Cardiol* **1**, 90-102.

MARSHALL JM (1987). Analysis of cardiovascular responses evoked following changes in peripheral chemoreceptor activity in the rat. *J Physiol* **394**, 393-414.

MARSHALL JM (1994). Peripheral chemoreceptors and cardiovascular regulation. *Physiol Rev* **74**, 543-594.

MARTIN GE & LIS EV, JR. (1985). Hypotensive action of 8-hydroxy-2-(di-N-propylamino)tetralin (8-OH-DPAT) in spontaneously hypertensive rats. *Arch Int Pharmacodyn Ther* **273**, 251-261.

MARTIN P, BENINGER RJ, HAMON M & PUECH AJ (1990). Antidepressant-like action of 8-OH-DPAT, a 5-HT1A agonist, in the learned helplessness paradigm: evidence for a postsynaptic mechanism. *Behav Brain Res* **38**, 135-144.

MASON P (2001). Contributions of the medullary raphe and ventromedial reticular region to pain modulation and other homeostatic functions. *Annu Rev Neurosci* **24**, 737-777.

MASSARI VJ, JOHNSON TA, LLEWELLYN-SMITH IJ & GATTI PJ (1994). Substance P nerve terminals synapse upon negative chronotropic vagal motoneurons. *Brain Res* **660**, 275-287.

MASUDA N, TERUI N, KOSHIYA N & KUMADA M (1991). Neurons in the caudal ventrolateral medulla mediate the arterial baroreceptor reflex by inhibiting barosensitive reticulospinal neurons in the rostral ventrolateral medulla in rabbits. *J Auton Nerv Syst* **34**, 103-117.

MATSUMOTO M, KOJIMA T, TOGASHI H, MORI K, OHASHI S, UENO K *et al.* (2002). Differential characteristics of endogenous serotonin-mediated synaptic transmission in the hippocampal CA1 and CA3 fields of anaesthetized rats. *Naunyn Schmiedebergs Arch Pharmacol* **366**, 570-577.

MAUAD H & MACHADO BH (2001). Pressor response to unilateral carotid chemoreceptor activation is not affected by ipsilateral antagonism of excitatory amino acid receptors in the rostral ventrolateral medulla of awake rats. *Auton Neurosci* **91**, 26-31.

MAURA G, FEDELE E & RAITERI M (1989). Acetylcholine release from rat hippocampal slices is modulated by 5-hydroxytryptamine. *Eur J Pharmacol* **165**, 173-179.

MCALLEN RM (1986). Action and specificity of ventral medullary vasopressor neurones in the cat. *Neuroscience* **18**, 51-59.

MCALLEN RM & DAMPNEY RA (1990). Vasomotor neurons in the rostral ventrolateral medulla are organized topographically with respect to type of vascular bed but not body region. *Neurosci Lett* **110**, 91-96.

MCALLEN RM & SPYER KM (1976). The location of cardiac vagal preganglionic motoneurones in the medulla of the cat. *J Physiol* **258**, 187-204.

MCALLEN RM & SPYER KM (1978). The baroreceptor input to cardiac vagal motoneurones. *J Physiol* **282**, 365-374.

MCBRIDE RL & SUTIN J (1976). Projections of the locus coeruleus and adjacent pontine tegmentum in the cat. *J Comp Neurol* **165**, 265-284.

MCCALL RB & AGHAJANIAN GK (1980). Pharmacological characterization of serotonin receptors in the facial motor nucleus: a microiontophoretic study. *Eur J Pharmacol* **65**, 175-183.

MCCALL RB & HARRIS LT (1988). 5-HT2 receptor agonists increase spontaneous sympathetic nerve discharge. *Eur J Pharmacol* **151**, 113-116.

MCCALL RB, PATEL BN & HARRIS LT (1987). Effects of serotonin1 and serotonin2 receptor agonists and antagonists on blood pressure, heart rate and sympathetic nerve activity. *J Pharmacol Exp Ther* **242**, 1152-1159.

MCCANN MJ, HERMANN GE & ROGERS RC (1989). Nucleus raphe obscurus (nRO) influences vagal control of gastric motility in rats. *Brain Res* **486**, 181-184.

MCLOUGHLIN DJ & STRANGE PG (2000). Mechanisms of agonism and inverse agonism at serotonin 5-HT1A receptors. *J Neurochem* **74**, 347-357.

MELLER E, GOLDSTEIN M & BOHMAKER K (1990). Receptor reserve for 5-hydroxytryptamine1A-mediated inhibition of serotonin synthesis: possible relationship to anxiolytic properties of 5-hydroxytryptamine1A agonists. *Mol Pharmacol* **37**, 231-237.

MELLIN C, VALLGARDA J, NELSON DL, BJORK L, YU H, ANDEN NE *et al.* (1991). A 3-D model for 5-HT1A-receptor agonists based on stereoselective methyl-substituted and conformationally restricted analogues of 8-hydroxy-2-(dipropylamino)tetralin. *J Med Chem* **34**, 497-510.

MENARD J & TREIT D (1999). Effects of centrally administered anxiolytic compounds in animal models of anxiety. *Neurosci Biobehav Rev* **23**, 591-613.

MENESES A (1999). 5-HT system and cognition. *Neurosci Biobehav Rev* **23**, 1111-1125.

MENESES A & TERRON JA (2001). Role of 5-HT(1A) and 5-HT(7) receptors in the facilitatory response induced by 8-OH-DPAT on learning consolidation. *Behav Brain Res* **121**, 21-28.

MENGOD G, NGUYEN H, LE H, WAEBER C, LUBBERT H & PALACIOS JM (1990a). The distribution and cellular localization of the serotonin 1C receptor mRNA in the rodent brain examined by in situ hybridization histochemistry. Comparison with receptor binding distribution. *Neuroscience* **35**, 577-591.

MENGOD G, POMPEIANO M, MARTINEZ-MIR MI & PALACIOS JM (1990b). Localization of the mRNA for the 5-HT2 receptor by in situ hybridization histochemistry. Correlation with the distribution of receptor sites. *Brain Res* **524**, 139-143.

METCALF MA, MCGUFFIN RW & HAMBLIN MW (1992). Conversion of the human 5-HT1D beta serotonin receptor to the rat 5-HT1B ligand-binding phenotype by Thr355Asn site directed mutagenesis. *Biochem Pharmacol* **44**, 1917-1920.

MEYERHOF W, OBERMULLER F, FEHR S & RICHTER D (1993). A novel rat serotonin receptor: primary structure, pharmacology, and expression pattern in distinct brain regions. *DNA Cell Biol* **12**, 401-409.

MICHELINI LC & BONAGAMBA LG (1988). Baroreceptor reflex modulation by vasopressin microinjected into the nucleus tractus solitarii of conscious rats. *Hypertension* **11**, I75-I79.

MIDDLEMISS DN, BLAKEBOROUGH L & LEATHER SR (1977). Direct evidence for an interaction of β-adrenergic blockers with the 5-HT receptor. *Nature* 289-290.

MIDDLEMISS DN & FOZARD JR (1983). 8-Hydroxy-2-(di-n-propylamino)-tetralin discriminates between subtypes of the 5-HT1 recognition site. *Eur J Pharmacol* **90**, 151-153.

MIFFLIN SW (1993a). Absence of respiration modulation of carotid sinus nerve inputs to nucleus tractus solitarius neurons receiving arterial chemoreceptor inputs. *J Auton Nerv Syst* **42**, 191-199.

MIFFLIN SW (1993b). Inhibition of chemoreceptor inputs to nucleus of tractus solitarius neurons during baroreceptor stimulation. *Am J Physiol* **265**, R14-R20.

MIFFLIN SW (2001). What does the brain know about blood pressure? *News Physiol Sci* **16**, 266-271.

MIFFLIN SW, SPYER KM & WITHINGTON-WRAY DJ (1988). Baroreceptor inputs to the nucleus tractus solitarius in the cat: modulation by the hypothalamus. *J Physiol* **399**, 369-387.

MILLAN MJ, GOBERT A, LEJEUNE F, NEWMAN-TANCREDI A, RIVET JM, AUCLAIR A *et al.* (2001). S33005, a novel ligand at both serotonin and norepinephrine transporters: I. Receptor binding, electrophysiological, and neurochemical profile in comparison with venlafaxine, reboxetine, citalopram, and clomipramine. *J Pharmacol Exp Ther* **298**, 565-580.

MILLAN MJ, NEWMAN-TANCREDI A, AUDINOT V, CUSSAC D, LEJEUNE F, NICOLAS JP *et al.* (2000). Agonist and antagonist actions of yohimbine as compared to fluparoxan at alpha(2)-adrenergic receptors (AR)s, serotonin (5-HT)(1A), 5-HT(1B), 5-HT(1D) and dopamine D(2) and D(3) receptors. Significance for the modulation of frontocortical monoaminergic transmission and depressive states. *Synapse* **35**, 79-95.

MILLAN MJ, NEWMAN-TANCREDI A, LOCHON S, TOUZARD M, AUBRY S & AUDINOT V (2002). Specific labelling of serotonin 5-HT(1B) receptors in rat frontal cortex with the novel, phenylpiperazine derivative, [3H]GR125,743. A pharmacological characterization. *Pharmacol Biochem Behav* **71**, 589-598.

MILLAN MJ, RIVET JM, CANTON H, MAROUILLE-GIRARDON S & GOBERT A (1993). Induction of hypothermia as a model of 5-hydroxytryptamine1A receptor-mediated activity in the rat: a pharmacological characterization of the actions of novel agonists and antagonists. *J Pharmacol Exp Ther* **264**, 1364-1376.

MILLHORN DE, HOKFELT T, VERHOFSTAD AA & TERENIUS L (1989). Individual cells in the raphe nuclei of the medulla oblongata in rat that contain immunoreactivities for both serotonin and enkephalin project to the spinal cord. *Exp Brain Res* **75**, 536-542.

MINER WD & SANGER GJ (1986). Inhibition of cisplatin-induced vomiting by selective 5-hydroxytryptamine M-receptor antagonism. *Br J Pharmacol* **88**, 497-499.

MINER WD, SANGER GJ & TURNER DH (1987). Evidence that 5-hydroxytryptamine3 receptors mediate cytotoxic drug and radiation-evoked emesis. *Br J Cancer* **56**, 159-162.

MIQUEL MC, DOUCET E & BONI C (1991). Central serotonin1A receptors: respective distributions of encoding nRNA, receptor protein and binding sites by in situ hybridisation histochemistry, radioimmunohistochemistry and autoradiographic mapping in the rat brain. *Neurochemistry International* **19**, 453-465.

MIQUEL MC, DOUCET E, RIAD M, ADRIEN J, VERGE D & HAMON M (1992). Effect of the selective lesion of serotoninergic neurons on the regional distribution of 5-HT1A receptor mRNA in the rat brain. *Brain Res Mol Brain Res* **14**, 357-362.

MIR AK & FOZARD JR (1987). Cardiovascular effects of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). In *Brain 5-HT1A Receptors*, eds. Dourish CT, Ahlenius S & Hutson PH, pp. 120-134. Ellis Horwood Series in Biomedicine, Chichester.

MITCHELL R & FLEETWOOD-WALKER S (1981). Substance P, but not TRH, modulates the 5-HT autoreceptor in ventral lumbar spinal cord. *Eur J Pharmacol* **76**, 119-120.

MOFFITT JA & JOHNSON AK (2004). Short-term fluoxetine treatment enhances baroreflex control of sympathetic nervous system activity after hindlimb unloading. *Am J Physiol Regul Integr Comp Physiol* **286**, R584-R590.

MOLINEAUX SM, JESSELL TM, AXEL R & JULIUS D (1989). 5-HT1c receptor is a prominent serotonin receptor subtype in the central nervous system. *Proc Natl Acad Sci U S A* **86**, 6793-6797.

MONACHON MA, BURKARD WP, JALFRE M & HAEFELY W (1972). Blockade of central 5-hydroxytryptamine receptors by methiothepin. *Naunyn Schmiedebergs Arch Pharmacol* **274**, 192-197.

MOORE SD & GUYENET PG (1983). Alpha-receptor mediated inhibition of A2 noradrenergic neurons. *Brain Res* **276**, 188-191.

MORGANE PJ & JACOBS MS (1979). Raphe projections to the locus coeruleus in the rat. *Brain Res Bull* **4**, 519-534.

MORIKAWA H, MANZONI OJ, CRABBE JC & WILLIAMS JT (2000). Regulation of central synaptic transmission by 5-HT(1B) auto- and heteroreceptors. *Mol Pharmacol* **58**, 1271-1278.

MORRISON JH, FOOTE SL, MOLLIVER ME, BLOOM FE & LIDOV HG (1982). Noradrenergic and serotonergic fibers innervate complementary layers in monkey primary visual cortex: an immunohistochemical study. *Proc Natl Acad Sci U S A* **79**, 2401-2405.

MORRISON SF (1993). Raphe pallidus excites a unique class of sympathetic preganglionic neurons. *Am J Physiol* **265**, R82-R89.

MOSQUEDA-GARCIA R, TSENG CJ, APPALSAMY M & ROBERTSON D (1989). Modulatory effects of adenosine on baroreflex activation in the brainstem of normotensive rats. *Eur J Pharmacol* **174**, 119-122.

MULLIGAN KA & TORK I (1988). Serotoninergic innervation of the cat cerebral cortex. *J Comp Neurol* **270**, 86-110.

MULLINS UL, GIANUTSOS G & EISON AS (1999). Effects of antidepressants on 5-HT7 receptor regulation in the rat hypothalamus. *Neuropsychopharmacology* **21**, 352-367.

NALIVAIKO E & BLESSING WW (2001). Raphe region mediates changes in cutaneous vascular tone elicited by stimulation of amygdala and hypothalamus in rabbits. *Brain Res* **891**, 130-137.

NANOPOULOS D, BELIN MF, MAITRE M, VINCENDON G & PUJOL JF (1982). Immunocytochemical evidence for the existence of GABAergic neurons in the nucleus raphe dorsalis. Possible existence of neurons containing serotonin and GABA. *Brain Res* **232**, 375-389.

NEEDLEMAN P (1976). The synthesis and function of prostaglandins in the heart. *Fed Proc* **35**, 2376-2381.

NEIL E & PALMER JF (1975). Effects of spontaneous respiration on the latency of reflex cardiac chronotropic responses to baroreceptor stimulation. *J Physiol* **247**, 16P.

NEUMAIER JF, SEXTON TJ, YRACHETA J, DIAZ AM & BROWNFIELD M (2001). Localization of 5-HT(7) receptors in rat brain by immunocytochemistry, in situ hybridization, and agonist stimulated cFos expression. *J Chem Neuroanat* **21**, 63-73.

NEVINS ME & ANTHONY EW (1994). Antagonists at the serotonin-3 receptor can reduce the fear-potentiated startle response in the rat: evidence for different types of anxiolytic activity? *J Pharmacol Exp Ther* **268**, 248-254.

NICHOLAS AP & HANCOCK MB (1990). Evidence for projections from the rostral medullary raphe onto medullary catecholamine neurons in the rat. *Neurosci Lett* **108**, 22-28.

NORTH RA & UCHIMURA N (1989). 5-Hydroxytryptamine acts at 5-HT2 receptors to decrease potassium conductance in rat nucleus accumbens neurones. *J Physiol* **417**, 1-12.

NOSAKA S (1986). Electrophysiologic identification of preganglionic neurons in rat dorsal motor nucleus and analysis of vagus afferent projections. *Exp Neurol* **91**, 366-381.

NOSAKA S, YAMAMOTO T & YASUNAGA K (1979). Localization of vagal cardioinhibitory preganglionic neurons with rat brain stem. *J Comp Neurol* **186**, 79-92.

NOSAKA S, YASUNAGA K & TAMAI S (1982). Vagal cardiac preganglionic neurons: distribution, cell types, and reflex discharges. *Am J Physiol* **243**, R92-R98.

NOSJEAN A, COMPOINT C, BUISSERET-DELMAS C, ORER HS, MERAHI N, PUIZILLOUT JJ *et al.* (1990). Serotonergic projections from the nodose ganglia to the nucleus tractus solitarius: an immunohistochemical and double labeling study in the rat. *Neurosci Lett* **114**, 22-26.

OCHI J & SHIMIZU K (1978). Occurrence of dopamine-containing neurons in the midbrain raphe nuclei of the rat. *Neurosci Lett* **8**, 317-320.

OGILVIE J, WIGGLESWORTH M, APPLEBY L, KINGSTON TO & CLARKE RW (1999). On the role of 5-HT1B/1D receptors in modulating transmission in a spinal reflex pathway in the decerebrated rabbit. *Br J Pharmacol* **128**, 781-787.

OLDFIELD BJ & MCLACHLAN EM (1981). An analysis of the sympathetic preganglionic neurons projecting from the upper thoracic spinal roots of the cat. *J Comp Neurol* **196**, 329-345.

OLIVAN MV, BONAGAMBA LG & MACHADO BH (2001). Involvement of the paraventricular nucleus of the hypothalamus in the pressor response to chemoreflex activation in awake rats. *Brain Res* **895**, 167-172.

ONIMARU H, ARATA A & HOMMA I (1988). Primary respiratory rhythm generator in the medulla of brainstem-spinal cord preparation from newborn rat. *Brain Res* **445**, 314-324.

ORER HS, MERAHI N, NOSJEAN A, FATTACCINI CM & LAGUZZI R (1991). Cardiovascular effects of the local injection of 5,7-dihydroxytryptamine into the nodose ganglia and nucleus tractus solitarius in awake freely moving rats. *Brain Res* **553**, 123-128.

OWENS MJ, MORGAN WN, PLOTT SJ & NEMEROFF CB (1997). Neurotransmitter receptor and transporter binding profile of antidepressants and their metabolites. *J Pharmacol Exp Ther* **283**, 1305-1322.

PAINTAL AS (1969). Mechanism of stimulation of type J pulmonary receptors. *J Physiol* **203**, 511-532.

PALKOVITS M, SAAVEDRA JM, JACOBOQITZ DM, KIZER JS, ZABORSZKY L & BROWNSTEIN MJ (1977). Serotonergic innervation of the forebrain: effect of lesions on serotonin and tryptophan hydroxylase levels. *Brain Res* **130**, 121-134.

PARNAVELAS JG & PAPADOPOULOS GC (1989). The monoaminergic innervation of the cerebral cortex is not diffuse and nonspecific. *Trends Neurosci* **12**, 315-319.

PARPURA V, BASARSKY TA, LIU F, JEFTINIJA K, JEFTINIJA S & HAYDON PG (1994). Glutamate-mediated astrocyte-neuron signalling. *Nature* **369**, 744-747.

PATEL KP & SCHMID PG (1988). Role of paraventricular nucleus (PVH) in baroreflex-mediated changes in lumbar sympathetic nerve activity and heart rate. *J Auton Nerv Syst* **22**, 211-219.

PATON JF, DEUCHARS J, AHMAD Z, WONG LF, MURPHY D & KASPAROV S (2001). Adenoviral vector demonstrates that angiotensin II-induced depression

of the cardiac baroreflex is mediated by endothelial nitric oxide synthase in the nucleus tractus solitarii of the rat. *J Physiol* **531**, 445-458.

PAUWELS PJ, VAN GOMPEL P & LEYSEN JE (1993). Activity of serotonin (5-HT) receptor agonists, partial agonists and antagonists at cloned human 5-HT1A receptors that are negatively coupled to adenylate cyclase in permanently transfected HeLa cells. *Biochem Pharmacol* **45**, 375-383.

PAXINOS G & WATSON C (1998). *The Rat Brain in Stereotaxic Coordinates*, 4th ed. Academic Press, San Diego.

PAZOS A & PALACIOS JM (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. *Brain Res* **346**, 205-230.

PEDIGO NW, YAMAMURA HI & NELSON DL (1981). Discrimination of multiple [3H]5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. *J Neurochem* **36**, 220-226.

PEHRSON R, OJTEG G, ISHIZUKA O & ANDERSSON KE (2002). Effects of NAD-299, a new, highly selective 5-HT1A receptor antagonist, on bladder function in rats. *Naunyn Schmiedebergs Arch Pharmacol* **366**, 528-536.

PEROUTKA SJ & SNYDER SH (1979). Multiple serotonin receptors: differential binding of [3H]5-hydroxytryptamine, [3H]lysergic acid diethylamide and [3H]spiroperidol. *Mol Pharmacol* **16**, 687-699.

PEROUTKA SJ, SWITZER JA & HAMIK A (1989). Identification of 5-hydroxytryptamine1D binding sites in human brain membranes. *Synapse* **3**, 61-66.

PERRY EB, BERMAN RM, SANACORA G, ANAND A, LYNCH-COLONESE K & CHARNEY DS (2004). Pindolol augmentation in depressed patients resistant to selective serotonin reuptake inhibitors: a double-blind, randomized, controlled trial. *J Clin Psychiatry* **65**, 238-243.

PETERS JA, MALONE HM & LAMBERT JJ (1992). Recent advances in the electrophysiological characterization of 5-HT3 receptors. *Trends Pharmacol Sci* **13**, 391-397.

PETRAS JM & CUMMINGS JF (1972). Autonomic neurons in the spinal cord of the Rhesus monkey: a correlation of the findings of cytoarchitectonics and

sympathectomy with fiber degeneration following dorsal rhizotomy. *J Comp Neurol* **146**, 189-218.

PHEBUS LA, JOHNSON KW, ZGOMBICK JM, GILBERT PJ, VAN BELLE K, MANCUSO V *et al.* (1997). Characterization of LY344864 as a pharmacological tool to study 5-HT1F receptors: binding affinities, brain penetration and activity in the neurogenic dural inflammation model of migraine. *Life Sci* **61**, 2117-2126.

PHILLIPS MA, SZABADI E & BRADSHAW CM (1999). The effects of the novel anxiolytic drug lesopitron, a full and selective 5-HT1A receptor agonist, on pupil diameter and oral temperature in man: comparison with buspirone. *J Psychopharmacol* **13**, 391-397.

PICKEL VM, JOH TH, CHAN J & BEAUDET A (1984). Serotoninergic terminals: ultrastructure and synaptic interaction with catecholamine-containing neurons in the medial nuclei of the solitary tracts. *J Comp Neurol* **225**, 291-301.

PICKERING AE, SIMMS AE & PATON JFR (2004). Dominant role of aortic baroreceptors in the cardiac baroreflex of the rat. *J Physiol* (Proceedings: in press).

PIGUET P & GALVAN M (1994). Transient and long-lasting actions of 5-HT on rat dentate gyrus neurones in vitro. *J Physiol* **481**, 629-639.

PIKE VW, MCCARRON JA, LAMMERSTMA AA, HUME SP, POOLE K, GRASBY PM *et al.* (1995). First delineation of 5-HT1A receptors in human brain with PET and [11C]WAY-100635. *Eur J Pharmacol* **283**, R1-R3.

PILOWSKY PM, DE CASTRO D, LLEWELLYN-SMITH I, LIPSKI J & VOSS MD (1990). Serotonin immunoreactive boutons make synapses with feline phrenic motoneurons. *J Neurosci* **10**, 1091-1098.

PINAULT D (1996). A novel single-cell staining procedure performed in vivo under electrophysiological control: morpho-functional features of juxtacellularly labeled thalamic cells and other central neurons with biocytin or Neurobiotin. *J Neurosci Methods* **65**, 113-136.

PIRES JG, SILVA SR, RAMAGE AG & FUTURO-NETO HA (1998). Evidence that 5-HT3 receptors in the nucleus tractus solitarius and other brainstem areas modulate the vagal bradycardia evoked by activation of the von Bezold-Jarisch reflex in the anesthetized rat. *Brain Res* **791**, 229-234.

PLASSAT JL, AMLAIKY N & HEN R (1993). Molecular cloning of a mammalian serotonin receptor that activates adenylate cyclase. *Mol Pharmacol* **44**, 229-236.

POMPEIANO M, PALACIOS JM & MENGOD G (1994). Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors. *Brain Res Mol Brain Res* **23**, 163-178.

PORRAS G, DI M, V, DE DEURWAERDERE P, ESPOSITO E & SPAMPINATO U (2002). Central serotonin4 receptors selectively regulate the impulse-dependent exocytosis of dopamine in the rat striatum: in vivo studies with morphine, amphetamine and cocaine. *Neuropharmacology* **43**, 1099-1109.

PORTER RH, BENWELL KR, LAMB H, MALCOLM CS, ALLEN NH, REVELL DF *et al.* (1999). Functional characterization of agonists at recombinant human 5-HT2A, 5-HT2B and 5-HT2C receptors in CHO-K1 cells. *Br J Pharmacol* **128**, 13-20.

POUZET B, DIDRIKSEN M & ARNT J (2002). Effects of the 5-HT(7) receptor antagonist SB-258741 in animal models for schizophrenia. *Pharmacol Biochem Behav* **71**, 655-665.

PRATT GD & BOWERY NG (1989). The 5-HT3 receptor ligand, [3H]BRL 43694, binds to presynaptic sites in the nucleus tractus solitarius of the rat. *Neuropharmacology* **28**, 1367-1376.

PRICE GW, BURTON MJ, COLLIN LJ, DUCKWORTH M, GASTER L, GOTHERT M *et al.* (1997). SB-216641 and BRL-15572--compounds to pharmacologically discriminate h5-HT1B and h5-HT1D receptors. *Naunyn Schmiedebergs Arch Pharmacol* **356**, 312-320.

PRITCHETT DB, BACH AW, WOZNY M, TALEB O, DAL TOSO R, SHIH JC *et al.* (1988). Structure and functional expression of cloned rat serotonin 5HT-2 receptor. *EMBO J* **7**, 4135-4140.

PROW MR, MARTIN KF & HEAL DJ (1996). 8-OH-DPAT-induced mydriasis in mice: a pharmacological characterisation. *Eur J Pharmacol* **317**, 21-28.

QIAN Y, MELIKIAN HE, RYE DB, LEVEY AI & BLAKELY RD (1995). Identification and characterization of antidepressant-sensitive serotonin transporter proteins using site-specific antibodies. *J Neurosci* **15**, 1261-1274.

RAMAGE AG (1985). The effects of ketanserin, methysergide and LY 53857 on sympathetic nerve activity. *Eur J Pharmacol* **113**, 295-303.

RAMAGE AG (1988). Examination of the effects of some 5-HT2 receptor antagonists on central sympathetic outflow and blood pressure in anaesthetised cats. *Naunyn Schmiedebergs Arch Pharmacol* **338**, 601-607.

RAMAGE AG (2000). Central 5-HT1A receptors and vagal tone to the airways. *Trends Pharmacol Sci* **21**, 201-203.

RAMAGE AG (2001). Central cardiovascular regulation and 5-hydroxytryptamine receptors. *Brain Res Bull* **56**, 425-439.

RAMAGE AG & DALY MB (1998). The central action of the 5-HT2 receptor agonist 1-(2,5-dimethoxy-4- iodophenyl)-2-aminopropane (DOI) on cardiac inotropy and vascular resistance in the anaesthetized cat. *Br J Pharmacol* **125**, 1172-1179.

RAMAGE AG & FOZARD JR (1987). Evidence that the putative 5-HT1A receptor agonists, 8-OH-DPAT and ipsapirone, have a central hypotensive action that differs from that of clonidine in anaesthetised cats. *Eur J Pharmacol* **138**, 179-191.

RAMAGE AG & MIFFLIN SW (1998). Vagal-evoked excitation of a sub-population of neurones in the nucleus of the solitary tract (NTS) involves 5-HT3 receptors in the anaesthetized rat. *J Physiol* **509**, 129P.

RAMAGE AG & MIRTSOU-FIDANI V (1995). Examination of the cardiovascular effects of WAY-100802, a selective 5-HT1A antagonist, in anaesthetized cats. *Br J Pharmacol* **116**, 289P.

RAMAMOORTHY S, BAUMAN AL, MOORE KR, HAN H, YANG-FENG T, CHANG AS *et al.* (1993). Antidepressant- and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization. *Proc Natl Acad Sci U S A* **90**, 2542-2546.

RANDALL WC & ARDELL JL (1985). Selective parasympathectomy of automatic and conductile tissues of the canine heart. *Am J Physiol* **248**, H61-H68.

RANDALL WC, ARDELL JL, CALDERWOOD D, MILOSAVLJEVIC M & GOYAL SC (1986). Parasympathetic ganglia innervating the canine atrioventricular nodal region. *J Auton Nerv Syst* **16**, 311-323.

RANDALL WC, ARDELL JL, WURSTER RD & MILOSAVLJEVIC M (1987). Vagal postganglionic innervation of the canine sinoatrial node. *J Auton Nerv Syst* **20**, 13-23.

RAPPORT MM, GREEN AA & PAGE IH (1948). Serum vasoconstrictor (serotonin). IV. Isolation and characterization. *Journal of Biological Chemistry* **176**, 1243-1251.

RATHNER JA, OWENS NC & MCALLEN RM (2001). Cold-activated raphe-spinal neurons in rats. *J Physiol* **535**, 841-854.

READ KE (2004). *The role of central 5-HT receptors in the control of the micturition reflex* Ph.D thesis, University of London.

READ KE, SANGER GJ & RAMAGE AG (2003). Evidence for the involvement of central 5-HT7 receptors in the micturition reflex in anaesthetized female rats. *Br J Pharmacol* **140**, 53-60.

READ KE, SANGER GJ & RAMAGE AG (2004). Can carboxamidotryptamine (5-CT) cause micturition in anaesthetized female rats? *Br J Pharmacol.*

REYNOLDS DJ, LOWENSTEIN PR, MOORMAN JM, GRAHAME-SMITH DG & LESLIE RA (1994). Evidence for cholinergic vagal afferents and vagal presynaptic M1 receptors in the ferret. *Neurochem Int* **25**, 455-464.

REYNOLDS DJM, LESLIE RA, GRAHAME-SMITH DG & HARVEY JM (1991). Autoradiographic localization of 5-HT3 receptor ligand binding in the cat brainstem. *Neurochem Int* **18**, 69-73.

REYNOLDS GP, MASON SL, MELDRUM A, DE KECZER S, PARNES H, EGLEN RM *et al.* (1995). 5-Hydroxytryptamine (5-HT)4 receptors in post mortem human brain tissue: distribution, pharmacology and effects of neurodegenerative diseases. *Br J Pharmacol* **114**, 993-998.

RICARDO JA & KOH ET (1978). Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. *Brain Res* **153**, 1-26.

RICHARDSON BP, ENGEL G, DONATSCH P & STADLER PA (1985). Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. *Nature* **316**, 126-131.

RICHE D, DE POMMERY J & MENETREY D (1990). Neuropeptides and catecholamines in efferent projections of the nuclei of the solitary tract in the rat. *J Comp Neurol* **293**, 399-424.

RICHERSON GB (1995). Response to CO2 of neurons in the rostral ventral medulla in vitro. *J Neurophysiol* **73**, 933-944.

RICHTER DW, BALLANYI K & SCHWARZACHER S (1992). Mechanisms of respiratory rhythm generation. *Curr Opin Neurobiol* **2**, 788-793.

ROBERTS AJ, KRUCKER T, LEVY CL, SLANINA KA, SUTCLIFFE JG & HEDLUND PB (2004a). Mice lacking 5-HT receptors show specific impairments in contextual learning. *Eur J Neurosci* **19**, 1913-1922.

ROBERTS C, ALLEN L, LANGMEAD CJ, HAGAN JJ, MIDDLEMISS DN & PRICE GW (2001a). The effect of SB-269970, a 5-HT(7) receptor antagonist, on 5-HT release from serotonergic terminals and cell bodies. *Br J Pharmacol* **132**, 1574-1580.

ROBERTS C, HATCHER P, HAGAN JJ, AUSTIN NE, JEFFREY P, WYMAN P *et al.* (2000). The effect of SB-236057-A, a selective 5-HT1B receptor inverse agonist, on in vivo extracellular 5-HT levels in the freely-moving guinea-pig. *Naunyn Schmiedebergs Arch Pharmacol* **362**, 177-183.

ROBERTS C, PRICE GW & MIDDLEMISS DN (2001b). Ligands for the investigation of 5-HT autoreceptor function. *Brain Res Bull* **56**, 463-469.

ROBERTS C, THOMAS DR, BATE ST & KEW JN (2004b). GABAergic modulation of 5-HT(7) receptor-mediated effects on 5-HT efflux in the guinea-pig dorsal raphe nucleus. *Neuropharmacology* **46**, 935-941.

ROMANIUK A, KOPROWSKA M, KROTEWICZ M, STRZELCZUK M & WIECZOREK M (2001). Effects of 8-OHDPAT administration into the dorsal raphe nucleus and dorsal hippocampus on fear behavior and regional brain monoamines distribution in rats. *Behav Brain Res* **120**, 47-57.

ROMERO L, HERVAS I & ARTIGAS F (1996). The 5-HT1A antagonist WAY-100635 selectively potentiates the presynaptic effects of serotonergic antidepressants in rat brain. *Neurosci Lett* **219**, 123-126.

ROOSE SP & GLASSMAN AH (1994). Antidepressant choice in the patient with cardiac disease: lessons from the Cardiac Arrhythmia Suppression Trial (CAST) studies. *J Clin Psychiatry* **55 Suppl A**, 83-87.

ROSS CA, RUGGIERO DA & REIS DJ (1985). Projections from the nucleus tractus solitarii to the rostral ventrolateral medulla. *J Comp Neurol* **242**, 511-534.

ROTH BL, CRAIGO SC, CHOUDHARY MS, ULUER A, MONSMA FJ, JR., SHEN Y *et al.* (1994). Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. *J Pharmacol Exp Ther* **268**, 1403-1410.

RUAT M, TRAIFFORT E, ARRANG JM, TARDIVEL-LACOMBE J, DIAZ J, LEURS R *et al.* (1993a). A novel rat serotonin (5-HT6) receptor: molecular cloning, localization and stimulation of cAMP accumulation. *Biochem Biophys Res Commun* **193**, 268-276.

RUAT M, TRAIFFORT E, LEURS R, TARDIVEL-LACOMBE J, DIAZ J, ARRANG JM *et al.* (1993b). Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT7) activating cAMP formation. *Proc Natl Acad Sci U S A* **90**, 8547-8551.

SALTZMAN AG, MORSE B, WHITMAN MM, IVANSHCHENKO Y, JAYE M & FELDER S (1991). Cloning of the human serotonin 5-HT2 and 5-HT1C receptor subtypes. *Biochem Biophys Res Commun* **181**, 1469-1478.

SANCHEZ-LOPEZ A, CENTURION D, VAZQUEZ E, ARULMANI U, SAXENA PR & VILLALON CM (2003). Pharmacological profile of the 5-HT-induced inhibition of cardioaccelerator sympathetic outflow in pithed rats: correlation with 5-HT1 and putative 5-ht5A/5B receptors. *Br J Pharmacol* **140**, 725-735.

SAPER C (2004). Central autonomic system. In *The Rat Nervous System (3rd Edition)*, ed. Paxinos G, Academic Press, San Diego.

SAPRU HN, GONZALEZ E & KRIEGER AJ (1981). Aortic nerve stimulation in the rat: cardiovascular and respiratory responses. *Brain Res Bull* **6**, 393-398.

SATTLER HD, RICHTER P, FRITZSCHE M, VON TURNER A & BARNETT W (2000). Neurophysiologic tests during antidepressive treatment - an exploratory study. *Pharmacopsychiatry* **33**, 229-233.

SAUDOU F, AMARA DA, DIERICH A, LEMEUR M, RAMBOZ S, SEGU L *et al.* (1994). Enhanced aggressive behavior in mice lacking 5-HT1B receptor. *Science* **265**, 1875-1878.

SAUER WH, BERLIN JA & KIMMEL SE (2001). Selective serotonin reuptake inhibitors and myocardial infarction. *Circulation* **104**, 1894-1898.

SAWYNOK J & REID A (1994). Spinal supersensitivity to 5-HT1, 5-HT2 and 5-HT3 receptor agonists following 5,7-dihydroxytryptamine. *Eur J Pharmacol* **264**, 249-257.

SAXENA PR & VILLALON CM (1991). 5-Hydroxytryptamine: a chameleon in the heart. *Trends Pharmacol Sci* **12**, 223-227.

SAXENA PR, VILLALON CM, DHASMANA KM & VERDOUW PD (1992). 5-Hydroxytryptamine-induced increase in left ventricular dP/dtmax does not suggest the presence of ventricular 5-HT4 receptors in the pig. *Naunyn Schmiedebergs Arch Pharmacol* **346**, 629-636.

SCHAFFAR N, JEAN A & CALAS A (1984). Radioautographic study of serotoninergic axon terminals in the rat trigeminal motor nucleus. *Neurosci Lett* **44**, 31-36.

SCHAFFAR N, KESSLER JP, BOSLER O & JEAN A (1988). Central serotonergic projections to the nucleus tractus solitarii: evidence from a double labeling study in the rat. *Neuroscience* **26**, 951-958.

SCHANEN NC, SCHERER SW, TSUI LC & FRANCKE U (1996). Assignment of the 5-hydroxytryptamine (serotonin) receptor 5A gene (HTR5A) to human chromosome band 7q36.1. *Cytogenet Cell Genet* **72**, 187-188.

SCHEUER DA & MIFFLIN SW (2001). Glucocorticoids modulate baroreflex control of renal sympathetic nerve activity. *Am J Physiol Regul Integr Comp Physiol* **280**, R1440-R1449.

SCHLOSSER R, ROSCHKE J, ROSSBACH W & BENKERT O (1998). Conventional and spectral power analysis of all-night sleep EEG after subchronic treatment with paroxetine in healthy male volunteers. *Eur Neuropsychopharmacol* **8**, 273-278.

SCHWABER JS, KAPP BS, HIGGINS GA & RAPP PR (1982). Amygdaloid and basal forebrain direct connections with the nucleus of the solitary tract and the dorsal motor nucleus. *J Neurosci* **2**, 1424-1438.

SEAGARD JL, HOPP FA, DRUMMOND HA & VAN WYNSBERGHE DM (1993). Selective contribution of two types of carotid sinus baroreceptors to the control of blood pressure. *Circ Res* **72**, 1011-1022.

SECKER AG (2004). *An Investigation into the Effects of Acute and Chronic Blockade of 5-HT1A Receptors Involved in the Control of Micturition* Ph.D Thesis, University of London.

SECKER AG, NAYLOR AM & RAMAGE AG (2002). A role for supraspinal 5-HT1A receptors in the control of micturition in urethane anaesthetized rats. *Pharmacologist* **44**, A186.

SEVOZ C, CALLERA JC, MACHADO BH, HAMON M & LAGUZZI R (1997). Role of serotonin3 receptors in the nucleus tractus solitarii on the carotid chemoreflex. *Am J Physiol* **272**, H1250-H1259.

SEVOZ C, NOSJEAN A, CALLERA JC, MACHADO B, HAMON M & LAGUZZI R (1996). Stimulation of 5-HT3 receptors in the NTS inhibits the cardiac Bezold-Jarisch reflex response. *Am J Physiol* **271**, H80-H87.

SEVOZ-COUCHE C, SPYER KM & JORDAN D (2000a). In vivo modulation of vagal-identified dorsal medullary neurones by activation of different 5-Hydroxytryptamine(2) receptors in rats. *Br J Pharmacol* **131**, 1445-1453.

SEVOZ-COUCHE C, WANG Y, RAMAGE AG, SPYER KM & JORDAN D (2000b). In vivo modulation of nucleus tractus solitarius (NTS) neurones by activation of 5-hydroxytryptamine(2) receptors in rats. *Neuropharmacology* **39**, 2006-2016.

SHAPIRO RE & MISELIS RR (1985). The central neural connections of the area postrema of the rat. *J Comp Neurol* **234**, 344-364.

SHARP T & HJORTH S (1990). Application of brain microdialysis to study the pharmacology of the 5-HT1A autoreceptor. *J Neurosci Methods* **34**, 83-90.

SHARP T, MCQUADE R, BRAMWELL S & HJORTH S (1993). Effect of acute and repeated administration of 5-HT1A receptor agonists on 5-HT release in rat brain in vivo. *Naunyn Schmiedebergs Arch Pharmacol* **348**, 339-346.

SHARP T, UMBERS V & HJORTH S (1996). The role of 5-HT1A autoreceptors and alpha 1-adrenoceptors in the inhibition of 5-HT release--II NAN-190 and SDZ 216-525. *Neuropharmacology* **35**, 735-741.

SHELDON PW & AGHAJANIAN GK (1991). Excitatory responses to serotonin (5-HT) in neurons of the rat piriform cortex: evidence for mediation by 5-HT1C receptors in pyramidal cells and 5-HT2 receptors in interneurons. *Synapse* **9**, 208-218.

SHEPHEARD S, EDVINSSON L, CUMBERBATCH M, WILLIAMSON D, MASON G, WEBB J *et al.* (1999). Possible antimigraine mechanisms of action of the 5HT1F receptor agonist LY334370. *Cephalalgia* **19**, 851-858.

SHEPHEARD SL, JORDAN D & RAMAGE AG (1990). Actions of 8-OH-DPAT on sympathetic and respiratory drives, blood pressure and heart rate in the rabbit. *Eur J Pharmacol* **186**, 267-272.

SHEPHEARD SL, JORDAN D & RAMAGE AG (1994). Comparison of the effects of IVth ventricular administration of some tryptamine analogues with those of 8-OH-DPAT on autonomic outflow in the anaesthetized cat. *Br J Pharmacol* **111**, 616-624.

SIEPMANN M, GROSSMANN J, MUCK-WEYMANN M & KIRCH W (2003). Effects of sertraline on autonomic and cognitive functions in healthy volunteers. *Psychopharmacology (Berl)* **168**, 293-298.

SILVA-CARVALHO L, DAWID-MILNER MS & SPYER KM (1995). The pattern of excitatory inputs to the nucleus tractus solitarii evoked on stimulation in the hypothalamic defence area in the cat. *J Physiol* **487**, 727-737.

SIMMS AE, PATON JFR & PICKERING AE (2004). Differences in the arterial pressure responsiveness of the sympathetic and parasympathetic baroreceptor reflex. *J Physiol* (Proceedings: in press).

SIPES TE & GEYER MA (1996). Functional behavioral homology between rat 5-HT1B and guinea pig 5-HT1D receptors in the modulation of prepulse inhibition of startle. *Psychopharmacology (Berl)* **125**, 231-237.

SKINGLE M, BEATTIE DT, SCOPES DI, STARKEY SJ, CONNOR HE, FENIUK W *et al.* (1996). GR127935: a potent and selective 5-HT1D receptor antagonist. *Behav Brain Res* **73**, 157-161.

SKINNER K, FIELDS HL, BASBAUM AI & MASON P (1997). GABA-immunoreactive boutons contact identified OFF and ON cells in the nucleus raphe magnus. *J Comp Neurol* **378**, 196-204.

SKINNER MR, RAMAGE AG & JORDAN D (2002). Modulation of reflexly evoked vagal bradycardias by central 5-HT1A receptors in anaesthetized rabbits. *Br J Pharmacol* **137**, 861-873.

SLEIGHT AJ, BOESS FG, BOS M, LEVET-TRAFIT B, RIEMER C & BOURSON A (1998). Characterization of Ro 04-6790 and Ro 63-0563: potent and selective antagonists at human and rat 5-HT6 receptors. *Br J Pharmacol* **124**, 556-562.

SMITH JC, ELLENBERGER HH, BALLANYI K, RICHTER DW & FELDMAN JL (1991). Pre-Botzinger complex: a brainstem region that may generate respiratory rhythm in mammals. *Science* **254**, 726-729.

SMITS JF, VAN ESSEN H & STRUYKER-BOUDIER AJ (1978). Serotonin-mediated cardiovascular responses to electrical stimulation of the raphe nuclei in the rat. *Life Sci* **23**, 173-178.

SORENSEN SM, KEHNE JH, FADAYEL GM, HUMPHREYS TM, KETTELER HJ, SULLIVAN CK *et al.* (1993). Characterization of the 5-HT2 receptor antagonist MDL 100907 as a putative atypical antipsychotic: behavioral, electrophysiological and neurochemical studies. *J Pharmacol Exp Ther* **266**, 684-691.

SPORTON SC, SHEPHEARD SL, JORDAN D & RAMAGE AG (1991). Microinjections of 5-HT1A agonists into the dorsal motor vagal nucleus produce a bradycardia in the atenolol-pretreated anaesthetized rat. *Br J Pharmacol* **104**, 466-470.

SPROUSE JS & AGHAJANIAN GK (1986). (-)-Propranolol blocks the inhibition of serotonergic dorsal raphe cell firing by 5-HT1A selective agonists. *Eur J Pharmacol* **128**, 295-298.

SPYER KM (1990). The central nervous organization of reflex circulatory control. In *Central Regulation of Autonomic Functions*, eds. Loewy AD & Spyer KM, pp. 168-188. Oxford University Press, New York.

SPYER KM (1994). Central nervous mechanisms contributing to cardiovascular control. *J Physiol* **474**, 1-19.

STANFORD SC (1999). *Selective Serotonin Reuptake Ihibitors (SSRIs): Past, Present and Future* Landes, Georgetown, TX.

STEAN TO, HIRST WD, THOMAS DR, PRICE GW, ROGERS D, RILEY G *et al.* (2002). Pharmacological profile of SB-357134: a potent, selective, brain penetrant, and orally active 5-HT(6) receptor antagonist. *Pharmacol Biochem Behav* **71**, 645-654.

STEIN PK, CARNEY RM, FREEDLAND KE, SKALA JA, JAFFE AS, KLEIGER RE *et al.* (2000). Severe depression is associated with markedly reduced heart rate variability in patients with stable coronary heart disease. *J Psychosom Res* **48**, 493-500.

STEINBUSCH HW (1981). Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience* **6**, 557-618.

STEINBUSCH HW & NIEUWENHUYS R (1983). The raphe nuclei of the rat braintsem: a cytoarchitectonic and immunohistochemical study. In *Chemical Neuroanatomy*, ed. Emson PC, pp. 131-207. Raven Press, New York.

STEWARD LJ, GE J, STOWE RL, BROWN DC, BRUTON RK, STOKES PR *et al.* (1996). Ability of 5-HT4 receptor ligands to modulate rat striatal dopamine release in vitro and in vivo. *Br J Pharmacol* **117**, 55-62.

STORER RJ & GOADSBY PJ (1997). Microiontophoretic application of serotonin (5HT)1B/1D agonists inhibits trigeminal cell firing in the cat. *Brain* **120**, 2171-2177.

STRACK AM, SAWYER WB, HUGHES JH, PLATT KB & LOEWY AD (1989). A general pattern of CNS innervation of the sympathetic outflow demonstrated by transneuronal pseudorabies viral infections. *Brain Res* **491**, 156-162.

STRACK AM, SAWYER WB, MARUBIO LM & LOEWY AD (1988). Spinal origin of sympathetic preganglionic neurons in the rat. *Brain Res* **455**, 187-191.

STREET JA, HEMSWORTH BA, ROACH AG & DAY MD (1979). Tissue levels of several radiolabelled β-adrenoceptor antagonists after intravenous administration in rats. *Arch int Pharmacodyn* **237**, 180-190.

STUESSE SL (1982). Origins of cardiac vagal preganglionic fibers: a retrograde transport study. *Brain Res* **236**, 15-25.

STUESSE SL & FISH SE (1984). Projections to the cardioinhibitory region of the nucleus ambiguus of rat. *J Comp Neurol* **229**, 271-278.

SU DF, CERUTTI C, BARRES C, JULIEN C, VINCENT M, PAULTRE C *et al.* (1992). Arterial baroreflex control of heart period is not related to blood pressure variability in conscious hypertensive and normotensive rats. *Clin Exp Pharmacol Physiol* **19**, 767-776.

SUMNER MJ, FENIUK W & HUMPHREY PP (1989). Further characterization of the 5-HT receptor mediating vascular relaxation and elevation of cyclic AMP in porcine isolated vena cava. *Br J Pharmacol* **97**, 292-300.

SUN MK & GUYENET PG (1986). Effect of clonidine and gamma-aminobutyric acid on the discharges of medullo-spinal sympathoexcitatory neurons in the rat. *Brain Res* **368**, 1-17.

SUN MK, HACKETT JT & GUYENET PG (1988). Sympathoexcitatory neurons of rostral ventrolateral medulla exhibit pacemaker properties in the presence of a glutamate-receptor antagonist. *Brain Res* **438**, 23-40.

SUN MK & SPYER KM (1991). Responses of rostroventrolateral medulla spinal vasomotor neurones to chemoreceptor stimulation in rats. *J Auton Nerv Syst* **33**, 79-84.

SUZUKI M, MATSUDA T, ASANO S, SOMBOONTHUM P, TAKUMA K & BABA A (1995). Increase of noradrenaline release in the hypothalamus of freely moving rat by postsynaptic 5-hydroxytryptamine1A receptor activation. *Br J Pharmacol* **115**, 703-711.

SVED AF, TSUKAMOTO K & SCHREIHOFER AM (1992). Stimulation of alpha 2-adrenergic receptors in nucleus tractus solitarius is required for the baroreceptor reflex. *Brain Res* **576**, 297-303.

SWANSON LW (1982). The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Res Bull* **9**, 321-353.

SWANSON LW & COWAN WM (1979). The connections of the septal region in the rat. *J Comp Neurol* **186**, 621-655.

SYKES RM, SPYER KM & IZZO PN (1994). Central distribution of substance P, calcitonin gene-related peptide and 5-hydroxytryptamine in vagal sensory afferents in the rat dorsal medulla. *Neuroscience* **59**, 195-210.

SZABO A, BUTZ BL & ALPER RH (1998). Further characterization of forebrain serotonin receptors mediating tachycardia in conscious rats. *Brain Res Bull* **45**, 583-588.

TABER E (1961). The cytoarchitecture of the brain stem of the cat. I. Brain stem nuclei of cat. *J Comp Neurol* **116**, 27-69.

TABER E, BRODAL A & WALBERG F (1960). The raphe nuclei of the brain stem in the cat. I. Normal topography and cytoarchitecture and general discussion. *J Comp Neurol* **114**, 161-187.

TAKAGI H, SHIOSAKA S, TOHYAMA M, SENBA E & SAKANAKA M (1980a). Ascending components of the medial forebrain bundle from the lower brain stem in the rat, with special reference to raphe and catecholamine cell groups. A study by the HRP method. *Brain Res* **193**, 315-337.

TAKAGI H, SHIOSAKA S, TOHYAMA M, SENBA E & SAKANAKA M (1980b). Ascending components of the medial forebrain bundle from the lower brain stem in the rat, with special reference to raphe and catecholamine cell groups. A study by the HRP method. *Brain Res* **193**, 315-337.

TAKEUCHI Y, KIMURA H & SANO Y (1982). Immunohistochemical demonstration of serotonin-containing nerve fibers in the cerebellum. *Cell Tissue Res* **226**, 1-12.

TALMAN WT, COLLING JM & ROBERTSON SC (1991). Glycine microinjected into nucleus tractus solitarii of rat acts through cholinergic mechanisms. *Am J Physiol* **260**, H1326-H1331.

TALMAN WT, PERRONE MH & REIS DJ (1980). Evidence for L-glutamate as the neurotransmitter of baroreceptor afferent nerve fibers. *Science* **209**, 813-815.

TALMAN WT, PERRONE MH, SCHER P, KWO S & REIS DJ (1981). Antagonism of the baroreceptor reflex by glutamate diethyl ester, an antagonist to L-glutamate. *Brain Res* **217**, 186-191.

TALMAN WT & ROBERTSON SC (1989). Glycine, like glutamate, microinjected into the nucleus tractus solitarii of rat decreases arterial pressure and heart rate. *Brain Res* **477**, 7-13.

TEPPER SJ, RAPOPORT AM & SHEFTELL FD (2002). Mechanisms of action of the 5-HT1B/1D receptor agonists. *Arch Neurol* **59**, 1084-1088.

TERREBERRY RR & NEAFSEY EJ (1983). Rat medial frontal cortex: a visceral motor region with a direct projection to the solitary nucleus. *Brain Res* **278**, 245-249.

TERRON JA (1997). Role of 5-ht7 receptors in the long-lasting hypotensive response induced by 5-hydroxytryptamine in the rat. *Br J Pharmacol* **121**, 563-571.

TERRY AV, JR., BUCCAFUSCO JJ, JACKSON WJ, PRENDERGAST MA, FONTANA DJ, WONG EH *et al.* (1998). Enhanced delayed matching performance in younger and older macaques administered the 5-HT4 receptor agonist, RS 17017. *Psychopharmacology (Berl)* **135**, 407-415.

TERUI N, MASUDA N, SAEKI Y & KUMADA M (1990). Activity of barosensitive neurons in the caudal ventrolateral medulla that send axonal projections to the rostral ventrolateral medulla in rabbits. *Neurosci Lett* **118**, 211-214.

TESTA R, GUARNERI L, POGGESI E, ANGELICO P, VELASCO C, IBBA M *et al.* (1999). Effect of several 5-hydroxytryptamine(1A) receptor ligands on the micturition reflex in rats: comparison with WAY 100635. *J Pharmacol Exp Ther* **290**, 1258-1269.

THOMAS DR, ATKINSON PJ, HASTIE PG, ROBERTS JC, MIDDLEMISS DN & PRICE GW (2002). [3H]-SB-269970 radiolabels 5-HT7 receptors in rodent, pig and primate brain tissues. *Neuropharmacology* **42**, 74-81.

THOMAS DR, GITTINS SA, COLLIN LL, MIDDLEMISS DN, RILEY G, HAGAN J *et al.* (1998a). Functional characterisation of the human cloned 5-HT7 receptor (long form); antagonist profile of SB-258719. *Br J Pharmacol* **124**, 1300-1306.

THOMAS DR & HAGAN JJ (2004). 5-HT7 receptors. *Curr Drug Target CNS Neurol Disord* **3**, 81-90.

THOMAS DR, LARMINIE CG, LYONS HR, FOSBERRY A, HILL MJ & HAYES PD (2004). Cloning and pharmacological characterisation of the guinea pig 5-ht5A receptor. *Eur J Pharmacol* (in press).

THOMAS DR, MELOTTO S, MASSAGRANDE M, GRIBBLE AD, JEFFREY P, STEVENS AJ *et al.* (2003). SB-656104-A, a novel selective 5-HT7 receptor antagonist, modulates REM sleep in rats. *Br J Pharmacol* **139**, 705-714.

THOMAS DR, MIDDLEMISS DN, TAYLOR SG, NELSON P & BROWN AM (1999a). 5-CT stimulation of adenylyl cyclase activity in guinea-pig hippocampus: evidence for involvement of 5-HT7 and 5-HT1A receptors. *Br J Pharmacol* **128**, 158-164.

THOMAS EA, CARSON MJ & SUTCLIFFE JG (1998b). Oleamide-induced modulation of 5-hydroxytryptamine receptor-mediated signaling. *Ann N Y Acad Sci* **861**, 183-189.

THOMAS EA, CRAVATT BF & SUTCLIFFE JG (1999b). The endogenous lipid oleamide activates serotonin 5-HT7 neurons in mouse thalamus and hypothalamus. *J Neurochem* **72**, 2370-2378.

THOR KB & HELKE CJ (1987). Serotonin- and substance P-containing projections to the nucleus tractus solitarii of the rat. *J Comp Neurol* **265**, 275-293.

THOREN P (1979). Role of cardiac vagal C-fibers in cardiovascular control. *Rev Physiol Biochem Pharmacol* **86**, 1-94.

THOREN P & JONES JV (1977). Characteristics of aortic baroreceptor C-fibres in the rabbit. *Acta Physiol Scand* **99**, 448-456.

TO ZP, BONHAUS DW, EGLEN RM & JAKEMAN LB (1995). Characterization and distribution of putative 5-ht$_7$ receptors in guinea-pig brain. *Br J Pharmacol* **115**, 107-116.

TODOROVIC S & ANDERSON EG (1990). 5-HT2 and 5-HT3 receptors mediate two distinct depolarizing responses in rat dorsal root ganglion neurons. *Brain Res* **511**, 71-79.

TOMASULO KC & EMMERS R (1972). Activation of neurons in the gracile nucleus by two afferent pathways in the rat. *Exp Neurol* **36**, 197-206.

TONINI M, GALLIGAN JJ & NORTH RA (1989). Effects of cisapride on cholinergic neurotransmission and propulsive motility in the guinea pig ileum. *Gastroenterology* **96**, 1257-1264.

TÖRK I (1985). Raphe nuclei and serotonin containing systems. In *The Rat Nervous System*, ed. Paxinos, pp. 43-78.

TRAVERS JB & NORGREN R (1983). Afferent projections to the oral motor nuclei in the rat. *J Comp Neurol* **220**, 280-298.

TREVETHICK MA, FENIUK W & HUMPHREY PP (1984). 5-hydroxytryptamine-induced relaxation of neonatal porcine vena cava in vitro. *Life Sci* **35**, 477-486.

TRIVEDI MH, RUSH AJ, ARMITAGE R, GULLION CM, GRANNEMANN BD, ORSULAK PJ *et al.* (1999). Effects of fluoxetine on the polysomnogram in outpatients with major depression. *Neuropsychopharmacology* **20**, 447-459.

TRULSON ME, PREUSSLER DW, HOWELL GA & FREDERICKSON CJ (1982). Raphe unit activity in freely moving cats: effects of benzodiazepines. *Neuropharmacology* **21**, 1045-1050.

TSENG CJ, APPALSAMY M, ROBERTSON D & MOSQUEDA-GARCIA R (1993). Effects of nicotine on brain stem mechanisms of cardiovascular control. *J Pharmacol Exp Ther* **265**, 1511-1518.

TSOU AP, KOSAKA A, BACH C, ZUPPAN P, YEE C, TOM L *et al.* (1994). Cloning and expression of a 5-hydroxytryptamine7 receptor positively coupled to adenylyl cyclase. *J Neurochem* **63**, 456-464.

TULEN JH, BRUIJN JA, DE MAN KJ, PEPPLINKHUIZEN L, VAN DEN MEIRACKER AH & MAN IN 'T VELD AJ (1996). Cardiovascular variability in major depressive disorder and effects of imipramine or mirtazapine (Org 3770). *J Clin Psychopharmacol* **16**, 135-145.

ULLMER C, ENGELS P, ABDEL'AL S & LUBBERT H (1996). Distribution of 5-HT4 receptor mRNA in the rat brain. *Naunyn Schmiedebergs Arch Pharmacol* **354**, 210-212.

UNDERWOOD MD, ARANGO V, BAKALIAN MJ, RUGGIERO DA & MANN JJ (1999). Dorsal raphe nucleus serotonergic neurons innervate the rostral ventrolateral medulla in rat. *Brain Res* **824**, 45-55.

UNDERWOOD MD, BAKALIAN MJ, ARANGO V & MANN JJ (1995). Effect of chemical stimulation of the dorsal raphe nucleus on cerebral blood flow in rat. *Neurosci Lett* **199**, 228-230.

UNDERWOOD MD, BAKALIAN MJ, ARANGO V, SMITH RW & MANN JJ (1992). Regulation of cortical blood flow by the dorsal raphe nucleus: topographic organization of cerebrovascular regulatory regions. *J Cereb Blood Flow Metab* **12**, 664-673.

URBANSKI RW & SAPRU HN (1988). Evidence for a sympathoexcitatory pathway from the nucleus tractus solitarii to the ventrolateral medullary pressor area. *J Auton Nerv Syst* **23**, 161-174.

VAN DE KAR LD & LORENS SA (1979). Differential serotonergic innervation of individual hypothalamic nuclei and other forebrain regions by the dorsal and median midbrain raphe nuclei. *Brain Res* **162**, 45-54.

VAN DEN HOOFF P & GALVAN M (1991). Electrophysiology of the 5-HT1A ligand MDL 73005EF in the rat hippocampal slice. *Eur J Pharmacol* **196**, 291-298.

VAN DEN HOOFF P & GALVAN M (1992). Actions of 5-hydroxytryptamine and 5-HT1A receptor ligands on rat dorso-lateral septal neurones in vitro. *Br J Pharmacol* **106**, 893-899.

VAN DEN WYNGAERT I, GOMMEREN W, VERHASSELT P, JURZAK M, LEYSEN J, LUYTEN W *et al.* (1997). Cloning and expression of a human serotonin 5-HT4 receptor cDNA. *J Neurochem* **69**, 1810-1819.

VAN DER KOOY D, KODA LY, MCGINTY JF, GERFEN CR & BLOOM FE (1984). The organization of projections from the cortex, amygdala, and hypothalamus to the nucleus of the solitary tract in rat. *J Comp Neurol* **224**, 1-24.

VAN HOOFT JA, SPIER AD, YAKEL JL, LUMMIS SC & VIJVERBERG HP (1998). Promiscuous coassembly of serotonin 5-HT3 and nicotinic alpha4 receptor subunits into Ca(2+)-permeable ion channels. *Proc Natl Acad Sci U S A* **95**, 11456-11461.

VAN HUIZEN F, BANSSE MT & STAM NJ (1993). Agonist-induced down-regulation of human 5-HT1A and 5-HT2 receptors in Swiss 3T3 cells. *Neuroreport* **4**, 1327-1330.

VAN WIJNGAARDEN I & SOUDIJN W (1997). 5-HT2A, 5-HT2B and 5-HT2C receptor ligands. In *Serotonin receptors and their ligands*, eds. Olivier B, van Wijngaarden I & Soudijn W, Elsevier, Amsterdam.

VAN WIJNGAARDEN I, TULP MT & SOUDIJN W (1990). The concept of selectivity in 5-HT receptor research. *Eur J Pharmacol* **188**, 301-312.

VANHOENACKER P, HAEGEMAN G & LEYSEN JE (2000). 5-HT7 receptors: current knowledge and future prospects. *Trends Pharmacol Sci* **21**, 70-77.

VARDHAN A, KACHROO A & SAPRU HN (1993). Excitatory amino acid receptors in the nucleus tractus solitarius mediate the responses to the stimulation of cardio-pulmonary vagal afferent C fiber endings. *Brain Res* **618**, 23-31.

VAYSSETTES-COURCHAY C, BOUYSSET F, LAUBIE M & VERBEUREN TJ (1997). Central integration of the Bezold-Jarish reflex in the cat. *Brain Res* **744**, 272-278.

VAYSSETTES-COURCHAY C, BOUYSSET F, VERBEUREN TJ, LAUBIE M & SCHMITT H (1991). Quipazine-induced hypertension in anaesthetized cats is mediated by central and peripheral 5-HT2 receptors: role of the ventrolateral pressor area. *Eur J Pharmacol* **192**, 389-395.

VELDMAN SA & BIENKOWSKI MJ (1992). Cloning and pharmacological characterization of a novel human 5-hydroxytryptamine1D receptor subtype. *Mol Pharmacol* **42**, 439-444.

VERBERNE AJ & GUYENET PG (1992). Medullary pathway of the Bezold-Jarisch reflex in the rat. *Am J Physiol* **263**, R1195-R1202.

VERBERNE AJ, SARTOR DM & BERKE A (1999). Midline medullary depressor responses are mediated by inhibition of RVLM sympathoexcitatory neurons in rats. *Am J Physiol* **276**, R1054-R1062.

VERBEUREN TJ (1989). Synthesis, storage, release and metabolism of 5-hydroxytryptamine in peripheral tissues. In *The Peripheral Actions of 5-Hydroxytryptamine*, ed. Fozard JR, pp. 1-25. Oxford University Press, Oxford.

VILLALON CM, CENTURION D, VALDIVIA LF, DE VRIES P & SAXENA PR (2002). An introduction to migraine: from ancient treatment to functional pharmacology and antimigraine therapy. *Proc West Pharmacol Soc* **45**, 199-210.

VILLALON CM, DEN BOER MO, HEILIGERS JP & SAXENA PR (1990). Mediation of 5-hydroxytryptamine-induced tachycardia in the pig by the putative 5-HT4 receptor. *Br J Pharmacol* **100**, 665-667.

VILLALON CM, DEN BOER MO, HEILIGERS JP & SAXENA PR (1991). Further characterization, by use of tryptamine and benzamide derivatives, of the putative 5-HT4 receptor mediating tachycardia in the pig. *Br J Pharmacol* **102**, 107-112.

VILLALON CM, HEILIGERS JP, CENTURION D, DE VRIES P & SAXENA PR (1997). Characterization of putative 5-HT7 receptors mediating tachycardia in the cat. *Br J Pharmacol* **121**, 1187-1195.

VILLALON CM, SANCHEZ-LOPEZ A, CENTURION D & SAXENA PR (2001). Unravelling the pharmacological profile of the canine external carotid vasodilator '5-HT1-like' receptors: coexistence of sympatho-inhibitory 5-HT1B and postjunctional 5-HT7 receptors. *Naunyn Schmiedebergs Arch Pharmacol* **363**, 73-80.

VINCENT SR & KIMURA H (1992). Histochemical mapping of nitric oxide synthase in the rat brain. *Neuroscience* **46**, 755-784.

VOIGT MM, LAURIE DJ, SEEBURG PH & BACH A (1991). Molecular cloning and characterization of a rat brain cDNA encoding a 5-hydroxytryptamine1B receptor. *EMBO J* **10**, 4017-4023.

WAEBER C, DIXON K, HOYER D & PALACIOS JM (1988). Localisation by autoradiography of neuronal 5-HT3 receptors in the mouse CNS. *Eur J Pharmacol* **151**, 351-352.

WAEBER C, HOYER D & PALACIOS JM (1989). 5-hydroxytryptamine3 receptors in the human brain: autoradiographic visualization using [3H]ICS 205-930. *Neuroscience* **31**, 393-400.

WALLACE JA, PETRUSZ P & LAUDER JM (1982). Serotonin immunocytochemistry in the adult and developing rat brain: methodological and pharmacological considerations. *Brain Res Bull* **9**, 117-129.

WANG Q & LI P (1988). Stimulation of the ventrolateral medulla inhibits the baroreceptor input to the nucleus tractus solitarius. *Brain Res* **473**, 227-235.

WANG Y, JONES JF, JEGGO RD, DE BURGH DM, JORDAN D & RAMAGE AG (2000a). Effect of pulmonary C-fibre afferent stimulation on cardiac vagal neurones in the nucleus ambiguus in anaesthetized cats. *J Physiol* **526 Pt 1**, 157-165.

WANG Y, JONES JF, RAMAGE AG & JORDAN D (1995). Effects of 5-HT and 5-HT1A receptor agonists and antagonists on dorsal vagal preganglionic neurones in anaesthetized rats: an ionophoretic study. *Br J Pharmacol* **116**, 2291-2297.

WANG Y & JORDAN D (1998). Involvement of nitric oxide (NO) in the excitatory vagal afferent input to nucleus tractus solitarii (NTS) neurones in anaesthetized rats. *J Physiol* **511**, 112P.

WANG Y & RAMAGE AG (2001). The role of central 5-HT(1A) receptors in the control of B-fibre cardiac and bronchoconstrictor vagal preganglionic neurones in anaesthetized cats. *J Physiol* **536**, 753-767.

WANG Y, RAMAGE AG & JORDAN D (1996). Mediation by 5-HT3 receptors of an excitatory effect of 5-HT on dorsal vagal preganglionic neurones in anaesthetized rats: an ionophoretic study. *Br J Pharmacol* **118**, 1697-1704.

WANG Y, RAMAGE AG & JORDAN D (1997). In vivo effects of 5-hydroxytryptamine receptor activation on rat nucleus tractus solitarius neurones excited by vagal C-fibre afferents. *Neuropharmacology* **36**, 489-498.

WANG Y, RAMAGE AG & JORDAN D (1998). Presynaptic 5-HT3 receptors evoke an excitatory response in dorsal vagal preganglionic neurones in anaesthetized rats. *J Physiol* **509**, 683-694.

WANG ZY, KEITH IM, BECKMAN MJ, BROWNFIELD MS, VIDRUK EH & BISGARD GE (2000b). 5-HT5a receptors in the carotid body chemoreception pathway of rat. *Neurosci Lett* **278**, 9-12.

WARDLE KA, ELLIS ES, BAXTER GS, KENNETT GA, GASTER LM & SANGER GJ (1994). The effects of SB 204070, a highly potent and selective 5-HT4 receptor antagonist, on guinea-pig distal colon. *Br J Pharmacol* **112**, 789-794.

WATKINS JC, KROGSGAARD-LARSEN P & HONORE T (1990). Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. *Trends Pharmacol Sci* **11**, 25-33.

WEINSHANK RL, ZGOMBICK JM, MACCHI MJ, BRANCHEK TA & HARTIG PR (1992). Human serotonin 1D receptor is encoded by a subfamily of two distinct genes: 5-HT1D alpha and 5-HT1D beta. *Proc Natl Acad Sci U S A* **89**, 3630-3634.

WICHEMS CH, HOLLINGSWORTH CK & BENNETT BA (1995). Release of serotonin induced by 3,4-methylenedioxymethamphetamine (MDMA) and other substituted amphetamines in cultured fetal raphe neurons: further evidence for calcium-independent mechanisms of release. *Brain Res* **695**, 10-18.

WIKLUND L, LEGER L & PERSSON M (1981). Monoamine cell distribution in the cat brain stem. A fluorescence histochemical study with quantification of indolaminergic and locus coeruleus cell groups. *J Comp Neurol* **203**, 613-647.

WILKINSON LO, MIDDLEMISS DN & HUTSON PH (1994). 5-HT1A receptor activation increases hippocampal acetylcholine efflux and motor activity in the guinea pig: agonist efficacy influences functional activity in vivo. *J Pharmacol Exp Ther* **270**, 656-664.

WILSON CG, ZHANG Z & BONHAM AC (1996). Non-NMDA receptors transmit cardiopulmonary C fibre input in nucleus tractus solitarii in rats. *J Physiol* **496**, 773-785.

WING LM & CHALMERS JP (1974). Effects of p-chlorophenylalanine on blood pressure and heart rate in normal rabbits and rabbits with neurogenic hypertension. *Clin Exp Pharmacol Physiol* **1**, 219-229.

WOOD M, CHAUBEY M, ATKINSON PJ & THOMAS DR (2000). Antagonist activity of meta-chlorophenylpiperazine and partial agonist activity of 8-OH-DPAT at the 5-HT7 receptor. *Eur J Pharmacol* **396**, 1-8.

WOOLLEY DC, MCWILLIAM PN, FORD TW & CLARKE RW (1987). The effect of selective electrical stimulation of non-myelinated vagal fibres on heart rate in the rabbit. *J Auton Nerv Syst* **21**, 215-221.

WOUTERS W, TULP MT & BEVAN P (1988). Flesinoxan lowers blood pressure and heart rate in cats via 5-HT1A receptors. *Eur J Pharmacol* **149**, 213-223.

WRIGHT DE, SEROOGY KB, LUNDGREN KH, DAVIS BM & JENNES L (1995). Comparative localization of serotonin1A, 1C, and 2 receptor subtype mRNAs in rat brain. *J Comp Neurol* **351**, 357-373.

WYSOWSKI DK, CORKEN A, GALLO-TORRES H, TALARICO L & RODRIGUEZ EM (2001). Postmarketing reports of QT prolongation and ventricular arrhythmia in association with cisapride and Food and Drug Administration regulatory actions. *Am J Gastroenterol* **96**, 1698-1703.

YAMAZAKI T & NINOMIYA I (1993). Noradrenaline contributes to modulation of the carotid sinus baroreflex in the nucleus tractus solitarii area in the rabbit. *Acta Physiol Scand* **149**, 1-6.

YATES WR & WALLACE R (1987). Cardiovascular risk factors in affective disorder. *J Affect Disord* **12**, 129-134.

YOCCA FD, IBEN L & MELLER E (1992). Lack of apparent receptor reserve at postsynaptic 5-hydroxytryptamine1A receptors negatively coupled to adenylyl cyclase activity in rat hippocampal membranes. *Mol Pharmacol* **41**, 1066-1072.

YU L, NGUYEN H, LE H, BLOEM LJ, KOZAK CA, HOFFMAN BJ *et al.* (1991). The mouse 5-HT1C receptor contains eight hydrophobic domains and is X-linked. *Brain Res Mol Brain Res* **11**, 143-149.

YU Y, RAMAGE AG & KOSS MC (2004). Pharmacological studies of 8-OH-DPAT-induced pupillary dilation in anesthetized rats. *Eur J Pharmacol* **489**, 207-213.

ZANDBERG P, DE JONG W & DE WIED D (1979). Effect of catecholamine-receptor stimulating agents on blood pressure after local application in the nucleus tractus solitarii of the medulla oblongata. *Eur J Pharmacol* **55**, 43-56.

ZGOMBICK JM, SCHECHTER LE, MACCHI M, HARTIG PR, BRANCHEK TA & WEINSHANK RL (1992). Human gene S31 encodes the pharmacologically defined serotonin 5-hydroxytryptamine1E receptor. *Mol Pharmacol* **42**, 180-185.

ZGOMBICK JM, WEINSHANK RL, MACCHI M, SCHECHTER LE, BRANCHEK TA & HARTIG PR (1991). Expression and pharmacological characterization of a canine 5-hydroxytryptamine1D receptor subtype. *Mol Pharmacol* **40**, 1036-1042.

ZHANG J & MIFFLIN SW (1997). Influences of excitatory amino acid receptor agonists on nucleus of the solitary tract neurons receiving aortic depressor nerve inputs. *J Pharmacol Exp Ther* **282**, 639-647.

ZHANG J & MIFFLIN SW (1998). Differential roles for NMDA and non-NMDA receptor subtypes in baroreceptor afferent integration in the nucleus of the solitary tract of the rat. *J Physiol* **511**, 733-745.

ZHANG W & MIFFLIN SW (1993). Excitatory amino acid receptors within NTS mediate arterial chemoreceptor reflexes in rats. *Am J Physiol* **265**, H770-H773.

ZHOU SY & GILBEY MP (1995). Sympathoexcitatory influence of a fast conducting raphe-spinal pathway in the rat. *Am J Physiol* **268**, R1230-R1235.

ZHU XO & MCNAUGHTON N (1994). The interaction of serotonin depletion with anxiolytics and antidepressants on reticular-elicited hippocampal RSA. *Neuropharmacology* **33**, 1597-1605.

9. APPENDIX

9.1 Chapter 3 data

9.1.1 Baselines

Mean (± s.e.m.) baseline MAP, R-R interval, integrated renal (IRNA) and integrated phrenic (IPNA) nerve activity before and after 10 µl i.c. test solution in anaesthetised, neuromuscular blocked and mechanically ventilated rats. † $P < 0.05$, ‡ $P < 0.01$ (compared to control), * $P < 0.05$, ** $P < 0.01$ (compared to i.c. saline). Numbers in parentheses show differences relative to the appropriate saline control.

Table 9.1 Saline (pH 5.8; $n = 5$)

time after injection (min)	baseline MAP (mmHg)	baseline R-R int (ms)	baseline IRNA (%)	baseline IPNA (%)
Control	112 ± 3	180 ± 7	100 ± 0	100 ± 0
5	116 ± 5	175 ± 7	96 ± 15	116 ± 13
15	118 ± 5	173 ± 7†	99 ± 18	121 ± 10
25	119 ± 5	171 ± 6†	95 ± 16	141 ± 19
35	120 ± 4	167 ± 5†	100 ± 15	140 ± 16

Table 9.2 SB-269970 (30 µg kg^{-1} i.c.; $n = 5$)

time after injection (min)	baseline MAP (mmHg)	baseline R-R int (ms)	baseline IRNA (%)	baseline IPNA (%)
Control	113 ± 10 **(+2 ± 12)**	177 ± 4 **(-2 ± 9)**	100 ± 0	100 ± 0
5	110 ± 11 **(-6 ± 16)**	175 ± 5 **(0 ± 11)**	99 ± 2 **(+3 ± 15)**	131 ± 18 **(+3 ± 19)**
15	114 ± 10 **(-4 ± 14)**	173 ± 5 **(+1 ± 10)**	103 ± 4 **(+4 ± 21)**	123 ± 21 **(-26 ± 20)**
25	115 ± 9 **(-4 ± 14)**	167 ± 6 **(-3 ± 11)**	106 ± 3 **(+11 ± 19)**	144 ± 34 **(-32 ± 16)**
35	115 ± 10 **(-5 ± 14)**	165 ± 6 **(-2 ± 9)**	107 ± 5 **(+7 ± 19)**	161 ± 18 **(+28 ± 29)**

Table 9.3 SB-269970 (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	baseline MAP (mmHg)	baseline R-R int (ms)	baseline IRNA (%)	baseline IPNA (%)
Control	120 ± 6 **(+8 ± 9)**	182 ± 3 **(+2 ± 8)**	100 ± 0	100 ± 0
5	119 ± 10 **(+3 ± 14)**	179 ± 3 **(+4 ± 8)**	102 ± 4 **(+5 ± 17)**	143 ± 18 **(+15 ± 24)**
15	126 ± 6 **(+10 ± 11)**	175 ± 3 **(+3 ± 8)**	103 ± 5 **(+5 ± 20)**	88 ± 18* **(-61 ± 24*)**
25	128 ± 7 **(+10 ± 10)**	175 ± 5 **(+4 ± 10)**	100 ± 5 **(+5 ± 17)**	101 ± 56* **(-75 ± 42*)**
35	133 ± 6 **(+13 ± 9)**	173 ± 5 **(+5 ± 9)**	101 ± 6 **(+1 ± 14)**	119 ± 19 **(-14 ± 31)**

Table 9.4 SB-269970 (300 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	baseline MAP (mmHg)	baseline R-R int (ms)	baseline IRNA (%)	baseline IPNA (%)
Control	111 ± 9 **(-1 ± 7)**	187 ± 5 **(+8 ± 11)**	100 ± 0	100 ± 0
5	116 ± 14 **(0 ± 10)**	183 ± 3 **(+8 ± 7)**	104 ± 4 **(+8 ± 18)**	56 ± 29* **(-72 ± 37*)**
15	131 ± 16 **(+14 ± 17)**	175 ± 2 **(+2 ± 7)**	115 ± 7 **(+16 ± 23)**	24 ± 12** **(-124 ± 18**)**
25	126 ± 11 **(+7 ± 7)**	173 ± 4 **(+2 ± 9)**	119 ± 8 **(+24 ± 17)**	42 ± 18** **(-133 ± 35**)**
35	138 ± 11 **(+19 ± 9)**	169 ± 3 **(+1 ± 7)**	119 ± 8 **(+19 ± 15)**	60 ± 23* **(-74 ± 33*)**

Table 9.5 SB-269970 (100 μg kg^{-1} i.v.; $n = 3$)

(Compared to *i.c.* control)

time after injection (min)	baseline MAP (mmHg)	baseline R-R int (ms)	baseline IRNA (%)
Control	107 ± 3 **(-6 ± 9)**	180 ± 7 **(+9 ± 4)**	100 ± 0
5	111 ± 6 **(-4 ± 12)**	179 ± 5 **(+10 ± 4)**	113 ± 4 **(+7 ± 9)**

Table 9.6 Vehicle for SB-656104 (10 μl i.c.; $n = 5$)

time after injection (min)	baseline MAP (mmHg)	baseline R-R int (ms)	baseline IRNA (%)	baseline IPNA (%)
Control	107 ± 2	190 ± 4	100 ± 0	100 ± 0
5	117 ± 1†	186 ± 5	100 ± 10	88 ± 10
15	118 ± 2†	184 ± 5	129 ± 29	114 ± 23
25	121 ± 1†	183 ± 5	116 ± 19	148 ± 41

Table 9.7 SB-656104 (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	baseline MAP (mmHg)	baseline R-R int (ms)	baseline IRNA (%)	baseline IPNA (%)
Control	105 ± 6 **(-1 ± 7)**	187 ± 4 **(+4 ± 3)**	100 ± 0	100 ± 0
5	115 ± 9 **(-2 ± 8)**	185 ± 4 **(-1 ± 3)**	145 ± 40 **(+46 ± 37)**	77 ± 8 **(-11 ± 6)**
15	128 ± 8 **(+10 ± 8)**	180 ± 4 **(-4 ± 5)**	139 ± 49 **(+10 ± 63)**	144 ± 38 **(+30 ± 19)**
25	137 ± 6 **(+13 ± 6)**	177 ± 3 **(-6 ± 5)**	164 ± 55 **(+48 ± 62)**	153 ± 21 **(+5 ± 17)**

Table 9.8 WAY-100635 (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	baseline MAP (mmHg)	baseline R-R int (ms)	baseline IRNA (%)	baseline IPNA (%)
Control	115 ± 3 **(+3 ± 5)**	170 ± 2 **(-9 ± 8)**	100 ± 0	100 ± 0
5	118 ± 4 **(+2 ± 7)**	170 ± 2 **(-5 ± 7)**	95 ± 4 **(-1 ± 15)**	93 ± 26 **(-35 ± 30)**
15	118 ± 3 **(+2 ± 5)**	165 ± 2 **(-7 ± 7)**	107 ± 5 **(+9 ± 15)**	87 ± 29 **(-62 ± 14)**
25	119 ± 3 **(+1 ± 6)**	164 ± 3 **(-7 ± 7)**	116 ± 5 **(+21 ± 16)**	103 ± 30 **(-73 ± 21)**

Table 9.9 Robalzotan (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	baseline MAP (mmHg)	baseline R-R int (ms)	baseline IRNA (%)	baseline IPNA (%)
Control	100 ± 4 **(-12 ± 4)**	177 ± 6 **(-2 ± 7)**	100 ± 0	100 ± 0
5	100 ± 7 **(-16 ± 8)**	177 ± 7 **(+3 ± 7)**	101 ± 2 **(+6 ± 13)**	86 ± 9 **(-27 ± 14)**
15	102 ± 6 **(-15 ± 7)**	176 ± 6 **(+3 ± 6)**	110 ± 7 **(+11 ± 12)**	96 ± 16 **(-40 ± 29)**
25	102 ± 6 **(-16 ± 9)**	174 ± 5 **(+3 ± 5)**	115 ± 9 **(+21 ± 10)**	93 ± 13 **(-33 ± 17)**

Table 9.10 (-)-Pindolol (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	baseline MAP (mmHg)	baseline R-R int (ms)	baseline IRNA (%)
Control	103 ± 2	170 ± 3	100 ± 0
	(-8 ± 2)	**(-9 ± 9)**	
5	110 ± 9	157 ± 4*	83 ± 15
	(-6 ± 8)	**(-18 ± 10*)**	**(-13 ± 15)**
15	111 ± 9	149 ± 4**	123 ± 47
	(-7 ± 8)	**(-23 ± 10**)**	**(+25 ± 37)**
25	117 ± 5	145 ± 4**	159 ± 63
	(-2 ± 6)	**(-26 ± 9**)**	**(+64 ± 61)**

Table 9.11 Cinanserin (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	baseline MAP (mmHg)	baseline R-R int (ms)	baseline IRNA (%)	baseline IPNA (%)
Control	113 ± 3	192 ± 3	100 ± 0	100 ± 0
	(+2 ± 5)	**(+13 ± 5)**		
5	120 ± 5	194 ± 4*	99 ± 2	141 ± 19
	(+4 ± 7)	**(+19 ± 3*)**	**(+3 ± 14)**	**(+13 ± 24)**
15	119 ± 3	186 ± 5	109 ± 3	150 ± 27
	(+2 ± 7)	**(+14 ± 2)**	**(+10 ± 19)**	**(+1 ± 37)**
25	119 ± 3	181 ± 4	131 ± 18*	150 ± 27
	(0 ± 7)	**(+10 ± 2)**	**(+36 ± 20*)**	**(-26 ± 49)**

Table 9.12 SB-204070 (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	baseline MAP (mmHg)	baseline R-R int (ms)	baseline IRNA (%)	baseline IPNA (%)
Control	113 ± 3	187 ± 5	100 ± 0	100 ± 0
	(+2 ± 5)	**(+8 ± 7)**		
5	114 ± 4	181 ± 8	112 ± 6	211 ± 14**
	(-2 ± 6)	**(+7 ± 10)**	**(+15 ± 14)**	**(+83 ± 23**)**
15	109 ± 4	182 ± 8	130 ± 14	168 ± 27
	(-9 ± 6)	**(+9 ± 11)**	**(+31 ± 14)**	**(+19 ± 35)**
25	112 ± 3	180 ± 7	126 ± 9	203 ± 18
	(-7 ± 6)	**(+10 ± 9)**	**(+31 ± 16)**	**(+28 ± 49)**

9.1.2 Cardiopulmonary reflex

Mean (± s.e.m.) effects of 10 µl saline i.c. on changes (Δ) in MAP, R-R interval, and integrated renal nerve activity (IRNA) in response to cardiopulmonary afferent stimulation with phenylbiguanide in anaesthetised, neuromuscular blocked and atenolol pretreated rats. † $P < 0.05$ (compared to control), * $P < 0.05$, ** $P < 0.01$ (compared to i.c. saline). Numbers in parentheses show differences relative to the appropriate saline control.

Table 9.13 Saline (10 µl pH 5.8 i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)
Control	-33 ± 5	41 ± 4	100 ± 0
5	-34 ± 5	40 ± 4	122 ± 7
15	-34 ± 6	39 ± 3	119 ± 8
25	-35 ± 6	39 ± 3	131 ± 11 †
35	-37 ± 6	37 ± 3	134 ± 9 †

Table 9.14 SB-269970 (30 µg kg^{-1} i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)
Control	-29 ± 3 **(+3 ± 3)**	43 ± 7 **(+1 ± 11)**	100 ± 0
5	-25 ± 2 **(+9 ± 6)**	27 ± 3* **(-13 ± 3*)**	83 ± 4** **(-39 ± 10**)**
15	-24 ±3 **(+10 ± 6)**	24 ± 3* **(-15 ± 5*)**	84 ± 9* **(-35 ± 14*)**
25	-27 ± 4 **(+9 ± 4)**	24 ± 6* **(-15 ± 8)**	106 ± 17 **(-25 ± 18)**
35	-34 ± 5 **(+2 ± 2)**	35 ± 5 **(-2 ± 8)**	85 ± 15** **(-49 ± 14**)**

Table 9.15 SB-269970 (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)
Control	-35 ± 3 **(-2 ± 5)**	41 ± 7 **(-1 ± 8)**	100 ± 0
5	-30 ± 5 **(+4 ± 8)**	23 ± 7* **(-17 ± 7*)**	94 ± 14 **(-27 ± 12)**
15	-26 ± 4 **(+9 ± 7)**	12 ± 3** **(-27 ± 2**)**	91 ± 18 **(-28 ± 19)**
25	-31 ± 4 **(+5 ± 7)**	17 ± 4** **(-22 ± 4**)**	90 ± 27* **(-40 ± 19*)**
35	-38 ± 5 **(-1 ± 7)**	23 ± 4* **(-14 ± 4)**	76 ± 22** **(-58 ± 21**)**

Table 9.16 SB-269970 (300 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)
Control	-28 ± 3 **(+5 ± 5)**	52 ± 11 **(+10 ± 12)**	100 ± 0
5	-6 ± 2** **(+30 ± 7**)**	7 ±2** **(-33 ± 4**)**	31 ± 16** **(-91 ± 15**)**
15	-1 ± 2** **(+36 ± 7**)**	4 ±2** **(-35 ± 4**)**	5 ± 2** **(-114 ± 7**)**
25	-3 ± 5** **(+35 ± 9**)**	5 ± 3** **(-34 ± 5**)**	18 ± 7** **(-112 ± 17**)**
35	-13 ± 3** **(+24 ± 5**)**	8 ± 3** **(-29 ± 5**)**	69 ± 18** **(-65 ± 23**)**

Table 9.17 SB-269970 (100 μg kg^{-1} i.v..; $n = 3$)

(differences relative to *i.c.* control)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)
Control	-32 ± 4 **(+3 ± 6)**	48 ± 14 **(+10 ± 8)**	100 ± 0
5	-37 ± 2 **(-2 ± 9)**	43 ± 13 **(+9 ± 10)**	115 ± 5 **(-3 ± 8)**

Table 9.18 Vehicle for SB-656104 (10 μl i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)
Control	-31 ± 7	61 ± 9	100 ± 0
5	-35 ± 12	55 ± 8	83 ± 16
15	-37 ± 12	52 ± 8	94 ± 25
25	-34 ± 12	50 ± 5	109 ± 23

Table 9.19 SB-656104 (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)
Control	-46 ± 8 **(-14 ± 8)**	56 ± 8 **(-5 ± 11)**	100 ± 0
5	-15 ± 5* **(+20 ± 11*)**	9 ± 5** **(-46 ± 8**)**	70 ± 15 **(-13 ±16)**
15	-18 ± 5* **(+20 ± 11*)**	9 ± 5** **(-42 ± 10**)**	64 ± 18 **(-30 ± 25)**
25	-19 ± 4 **(+14 ± 12)**	9 ± 6** **(-41 ± 7**)**	78 ± 14 **(-31 ± 23)**

Table 9.20 WAY-100635 (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)
Control	-36 ± 2 **(-3 ± 5)**	48 ± 7 **(+7 ± 8)**	100 ± 0
5	-22 ± 3 **(+11 ± 4)**	20 ± 3** **(-20 ± 6**)**	87 ± 12 **(-35 ± 13)**
15	-30 ± 5 **(+5 ± 7)**	30 ± 4 **(-9 ± 5)**	125 ± 28 **(+6 ± 32)**
25	-40 ± 4 **(-4 ± 8)**	43 ± 4 **(+4 ± 4)**	123 ± 10 **(-8 ± 18)**

Table 9.21 Robalzotan (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)
Control	-28 ± 4 **(+5 ± 4)**	57 ± 14 **(+15 ± 16)**	100 ± 0
5	-29 ± 5 **(+5 ± 6)**	47 ± 8 **(+7 ± 11)**	99 ± 5 **(-23 ± 8)**
15	-30 ± 4 **(+4 ± 6)**	61 ± 14 **(+22 ± 13)**	111 ± 6 **(-7 ± 7)**
25	-31 ± 5 **(+5 ± 4)**	60 ± 12 **(+21 ± 11)**	114 ± 11 **(-16 ± 16)**

Table 9.22 Cinanserin (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)
Control	-34 ± 1 **(-1 ± 5)**	51 ± 5 **(+9 ± 4)**	100 ± 0
5	-28 ± 2 **(+5 ± 6)**	45 ± 5 **(+5 ± 4)**	90 ± 7* **(-32 ± 14*)**
15	-34 ± 2 **(0 ± 7)**	50 ± 8 **(+11 ± 7)**	128 ± 8 **(+9 ± 8)**
25	-36 ± 1 **(-1 ± 6)**	56 ± 8* **(+17 ± 8*)**	131 ± 14 **(0 ± 11)**

Table 9.23 SB-204070 (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)
Control	-39 ± 3 **(-6 ± 8)**	62 ± 12 **(+21 ± 12)**	100 ± 0
5	-32 ± 5 **(+2 ± 9)**	42 ± 10 **(+2 ± 11)**	101 ± 17 **(-21 ± 23)**
15	-39 ± 4 **(-5 ± 9)**	54 ± 13 **(+15 ± 14)**	126 ± 8 **(+7 ± 13)**
25	-42 ± 3 **(-6 ± 7)**	56 ± 11 **(+17 ± 12)**	130 ± 10 **(-1 ± 9)**

9.1.3 Chemoreflex

Mean (± s.e.m.) effects of test solutions (10 μl i.c.) on changes (Δ) in MAP, R-R interval,, integrated renal nerve activity (IRNA) and integrated phrenic nerve activity (IPNA) in response to chemoreceptor stimulation with sodium cyanide in anaesthetised, neuromuscular blocked and atenolol pretreated rats. † $P < 0.05$, ‡ $P < 0.01$ (compared to control), * $P < 0.05$, ** $P < 0.01$ (compared to i.c. saline). Numbers in parentheses show differences relative to the appropriate saline control.

Table 9.24 10 μl saline (pH 5.8 i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)	reflex ΔIPNA (%)
Control	-30 ± 2	22 ± 2	100 ± 0	100 ± 0
10	-25 ± 4	29 ± 3†	159 ± 21	92 ± 30
20	-23 ± 3	29 ± 4†	113 ± 31	96 ± 10
30	-26 ± 5	27 ± 2	152 ± 43	84 ± 18

Table 9.25 SB-269970 (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)	reflex ΔIPNA (%)
Control	-31 ± 5 **(-1 ± 7)**	30 ± 4 **(8 ± 5)**	100 ± 0	100 ± 0
10	-32 ± 7 **(-7 ± 8)**	11 ± 2** **(-19 ± 5**)**	42 ± 6** **(-118 ± 16**)**	103 ± 21 **(+10 ± 41)**
20	-33 ± 6 **(-10 ± 7)**	11 ± 3** **(-18 ± 3**)**	54 ± 10 **(-59 ± 29)**	93 ± 15 **(-4 ± 15)**
30	-26 ± 3 **(0 ± 3)**	20 ± 7 **(-7 ± 6)**	90 ± 13 **(-63 ± 40)**	84 ± 18 **(0 ± 20)**

Table 9.26 WAY-100635 (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)	reflex ΔIPNA (%)
Control	-34 ± 4 **(-4 ± 4)**	33 ± 4 **(+11 ± 2)**	100 ± 0	100 ± 0
10	-34 ± 5 **(-7 ± 8)**	36 ± 5 **(+6 ± 8)**	93 ± 9* **(-55 ± 26*)**	141 ± 47 **(+67 ± 27)**
20	-32 ± 5 **(-9 ± 6)**	34 ± 5 **(+3 ± 7)**	127 ± 12 **(+17 ± 35)**	167 ± 64 **(+96 ± 56)**
30	-31 ± 5 **(-3 ± 5)**	38 ± 7 **(+9 ± 3)**	137 ± 14 **(-42 ± 31)**	143 ± 49 **(+70 ± 33)**

Table 9.27 Saline: Protocol 3 (10 μl i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)	reflex ΔIPNA (%)
Control	-38 ± 4	37 ± 7	100 ± 0	100 ± 0
2	-41 ± 3	43 ± 10	193 ± 40	120 ± 16
5	-43 ± 4	40 ± 9	182 ± 60	113 ± 25

Table 9.28 WAY-100635: Protocol 3 (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)	reflex ΔIRNA (%)	reflex ΔIPNA (%)
Control	-41 ± 3 **(-3 ± 7)**	35 ± 8 **(-1 ± 5)**	100 ± 0	100 ± 0
2	-38 ± 2 **(+3 ± 5)**	18 ± 7* **(-24 ± 5*)**	101 ± 8* **(-92 ± 41*)**	126 ± 17 **(+4 ± 11)**
5	-41 ± 4 **(+2 ± 6)**	31 ± 7 **(-9 ± 5)**	133 ± 27 **(-49 ± 80)**	110 ± 15 **(-3 ± 18)**

9.1.4 Aortic nerve stimulation

Mean (± s.e.m.) effects of test solutions (10 µl i.c.) on changes (Δ) in MAP and R-R interval in response to electrical stimulation of the right aortic depressor nerve in anaesthetised, neuromuscular blocked and atenolol pretreated rats. † $P < 0.05$ (compared to control), * $P < 0.05$, ** $P < 0.01$ (compared to i.c. saline). Numbers in parentheses show differences relative to the appropriate saline control.

Table 9.29 10 µl saline i.c. (pH 5.8; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)
Control	-33 ± 3 **(-10 ± 4)**	50 ± 4 **(-3 ± 15)**
5	-23 ± 5 **(+2 ± 10)**	24 ± 6* **(-29 ± 18*)**
15	-20 ± 4 **(+7 ± 8)**	23 ± 7 **(-22 ± 13)**
25	-24 ± 4 **(+2 ± 8)**	24 ± 8 **(-14 ± 13)**

Table 9.30 SB-269970 (100 µg kg^{-1} i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)
Control	-33 ± 3 **(-10 ± 4)**	50 ± 4 **(-3 ± 15)**
5	-23 ± 5 **(+2 ± 10)**	24 ± 6* **(-29 ± 18*)**
15	-20 ± 4 **(+7 ± 8)**	23 ± 7 **(-22 ± 13)**
25	-24 ± 4 **(+2 ± 8)**	24 ± 8 **(-14 ± 13)**

Table 9.31 WAY-100635 (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)
Control	-30 ± 7 **(-8 ± 8)**	78 ± 21 **(+25 ± 11)**
5	-27 ± 8 **(-2 ± 8)**	68 ± 18 **(+15 ± 10)**
15	-31 ± 8 **(-4 ± 10)**	62 ± 13 **(+17 ± 10)**
25	-35 ± 6 **(-9 ± 9)**	76 ± 16 **(+38 ± 13)**

Table 9.32 WAY-100635 (100 followed by 200 μg kg^{-1}; $n = 6$)

Time after injection (min)		reflex Δ MAP (mmHg)	reflex Δ R-R interval (ms)
	Control	-35 ± 5	59 ± 7
100 μg kg^{-1}	**2**	-25 ± 6	42 ± 9
	5	-27 ± 5	45 ± 8
200 μg kg^{-1}	**2**	-16 ± 4 †	25 ± 8 †
	3	-17 ± 5 †	37 ± 11
	4	-20 ± 8 †	45 ± 12
	5	-26 ± 7	57 ± 15

Table 9. 33 (-)-Pindolol (100 μg kg^{-1} i.c.; n = 5)

time after injection (min)	reflex ΔMAP (mmHg)	reflex ΔR-R interval (ms)
Control	-22 ± 5 **(0 ± 4)**	50 ± 11 **(-2 ± 19)**
5	-18 ± 4 **(+6 ± 3)**	32 ± 7 **(-22 ± 18)**
15	-25 ± 7 **(+2 ± 4)**	44 ± 6 **(-1 ± 11)**
25	-37 ± 4 **(-10 ± 5)**	55 ± 6 **(+17 ± 7)**

9.1.5 Baroreflex (phenylephrine)

Mean (± s.e.m.) effects of i.c. saline on changes (Δ) in MAP, R-R interval, reflex gain, and reflex changes in integrated renal nerve activity (IRNA), in response to intravenous phenylephrine in anaesthetised, neuromuscular blocked and atenolol pretreated rats. † $P < 0.05$ (compared to control), * $P < 0.05$, ** $P < 0.01$ (compared to saline). Numbers in parentheses show differences relative to the appropriate saline control.

Table 9.34 10 μl saline i.c. (pH 5.8; $n = 5$)

time after injection (min)	Δ MAP (mmHg)	reflex ΔR-R interval (ms)	reflex gain (ms mmHg^{-1})	reflex ΔIRNA (%)
Control	77 ± 4	26 ± 4	0.25 ± 0.04	100 ± 0
5	73 ± 5	28 ± 3	0.34 ± 0.06†	114 ± 12
15	75 ± 6	27 ± 5	0.27 ± 0.05	130 ± 23
25	64 ± 4	21 ± 5	0.23 ± 0.04	135 ± 33

Table 9.35 SB-269970 (100 μg kg^{-1} i.c.; $n = 5$)

time after injection (min)	Δ MAP (mmHg)	reflex ΔR-R interval (ms)	reflex gain (ms mmHg^{-1})	reflex ΔIRNA (%)
Control	78 ±3 **(0 ± 5)**	19 ± 4 **(-4 ± 6)**	0.22 ± 0.04 **(-0.04 ± 0.07)**	100 ± 0
5	77 ± 6 **(+4 ± 6)**	10 ± 3** **(-18 ± 4**)**	0.15 ± 0.06** **(-0.2 ± 0.1**)**	96 ± 9 **(-17 ± 15)**
15	73 ± 7 **(-2 ± 10)**	9 ± 1** **(-17 ± 4**)**	0.1 ± 0.03* **(-0.17 ± 0.06*)**	87 ± 10 **(-42 ± 21)**
25	62 ± 5 **(-2 ± 7)**	13 ± 3 **(-9 ± 3)**	0.11 ± 0.04 **(-0.12 ± 0.05)**	87 ± 10 **(-48 ± 32)**

9.2 Chapter 4 data

9.2.1 Raphe stimulation

Mean (± s.e.m.) changes (Δ) evoked by microinjection of DL-homocysteic acid (DLH; 2.5 nmol) into raphe at various depths from the brain surface, and 1 mm lateral to the midline (lateral), in anaesthetised, neuromuscular blocked and atenolol pretreated rats. Rostral location is relative to the calamus scriptorius. * $P < 0.05$, ** P 0.01 (relative to baseline, Student's paired t-test). ROb: raphe obscurus, RPa: raphe pallidus, RMg: raphe magnus, Gi: gigantocellular reticular nucleus, pontam.: pontamine.

Table 9.36 1.5 mm rostral

Depth (mm)	Nucleus	*n*	Δ MAP (mmHg)	Δ HR (bpm)	Δ IRNA (%)	Δ IPNA (%)
2.0	ROb	6	4 ± 2	2 ± 2	-45 ± 8 **	116 ± 21 **
2.5	RPa	5	3 ± 3	3 ± 1	-40 ± 8 **	123 ± 46 *
lateral	Gi	3	-2 ± 8	0	-6 ± 6	20 ± 48
saline	RPa	3	0	0	0	0

Table 9.37 2.5 mm rostral

Depth (mm)	Nucleus	*n*	Δ MAP (mmHg)	Δ HR (bpm)	Δ IRNA (%)	Δ IPNA (%)
1.5	ROb	9	7 ± 4	1 ± 3	-35 ± 3**	38 ± 20
2.0	ROb	7	11 ± 1	7 ± 1	-29 ± 8**	15 ± 15
2.5	RPa	8	12 ± 1	8 ± 1	-20 ± 9*	41 ± 18
lateral	Gi	4	22 ± 3**	0 ± 7	36 ± 3**	–

Table 9.38 3.5 mm rostral

Depth (mm)	Nucleus	*n*	Δ MAP (mmHg)	Δ HR (bpm)	Δ IRNA (%)	Δ IPNA (%)
2.0	RMg	6	8 ± 3	-30 ± 7**	31 ± 6**	-53 ± 7**
2.5	RMg/Pa	7	9 ± 1	-42 ± 6**	46 ± 6**	-60 ± 6**
lateral	Gi	5	13 ± 1*	-8 ± 5	33 ± 10	-47 ± 8**
pontam.	RMg/Pa	7	0 ± 1	0 ± 1	-1 ± 4	-3 ± 3

9.2.2 Pharmacology

Table 9.39 5-HT receptor antagonists: effects on baselines

Mean (± s.e.m.) baseline MAP, HR, IRNA and IPNA before (control) and 5 min after intravenous injection of saline or various 5-HT receptor antagonists in anaesthetised, neuromuscular blocked and atenolol pretreated rats. * $P < 0.05$, ** P 0.01 (relative to saline, 2-way ANOVA followed by LSD test). † no statistical comparison.

DRUG	*n*	MAP (mmHg)		HR (bpm)		IRNA (%)		IPNA (%)	
		control	*5 min*	*control*	*5 min*	*control*	*5 min*	*control*	*5 min*
Saline 1 ml kg^{-1} i.v.	5	122 ± 5	124 ± 6	345 ± 4	352 ± 6	100 ± 0	137 ± 15	100 ± 0	154 ± 29
Methiothepin 1 mg kg^{-1} i.v.	5	128 ± 6	118 ± 8	348 ± 10	347 ± 9	100 ± 0	103 ± 19	100 ± 0	124 ± 43
Methiothepin 3 mg kg^{-1} i.v.	4	123 ± 2	120 ± 5	320 ± 10*	314 ± 9**	100 ± 0	93 ± 7	100 ± 0	229 ± 63
Granisetron † 0.3 mg kg^{-1} i.v.	2	119 ± 1	118 ± 4	341 ± 7	340 ± 12	100 ± 0	132 ± 9	100 ± 0	122 ± 39
SB-204070 † 3 mg kg^{-1} i.v.	2	126 ± 6	130 ± 2	351 ± 5	329 ± 9	100 ± 0	67 ± 34	100 ± 0	117 ± 23

Table 9.40 5-HT receptor antagonists: effects on raphe stimulation

Mean (± s.e.m.) changes (Δ) in MAP, HR, IRNA and IPNA evoked by microinjection of DLH (2.5 nmol) into raphe magnus/pallidus, both before (control) and 5 min after intravenous injection of saline or various 5-HT receptor antagonists in anaesthetised, neuromuscular blocked and atenolol pretreated rats. * $P < 0.05$, ** P 0.01 (relative to saline, 2-way ANOVA followed by LSD test). † no statistical comparison.

DRUG	*n*	Δ MAP (mmHg)		Δ HR (bpm)		Δ IRNA (%)		Δ IPNA (%)	
		control	*5 min*	*control*	*5 min*	*control*	*5 min*	*control*	*5 min*
Saline 1 ml kg^{-1}	5	5 ± 4	4 ± 4	-29 ± 5	-28 ± 5	101 ± 29	80 ± 29	-81 ± 5	-78 ± 5
Methiothepin 1 mg kg^{-1} i.v.	5	2 ± 5	-2 ± 3	-16 ± 1	-22 ± 3	120 ± 20	155 ± 55	-88 ± 6	-65 ± 7
Methiothepin 3 mg kg^{-1} i.v.	4	-9 ± 1	-4 ± 2	-25 ± 7	-18 ± 6	197 ± 50	355 ± 100**	-93 ± 4	-66 ± 17
Granisetron † 0.3 mg kg^{-1} i.v	2	-3 ± 1	1 ± 5	-25 ± 6	-20 ± 6	77 ± 37	37 ± 37	-67 ± 0	-59 ± 1
SB-204070 † 3 mg kg^{-1} i.v.	2	-5 ± 2	-7 ± 1	-46 ± 14	-39 ± 5	175 ± 19	275 ± 108	-72 ± 9	-70 ± 10

9.2.3. 5-HT depletion

Table 9.41 p-CPA: effect of raphe stimulation

Mean (± s.e.m.) changes (Δ) in MAP, HR, IRNA and IPNA in response to microinjection of DLH (2.5 nmol) into parts of the raphe at various points relative to the calamus scriptorius (rostral) and brain surface (depth) in anaesthetised, neuromuscular blocked and atenolol pretreated rats, treated with either saline or p-CPA. * $P < 0.05$, ** P 0.01 (relative to control, Student's paired t-test).

Rostral	**Depth**	***n***	**Δ MAP (mmHg)**		**Δ HR (bpm)**		**Δ IRNA (%)**		**Δ IPNA (%)**	
			saline	***p-CPA***	***saline***	***p-CPA***	***saline***	***p-CPA***	***saline***	***p-CPA***
1.5	1.5	5	0 ± 2	-10 ± 3*	10 ± 2	6 ± 1	-26 ± 21	-40 ± 7	220 ± 75	263 ± 43
	2.5	5	2 ± 2	1 ± 5	10 ± 2	9 ± 2	20 ± 37	-24 ± 12	227 ± 68	75 ± 19
2.5	1.5	5	11 ± 2	-3 ± 4**	7 ± 3	3 ± 1	-41 ± 7	-39 ± 8	120 ± 36	63 ± 30
	2.5	5	7 ± 3	18 ± 1**	8 ± 2	13 ± 2	-8 ± 11	-54 ± 5**	182 ± 31	123 ± 34
3.5	1.5	5	0 ± 2	-4 ± 6	-7 ± 2	-4 ± 3	-13 ± 12	-5 ± 8	-18 ± 23	-19 ± 12
	2.5	5	2 ± 4	-1 ± 1	-29 ± 6	-27 ± 8	113 ± 16	59 ± 36	-51 ± 13	-54 ± 8

Table 9.42 p-CPA: effect on baselines and reflexes

Mean (± s.e.m.) baseline MAP and HR, changes (Δ) in MAP and R-R interval, and baroreflex slope and correlation, evoked by cardiopulmonary reflex activation with intra-atrial phenylbiguanide (2.5 μg) or baroreflex activation with intravenous phenylephrine (10 μg), in anaesthetised, neuromuscular blocked and atenolol pretreated rats, treated with either saline or p-CPA.

* $P < 0.05$, ** $P < 0.01$ (relative to control, Student's paired t-test).

	n	**Saline**	**p-CPA**
Baseline MAP (mmHg)	7	107 ± 5	86 ± 7*
Baseline HR (bpm)	7	311 ± 5	321 ± 6
Cardiopulmonary reflex			
Δ R-R interval (ms)	7	130 ± 31	38 ± 7**
Δ MAP (mmHg)	7	-31 ± 6	-27 ± 4
Baroreflex (phenylephrine)			
Δ MAP (mmHg)	5	72 ± 6	77 ± 7
Δ R-R interval (ms)	5	82 ± 12	55 ± 12
Slope (ms $mmHg^{-1}$)	5	0.66 ± 0.07	0.19 ± 0.05**
$\Delta P/\Delta t$ (mmHg s^{-1})	5	28 ± 2	27 ± 3
Correlation (r)	5	0.82 ± 0.02	0.76 ± 0.08

9.3 Chapter 5 data

9.3.1 Iontophoresis

Table 9.43 Effects of agonists

Effects of AMPA (20 – 120 nA), NMDA (40 – 180 nA) and PBG (80 – 300 nA) on baseline firing rate (spikes s^{-1}) of vagally-evoked NTS neurones. ‡ $P < 0.01$ (Mann-Whitney Rank Sum test: before *cf.* after)

Drug	n	Before	During
AMPA	13	0.6 ± 0.3	8.2 ± 0.8 ‡
NMDA	13	0.5 ± 0.2	6.4 ± 0.9 ‡
PBG	7	2.7 ± 1.4	4.2 ± 1.6

Table 9.44 Effects of DNQX on AMPA & PBG

Effects of DNQX (10 – 80 nA) on baseline and agonist-evoked neuronal firing (spikes s^{-1}). Rec: recovery. ‡ $P < 0.01$ (Mann-Whitney Rank Sum test: agonist *cf.* agonist + DNQX)

Agonist	n	baseline	agonist	baseline + DNQX	agonist + DNQX	agonist (rec)	n (rec)
AMPA	13	0.3 ± 0.1	8.2 ± 0.8	0.3 ± 0.1	2.6 ± 0.7 ‡	6.4 ± 0.7	11
PBG	5	2.4 ± 1.9	3.5 ± 2.1	2.3 ± 1.6	2.2 ± 1.7	3.4 ± 2.1	4

Table 9.45 Effects of DNQX on NMDA

Different effects of DNQX (10 – 80 nA) on baseline and NMDA-evoked neuronal firing (spikes s^{-1}). ↓ > 20 % inhibition; ↑ > 20 % potentiation; NE: no change. Rec: recovery. Bottom row shows mean of whole group (Total).

Effect	n	baseline	NMDA	baseline + DNQX	NMDA + DNQX	NMDA (rec)	n (rec)
↓	6	0.7 ± 0.4	6.6 ± 0.9	0.4 ± 0.1	4.4 ± 1.2	9.3 ± 1.6	6
↑	3	0.3 ± 0.2	5.3 ± 1.6	0.5 ± 0.4	7 ± 2.2	4.9 ± 0.1	2
NE	4	0.3 ± 0.3	7 ± 2.7	0.3 ± 0.3	7.3 ± 2.9	11.2 ± 4.7	4
Total	13	0.5 ± 0.2	6.4 ± 0.9	0.4 ± 0.1	5.9 ± 1.1	9.2 ± 1.7	12

Table 9.46 Effects of DNQX on cardiopulmonary reflex

Effects of iontophoretically applied DNQX (30 – 80 nA) on cardiopulmonary afferent-evoked discharge of NTS neurones evoked by intra-atrial (i.a.) PBG. Rec: recovery.

	n	Reflex	Reflex + DNQX	Reflex (rec)	n (rec)
PBG i.a.	5	57 ± 25	33 ± 20	51 ± 24	4

Table 9.47 Effects of DNQX on vagus-evoked activity

Effects of iontophoretically applied DNQX (30 – 100 nA) on discharge of NTS neurones evoked by electrical stimulation of the vagus (number of spikes per 20 s). ‡ $P < 0.01$ (Mann Whitney Rank Sum test: Vagus *cf.* DNQX).

	n	Vagus	Vagus + DNQX	Vagus (rec)	n (rec)
Vagus 1 Hz	12	35.8 ± 7.5	15.3 ± 1.9‡	34 ± 9.8	11

9.3.2 Topical SB-269970

Mean (± s.e.m.) effects of topically applied saline (10 µl) and SB-269970 (100 µg kg^{-1}) on neuronal activity in anaesthetised and neuromuscular blocked rats. Time is relative to administration (data after 10 min not shown). * $P < 0.05$, ** $P < 0.01$ (compared to saline).

Table 9.48 Vagally-evoked NTS neuronal activity (evoked spikes 50 $sweeps^{-1}$; $n = 5$)

Time (min)	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Saline	51±1	49±1	48±2	49±2	47±2	48±1	46±22	48±2	47±1	47±2	47±3	47±2	46±2
SB-269970	48±1	48±1	48±1	46±2	36±9	35±9	32±9	33±9*	28±8**	24±8**	25±8**	25±9**	25±7**

Table 9.49 Baseline DVN neuronal activity (spikes s^{-1}; $n = 6$ (drug) and $n = 4$ (saline))

Time (min)	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Saline	4.3±0.4	4±0.4	4.3±0.7	3.6±0.6	3.7±0.9	3.9±0.8	4.4±1.2	4.4±1.6	4.3±1.5	4.5±1.5	3.8±0.9	3.7±0.8	3.7±0.8
SB-269970	3.9±0.7	3.9±0.7	4±0.9	3.1±0.8	3.6±0.7	3.5±0.7	3.2±0.8	3.6±0.8	3.8±0.9	4±0.8	3.6±0.8	3.6±0.8	3.6±1.1

Table 9.50 Touch-evoked Gracile neuronal activity (evoked spikes 10 stimuli^{-1}; n = 7)

Time (min)	-2	-1	0	1	2	3	4	5	6	7	8	9	10
Saline	318±99	295±85	316±102	334±108	320±101	333±109	300±103	298±98	302±109	327±109	332±116	341±115	306±98
SB-269970	237±48	236±44	224±43	203±37	184±42	166±38	164±46	179±50	176±48	187±50	171±50	174±46	161±43

Table 9.51 Touch-evoked Gracile neuronal activity (% of control; n = 7)

Time (min)	Control	1	2	3	4	5	6	7	8	9	10
Saline	100±0	120±14	113±15	114±14	110±18	105±13	105±13	117±18	109±10	126±8	115±9
SB-269970	100±0	89±6*	89±6	80±12*	78±13*	82±13	82±14	86±14*	77±14*	80±13**	72±11**

Table 9.52 Baseline gracile neuronal activity (spikes s^{-1}; n = 7 (drug) and n = 5 (saline))

Time (min)	-2	-1	0	1	2	3	4	5	6	7	8	9	10
Saline	3.4±1.7	3.8±1.7	4.7±1.8	5.5±2	5.4±1.8	5.6±2.2	5.8±2.5	5.4±2.5	4.5±1.9	4.5±1.9	4.9±2	3.6±2.2	4±2.2
SB-269970	3.6±1.4	3.2±1.1	3.6±1.2	2.7±0.8	2.6±0.5	3.7±1.3	2.9±1	2.1±0.5	2.7±0.9	2.8±0.9	2.6±0.7	2.3±0.8	2.7±1

Table 9.53 Baseline gracile neuronal activity (% of control; n = 7 (drug) and n = 5 (saline))

Time (min)	Control	1	2	3	4	5	6	7	8	9	10
Saline	100±0	150±35	153±16	151±37	174±46	132±16	135±29	139±31	134±21	132±26	155±33
SB-269970	100±0	99±17	126±39	140±32	128±35	102±35	118±44	131±48	110±36	103±42	134±51

9.4 Chapter 6 data

Mean (± s.e.m.) baseline or reflex-evoked changes (Δ) in MAP and HR before (control) and after 5 µl i.c. test solution in awake unrestrained rats.

$P < 0.05$, ** $P < 0.01$ (compared to saline).

Numbers in parentheses show differences relative to the appropriate saline control.

Table 9.54 5 µl i.c. saline ($n = 5$)

Effects on baselines

time after injection (min)	baseline MAP (mmHg)	baseline HR (bpm)
Control	97 ± 6	352 ± 18
5	101 ± 5	370 ± 20
10	102 ± 7	360 ± 27
15	100 ± 6	354 ± 19
20	99 ± 7	360 ± 23
25	100 ± 6	344 ± 19
30	101 ± 5	358 ± 17

Table 9.55 Saline (5 µl i.c.; $n = 5$)

Effects on cardiopulmonary reflex

time after injection (min)	reflex Δ MAP (mmHg)	reflex Δ HR (bpm)
Control	-56 ± 8	-265 ± 52
5	-64 ± 9	-296 ± 68
10	-59 ± 12	-288 ± 71
15	-57 ± 12	-268 ± 64
20	-60 ± 14	-278 ± 64
25	-57 ± 12	-260 ± 59
30	-60 ± 9	-266 ± 60

Table 9.56 SB-269970 (100 μg kg^{-1} i.c.; $n = 5$)

Effects on baselines

time after injection (min)	baseline MAP (mmHg)	baseline HR (bpm)
Control	112 ± 5	399 ± 27
	(+15 ± 5)	**(+47 ± 25)**
5	111 ± 8	386 ± 9
	(+10 ± 3)	**(+16 ± 26)**
10	112 ± 8	400 ± 26
	(+10 ± 5)	**(+40 ± 28)**
15	111 ± 8	394 ± 25
	(+11 ± 4)	**(+40 ± 26)**
20	107 ± 6	370 ± 15
	(+8 ± 7)	**(+10 ± 25)**
25	109 ± 4	378 ± 17
	(+9 ± 4)	**(+34 ± 21)**
30	106 ± 4	358 ± 15
	(+5 ± 2)	**(0 ± 21)**

Table 9.57 SB-269970 (100 μg kg^{-1} i.c.; $n = 5$)

Effects on cardiopulmonary reflex

time after injection (min)	reflex Δ MAP (mmHg)	reflex Δ HR (bpm)
Control	-56 ± 8 **(+1 ± 7)**	-290 ± 22 **(-25 ± 20)**
5	-22 ± 7** **(+42 ± 15**)**	-142 ± 54** **(+154 ± 61**)**
10	-33 ± 5* **(+26 ± 15*)**	-184 ± 37* **(+104 ± 46*)**
15	-39 ± 5 **(+18 ± 15)**	-226 ± 28 **(+42 ± 50)**
20	-39 ± 7 **(+21 ± 14)**	-238 ± 18 **(+40 ± 19)**
25	-46 ± 7 **(+11 ± 9)**	-238 ± 18 **(+22 ± 18)**
30	-55 ± 7 **(+5 ± 7)**	-272 ± 21 **(-6 ± 27)**

Table 9.58 SB-269970 (100 μg kg^{-1} i.c.; $n = 5$)

Effects on chemoreflex

time after injection (min)	reflex Δ MAP (mmHg)	reflex Δ HR (bpm)
Control	41 ± 4	-188 ± 23
5	9 ± 18	-88 ± 51
10	16 ± 26	126 ± 44
15	21 ± 23	-124 ± 45
20	41 ± 4	-193 ± 28
25	25 ± 15	-118 ± 52
30	23 ± 14	-124 ± 50

9.5 Baroreflex gain script

Spike2 script to measure beat-by-beat arterial pressure and R-R interval from a one channel of BP and one channel of discriminated R waves (from ECG). The script plots peak and trough (systolic and diastolic) arterial pressure pressure and corresponding heart rate, also time of each systolic and diastolic peak, and time of each R wave peak (from which R-R interval is calculated). Data are plotted to a Log file.

```
var v12%;
var v19%;
var v10%;
var v20%;
var v21%;
var tv%,sTime,eTime;
v12%:=ViewFind("*.SMR");
v10%:=ViewFind("Log1");
FrontView(v12%);
CursorSet(0);
CursorLabel(2);
CursorVisible(0,1);

CursorActive(0); ',14,trig%,0.4,"","",0,0,0);
 Interact("Select analysis start position, press
'control + 0' for cursor",128);
CursorSearch(0);
sTime := Cursor(0);
eTime := Cursor(1);
'CursorCheck();

v19%:=MeasureToXY(4,2,0,10,0,0);
MeasureX(102,1,"Cursor(0)","0",0);
MeasureY(100,2,"Cursor(0)","0",0);
MeasureChan(1,"Systolic",0);
WindowVisible(1);
Process(sTime,eTime,0,1);
EditCopy(4);
FrontView(v10%);
Window(0,0,65.25,40.5405);
EditPaste();
WindowVisible(2);
FrontView(v19%);
WindowVisible(2);
FrontView(v12%);
v20%:=MeasureToXY(5,2,0,10,0,0);
MeasureX(102,1,"Cursor(0)","0",0);
MeasureY(100,2,"Cursor(0)","0",0);
MeasureChan(1,"Diastolic",0);
WindowVisible(1);
Process(sTime,eTime,0,1);
EditCopy(4);
FrontView(v10%);
Window(0,0,65.25,40.5405);
EditPaste();
WindowVisible(2);
FrontView(v20%);
WindowVisible(2);
FrontView(v12%);
v21%:=MeasureToXY(14,10,0,10,0,0);
MeasureX(102,1,"Cursor(0)","0",0);
MeasureY(100,10,"Cursor(0)","0",0);
MeasureChan(1,"Heart rate",0);
WindowVisible(1);
Process(sTime,eTime,0,1);
EditCopy(4);
FrontView(v10%);
Window(0,0,65.25,40.5405);
EditPaste();
WindowVisible(2);
FrontView(v21%);
WindowVisible(2);
```