5-HT receptors and brainstem cardio-respiratory neurones

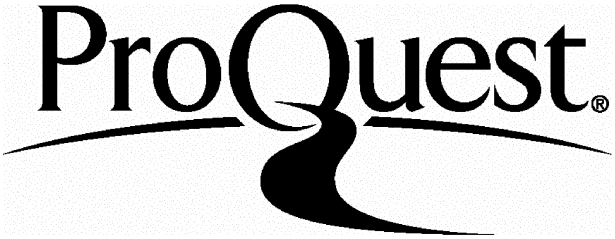
A thesis submitted for the degree of Doctor of Philosophy to the Faculty of Science at the University of London

by

Ross David Jeggo B.Sc. (Hons.)

March 2003

Department of Pharmacology
University College London, Royal Free Campus
Rowland Hill Street, Hampstead
London, NW3 2PF



ABSTRACT

Experiments were performed on anaesthetized rats and cats to examine the effects of cardiopulmonary afferent activation on neuronal activity in the nucleus ambiguus (NA) and the nucleus tractus solitarius (NTS). Furthermore, the role of 5-$HT_{1B/1D}$ and 5-HT_3 receptors in the NTS was studied in rats including examinations into their role on identified inputs, such as cardiopulmonary afferent activation. Anaesthetized animals were instrumented to allow recording of cardiovascular and respiratory variables, neurones were recorded using glass microelectrodes and the central administration of compounds was carried out using ionophoresis. Cardiopulmonary afferents were activated by right atrial injections of the 5-HT_3 receptor agonist phenylbiguanide (PBG), and NA and NTS neurones were identified by a combination of their response to vagus nerve stimulation and histological localisation.

The majority of B-fibre cardiac vagal preganglionic neurones (CVPNs) of the NA were excited by right atrial PBG administration and this excitation was maintained in the absence of respiratory drive. The majority of NTS neurones recorded responded to right atrial PBG, with the highest proportions of these neurones (70%) excited by this stimulus. The 5-$HT_{1B/1D}$ receptor agonist sumatriptan decreased the activity of the majority of NTS neurones recorded and also attenuated both the vagal-evoked and cardiopulmonary afferent-evoked activation of NTS neurones. In contrast, the 5-HT_{1B} receptor agonist CP-93,129 increased the activity of the majority of NTS neurones, and excited both vagal-evoked and cardiopulmonary afferent-evoked activation. The inhibitory action of sumatriptan on neuronal activity was attenuated in the majority of neurones in the presence of the 5-HT_{1D} receptor antagonist ketanserin, whilst it was potentiated in the majority of neurones in the presence of the 5-HT_{1B} receptor antagonist GR55562B. Ionophoretic PBG increased the activity of the majority of NTS neurones and potentiated the vagal-evoked and cardiopulmonary afferent-evoked activation of neurones. The 5-HT_3 receptor antagonists granisetron and ondansetron and the NMDA receptor antagonist AP-5 attenuated this PBG-evoked increase in activity in the majority of neurones. Granisetron and AP-5 also attenuated the cardiopulmonary afferent-evoked activation of the majority of NTS neurones.

These data suggest that the cardiopulmonary reflex-evoked bradycardia is mediated in part by B-fibre CVPNs in the NA. In addition, 5-HT_{1B} and 5-HT_3 receptors play an excitatory role, whilst 5-HT_{1D} receptors play an inhibitory role in the functioning of the NTS. Furthermore, the excitatory action of 5-HT_3 receptors is mediated in part via the release of glutamate, acting at NMDA receptors, and these 5-HT_3 receptors are tonically active during cardiopulmonary afferent transmission in the NTS.

ACKNOWLEDGEMENTS

I would like to thank my supervisors Dr Andy Ramage and Professor David Jordan for all their invaluable help, encouragement and constructive criticism throughout the duration of my study. I would also like to thank Dr Yun Wang for educating me in the ways of electrophysiological recording, and Steve Wilkinson for all his assistance in the lab.

In addition, I am grateful to the British Heart Foundation, whose financial support made this research possible.

Finally, I would like to take this opportunity to thank all my family and friends, for their considerable understanding and support throughout the last four years.

DEDICATION

I would like to dedicate this thesis to my three remaining grandparents and to the memory of my late grandfather.

PUBLICATIONS

JEGGO, R.D., WANG, Y., JORDAN, D. & RAMAGE, A.G. (2000). The effects of 5-$HT_{1B/1D/1F}$ 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, R.D., WANG, Y., JORDAN, D. & RAMAGE, A.G. (2000). The role of 5-HT_3 receptors in the cardiopulmonary afferent-evoked response of NTS neurones in the anaesthetized rat. *J. Physiol.*, **523,** 258P.

WANG, Y., JONES, J.F.X., JEGGO, R.D., DALY, M. DE B., JORDAN, D. & RAMAGE, A.G. (2000). Effect of pulmonary C-fibre afferent stimulation on cardiac vagal neurones in the nucleus ambiguus in anaesthetized cats. *J. Physiol.*, **526,** 157-165.

JEGGO, R.D., WANG, Y., JORDAN, D. & RAMAGE, A.G. (2001). Evidence to suggest that presynaptic 5-HT_3 receptors are involved in the excitation of NTS neurones in anaesthetized rats. *J. Physiol.*, **533,** 92P.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

A5	A5 noradrenergic group
ADN	aortic depressor nerve
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANGII	angiotensin II
AP-5	2-amino-5-phosphonovalerate
AT_1	angiotensin receptor
ATP	3',5'-adenosine triphosphate
AV3V	anteroventral third ventricular area
B_2	bradykinin receptor
BRL-15572	3-[4-(3-chlorophenyl) piperazin-1-yl]-1,1-diphenyl-2-propanol
BST	bed nucleus of the stria terminalis
cAMP	cyclic 3'5'-adenosine monophosphate
CCK	cholecystokinin
CEA	central nucleus of the amygdala
CP-93,129	3-(1,2,5,6-Tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one
CSN	carotid sinus nerve
5-CT	5-carboxyamidotryptamine maleate
CVLM	caudal ventrolateral medulla
CVPN	cardiac vagal preganglionic neurone
DOI	()-2-5-dimethoxy-4-iodoamphetamine HCl
DVN	dorsal vagal motor nucleus
DLH	d,l-homocysteic acid
eNOS	endothelial nitric oxide synthase
ECG	electrocardiogram
GABA	γ-aminobutyric acid
GR127935	2'-methyl-4'-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxyli c acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide
GR55562	3-[3-(N,N-dimethylamino)propyl]-4-hydroxy- N-[4-(pyridin-4-yl)phenyl]benzamide

5-HT	5-hydroxytryptamine
i.c.	intra-cisternal
i.c.v.	intra-cerebro-ventricular
ILC	infralimbic cortex
IML	intermediolateral cell column
INS	insular cortex
i.v.	intra-venously
LC	locus coeruleus
LH	tuberal hypothalamic cell groups
LHAp	posterior lateral hypothalamus
LY334370	5-(4-fluorobenzoyl)amino-3-(1-methylpiperidin-4-yl)-1H-indole fumarate
MAP	mean arterial blood pressure
MDL-100907	R(+)-alpha-(2,3-dimethoxyphenyl)-1- [2-(4-fluorophenylethyl)]-4-piperidine-methanol
MDL-72222	tropanyl 3,5-dichlorobenzoate
NA	nucleus ambiguus
NG	nodose ganglion
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NOS	nitric oxide synthase
NPY	neuropeptide Y
NTS	nucleus tractus solitarius
8-OH-DPAT	8-Hydroxy-2-(di-n-propylamino)tetralin
PAG	periaqueductal grey
PB	parabrachial nucleus
PBG	phenylbiguanide
PVN	paraventricular nucleus
RVLM	rostral ventrolateral medulla
SAR	slowly adapting pulmonary afferents
SB-224289	1'-methyl-5-[[2'-methyl-4'-(5-methyl-1,2, 4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl]-2,3,6,7-tetrahydro- spiro[furo[2,3-f]indole-3,4'-piperidine]

SB-204741	N-(1-methyl-1H-indo-5yl)-N'-(3-methyl-5-isothiazolyl)urea
SB-242084	6-chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]- indoline
SB-204070	(1-butyl-4-piperidinyl)methyl 8-amino-7-chloro-1,4-benzodioxane-5-carboxylate hydrochloride
SLN	superior laryngeal nerve
SNP	sodium nitroprusside
SSRIs	selective serotonin reuptake inhibitors
V_1	vasopressin receptor
VLM	ventrolateral medulla
VPpc	ventroposterior parvocellular nucleus of the thalamus
WAY-100135	N-tert-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide
WAY-100635	N-[2-]4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2- pyridinyl)cyclohexanecarboxamide

Chapter 1 General Introduction

1.1 Neurophysiology of the vagus nerve and cardiovascular system

1.1.1 Vagus nerve

The vagus nerve is the tenth cranial nerve. It is the longest nerve in the body and derives its name from the latin for wandering. As its name suggests the vagus wanders throughout the body and contains axons that carry out a huge number of functions. The origin of peripherally projecting axons of the vagus is the brainstem, and the nerve departs the brain via the jugular foramen. The targets for the vagus are diverse and range from the pharynx to the heart to the small intestine. It also carries sensory information from areas such as the heart, the lungs and abdominal viscera. The vagus has been targeted for research since it has been implicated in cardiovascular diseases including hypertension and heart failure, where vagal activity is diminished and unresponsive (Vanoli *et al.*, 1991).

The nerve has been shown, in the cat, to be composed of approximately thirty thousand fibres of which 80% are afferent and predominantly non-myelinated, although both afferent and efferent functions have been shown to be carried out by a combination of myelinated and unmyelinated fibres (see Daly, 1997). In addition, the nodose ganglion (NG) has been identified as a site through which primary vagal afferents pass (Agostoni *et al.*, 1957). In contrast, no efferent fibres of the vagus are thought to have their cell bodies located in the NG (see Daly, 1997).

One of the more important functions of the vagus is its role in the maintenance of a cardiovascular homeostasis. It contains both afferent and efferent fibres

involved in central cardiovascular reflexes and the termination of the afferent information is within, or in close proximity to, the nucleus of the tractus solitarii (NTS) near to the dorsal surface of the medulla oblongata (Kalia & Mesulam, 1980). This nucleus is a key component of these reflexes acting as both a relay and integrative centre. Vagal afferents arrive at the nucleus before being either transmitted to higher centres; including the hypothalamus, transmitted to other NTS neurones (local interneurones), or passed directly onto medullary motor nuclei; including the nucleus ambiguus (NA). Preganglionic neurones are then activated either directly or after central integration, carrying efferent information to peripherally located ganglia.

The motor fibres in the vagus nerve form part of the parasympathetic branch of the autonomic nervous system, the other being the sympathetic branch. The roles of these two branches and their co-ordination are important in achieving cardiovascular homeostasis. One example of the functional dichotomy of the two branches of the autonomic nervous system can be seen in their projections to the heart, which individually have opposing effects. Stimulation of the sympathetic innervation of the heart increases heart rate whereas stimulation of the parasympathetic component (the cardiac branch of the vagus) decreases heart rate. Both branches have also been shown to exert tonic influence on cardiac activity at rest, which is known to be in phase with respiration. Sympathetic nerve activity to the heart increases during phrenic nerve activity, or inspiration, whilst parasympathetic nerve activity increases between phrenic bursts, during expiration. This results in oscillations in heart rate known as respiratory sinus arrhythmia. Parasympathetic postganglionic neurones release acetylcholine, which is broken down rapidly by acetycholinesterase whereas sympathetic preganglionic neurones release noradrenaline, which is taken back up into the presynaptic terminal (a slower process). This results in a greater beat by beat effect of vagal, parasympathetic, activity on heart rate.

The integration of the parasympathetic and sympathetic components of the autonomic nervous system can also be demonstrated in the characteristics of a number of reflexes important in cardiovascular regulation; some of these are briefly described below.

1.1.2 Autonomic reflexes

In 1915 Bainbridge reported one such reflex, where infusions of blood or saline increased heart rate irrespective of blood pressure, known as the Bainbridge reflex. This effect is mediated by stretch receptors located in the pulmonary vein-atrial junction which are mechanically activated by increases in blood volume. The resultant increase in blood pressure subsequently activates arterial baroreceptors, which in turn has a negative effect on heart rate. This represents an example of two reflexes with opposing and therefore balancing effects on heart rat and blood pressure.

In the baroreceptor reflex the change in blood pressure is detected by stretch-activated receptors located within the aortic arch and carotid sinus. Baroreceptor afferents are carried within the aortic depressor nerve (ADN) and carotid sinus nerve (CSN) terminating in the NTS (Ciriello *et al.*, 1981), where neurones integrate/relay this information to other central areas and finally transmit it to sympathetic and parasympathetic preganglionic output neurones. The systemic effects of evoking the baroreflex are seen when α-adrenoceptors are activated by an i.v. injection of phenylephrine, resulting in vasoconstriction and an increase in blood pressure. As mentioned above this results in the stretch activation of baroreceptors, subsequently causing a reduction in blood pressure and heart rate.

The ADN and CSN have also been shown to carry chemoreceptor afferents in both the cat (Ciriello *et al.*, 1981), and the rat (Brophy *et al.*, 1999). They form the afferent limb of the arterial chemoreflex, and are activated by chemical stimulation of receptors located in the carotid and aortic bodies. Activation of these chemoreceptors by increasing PCO_2 and, to a certain extent, decreasing PO_2 can result in either a decrease or increase in heart rate. The direction depends on the differential effects of two responses to chemoreflex activation. The primary response on heart rate is brought about by increased drive to cardiac vagal preganglionic neurones, resulting in bradycardia. The secondary response is caused by increased drive to breathing centres, and subsequently increasing ventilatory rate and depth, resulting in inhibition of cardiac vagal

preganglionic neurones and tachycardia. Therefore, the net cardiovascular effects of chemoreceptor stimulation depend on the level of ventilation (Daly *et al.*, 1987). The cardiac effects of baroreceptor activation have also been shown to be respiratory modulated (Spyer, 1994).

1.1.3 Cardiopulmonary reflex

There are a large number of unmyelinated mechano- and chemo-sensitive afferents in the cardiopulmonary area. The cardiac mechanoreceptors are thought to monitor ventricular/atrial stretch (Thoren, 1979), whereas the cardiac chemoreceptors may have a role during pathological conditions such as myocardial ischaemia (Mark, 1983). One reflex which involves the activation of chemosensitive vagal afferents located in the heart and lungs is the Bezold-Jarisch reflex. This reflex, first described by von Bezold and Hirt in 1867, and further studied by Jarisch and Richter, is characterised by a pronounced bradycardia, sympathoinhibition and hypotension (Krayer, 1961). Classically the Bezold-Jarisch reflex is elicited by intra-coronary administration of substances such as the veratrum alkaloids (Krayer, 1961).

A similar, 'cardiopulmonary,' reflex can be achieved by a right atrial injection of the 5-HT_3 receptor agonist phenylbiguanide (PBG), which, along with the reduction in heart rate and blood pressure also produces an apnoea followed by a period of rapid, shallow breathing. This reflex is called the 'cardiopulmonary' reflex due to the activation of afferents in the pulmonary and cardiac circulations, although afferents in the systemic circulation are also likely to be stimulated. Physiologically, this reflex is induced by the mechanical activation of pulmonary afferents in the alveoli of the lungs in, for example, a situation of pulmonary oedema, when a bradycardia, fall in blood pressure and rapid shallow breathing would be beneficial (Paintal, 1995). Other authors have classified the response of irritant chemical administration into the pulmonary arteries via the right atrium as the 'pulmonary chemoreflex' (see Coleridge *et al.*, 1991), referring to the specific activation of pulmonary C-fibres. One distinction in the differential classification of the Bezold-Jarisch reflex and cardiopulmonary reflex is the likely different populations of afferents activated

after intra-coronary administration of veratridine and intra-atrial administration of PBG (see Verberne & Guyenet, 1992; Hainsworth, 1991). However, the neural pathways of the vagal component of these reflexes are thought to be the same, and indeed, very closely matched to that of the baroreceptor reflex (see Verberne & Guyenet, 1992).

1.2 Central cell groups and the processing of cardiovascular reflexes

As mentioned previously, the brainstem is the site of arrival and departure of autonomic and respiratory nervous information and is, therefore, fundamental in the functioning of cardiovascular control. Studies have shown that 'vasomotor' tone is generated within this area, although there was thought to be a distinct topographical differentiation controlling pressor and depressor responses (Alexander, 1946). However, modern axonal tracer methods have revealed the far more complex central organisation of autonomic regulation (see Saper, 1995; figure 1.1).

The role of higher centres in modulating brainstem reflexes has now been widely demonstrated. For example, stimulation of the hypothalamic defence area has been shown to affect both ventilation, via pathways to medullary respiratory neurones (Ballantyne *et al.*, 1988), and baroreflex, via an inhibitory pathway to NTS neurones (Mifflin *et al.*, 1988). Further, the projections of arterial chemoreceptors and baroreceptors to the hypothalamus have been shown to modulate defensive responses (Marshall, 1994).

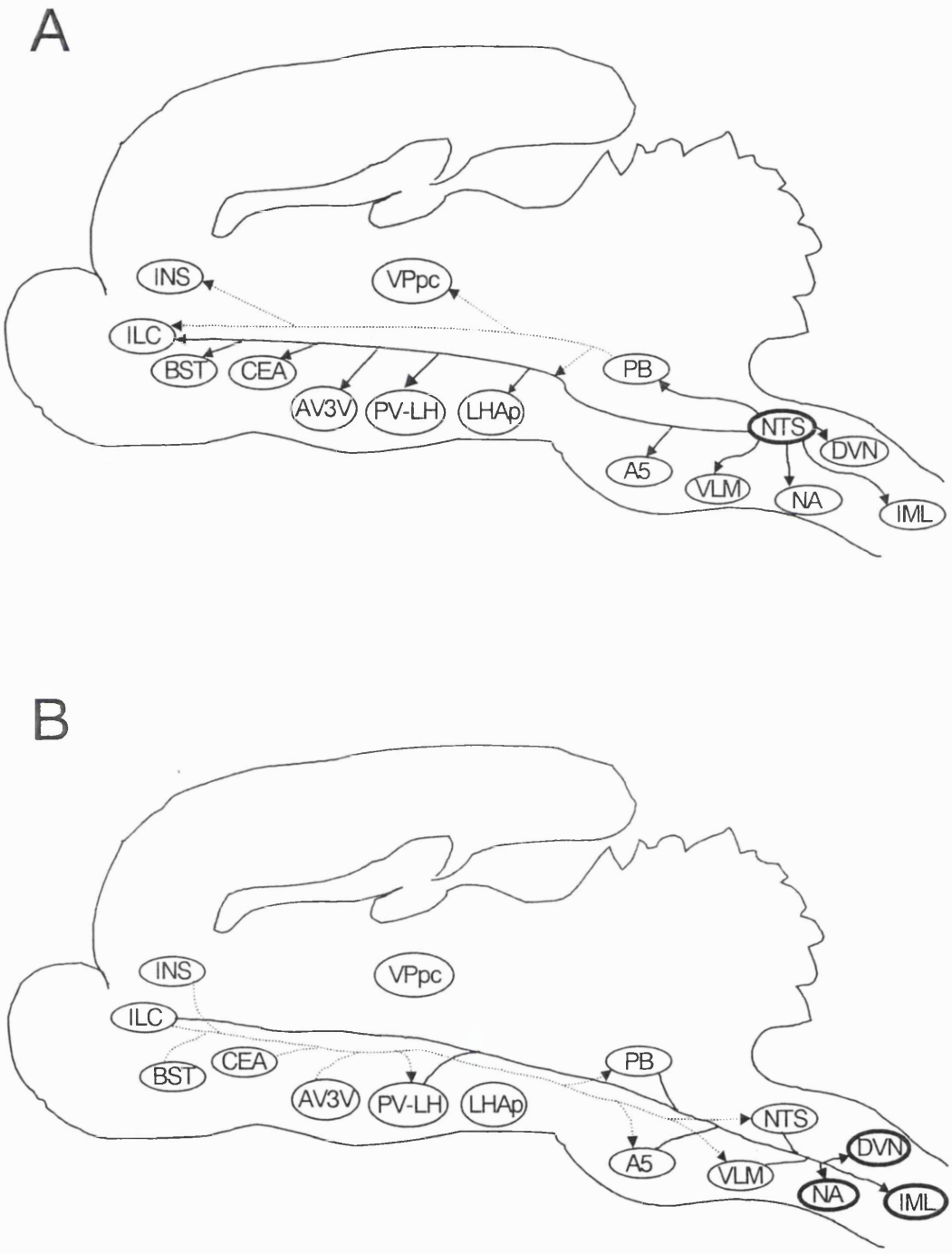
The following section describes some of the known anatomical connections and roles of specific nuclei involved in autonomic regulation within the CNS, see also figure 1.1.

Figure 1.1 Central autonomic afferent and efferent systems (Adapted from Saper, 1995)

A Central cell groups receive afferent information either from the NTS or via a relay in the parabrachial nucleus (PB). The projection to the ventroposterior parvocellular nucleus of the thalamus (VPpc) and the insular cortex (INS), the dashed line, originates exclusively from the parabrachial nucleus in the rat, whereas projections to the hypothalamus, infralimbic cortex (ILC), and basal forebrain originate from both parabrachial and NTS sources.

B Descending, efferent, projections to the preganglionic cell groups of the brainstem are shown, with direct projections as a solid line, and indirect, via premotor sites as a dashed line.

A5, A5 noradrenergic group; AV3V, anteroventral third ventricular area, BST, bed nucleus of the stria terminalis; CEA, central nucleus of the amygdala; DVN, dorsal vagal motor nucleus; IML, intermediolateral cell column; LHAp, posterior lateral hypothalamus; NA, nucleus ambiguus; PV-LH, tuberal hypothalamic cell groups, including paraventricular and dorsomedial nuclei and dorsal, perifornical, lateral tuberal and retrochiasmatic areas; VLM, ventrolateral medulla.


A
INS
VPpc
ILC
BST
CEA
AV3V
PV-LH
LHAp
PB
NTS
DVN
A5
VLM
NA
IML
B
INS
VPpc
ILC
BST
CEA
AV3V
PV-LH
LHAp
PB
NTS
DVN
A5
VLM
NA
IML

1.2.1 Cortex

Cortical areas, known to receive vagal projections, such as the insular cortex (INS) have been shown to produce a bradycardia and apnoea upon electrical stimulation. These areas have been termed 'vagal zones' by Kaada (1960) due to similarities seen with stimulation of certain vagal afferent fibres, specifically, the chemical activation of pulmonary C-fibres.

Anatomical evidence has also revealed that the INS has a number of projections to cell groups known to be important in cardiovascular control, such as the amygdala, lateral hypothalamus, NTS and parabrachial complex (see Yasui *et al.*, 1991). Electrical and chemical stimulation can produce both depressor responses, from more caudal areas, and pressor responses from more rostral regions (Ruggiero *et al.*, 1987; Yasui *et al.*, 1991), suggesting a topographical functional organisation.

A second cortical cell group involved in autonomic operation is the infralimbic cortex (ILC). The ILC receives afferents from areas such as the parabrachial nucleus (Saper, 1982) and projects to a large number of nuclei, including the NTS, parabrachial nucleus (PB), rostral ventrolateral medulla (RVLM) and intermediolateral cell column (IML; Hurley *et al.*, 1991).

There are also thought to be other structures including the motor and sensory cortex that can modulate cardiovascular function, revealed from the work of Hoff *et al* (1983). However, the general understanding is that the cortex provides an interface between behavioural and mood states and the subsequent readjustment of cardiovascular homeostasis required.

1.2.2 Central nucleus of the amygdala

The central nucleus of the amygdala is one forebrain structure involved in cardiovascular function. Specifically, electrical stimulation evokes a pattern of response similar to that produced by exposure to a threat. Interestingly, the cardiovascular component can take the form of a bradycardia and depressor

response in the rabbit (Kapp *et al.*, 1982), or the opposite in the cat, including vasodilation of skeletal muscle vascular beds (Timms, 1981), which concurs with behavioural differences between these species in response to threatening stimuli. Anatomical studies have shown that the amygdala receives projections from the NTS, either direct (Ricardo & Koh, 1978) or via the parabrachial nucleus (Saper & Loewy, 1980), and neurones recorded in this nucleus responded to activation of baro-afferents (Cechetto & Calaresu, 1983). Other anatomical evidence has also revealed a direct descending pathway from the amygdala to the NTS (Schwaber *et al.*, 1982).

1.2.3 Thalamus

The ventroposterior parvocellular nucleus of the thalamus (VPpc) has been shown to receive projections from the NTS via an indirect pathway through the parabrachial nucleus (Norgren, 1976). The cardiovascular and respiratory projections terminate in the lateral regions of this nucleus, whilst gustatory and gastrointestinal afferents terminate more medially (Loewy & Neil, 1981). One of the functions of the medial VPpc is thought to be the relay of gustatory information involved in taste discrimination.

1.2.4 Hypothalamus

The anteroventral third ventricle region (AV3V) is an area thought to be important in fluid and electrolyte balance, and the development of some forms of hypertension (Brody & Johnson, 1980). In addition, it is one of a number of forebrain areas shown to contain neurones that respond to peripheral baroreceptor stimulation (Knuepfer *et al.*, 1985). The AV3V is also known to receive projections from the NTS (Ricardo & Koh, 1978), and to contain neurones that project to both parabrachial and NTS cell groups (Saper & Levisohn, 1983). Thus, an important role for the AV3V in the maintenance of cardiovascular regions seems likely.

The paraventricular nucleus (PVN) receives both a direct and indirect (via the parabrachial nucleus) projection from the NTS (see figure 1.1; Sawchenko &

Swanson, 1982; Fulwiler & Saper, 1984). The PVN is also known to project to many autonomic cell groups within the CNS, including the PB, VLM, NTS, DVN, NA and periaqueductal grey (PAG; Luiten *et al.*, 1985) and has been the subject of a review in relation to its function within the cardiovascular system (Coote, 1995). This review describes spinal projecting neurones of the PVN which are inhibited by baroreceptors and excited or inhibited by pulmonary and cardiac vagal afferents. Electrical and chemical stimulation of the PVN does produce cardiovascular changes, however, these responses have been found to be variable with both depressor and pressor responses reported (Gilbey *et al.*, 1982; Katafuchi *et al.*, 1988; Dampney, 1994b). In addition, a recent study has described the ability of different populations of PVN neurones to elicit both decreases and increases in blood pressure via connections to vasomotor neurones in the rostral ventrolateral medulla (Yang & Coote, 1998). These authors also demonstrated that PVN neurones can modulate caudal ventrolateral medullary neurones (Yang & Coote, 1999), a further mechanism through which the PVN could mediate depressor effects. Furthermore, lesion studies have revealed that although the integrity of the baroreflex is maintained in the absence of the PVN, an increase in baroreflex-evoked sympathoinhibition can be induced (Patel & Schmid, 1988). It has also been suggested to be important in pathological conditions such as neurogenic hypertension (see Dampney, 1994), further indicating a strong autonomic neuromodulatory role for this nucleus.

1.2.5 Mesopontine

The periaqueductal gray (PAG) is known to be important in antinociception and defensive behaviour (Basbaum & Fields, 1984). It has more recently been understood to play a role in cardiovascular control with projections to and from the NTS, and is thought to co-ordinate complex autonomic responses in different behavioural states. Stimulation in medial and dorsal subnuclei of the PAG has been shown to cause predominantly hypertensive effects (Carrive *et al.*, 1989), although more ventrolateral regions mediate hypotensive responses (Carrive & Bandler, 1991). Furthermore, these ventrolateral areas have been shown to modulate components of the cardiovascular defence response, via

neurones of the rostral ventrolateral medulla (Lovick, 1992), and receive a dense projection from the NTS (Kwiat & Basbaum, 1990).

Chemical stimulation of the A5 noradrenergic group, located in the pons, evokes a hypotension (Neil & Loewy, 1982). There is also evidence for a descending pathway to the NTS (Loewy *et al.*, 1986); nevertheless, the role of these neurones remains to be determined.

The parabrachial nucleus is another cell group where stimulation produces cardiovascular and respiratory effects. It acts as a relay centre for autonomic reflex afferent information to forebrain levels, and is also included in the descending pathway from these higher areas. There are 13 distinct subdivisions of the parabrachial, although the Kolliker-Fuse subnucleus and the lateral margin of the external lateral nucleus, which forms a crescent shape, contain the neurones that project to the NA, VLM, NTS and spinal cord (Saper & Loewy, 1980). Stimulation of the lateral margin causes a significant fall in blood pressure (Chamberlin & Saper, 1992). However, the characterisation of the parabrachial nucleus is far from complete and it remains an important target for research into the functioning of the autonomic nervous system

There is also evidence that the midbrain raphe nuclei, which receive a dense afferent projection from the parabrachial nucleus (Saper & Loewy, 1980), may modulate the activity of cell groups in the thalamus that are involved in autonomic control (Petrov *et al.*, 1992). The midbrain raphe nuclei also provide the major serotonergic pathway to the forebrain (Azmitia, 1978).

1.2.6 Medullary nuclei

As mentioned above, the brainstem is a focal point for research into mechanisms of cardiovascular control, as a result there are a large number of reviews of the area. However, despite the amount of studies undertaken on nuclei contained within the medulla, their mechanisms are still only partially understood. The medulla contains: the NTS - the central termination of the

vast majority of cardiovascular afferents; the motor, or premotor nuclei - supplying the efferent pathways of autonomic outflow, including the NA, RVLM & DVN; and other structures known to be important in the integration and/or relay of cardiovascular information - including the caudal raphe and caudal ventrolateral medulla (CVLM). Some of the inputs and projections of these cell groups, in addition to existing interpretations of their roles in both physiological and pharmacological aspects of cardiovascular regulation are detailed below.

Caudal raphe

Both electrical (Adair *et al.*, 1977) and chemical (Dreteler *et al.*, 1991) stimulation of caudal raphe nuclei, including raphe pallidus, raphe obscurus and raphe magus, has been shown to cause an increase and a decrease in blood pressure dependent on the site stimulated. In addition, some raphe neurones are known to receive baroreceptor inputs (see Dampney, 1994a), although the baroreflex is maintained following lesions of this area, indicating that these neurones are not vital in the transmission of the reflex (McCall & Harris, 1987). Another characteristic of caudally-located raphe nuclei is the high density of serotonergic neurones located there and their projection to (Schaffer *et al.*, 1988) and from (Steinbusch, 1981) the NTS. Serotonin containing neurones are also known to project from caudal raphe nuclei to the IML (Loewy & Neil, 1981, see Dampney, 1994a)

Rostral ventrolateral medulla

The rostral ventrolateral medulla (RVLM) has been widely studied, and is therefore one of the more understood of brainstem structures. It has a vital role in providing the tonic and phasic regulation of blood pressure, projecting to sympathetic preganglionic neurones in the IML (Guyenet, 1990). This cell group also contains neurones known to be important in pain modulation and in respiration, and other projections of RVLM neurones include the parabrachial nucleus, the PAG, and the PVN (Guyenet & Young, 1987). Both electrical and chemical (using the excitatory amino acid glutamate) stimulation in this area produces sympathoexcitation and a pressor response (Dampney *et al.*, 1982). The RVLM receives afferent input from a large number of sources including the

NTS, CVLM, PB, medullary raphe, PAG and PVN (Dampney *et al.*, 1987; Ross *et al.*, 1985). The highest density of some of these projections to the RVLM, from the NTS particularly, has been shown to be in the same location, in the RVLM, as the premotor neurones which project to the IML (Dampney *et al.*, 1987; Ross *et al.*, 1985). These anatomical connections suggest that direct pathways involving the RVLM are important in cardiovascular reflex transmission. Further evidence for this comes from electrophysiological data showing the strong inhibitory response of RVLM neurones to baroreceptor activation (Guyenet, 1990). Another group of cells in the VLM known to have effects on blood pressure is contained within the CVLM. These neurones receive input from the NTS (Ross *et al.*, 1985) and project to the RVLM. The current understanding is that baroreceptor and cardiopulmonary C-fibre afferent information arrives at NTS neurones, is then passed, via an excitatory pathway, to the CVLM which, in turn, inhibits RVLM premotor neurones, resulting in sympatho-inhibition (Agarwal *et al.*, 1990). Further, on the basis of anatomical and functional studies, chemoreceptor activation is thought to cause sympatho-excitation via a direct excitatory pathway between NTS and RVLM, although this mechanism is by no means completely understood (Koshiya & Guyenet, 1996; Aicher *et al.*, 1996). In addition, neurones of the RVLM, which are known to be responsible for the generation of sympathetic vasomotor tone, are also under tonic inhibition from GABAergic neurones in the CVLM which may be important in the understanding of the long term maintenance of blood pressure (see Schreihofer & Guyenet, 2002).

Nucleus ambiguus

The nucleus ambiguus (NA) contains, amongst others, cardiac vagal preganglionic neurones (CVPNs; McAllen & Spyer, 1976; Nosaka *et al.*, 1979). These neurones are also found in the dorsal vagal motor nucleus (DVN) and the regions between these two nuclei to a lesser extent (Nosaka *et al.*, 1979). However, in the cat, the NA has been shown to be the location of CVPNs that exert chronotropic control of heart rate. (McAllen & Spyer, 1976; 1978a). CVPNs receive a tonic excitatory input from arterial baroreceptors, resulting in a pulse correlated activity (McAllen & Spyer, 1978b). This is thought to be mediated by a direct excitatory pathway from the NTS (Agarwal & Calaresu,

1992). This direct pathway can be demonstrated in the response of these CVPNs to baro-afferent activation, which affects these neurones with a relatively short latency, although there is a longer latency component as well (McAllen & Spyer, 1978b). This longer latency component likely reflects a slower pathway through higher centres, possibly including the hypothalamus (see Spyer, 1982). These CVPNs fire with a respiratory rhythm, firing most often during the post-inspiratory phase of respiration, and less often during inspiration and phase two expiration. As mentioned previously this rhythmical activity leads to the appearance of respiratory sinus arrhythmia, and is thought to be driven by a respiratory modulation from medullary inspiratory neurones (McAllen & Spyer, 1978b; Gilbey *et al.*, 1984). This respiratory modulation of CVPNs also shows itself in the bradycardia evoked by most, but not all, cardioinhibitory reflexes. Reflex cardiac slowing is greatest during expiration when CVPNs are most active, due to coupling between the brainstem respiratory and cardiovascular system (see Taylor *et al.*, 1999). However, in cats the bradycardia evoked by administration of phenylbiguanide into the right atrium (the pulmonary chemoreflex) is not influenced by central respiratory drive or lung inflation so it was suggested that it was not mediated by CVPNs located in nucleus ambiguus (Daly, 1991; Daly *et al.*, 1992).

Dorsal vagal motor nucleus

As its name suggests the dorsal vagal motor nucleus (DVN) is located in the dorsal region of the brainstem, directly ventral to the NTS, and contains preganglionic neurones of the vagus. Projections of these vagal preganglionic neurones from the DVN include the gastrointestinal tract and the heart, as mentioned above. Cardiac vagal preganglionic neurones have been described in the DVN, which have non-myelinated axons (Donoghue *et al.*, 1981, Jones *et al.*, 1998). These C-fibre axons have been suggested to mediate negative chronotropic, ionotropic and dromotropic effects on the heart (Jones *et al.*, 1995; Garcia & Jordan, 2001). Moreover, other authors have proposed a role for these neurones in the bradycardia evoked by pulmonary C-fibre stimulation (Daly, 1991).

The DVN receives a dense projection from the NTS, and the relationship between these two nuclei is an important target for research into gastric reflex integration, as well as giving a better understanding of the mechanisms of neural networks in the brainstem.

1.3 Nucleus tractus solitarius

The extent of afferent inputs and efferent projections of the nucleus tractus solitarius (NTS) have been reviewed above and show the importance of this group of cells in the transmission and modulation of cardiovascular reflexes. Indeed a clinical example of the importance of this nucleus in autonomic function has been described (Biaggioni *et al.*, 1994). In this case a patient presented who was totally deficient of a cardiac response to a drop in blood pressure evoked by peripherally administered nitroprusside. The post mortem showed bilateral infarctions and gliosis of the NTS, and no other autonomic nuclei. However, the NTS is not solely a centre for cardiovascular and respiratory function, it also serves crucial functions in a wide variety of other areas, ranging from nociception to gastro-intestinal tract function to liver and kidney function.

1.3.1 Anatomy of the NTS

Early histological studies (Foley & Dubois, 1934; Ingram & Dawkins, 1945) have described how the tracts of the NTS enter from the rostral end of the medulla oblongata as two symmetrical, bilateral nuclei containing afferents of the glossopharyngeal and vagus nerve. They descend caudally through the brainstem, although at the level of obex they combine into one single tract. Throughout this passage of the tracts collaterals of both groups of afferent fibres are sent to, and terminate in, subnuclei of the NTS itself. One of the more important distinctions in the functional layout of the NTS is the distinction in the termination of glossopharyngeal nerve collaterals in the rostral two thirds of the nucleus and the termination of vagus nerve collaterals in the caudal two thirds of the nucleus (Cottle, 1964). There is therefore an area of overlap that both

nerves afferents target termed the intermediate zone, a likely important site in the integration of cardiovascular afferent information.

However, further research has provided a much more detailed description of the termination of identified cardiovascular and respiratory afferent information into individual subnuclei of the NTS (see Loewy & Burton, 1979; Kalia & Mesulam, 1980; Jordan & Spyer, 1986; figure 1.2). For this the nucleus was split into subnuclei based on anatomical differentiation, thus areas included: commisural nucleus, medial solitary nucleus, dorsomedial solitary nucleus, parvocellular solitary nucleus, lateral solitary nucleus, dorsolateral solitary nucleus, ventrolateral solitary nucleus, intermediate nucleus and interstitial nucleus (Loewy & Burton, 1979). In summary, baroreceptor afferents arising from both the aortic and carotid sinus have been shown to terminate principally in dorsal regions of the lateral and medial areas, with the densest afferentation being rostral to obex (Donoghue *et al.*, 1982; Donoghue *et al.*, 1984). Carotid chemoreceptors on the other hand have been shown to project to more dorsomedial, medial and commissural regions (Donoghue *et al.*, 1984). Both unmyelinated bronchial and pulmonary afferents also terminate within medial and commissural subnuclei, with very few projections to lateral or ventrolateral regions (Kubin *et al.*, 1991). In addition, a major difference has been shown in the projections of slowly and rapidly adapting pulmonary afferents with the highest density of cells responding to rapidly adapting afferents found in more caudal regions the commisural subnucleus of the NTS (Kubin & Davies 1985). However, neither groups of afferents have been shown to have a significant projection to more lateral subnuclei of the NTS.

In light of this evidence there is no one region of the NTS thought to receive only one type of afferent input. Further, the degree of overlapping of these inputs to the NTS has, understandably, been implicated in the integrative function of the NTS (Jordan, 1995). As mentioned above a monosynaptic pathway between NTS neurones and both premotor neurones of the RVLM and vagal preganglionic neurones of the NA and DVN has been shown (Luiten *et al.*, 1987). However, direct connections are more likely to be far fewer than indirect pathways due to a number of reasons. Firstly, simply the complexity of the many reflex responses, and secondly the latency of response between

afferent stimulation and efferent activation being longer than expected if a solely monosynaptic pathway existed (Jordan, 1995). Therefore, the inclusion of higher centres (as discussed in earlier sections) and also interneurones within the NTS itself, which may themselves be modulated by descending inputs, in these reflex pathways would seem the likely predominant mechanism of central autonomic co-ordination and transmission.

Figure 1.2 The termination densities of cardiovascular and respiratory afferents (taken from Jordan, 1995)

Summary of the major regions of termination within the NTS of the cat of cardiovascular and pulmonary afferents as determined by antidromic mapping studies. The relative density of ipsilateral (●) and contralateral (○) regions of termination is denoted by the number of dots; the most extensive regions of termination are shaded.

Receptor Type	Divisions of the NTS		
	Medial	Commissural	Lateral
Myelinated aortic baroreceptor	● ● ● ○	●	● ● ● ●
Myelinated carotid baroreceptor	● ● ●	●	● ● ● ● ● ●
Unmyelinated aortic baroreceptor	● ● ●	●	● ● ● ●
Unmyelinated carotid chemoreceptor	● ● ● ○ ● ● ●	● ● ● ○ ● ● ○	● ●
Myelinated lung SAR	● ● ● ● ● ●		● ●
Myelinated lung RAR	● ● ○ ● ● ○ ○	● ● ● ○ ● ● ○ ○	● ●
Unmyelinated bronchial receptor	● ● ● ○ ● ● ●	● ● ● ○ ● ● ○	
Unmyelinated pulmonary receptor	● ● ● ○ ● ●	● ● ○ ● ●	

1.3.2 The roles of synaptic connections

An important way in which afferent information is modulated in the NTS is mediated via presynaptic mechanisms. To this effect, a lot of the work was done on vagal afferents and studied changes in the terminal excitability of peripheral nerve upon stimulation of other branches of the vagus, and of the NTS itself (Rudomin, 1967; Barillot, 1970; Jordan & Spyer, 1979). It was demonstrated that the activation of superior laryngeal nerve (SLN) afferents by stimulation of the NTS (Rudomin, 1967) could be modulated by activation of slowly adapting pulmonary afferents (SARs; Jordan & Spyer, 1979). The opposite is also true in that the terminal excitability of SARs has been shown to be influenced by stimulation of both the NTS and the SLN (Jordan & Spyer, 1979). A more recent study recorded SAR fibre membrane potentials in response to SLN stimulation (Ballantyne *et al.*, 1986). Two waves of depolarisation were recorded in response to SLN stimulation, the latter of the two waves was found to be maximal during phrenic nerve activity, confirming an inspiratory correlated activity. Further effects of lung inflation were found to be superimposed over this response, revealing a presynaptic interaction of afferent input (Ballantyne *et al.*, 1986). This particular example of a presynaptic interaction can be related to a whole body function, i.e. laryngeal afferents are mechanically activated by the introduction of foreign bodies into the airways, and subsequently suppress inspiration (Jordan and Spyer, 1986).

Anatomical data has also revealed that axo-axonal synapses have been found in commissural (Chiba & Doba, 1976), but not intermediate (Chiba & Doba, 1975) regions of the NTS. In addition, SAR terminals forming the postsynaptic element of axo-axonal synapses have been described within ventral and ventrolateral regions of the NTS (Kalia & Richter, 1985), which adds further evidence for the presence and functional significance of presynaptic contacts.

However, it has been suggested that there is a topographical differentiation within the NTS of these effects (Ballantyne *et al.*, 1986; Jordan & Spyer, 1986). Baroreceptor afferents, which terminate, at their highest density, more laterally, dorsally and rostrally to SAR afferents (see figure 1.2 and above) differ in their

response. They have been shown to have no respiratory related activity (Ballantyne *et al.*, 1986), and the threshold for antidromic activation of these afferents from stimulation of NTS is not affected by stimulation of other NTS sites (Rudomin, 1967; Jordan & Spyer, 1979). This suggests that the respiratory modulation of the cardiac response of both baro- and chemo-reflexes mentioned earlier must happen further on in their respective reflex pathways. This evidence also suggests that presynaptic interactions are not necessarily universal within the NTS.

The next important understanding of NTS function is in the degree of convergence of afferent inputs to the nucleus, or postsynaptic mechanisms. There are a number of issues; firstly the NTS is not a simple relay station and as mentioned previously the involvement of second and third order neurones within the nucleus is commonplace. Secondly, the allowance for independent reflex transmission to pass through the NTS without the involvement of other afferent inputs must be there, however rarely this occurs physiologically. Early extracellular experiments demonstrated that the occurrence of convergent inputs, from ADN, CSN and vagus nerves, to NTS neurones is rare, in contrast to neurones surrounding the nucleus, and involves only excitatory connections (see Spyer, 1994). However, some of these findings did suggest that convergence was more likely between fibres of similar conducting velocities, myelinated and unmyelinated fibres not converging on the same neurone very often (see Coleridge *et al.*, 1991). The limits for extracellular recordings in determining physiologically convergent post- or presynaptic effects are that subthreshold afferent inputs cannot be measured, neither can inhibitory connections in quiescent cells.

Recent evidence for convergent inputs to NTS neurones has come from a number of studies (Mifflin, 1996; Paton *et al.*, 1999; Hines *et al.*, 1994, Silva-Carvalho *et al.*, 1998). One such study described inputs, some of which were found to be monosynaptic, from arterial baroreceptors and carotid chemoreceptors terminating onto the same neurone (Mifflin, 1996). A second study showed a high proportion of NTS cells that received pharyngoesophageal inputs also responded to carotid chemoreceptor activation (Paton *et al.*, 1999).

Interestingly, as regards this thesis, atrial volume receptors have been shown to be unlikely to project to the same cells as pulmonary C-fibre afferents (Hines *et al.*, 1994). Moreover, cells receiving pulmonary C-fibre input and/or carotid body chemoreceptor input did not receive projections from arterial baroreceptors but did receive afferentation from chemosensitive cardiac afferents (Silva-Carvalho *et al.*, 1998). However, taken together these studies again highlight the complexity of neuronal connections within the NTS.

1.3.3 NTS neuropharmacology

Glutamate

L-Glutamate was first suggested as the primary neurotransmitter at baroreceptor afferent terminals within the NTS in 1980 (Talman *et al.*, 1980). There is a now a large amount of evidence that glutamate clearly has a major role in neurotransmission but is unlikely to be the only neurotransmitter released from primary afferents arriving in the NTS. Sectioning of the vagus and glossopharyngeal nerves has been shown to cause a reduction in glutamate levels within the NTS (Dietrich *et al.*, 1982), whereas stimulation of vagus nerve in the cat has been reported to increase glutamate release in the NTS (Allchin *et al.*, 1994). Further, blockade of glutamate receptors, including NMDA and non-NMDA receptors has been shown to eliminate synaptically mediated aortic baroreflexes (Leone & Gordon, 1989), although the response to exogenously applied glutamate was unaffected by kynurenate, a glutamate receptor antagonist (Talman, 1989). An explanation for this seeming contradiction is that the high concentrations of microinjected glutamate allowed the substance to reach neurones and receptors not accessible to endogenously released neurotransmitter including kynurenate–insensitive receptors (Lawrence & Jarrott, 1996). Intracellular recordings have now suggested that glutamate released from synaptic terminals activates primarily non-NMDA receptors, with NMDA receptors playing a more modulatory role (Andresen & Yang, 1990).

When glutamate is microinjected into the NTS of anaesthetized rats, a depressor response and bradycardia occurs (Talman *et al.*, 1980), interestingly,

this is not mirrored in the conscious preparation where a pressor response is observed (Machado & Bonagamba, 1992). Additionally, as mentioned earlier blockade of glutamate receptors has been shown to abolish the baroreflex, although again this response does not appear to be mirrored in conscious rats (Colombari *et al.*, 1994). There is a growing amount of research being done in conscious animals and these are not the only examples of differing results compared to anaesthetized animals. However, so far these apparently conflicting results remain unexplained in the theory of glutamate as the likely transmitter at baroreceptor afferent terminals.

There is now also evidence for glutamate as the primary NTS neurotransmitter for chemoreceptor and cardiopulmonary reflexes. Microinjections of NMDA and non-NMDA receptor antagonists into the NTS have been shown to block the pressor effects of carotid chemoreceptor activation (Zhang & Mifflin, 1993). Further, the apnoea, hypotension and bradycardia associated with the cardiopulmonary reflex has been blocked with glutamate receptor antagonism in the NTS (Bonham *et al.*, 1993; Vardhan *et al.*, 1993). This confirms glutamate as a major player within the NTS acting via both NMDA and non-NMDA receptors.

GABA

γ-amino-butyric acid (GABA) is considered to be the major inhibitory neurotransmitter, and is known to be present in the NTS at high concentrations (Dietrich *et al.*, 1982). Initial studies showed that bilateral microinjections of GABA into the NTS produced a drop in blood pressure with little effect on heart rate (Catelli *et al.*, 1987). More recent electrophysiological evidence from brain slices has revealed that NTS cells inhibited by solitary tract stimulation are 90% likely to be GABAergic (Fortin & Champagnat, 1993). The actions of individual GABA receptors has also been studied, with both $GABA_A$ and $GABA_B$ receptor antagonists potentiating the evoked responses of NTS neurones sensitive to baroreflex inputs (Suzuki *et al.*, 1993). This study also revealed that the $GABA_A$ antagonist bicuculline increased the ongoing activity of these NTS neurones. GABA has also been suggested to be released from vagal afferent neurones from studies looking at GABA release *in vitro* after nodose ganglion removal

(Meeley *et al.*, 1989). This was supported by work done using monoclonal antibodies, which identified GABA in nodose ganglion perikarya (Szabat *et al.*, 1992). One of the more important aspects of GABA is in its actions as an inhibitory neuromodulatory gate on strong, excitatory, afferent input arriving at NTS. An example of this has been shown in the NTS where somatic noxious stimulation attenuates the bradycardic component of the chemoreceptor reflex via a $GABA_A$ dependent mechanism (Boscan & Paton, 2002). GABA is also thought likely to have interactions with other neurotransmitters within the NTS. One such study has reported that stimulation of 5-HT_3 receptors in the NTS, via the activation of $GABA_A$ receptors, reduces the cardiac response evoked by NMDA microinjection into the NTS (Bonagamba *et al.*, 2000).

Glycine

Glycine must also be mentioned in relation to the neuropharmacology of the NTS. It is found in high concentrations in the NTS (Meeley *et al.*, 1989), in addition to a high density of glycine receptors, some of which have been shown to be presynaptic (Cassell *et al.*, 1992). Some cardiovascular responses have been shown after microinjection of glycine into the NTS, although strychnine, a glycine receptor antagonist, did not produce any systemic effect, or alter responses to baroreflex after microinjection into the nucleus (Talman & Robertson, 1989). Therefore, although there does appear to be a large amount of glycine in the NTS and thus, an important role for the neurotransmitter, its actions in relation to autonomic control are still not clear.

Neuropeptides

Neuropeptide Y

There are a number of neuropeptides that are thought to be important within the neuropharmacology of the NTS, one of these is neuropeptide Y (NPY). The locus coeruleus (LC) has been shown to have a high density of NPY-containing neurones, and interestingly the attenuation of baroreceptor reflex seen upon stimulation of LC can be blocked by microinjection of NPY antisera into the NTS (Chan *et al.*, 1993). Anatomical data has also revealed the presence of NPY-positive neurones in a number of NTS subnuclei that are known to be important

in cardiovascular control (Harfstrand *et al.*, 1987). Currently the understanding is that NPY acts as a neuromodulator of baroreflex transmission and may affect glutamate efficacy within NTS (see Lawrence & Jarrott, 1996).

Angiotensin II (ANGII), Bradykinin, & Substance P

Angiotensin administered systemically causes a pressor response but no bradycardia, this unusual response is possibly caused by actions within the NTS (see Reid, 1992). The nucleus is known to have a high density of angiotensin AT_1 receptors located on interneurones and presynaptically on vagal and carotid sinus afferents (Healy *et al.*, 1989). In addition, microinjection of ANGII into the NTS attenuated baroreceptor reflex in anaesthetized rat (Casto & Phillips, 1986), whilst AT_1 receptor blockade augmented reflex response (Campagnole-Santos *et al.*, 1988). However, the story of ANGII is not complete, as a number of studies have found contradicting effects of ANGII microinjected into the NTS at different doses (see Paton & Kasparov, 1999). This is thought to be due to the exogenously applied substance acting at functionally diverse cells within the NTS. Another facet of ANGII function in the NTS is with possible interactions between AT_1 receptors and bradykinin B_2 receptors (Fior *et al.*, 1993), which are thought to mediate, in part, the pressor actions of bradykinin.

There is also evidence that ANGII causes release of substance P, which may modulate chemoreceptor reflex acting at NK_1 receptors (Paton & Kasparov, 1999). Substance P was first thought to play a role in NTS neuropharmacology in experiments that showed a marked decrease in levels of the substance upon destruction of the vagal and glossopharyngeal nerves (Gilles *et al.*, 1980). Microinjection of substance P into the NTS has been shown to produce a vagally mediated bradycardia (Hall *et al.*, 1989). Again there is contradictions in the findings on substance P to date which may be due to different receptor subtypes mediating different functions (see Lawrence & Jarrott, 1996). However, a clear role for both substance P and ANGII in NTS function seems likely.

Vasopressin

Vasopressin has been shown to have cardiovascular effects, mediated by the V_1 receptor, upon microinjection into the NTS, but as with ANGII, the nature of the response varies according to dose (Matsuguchi *et al.*, 1982; Brattstrom *et al.*, 1988). Anatomical data confirms that there is a population of V_1 receptors in the NTS and that some of these are indeed presynaptically located (Gao *et al.*, 1992). In addition, blockade of these receptors has been shown to attenuate systemic effects of ADN stimulation (Hegarty & Felder, 1993). The area postrema has also been linked with the role of vasopressin in modulating cardiovascular reflex pathways (Zhang *et al.*, 1992), as have interactions with other neurotransmitters (see Ramage, 2001), discussed later.

Other Neuropeptides

There is a host of other neuropeptides implicated in autonomic function within the NTS. For example, natriuretic peptides are known to be contained within the NTS, and they have been shown to have cardiovascular effects although the exact receptor subtypes and mechanisms involved remain to be determined (see Lawrence & Jarrott, 1996). Opioidergic terminals have also been described in the NTS (Kalia *et al.*, 1984), as well as actions of cholecystokinin, endothelins and others, however, their roles have yet to be fully elucidated, and are unimportant for the purposes of this thesis.

Nitric oxide

Nitric oxide (NO), the production of which is catalysed by the enzyme nitric oxide synthase (NOS), is another substance that has been shown to have an important role in modulation of autonomic function at the level of the NTS. However, studies have revealed that blockade of the guanylate cyclase effector pathway in the NTS had no effects systemically (Machado & Bonagamba, 1992), therefore tonic release of NO appears not to have a crucial role in central cardiovascular regulation. Nevertheless, S-nitrocysteine caused depressor and bradycardic responses when injected into the NTS (Machado & Bonagamba, 1992), and NOS inhibitors have been shown to decrease the ongoing firing rate of NTS neurones (Ma *et al.*, 1995). In addition, NOS inhibitors in the NTS have been shown to decrease baroreflex gain in both conscious (Pontieri *et al.*, 1998)

and anaesthetized rats (Li *et al.*, 2002). Another recent study used an adenoviral vector to inhibit specifically endothelial NOS (eNOS) in the NTS and found that this enhanced the baroreceptor reflex in conscious rats (Waki *et al.*, 2003), suggesting that eNOS is actively involved in the regulation of blood pressure. This group has also previously described how the ability of exogenously applied ANGII to depress the cardiac component of the baroreceptor reflex is lost when eNOS is inactivated within the NTS (Paton *et al.*, 2001). Therefore, NO clearly plays an important role in autonomic regulation within the NTS, although exact mechanisms are still being uncovered.

Purine nucleotides

There is now ample evidence that both ATP and adenosine are capable of modulating cardio-respiratory responses (Spyer & Thomas, 2000). Indeed, the highest central density of adenosine uptake sites in rat has been shown to be contained within the NTS (Bisserbe *et al.*, 1985). Adenosine is produced in the NTS from the breakdown of ATP or by its release via nucleoside transporters and it is another substance shown to have differential dose-related responses after microinjection into the NTS (see Lawrence & Jarrott, 1996). It has been demonstrated that microinjection of adenosine antagonists into the NTS attenuates the baroreceptor heart rate reflex (Mosqueda-Garcia *et al.*, 1989). Recent evidence has also now revealed that adenosine levels in the NTS rise during stimulation of the hypothalamic defence area (Dale *et al.*, 2002). These effects of adenosine have been shown to be blocked by $\alpha\beta$-methylene ADP which prevents the breakdown of ATP to adenosine via AMP, therefore the source of adenosine appears to be via released ATP (see Spyer & Thomas, 2000).

In addition, ATP is able to mediate strong cardiovascular effects in NTS (see Lawrence & Jarrott). Studies have shown that ATP can cause a drop in blood pressure mediated by P_2 purinoceptors within the NTS (Ergene *et al.*, 1994). These purinergic receptors are classified into P_1 (adenosine) and P_2 (ATP) receptors, and there is currently a great deal of interest in the actions of the many subtypes of both P_1 and P_2 purinoceptors throughout the nervous system.

However, the current poor availability of selective compounds acting at these receptors, has made this research and therefore the understanding of the exact nature of the roles of purinoceptors difficult.

Catecholamines

Dopamine was shown to mediate both depressor and bradycardic as well as pressor and tachycardic effects in two separate studies 20 years ago (Granata & Woodruff, 1982; Zandberg *et al.*, 1979). Recent histological data has revealed the presence of D_2 receptors, some of which are located on rat vagal afferent terminals (Lawrence *et al.*, 1995). The density of receptors matches, to a certain extent, the density of baroreceptor afferent input to the NTS, and the current understanding is that dopamine serves a modulatory function with respect to chemo- and baro-receptor reflexes (see Lawrence & Jarrott, 1996). It may also play a role in a situation of severe hypoxia (Goiny *et al.*, 1991).

Noadrenaline is another catecholamine known to be present in the NTS. Studies have demonstrated an inhibitory neuromodulatory role for NA, particularly with respect to baroreflex, acting at α_1 and α_2 adrenoceptors (Feldman & Felder, 1989). Interestingly, noradrenaline is another NTS neurotransmitter that has been shown to be involved in interactions with other chemo-active substances in the NTS, including vasopressin and bradykinin. In the first case by modulation of vasopressin release, and in the case of bradykinin interactions were seen between α_2-adrenoceptors and B_2 receptors (see Lawrence & Jarrott, 1996). More recently, a tonic role for α_2-adrenoceptors has been established after the systemic response to ADN stimulation was attenuated by the administration of yohimbine and idazoxan into the NTS (Sved *et al.*, 1992). Less is known about the actions of adrenaline in the NTS, although the administration of isoprenaline into NTS causes respiratory changes and a drop in blood pressure (Barraco & Janusz, 1988), suggesting a functional role, possibly at β_2 -adrenoceptors. This may be important in the clinical understanding of the actions of β-adrenoceptor antagonists.

Acetylcholine

Acetylcholine, acting at both nicotinic and muscarinic receptors, is yet another transmitter that seems to have a role in NTS cardiovascular regulation. Microinjection of atropine into NTS has been shown to block baroreceptor reflex (Criscione *et al.*, 1983), whilst nicotine applied to the nucleus drops both blood pressure and heart rate. (Tseng *et al.*, 1993). Importantly, nicotine-binding sites, including those for α-bungarotoxin, have been found throughout NTS, with the sites sensitive to α-bungarotoxin located more caudally (Maley & Seybold, 1993). The exact nature of the modulation acetylcholine exerts on cardiovascular and other inputs to NTS neurones is still not fully understood.

5-HT

One other neurotransmitter that is known to be present in the NTS and has been shown to be important in the functioning of NTS cells is 5-hydroxytryptamine (5-HT). Analysis of the role of 5-HT in NTS and other autonomic areas is contained below together with descriptions of the receptor subtypes involved.

1.4 5-HT

1.4.1 History and pharmacology

Introduction

5-HT was first characterised as serotonin in 1948, when this substance was found to have vasoconstrictor properties (Rapport *et al.*, 1948), the name being derived from its actions as a **ser**um vasoconstrictor increasing blood pressure **ton**e. However, after the description of another substance with similar characteristics, enteramine, the two were found to be identical and were called 5-hydroxytryptamine (Amin *et al.*, 1954). It was not until a few years later that suggestions were made about 5-HT as a possible neurotransmitter (Brodie & Shore, 1957).

5-HT is thought to be important in the aetiology of a large number of disease states. Particular emphasis, especially in the public eye, is made on the role of 5-HT in mental illness, and the use of selective serotonin re-uptake inhibitors (SSRIs) such as fluoxetine (Prozac) in their treatment. However, there are many other conditions such as migraine and disorders of the gastro-intestinal and cardiovascular systems in which changes in 5-HT levels, or serotonergic tone, may be important. It is therefore, an important target for medical research to further understanding of not only peripheral, but also central mechanisms.

5-HT synthesis

5-HT belongs to a group of transmitters known as the biogenic amines. This includes: - the catecholamines, which are derived from the amino acid tyrosine; histamine; and the indolamine 5-HT, the precursor of which is the amino acid tryptophan. The term indolamine comes from 5-HT belonging to the group known as indoles, identified by their structure including a five membered ring containing nitrogen, connected to a benzene ring. Two enzymes are required for the synthesis of 5-HT, the rate limiting catalyst being tryptophan hydroxylase, the other being 5-hydroxytryptophan decarboxylase. Interestingly,

this second enzyme is thought to be identical in structure to the enzyme required for the synthesis of the catecholamine, dopamine.

1.4.2 Peripheral 5-HT

Peripherally, 5-HT is found in enterochromaffin cells and platelets throughout the body. In addition, it has been found in significant amounts in the gastrointestinal tract, urinary system, kidneys, liver, heart and in sympathetic nerves. It has also been shown that 5-HT does not cross the blood-brain barrier readily (Lexchin *et al.*, 1977). 5-HT is rapidly broken down in the periphery by oxidative deamination, which is catalysed by monoamine oxidase and produces 5-hydroxyindole acid. This occurs primarily in the endothelial cells of the lungs and liver (Verbeuren, 1989).

1.4.3 Central 5-HT

In the central nervous system the presence of 5-HT was first demonstrated in 1964 (Dahlstrom & Fuxe, 1964). This initial work identified the close association between a high number of serotonergic positive neurones and the raphe nuclei of the medulla and mesopontine levels as mentioned above. 14 years later more modern immunohistochemical techniques have furthered the understanding of the central distribution of 5-HT (Steinbusch *et al.*, 1978). These studies, and others (Steinbusch, 1981) revealed that 5-HT is found in neurones and fibres in defined areas throughout the CNS. In contrast to the periphery, removal of central 5-HT is primarily by re-uptake into the presynaptic terminal via active transport where it is either metabolised by oxidative deamination, or taken back into presynaptic vesicles.

In addition to raphe nuclei, serotonin positive terminals and fibres have been described in significant numbers in caudal regions of the medial NTS (Calza *et al.*, 1985). As mentioned both ascending and descending serotonergic projections between caudal raphe nuclei and the NTS have been described (section 1.2.6). Using a retrograde tracer in rat, serotonergic positive neurones located peripherally, in the nodose ganglia, have also been shown to project to

the NTS (Nosjean *et al.*, 1990), whilst a nodose ganglionectomy caused a reduction in the cardiovascular response to serotonin, suggesting that 5-HT is acting on primary afferent terminals (Merahi *et al.*, 1992). Specifically, as regards the raphe nuclei of the brainstem, the highest density of serotonergic projections to the NTS originates from raphe magnus, with the paragigantocellular nucleus also sending a significant projection (Schaffer *et al.*, 1988). In addition, this study found a minor projection from nucleus raphe dorsalis, and confirmed the presence of non-serotonergic projections from this subnuclei and raphe magnus to NTS. Another point brought up by these, and other authors (Steinbusch, 1984) is the likely high degree of collateralisation of serotonergic neurones, with collateral axons being sent to other known projection targets of raphe nuclei, including hypothalamus (source: nucleus raphe dorsalis) and the intermediate zone of the spinal cord (source: raphe magnus; Steinbusch, 1984).

Other ascending central serotonergic projections of raphe nuclei arise from the more rostrally located subnuclei, travelling via the dorsal and median raphe forebrain tract to terminate in areas including the basal ganglia, central nucleus of the amygdala, thalamus and hippocampus (Azmitia, 1978; Azmitia & Segal, 1978). The midbrain raphe nuclei also project, via separate tracts, to hypothalamic structures such as the paraventricular nucleus and suprachiasmatic nucleus as well as some cortical regions (Azmitia, 1978). A number of the sites of serotonin immunoreactivity have also been implicated in autonomic function (see above), indicating that 5-HT plays a strong role in cardiovascular regulation (see Ramage, 2001).

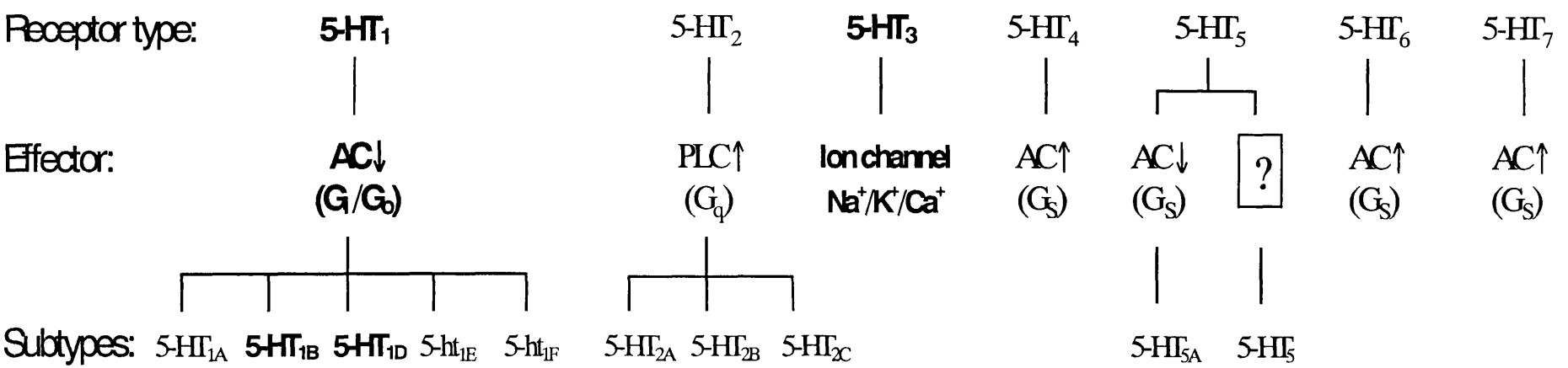
1.5 5-HT receptors

The first major classification of 5-HT receptors was put forward in a review published 17 years ago (Bradley *et al.*, 1986). At this time there were thought to be three families of 5-HT receptor (5-HT_{1-3}), with 5-HT_1 receptors having an autoreceptor role, and 5-HT_2 receptors mediating a depolarising effect. However, even at this time the classification was thought to be far from complete and a further review 8 years post (Hoyer *et al.*, 1994) reported seven 5-HT receptor families (5-HT_{1-7}), a description which still stands now (see figure 1.3). The use of molecular biological techniques, such as recombinant receptor models, has revealed much about the functional characteristics of individual receptor subtypes. The odd one out in terms of function is the 5-HT_3 receptor, which acts as a ligand gated ion channel. The 5-HT_3 receptor has also yet to be split into separate subtypes although their presence is becoming increasingly popular (see below). The remainder of 5-HT receptor subtypes are G-protein coupled metabotropic receptors with a seven transmembrane spanning domain. With new pharmacological tools slowly emerging, which are able to differentiate individual receptor subtypes, the actions of 5-HT receptors both centrally and peripherally are becoming easier to study. However, there still remains a long way to go in producing selective agonists and antagonists for each 5-HT receptor subtype. Contained below is a more detailed analysis of 5-HT receptor subtypes and their central location and function with, for the purposes of this thesis, more emphasis being placed on the 5-HT_1-like receptors and the 5-HT_3 receptor.

Figure 1.3 5-HT receptor classification (Taken from Kennett, 1999)

5-HT receptors are at present divided into 7 classes, based upon their pharmacological profiles, cDNA-deduced primary sequences and signal transduction mechanisms. With the exception of the 5-HT_3 receptor which forms a ligand-gated ion channel, all 5-HT receptors belong to the superfamily of G-protein coupled receptors containing a predicted seven-transmembrane domain structure.

Current classification of 5-HT Receptors



1.5.1 5-HT_1 receptors

There are five 5-HT_1 receptor subtypes identified at present, all of which are negatively coupled to adenylyl cyclase via G proteins. One obvious point to note about 5-HT_1 receptors (see fig. 3) is the absence of 5-HT_{1C} receptors. This can be explained by the reclassification of this receptor subtype as 5-HT_{2C} (see Hoyer *et al.*, 1994). Originally, one of the differentiating points of 5-HT_1 receptors was the ability of 5-CT to produce similar effects to activation of these receptor subtypes (Bradley *et al.*, 1986). However, 5-ht_{1E} and 5-ht_{1F} receptors don't follow this pattern, indeed the 5-ht_{1E} subtype, which is a high affinity binding site in human cortical membranes, has a low affinity for 5-CT (Leonhardt *et al.*, 1989). In addition, the classification of 5-HT_{1B} and 5-HT_{1D} receptors has changed over the past five years or so, the details of which are contained below.

5-HT_{1A} receptors

The 5-HT_{1A} receptor subtype has been widely studied. This work has been primarily done centrally where there is known to be a high density of 5-HT_{1A} receptors. One of the explanations for this interest is due to the discovery of 8-OH-DPAT (Hjorth *et al.*, 1982), a selective agonist, and more recently WAY-100135 and WAY-100635 (Fletcher *et al.*, 1993; Fletcher *et al.*, 1996), the latter of which is still the best selective high affinity antagonist. There are also a host of other substances which have been reported to have a potent affinity at the 5-HT_{1A} receptor, including agonists buspirone (Dourish *et al.*, 1986) and 5-CT (see Bradley *et al.*, 1986), antagonists methiothepin (Fozard *et al.*, 1987), and metergoline (Fozard *et al.*, 1987) and the β-adrenceptor antagonist pindolol (Middlemiss *et al.*, 1977, see Hoyer, 1991). However, these compounds are not particularly selective, for example pindolol has been shown to have affinity for 5-HT_{1B} receptors, and methiothepin is known to interact with α-adrenoceptors and histamine receptors. Indeed even 8-OH-DPAT has been shown to act as a partial agonist at 5-HT_7 receptors (see Barnes & Sharp, 1999). Another important aspect of 5-HT_{1A} receptor pharmacology is the potential variation in

ligand affinity dependent on central location, functional model and species used (see Zifa & Fillion, 1992).

The use of autoradiography has provided a great deal of information as to the pattern of 5-HT_{1A} receptor location throughout the CNS (Pazos & Palacios, 1985; Hoyer *et al.*, 1986). Inter species differences in this receptor subtype are few although the organisation of laminar regions in the cortex and hippocampus of rat has been shown to be different to that found in humans (Burnet *et al.*, 1995). Levels of the 5-HT_{1A} receptor are high in dorsal and median raphe nuclei, together with limbic brain areas such as the hippocampus, central nucleus of the amygdala and cortical regions. Binding sites have also been found throughout the brainstem including two vagal motor nuclei, the DVN and NA (Pazos & Palacios, 1985), and the NTS (Dashwood *et al.*, 1988). However, areas including the basal ganglia and cerebellum contain very few binding sites. Both presynaptic and postsynaptic location of this receptor occurs. At forebrain levels postsynaptically located receptors have been identified whilst at the level of the raphe nuclei presynaptic location on the soma and dendrites of serotonergic neurones has been demonstrated by a number of methods (Miquel *et al.*, 1991, Miquel *et al.*, 1992). These presynaptic receptors are thought to function as somatodendritic autoreceptors, with selective agonists attenuating the ongoing activity of serotonergic neurones in dorsal raphe, whilst antagonism of the receptor reverses this attenuation (Sprouse & Aghajanian, 1986). Another functional characteristic of 5-HT_{1A} receptors is their tonic activation in conscious cats, demonstrated by the ability of the antagonist WAY 100635 to increase serotonergic cell firing (Fornal *et al.*, 1996). Further detail on the functioning of the 5-HT_{1A} receptor can be found in a recent review (see Barnes & Sharp, 1999), including the role that these receptors may play in the modulation of other neurotransmitters such as acetylcholine and noradrenaline. Other topics included are the behavioural responses to 5-HT_{1A} receptor activation, however, one important aspect missing from this review is the important role that this and other 5-HT receptor subtypes play in the modulation of cardiovascular control (see Ramage, 2001; section 1.6).

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

The 5-$HT_{1B/1D}$ receptor classification has undergone a significant change over the last five years. The originally defined 5-HT_{1D} receptor, characterised in bovine tissue 15 years ago (Heuring & Peroutka, 1987), is now known to be a species variant of the rat 5-HT_{1B} receptor (Hartig *et al.*, 1996). The similar central distribution of these two receptor subtypes suggested a close homology and indeed more recently the amino acid sequence has been characterised and reveals they are 93% identical overall (see Hoyer *et al.*, 1994). There was also the identification of a further receptor subtype in higher species with similar pharmacology, which was given the classification 5-$HT_{1D\alpha}$, with the previous subtype classified as 5-$HT_{1D\beta}$, due to the similarities with rat 5-HT_{1B}. Subsequently, the 5-$HT_{1D\alpha}$ receptor was isolated in rat and the recent reassessment of the nomenclature began from there (Hamblin *et al.*, 1992). This now stands as the original 5-HT_{1D} receptor being reclassified as 5-HT_{1B}, however, this must be prefixed with the species subtype, i.e. r5-HT_{1B} for rodent and h5-HT_{1B} for human. Furthermore, the 5-$HT_{1D\alpha}$ receptor found in both rat and human has been reclassified as the 5-HT_{1D} receptor. This has led to a certain degree of confusion in the use of selective pharmacological tools in research involved with these subtypes, possibly requiring a reappraisal of some of the relevant published work.

Receptor Ligands at 5-HT_{1B} and 5-HT_{1D} receptors

One of the recent advances in research into the individual characteristics of function of 5-HT_{1B} and 5-HT_{1D} receptors is the availability of selective ligands. One group of selective agonists at these binding sites is the triptans, including sumatriptan, zolmitriptan and others. Sumatriptan has high affinities specifically at 5-HT_{1B} and 5-HT_{1D} and 5-HT_{1F} receptors (pK_i's of 8.0, 8.3 and 7.6 respectively, see Barnes & Sharp, 1999, value given for h5-HT_{1B}, data for r5-HT_{1B} - pK_i 6.3, see Hoyer *et al.*, 1994) and to a lesser extent 5-HT_{1A} receptors (pK_i 6.1, see Hoyer *et al.*, 1994) and 5-HT_7 receptors (pK_i 6.2, see Hoyer *et al.*, 1994). One of the common attributes of 5-HT_{1B} and 5-HT_{1D} receptor ligands is their high selectivity for the 5-HT_{1A} receptor as well, however, a more recently developed agonist for the 5-HT_{1D} receptor, MK 464, does have pronounced selectivity over the 5-HT_{1A} receptor (Street *et al.*, 1995).

In addition, CP-93,129 is a potent 5-HT_{1B} receptor agonist, which also has high selectivity over other 5-HT receptor subtypes (Koe *et al.*, 1992). Whilst the compound GR127935, reported to be an antagonist with high affinity for both 5-HT_{1B} and 5-HT_{1D} receptor binding sites (Skingle *et al.*, 1993), has been demonstrated to have partial agonist activity at the 5-HT_{1B} receptor (Walsh *et al.*, 1995). It has also been shown to have agonist properties at the 5-HT_{1D} receptor (Starkey & Skingle, 1994; Pauwels & Colpaert, 1995). One ligand whose actions have been confused in light of the reclassification of 5-HT_{1B} and 5-HT_{1D} receptors, is GR55562. This compound is reported to act as an antagonist at 5-$HT_{1D\beta}$ (or according to the new classification h5-HT_{1B}) receptors (Walsh *et al.*, 1995).

In addition, a selective 5-HT_{1B} receptor antagonist (SB-224289) has recently been developed which has been shown to be unable to induce 5-HT release in the frontal cortex (Roberts *et al.*, 1997). Whilst another new compound, the 5-HT_{1D} receptor antagonist BRL-15572, is reported to have a 60-fold higher affinity for the 5-HT_{1D} versus the 5-HT_{1B} receptor. Thus, pharmacological tools are slowly beginning to emerge to differentiate these two similar receptor subtypes in research at least. Finally, ketanserin, a 5-HT_{2A} receptor antagonist, is able to differentiate between 5-HT_{1D} and 5-HT_{1B} receptors, having 15-30 fold selectivity for the 5-HT_{1D} over the 5-HT_{1B} receptor (Kaumann *et al.*, 1994; Pauwels & Colpaert, 1996).

5-HT_{1B} receptors

The highest concentrations of central 5-HT_{1B} receptor distribution are found in basal ganglia, striatum and frontal cortex (Pazos & Palacios, 1985). However, there are many other regions known to contain an appreciable density of this receptor, including the NTS (Manaker & Verderame, 1990) and raphe nuclei (Doucet *et al.*, 1995). As described for 5-HT_{1A} receptors there is a reduction in 5-HT_{1B} mRNA in raphe nuclei after a 5-HT neuronal lesion (Doucet *et al.*, 1995). Evidence for the presence of a 5-HT_{1B} autoreceptor has been demonstrated by strong correlations between the affinity of 5-HT receptor agonists at the 5-HT_{1B} binding site and the potency with which these agonists inhibit 5-HT release (see Middlemiss & Hutson, 1990). Additionally, *in vivo*, the selective 5-HT_{1B} receptor

agonist CP-93,129 has also evoked a marked reduction in 5-HT release (Hjorth & Tao, 1991). Furthermore, there is preliminary evidence for a small population of 5-HT_{1B} somatodendritic autoreceptors in the raphe nuclei (Davidson and Stamford, 1995). However, the ability of 5-HT_{1B} receptors to act as autoreceptors and the tonic level of their activation may well vary according to brain region (see Barnes & Sharp, 1999).

There is growing evidence that the 5-HT_{1B} receptor also acts as a heteroreceptor in some brain regions (Bruinvels *et al.*, 1993; Bruinvels *et al.*, 1994; see Barnes & Sharp, 1999). One electrophysiological study demonstrated a 5-HT_{1B} receptor-mediated reduction in glutamate release in locus coeruleus (Bobker & Williams, 1989), whilst heteroreceptors have also been implicated in the 5-HT-induced attenuation of $GABA_B$ receptor-mediated IPSPs in dopamine neurones in rat midbrain in vitro (Johnson *et al.*, 1992). One other microdialysis study also reported an augmentation of acetylcholine release in rat frontal cortex by endogenous 5-HT mediated by 5-HT_{1B} receptors (Consolo *et al.*, 1996).

Interest in the 5-HT_{1B} receptor is high at the moment after the discovery of the antimigraine properties of the 'triptans' (e.g. sumatriptan). There are currently thought to be two components of the therapeutic effect of these compounds; by activation of 5-HT_{1B} receptors on cerebral arteries resulting in vasoconstriction, or by 5-$HT_{1D/1F}$ receptor mediated presynpatic inhibition of trigeminovascular inflammatory responses, reducing nociception and neurogenic inflammation (see Villalon *et al.*, 2002). The latter of which is supported by the ability of 5-HT_{1B} selective ligands to attenuate dural plasma protein extravasation evoked by trigeminal ganglion stimulation in mice, a response absent in 5-HT_{1B} receptor knockout mice (Yu *et al.*, 1996). However, the success of these compounds in the treatment of migraine is unlikely to be solely due to 5-HT_{1B} receptor activation, but probably includes the activation of other 5-HT_1 receptor subtypes e.g. the 5-HT_{1F} receptor which has been implicated due to the affinity of the triptans for this receptor subtype. 5-HT_{1B} receptors are also thought to have an important role in a number of other physiological and pathological states, such as locomotion and hypothermia (see Barnes & Sharp, 1999).

5-HT$_{1D}$ receptors

As mentioned previously the originally defined 5-HT$_{1D}$ receptor is now considered a species homologue of the 5-HT$_{1B}$ receptor and what is now termed the 5-HT$_{1D}$ receptor of rat and human (and other species) was discovered using gene sequencing methods (Hartig *et al.*, 1996; Hamblin *et al.*, 1992). Using predominantly in situ hybridisation techniques 5-HT$_{1D}$ receptor levels have been shown to be highest in areas such as the basal ganglia, hippocampus, parts of the cortex, dorsal raphe and locus coeruleus (Hoyer *et al.*, 1994). However, there has been shown to be low densities of 5-HT$_{1D}$ receptors located throughout the CNS, including the NTS, and it has been suggested that these are primarily located on the axon terminals of both 5-HT and non-5-HT neurones (see Barnes & Sharp, 1999).

Due to the problems in pharmacologically distinguishing between 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (see above), there have been difficulties in classifying individual receptor functions. An example of this is shown in the conflicting results of studies using 5-HT$_{1B}$ knock-out mice. One study showed that the reduction of 5-HT release by administration of 5-HT receptor agonists was maintained in this model (Pineyro *et al.*, 1995), whilst another, which used 5-HT$_{1B/1D}$ receptor agonists, found this response to be absent (Trillat *et al.*, 1997). Both these studies examined hippocampus and cortex, although the latter study also examined dorsal raphe, but their conflicting results clouded confirmation of the presence of a 5-HT$_{1D}$ autoreceptor. A more recent study in guinea pig and human cortex, using more selective ligands, has shown that the 5-HT autoreceptor can be blocked by the h5-HT$_{1B}$ receptor antagonist SB-216641, but not by the 5-HT$_{1D}$ receptor antagonist BRL-15572 (Schlicker *et al.*, 1997). However, this evidence does not rule out the possibility of 5-HT$_{1D}$ autoreceptors in other central regions.

The identification of a 5-HT$_{1D}$ heteroreceptor has faced the same problem but the emergence of new selective ligands is helping to differentiate and identify the functions of this subtype. A number of studies have demonstrated the ability of 5-HT$_{1D}$-like receptors to modulate the release of other

neurotransmitters, including the inhibition of glutamate release from rat cerebellar synaptosomes, mediated by 5-HT_{1D} receptors (Maura & Raiteri, 1996), and the inhibition of GABA release in human cortex by 5-HT_{1D}-like receptors (Feuerstein *et al.*, 1996). Thus, the presence of 5-HT_{1D} heteroreceptors seems likely, although their distribution and inter-species differences have yet to be clarified.

5-ht_{1E} and 5-ht_{1F} receptors

5-ht_{1E} receptors were first isolated by their response to [^{3}H]5-HT in the presence of 5-CT, which masked the responses of activation of other 5-HT receptor subtypes (5-HT_{1A}, 5-HT_{1D} and others; Leonhardt *et al.*, 1989). The insensitivity of this receptor subtype to 5-CT is one of its novel aspects, although there are at present no selective ligands. The highest central distribution of the receptor is seen in the caudate putamen, the central nucleus of the amygdala, the globus pallidus and frontal cortex regions (Hoyer *et al.*, 1994). Human brain binding studies have also suggested that the 5-ht_{1E} receptor may represent up to 60% of all 5-HT_1 receptor binding. The lack of ligands to date has prevented any functional characteristics of this receptor to be classified to date.

More is known about the 5-ht_{1F} receptor as there is currently a selective agonist, LY334370, which has been shown to have high selectivity over 5-$HT_{1B/1D}$ receptor subtypes (pK_i 8.8 for 5-ht_{1F} vs. pK_i 6.9 for both 5-$HT_{1B/1D}$ receptors, see Barnes & Sharp, 1999). This is important because another ligand known to have a high affinity for the 5-ht_{1F} binding site is sumatriptan. As mentioned previously sumatriptan has a nearly equivalent affinity for these three receptor subtypes (5-HT_{1B}, 5-HT_{1D} and 5-ht_{1F}), which is important in research concerning the antimigraine properties of the triptans. Indeed, the presence of 5-ht_{1F} mRNA in trigeminal ganglia initiated experiments that have found that the selective agonist LY334370 may have important antimigraine properties (Shepheard *et al.*, 1999), suggesting an important role for these receptors in this condition. Other areas thought to contain 5-ht_{1F} receptors include the hippocampus, striatum and cortex, and it is thought to play a role in certain behavioural states, as well as possibly acting as an autoreceptor (see Barnes & Sharp, 1999).

1.5.2 5-HT_2 receptors

The three 5-HT_2 receptor subtypes, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} are thought to act via a phosphoinositol hydrolysis signal transduction mechanism utilising the G_q GTP binding protein, however, this has yet to be proven for 5-HT_{2B} receptors in native tissue (Hoyer *et al.*, 1994). 5-HT_2 receptor ligands are currently being assessed as a possible treatment of various psychological disorders (Baxter *et al.*, 1995). In addition, 5-HT_2 receptors have also been implicated in playing a role in the maintenance of central sympathetic outflow (see Ramage, 2001), including actions mediated via the RVLM (Mandal *et al.*, 1990), described in further detail in section 1.6.3.

5-HT_{2A} receptors

There are a large number of 5-HT_{2A} receptor ligands although the majority have high affinity for other receptors e.g. ketanserin, a 5-HT_{2A} receptor antagonist, has high selectivity over other 5-HT_2 receptor subtypes, but has high affinity at the 5-HT_{1D} receptor (as mentioned previously), and is also known to be a potent α_1 adrenoceptor antagonist (Van Neuten *et al.*, 1981). Other compounds known to have affinity at the 5-HT_{2A} receptor binding site include DOI and LSD, as agonists (see Baxter *et al.*, 1995), and the relatively new antagonist MDL-100907 (Sorensen *et al.*, 1993). Histological studies have revealed the distribution of central 5-HT_{2A} receptors within the basal ganglia, claustrum and cortex, although there is a high density of peripherally located receptors in, particularly, gastrointestinal and urinary areas (Hoyer *et al.*, 1994). In addition, autoradiographic studies have revealed that NTS neurones have binding sites for 5-HT_2 receptors (Dashwood *et al.*, 1988). Activation of 5-HT_2 receptors has been shown to produce head shaking in rodents, and this was subsequently shown to be mediated by 5-HT_{2A} receptors using selective antagonists (Schreiber *et al.*, 1995).

5-HT_{2B} receptors

There are few ligands selective for the 5-HT_{2B} receptor, although α-methyl 5-HT is a full agonist at this binding site, with selectivity over the 5-HT_{2A} and 5-HT_{2C} sites. In addition, SB-204741 is reported to be an antagonist selective for the 5-HT_{2B} receptor binding site (see Baxter *et al.*, 1995). This 5-HT_2 receptor is thought to be present in much higher densities in the periphery than in the central nervous system. Outside of the CNS these receptors are thought to mediate contractions of stomach fundus, specifically in rat, as well as relaxations of the jugular vein, specifically in the rat and cat model (see Baxter *et al.*, 1995). A recent study has highlighted the central distribution of these receptors (Duxon *et al.*, 1997a), and activation of these receptors has been shown to cause anxiolysis (Duxon *et al.*, 1997b), hyperphagia and reduced grooming (Kennett *et al.*, 1997). Centrally the receptor has been demonstrated to be located in the septum, hypothalamus, cerebellum and central nucleus of the amygdala (Duxon *et al.*, 1997a), in addition to the NTS (Dashwood *et al.*, 1988).

5-HT_{2C} receptors

Much of the original research into the actions of the 5-HT_{2C} receptor has employed ligands such as mCPP, a non-selective agonist, and ritanserin, a non-selective antagonist. However, more selective compounds are becoming available, such as SB-242084 (Kennett *et al.*, 1997; see below), which are better able to differentiate between individual 5-HT_2 receptor subtypes. In contrast to 5-HT_{2B} receptors, 5-HT_{2C} receptors are found almost exclusively within the central nervous system. One of the defining anatomical characteristics of this subtype is the high density found in the choroid plexus, leading to suggestions that it plays a role in cerebrospinal fluid production. In addition, significant numbers were found in the limbic system, the basal ganglia, throughout the cortex (Palacios *et al.*, 1991) and in the NTS (Dashwood *et al.*, 1988). The selective 5-HT_{2C} antagonist SB 242084 has been shown to have anxiolytic-like actions in animal models (Kennett *et al.*, 1997), an action which has been suggested to explain part of the mode of action of SSRI's, which desensitise 5-HT_{2C} receptors (Kennett *et al.*, 1993).

1.5.3 5-HT_3 receptors

The 5-HT_3 receptor has been recognised for nearly half a century, leading to its cloning in 1991, and as such is one of the more researched and understood of the 5-HT family. Functionally, the 5-HT_3 receptor is a ligand gated ion channel, which is made up of a pentameric cation channel selectively permeable to sodium, potassium and calcium ions, resulting in depolarisation (Hoyer *et al.*, 1994). There is inter species variation both in the receptor formation and distribution, and, due to variations in ligand affinities, the receptor has been suggested as having multiple binding sites (Steward *et al.*, 1995).

There are a host of 5-HT_3 receptor ligands currently available. However, 5-HT_3 receptors are closely related to other members of the ligand gated ion channel family, particularly the nicotinic receptor (see Ortells & Lunt, 1995). As a result a number of compounds are thought to interact allosterically with the receptor such as ethanol and some anaesthetic agents (Parker *et al.*, 1996). However, there is still a wide availability of potent 5-HT_3 receptor antagonists including granisetron (Nelson & Thomas, 1989; Sanger & Nelson, 1989), ondansetron (Butler *et al.*, 1988) and MDL-72222 (Fozard, 1984). Agonists at the 5-HT_3 receptor include phenylbiguanide (Richardson *et al.*, 1985) and chlorophenylbiguanide (Kilpatrick *et al.*, 1990), the latter of which has the highest affinity. An important characteristic of these 5-HT_3 receptor agonists is their ability to block dopamine uptake (see Hoyer *et al.*, 1994).

5-HT_3 receptors have received clinical recognition in the use of 5-HT_3 receptor antagonists as antiemetic agents. Increased 5-HT_3 receptor activation is thought to underlie the nausea and vomiting induced by the use of radiotherapy and chemotherapy in the treatment of cancer. Importantly this anti-emetic characteristic has not yet been defined as being mediated by 5-HT_3 receptors in peripheral or central locations, or at combination of both (see Leslie *et al.*, 1994). The highest density of central 5-HT_3 receptors is located in the brainstem, particularly in more dorsal areas such as the DVN, area postrema and NTS, suggesting an important role in autonomic regulation for this receptor (see section 1.6.4). In addition, these three nuclei are known to play a crucial

role in the production of a vomiting reflex, therefore it seems likely that 5-HT_3 receptors located there are activated by these antiemetic agents. However, there is also a high density of 5-HT_3 receptors in the gastrointestinal tract itself (Glatzle *et al.*, 2002) and on pre- and postganglionic autonomic neurones (see Leslie *et al.*, 1994), which are also likely to be activated by these agents.

Other peripheral locations of 5-HT_3 receptors include large areas of the peripheral nervous system. They have been shown to be located within the enteric nervous system as mentioned above, on sensory nerves, such as somatic primary afferent fibres, where intradermal application of 5-HT causes pain, mediated by 5-HT_3 receptors (Richardson *et al.*, 1985). In addition, they are found on pulmonary C-fibre afferents, where activation of 5-HT_3 receptors activates the cardiopulmonary reflex, an effect which has been used to characterise new 5-HT_3 receptor antagonists (see Leslie *et al.*, 1994). Another significant location for these receptors in the periphery is within the dorsal and ventral horn of the spinal cord (Morales *et al.*, 1998).

Centrally the 5-HT_3 receptor has also been found in the hippocampus, the central nucleus of the amygdala, and regions of the cortex. Further study found an association between 5-HT_3 receptors location and the location of GABAergic interneurones in regions CA1-CA3 of the hippocampus (Morales & Bloom, 1997). Cholecystokinin (CCK) has also been demonstrated to be co-localised with 5-HT_3 receptors in cortical, hippocampal, and amygdaloid areas using double labelling methods (Morales & Bloom, 1997). Further, 5-HT, acting at 5-HT_3 receptors, is also thought to modulate the release of a number of other neurotransmitters, including glutamate (see Lawrence & Jarrott, 1996).

Systemic effects evoked by 5-HT_3 receptor ligand administration include modulation of cardiac activity, vasodilatatory effects, disturbances in lung and intestinal function and even anxiolysis and enhanced cognitive function (see Hoyer *et al.*, 1994), supporting the idea that the 5-HT_3 receptor serves a crucial role in both the central nervous system and the periphery.

1.5.4 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 receptor subtypes

5-HT_4 receptors

The 5-HT_4 receptor is positively coupled to adenylyl cyclase, and there are highly selective antagonists currently available, such as SB-204070 (Kennett *et al.*, 1997), although, to date, 5-HT_4 receptor agonists do not have significant selectivity over 5-HT_3 receptors. The 5-HT_4 receptor has been shown to be located centrally in areas including the nucleus accumbens, basal ganglia and striatum in rat brain (Patel *et al.*, 1995). It has been associated with dopamine function, and co-localised, using double labelling methods, with cholinergic and GABAergic interneurones, together with a suggested location on GABAergic fibres projecting to the substantia nigra (Patel *et al.*, 1995). Indeed, 5-HT_4 receptor activation has been shown to facilitate acetylcholine release in rat cortex (Consolo *et al.*, 1994). This receptor has also been demonstrated to modulate 5-HT release in rat hippocampus (Ge & Barnes, 1996). Another study has also reported anxiolytic-type actions of selective 5-HT_4 receptor antagonists, including SB-204070 (Kennett *et al.*, 1997). Peripherally, modulation of the myenteric plexus by 5-HT_4 receptors has been demonstrated in animal models, leading to suggestions for a role for this receptor in irritable bowel syndrome (Bockaert *et al.*, 1994). Other actions of 5-HT_4 receptors include positive iono- and chronotropic effects on the heart, involvement in bladder contraction, cortisol secretion, activation of vagal afferents and possible secretion from rat mucosa (see Bockaert *et al.*, 1994).

5-HT_5 receptors

5-HT_5 receptor subtypes 5-HT_{5A} and 5-HT_{5B} were first described in rat using brain cDNA libraries (Erlander *et al.*, 1993). The 5-HT_{5A} receptor has been shown to be negatively linked to adenylyl cyclase and may function as a terminal autoreceptor in mouse cortex (Carson *et al.*, 1995), whilst less is known about the 5-HT_{5B} receptor mechanism of action. 5-HT_{5A} receptor distribution has been identified in rat cerebellum, hippocampus and cortex, whilst 5-HT_{5B} receptors are thought to be located, in mouse brain at least, in hippocampus, habenula and dorsal raphe (Matthes *et al.*, 1993). Little is known

about the functional effects of 5-HT_5 receptor activation in animal models, or the changes seen in knock-out mice (see Barnes & Sharp, 1999).

5-HT_6 receptors

The 5-HT_6 receptor is another member of the 5-HT family about which little is known. It is positively linked to adenylyl cyclase via a G protein (Sebben *et al.*, 1994), and functional studies using antisense techniques produced a behavioural syndrome of yawning, stretching and scratching (Bourson *et al.*, 1995). Interestingly antipsychotic and antidepressant agents such as clozapine and clomipramine have been demonstrated to act as antagonists at the 5-HT_6 receptor (Kohen *et al.*, 1996). Central distribution of 5-HT_6 receptor mRNA includes striatum, nucleus accumbens, hippocampus, the central nucleus of the amygdala and cortical areas (Kohen *et al.*, 1996).

5-HT_7 receptors

The 5-HT_7 receptor is the most recently cloned receptor and as with the 5-HT_6 receptor is positively coupled to adenylyl cyclase. There are a number of receptor agonists developed for other 5-HT receptor subtypes, including 8-OH-DPAT, which have affinity at the 5-HT_7 receptor binding site. Furthermore, ritanserin and metergoline are antagonists with a high affinity for this receptor; although, at present there are no selective ligands. Many responses previously attributed to other 5-HT receptor subtypes are now thought to be due to the 5-HT_7 receptor (Eglen *et al.*, 1997). In rat and guinea pig, the density of 5-HT_7 receptor distribution is relatively consistent and concentrates primarily in hypothalamus, thalamus, hippocampus and brainstem, and in man, peripherally, it has been described in coronary artery (Hoyer *et al.*, 1994). Smooth muscle relaxation is one functional characteristic of this receptor subtype, whilst activation of the subtype may cause prolonged hypotension (Eglen *et al.*, 1997). 5-HT_7 receptor activity may also mediate changes in circadian rhythm; a theory proposed after ligands with high affinity at this site increased neuronal firing in hypothalamic cell cultures (see Hoyer *et al.*, 1994).

1.6 Central cardiovascular actions for 5-HT receptors

A detailed review on the role of 5-HT receptors in cardiovascular regulation has been recently published (Ramage, 2001). Taken together, there is ample evidence described above suggesting an important regulatory function for the central serotonergic system in autonomic control, an action proposed over 20 years ago (Kuhn *et al.*, 1980). The location of both serotonergic neurones and 5-HT receptor subtypes in areas known to modulate and direct the cardiovascular system supports this. Further, the systemic effects of central 5-HT administration on blood pressure and other cardiovascular variables, although not necessarily clear cut, are another important indicator (see Kuhn *et al.*, 1980). What is now becoming clear is that the interaction between 5-HT and both parasympathetic and sympathetic branches of the autonomic nervous system is a complex system, involving most, or all, of the individual 5-HT receptor subtypes. It must be stated also that peripherally serotonin plays a strong role in cardiovascular functions, most notably in vascular tissue (see Marwood & Stokes, 1984), but also in other organs under autonomic control, such as the bladder (see Ramage, 2001).

The varied and sometimes conflicting cardiovascular responses achieved by central administration of 5-HT is now thought to be due to a number of factors including:- activation of different 5-HT receptor populations; inter-species differences; dose of 5-HT administered; site of administration; use or not of anaesthetic agents and even type of anaesthetic used. One early study described a pressor effect and bradycardia associated with 5-HT administration intra-cerebro ventricularly (i.c.v.) in the conscious rat, which could be blocked by pre-treatment with a non-selective 5-HT receptor antagonist (Sukamoto *et al.*, 1984). It has also been reported that changes in heart rate caused by i.c.v. administration of 5-HT are dose related, with lower doses causing tachycardia and higher doses resulting in bradycardia (Dedeoglu & Fisher, 1991). These dose-related issues may be a result of increased diffusion of 5-HT to brain areas distal to the administration site at higher doses (Coote, 1990).

Specifically, the administration of 5-HT into the NTS has also been shown to produce a variety of responses, perhaps unsurprisingly due to the diverse integrative functions of this nucleus. Low doses produced a depressor and bradycardic response in anaesthetized (Laguzzi *et al.*, 1984) and conscious (Callera *et al.*, 1997a) rat models, whilst higher doses have produced both an increase (Merahi *et al.*, 1992) and a decrease (Feldman & Galliano, 1995) in blood pressure. Another study in rats demonstrated that 5-HT injected into the NTS always produced a depressor and bradycardic response which was enhanced in spontaneously hypertensive rats (Okada & Bunag, 1994), a pattern that is mirrored by glutamate (see Okada & Bunag, 1994). These responses are likely to involve more than one 5-HT receptor subtype due to the demonstrated location of a number of these subtypes within the NTS (at varying density, see above). Furthermore clear conclusions are difficult to make due to the limitations of microinjection studies in not being able to target functionally identified neurones.

However, the development of selective compounds, and more refined techniques, such as in vivo extracellular recordings, is significantly furthering the understanding of which subtypes of 5-HT receptor and which central areas are involved in these responses. Below are descriptions of some of the recent advances into the functioning of the central serotonergic system with respect to cardiovascular regulation, and where the current understanding lies.

1.6.1 5-HT_{1A} receptors

Central activation of 5-HT_{1A} receptors by i.c.v. administration of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT has been shown to cause a depressor effect and bradycardia in all species tested (see McCall & Clement, 1994), a response that was mirrored in conscious and spontaneously hypertensive rats (Dreteler *et al.*, 1990; Gradin *et al.*, 1985). In cats a differential effect of central 5-HT_{1A} receptor activation on sympathetic outflow has now been described (Ramage *et al.*, 1988). This was demonstrated by the absence of a vasodilation of the hindlimb and a delay in the activation of thoracic preganglionic neurones, after a 5-HT_{1A} agonist-evoked hypotension. Further, the depressor response to

5-HT_{1A} receptor activation has implicated the major sympathoexcitatory area (located in the RVLM) as a possible zone for this serotonergic modulation (Gillis *et al.*, 1989; Laubie *et al.*, 1989). In addition, one study reported no reduction in sympathetic outflow to the heart after activation of 5-HT_{1A} receptors in the region of the RVLM (King & Holtman, 1989), again supporting further differential functions for this subtype. One explanation for this latter observation is the proposed topographical organisation of RVLM neurones with respect to their end target (Dampney, 1994), suggesting that 5-HT_{1A} receptors may only modulate outflows to specific vascular beds.

In the anaesthetized rat, sympathoexcitatory effects of 5-HT_{1A} receptor activation have also been described following low dose i.c.v. administration of a selective agonist (Anderson *et al.*, 1992). This is thought to be mediated by increased release of adrenaline, although due to the presence of this response in the conscious model, some doubt about this mechanism remains (see Ramage, 2001). One possible important distinction in these apparently opposing effects of 5-HT_{1A} receptor activation is the different central nuclei from which they can be evoked. Depressor responses have been described from the raphe magnus and pallidus, the RVLM and even the dorsal raphe (see McCall & Clement, 1994), whereas pressor responses have been reported from raphe obscurus, the preoptic area but not the paraventricular nucleus in the rat (see Ramage, 2001). With respect to the maintenance of sympathetic outflow i.v. administration of a selective 5-HT_{1A} receptor antagonist was unable to significantly alter resting blood pressure in the anaesthetized cat (Ramage & Mirtsou-Fidani, 1995), indicating that, under these conditions, 5-HT_{1A} receptors capable of modulating the sympathetic nervous system are not under tonic activation.

The ability of 5-HT_{1A} receptors to modulate the parasympathetic nervous system (Shepheard *et al.*, 1994) can be partially explained by the demonstrated tonic activation of 5-HT_{1A} receptors during cardiac vagal reflex activation in the nucleus ambiguus of the cat (Wang & Ramage, 2001). This study confirmed data that described the attenuation of reflex responses to cardiopulmonary receptor activation and upper airway receptor activation by 5-HT_{1A} receptor

blockade (see Dando *et al.*, 1998). Indeed, application of a 5-HT_{1A} receptor agonist into the IVth ventricle was shown to increase cardiac vagal tone (Shepheard *et al.*, 1994). The site of this modulation by 5-HT_{1A} receptors was assumed to be close to the location of the vagal preganglionic neurones, i.e. the DVN and NA in the medulla. In support of this a study in the rat has demonstrated the predominant decrease in the activity of these neurones by 5-HT_{1A} receptor ligands (Wang *et al.*, 1995). In addition, a recent study examining functionally identified neurones of the nucleus ambiguus in anaesthetized cats confirmed the modulation of cardiac vagal preganglionic neurones (CVPNs) by 5-HT_{1A} receptors using the highly selective antagonist WAY-100635 (Wang & Ramage, 2001). Interestingly, activation of 5-HT_{1A} receptors was able to excite these neurones, which, considering the mechanism of action these of receptors, must occur via interneurones due to the known hyperpolarising action of 5-HT_{1A} receptors. This has been proposed to be a result of 'disinhibition', which involves the switching off of GABAergic interneurones by activation of 5-HT_{1A} receptors, resulting in reduced inhibitory drive to the CVPNs and their subsequent increased activity (see Ramage, 2000). A GABAergic modulation of CVPNs has been demonstrated before (DiMicco *et al.*, 1979), and in respect of the serotonergic modulation via 5-HT_{1A} receptors this is not restricted to parasympathetic control of the heart, but also includes modulation of parasympathetic control of the bladder, airways and possibly the eye (see Ramage, 2000). Furthermore, although this data indicates an indirect serotonergic modulation of CVPNs there is some anatomical evidence for fibres projecting to vagal preganglionic neurones in the DVN (Sykes *et al.*, 1994) which may well involve the activation of other serotonergic receptor subtypes (see below).

In addition, a role for 5-HT_{1A} receptors in NTS has also been implicated (Wang *et al.*, 1997), with 8-OH-DPAT affecting the ongoing activity of almost all vagally identified NTS neurones, although roughly half these neurones were excited, and half were inhibited by this receptor ligand. Interestingly, out of five neurones that were functionally identified as receiving cardiac vagal afferent input, four were excited by the 5-HT_{1A} receptor agonist. Although this demonstrates a strong role for this subtype in the NTS, the high affinity of

8-OH-DPAT for the 5-HT_7 receptor, cannot be ignored (pK_i 8.7 for 5-HT_{1A}, pK_i 7.4 for 5-HT_7, see Hoyer, 1994).

1.6.2 5-$HT_{1B/1D}$ receptors

Activation of 5-HT_{1D} receptors by i.c.v. injection of selective receptor ligands has been shown to cause a depressor response, although there is also some evidence demonstrating that activation of central 5-HT_{1B} receptors might causes the opposite, a rise in blood pressure in anaesthetized rats (Gallacher & Ramage, 1984). In the presence of WAY-100635, sumatriptan (a 5-$HT_{1B/1D}$ receptor agonist) evoked a depressor response associated with hindquarter vasodilation, whilst the 5-HT_{1B} receptor agonist CP-93,129 caused a small tachycardia and pressor response. The sumatriptan response was sensitive to selective 5-HT_{1D} receptor antagonism, and was also delayed, consistent with the increased diffusion time needed for an i.c.v. injection to reach hindbrain regions.

In addition, preliminary studies have revealed a similar differential effect of 5-HT_{1B} and 5-HT_{1D} receptor activation in the NTS, with the ligand CP-93,129 increasing vagal afferent evoked activity and sumatriptan attenuating this activity (Wang *et al.*, 1998b). A role for 5-$HT_{1B/1D}$ receptors in the modulation of afferent relay and integration within the NTS therefore seems likely, although they may also play a role in other autonomic nuclei of the brainstem.

1.6.3 5-HT_2 receptors

There is now a large amount of evidence implicating 5-HT_2 receptor subtypes in the control of blood pressure (see Ramage, 2001). Original interest in a role for these receptors was triggered by the ability of ketanserin to induce central sympathoinhibition, however, this has been demonstrated to be largely due to the blockade of central α_1-adrenoceptors (see McCall & Clement, 1994). However, the selective 5-HT_2 receptor agonist DOI , administered i.c.v., has recently been shown to produce a profound sympathoexcitation (Anderson *et al.*, 1995), and this has confirmed an important role for this receptor subtype.

One significant aspect when investigating the role of central 5-HT_2 receptors is the response to peripheral activation of these receptors. Experiments have shown that i.v. administration of DOI causes a generalised sympathoexcitation, a pressor response, but no tachycardia. Of these, a vasoconstriction-induced rise in blood pressure and bronchoconstriction has been shown to be mediated by peripheral 5-HT_2 receptors (see Ramage *et al.*, 1993). These peripheral responses must therefore be taken into account when studying centrally mediated effects of 5-HT_2 receptor activation.

Activation of 5-HT_2 receptors in the rostral ventrolateral medulla (RVLM) produces a pressor response but no change in heart rate (Mandal *et al.*, 1990), explained by the differential effects of specific populations of RVLM neurones capable of modulating ventricular contraction independent of heart rate. However, due to the rise in blood pressure, the increased force of contraction could be due to a phenomenon known as the Anrep effect which describes the association between these two variables (see Ramage, 2001). Experimentation has revealed that administration of 5-HT_2 receptor ligands to the ventral surface of the brainstem (adjacent to the RVLM), in a paced heart preparation produced no increase in ventricular contraction (Ramage & Daly, 1998). This has led to the conclusion that 5-HT_2 receptors in the RVLM mediate different effects on sympathetic premotor neurones projecting to the heart, than to other sympathetic premotor neurones, a characteristic also seen with the 5-HT_{1A} receptor ligand 8-OH-DPAT (see Ramage, 2001).

Activation of central 5-HT_2 receptors by i.c.v. administration of selective agonists has also now been shown to cause the release of vasopressin (Anderson *et al.*, 1991; Pergola *et al.*, 1993; Knowles & Ramage, 1999). This vasopressin release has been shown to be induced by the activation of, specifically, 5-HT_{2A} receptors and involves the stimulation of a central angiotensinergic pathway (Knowles & Ramage, 1999). In addition, the expected 5-HT_{2A} receptor-mediated sympathoexcitation is inhibited by a feedback system indirectly or directly mediated via the released vasopressin and, furthermore, this sympathoinhibitory feedback system involves the

activation of 5-HT_{2B} receptors, as it is sensitive to blockade by the selective 5-HT_{2B} receptor antagonist SB204741 (Knowles & Ramage, 1999). Whether the 5-HT_{2B} receptor is activated by the vasopressin-induced baroreceptor activation or directly by a central action of vasopressin remains to be determined. Surprisingly, selective central 5-HT_{2B} receptor activation, via i.c.v. administration of a selective agonist, has also been shown to cause renal sympathoexcitation and a small fall in blood pressure (Knowles & Ramage, 2000). A role for central 5-HT_2 receptors in the mechanisms by which the brain regulates blood volume therefore clearly exists, although more research is required to fully understand the functions of the individual receptor subtypes in this mechanism. The current understanding is that the central regions thought to mediate these effects of midbrain 5-HT_2 receptor activation include the paraventricular nucleus, the anterior hypothalamus and preoptic area, and the subfornical organ (see Ramage, 2001). This would explain the successful activation of these receptors within relatively short onset times using i.c.v. injections, it is also corroborated by the known activation of AT_1 receptors within the subfornical region by vasopressin (Iovino & Steardo, 1985). These areas are known to receive serotonergic projections from dorsal and median raphe nuclei (see above).

As mentioned previously 5-HT_2 receptor binding sites have also been described in the brainstem, specifically the NTS (Dashwood, 1988). Electrophysiological studies examined the effects of the 5-HT_2 receptor agonist DOI on the excitability of NTS neurones (Sévoz-Couche *et al.*, 2000a). Variable effects were described upon 5-HT_2 receptor activation, although the use of more selective ligands, has now demonstrated that 5-HT_{2B} receptors are more likely to mediate excitatory responses, whilst 5-HT_{2C} receptors appear to mediate inhibitory responses in the NTS (Sévoz-Couche et al., 2000b).

1.6.4 5-HT_3 receptors

As described previously there is a high density of 5-HT_3 receptors within the brainstem, suggesting an important role for these receptors in the complex interaction of brainstem nuclei. These receptors have been shown to be predominantly located presynaptically within the NTS (Pratt & Bowery, 1989; Leslie *et al.*, 1990), and activation of these receptors using microinjections and intracisternal (i.c.) injections has been shown to affect both resting cardiovascular variables and also baroreflex and cardiopulmonary reflex-evoked responses (see below). The hypotensive effects evoked by microinjection of low (pmol) doses of 5-HT have been suggested to be partially mediated by activation of 5-HT_3 receptors since it is mimicked by application of 5-HT_3 receptor agonists and attenuated by selective antagonists (Merahi *et al.*, 1992; Merahi & Laguzzi, 1995). In addition, a number of studies in the NTS have also reported that microinjection of a 5-HT_3 receptor agonist abolishes the vagally-mediated fall in heart rate caused by baroreflex (Merahi *et al.*, 1992; Callera *et al.*, 1997b), chemoreflex (Sévoz *et al.*, 1996; Callera *et al.*, 1997b) and cardiopulmonary reflex (Sévoz *et al.*, 1996). However, in contrast other work has described an attenuation in the vagally-mediated bradycardia caused by upper airway and cardiopulmonary reflex activation after i.c. administration of a 5-HT_3 receptor antagonist (Dando *et al.*, 1995; Pires *et al.*, 1998). The apparent conflicts in these results have yet to be defined experimentally and remain, to date, unanswered.

Other *in vitro* research has demonstrated the ability of 5-HT_3 receptor activation to affect both spontaneous and evoked synaptic transmission as well as causing a postsynaptic depolarisation in neurones throughout the NTS (Glaum *et al.*, 1992). Interestingly the magnitude of the depolarising response to 2-CH_3-5-HT was dependent on the concentration administered, with higher concentrations mediating a smaller depolarising effect. This patch clamp study also stated that 5-HT_3 receptors might produce localised depolarisation of the presynaptic terminal mediated via axonally or presynaptically located receptors, suggested to be present on glutamatergic and GABAergic neurones of the NTS. A further study confirmed this, at least with glutamate, when the 5-HT_3 receptor

agonist phenylbiguanide, administered into the NTS, increased levels of glutamate release, a response shown to be sensitive to antagonism with the selective ligand ondansetron (Ashworth-Preece *et al.*, 1995). Furthermore, ionotropic glutamate receptor blockade in the NTS abolishes the bradycardia, hypotension and apnoea evoked by the activation cardiopulmonary afferents (Bonham *et al.*, 1993; Vardhan *et al.*, 1993), implicating 5-HT_3 receptors in the cardiovascular reflex modulation of heart rate.

As a result of some of the above work suggestions have been made for two populations of 5-HT_3 receptor in the NTS. Firstly, a postsynaptic population, which mediates a pressor response, located on, and able to depolarise, GABAergic neurones. Secondly, a population of presynaptically located 5-HT_3 receptors which depolarise glutamatergic presynaptic terminals facilitating glutamate release, and subsequently mediating a depressor response (see Lawrence & Jarrott, 1996). However, at the cellular level these, and other, proposed mechanisms have yet to be clarified, although it has been demonstrated that the ongoing activity of the majority of vagally identified NTS neurones is increased after ionophoretic administration of the selective 5-HT_3 receptor agonist phenylbiguanide (Wang *et al.*, 1997).

Adjacent to the NTS, the DVN also contains 5-HT_3 receptors and electrophysiological extracellular recordings have demonstrated an excitatory action for these receptors on DVN neurone activity, mediated by both pre- and postsynaptic receptors (Wang *et al.*, 1998a; Albert *et al.*, 1996). Indeed, 5-HT_3 receptor activation of DVN neurones has been shown to be sensitive to glutamate receptor antagonists, demonstrating a presynaptic location of 5-HT_3 receptors on glutamatergic terminals (Wang *et al.*, 1998a), potentially supporting theories for the same mechanism in NTS. Finally, a recent paper demonstrated that CVPNs of the nucleus ambiguus are also sensitive to 5-HT_3 receptor activation in the anaesthetized cat (Wang & Ramage, 2001). As demonstrated in the DVN and NTS (to a certain extent) these neurones demonstrated a predominantly excitatory action for 5-HT_3 receptors.

1.6.5 5-HT_4 receptors

A recent paper has reported that the cAMP-protein kinase A pathway modulation of the cardiopulmonary reflex at the level of the NTS is mediated by the activation of 5-HT_4 receptors (Edwards & Paton, 1999). This study described, in anaesthetized rats, an attenuation of the bradycardia and tachypnea produced in response to cardiopulmonary afferent activation, by the prior microinjection of the non-specific 5-HT receptor agonist 5-methoxytryptamine. This response was blocked by microinjection into the NTS of a 5-HT_4 selective antagonist, demonstrating that 5-HT acting at 5-HT_4 receptors is able to attenuate the reflex bradycardic component of the cardiopulmonary reflex. However, this selective antagonist did not alter baseline values for blood pressure or heart rate, suggesting that 5-HT_4 receptors are not tonically active in this preparation. Other functions for this receptor subtype in relation to cardiovascular regulation remain to be determined.

1.7 Aims of this thesis

Experiments were carried out to:-

- examine, in cats, the response of respiratory modulated CVPNs in the region of the NA to stimulation of the pulmonary chemoreflex. Secondly, in the rat, the vagal and cardiopulmonary afferent-evoked responses of NTS neurones was studied, providing a basis for, in subsequent experiments, examinations into the role of 5-HT receptor subtypes in any modulation of these afferents.

- investigate the effects of ligands selective for 5-HT_{1B} and 5-HT_{1D} receptor binding sites on the ongoing activity of NTS neurones in the anaesthetized rat. In addition, the ability of these ligands to modulate vagal or cardiopulmonary afferent input to NTS neurones was also examined.

- examine the role of 5-HT_3 receptors in the NTS at the neuronal level. Firstly, confirming the predominant excitatory effect that activation of 5-HT_3 receptors has on NTS neuronal activity and secondly examining any role that these receptors may have on the vagal afferent-evoked and cardiopulmonary afferent-evoked activation of NTS neurones in the anaesthetized rat.

- investigate interactions between 5-HT_3 receptors and NMDA receptors in the NTS at the neuronal level in the anaesthetized rat. In addition, the role of NMDA receptors in the cardiopulmonary afferent-evoked activation of NTS neurones was investigated.

Chapter 2 Methods

Experiments were carried out under the Animals (Scientific Procedures) Act, 1986, and, upon completion of each experiment, animals were killed by an overdose of anaesthetic and exsanguination.

2.1 Anaesthetized rat preparation

Adult male Sprague-Dawley rats (275-450 g) were anaesthetized with an intra-peritoneal injection of either pentobarbitone sodium (Sagatal, 60 mg kg^{-1}) or thiobutabarbitone sodium (Inactin, 120 mg kg^{-1}). Additional anaesthetic was administered when required (10 mg kg^{-1} and 30 mg kg^{-1} respectively, i.v.), assessed by cardiovascular responses to paw-pinch and the stability of measured cardiovascular and respiratory variables.

Core body temperature was monitored and maintained at 37 °C via a homeothermic blanket and rectal probe (Harvard). The right femoral vein was cannulated with silicone tubing of external diameter 0.96 mm for the administration of drugs. The right femoral artery was also cannulated, using the same tubing, for the withdrawal of fluids and for the recording of arterial blood pressure via a pressure transducer (Gould Statham P23XL). In addition, the trachea was exposed, posterior to the larynx, allowing for its cannulation using a short length of silicone tubing of diameter 3 mm.

Two wires tipped with thin gauge needles were then inserted into two opposing paws, and connected to an amplifier (x 5 000; Neurolog AC preamplifier, model NL104) and filter (0.5-5 kHz; Neurolog filter; model NL125) to allow for the recording of ECG activity, followed by cannulation of the right jugular vein with silicone tubing of external diameter 0.96 mm. This cannula was then slowly advanced further into the jugular vein until a small interruption in ECG activity was observed, indicating the location of the tip of the cannula as being within or in close proximity to the right atrium of the heart. The subsequent

administration of phenylbiguanide (PBG, 12-24 μg kg^{-1}) through this cannula could then activate cardiopulmonary afferents, confirmed by the production of a bradycardia, hypotension and apnoea in response to this stimulus. The inter-administration period of PBG was maintained at a minimum of 5 min to avoid tachyphylaxis and, in order to eliminate the activation of atrial stretch receptors, the concentration of PBG administered was 200 μg ml^{-1}, producing a volume range for injection of 60-120 μl kg^{-1}. In addition, in some experiments a dual lumen cannula was advanced into the right atrium via the right jugular vein. Lumens contained PBG and saline allowing volume controls to be carried out on central and peripheral responses.

Animals were then placed in a stereotaxic frame and artificially ventilated (rate 50-70 strokes min^{-1}, stroke volume - 6-8 ml kg^{-1}) with oxygen-enriched room air via a positive pressure pump (Harvard rodent ventilator, model 683). Tracheal pressure was recorded and monitored via a pressure transducer (Gould) and, in some experiments, animals were neuromuscular blocked with either repeated doses of decamethonium bromide (3 mg kg hr^{-1} i.v.) or a single dose of α-bungarotoxin (140 μg kg^{-1} i.v.). In addition, in some experiments a pneumothorax was carried out on the right side for increased stability during central recording, consequently end-expiratory pressure was raised to 1-2 cm of H_2O to prevent collapse of the lungs. In all experiments arterial blood samples were taken regularly from the femoral artery to monitor blood gases and blood pH using a Corning Blood Gas Analyser (model 238). Blood gas levels and blood pH were maintained within physiological levels by varying the volume and rate of ventilation and administering sodium bicarbonate (1M; i.v.) where necessary.

The dorsal brainstem surface was exposed by removal of the nucchal muscles and subsequent removal of the occipital bone. The dural layers overlying the brain were cut along the midline and reflected laterally allowing for removal of the pia matter directly adjacent to the dorsal brainstem surface. Removal of the pia matter was restricted to within 2 mm rostrally, caudally and laterally to obex, on the side ipsilateral to vagal stimulation (see below) to limit bleeding and surface damage.

Isolation of the right phrenic nerve was achieved from a dorsolateral approach by deflection of the right scapula forwards. The nerve was then cut peripherally and desheathed, with the central cut end subsequently placed on a bipolar platinum recording electrode. The right cervical vagus was isolated from the same approach and placed on a bipolar silver stimulating electrode connected to an isolated stimulator (Digitimer; model DS2A) triggered by a Digitimer programmer (model D4030). Identification of the vagus nerve was confirmed by the generation of a cardiac arrest upon stimulation (1 ms pulses at 100 μA, 50 Hz). The exposed lengths of both nerves were subsequently covered in paraffin wax, and fixed in place with dental impression material (President light body dental polyvinylsiloxane – Coltene®).

2.2 Anaesthetized cat preparation

Experiments were performed on seven adult cats of either sex (2.5-4.5kg). Anaesthesia was induced by an i.v. injection of a mixture of α-chloralose (70 mg kg^{-1}) and pentobarbitone sodium (6 mg kg^{-1}), and supplemented as required with additional α-chloralose (10-15 mg kg^{-1}; i.v.). Depth of anaesthesia was assessed by the withdrawal reflex, cardiovascular response to paw-pinch, stability of cardiovascular and respiratory variables and pupil size.

Core body temperature was monitored and maintained at 37 °C using a homeothermic blanket and probe (Harvard). Upon achieving surgical anaesthesia both brachial veins and one femoral vein were cannulated using silicone tubing of external diameter 1.52 mm for the administration of drugs. In addition, both brachial arteries were cannulated using silicon tubing of external diameter 0.96 mm for the withdrawal of fluids and for recording arterial blood pressure via a pressure transducer (Gould Statham P23XL). Two leads tipped with thin gauge needles were then inserted into each forepaw and connected to an amplifier (x 5 000; Neurolog AC preamplifier; model NL104) and filter (0.5-5 kHz; Neurolog filter; model NL125) in order to monitor ECG activity. Exposure and cannulation of the trachea was then carried out using a brass cannula with a diameter of between 4 and 8 mm. In addition, a cannulation of

the bladder was also carried out using 3 mm diameter silicon tubing inserted via the urethra. This allowed urine to drain freely and prevented any reflex changes associated with bladder distension (de Groat *et al.*, 2001).

To stimulate pulmonary C-fibre afferents the right jugular was cannulated using silicon tubing of external diameter 1.52 mm containing a solution of PBG (400 $\mu g\ ml^{-1}$; 14-32 $\mu g\ kg^{-1}$). This cannula was then slowly advanced into the right atrium of the heart, confirmed by a small interruption in ECG activity. The volume of PBG administered was limited to less than 200 μl per injection to prevent the activation of atrial stretch receptors and five minutes was the minimum period between adjacent PBG injections.

Once placed in a stereotaxic frame the animal was connected to a positive pressure ventilator (Harvard ventilator; model 551) and ventilated with oxygen-enriched room air. Tracheal pressure was recorded and monitored via a pressure transducer (Gould) and end-expiratory pressure was maintained between 1-2 cm H_2O. Neuromuscular blockade was then achieved using a bolus injection of vecuronium bromide (200 $\mu g\ kg^{-1}$; i.v.), this was maintained via a continuous infusion (480 mg $kg^{-1}\ hr^{-1}$ at an infusion rate of 6 ml kg hr^{-1}). Other components of the infusion include 2g of glucose, 8.4g of sodium bicarbonate and 80mg of vecuronium bromide made up in 500ml of H_2O and 500ml plasma substitute gelofusine. This was designed to maintain blood pH and volume and prevent the development of non-respiratory acidosis for the duration of the experiment. In support of this arterial blood samples were taken regularly from the femoral artery to monitor both blood gases and blood pH using a Corning Blood Gas Analyser (model 238). Where necessary supplementary doses of sodium bicarbonate (1M; i.v.) were administered in addition to any necessary modulation of ventilatory rate and volume. All animals were pretreated with the β_1-adrenoceptor antagonist atenolol (1 mg kg^{-1}; i.v.), to block sympathetic drive to the heart. Thus, all cardiac chronotropic changes could be attributed to alterations in vagal drive to the heart.

The right phrenic nerve was isolated from a dorsolateral approach after forward deflection of the right scapula, cut peripherally, desheathed, and mounted on a bipolar silver wire recording electrode. In addition, clamps were placed on the spine at positions C5 and L2-3 to ensure the stability of central recording sites. Exposure of the brainstem was then carried out by removal of the nucchal muscles and subsequent removal of the occipital bone. In some experiments the rostral displacement of the cerebellum by means of a small retractor was necessary, allowing easier access to the area overlying the nucleus ambiguus (NA). In addition, in the cat, removal of the pia matter was not found necessary in all experiments.

On the right side of the animal a thoracotomy was performed between the fourth and sixth ribs exposing the thoracic cavity, allowing identification and isolation of the right pulmonary and both the caudal and cranial cardiac branches of the vagus (as described in McAllen & Spyer, 1976). Bipolar silver electrodes were constructed using fine silver wire (0.125mm) and insulated copper wire, with the paired electrodes held together and insulated from each other with wax. Electrodes were then placed on the vagus between caudal and cranial cardiac branches, on the pulmonary branch and on the intact cardiac branch, and fixed in place using dental impression material (President light body dental polyvinylsiloxane – Coltene®). The protruding insulated copper tails were subsequently attached to the thorax of the animal and connected to an isolated stimulator (Digitimer; model DS2A) and triggered using a Digitimer programmer (model D4030). Functional identification of the cardiac and pulmonary branches was demonstrated by the cessation of ECG activity during cardiac branch stimulation and by the modulation of tracheal activity independent of heart rate changes seen upon selective stimulation of the pulmonary branch (1 ms pulses at 100 μA, 50 Hz).

2.3 Extracellular recording

Extracellular recordings were made from brainstem neurones using a combination of single barrel, multi-barrelled (5-7) and compound 'piggyback' glass microelectrodes. These 'piggyback' electrodes are constructed from a single barrel glass microelectrode (tip diameter ~1 μm) cemented to a multi-barrel electrode (tip diameter 3-7 μm), with the tips of the two electrodes, glued together with cyano-acrylate adhesive, less than 10 μm apart (see figure 2.1; Wang *et al.*, 1998).

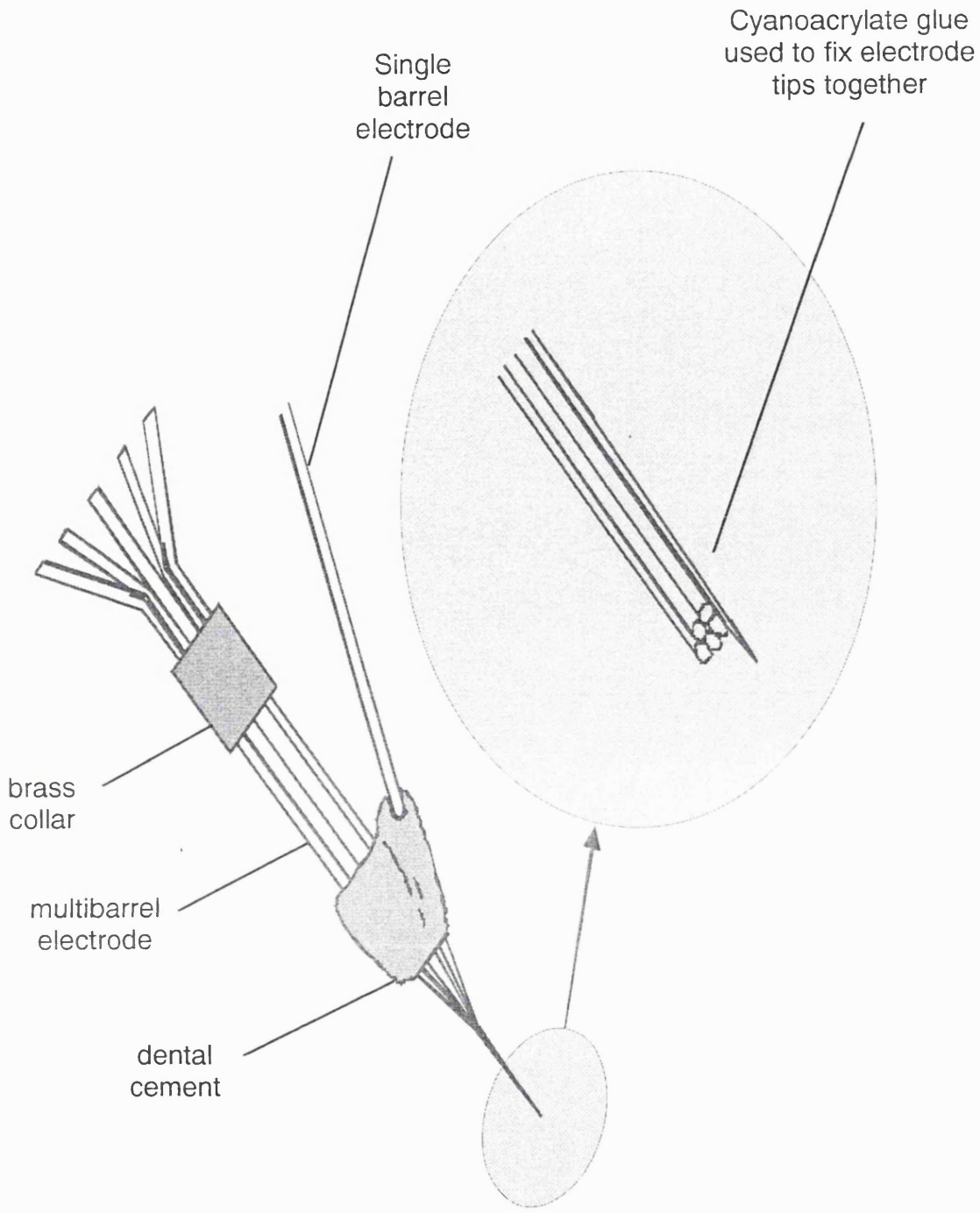
Recording barrels contained a solution of sodium chloride with a concentration of between 0.5 M and 4 M. In experiments using multi-barrel electrodes, other barrels contained Pontamine Sky Blue (PSB, 2% dissolved in 0.5 M sodium acetate) and a combination of 5-HT and glutamate receptor ligands. These compounds were ejected via ionophoresis (Neurophore, Medical Systems) with the PSB solution-containing barrel providing the balancing for this current ejection.

2.4 Histological localisation

In some experiments PSB was injected via ionophoresis as a marker into the brainstem after the termination of recording for the identification and localisation of specific neurones. At the end of these experiments the marked brainstems were removed and fixed in 10% formal saline, followed by serial cryo-sectioning of the brainstem region and staining of these sections with neutral red. The PSB marked recording sites were then visualised under light microscope and mapped onto a standard stereotaxic atlas of the appropriate region of the cat or rat brainstem, confirming and visually demonstrating the location of specific neurones.

Figure 2.1 Compound 'piggyback' glass micorelectrode construction

A constructed 'piggyback' glass microelectrode. The tips of the multibarrel (3-7 μm tip diameter) and single (~ 1 μm tip diameter) glass electrodes are glued together under a light microscope using cyanoacrylate glue, ensuring that the distance between the tips of the two electrodes remains less than 10 μm. Dental cement is then placed over the necks of the two electrodes to provide further structural support during their use.



* Multibarrel tip 3-7 μm
* Single electrode tip ~1 μm
* Distance between tips <10 μm

2.5 Electrophysiological identification of neurones

Nucleus tractus solitarius (NTS) neurones were recorded from the anaesthetized rat preparation and were identified by the presence of a cellular response evoked following vagus nerve stimulation (50-500 μA, 1 ms pulse width, 0.3-1 Hz). In addition, identification was aided by the neurones approximate location of between 200μm and 800 μm below the dorsal surface of the brainstem at the level of obex. Excitatory evoked responses were identified as being orthodromic (see figure 3.4) in order to distinguish from antidromically activated (see below) neurones of the dorsal vagal motor nucleus (DVN) located directly ventral to the NTS, which contains vagal preganglionic neurones.

Cardiac vagal preganglionic neurones (CVPNs) of the NA were recorded from the anaesthetized cat preparation and were identified by antidromic activation following stimulation of the cardiac branches of the vagus (100-500 μA, 1ms pulses, 0.2-1.0 Hz) as described previously (see McAllen & Spyer, 1976; 1978a). Antidromic activation is characterised firstly by the constant latency of the evoked response. In addition, it is defined by the ability of spontaneous action potentials to pass down the axon of the neurone and collide with the antidromic-conducting stimulation-evoked action potential, in the appropriate time band. Current spread from stimulation of the cardiac branch of the vagus to the main trunk of the vagus was ruled out by stimulation of the vagus, posterior to the pulmonary branch, not producing an evoked response in at least one neurone per experiment.

2.6 Data capture and protocols

2.6.1 Anaesthetized rat - data capture

Tracheal pressure and blood pressure were both displayed on a polygraph pen recorder (Grass, model 7D), along with heart rate, which was derived electronically from blood pressure in this instance (Gould Biotach Amplifier).

This was maintained for the duration of each experiment, providing a long term record of systemic variables and thus indicating experimental stability.

Phrenic nerve activity and ECG activity was amplified (x 20 000 and x 5 000 respectively; Neurolog AC preamplifier, model NL104) and filtered (0.5-5 kHz; Neurolog filter, model NL125). Subsequently, phrenic nerve activity, ECG activity, blood pressure, tracheal pressure and neuronal activity (see below) was captured on computer (PC) using a 1401 plus interface (Cambridge Electronic Design, CED) and Spike2 software (CED). An on-line derivation of heart rate was then made from the blood pressure wave using a script program (see Appendix) and Spike2.

Neuronal activity was recorded using one of two methods. Initial experiments used a Neurolog headstage (model NL105), connected to a Neurolog amplifier (x 1 000-5 000; Neurolog AC preamplifier NL104) and filter (0.5-5 kHz; Neurolog filter, model NL125). However, in later experiments an Axoclamp headstage (model HS-2A (0.1)) connected to an Axoclamp centre unit (model 2A) was used. This was amplified (x 1 000-5 000; NL104) and filtered (0.5-5 kHz; NL125) in the same way. In both cases the output was transmitted to the PC via the 1401 plus interface.

Furthermore, all variables were also recorded, via a digital data recorder (Instrutech, model VR100B), onto videotape in the majority of experiments. This provided a digital record of each experiment and also allowed off-line replaying of experimental data through Spike2.

2.6.2 Anaesthetized rat - protocols

Systemic variables

Resting systemic data for the instrumented and stabilised preparation was examined over a 2 min period to give mean values for arterial blood pressure (MAP), heart rate (HR), tracheal pressure-inflation and deflation. In addition, blood pH, pO_2 and pCO_2 levels were taken from blood sample results from each experiment providing mean data for respiratory variables.

Cardiopulmonary reflex analysis-peripheral

For analysis of the peripheral response to right atrial injections of PBG and saline, cardiovascular variables (MAP and HR) were measured over a period of 40 s prior to injection and mean levels were compared to the evoked response. This evoked response was taken as the maximum change in MAP and HR following right atrial injection of either PBG or saline. Any reduction in phrenic nerve activity evoked by injections was also noted.

In some experiments both vagi were isolated at the cervical level the responses to right-atrial PBG injection were recorded before and following bilateral vagotomy.

Neuronal identification and characterisation

Using low frequency vagal stimulation (0.3-1 Hz; 50-500 μA; 1 ms pulse width) as a search stimulus, evoked NTS neurones were recorded by descending through the brainstem from dorsal to ventral areas perpendicular to the surface of the brain using a nano-stepper (Burleigh, model 6100) with the step size set to 3 μm through the depths of interest (see section 2.3). In some experiments, in which multi-barrel electrodes were used, a low current of a glutamate receptor agonist (DL-homocysteic acid (DLH) or AMPA) was continuously ejected ionophoretically whilst searching for neurones. This provided an excitatory drive for cells which had no spontaneous activity and/or were too hyperpolarised to fire an action potential evoked by vagus nerve stimulation at a level supra-threshold for unmyelinated fibre recruitment. It therefore aided identification and also allowed further characterisation of NTS neurones. Single unit activity was discriminated and counted using a window discriminator (Digitimer, model D130), and, using Spike2, presented as a rate histogram with units of spikes s^{-1}.

After the termination of experiments all analysis of neuronal activity was carried out using Spike 2 software. A Post-stimulus-triggered histogram (PSTH) was produced for each neurone using a Spike 2 script program, with a bin size of 1 ms over a minimum of 50 sweeps. The PSTH was triggered by vagus nerve

stimulation and demonstrated any modulation of ongoing neurone activity by this stimulus. The latency of evoked excitatory spikes was measured, in addition to the variability in this latency over a minimum of 50 sweeps.

Any correlation of neuronal activity to MAP, phrenic nerve activity and tracheal pressure was also examined in each neurone. The trigger for each correlation was produced from spike discrimination of ECG activity, phrenic nerve activity and each peak of inspiration. Correlation histograms were produced using a standard Spike 2 script program, with bin sizes of between 2 and 10 ms over a maximum period of stable neuronal firing with vagal stimulation switched off. Assessment of any correlation was carried out by comparing each correlogram with a waveform average of the related trigger, produced once more using a spike 2 script program with the same bin width over the same time period.

Cardiopulmonary reflex analysis-neuronal

NTS neuronal responses to right atrial injections of PBG were observed, and neurones were classified into groups based on the characteristics of this response. The latency of both the cellular and peripheral changes evoked by right atrial PBG was measured using on-screen cursors within Spike 2 and compared. Neuronal responses were measured by counting the number of spikes in the evoked burst of excitation, until neuronal activity returned to pre-injection levels. Or in the case of an inhibitory response to right atrial PBG injection, the time period of this inhibition was measured, until neuronal activity returned to pre-injection levels.

In addition, the stability of excitatory neuronal responses to right atrial PBG was examined in neurones in which multiple injections of PBG were made. This would establish the potential for studying the effects of ionophoretically applied receptor ligands on neuronal response to right atrial PBG injections.

Single ligand administration analysis

Initial examination into the effects of ionophoresis of glutamate and 5-HT receptor ligands was carried out on the ongoing activity of NTS neurones. However, some neurones were quiescent, with no ongoing activity and so in some cases the membrane potential of neurones was depolarised by ionophoretic ejection of either DLH or AMPA to allow the inhibitory effects of drugs to be observed. This activity level, on which drugs were tested, whether ongoing or DLH-evoked is referred to as the baseline activity of the neurone. Figure 2.2 describes the protocol for the administration and analysis of the effects of drugs on the baseline activity of neurones. Baseline activity was measured as the initial firing rate of neurones before, during and, where possible, after ionophoretic administration of the test drug. All activity was calculated from a period of neurone firing of between 10 and 20 seconds from which a mean was taken to give spikes s^{-1}. Drugs were defined as causing excitation or inhibition if they altered baseline firing rates of neurones by more than 20%, as has been used previously (Wang *et al.*, 1996). Neurones were subsequently grouped together into one of three groups; excitatory effect, inhibitory effect or no effect. The mean data for each group (baseline, test & recovery) was then obtained in spikes per second and comparisons were made.

Dual ligand administration analysis

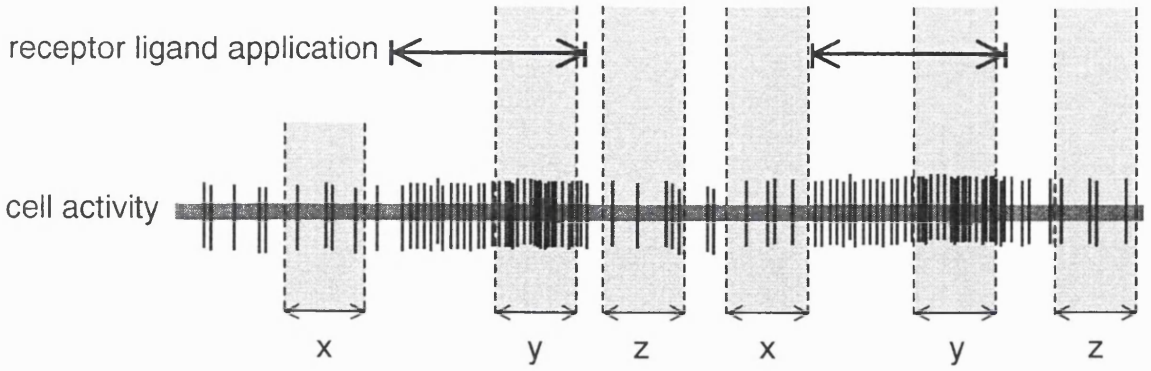
The effects of ionophoretic administration of certain drugs were subsequently tested during the ionophoretic administration of a second drug. Any modulation of the control effect of the ligand was then examined; responses were again classified on the basis of the control effect being altered by a magnitude of more than 20% in the presence of the second drug e.g. an excitatory ligand tested in the presence of a second ligand could have this excitation either potentiated, attenuated or unaffected. However, in some cases the second drug altered baseline firing itself and therefore, if possible, activity was returned to control levels by varying the current of ejection of DLH (or AMPA) before test application of the first drug in the presence of the second drug.

Figure 2.2 Ligand ionophoresis - protocols and analysis

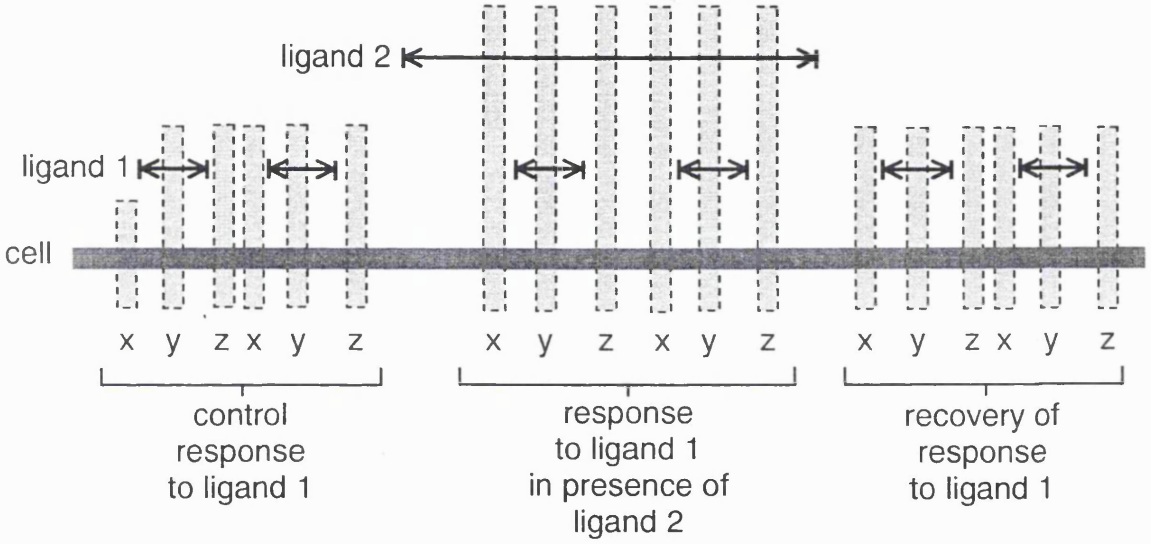
(i) Effects of single compound administration. Example given for an excitatory response of an NTS neurone to a receptor ligand or vehicle control. Neurones were classified into three groups; excitation if compounds increase baseline activity by more than 20%; inhibition if compounds decrease baseline activity by more than 20%; and none if baseline activity is altered by 20 % or less.

(ii) Effects of administration of one compound in the presence of another. The effect of one drug tested in the presence of a second. This effect is then classified into three groups; potentiation of effect if the predominant effect of the ligand is augmented by more than 20%; attenuation of effect if the predominant effect is attenuated by more than 20%; and none if the predominant effect is altered by 20% or less.

(i)



(ii)



⟷ – time period of ionophoretic compound administration

▯ – areas analysed (time width 10-20 s, see below)

All neuronal activity taken as spikes s^{-1} averaged over a period of either 10, 15 or 20 s and in the large majority of neurones over two periods of ligand administration (as above)

x – control, baseline, response pre-ligand administration (10-20 s)
y - max^{m} response during ligand administration (10-20 s)
z - recovery of baseline response post-ligand administration (10-20 s)

Effects of ligand administration on vagal-evoked activity

The effect of ionophoresis of receptor ligands was also examined on the vagus nerve stimulation-evoked response of NTS neurones. The control response was calculated by producing a PSTH over a minimum of 20 sweeps of vagal stimulation. This showed the total number of evoked spikes, and if the number of sweeps exceeded 20, then the response was adjusted to give a value of number of evoked spikes (20 sweeps)$^{-1}$. This was subsequently compared with the evoked response (20 sweeps)$^{-1}$ during and after ionophoretic ligand administration. As before, neurones were classified into three groups, potentiation of the evoked response if drug administration increased the number of spikes (20 sweeps)$^{-1}$ by more than 20%, attenuation if the response was decreased by more than 20% and none if the response was altered by 20% or less.

Effects of ligand administration on cardiopulmonary reflex-evoked activation

The effect of ionophoretic administration of drugs was tested on the excitatory response of some neurones to right atrial PBG administration. The total number of evoked spikes (from the start of the excitatory burst until the cell returns to pre-PBG administration activity) was compared with the response during the ionophoresis of test ligands. Where possible the response to right atrial PBG administration after the test response was also recorded and compared.

In order to minimise the effects of ligands on the baseline firing rate of neurones only low currents of ejection were used during comparisons of the response to right atrial PBG administration and vagus nerve stimulation.

Cardiopulmonary reflex-neuronal controls

In a sample population of NTS neurones the response to right atrial administration of PBG was examined and compared to the response of the same neurone evoked by right atrial administration of the same volume of saline. Furthermore, a bilateral vagotomy was performed during the recording of a small group of NTS neurones to confirm that the excitatory cellular

responses evoked by right atrial PBG administration were mediated by vagal afferents.

Additionally, in some experiments a dose of sodium nitroprusside (0.4-0.8 μg per animal, i.v.) which produced a depressor response of similar magnitude to that evoked by right atrial PBG administration was administered during neuronal recording, in order to demonstrate whether neurones were modulated by baroreceptor afferents.

All data are presented as mean ± standard error mean (s.e.mean) except where indicated. All comparisons of mean were made with the Student's paired *t* -test, with the significant levels set at: * $P < 0.05$ ** $P < 0.01$

2.6.3 Anaesthetized cat - data capture

Tracheal pressure and blood pressure were both displayed on a polygraph pen recorder (Grass, model 7D), along with heart rate, which was derived electronically from blood pressure (Gould Biotach Amplifier). This was maintained for the duration of each experiment, providing a long term record of systemic variables and thus indicating experimental stability.

Phrenic nerve activity and ECG activity was amplified (x 20 000 and x 5 000 respectively; Neurolog AC preamplifier, model NL104) and filtered (0.5-5 kHz; Neurolog filter, model NL125). In addition, phrenic nerve activity was integrated using an EMG integrator (Neurolog, model NL703). Subsequently, ECG activity, blood pressure, tracheal pressure, raw phrenic nerve activity, integrated phrenic nerve activity and neuronal activity (see below) was captured on computer (PC) using a 1401 plus interface (Cambridge Electronic Design, CED) and Spike2 software (CED).

Neuronal activity was captured using a Neurolog headstage (model NL105), connected to a Neurolog amplifier (x 2 000; Neurolog AC preamplifier NL104) and filter (0.5-5 kHz; Neurolog filter, model NL125). The output was then transmitted to the PC via the 1401 plus interface.

Furthermore, all variables were also recorded, via a digital data recorder (Instrutech, model VR100B), onto videotape in the majority of experiments. This provided a digital record of each experiment and also allowed off-line replaying of experimental data through Spike2.

2.6.4 Anaesthetized cat - protocols

Systemic variables

Resting systemic variables (MAP, HR, tracheal pressure-inflation and deflation, arterial blood pH, pO_2 and pCO_2) were measured over a period of 40 seconds and the mean was calculated for each variable in all animals.

Cardiopulmonary reflex analysis-peripheral

Peripheral responses to intra-atrial PBG administration were analysed by comparing the mean heart rate and mean arterial blood pressure (MAP) over a 40 second period prior to PBG injection with the maximal effect on heart rate and blood pressure induced by right atrial PBG injection.

Neuronal identification and characterisation

Using low frequency vagal stimulation (0.2-1.0 Hz, 1 ms pulses, 100-500 μA) as a search stimulus, antidromically activated CVP neurones were recorded by descending through the brainstem from dorsal to ventral areas perpendicular to the surface of the brain using a nano-stepper (Burleigh, model 6100) with the step size set to 3 μm through the region of the nucleus ambiguus. In some experiments, in which multi-barrel electrodes were used, a low current of DLH was continuously ejected ionophoretically whilst searching for neurones, providing an excitatory drive for hyperpolarised neurones. This also allowed the further characterisation of CVPNs by raising their activity to high levels and examining any modulation of cardiac activity. Single unit activity was discriminated and counted using a window discriminator (Digitimer, model D130), and, using Spike2, presented as a rate histogram with units of spikes s^{-1}.

After the termination of experiments all analysis of neuronal activity was carried out using Spike 2 software. A post-stimulus-triggered histogram (PSTH) was produced for each neurone using a Spike 2 script program, with a bin size of 1 ms over a minimum of 50 sweeps. The PSTH was triggered by stimulation of the cardiac branch of the vagus and demonstrated the latency of the antidromic spike in order for fibre conduction velocities to be calculated. This also confirmed the stability of the antidromic spike and allowed examinations into the presence of orthodromic afferent inputs to these CVPNs from both cardiac and pulmonary branches of the vagus.

Any correlation of neuronal activity to MAP, phrenic nerve activity and tracheal pressure was also examined in each neurone. The trigger for each correlation was produced from spike discrimination of ECG activity, phrenic nerve activity and each peak of inspiration. Correlation histograms were produced using a standard Spike 2 script program, with bin sizes of between 10 and 50 ms over a maximum period of stable neuronal firing with nerve stimulation switched off. Assessment of any correlation was carried out by comparing each correlogram with a waveform average of the related trigger, produced once more using a spike 2 script program with the same bin width and time period as the correlogram.

Cardiopulmonary reflex analysis-neuronal

The ongoing activity of recorded CVPNs was measured over a period of forty seconds prior to right atrial PBG administration and mean activity was calculated. However, these neurones fired in bursts in phase with the respiratory cycle, specifically during the post-inspiratory and stage two expiratory segments (Gilbey *et al.*, 1984). Therefore, the mean number of spikes burst^{-1} and mean burst duration in the four cycles prior to PBG administration was also recorded, acting as the control for the first burst of cellular activity post-PBG administration. PBG-evoked neuronal excitation was defined by an increase of greater than 20% in either burst density or duration.

One important consideration in this analysis was the observation that neuronal responses, in the cat, occurring within five seconds of the administration of PBG could be attributed solely to the activation of pulmonary C-fibres (Daly & Kirkman, 1988; Jones *et al.*, 1998). Neuronal excitation occurring later than 5 seconds could be mediated by activation of cardiac afferent, or afferents in other parts of the systemic circulation (Daly & Kirkman, 1988). Consequently, neuronal activation was defined as to whether it occurred within five seconds of the right atrial administration of PBG. In the majority of cases the evoked neuronal excitation was within, but exceeded, this five second period and so a further analysis of the response was carried out. This was achieved by counting the number of spikes seen in the first second of the evoked neuronal response within the five second period post-PBG administration and comparing it to the first second of the burst for the four previous respiratory cycles.

All data are presented as means ± standard error mean (s.e.mean) except where indicated, and all comparisons of mean were made with the Student's paired *t* test, with the significant level set at: $P < 0.01$

2.7 Drugs and solutions

Ionophoretic drug recipes

In order to ionophorese compounds it was necessary to alter the pH of solutions. Some compounds were adjusted to pH 4, some to pH 8.5 and one compound was adjusted to pH 10. The pH of solutions was adjusted by adding drops of either 0.1 M HCl or 0.1 M NaOH.

The following ionophoretically applied drugs were dissolved in 0.9% saline:-

pH 4		
	granisetron	(10 mM)
	GR55562B	(10 mM)
	ondansetron	(10 mM)
	sumatriptan	(20 mM)
	ketanserin	(10 mM)
pH 8.5		
	AMPA	(20 mM)
	AP-5	(20 mM)
	DLH	(100 mM)
	NMDA	(20 mM)
pH 10		
	PBG	(10 mM)

In addition the compound CP-93,129 (10 mM) was dissolved in 50μl of 1% ascorbic acid, then made up to 1 ml with 0.9% saline and the solution was then adjusted to pH 4.

Drug sources

Drugs were obtained from the following sources: α-bungarotoxin, α-chloralose, AMPA, AP-5 and atenolol from Sigma Aldrich Chemical Co. (Poole, Dorset, U.K.); CP-93,129 from Tocris Cookson Ltd. (Northpoint, Avonmouth, U.K.); decamethonium bromide and DL-homocysteic acid from Sigma Aldrich Chemical Co. (Poole, Dorset, U.K.); Gelofusine from Braun Medical Ltd (Aylesbury, Bucks, U.K.); GR55562B and granisetron from Glaxo-Smithkline (Harlow, Essex, U.K.); ketanserin from Tocris Cookson Ltd. (Northpoint, Avonmouth, U.K.); NMDA from Sigma Aldrich Chemical Co. (Poole, Dorset, U.K.); ondansetron from Glaxo Group Research (Ware, Hertfordshire, U.K.); pentobarbitone sodium from Rhône Mérieux Ltd (Harlow, Essex, U.K.), phenylbiguanide from Research Biochemicals Inc., Semat Technical Ltd (St. Albans, Herts, UK); Pontamine Sky Blue dye from BDH (Poole, Dorset, U.K.); sodium nitroprusside from Sigma Aldrich Chemical Co. (Poole, Dorset, U.K.); sumatriptan from Glaxo-Smithkline (Harlow, Essex, U.K.) ; thiobutabarbitone sodium from Sigma Aldrich Chemical Co. (Poole, Dorset, U.K.) and vecuronium bromide from Organon Technika Ltd (Cambridge, U.K.).

All drugs given i.v. were dissolved in 0.9% saline.

Chapter 3 Cardiopulmonary reflex and brainstem neuronal responses

3.1 Introduction

The cardiopulmonary reflex is characterised by a bradycardia, hypotension and apnoea mediated by vagal afferent C-fibres with receptors in the pulmonary and cardiac circulations (see Coleridge & Coleridge, 1980). Electrolytic lesions of the nucleus tractus solitarii (NTS) or nucleus ambiguus (NA) abolished the depressor and bradycardic components of this reflex (Lee *et al.*, 1972), confirming the role of these two nuclei in this reflex pathway. Furthermore, caudal ventrolateral medullary neurones have also been suggested to be important in mediating the sympathetic component of this reflex (Verberne *et al.*, 1989). However, the actions of supramedullary structures on the integrity of this reflex appear to be minimal (see Verberne & Guyenet, 1992), although these structures are certainly capable of modulating vagal reflexes.

With respect to the NTS it is important to note that although discrete pathways for individual reflexes through the nucleus exist, it is also known that a high degree of modification of reflex input occurs at this site (see Spyer, 1991). In any given physiological situation there are likely to be inputs to the nucleus from a host of different peripheral sites, some converging onto the same NTS neurone, resulting in numerous temporal and spatial interactions (see Mifflin & Felder, 1990). For instance, the excitatory response to chemoreflex activation evoked in a population of NTS neurones has been shown to be attenuated by the simultaneous activation of baroreceptor afferents (Mifflin, 1993). Modulation of NTS neuronal activity by cardiopulmonary reflex activation has also recently been examined (Sévoz-Couche *et al.*, 2000a; Sévoz-Couche *et al.*, 2000b) and the authors described a correlation between this response and the vagal

stimulation-evoked response of each neurone. Their initial classification placed NTS neurones receiving vagal afferent input into three groups dependent on the variability of the latency of the C-fibre input (Sévoz-Couche *et al.*, 2000a; Sévoz-Couche *et al.*, 2000b). Group 1 neurones had an onset latency variability of <3ms, group 2 from 3-5ms and group 3 neurones had a variability of greater than 5ms. Thus, neurones in group 1 were most likely receiving a monosynaptic unmyelinated vagal afferent input, group 2 neurones were of a higher order receiving at least some polysynaptic input, whilst group 3 neurones were deemed to be an even higher order, possibly output neurones, receiving polysynaptic vagal afferent input. Those studies then demonstrated that group 3 neurones were excited by activation of cardiopulmonary afferents, in contrast to neurones of group 1 which were mainly inhibited by these afferents. This inhibition of first order neurones by cardiopulmonary afferents was suggested to mediate a protective effect over other vagal reflexes mediating a depressor response (Sévoz-Couche *et al.*, 2000a).

A component of this excitatory output response subsequently activates cardiac vagal preganglionic neurones (CVPNs) in either the NA or dorsal vagal motor nucleus (DVN) resulting in the associated reflex bradycardia. Most vagal reflexes display decreased slowing of the heart during inspiration; a response thought to be mediated by disfacilitation of baroreceptor and chemoreceptor inputs by lung stretch afferents (Potter, 1981) and the inhibition of CVPN's by central respiratory neurones (Gilbey *et al.*, 1984). In addition, the CVPNs that mediate this respiratory modulated bradycardia have been demonstrated to be located in the NA, having a baroreceptor input (McAllen & Spyer, 1978a), and small myelinated axons (McAllen & Spyer, 1976; McAllen & Spyer, 1978b; Gilbey *et al.*, 1984). However, the pulmonary chemoreflex, evoked by specific activation of pulmonary C-fibres in the cat, is one vagal reflex whose bradycardia is not under respiratory modulation (Daly, 1991; Daly *et al.*, 1992; Daly & Kirkman, 1988, 1989). This bradycardia was suggested to be mediated by CVPNs located in the DVN (Daly, 1991). These neurones have C-fibre axons (Donoghue *et al.*, 1981; Jordan *et al.*, 1986; Ford *et al.*, 1990), an input from C-fibre pulmonary afferents (Bennett *et al.*, 1985), a negative chronotropic effect on heart rate (Jones *et al.*, 1995) and are excited during activation of the

cardiopulmonary reflex in the rat (Jones *et al.*, 1998). However, the excitation of CVPN's in the DVN by cardiopulmonary afferent activation is of shorter duration than the associated bradycardia (Jones *et al.*, 1995). Secondly, in the cat the magnitude of the bradycardia evoked by selective C-fibre vagal efferent stimulation is 14 bpm (Jones *et al.*, 1995), whereas the pulmonary chemoreflex-evoked bradycardia is in the order of 55 bpm (PBG dose 6-15μg kg^{-1}, Daly & Kirkman, 1988). These findings are not consistent with C-fibre CVPN's providing the sole negative chronotropic cardiac component of the pulmonary chemoreflex, and the question remains as to whether respiratory modulated B-fibre CVPN's located in the NA are activated by this reflex, therefore contributing to the associated cardiac slowing.

One important point concerning the activation of these cardiopulmonary reflexes evoked by right atrial administration of phenylbiguanide (PBG) is the ability in the cat to differentiate the activation of afferents from the pulmonary circulation with those activated from the heart and systemic circulation (Daly & Kirkman, 1988). Consequently, in the cat, responses to the administration of PBG occurring within five seconds of the injection are deemed as resulting from solely pulmonary C-fibre stimulation (pulmonary chemoreflex; see section 1.1.4). Unfortunately, in the rat, because of the much shorter pulmonary circulation time, this differentiation cannot be made and the activation of cardiac chemo-sensitive afferents and/or afferents within the systemic circulation can therefore not be ruled out. However, in rats the effects of pericardial-administered PBG have been examined (Veelken *et al.*, 1990), this study used a dose of 90 μg per animal to activate cardiac chemoreceptors, which may indicate that at lower doses significant cardiac afferent activation is unlikely.

Aims:

- Experiments were carried out to examine the response of respiratory modulated CVPNs in the region of the NA to stimulation of the pulmonary chemoreflex in the cat. Secondly, in the rat, the vagal and cardiopulmonary afferent-evoked responses of NTS neurones was studied, providing a basis for, in subsequent experiments, examinations into the role of 5-HT receptor subtypes in any modulation of these afferents.

3.2 Results - anaesthetized rat

Baseline values for systemic variables were measured and averaged for a total of 38 anaesthetized rats. They were (mean ± S.E.): - mean arterial pressure (MAP) 99 ± 2 mmHg; heart rate 326 ± 5 bpm; tracheal pressure - inflation 7.0 ± 0.3 mmHg, deflation -0.4 ± 0.1 mmHg; blood pH 7.35 ± 0.01; blood PO_2 155 ± 5 mmHg; blood pCO_2 37 ± 1 mmHg.

3.2.1 Cardiopulmonary Reflex (systemic response)

In 38 animals right atrial injection of phenylbiguanide (PBG; 12-24 μg kg^{-1}, 20-40 μl) evoked a significant bradycardia of 92 ± 12 bpm (326 ± 4 to 234 ± 12 bpm, $P<0.01$) and a significant fall in blood pressure of 37 ± 2 mmHg (MAP, 99 ± 2 to 63 ± 2 mmHg, $P<0.01$). The left panel of figure 3.1 shows a typical example of this response and also illustrates the reduction in phrenic nerve activity following right atrial PBG injection, a response that was seen in 31 of the 38 animals.

Comparisons of cardiopulmonary reflex (systemic response)

Using a dual lumen cannula, it was possible to carry out right atrial injections of both PBG and the PBG vehicle 0.9% saline to the same animal (figure 3.1). In a group of 8 animals PBG (12 μg kg^{-1}, 20 μl) evoked a bradycardia of 83 ± 25 bpm (344 ± 8 to 261 ± 25 bpm, $P<0.05$), a hypotension of 35 ± 4 mmHg (MAP, 99 ± 7 to 64 ± 6 mmHg, $P<0.01$) and a reduction in phrenic nerve activity in 7 of the 8 animals. In contrast saline (20 μl) produced a bradycardia of just 12 ± 3 bpm (345 ± 6 to 333 ± 4 bpm, $P<0.01$), and a small hypotension of 4 ± 1 mmHg (MAP, 96 ± 8 to 92 ± 8 mmHg, $P<0.05$) but no change in phrenic nerve activity. However, the bradycardia and hypotension produced by PBG was significantly bigger ($P<0.05$ and $P<0.01$ respectively) than that produced by saline (figure 3.1).

The cardiac response to right atrial PBG injection was abolished following a bilateral vagotomy, at the cervical level, (figure 3.2) in all 3 animals tested

(bradycardia – 100 ± 53 to 5 ± 3 bpm, n=3). In addition, the reflex-evoked apnoea, which occurred in 2 of the 3 animals, was also abolished, confirming that the evoked cardiac and respiratory changes are mediated by vagus nerve transmission. However, the associated drop in blood pressure was maintained, although it was found to be smaller in magnitude in all 3 animals (mean hypotension: 34 ± 4 to 23 ± 3 mmHg, n=3; figure 3.2), suggesting a non-vagal component of the reflex-evoked hypotension.

3.2.2 Nucleus tractus solitarius neurones

Single barrel, multibarrel and compound 'piggyback' glass microelectrodes were used to record a total of 360 nucleus tractus solitarius (NTS) neurones from 116 animals. These neurones responded to electrical activation of afferents at the cervical level of the vagus. Stimulation evoked an orthodromic action potential in 342 (95% of the population) of these neurones (figure 3.3 A). In 10 neurones vagus nerve stimulation evoked solely an inhibitory response, and the location of these 10 and the remaining 8 neurones was confirmed as within the NTS using histological methods after ionophoretic Pontamine Sky Blue ejection at their recording sites.

314 neurones (87% of the population) received vagal afferent projection with a conduction velocity in the C-fibre range (0.6-2.7 m s^{-1}; mean: 1.31 ± 0.01 m s^{-1}; figure 3.3 A). In addition, 67 neurones (19% of the population) received inputs with a conduction velocity consistent with transmission along small myelinated fibres (3.1-13.3 m s^{-1}; mean: 6.9 ± 0.3 m s^{-1}), and of these neurones 39 (11%) received both a short and long latency evoked potential. As mentioned above 10 neurones presented with only a post-stimulation period of inhibition, this inhibition was also seen in a further 136 neurones which received an initial excitatory vagal afferent input and had ongoing, or ionophoretically-induced activity (range of inhibition: 50 to 750 ms (post-stimulation), mean: 319 ± 14 ms; figure 3.4 B).

In addition, in many neurones it was possible to vary the number of evoked spikes per stimulus (sweep^{-1}) by altering the intensity of current stimulation of

the vagus nerve, i.e. at low currents over a period of 20 stimulations (sweeps) of the vagus there might only be 10 evoked spikes. This observation allowed examinations into the effects of various ligands on the excitability of this vagal-evoked response in later experiments.

Correlation of NTS neurone activity

183 of the 360 neurones recorded had no ongoing activity, 47 had a low (<1Hz) ongoing activity, whilst the remaining 130 had an ongoing activity greater than 1 Hz. Therefore, in order to examine the correlation of neuronal activity to systemic variables it was necessary to apply excitatory compounds, using ionophoresis, to raise the baseline firing rates of some neurones.

313 neurones showed neuronal activity related to ECG activity, 307 showed activity correlated to tracheal pressure and 166 neurones fired in phase with phrenic nerve activity. Of these, 148 of the 360 neurones were correlated to all three systemic variables (figure 3.3 B), whilst 154 were correlated to two variables (141 to tracheal pressure and ECG activity, 10 to phrenic nerve activity and ECG activity, and 3 to tracheal pressure and phrenic nerve activity). Only 34 neurones were correlated to just one variable (15 to tracheal pressure, 14 to ECG activity and 5 to phrenic nerve activity), whilst the neuronal activity of the remaining 24 neurones was not correlated to any of the measured systemic variables. In some cases, in these neuromuscular blocked animals, phrenic nerve activity was locked in phase with tracheal pressure and therefore attributing the correlation of neuronal activity to tracheal pressure or phrenic nerve activity is difficult. However, the correlations of the neurone shown in figure 3.3B suggests that neuronal activity is linked to both phrenic nerve activity and tracheal pressure despite their synchronicity, demonstrated by the biphasic peak of the correlogram.

Variability (jitter) of the orthodromic evoked response

All inputs to NTS neurones were identified as orthodromic and, as such, the latency of the evoked action potential varied between stimuli. For the purposes

of this thesis this variability is referred to as the jitter of the vagal-evoked response.

Electrical stimulation of the cervical vagus was carried out using a bipolar silver stimulating electrode, thus stimulation could be carried out at two different polarities. A study was carried out to determine if the jitter of the evoked response of an NTS neurone varied according to polarity of stimulation or to the magnitude of current used. The minimum current used was 2 x the threshold of the evoked response and the maximum was 6 x threshold.

In 7 of 11 neurones tested the jitter of the evoked response, over 50 sweeps, was different according to polarity, with a higher jitter in 5 of the 7 neurones when stimulation polarity was reversed (figure 3.4 A). These 11 neurones all had a jitter of 5 ms or less (mean: 2.6 ms), and the range of variation was 1-3 ms (mean: 1.6 ms, n= 7).

Furthermore, in 4 of 7 neurones the jitter, over 50 sweeps, was different according to the intensity of the current used to stimulate the vagus nerve (figure 3.4 A). These neurones all had a jitter of 5 ms or less (mean: 2.6 ms) and the maximum variation was 1 ms. Surprisingly, the jitter was greater in 3 of the 4 neurones when current was increased.

Finally, in one neurone the evoked pattern was tested before and after the administration of supplementary anaesthetic (pentobarbitone sodium I.V.; 10 mg kg^{-1}), and the jitter was found to be greater after the addition of the anaesthetic. Unsurprisingly, spontaneous activity, in this neurone, was also inhibited by anaesthetic administration.

On the basis of the above data, the jitter of the total population of NTS neurones was used to classify neurones into two groups; those receiving an excitatory, synaptic, input with a jitter <5 ms and those receiving an input with a jitter >5 ms. In 342 neurones the evoked response of 43 had a jitter <5 ms, whilst the remaining 299 neurones had a mean jitter of 2.96 ± 0.06 ms.

3.2.3 Cardiopulmonary reflex (NTS neuronal response)

The response of 204 NTS neurones to right atrial injections of PBG was examined. In 129 neurones activation of cardiopulmonary afferents produced a burst of excitation in neuronal activity of 43 ± 7 spikes (figure 3.5 A), in contrast, in a further 34 neurones a period of inhibition was evoked of duration 8.5 ± 1.8 s (figure 3.5 B). The remaining neurones were either unaffected by right atrial PBG injection (28) or responded in a biphasic manner, with a period of excitation followed by a period of inhibition (10; figure 3.5 C) or a period of inhibition followed by a period of excitation (3).

24% (31 of 129) of the neurones excited by activation of cardiopulmonary afferents had a myelinated vagal afferent fibre input, whereas only 12% (4 of 34) of the neurones inhibited by afferent activation had a myelinated fibre input. In addition, only 6% (8 of 129) of the neurones excited by right atrial PBG administration had a jitter <5 ms, in contrast to 12% (4 of 34) of the neurones inhibited by PBG. Further analysis also revealed that of 62 neurones with a jitter [2 ms 49 were excited by PBG administration, whilst 6 were inhibited and 7 were unaffected.

The onset latency of the evoked cellular and depressor response to right atrial PBG administration was also examined (i.e. the time at which neuronal activity first changes from baseline and the point at which blood pressure first begins to fall). For the 129 neurones which responded with excitation the mean latency of the neuronal cellular response was significantly longer ($P<0.01$) than the mean latency of the systemic response (3.1 ± 0.3 ms vs. 2.3 ± 0.1 ms respectively). However, in 52 of these neurones the latency of the neuronal response was significantly shorter ($P<0.01$) than that of the systemic response (mean data: 1.7 ± 0.1 ms vs. 2.7 ± 0.1 ms respectively). Additionally, the mean latencies for the 34 neurones that responded to right atrial PBG with a period of inhibition was not significantly different (cell 2.6 ± 0.2 ms vs. sys 2.3 ± 0.2 ms), although again in 14 (40%) of these neurones the latency of the neuronal response was significantly shorter than the systemic response (mean data: 1.8 ± 0.2 ms vs. 2.7 ± 0.2 ms respectively, $P<0.01$).

To examine the effects of receptor ligands on the right atrial PBG injection-evoked excitation of NTS neurones it was necessary to ensure that this neuronal response was consistent between injections (figure 3.6 & 3.7). In a control group of 6 neurones the evoked excitatory response varied by less than 20% in all 6 (mean variation: -3.8 ± 3.1%).

Comparisons of cardiopulmonary reflex (neuronal response)

In 2 NTS neurones sodium nitroprusside (SNP; 1 $\mu g\ kg^{-1}$, I.V.) was used to lower blood pressure to a level equivalent to that evoked by right atrial PBG injection (figure 3.7). SNP evoked no significant change in the activity of either neurone.

Right atrial administration of volume-matched saline was carried out on 10 NTS neurones excited by intra-atrial PBG administration. In 9 of 10 neurones saline evoked no change in neuronal activity (figure 3.8); in the remaining neurone PBG evoked a burst of excitation of 44 spikes, whereas saline evoked an excitation of only 4 spikes.

The excitatory response of 2 NTS neurones to right atrial PBG administration before and after a bilateral vagotomy at the cervical level was also examined; in both neurones the excitation was abolished (figure 3.9; mean: 71.5 (before) to 0 (after) spikes).

Figure 3.1 Cardiopulmonary reflex I – systemic responses to right atrial administration of phenylbiguanide and saline in the anaesthetized rat

Illustration of the systemic responses to the right atrial administration of phenylbiguanide (PBG) and 0.9% saline. From the top, traces show heart rate (HR; bpm - beats min^{-1}), raw phrenic nerve activity (PNA; μV) and blood pressure (BP; mmHg). PBG and saline were administered at the horizontal line (▬). There is a gap in recording signified by the symbol ∿ and of the duration stated.

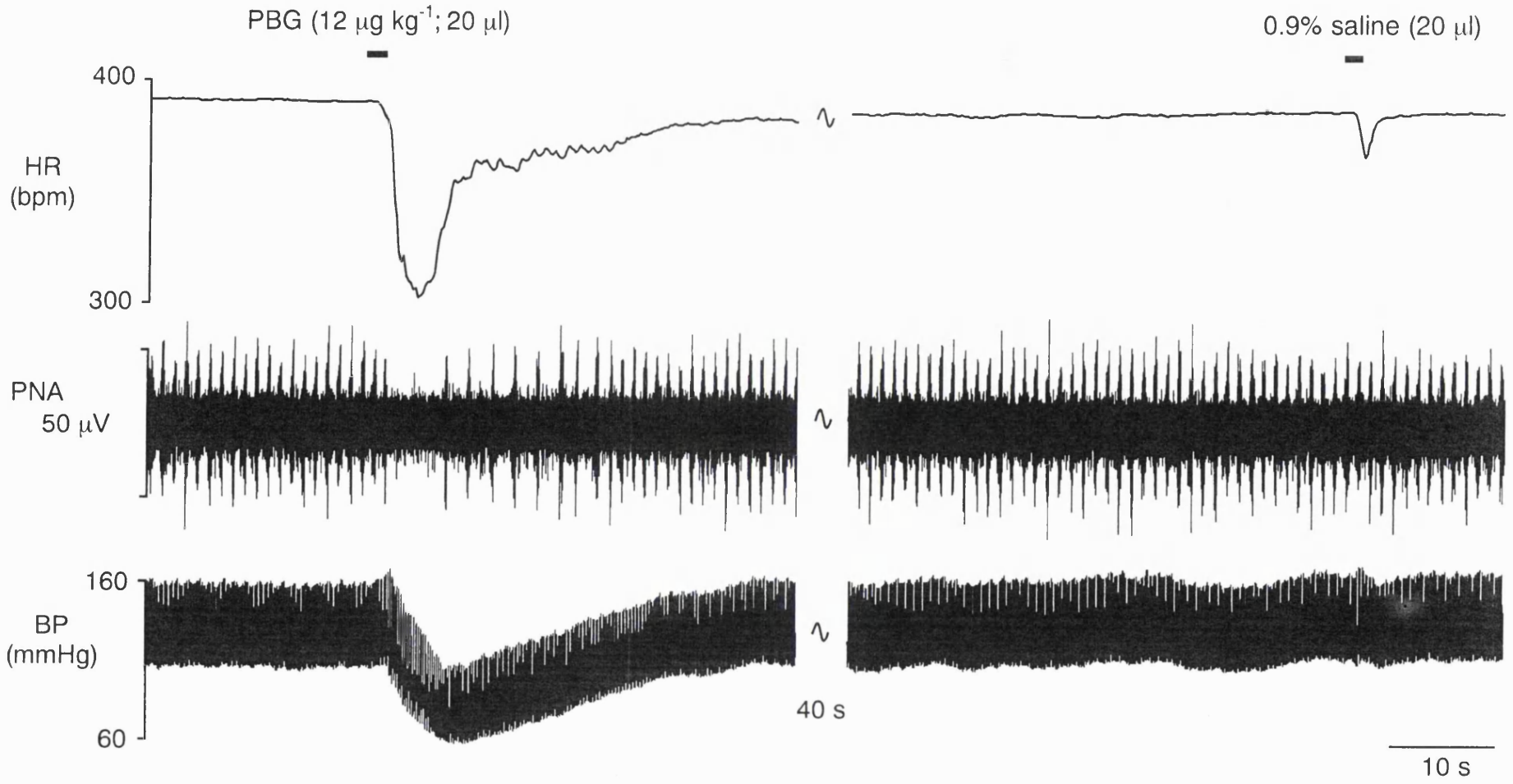

PBG (12 μg kg-1; 20 μl)
0.9% saline (20 μl)
400
HR
(bpm)
300
PNA
50 μV
160
BP
(mmHg)
60
40 s
10 s

Figure 3.2 Cardiopulmonary reflex II – systemic responses to right atrial administration of phenylbiguanide prior to and post a bilateral vagal transection at the cervical level in the anaesthetized rat

Shown are the systemic responses to the right atrial administration of phenylbiguanide (PBG) before and after a vagal transection at the cervical level. From the top, traces show heart rate (HR; bpm - beats min^{-1}), raw phrenic nerve activity (PNA; μV) and blood pressure (BP; mmHg). The administration of PBG is highlighted by a solid horizontal line (▬ ; 12 μg kg^{-1}; 20μl). There is a gap in recording signified by the symbol ∿ and of the duration stated.

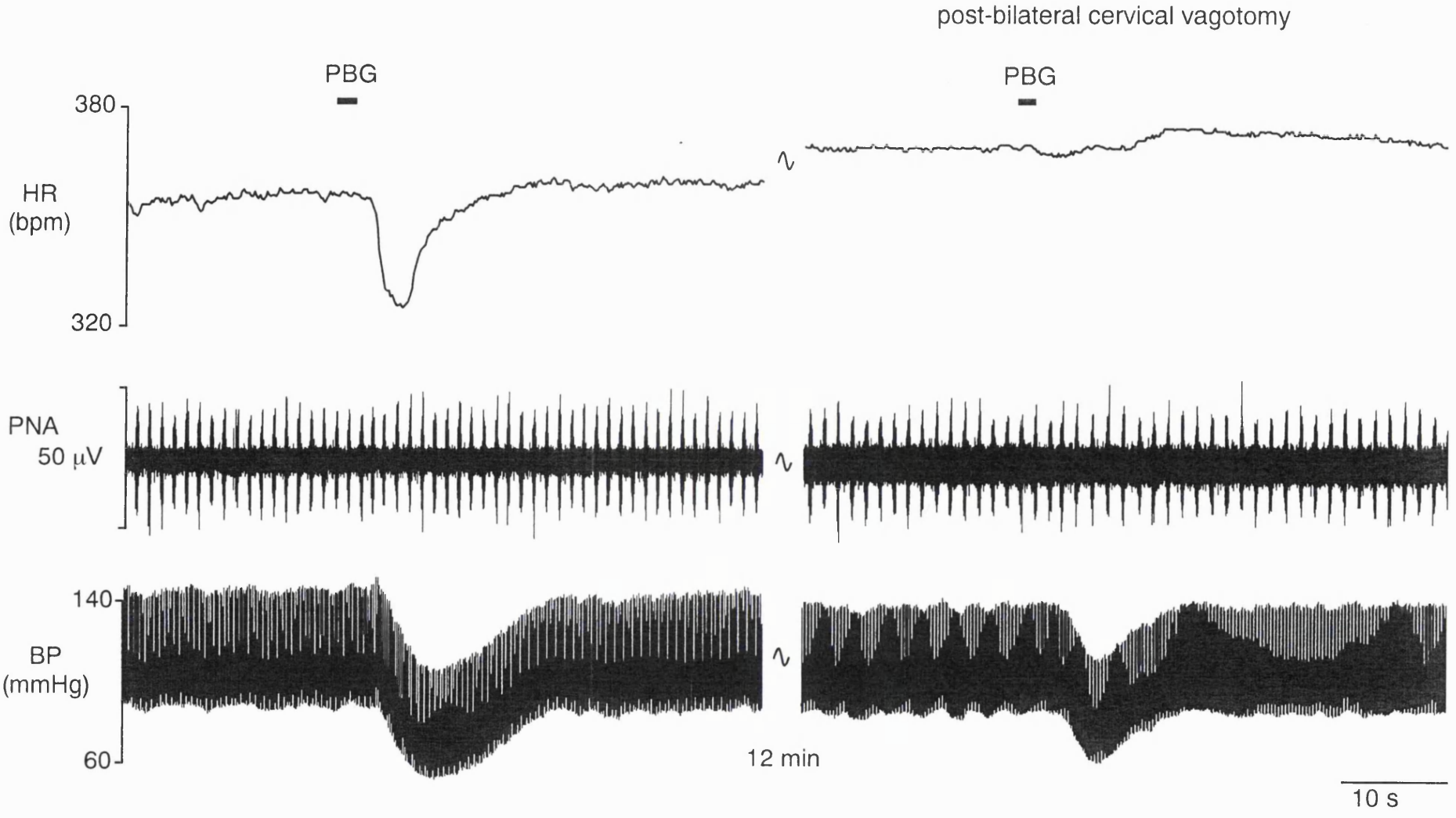

post-bilateral cervical vagotomy
PBG
PBG
380
HR
(bpm)
320
PNA
50 μV
140
BP
(mmHg)
60
12 min
10 s

Figure 3.3 Identification of an NTS neurone in the anaesthetized rat

A The orthodromic activation of an NTS neurone by single shot electrical stimulation of the cervical vagus (150 μA, 1ms, 0.9 Hz). Traces show the raw extracellular recording of neuronal activity (μV). **(i)** Five superimposed sweeps, showing a jitter of 3ms in the evoked response (latency - 32 ms). **(ii)** Three consecutive sweeps indicating the inability of a spontaneous spike in the middle trace to cancel the evoked response. ● - stimulus artefact.

NB. The presence of a second neurone in the background, the evoked response of which is cancelled in the middle trace, suggesting antidromic activation.

B Correlation of the ongoing activity of the same NTS neurone as shown in **A** to blood pressure, tracheal pressure and phrenic nerve activity. The top panel shows an ECG-triggered average of arterial blood pressure (BP, mmHg; 720 sweeps) superimposed above a histogram of neuronal activity triggered with ECG activity (2 ms bin width; 720 sweeps). The bottom panel shows an average of tracheal pressure (TP, mmHg; 54 sweeps) and a simultaneous recording of phrenic nerve activity (PNA; μV) superimposed above a histogram of neuronal activity triggered with tracheal pressure (4 ms bin width; 54 sweeps).

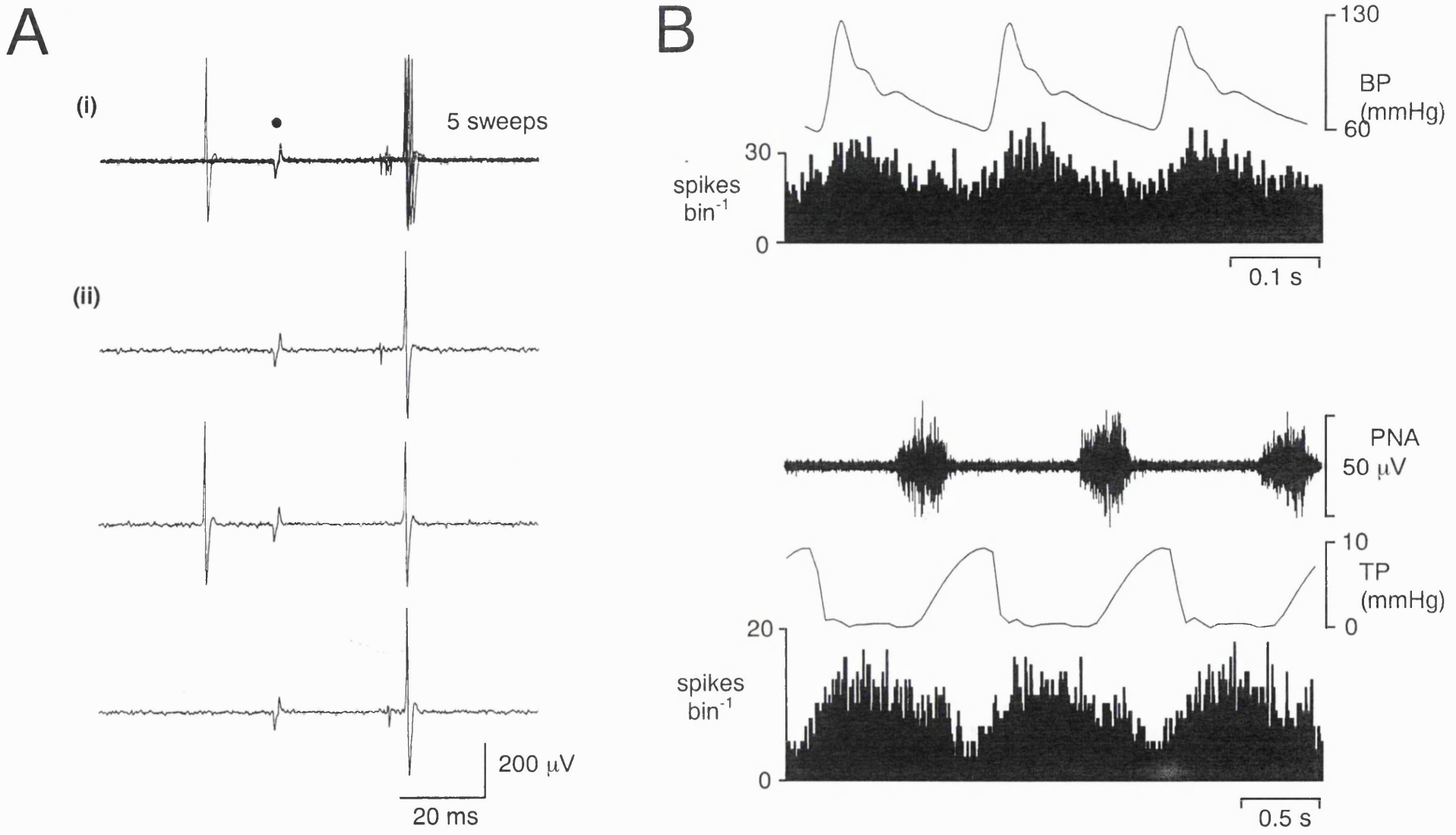
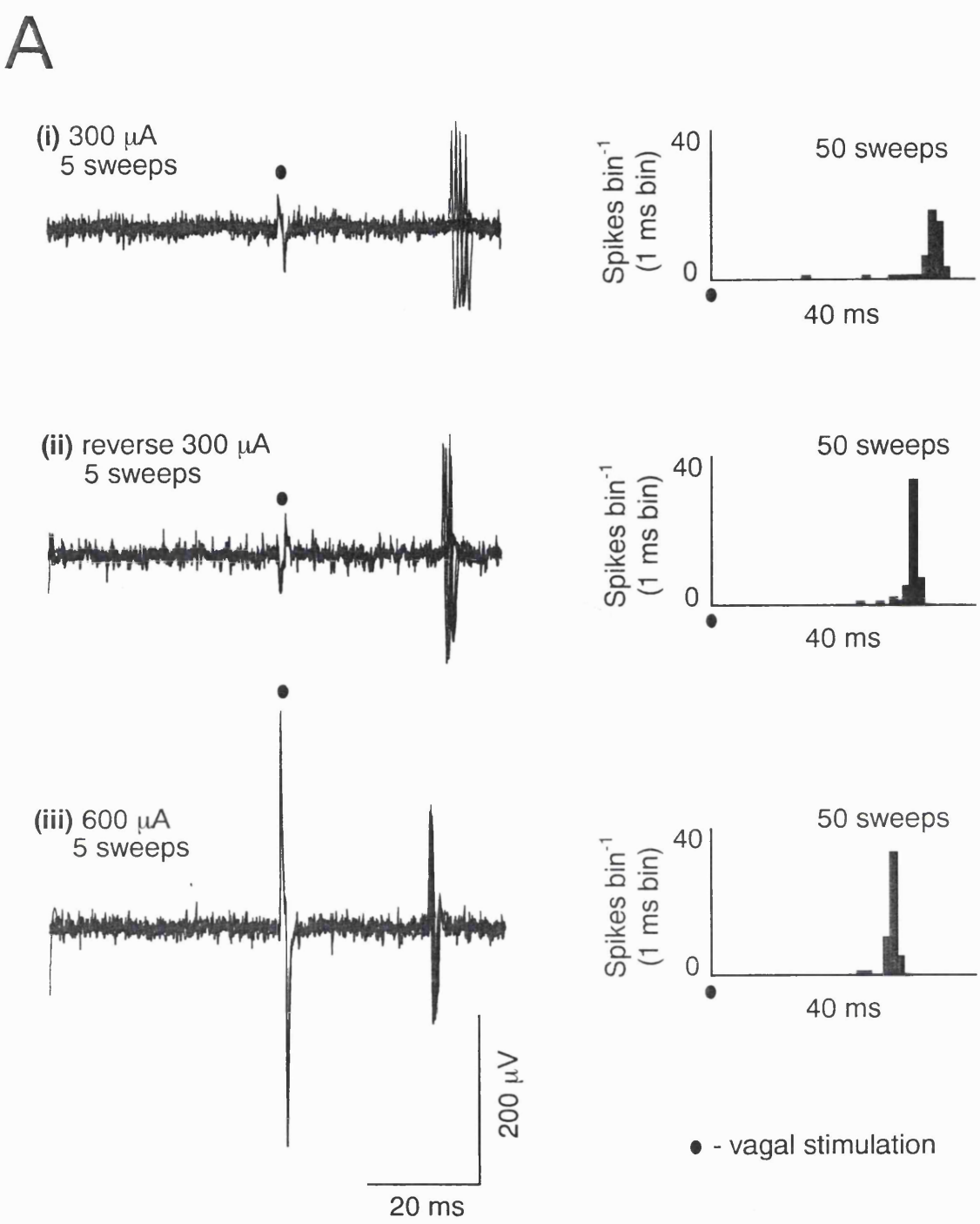

A
(i)
5 sweeps
(ii)
200 μV
20 ms
B
130
BP
(mmHg)
60
30
spikes
bin-1
0
0.1 s
PNA
50 μV
10
TP
(mmHg)
0
20
spikes
bin-1
0
0.5 s

Figure 3.4 Electrophysiological characteristics of an NTS neurone in the anaesthetized rat

A The excitation of an NTS neurone by single shot electrical stimulation of the cervical vagus at different currents and polarities of stimulation (300-600 μA, 1 ms, 0.91 Hz). Traces show 5 superimposed sweeps of neuronal activity (left panels) and post stimulation time histograms (PSTHs) of neuronal activity (right panels) during different vagal stimulation conditions. **(i)** The response to stimulation of the vagus nerve at 2x threshold (300mA; latency 34 ms). **(ii)** The response to stimulation at reverse polarity (300mA; latency 31 ms). **(iii)** The response to stimulation at higher current, 4x threshold (600mA; latency 28 ms).

B Demonstration of inhibition in the ongoing activity of an NTS neurone evoked by electrical stimulation of the cervical vagus (300 μA, 1 ms, 0.91 Hz). A PSTH of the neuronal activity of the same neurone as in **A** is shown, and an inhibitory period occurring after the excitatory response (see above) and lasting 400 ms can be seen .


A
(i) 300 μA
5 sweeps
Spikes bin-1
(1 ms bin)
40
0
50 sweeps
40 ms
(ii) reverse 300 μA
5 sweeps
Spikes bin-1
(1 ms bin)
40
0
50 sweeps
40 ms
(iii) 600 μA
5 sweeps
Spikes bin-1
(1 ms bin)
40
0
50 sweeps
40 ms
200 μV
20 ms
● - vagal stimulation

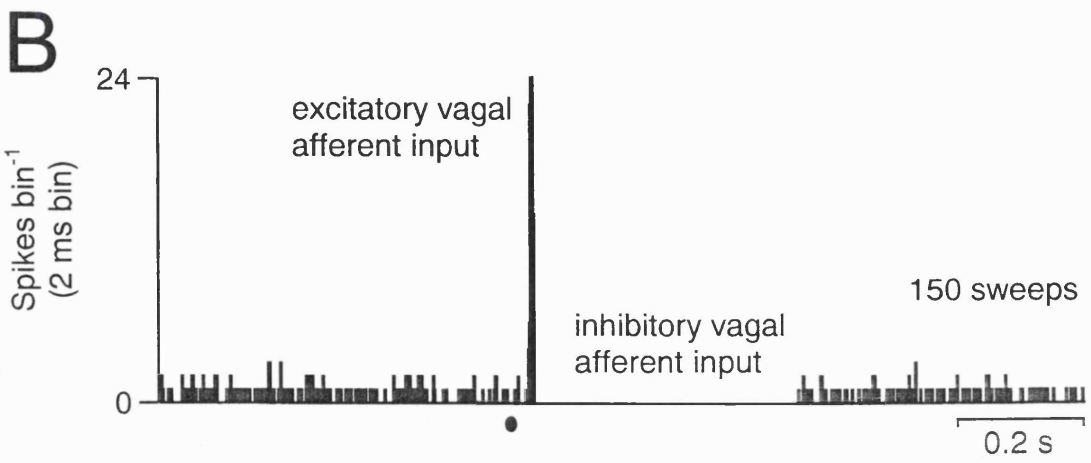

B
24
Spikes bin-1
(2 ms bin)
0
excitatory vagal
afferent input
inhibitory vagal
afferent input
150 sweeps
0.2 s

Figure 3.5 Responses of NTS neurones to activation of cardiopulmonary afferents in the anaesthetized rat

A, B & **C** Responses of three NTS neurones to activation of cardiopulmonary afferents by right atrial administration of phenylbiguanide (PBG). From the top, each set of traces show blood pressure (BP; mmHg), a continuous rate histogram of neuronal activity (spikes bin^{-1}) and the raw recording of neuronal activity (μV). The administration of PBG is highlighted by a solid horizontal line (▬; **A** - 12 μg kg^{-1}, 20 μl; **B** – 18 μg kg^{-1}, 30 μl; **C** – 12 μg kg^{-1}, 20 μl). Of a total population of 204 neurones tested with cardiopulmonary afferent activation 129 were excited (**A**), 34 were inhibited (**B**) and 10 showed a period of excitation followed by a period of inhibition (**C**). Of the remaining 31 neurones, 3 responded with a period of inhibition followed by a period of excitation and 28 neurones were unaffected by cardiopulmonary afferent activation.

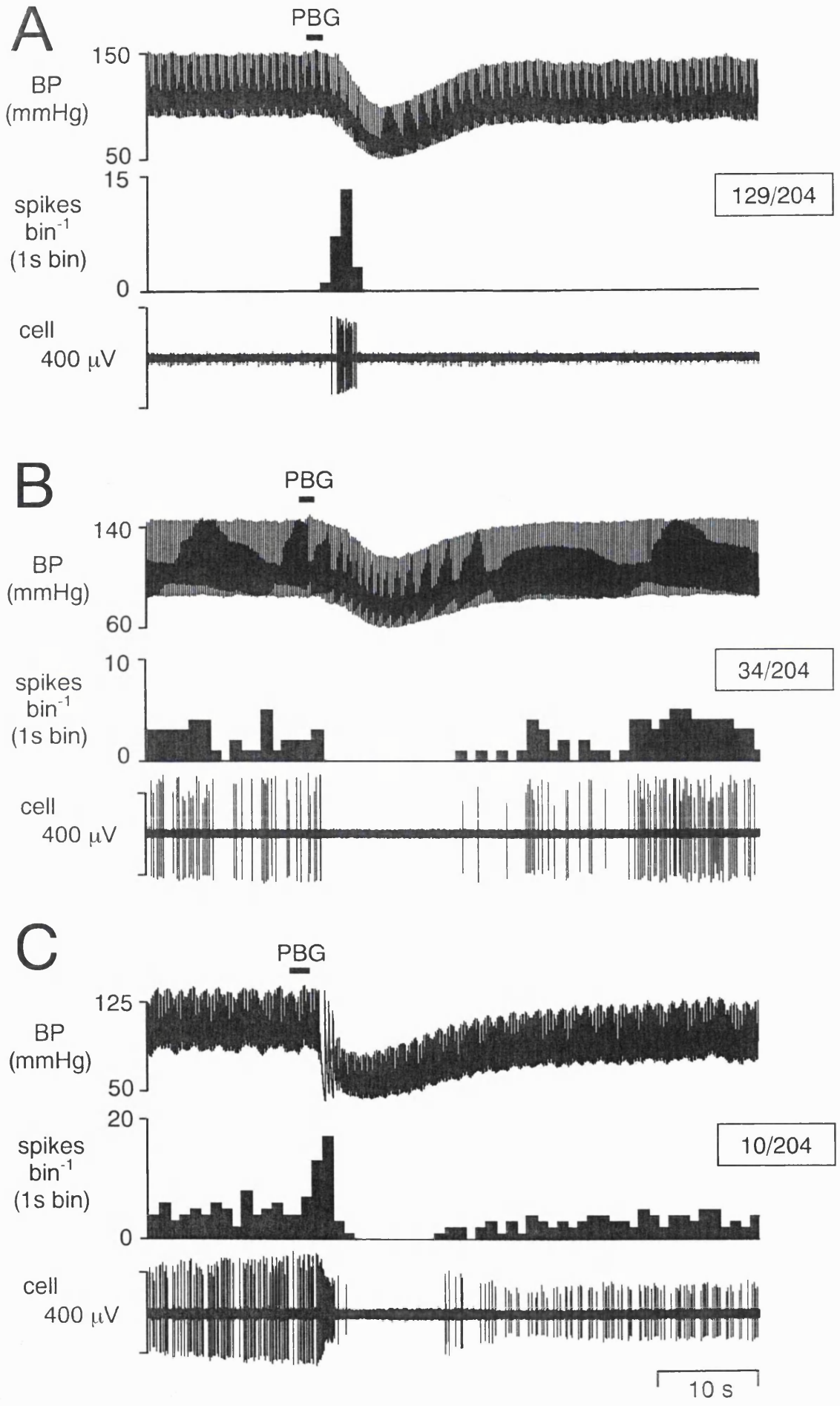

A
PBG
150
BP
(mmHg)
50
15
spikes
bin-1
(1s bin)
0
129/204
cell
400 μV
B
PBG
140
BP
(mmHg)
60
10
spikes
bin-1
(1s bin)
0
34/204
cell
400 μV
C
PBG
125
BP
(mmHg)
50
20
spikes
bin-1
(1s bin)
0
10/204
cell
400 μV
10 s

Figure 3.6 Stability of the neuronal and systemic response to activation of cardiopulmonary afferents in a single NTS neurone in the anaesthetized rat

The response of an NTS neurone to activation of cardiopulmonary afferents by right atrial administration of phenylbiguanide (PBG) on two separate occasions (expanded below). From the top, each set of traces show blood pressure (BP; mmHg), a continuous rate histogram of neuronal activity (spikes bin^{-1}) and the raw recording of neuronal activity (μV), also shown in the expanded sections is raw phrenic nerve activity (μV). The administration of PBG is highlighted by a solid horizontal line (▬ ; 12 μg kg^{-1} ; 20 μl).

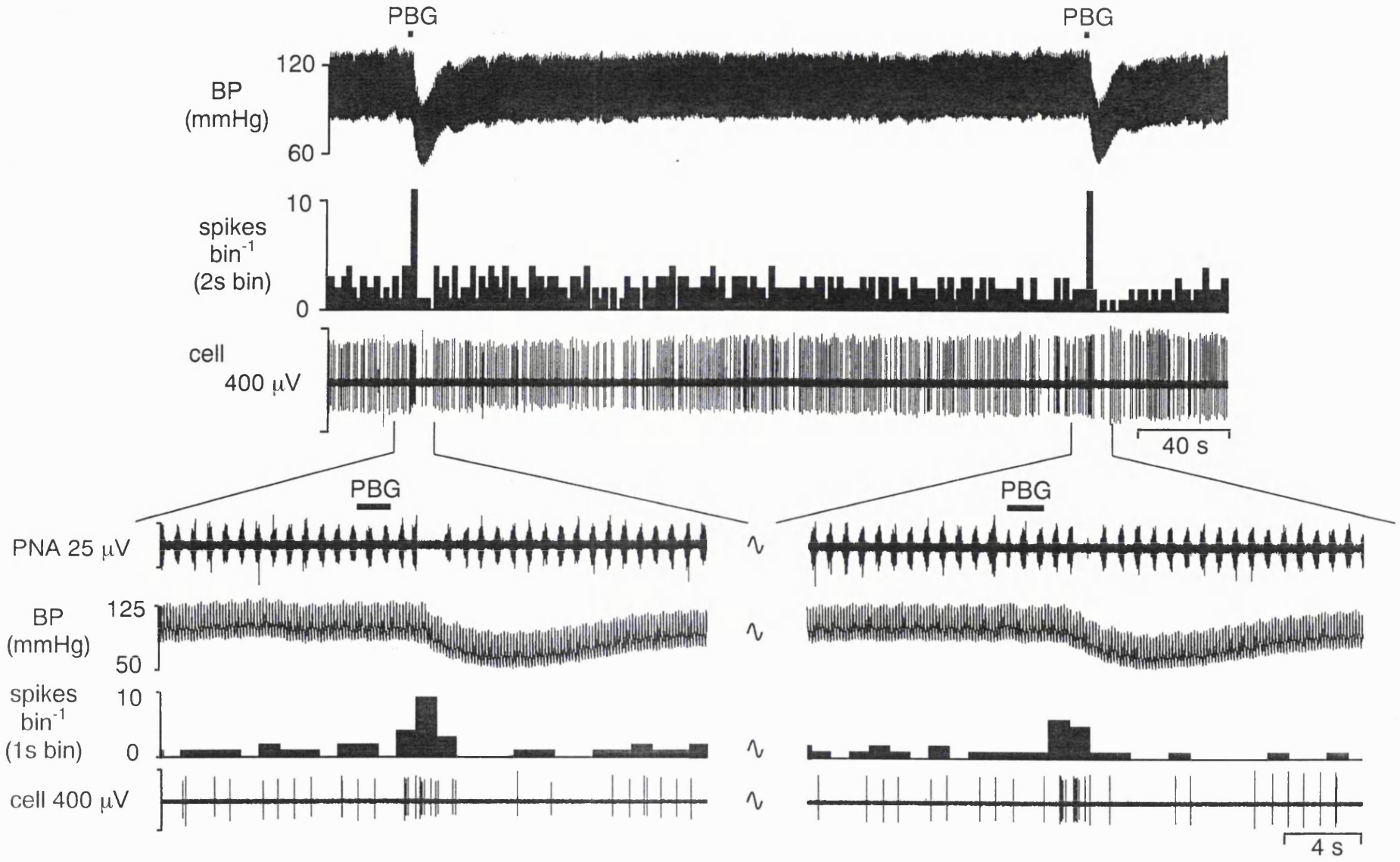

PBG
PBG
120
BP
(mmHg)
60
10
spikes
bin-1
(2s bin)
0
cell
400 μV
40 s
PBG
PBG
PNA 25 μV
125
BP
(mmHg)
50
spikes
bin-1
(1s bin)
10
0
cell 400 μV
4 s

Figure 3.7 Stability of the neuronal and systemic responses to activation of cardiopulmonary afferents, and the response to sodium nitroprusside of a single NTS neurone in the anaesthetized rat

The response of an NTS neurone to activation of cardiopulmonary afferents by right atrial administration of phenylbiguanide (PBG) on two separate occasions, and the response of the same neurone to sodium nitroprusside (SNP). From the top, traces show blood pressure (BP; mmHg), a continuous rate histogram of neuronal activity (spikes bin^{-1}) and the raw recording of neuronal activity (μV). The administration of PBG and SNP is highlighted by a solid horizontal line (▬; PBG - 18 μg kg^{-1}, 30 μl; SNP - 1 μg kg^{-1}, i.v.). The boxes show the total number of spikes in the burst of excitation evoked by cardiopulmonary afferent activation.

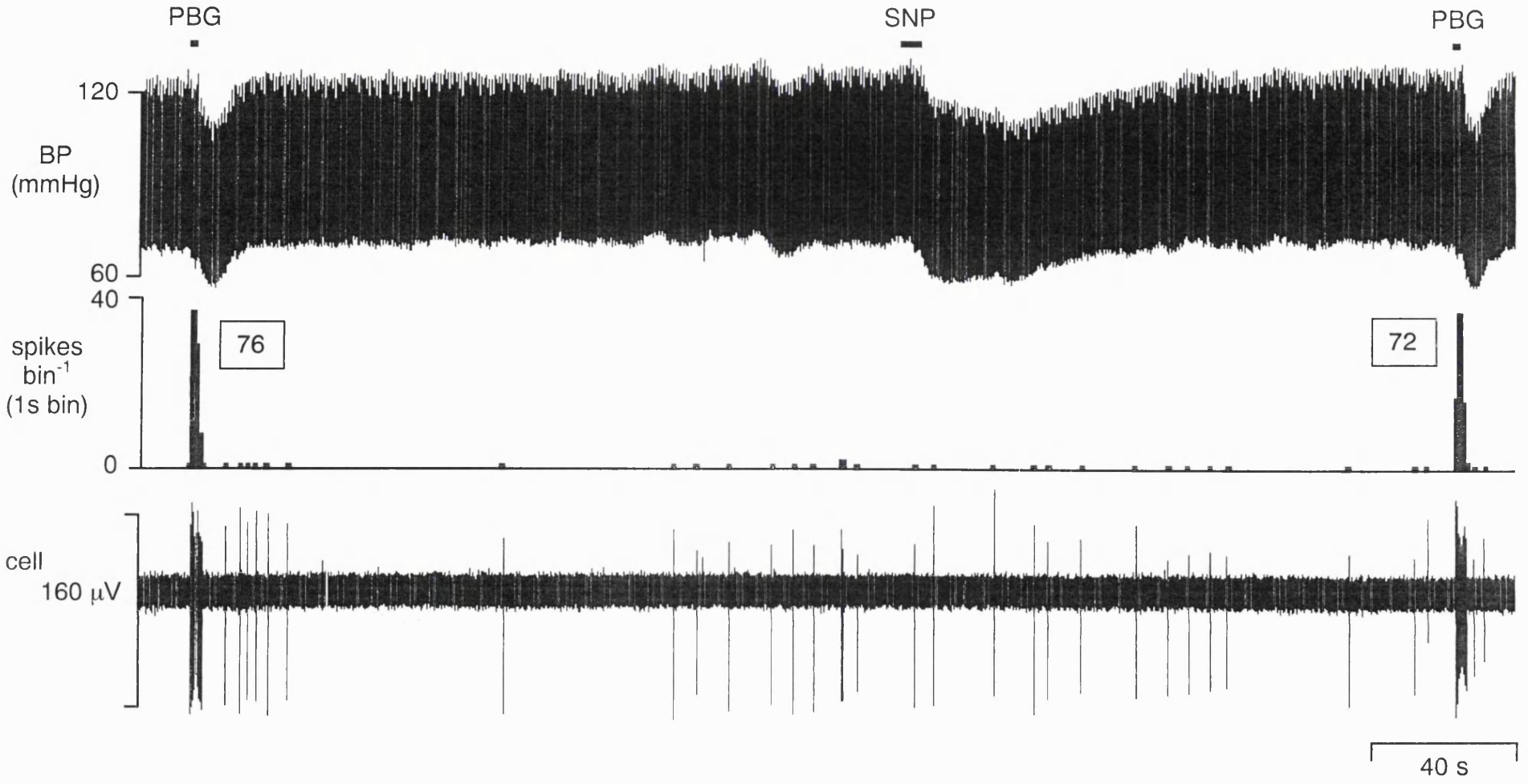

PBG
SNP
PBG
120
BP
(mmHg)
60
40
spikes
bin-1
(1s bin)
0
76
72
cell
160 μV
40 s

Figure 3.8 The response of a single NTS neurone to right atrial injections of phenylbiguanide and saline in the anaesthetized rat

The response of an NTS neurone to activation of cardiopulmonary afferents by right atrial administration of phenylbiguanide (PBG) and the response of the same neurone to right atrial administration of saline. From the top, traces show blood pressure (BP; mmHg), a continuous rate histogram of neuronal activity (spikes bin^{-1}) and the raw recording of neuronal activity (μV). The administration of PBG and saline is highlighted by a solid horizontal line (▬ ; PBG - 12 μg kg^{-1}, 20 μl; saline - 20μl).

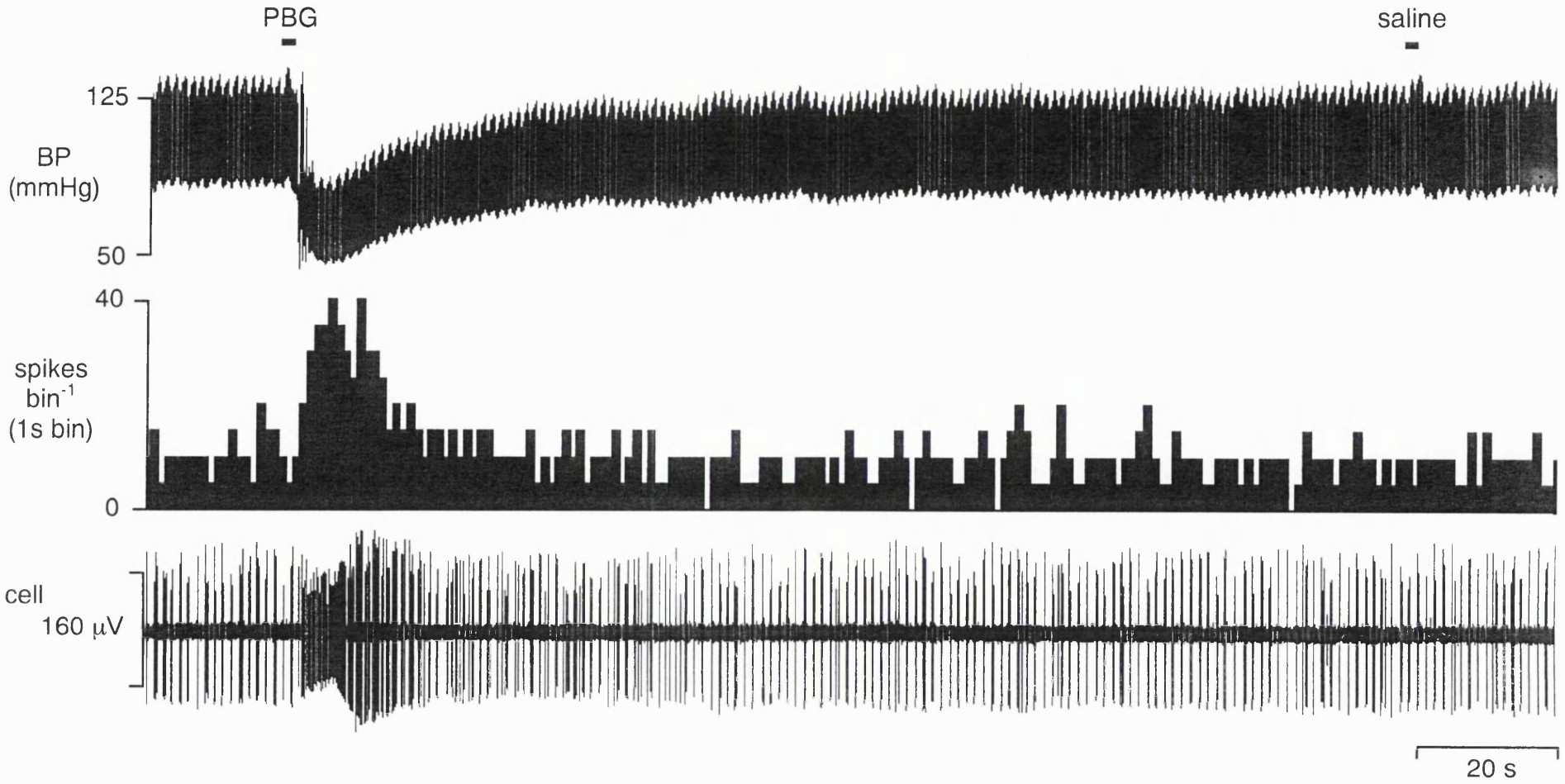
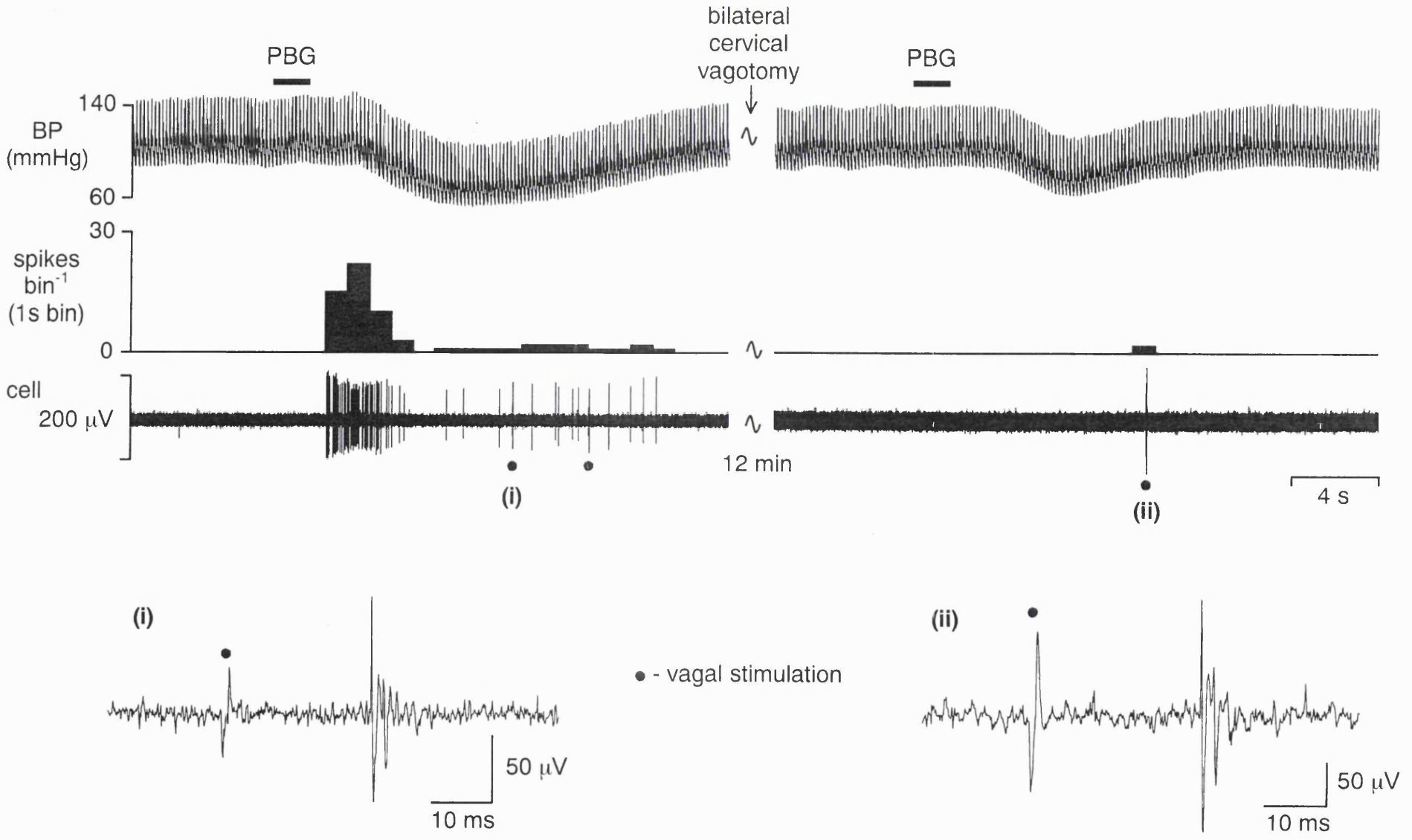

PBG
saline
125
BP
(mmHg)
50
40
spikes
bin-1
(1s bin)
0
cell
160 μV
20 s

Figure 3.9 The response of a single NTS neurone to a right atrial injection of phenylbiguanide is abolished by a bilateral cervical vagotomy in the anaesthetized rat

The response of an NTS neurone to activation of cardiopulmonary afferents by right atrial administration of phenylbiguanide (PBG; left) and the response of the same neurone to right atrial administration of PBG following bilateral cervical vagotomy (right). Top panels - from the top, traces show blood pressure (BP; mmHg), a continuous rate histogram of neuronal activity (spikes bin^{-1}) and the raw recording of neuronal activity (μV). The administration of PBG is highlighted by a solid horizontal line (▬; PBG - 12 μg kg^{-1}, 20 μl). There is a gap in recording signified by the symbol ∿ and of the duration stated. Bottom panels – vagal stimulation was carried out at the identified points (●) and neuronal identity was confirmed between PBG injections by the similar shape of the evoked spike.


PBG
bilateral
cervical
vagotomy
PBG
140
BP
(mmHg)
60
30
spikes
bin-1
(1s bin)
0
cell
200 μV
12 min
(i)
(ii)
4 s
(i)
50 μV
10 ms
● - vagal stimulation
(ii)
50 μV
10 ms

3.3 Results – anaesthetized cat

Baseline values for systemic variables were measured and averaged for all 7 anaesthetized cats. They were (mean ± S.E.): - mean arterial pressure (MAP) 101 ± 5 mmHg; heart rate 160 ± 23 bpm; tracheal pressure - inflation 5.4 ± 2.5 mmHg, deflation 2.1 ± 0.7 mmHg; blood pH 7.32 ± 0.05; blood PO_2 141 ± 27 mmHg; blood pCO_2 38 ± 10 mmHg.

3.3.1 Cardiac vagal preganglionic neurones

Compound 'piggyback' glass microelectrodes were used to record a total of 11 cardiac vagal preganglionic neurones (CVPNs). These neurones had axons located in the cardiac branches of the vagus, confirmed by antidromic activation (figure 3.10 A), with conduction velocities in the B-fibre range (5.9-18.0 m s^{-1}; mean: 11.4 ± 0.9 m s^{-1}). The recording sites of 6 of these neurones were marked by ionophoretic Pontamine Sky Blue ejection (figure 3.11), a further 2 neurones were recorded in close proximity to one of these recording sites. In addition, based on the depth of recording and position of entry of the electrode through the dorsal surface of the brainstem, the positions of the remaining CVPNs were also judged as being close to the marked sites. Thus, the location of at least the majority of neurones recorded was found to be within or ventrolateral to the nucleus ambiguus (figure 3.11).

Correlation of CVPN activity

10 of the 11 CVPNs recorded had little or no ongoing activity, the remaining neurone had an ongoing activity of 6.3 spikes s^{-1}. Therefore, in order to examine correlation patterns of neuronal activity the excitatory amino acid DL-homocysteic acid (DLH; 10-120 nA) was ionophoretically applied to 7 of the 11 neurones.

All 11 CVPNs showed a strong correlation to ECG, with activity at its highest during the peak of the blood pressure wave (figure 3.10 B). Furthermore, all neurones had activity correlated to central respiratory drive, with the highest

period of activity in the post-inspiration and stage 2 expiration phases (figure 3.10 B). This correlation was maintained even in periods of high excitability evoked by ionophoretic DLH administration (60-120 nA; figure 3.12 C). However, artificial removal of central respiratory drive by hyperventilation (data not shown) or low current (10 μA) pulmonary vagal branch stimulation (figure 3.13 B), which also considerably increased ongoing activity, did remove the respiratory-related modulation of these neurones. Finally, the ongoing activity of only 3 of these 11 neurones was correlated to tracheal pressure, at its highest during the period of lung deflation, the remaining 8 neurones did not display any tracheal pressure related rhythm (figure 3.10 B).

Ionophoretic application of DLH at high currents (30-160 nA) also produced a significant fall in both blood pressure and heart rate (MAP – 106 ± 4 to 90 ± 3 mmHg, $P<0.05$; heart rate – 156 ± 6 to 139 ±6 bpm, $P<0.01$; n=6) at 6 of 8 recording sites. In 3 cases CVPN activity was also recorded and increased inversely with the associated decreases in heart rate and blood pressure (figure 3.12).

3.3.2 Cardiopulmonary reflex (CVPN response)

Right atrial injections of phenylbiguanide (PBG; 14-32 μg kg^{-1}, 100-200 μl) excited 9 of 11 CVPNs tested (figure 3.13 A). Increases occurred in both the number of spikes in each respiratory-related burst of activity (11 ± 4 to 27 ± 6; $P<0.01$), as well as in the duration of each burst (1.9 ± 0.5 to 3.6 ± 0.8 ms; $P<0.01$). In these 9 neurones right atrial PBG also caused a significant bradycardia of 69 ± 6 bpm (159 ± 7 to 90 ± 6 bpm, $P<0.01$), a significant fall in blood pressure of 22 ± 1 mmHg (MAP, 96 ± 5 to 74 ± 4 mmHg, $P<0.01$), and a reduction in phrenic nerve activity (figure 3.13 A).

Furthermore, there was a significant ($P<0.01$) difference between the onset latency of the CVPN response (1.8-4.5 s; 3.4±0.3 s) and that of the bradycardic response (2.5-4.5 s; mean 3.7 ± 0.3 s) to right atrial PBG, the excitatory neuronal response commencing before the bradycardia in 6 of the 9 neurones.

Further analysis of this neuronal response revealed that in 8 of the 9 neurones the 1st second of the evoked burst of activity (10 ± 1), which occurred within a 5 second window post-PBG injection, was significantly bigger ($P<0.01$) than the mean of the 1st second of the previous four control bursts (4 ± 1; figure 3.13 A). This excitation is therefore likely to be mediated by activation of C-fibres in the pulmonary circulation as it occurs inside the 5 second window i.e. inside the pulmonary circulation time. Additionally, although the 1st second of the response of the remaining neurone was unaffected, the total intensity and duration of the first burst post-PBG injection was increased.

As mentioned previously low current (10 μA) pulmonary vagal branch stimulation increased the ongoing activity and removed the respiratory phase pattern of firing of a single CVPN, in addition to inhibiting phrenic nerve activity (figure 3.12 B). However, right atrial PBG injection still caused excitation in this neurone (figure 3.12B). This was also found to be the case in a second CVPN, where respiratory modulation was removed by hyperventilation (data not shown).

3.3.3 Synaptic inputs to recorded CVPNs

In 6 of the 8 CVPNs excited by pulmonary C-fibre activation, stimulation of cardiac or pulmonary vagal branches evoked an excitatory synaptic input (figure 3.14). Three of these neurones received an input from both cardiac and pulmonary branches, two received an input solely from the cardiac branch, and the remaining neurone received an input solely from the pulmonary branch.

The latency of these orthodromic inputs (125-230 ms; figure 3.14) was also found to be much greater than that observed for antidromic responses (11-21 ms; figures 3.10 and 3.14). However, one neurone did receive both a short latency and long latency orthodromic input from pulmonary branch stimulation (figure 3.14 A).

Figure 3.10 Identification of a B-fibre cardiac vagal preganglionic neurone in the nucleus ambiguus of an anaesthetized cat

A The antidromic activation of a cardiac vagal preganglionic neurone by electrical stimulation of the right cardiac branch of the vagus (200 μA, 1ms, 0.5 Hz). Traces show the raw extracellular recording of neuronal activity (μV). **(i)** shows five superimposed sweeps, showing the constant latency of the evoked response (15 ms). **(ii)** shows three consecutive sweeps indicating the presence of the evoked response in the top and bottom trace and the cancellation of this response by spontaneous activity as shown in the middle trace. ● - stimulus artefact.

B shows the correlation of the DL-homocysteic acid (20 nA)-evoked activity of the same cardiac vagal preganglionic neurone as shown in **A** to integrated phrenic nerve activity, blood pressure and tracheal pressure. From the top, traces show a histogram of neuronal activity triggered with integrated phrenic nerve activity (50 ms bin width; 330 sweeps), ECG activity (10 ms bin width; 4724 sweeps) and tracheal pressure (10 ms bin width; 1536 sweeps) respectively. Superimposed above each respective histogram is an average of integrated phrenic nerve activity (Int-phre), ECG triggered arterial blood pressure (BP; mmHg) and tracheal pressure (TP; mmHg) with the same number of sweeps taken as for the respective histogram.

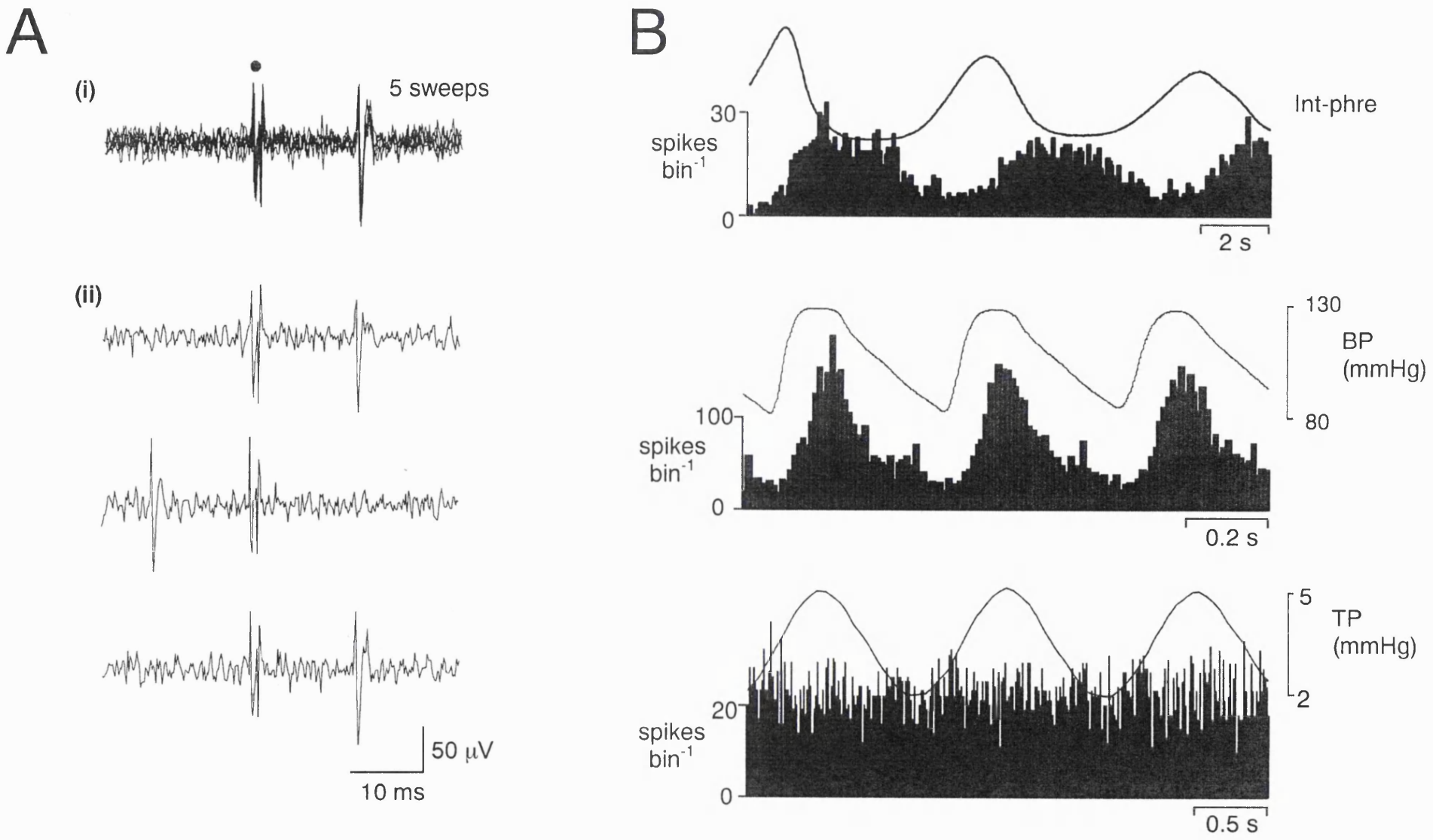

A
(i)
5 sweeps
(ii)
50 μV
10 ms
B
30
spikes
bin-1
0
Int-phre
2 s
130
BP
(mmHg)
80
100
spikes
bin-1
0
0.2 s
5
TP
(mmHg)
2
20
spikes
bin-1
0
0.5 s

Figure 3.11 Histological localisation of cardiac vagal preganglionic neuronal recording sites in the anaesthetized cat

Recording sites marked by Pontamine Sky Blue ejection for six B-fibre cardiac vagal preganglionic neurones mapped onto five standard serial sections of the medulla oblongata from –1 to +2.5 mm caudal to rostral levels relative to obex.

AP – area postrema; NA – nucleus ambiguus; NTS – nucleus tractus solitarius; X – dorsal vagal motor nucleus; XII – hypoglossal nucleus.

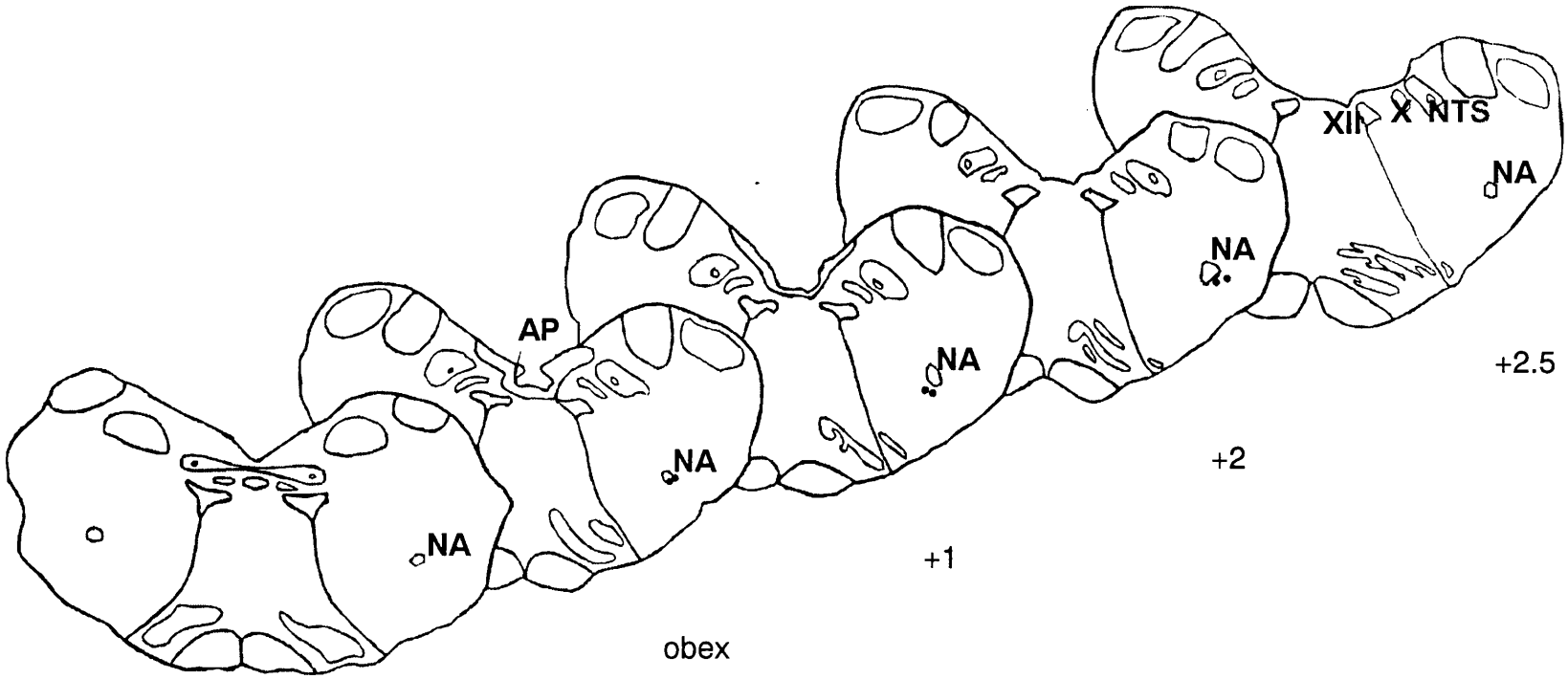

XII
X
NTS
NA
NA
NA
AP
NA
NA
+2.5
+2
+1
obex
-1
• recording sites of B-fibre CVPNs

Figure 3.12 The effect of ionophoretic administration of an excitatory ligand onto a single cardiac vagal preganglionic neurone in the nucleus ambiguus of an anaesthetized cat

A, B & C show the activity of a B-fibre cardiac vagal preganglionic neurone (CVPN) at differing currents of ionophoretic application of the excitatory amino acid DL-homocysteic acid (DLH) in the nucleus ambiguus of an atenolol-pretreated (1 mg kg^{-1}, i.v.) anaesthetized cat. Traces show, from the top, integrated phrenic nerve activity (IPNA), blood pressure (mmHg), heart rate (HR, bpm - beats min^{-1}), a continuous rate histogram of neuronal activity (spikes bin^{-1}; 0.5s bin) and the raw recording of neuronal activity (CVPN, μV). **A** – CVPN activity with no DLH application, **B** – CVPN activity during 20 nA DLH application, **C** – CVPN activity during 60 nA DLH application.

NB. As CVPN activity increases there is an associated fall in heart rate, with the dotted line representing mean heart rate in the absence of DLH application. In addition, respiratory related activity remains even at high levels of activity.

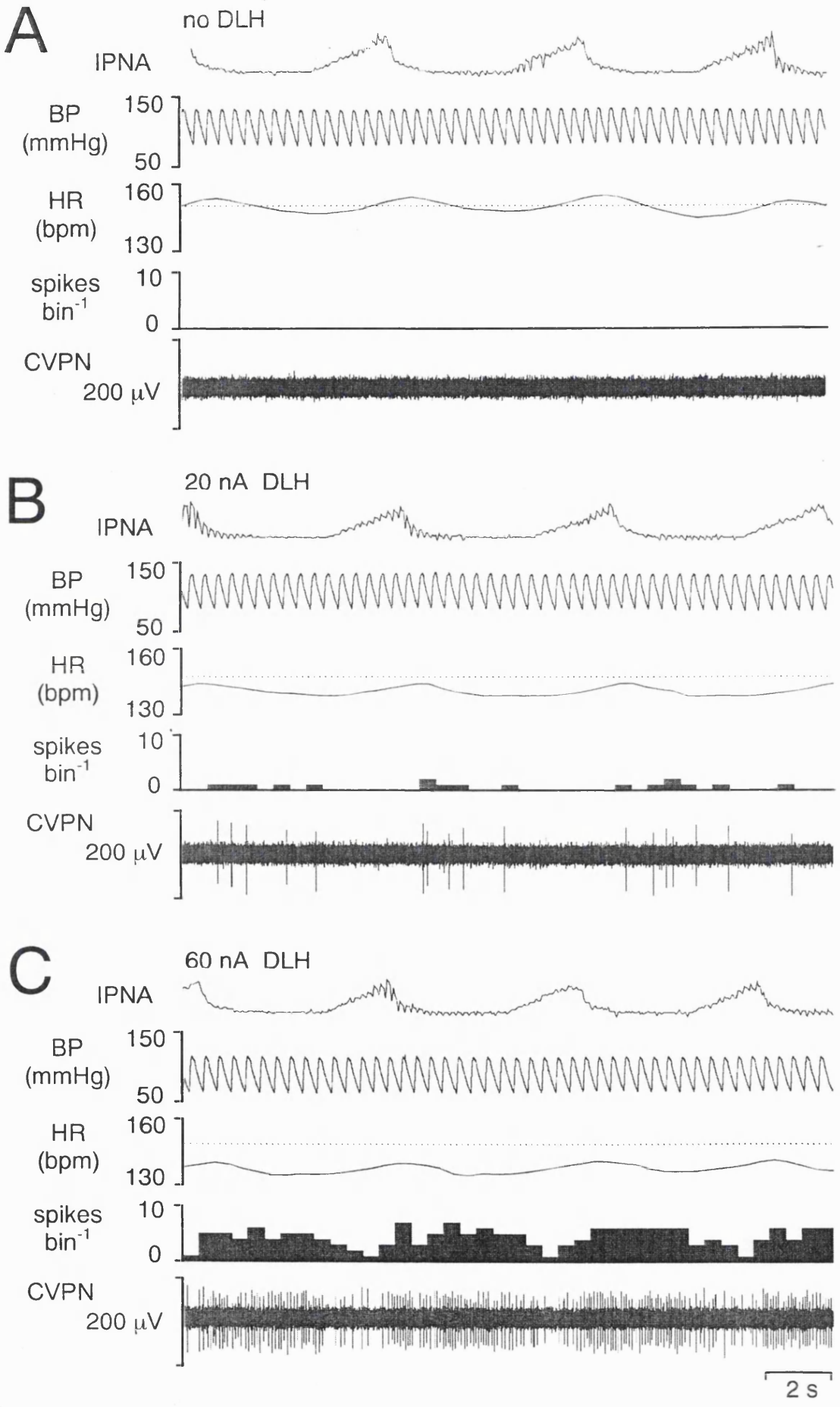

A
no DLH
IPNA
BP
(mmHg)
150
50
HR
(bpm)
160
130
spikes
bin-1
10
0
CVPN
200 μV
B
20 nA DLH
IPNA
BP
(mmHg)
150
50
HR
(bpm)
160
130
spikes
bin-1
10
0
CVPN
200 μV
C
60 nA DLH
IPNA
BP
(mmHg)
150
50
HR
(bpm)
160
130
spikes
bin-1
10
0
CVPN
200 μV
2 s

Figure 3.13 The effect of right atrial injections of phenylbiguanide in the presence and absence of central respiratory drive on the activity of a single cardiac vagal preganglionic neurone in the nucleus ambiguus of an anaesthetized cat

A & B shows the ongoing activity of a B-fibre cardiac vagal preganglionic neurone (CVPN) prior to, during and after an intra-atrial injection of phenylbiguanide (PBG) in the presence (**A**) and absence (**B**) of central respiratory drive in an atenolol-pretreated anaesthetized cat. Traces show, from the top, integrated phrenic nerve activity (IPNA), blood pressure (mmHg), heart rate (HR, bpm - beats min^{-1}), a continuous rate histogram of neuronal activity (spikes bin^{-1}; 0.5s bin) and the raw recording of neuronal activity (CVPN, μV). DL-homocysteic acid is applied at 20 nA during recording. In addition, vertical lines represent a 5 second window following PBG injection (see Methods), and this time period is expanded in panels **(i)** and **(ii)**.

NB. In the bottom panel low intensity electrical stimulation of the pulmonary vagal branch inhibits central respiratory drive, as indicated by the lack of phrenic nerve activity, and the increase seen in neuronal activity during this stimulation is therefore due to inhibition of central respiratory drive.

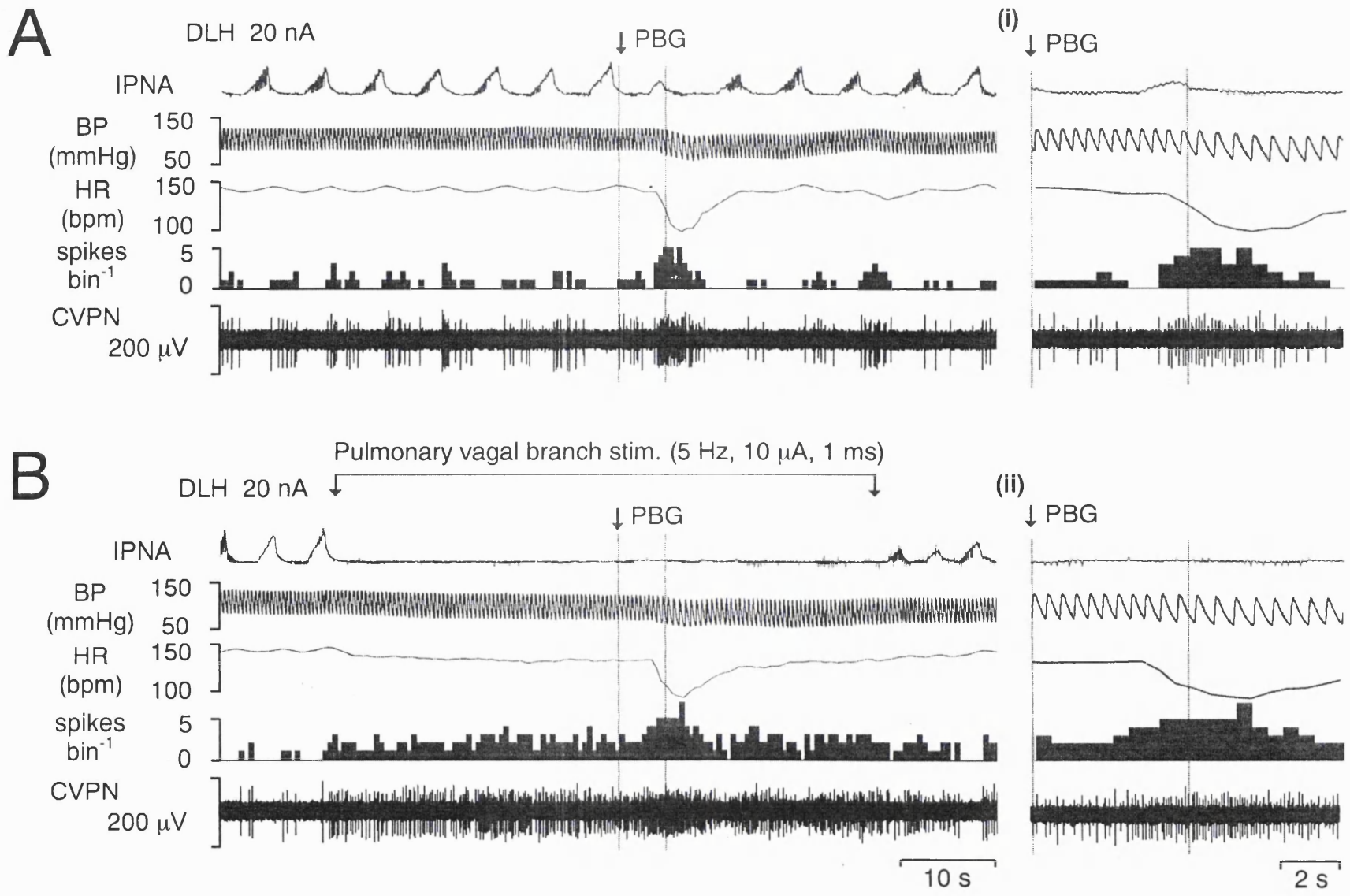
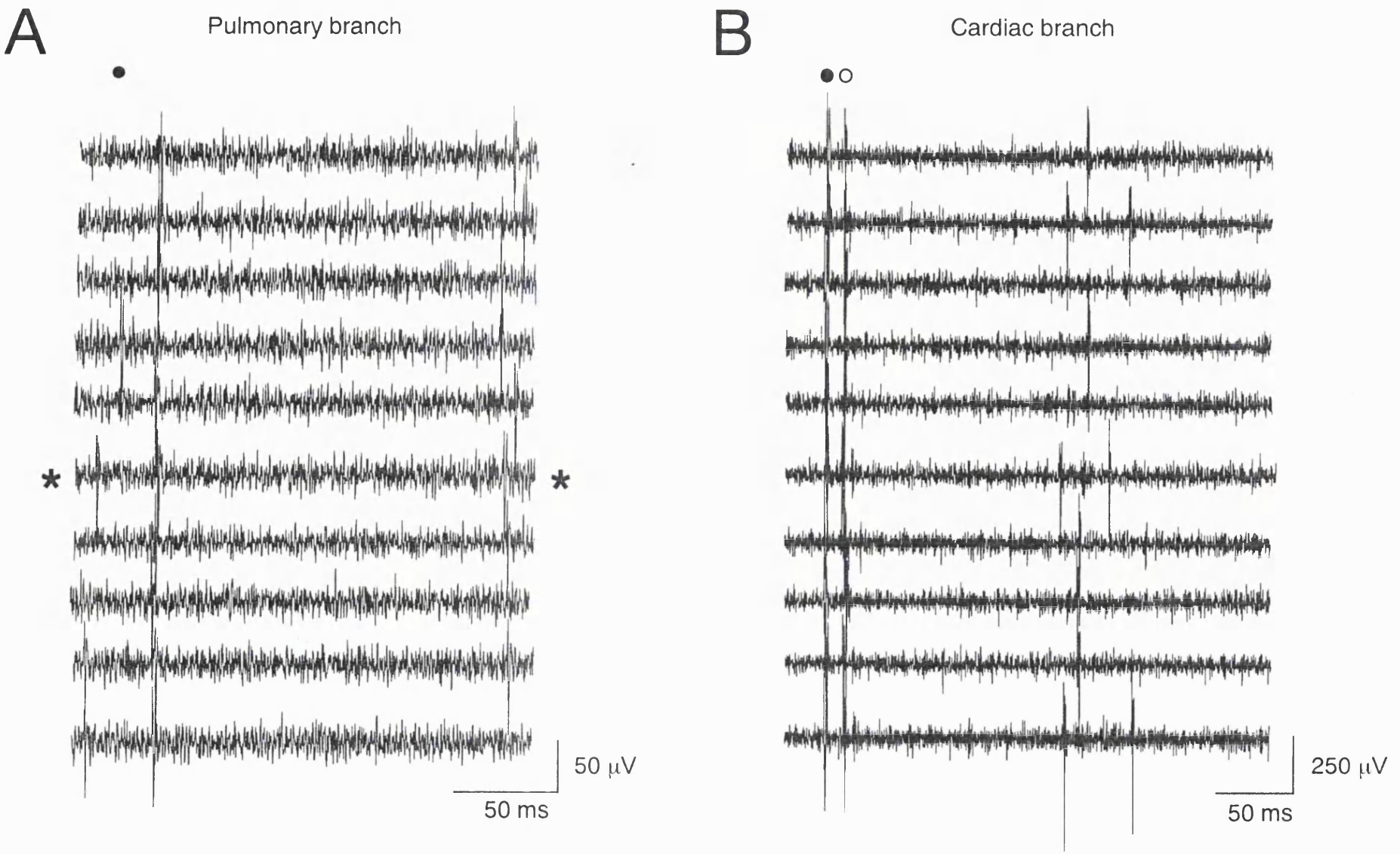

A
DLH 20 nA
↓ PBG
(i)
↓ PBG
IPNA
BP
(mmHg)
150
50
HR
(bpm)
150
100
spikes
bin-1
5
0
CVPN
200 μV
B
Pulmonary vagal branch stim. (5 Hz, 10 μA, 1 ms)
DLH 20 nA
↓ PBG
(ii)
↓ PBG
IPNA
BP
(mmHg)
150
50
HR
(bpm)
150
100
spikes
bin-1
5
0
CVPN
200 μV
10 s
2 s

Figure 3.14 Synaptic activation of cardiac vagal preganglionic neurones from stimulation of thoracic vagal branches in the anaesthetized cat

Two sets of traces containing ten consecutive sweeps each, showing the effect of stimulation (●) of the pulmonary branch (200 μA, 1 Hz, 1 ms; **A**) and the cardiac branch (150 μA, 0.5 Hz, 1 ms; **B**)

A stimulation of the pulmonary branch evoked both a short and a long latency orthodromic excitatory input (NB. In the 6th trace (*) the spontaneous spike did not cancel the short latency input from the pulmonary nerve, identifying it as a synaptic input)

B stimulation of the cardiac branch evoked a short latency antidromic spike (o) and a long latency synaptic input.


A
Pulmonary branch
*
*
50 μV
50 ms
B
Cardiac branch
250 μV
50 ms

3.4 Discussion

Main findings

Rat:-

Neurones located in caudal medial areas of the NTS received excitatory and inhibitory vagal afferent input and had activity correlated to cardiovascular and respiratory variables. The majority of these neurones responded to right atrial administration of PBG but were unaffected by i.v. administration of sodium nitroprusside. In addition, the neuronal activation of neurones evoked by right atrial PBG administration was abolished by a bilateral cervical vagotomy.

Cat:-

Neurones located within or ventrolateral to the NA were antidromically activated by stimulation of the cardiac branch of the vagus, with a latency of response consistent with small myelinated fibre transmission. These neurones had activity correlated to respiration and the oscillations of arterial blood pressure and were activated by right atrial administration of PBG. In addition, a large proportion of neurones received afferent input from cardiac and pulmonary branches of the vagus nerve.

Systemic data

Right atrial administration of a 5-HT_3 receptor agonist, PBG in these experiments, consistently produced a bradycardia, hypotension and apnoea in both rats and cats, as has been previously demonstrated (Coleridge *et al.*, 1991). In a small number of rats a fall in phrenic nerve activity did not occur, an observation most likely explained by the relatively low dose of PBG administered (12-24 $\mu g\ kg^{-1}$).

Volume-matched controls of right atrial PBG injections, using the PBG vehicle saline, were carried out in the rat and demonstrated that PBG was indeed responsible for the apnoea since saline did not induce any reduction in phrenic nerve activity. Furthermore, the large majority of the bradycardia and hypotension evoked by PBG was also not mediated by PBG vehicle or by the

activation of atrial stretch afferents since saline only evoked a very small reduction in blood pressure and heart rate. These small alterations in cardiovascular variables evoked by right atrial saline administration might be mediated via temperature i.e. saline (and PBG) was held in the cannula at room temperature and this may be cold enough to affect node conduction in the heart, marginally lowering cardiac output and subsequently blood pressure. However, due to the low volumes of fluid injected in the rat (20-40 μl) these responses are unlikely to be mediated via the activation of stretch receptors in the right atrium. Thus, the majority of the cardiovascular response and the entire respiratory response to right atrial administration of PBG is mediated via the actions of PBG and not by PBG vehicle or by volume effects in the heart.

In the cat, the β-adrenoceptor antagonist atenolol was used to prevent alterations in heart rate via changes in the sympathetic nervous system, ensuring that the bradycardic response to right atrial PBG administration was mediated solely via increases in cardiac vagal activity. In the rat, a bilateral cervical vagotomy was carried out between reflexes to demonstrate the vagal component of the response to right atrial PBG administration. The bradycardic and respiratory components of the response were almost entirely abolished by bilateral cervical vagotomy, although the hypotension was reduced by only a third. This is in agreement with a number of other studies (Sévoz *et al.*, 1996; Verberne & Guyenet, 1992) in the demonstration of a non-vagal component of the hypotension, although in conscious rats complete inhibition of the hypotensive and bradycardic responses during cholinergic blockade has been shown (Chianca & Machado, 1996). Thus, in these experiments at least, the bradycardia, apnoea and a small component of the hypotension evoked by right atrial administration of PBG is mediated via the vagus nerve.

Nucleus tractus solitarius neurones

Neuronal characteristics

Extracellular recordings were made of a large population of neurones in the nucleus tractus solitarii of anaesthetized rats. In the vast majority of neurones an action potential was evoked by cervical vagus nerve stimulation with a latency consistent with unmyelinated fibre transmission. A significant number

(~20%) of neurones also received an excitatory input with a latency consistent with small myelinated fibre passage, a similar proportion to that described previously (Wang *et al.*, 1997). In addition, vagal afferent stimulation evoked an inhibitory response in over a third of all neurones recorded, although whether this was mediated by unmyelinated or myelinated fibres remains to be determined.

Initial examinations were made into the relative position of recorded neurones within their afferent pathway through the NTS. *In vitro* studies have described how the variability of the evoked action potential between multiple stimuli (known as synaptic jitter) is the most reliable method of discriminating mono- versus polysynaptic pathways (Doyle & Andresen, 2001). Furthermore, an *in vivo* study has classified neurones into 3 groups: those receiving vagal afferent input with a jitter >5 ms, those with a jitter of 3-5ms and those with a jitter <3ms (Sévoz-Couche *et al.*, 2000b). Neurones with low jitter were assumed to be likely monosynaptic, 2nd order neurones, and those with a high jitter were deemed as higher order, polysynaptic neurones. However, in these experiments it was found that the synaptic jitter could vary during different afferent stimulation conditions which could result in neurones being classified incorrectly. Figure 3.4 is an example of this since the jitter of the unmyelinated input to this neurone is <3 ms at high current stimulation but at lower current stimulations this jitter rises to 4 ms. Other factors were also found to affect the jitter of the vagal input, including increased jitter after the addition of supplementary anaesthetic. Thus, in the present experiments two groups were used to classify neurones, those with jitter >5 ms, which were deemed as polysynaptic, possibly output, neurones, and those with a jitter of <5 ms which would include 2nd order and higher order NTS neurones.

Half of all NTS neurones recorded had no ongoing activity in these pentobarbitone sodium anaesthetized rats, a proportion found to be similar to that described in previous studies using the same anaesthetic (Wang *et al.*, 1997; Sévoz-Couche *et al.*, 2000b). This is unlikely to reflect the proportion of NTS neurones with ongoing activity in conscious rats, and may well be related to the level of anaesthesia, confirmed by the observation that ongoing activity

was suppressed after supplementary anaesthetic administration during neuronal recording.

Almost 90% of neurones were correlated to cardiovascular and respiratory variables, with almost half of all neurones correlated to both, suggesting that these neurones play some role in cardio-respiratory function. When comparing the regions in which NTS neurones were recorded (0.5 mm rostral and caudal to obex, <0.5 mm lateral to midline) with the afferent termination density map shown in figure 1.2 this result is perhaps not surprising. Generally, neurones were located in medial and relatively caudal zones of the nucleus although distinctions were not made regarding accurate localisation in specific subnuclei. These areas are thought to have the highest density of vagal afferent termination (Cottle, 1964; section 1.3.1) and unmyelinated chemo-, bronchial and pulmonary receptor afferent termination (see Loewy & Burton, 1979; Kalia & Mesulam, 1980; Jordan & Spyer, 1986). However, these studies have also described how the highest density of baroreceptor afferent termination was in more lateral areas, which may explain the lack of a neuronal response to the vasodilator sodium nitroprusside in the present experiments.

Right atrial administration of PBG

A high proportion of NTS neurones (86%) responded to the activation of cardiopulmonary afferents evoked by right atrial administration of PBG. The majority of neurones were excited by this stimulus, although some responded either with inhibition or in a biphasic manner. Bilateral cervical vagotomy abolished the excitatory response of neurones, confirming that at least the activation of neurones is mediated via vagal transmission in the present experiments. Furthermore, the lack of a neuronal response to volume-matched right atrial administration of saline demonstrated that the response to PBG cannot be accounted for by the activation of atrial stretch receptors.

In a large proportion of neurones the latency of the neuronal response was shorter than the onset of the hypotension evoked by right atrial PBG administration. This suggests that these neurones were activated by, and possibly mediating, the vascular response to PBG, as opposed to being reflexly

activated by the systemic response to right atrial PBG. In some neurones the latency of the neuronal response was very short (<1 s), which may suggest that at least part of the response is mediated within the pulmonary circulation time, i.e. is due to the activation of pulmonary C-fibres. Further, a recent study has examined the response of NTS neurones to right atrial and left ventricular injections of PBG and described how right atrial PBG evoked a significantly greater excitatory neuronal response (27 spikes s^{-1}) than left ventricular PBG (11 spikes s^{-1}; Wilson *et al.*, 1996), suggesting that the difference is due to the activation of afferents in the pulmonary circulation. In addition, another study used far higher doses (90 μg) of PBG than the present studies (4-8 μg) to activate cardiac chemoreceptors via pericardial administration, which may indicate the amount of PBG used in this investigation is insufficient to activate chemoreceptors in the heart. However, the activation of vagal afferents in both the heart and the systemic circulation cannot be wholly ruled out, indeed there are also a high number of 5-HT_3 receptors located throughout the digestive tract (another vagal afferent source; Glatzl *et al.*, 2002) which may be activated by right atrial PBG administration. In summary, the excitatory neuronal response to right atrial administration of PBG is mediated via chemoreceptors, which subsequently activate vagal afferents and, at least a proportion of this excitation is likely to be directly resulting from afferent activation in the pulmonary and/or cardiac circulation.

Interestingly, a previous study reported that neurones with an afferent input jitter >5 ms were excited by cardiopulmonary reflex, as opposed to neurones with an afferent input jitter <5 ms which were inhibited by this stimulus (Sévoz-Couche *et al.*, 2000b). Those authors suggested that this observation might underlie a protective effect of cardiopulmonary afferents on inputs from other depressor cardiovascular reflexes such as the baroreflex. However, in this study only 6% of neurones excited by right atrial PBG administration had a jitter >5 ms, compared with 12% of inhibitory responding neurones. These conflicting data may reflect the responses of different sub-populations of NTS neurones although the anatomical regions of the NTS in which recordings were made are similar. In fact, in the present experiments nearly half of all neurones excited by PBG had a synaptic jitter of <2 ms, suggesting that these are 2nd order NTS

neurones receiving monosynaptic vagal afferent input. Interestingly, the latency of the excitatory neuronal response to PBG was very short in the majority of these neurones which, once more, may indicate that this excitation is mediated via the activation of vagal afferents in the pulmonary circulation.

Unfortunately, whether these neurones (or indeed any of the NTS neurones recorded in these experiments) project to other neurones within the NTS, to higher cell groups or directly to preganglionic cell groups in the medulla cannot be determined. Therefore, the nature of the response (excitatory or inhibitory) to cardiopulmonary afferent activation may not reflect that which is transmitted to other neurones outside the NTS.

Conclusions

In conclusion, this study describes a population of NTS neurones located primarily in caudo-medial areas of the nucleus which have activity related to resting autonomic variables and are insensitive to baroreceptor modulation. The majority of these neurones are sensitive to activation of cardiopulmonary afferents although whether the origin of the neuronal response to right atrial PBG administration is entirely from the pulmonary and/or cardiac circulations or includes the activation of afferents from further along the systemic circulation cannot be wholly determined in these experiments. However, the excitatory neuronal response is mediated via the vagus and further evidence suggests that a proportion of the excitation is mediated via cardiopulmonary afferent activation. In addition, a large number of the excitatory-responding neurones may well be 2nd order NTS neurones receiving monosynaptic vagal afferent input.

Cardiac vagal preganglionic neurones

Neuronal characteristics

The characteristics of neurones recorded within or ventrolateral to the nucleus ambiguus that were antidromically activated following stimulation of one of the cardiac branches of the vagus nerve were also examined in the anaesthetized cat. These neurones had respiratory-related activity which was also positively correlated to the oscillations of arterial blood pressure. This activity was highest

in the post-inhibitory and stage 2 expiratory phases of the respiratory cycle. The combination of these characteristics confirm their identity as cardiac vagal preganglionic neurones as described previously (McAllen & Spyer, 1976, 1978a,b; Gilbey *et al.*, 1984). Subsequent high current ionophoretic ejection of DLH at six recording sites of CVPNs in these atenolol-pretreated animals evoked a bradycardia confirming the vagally-mediated chronotropic capabilities of these neurones. In addition, the bradycardia evoked by DLH administration was paralleled by increasing CVPN activity at two of these sites, confirming similar observations made by McAllen & Spyer (1978a). However, atrial ionotropic, dromotropic or coronary vasomotor changes were not identified, therefore the precise cardiac functions of these neurones cannot be determined.

Right atrial administration of PBG

Right atrial administration of PBG activated 9 of the 11 CVPNs indicating a synaptic input from cardiopulmonary C-fibres to these neurones. In addition, it is important to remember that right atrial injections of PBG may also activate afferents in the systemic circulation (Coleridge & Coleridge, 1979). However, in the cat, it has been demonstrated that the latency for right atrial PBG to evoke a bradycardia due to activation of pulmonary C-fibre afferent stimulation alone ranges between 2 and 5 seconds (Daly & Kirkman, 1988). Thus, using a 5 second window to discriminate neuronal activity following PBG injection, an increase was recorded in the activity of 8 CVPNs, and this excitation was therefore mediated solely by pulmonary C-fibre afferents within this time period. Furthermore, an excitatory orthodromic spike was evoked in 6 of these 8 neurones by electrical stimulation of the cardiac and pulmonary branches of the vagus, confirming that these neurones received an input from C-fibre afferents running in these branches. The failure to see evoked spikes in the remaining 2 neurones may be a result of the afferents supplying these neurones running in cardiac or pulmonary branches other than those which were stimulated.

Since right atrial injections of PBG inhibit inspiration then another potential component of the evoked excitation of CVPNs may be mediated by indirect disinhibition of these neurones resulting from inhibition of central respiratory

drive. However, during periods of central apnoea, induced by pulmonary vagal branch stimulation, right atrial PBG injection still evoked an excitation in recorded CVPNs, suggesting that the observed neuronal excitation is independent of the inhibition of central respiratory drive. As described, ongoing or DLH-evoked CVPN activity was respiratory modulated, therefore it may be expected that the PBG-evoked excitation was also modulated by respiratory activity. However, due to the short duration of the evoked excitation and the variability in the size and occurrence of phrenic nerve activity this could not be examined in these experiments.

Conclusions

In conclusion, this study demonstrates that CVPNs with B-fibre axons, located in the region of the nucleus ambiguus are activated by the stimulation of pulmonary C-fibre afferents and at least part of this excitation was not due to the inhibition of central respiratory drive by these afferents. In combination with the findings of other studies (Jones *et al.*, 1998), this indicates that the stimulation of pulmonary C-fibre afferents activates simultaneously two groups of CVPNs, those in the region of the nucleus ambiguus described above and also CVPNs with C-fibre axons located in the dorsal vagal motor nucleus. The question therefore remains as to how the vagal bradycardia evoked by stimulation of pulmonary C-fibres is not respiratory modulated (Daly & Kirkman, 1988, 1989; Daly, 1991)? As mentioned the activity of B-fibre CVPNs is respiratory modulated therefore it would be expected that pulmonary C-fibre evoked increases in activity, and indeed baroreceptor and chemoreceptor evoked changes are also respiratory modulated. However, since this was not tested in these experiments this may or may not be the case. It is unlikely that the high firing rate of CVPNs evoked by pulmonary C-fibre stimulation (figure 3.13Ab) is overriding the respiratory modulation since CVPNs, in which the firing was raised to similar levels with DLH, still showed respiratory modulation (figure 3.12C). However, it may be possible that respiratory modulation might be prevented if the activation of pulmonary C-fibre afferents also inhibited the inspiratory input to CVPNs, in addition to causing the excitation described above. Another possibility is that an interaction may occur between the activity of CVPNs located in the dorsal vagal motor nucleus with C-fibre axons and the

activity of B-fibre CVPNs in the region of the nucleus ambiguus at the level of the cardiac ganglia and/or postganglionic nerve terminals in the sino-atrial node (Jones, 1993; Jones *et al.*, 1998). However, the exact mechanism by which the pulmonary C-fibre-evoked bradycardia remains non-respiratory modulated is still unclear.

Chapter 4 5-$HT_{1B/1D}$ receptor modulation of NTS neuronal activity

4.1 Introduction

Activation of centrally located 5-$HT_{1A/1B/1D\ \&\ 1F}$ receptor subtypes using i.c.v. injection has been shown to mediate cardiovascular responses *in vivo* (see sections 1.6.1 & 1.6.2 and Ramage, 2001). Studies have shown that i.c.v. administration of the archetypal 5-$HT_{1B/1D}$ receptor agonist sumatriptan produced a depressor response in the presence of a 5-HT_{1A} antagonist (Gallacher & Ramage, 1996). This response could be attenuated by pre-treatment with the selective 5-HT_{1D} receptor antagonist GR127935. In contrast, i.c.v. administration of CP-93,129, a selective 5-HT_{1B} receptor agonist, produced a pressor response (Gallacher & Ramage, 1996), suggesting opposing effects for 5-HT_{1B} versus 5-HT_{1D} receptors.

Some of these 5-HT_1 receptor ligand-mediated effects on the cardiovascular system have now been attributed to a brainstem location of action. Microinjection techniques have now demonstrated the ability of 5-HT to produce both pressor and depressor effects when administered to the NTS (see Lawrence & Jarrott, 1996). These contradictory observations may be explained by the activation of a number of different 5-HT receptor subtypes as described above with distinct functional roles. It is also important to note the limitations of microinjection techniques in accurately targeting specific cell groups, with injected compounds diffusing to adjacent central nuclei.

Autoradiographic studies have demonstrated binding sites for both 5-HT_{1B} and 5-HT_{1D} receptors in the NTS (Manaker & Verderame, 1990). Therefore, it is likely that a proportion of these cardiovascular responses are mediated by an

action within this nucleus. Furthermore, recent studies have suggested a possible excitatory action for the 5-HT_{1B} selective ligand CP-93,129, and an inhibitory action for the 5-$HT_{1B/1D}$ ligand sumatriptan on the excitatory vagal-evoked activity of NTS neurones in the anaesthetized rat (Wang *et al.*, 1998b).

Aims:

- Experiments were carried out to further investigate the effects of ligands selective for 5-HT_{1B} and 5-HT_{1D} receptor binding sites on the baseline activity of NTS neurones in the anaesthetized rat. In addition, their modulatory actions on vagal or cardiopulmonary afferent input to NTS neurones was also examined.

4.2 Results

4.2.1 Effects of 5-$HT_{1B/1D}$ receptor ligands on neuronal activity

The effect of ionophoretic administration of 5-HT_{1B} and 5-HT_{1D} receptor ligands and their vehicles on the activity of NTS neurones was tested in a total population of 108 cells. A proportion of these neurones showed little or no ongoing activity therefore in some cases it was necessary to raise the activity of these cells using ionophoretic application of the excitatory amino acid DL-homocysteic acid (DLH) to examine the effects of these 5-HT receptor ligands. Excitation or inhibition of the activity of each neurone was classified by a change in this baseline firing rate of greater than 20%. Recording of the baseline activity, post-ligand administration, was not achieved in all neurones. However, mean firing rates (spikes s^{-1}) for both the full populations tested and the proportions with a recorded post-ligand response are given.

Sumatriptan

Ionophoretic administration of the 5-$HT_{1B/1D}$ receptor agonist sumatriptan caused inhibition in 54 of 64 NTS neurones tested and this reduction in baseline firing rate had an average magnitude of 66% (figure 4.1; table 4.1). Sumatriptan did not affect the activity of 8 of the remaining 10 neurones but increased the baseline firing rate of the other 2 neurones by more than 20% (table 4.1).

Sumatriptan in the presence of ketanserin

The predominant inhibitory response evoked by ionophoretic administration of sumatriptan was examined during the ionophoretic administration of the 5-$HT_{2A/1D}$ receptor antagonist ketanserin (see table 4.1). In 11 of the 14 neurones tested the inhibitory response to sumatriptan was attenuated in the presence of ketanserin (figures 4.2 and 4.6). In these 11 neurones sumatriptan evoked a mean decrease in baseline activity of 71% which was reduced to a 28% decrease in the presence of ketanserin. In addition, the response to sumatriptan post-ketanserin administration was recorded in 5 neurones and

partial recovery of the control sumatriptan response was observed. Interestingly, in 3 of the 11 neurones the inhibition in baseline activity evoked by sumatriptan was reversed to an excitation in the presence of ketanserin (figure 4.3). This excitatory response to sumatriptan was not found to be statistically significant, although it did return to an inhibition in both neurones where the post-ketanserin response was recorded.

In 2 of the remaining 3 neurones ketanserin had no effect on the inhibitory response evoked by sumatriptan whilst in 1 neurone it was potentiated (table 4.1).

The effect of ketanserin on the baseline activity of NTS neurones was also examined (see table 4.4). In 7 of 14 neurones ketanserin reduced baseline firing rates (figure 4.6), although the group data showed that this was not statistically significant. In a further 6 neurones ketanserin had no effect on baseline firing rates, whilst the firing rate of the remaining neurone was increased by more than 20%.

Sumatriptan in the presence of GR55562B

The inhibitory response evoked by sumatriptan was also examined during the ionophoretic administration of the h5-HT_{1B} receptor antagonist GR55562B (see table 4.2). In 6 of 11 NTS neurones a 58% reduction in baseline activity evoked by sumatriptan was significantly increased to 82% in the presence of GR55562B (figure 4.4). In 4 of these neurones the sumatriptan response post-GR55562B administration was recorded and a partial recovery of the control inhibition was observed (figures 4.4).

Of the remaining 5 neurones the inhibition evoked by sumatriptan was attenuated in 2 and unaffected in 3 neurones in the presence of GR55562B.

In addition, the effect of ionophoretic administration of GR55562B on the baseline activity of NTS neurones was examined in 32 neurones (see table 4.4). In 20 neurones GR55562B caused a mean decrease of 45% in the baseline

activity (figure 4.7). The activity of the remaining 12 neurones was unaffected by GR55562B.

CP-93,129

Ionophoretic administration of the selective 5-HT_{1B} receptor agonist CP-93,129 was examined in 41 NTS neurones (see table 4.2). In the majority of neurones (31 of 41) CP-93,129 caused excitation, increasing the mean baseline activity of these neurones by 100% (figure 4.5). However, CP-93,129 also evoked a significant decrease in the ongoing activity of 5 of 41 neurones (figure 4.5B), whilst the remaining 5 neurones were unaffected by CP-93,129 administration. In addition, the post-CP-93,129 activity was recorded in 28 of the 31 neurones excited by CP-93,129 and complete recovery of control baseline activity was observed.

Figure 4.1 The effect of ionophoretic administration of sumatriptan on the activity of NTS neurones in the anaesthetized rat

A Current-dependent inhibition of the activity of an NTS neurone evoked by ionophoretic administration of sumatriptan (during the bars & at the currents stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histogram of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. From left to right: baseline activity (■; CON), activity evoked by ionophoretic administration of sumatriptan (20-160 nA; □; SUM), and recovery of baseline activity (▨; REC). 54 of 64 neurones were inhibited by the ionophoretic administration of sumatriptan. Of the remaining 10 neurones 8 were unaffected whilst 2 were excited by sumatriptan (figure 8.1).

** $P<0.01$

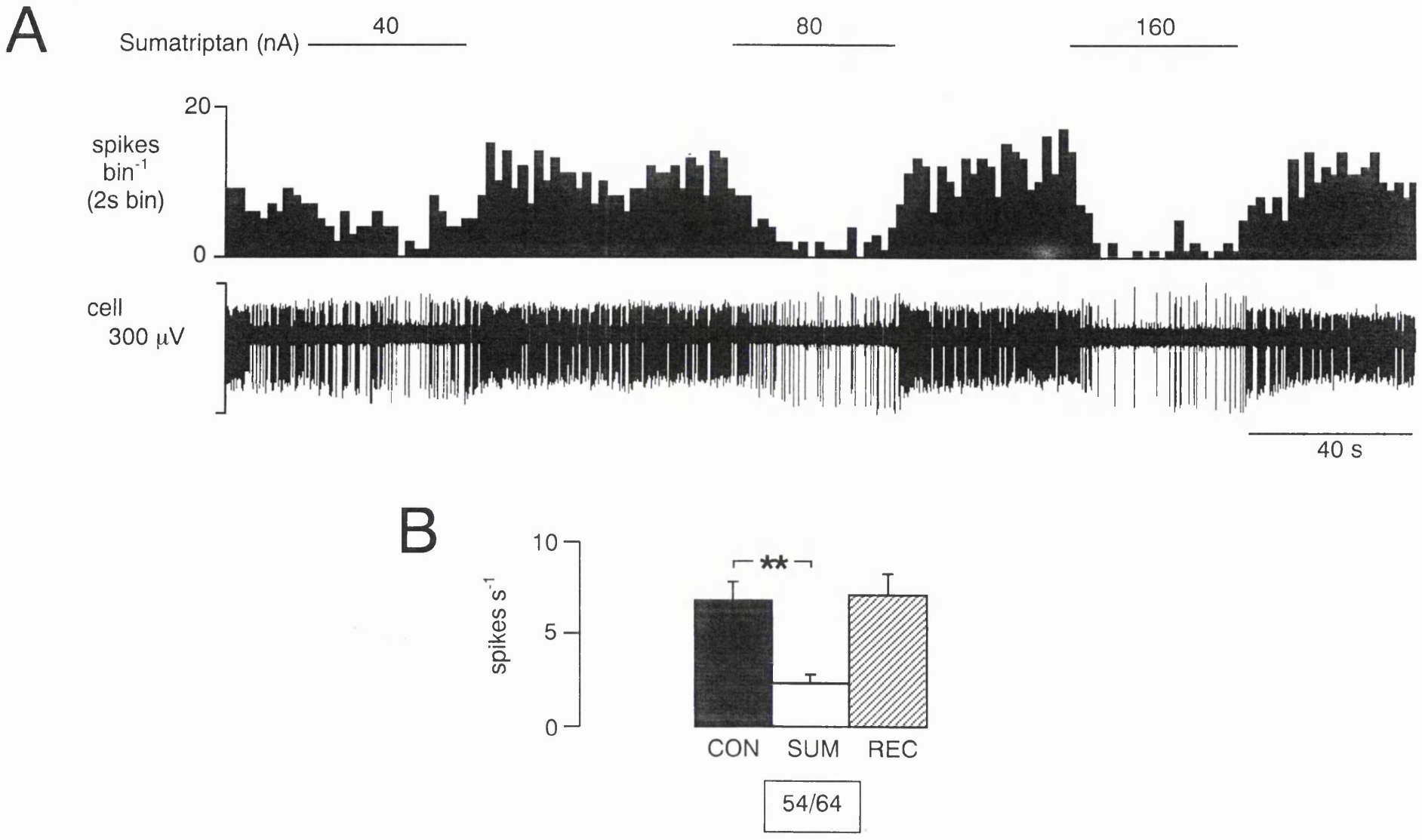
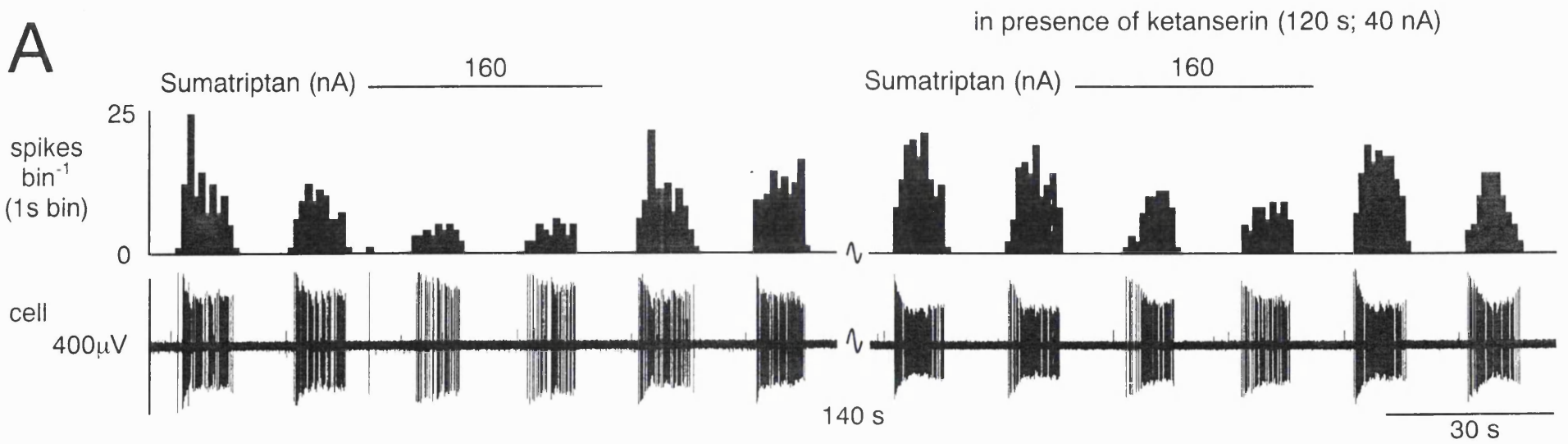

A
Sumatriptan (nA)
40
80
160
20
spikes
bin-1
(2s bin)
0
cell
300 μV
40 s
B
10
5
0
spikes s-1
**
CON
SUM
REC
54/64

Figure 4.2 The effect of ionophoretic administration of ketanserin on the inhibitory effect evoked by ionophoretic administration of sumatriptan in the anaesthetized rat

A The response of an NTS neurone to the ionophoretic administration of sumatriptan before and during the ionophoretic administration of ketanserin (during the bars and at the currents stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. From left to right: activity evoked by ionophoretic administration of sumatriptan (20-160 nA; ☐; SUM), activity evoked by sumatriptan in the presence of ketanserin (10-40 nA; ▤; SUM + KET), baseline activity (■; CON) and recovery of baseline activity (▨; REC). The inhibitory response to sumatriptan was attenuated by ketanserin in 11 of 14 neurones, with the baseline firing rates of 10 of these neurones shown. The post-ketanserin response to sumatriptan was recorded in 5 of these 11 neurones (figure 8.2). Of the remaining 3 of the 14 neurones the inhibitory response to sumatriptan was unaffected in 2 and potentiated in one in the presence of ketanserin (figure 8.2).

** $P<0.01$


A
in presence of ketanserin (120 s; 40 nA)
Sumatriptan (nA)
160
Sumatriptan (nA)
160
25
spikes
bin-1
(1s bin)
0
cell
400μV
140 s
30 s

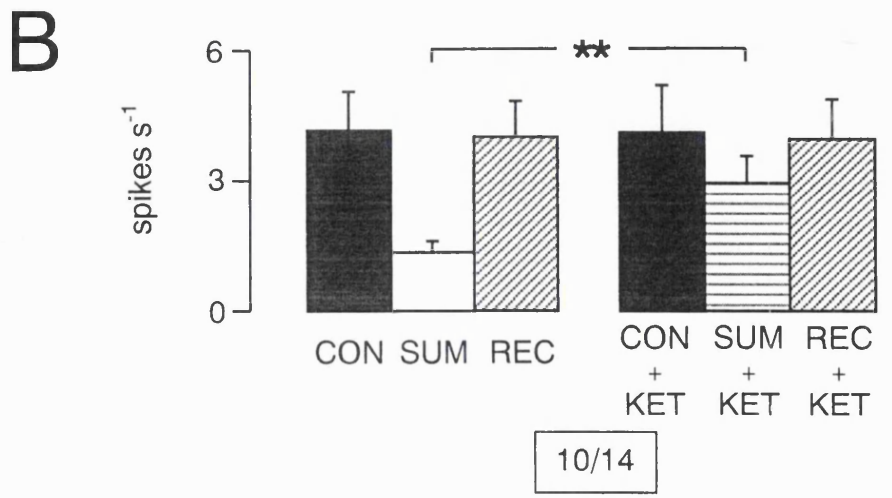

B
**
6
3
0
spikes s-1
CON SUM REC
CON + KET
SUM + KET
REC + KET
10/14

Figure 4.3 An excitatory response to the ionophoretic administration of sumatriptan during the ionophoretic administration of ketanserin in the anaesthetized rat

A The response of an NTS neurone to the ionophoretic administration of sumatriptan (during the bars and at the currents stated) before, during & after the ionophoretic administration of ketanserin. The trace shows a rate histogram of neuronal activity (spikes bin^{-1}).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. From left to right: activity evoked by ionophoretic administration of sumatriptan (40-80nA; ☐; SUM), activity evoked by sumatriptan in the presence of ketanserin (10-40 nA; ▤; SUM + KET), baseline activity (■; CON) and recovery of baseline activity (▨; REC). In 14 neurones tested with sumatriptan and ketanserin (see figure 4.2), 3 neurones were excited by sumatriptan in the presence of ketanserin with the post-ketanserin response to sumatriptan recorded in 2 of these 3 neurones (figure 8.2).

NS - not significant

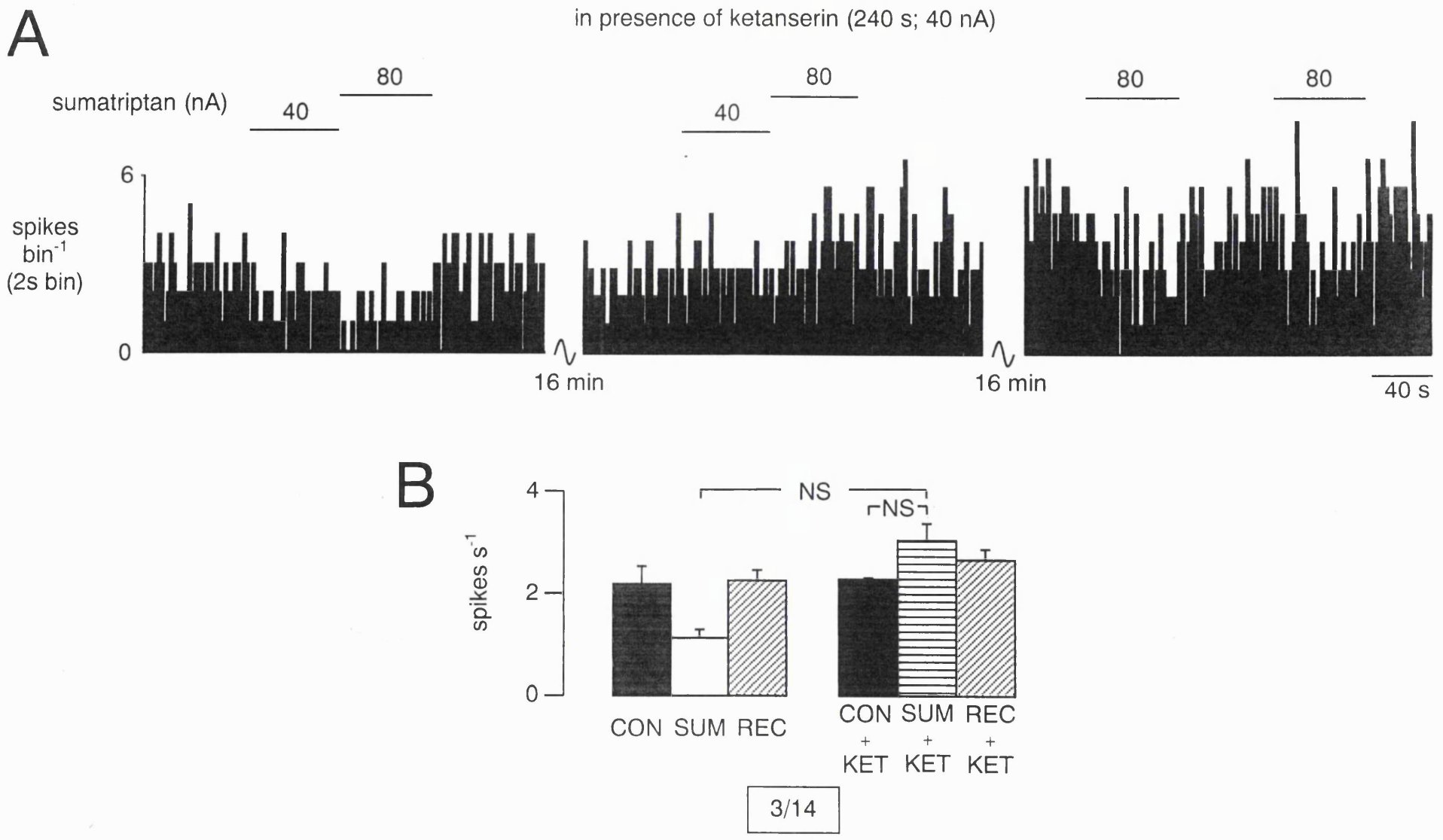

A
in presence of ketanserin (240 s; 40 nA)
sumatriptan (nA)
40
80
40
80
80
80
6
0
spikes bin-1 (2s bin)
16 min
16 min
40 s
B
4
2
0
spikes s-1
NS
NS
CON SUM REC
CON SUM REC
+ KET
+ KET
+ KET
3/14

Figure 4.4 The effect of ionophoretic administration of GR55562B on the inhibitory effect evoked by ionophoretic administration of sumatriptan in the anaesthetized rat

A The response of an NTS neurone to the ionophoretic administration of sumatriptan before, during and after the ionophoretic administration of GR55562B (during the bars and at the currents stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. From left to right: activity evoked by ionophoretic administration of sumatriptan (20-80 nA; □; SUM), activity evoked by sumatriptan in the presence of GR55562B (10-80 nA; ▤; SUM + GR5), baseline activity (■; CON) and recovery of baseline activity (▨; REC). In 6 of 11 neurones the inhibition evoked by sumatriptan was potentiated in the presence of GR55562B. The post-GR55562B response to sumatriptan recorded in 4 of these 6 neurones (figure 8.3). Of the remaining 5 neurones the inhibitory response to sumatriptan was attenuated in 3 and unaffected in 2 in the presence of GR55562B (figure 8.3).

* $P<0.05$

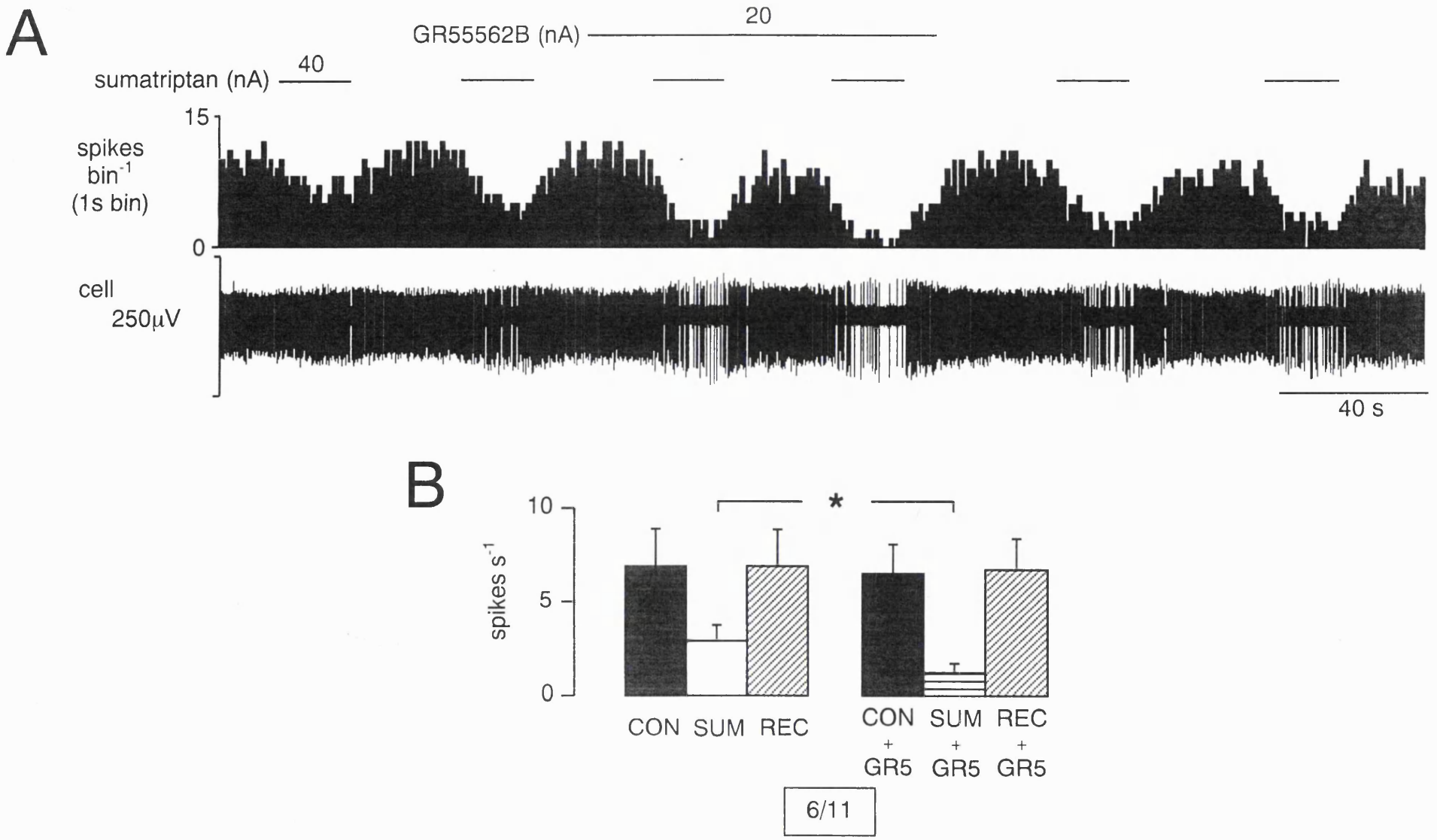

A
GR55562B (nA)
20
sumatriptan (nA)
40
15
spikes
bin-1
(1s bin)
0
cell
250μV
40 s
B
10
5
0
spikes s-1
*
CON SUM REC
CON SUM REC
+ + +
GR5 GR5 GR5
6/11

Figure 4.5 The effect of ionophoretic application of CP-93,129 on the activity of NTS neurones in the anaesthetized rat

A The response of an NTS neurone to the ionophoretic administration of CP-93,129 (during the bars and at the currents stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. **(i)** & **(ii)** From left to right: baseline activity (■; CON), activity evoked by ionophoretic administration of CP-93,129 (40-240 nA; □; CP), and, where shown, recovery of baseline activity (▨; REC). 31 of 41 neurones were excited by the ionophoretic administration of CP-93,129 **(i)**, with the post-CP-93,129 activity recorded in 28 neurones (figure 8.1). Of the remaining 10 neurones 5 were inhibited **(ii)**, and 5 were unaffected by CP-93,129 (figure 8.1).

* $P<0.05$ ** $P<0.01$

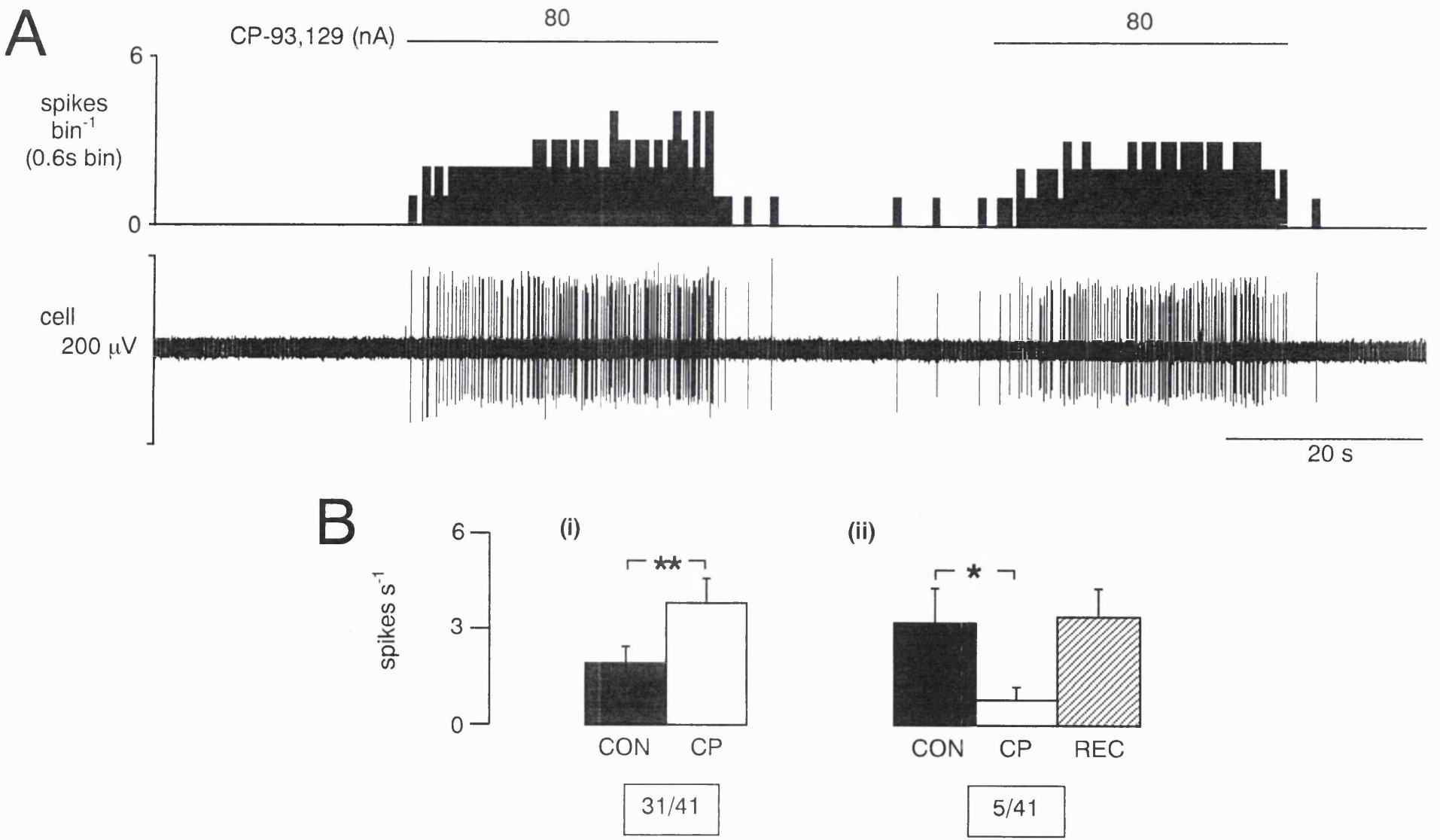

A
CP-93,129 (nA)
80
80
6
spikes
bin-1
(0.6s bin)
0
cell
200 μV
20 s
B
6
3
0
spikes s-1
(i)
**
CON
CP
31/41
(ii)
*
CON
CP
REC
5/41

Figure 4.6 The effect of ionophoretic administration of ketanserin on the activity of NTS neurones in the anaesthetized rat

A The activity of a single NTS neurone prior to (left panel) and during (right panel) the ionophoretic administration of ketanserin. Also shown is the response of this neurone to sumatriptan (during the bars and at the current stated) prior to (left) and during (right) the period of ketanserin administration. From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. **(i)** & **(ii)** From left to right: baseline activity (■; CON) and activity evoked by ionophoretic administration of ketanserin (10-40 nA; □; KET). 7 of 14 neurones were inhibited by the ionophoretic administration of ketanserin **(i)** whilst 6 neurones were unaffected **(ii)**. The post-ketanserin activity was recorded in 6 of these neurones (figure 8.1). The activity of the remaining neurone was increased in the presence of ketanserin.

NS - not significant

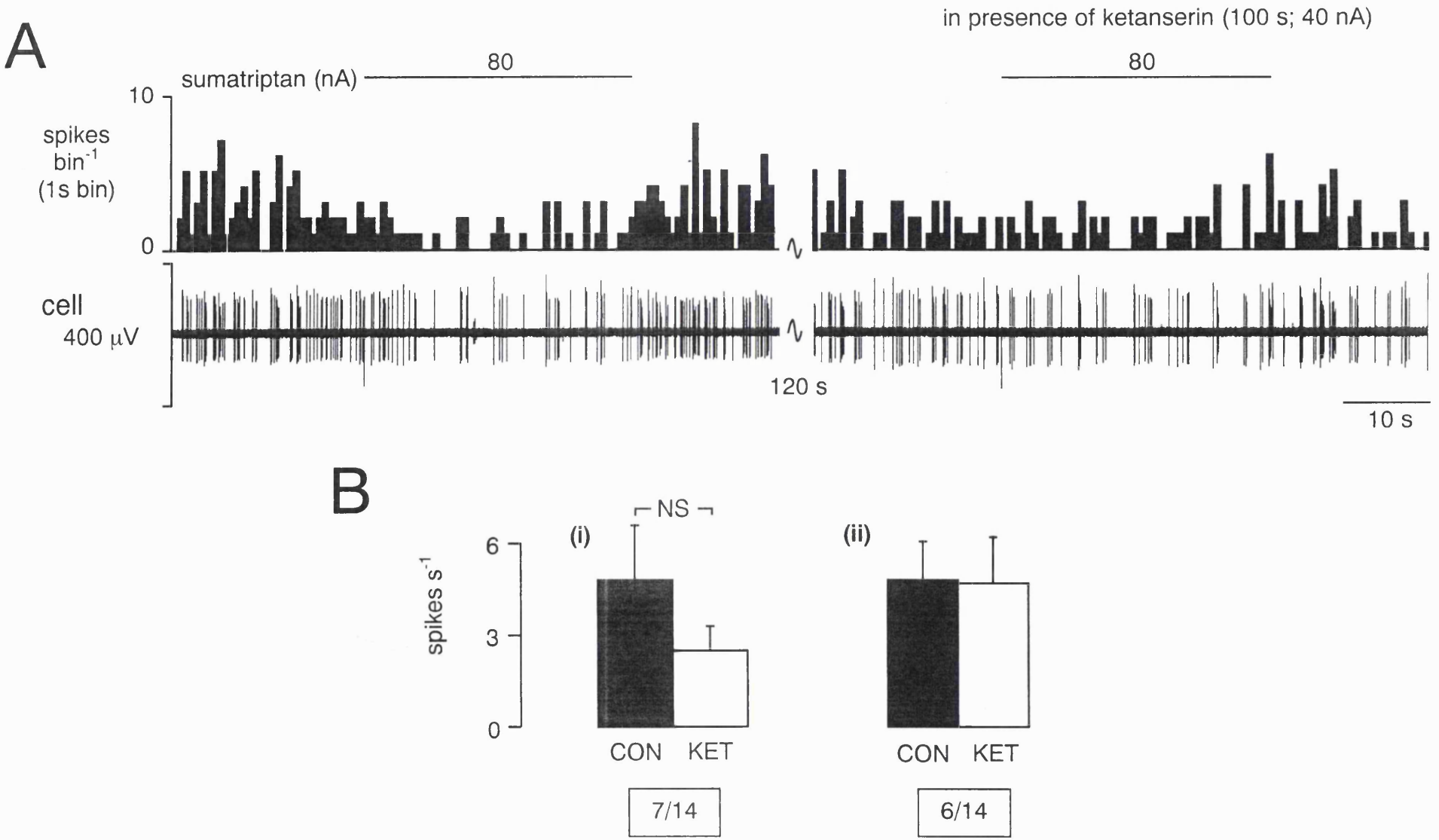
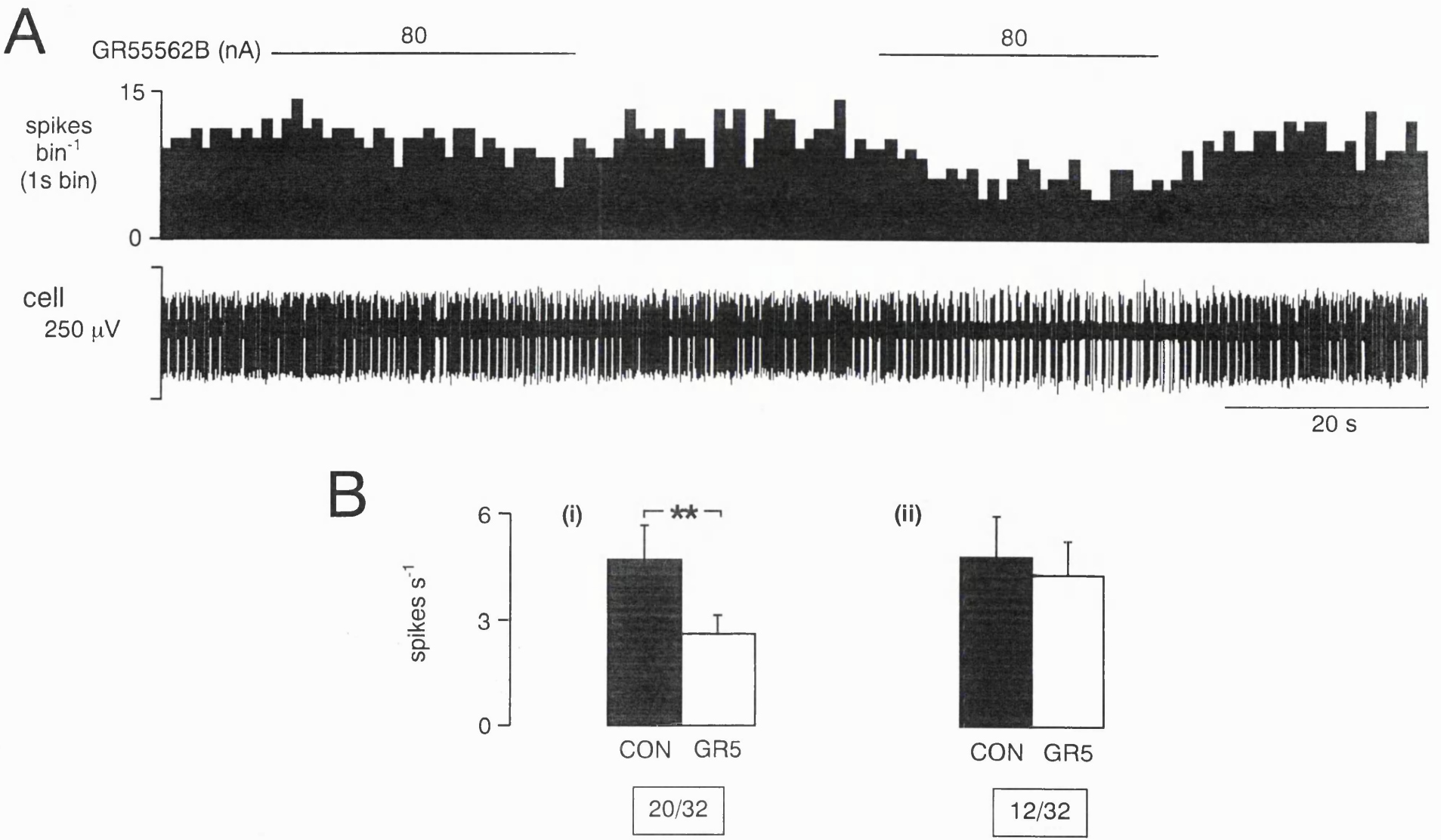

A
in presence of ketanserin (100 s; 40 nA)
sumatriptan (nA)
80
80
10
spikes bin-1 (1s bin)
0
cell
400 μV
120 s
10 s
B
NS
(i)
(ii)
spikes s-1
6
3
0
CON
KET
CON
KET
7/14
6/14

Figure 4.7 The effect of ionophoretic administration of GR55562B on the activity of NTS neurones in the anaesthetized rat

A Inhibition in the ongoing activity of an NTS neurone evoked by the ionophoretic administration of GR55562B (during the bars & at the currents stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. **(i)** & **(ii)** From left to right: baseline activity (■; CON) and activity evoked by ionophoretic administration of GR55562B (40-240 nA; □; GR5). Of 32 neurones, 20 were inhibited by the ionophoretic administration of GR55562B **(i)** and 12 neurones were unaffected **(ii)**. The post-GR55562B activity was recorded in 28 of these neurones (figure 8.1).

** $P<0.01$


A
GR55562B (nA)
80
80
15
spikes
bin-1
(1s bin)
0
cell
250 μV
20 s
B
6
3
0
spikes s-1
(i)
**
CON
GR5
20/32
(ii)
CON
GR5
12/32

4.2.2 5-$HT_{1B/1D}$ receptor ligands and identified inputs

The effects of the 5-$HT_{1B/1D}$ receptor agonist sumatriptan and the selective 5-HT_{1B} receptor agonist CP-93,129 were examined on both the vagal afferent-evoked and cardiopulmonary afferent-evoked activation of NTS neurones (see table 4.3).

Vagal afferent activation of neurones was measured as the total number of spikes evoked by vagus nerve stimulation, including both short and long latency spikes over a time period of a minimum of 20 sweeps of nerve stimulation. The mean response of neurones was subsequently calculated per 20 sweeps of stimulation. In addition, cardiopulmonary afferent activation was measured as the total number of spikes in the burst of neuronal activity evoked by right atrial administration of phenylbiguanide. Responses prior to, during and, where possible, after the ionophoretic administration of each receptor ligand were then compared.

Vagal-afferent-evoked activation

Ionophoretic administration of sumatriptan decreased vagal afferent-evoked activity by a mean of 24% in 20 out of 23 neurones (figure 4.8). The post-sumatriptan response was recorded in 12 of these neurones and partial recovery of control evoked activity was observed. Of the remaining 3 neurones 2 were unaffected, whilst the vagal afferent-evoked activity of one neurone was potentiated by sumatriptan.

In contrast, ionophoretic administration of CP-93,129 increased vagal afferent-evoked activation by a mean of 33% in 22 out of 25 neurones (figure 4.9). In addition, the post-CP-93,129 response was recorded in 15 of these neurones and partial recovery of control evoked activity was observed. The total vagal afferent-evoked activity of the remaining 3 neurones was unaffected by CP-93,129.

Cardiopulmonary afferent-evoked activation

Ionophoretic application of sumatriptan also decreased the cardiopulmonary afferent-evoked activation of NTS neurones by a mean of 42% in 2 out of 3 neurones tested (figures 4.10). The activation of the third neurone was unaffected by sumatriptan.

In contrast, CP-93,129 increased the cardiopulmonary afferent-evoked activation by a mean of 240% in 3 of 5 neurones tested (figures 4.11). The activation of the remaining 2 neurones was unaffected by CP-93,129 administration.

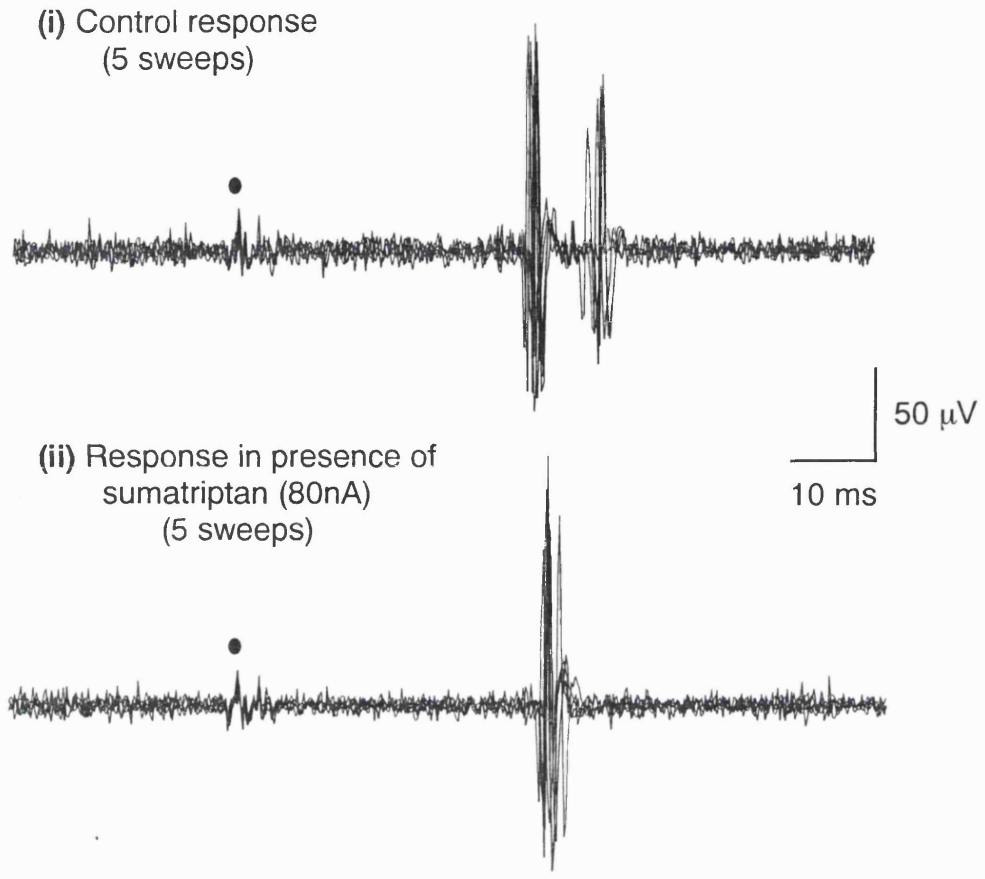
Figure 4.8 Attenuation of the vagal afferent-evoked activation of NTS neurones by ionophoretic administration of sumatriptan in the anaesthetized rat

A Attenuation of the vagal afferent-evoked activity of an NTS neurone by the ionophoretic administration of sumatriptan (at the current stated). From the top, traces show the raw extracellular recording of neuronal activity (5 sweeps; μV) before **(i)** and during **(ii)** the administration of sumatriptan. ● - stimulus artefact.

B Histogram of the mean data of vagal afferent-evoked activity with vertical bars representing s.e.mean. From left to right: control evoked activity (mean response per 20 sweeps; ■; CON) and evoked activity during the ionophoretic administration of sumatriptan (30-160 nA; □; SUM). In 23 neurones, the total evoked activity was inhibited by sumatriptan in 20 with the post-sumatriptan activity recorded in 12 of these neurones (figure 8.4). Of the remaining 3 neurones the total evoked response was unaffected in 2 and potentiated in one by sumatriptan (figure 8.4).

** $P<0.01$

A


(i) Control response
(5 sweeps)
50 μV
10 ms
(ii) Response in presence of
sumatriptan (80nA)
(5 sweeps)

B

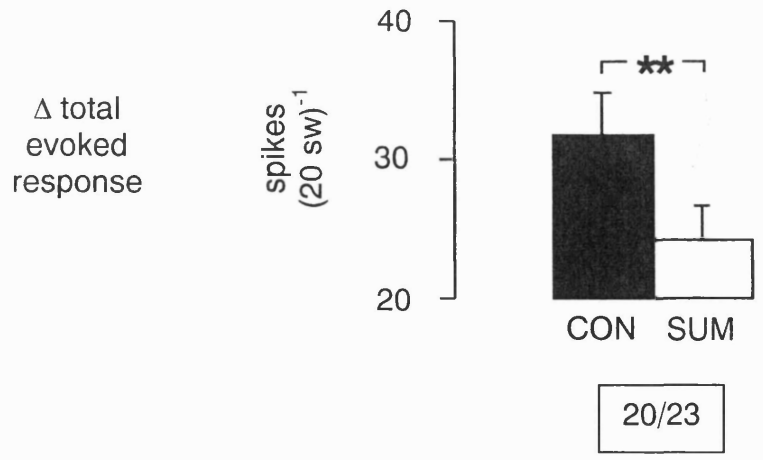

Δ total
evoked
response
spikes
(20 sw)-1
40
30
20
**
CON
SUM
20/23

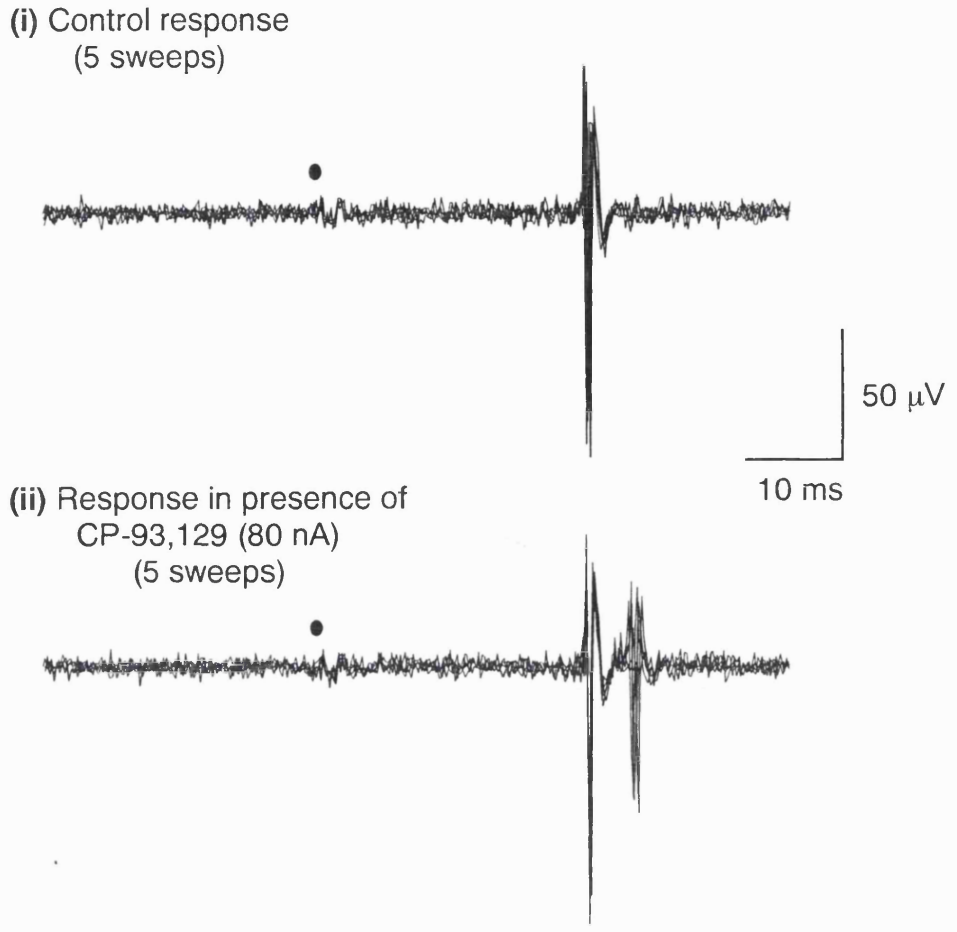
Figure 4.9 Potentiation of the vagal afferent-evoked activation of NTS neurones by ionophoretic administration of CP-93,129 in the anaesthetized rat

A Potentiation of the vagal afferent-evoked activity of an NTS neurone by the ionophoretic administration of CP-93,129 (at the current stated). From the top, traces show the raw extracellular recording of neuronal activity (5 sweeps; μV) and before **(i)** and during **(ii)** the administration of CP-93,129. ● - stimulus artefact.

B Histogram of the mean data of vagal afferent-evoked activity with vertical bars representing s.e.mean. From left to right: control evoked activity (mean response per 20 sweeps; ■; CON) and evoked activity during the ionophoretic administration of CP-93,129 (40-160 nA; □; CP). In 25 neurones, the total evoked activity of was potentiated by CP-93,129 in 22 with the post-CP-93,129 activity recorded in 15 of these neurones (figure 8.4). The total evoked response of the remaining 3 neurones was unaffected by CP-93,129 (figure 8.4).

** $P<0.01$

A


(i) Control response
(5 sweeps)
50 μV
10 ms
(ii) Response in presence of
CP-93,129 (80 nA)
(5 sweeps)

B

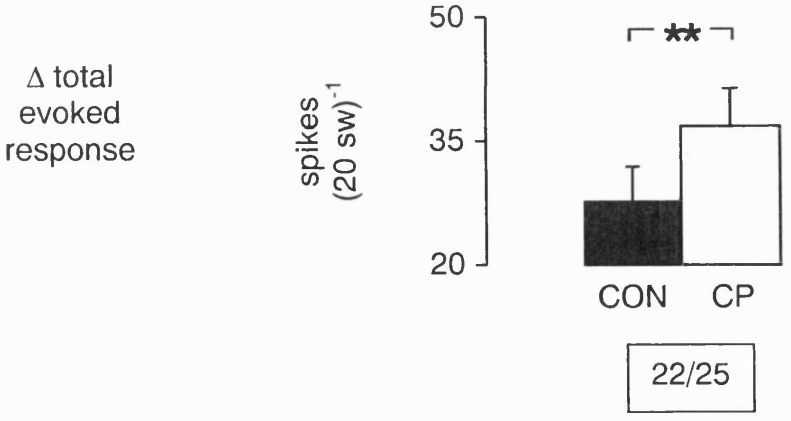

Δ total
evoked
response
spikes
(20 sw)-1
50
35
20
**
CON
CP
22/25

Figure 4.10 Attenuation of the cardiopulmonary afferent-evoked activation of NTS neurones by ionophoretic administration of sumatriptan in the anaesthetized rat

The response of an NTS neurone to activation of cardiopulmonary afferents by right atrial administration of PBG prior to (left panel) and during (right panel) the ionophoretic administration of sumatriptan. From the top, traces show blood pressure (BP; mmHg), a continuous rate histogram of neuronal activity (spikes bin^{-1}) and the raw recording of neuronal activity (μV). The administration of PBG is highlighted by a solid horizontal line (▬; PBG - 18 μg kg^{-1}, 30 μl). Circled numbers show the number of spikes in the corresponding burst of activity. In 3 neurones, the cardiopulmonary afferent-evoked activation of 2 was attenuated in the presence of sumatriptan (80 nA), whilst the activation of the remaining neurone was unaffected (figure 8.5).

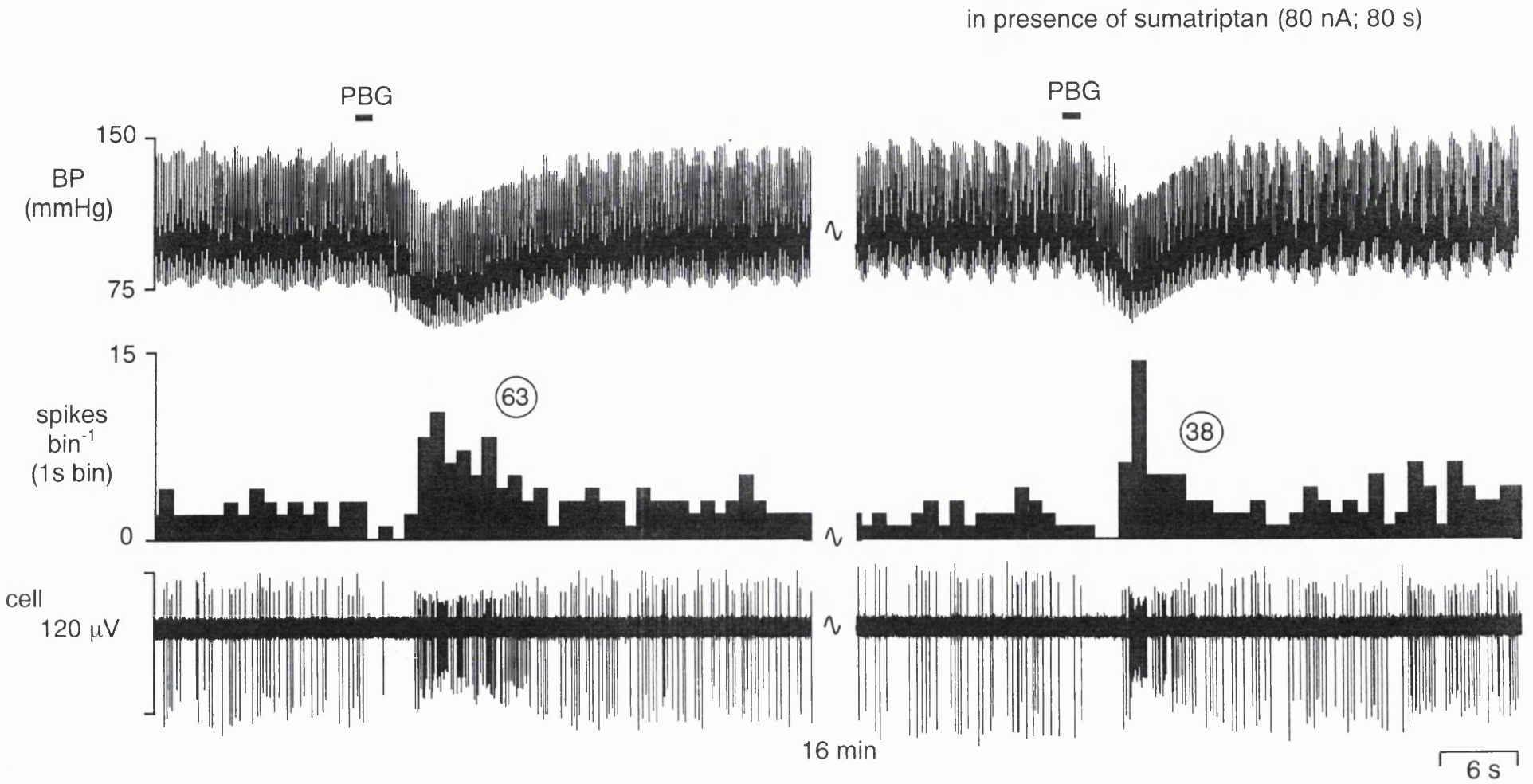
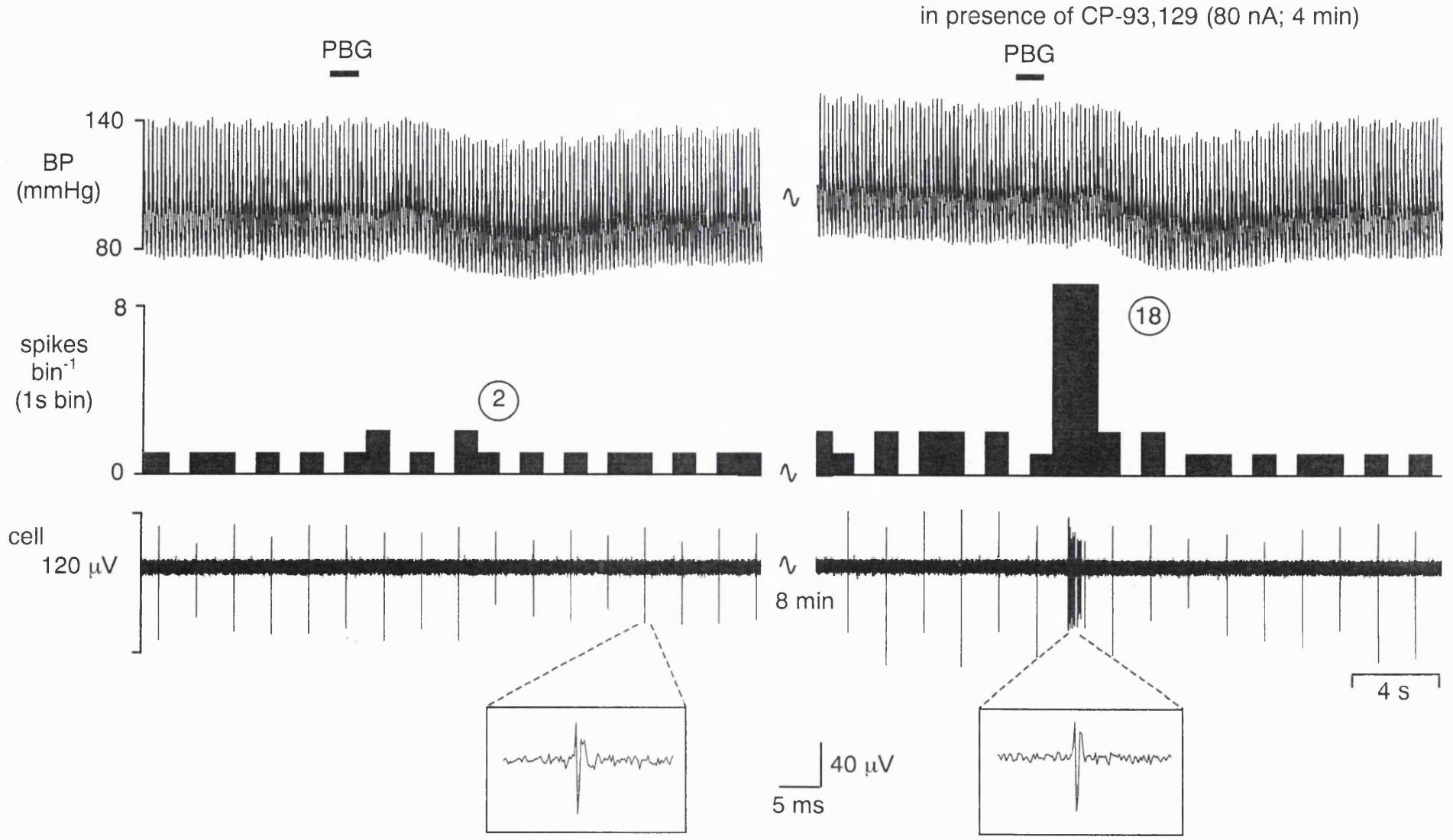

in presence of sumatriptan (80 nA; 80 s)
PBG
PBG
150
BP
(mmHg)
75
15
spikes
bin-1
(1s bin)
0
63
38
cell
120 μV
16 min
6 s

Figure 4.11 Potentiation of the cardiopulmonary afferent-evoked activation of NTS neurones by ionophoretic administration of CP-93,129 in the anaesthetized rat

The response of an NTS neurone to activation of cardiopulmonary afferents by right atrial administration of phenylbiguanide (PBG) prior to (left panel) and during (right panel) the ionophoretic administration of CP-93,129. From the top, traces show blood pressure (BP; mmHg), a continuous rate histogram of neuronal activity (spikes bin^{-1}) and the raw recording of neuronal activity (μV). The administration of PBG is highlighted by a solid horizontal line (▬; PBG - 18 μg kg^{-1}, 30 μl). Circled numbers highlight the number of spikes in the corresponding burst of activity. In 3 of 5 neurones, the cardiopulmonary afferent-evoked activation was potentiated in the presence of CP-93,129 (40-80 nA), whilst the activation of the remaining 2 neurones was unaffected (figure 8.5).

NB. Background activity is vagal-evoked activity (0.6 Hz), and is potentiated by CP-93,129 in this neurone, in addition cell identity between panels is confirmed by the expanded sections at the bottom of the page.


in presence of CP-93,129 (80 nA; 4 min)
PBG
PBG
140
BP
(mmHg)
80
8
spikes
bin-1
(1s bin)
0
2
18
cell
120 μV
8 min
4 s
40 μV
5 ms

4.2.3 Effects of vehicle controls on neuronal activity

The effects of the vehicles for the above ligands were examined on the ongoing or DLH-evoked activity of NTS neurones. Sumatriptan, ketanserin and GR55562B were all dissolved in saline which was adjusted to pH 4, whereas CP-93,129 was dissolved in 1% ascorbic acid then made up to volume with saline and adjusted to pH 4. Therefore ionophoretic administration of saline at pH 4 and 1% ascorbic acid at pH 4 was tested on the activity of NTS neurones.

Saline at pH 4

Figure 4.12 shows that ionophoretic administration of saline at pH 4 does not affect the DLH-evoked activity of a single NTS neurone whilst ionophoretic administration of sumatriptan to the same neurone is able to strongly inhibit DLH-evoked activity. Indeed, in 12 neurones the baseline activity of 11 was unaffected by the ionophoretic administration of saline at pH 4 (figure 4.12B; table 4.4). The activity of the remaining neurone was increased by more than 20%.

1% ascorbic acid

The baseline activity of 5 of 6 NTS neurones was unaffected by the ionophoretic administration of a 1% solution of ascorbic acid at pH 4 (figure 4.13B; table 4.4). Figure 4.13 shows the ongoing activity of the remaining neurone which is decreased by 58% by the ionophoretic administration of ascorbic acid, however, ionophoretic administration of the 5-HT_{1B} receptor ligand CP-93,129 to the same neurone, despite showing a brief period of inhibition, significantly increased baseline activity by 163%.

Figure 4.12 The effect of ionophoretic administration of saline at pH 4 on the activity of NTS neurones in the anaesthetized rat

A The response of an NTS neurone to the ionophoretic administration of DLH in the presence of sumatriptan and saline at pH 4 (during the bars and at the currents stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. From left to right: baseline activity (■; CON), activity evoked by ionophoretic administration of saline at pH 4 (40-120 nA; □; pH 4 SAL), and recovery of baseline activity (▨; REC). In 12 neurones, 11 were unaffected by the ionophoretic administration of saline at pH 4. The activity of the remaining neurone was increased by saline at pH 4.

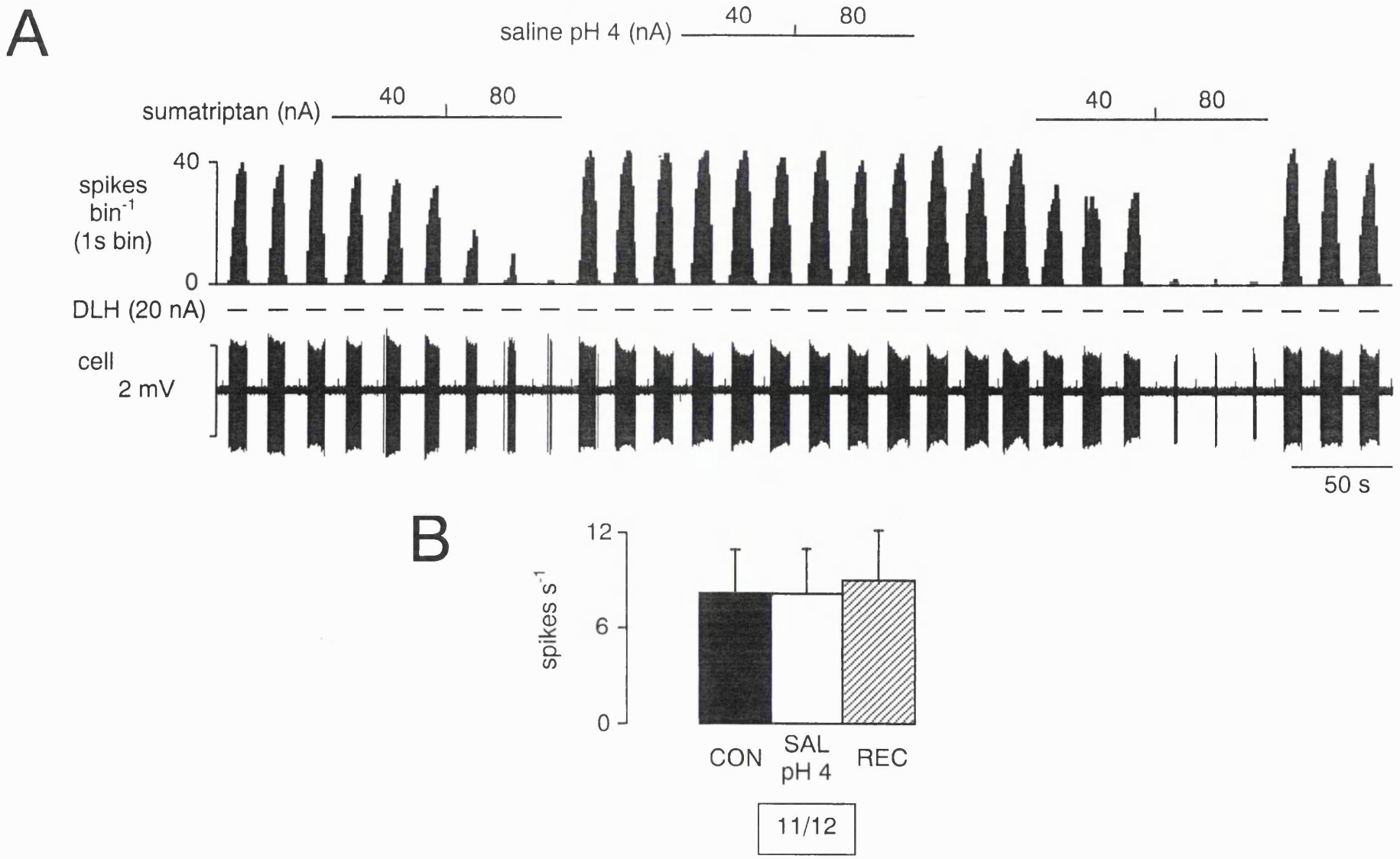

A
saline pH 4 (nA)
40
80
sumatriptan (nA)
40
80
40
80
spikes bin-1 (1s bin)
40
0
DLH (20 nA)
cell
2 mV
50 s
B
spikes s-1
12
6
0
CON
SAL pH 4
REC
11/12

Figure 4.13 The effect of ionophoretic administration of the CP-93,129 vehicle ascorbic acid on the activity of NTS neurones in the anaesthetized rat

A The response of an NTS neurone to the ionophoretic administration of CP-93,129 and ascorbic acid, the CP-93,129 vehicle (during the bars and at the currents stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. From left to right: baseline activity (■; CON), activity evoked by ionophoretic administration of CP-93,129 vehicle (60-80 nA; □; VEH), and recovery of baseline activity (▨; REC). In 5 of 6 neurones the baseline activity was unaffected by the ionophoretic administration of CP-93,129 vehicle whilst the activity of one neurone was inhibited (shown in **A**).

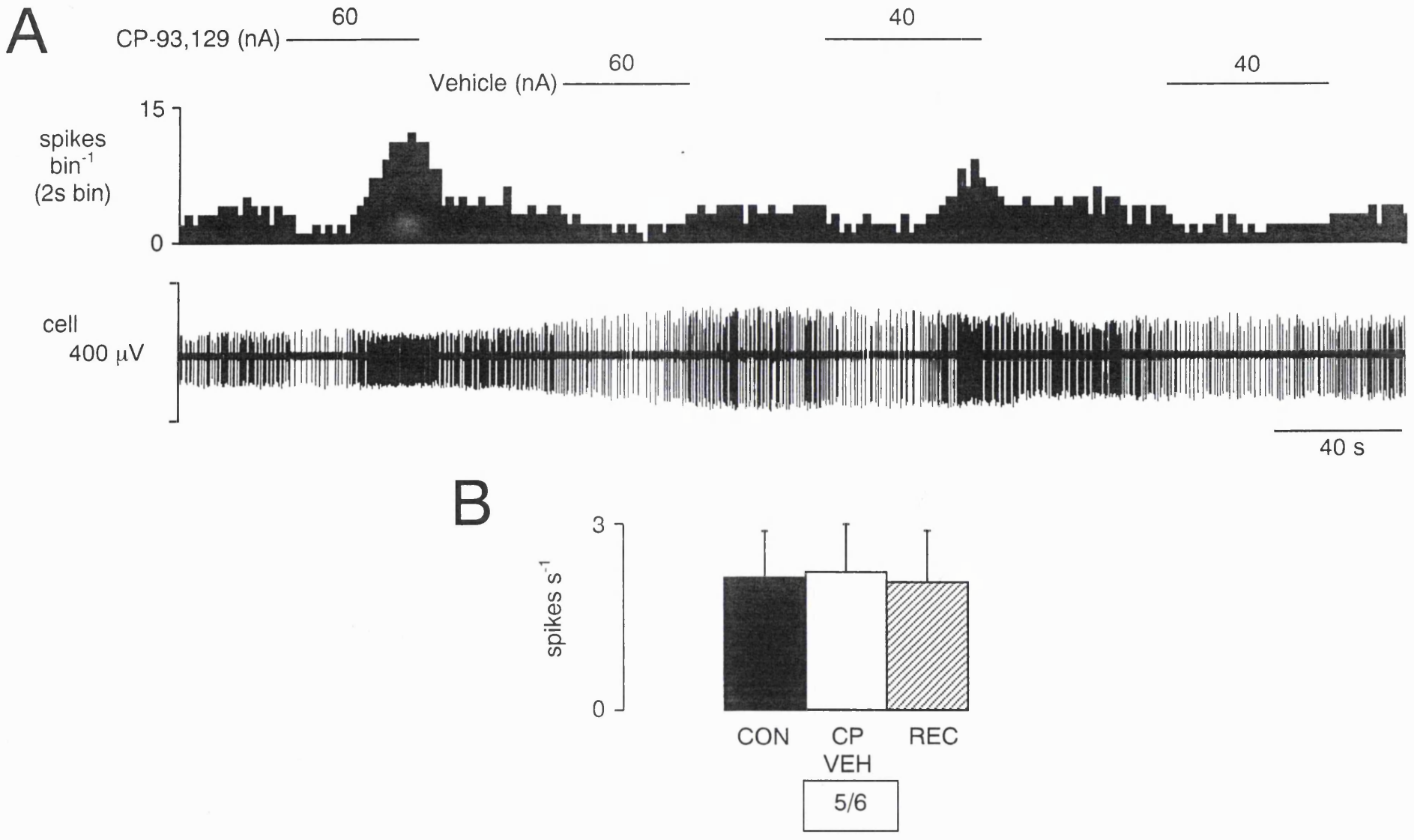

A
CP-93,129 (nA)
60
40
Vehicle (nA)
60
40
15
spikes
bin-1
(2s bin)
0
cell
400 μV
40 s
B
3
spikes s-1
0
CON
CP
VEH
REC
5/6

Table 4.1 The effect of ionophoretic administration of sumatriptan, in addition to the effect of ionophoretic administration of ketanserin on the inhibition evoked by sumatriptan, on the activity of NTS neurones

Numbers reflect mean firing rates of neurones in spikes s^{-1}

(i) The effects of ionophoretic administration of sumatriptan on the activity of NTS neurones

(ii) The effects of ionophoretic administration of sumatriptan before, during, and after the ionophoretic administration of ketanserin, on the activity of NTS neurones

(iii) Further effects of ionophoretic administration of sumatriptan before, during, and after the ionophoretic administration of ketanserin, on the activity of NTS neurones

CON-baseline activity, SUM-activity in the presence of sumatriptan, REC-recovery of baseline activity, + KET-in the presence of ketanserin, Att. Inh.-attenuated inhibition, none-activity unaffected, Pot. Inh.-inhibition potentiated, to Exc.-inhibition reversed to excitation.

* $P<0.05$ ** $P<0.01$ compared to CON
† $P<0.05$ †† $P<0.01$ compared to SUM
‡ $P=0.057$ compared to SUM

(i) The effects of sumatriptan

Number of cells	Effect	CON	SUM	REC
		spikes s^{-1}		
54/64	inhibition	6.8±1.0	2.3±0.5**	7.1±1.2
8/64	none	5.3±0.8	5.1±0.8	5.3±1.0
2/64	excitation	1.7	4.3	1.7

(ii) The effects of sumatriptan in the presence of ketanserin

Number of cells	Effect	CON	SUM	REC	CON +KET	SUM +KET	REC +KET	CON	SUM	REC
		spikes s^{-1}								
11/14	Att. Inh.	4.1 ±0.8	1.2** ±0.3	3.9 ±0.8	3.9 ±1.0	2.8†† ±0.6	-	-	-	-
10/14	Att. Inh.	4.2 ±0.9	1.3** ±0.3	4.0 ±0.8	4.1 ±1.1	3.0†† ±0.6	4.0 ±0.9	-	-	-
5/14	Att. Inh.	4.2 ±1.2	1.3* ±0.4	3.6 ±0.8	3.5 ±1.1	3.3† ±0.9	3.4 ±1.0	3.0 ±0.3	1.3* ±0.4	2.8 ±0.2
2/14	none	9.8	1.8	10.5	7.5	1.6	8.6	-	-	-
1/14	Pot. Inh.	6.5	1.8	7.7	4.0	0.3	3.9	2.3	0.6	2.0

(iii) An excitatory response to sumatriptan in the presence of ketanserin

Number of cells	Effect	CON	SUM	REC	CON +KET	SUM +KET	REC +KET	CON	SUM	REC
		spikes s^{-1}								
3/14	to Exc.	2.2 ±0.4	1.1 ±0.2	2.3 ±0.2	2.3 ±0.0	3.0‡ ±0.3	2.7 ±0.2	-	-	-
2/14	to Exc.	2.5	1.1	2.3	2.3	3.3	2.6	3.0	2.2	2.8

Table 4.2 The effect of ionophoretic administration of GR55562B on the inhibition evoked by ionophoretic administration of sumatriptan, in addition to the effect of ionophoretic administration of CP-93,129, on the activity of NTS neurones

Numbers reflect mean firing rates of neurones in spikes s^{-1}

(i) The effects of ionophoretic administration of sumatriptan before, during, and after the ionophoretic application of GR55562B, on the activity of NTS neurones

(ii) The effects of ionophoretic administration of CP-93,129 on the activity of NTS neurones

CON-baseline activity, SUM-activity in the presence of sumatriptan, CP- in the presence of CP-93,129, REC-recovery of baseline activity, + GR5-in the presence of GR5562B, Pot. Inh.-inhibition potentiated, Att. Inh.-attenuated inhibition, none-activity unaffected.

* $P<0.05$ compared to CON
** $P<0.01$ compared to CON
† $P<0.05$ compared to SUM

(i) The effects of sumatriptan in the presence of GR55562B

Number of cells	Effect	CON	SUM	REC	CON +GR5	SUM +GR5	REC +GR5	CON	SUM	REC
		spikes s^{-1}								
6/11	Pot. Inh.	6.9 ±2.0	2.9* ±0.8	6.9 ±2.0	6.5 ±1.6	1.2**† ±0.5	6.7 ±1.7	-	-	-
4/11	Pot. Inh.	6.0 ±1.6	3.0* ±1.0	6.1 ±1.6	6.0 ±1.3	1.2*† ±0.6	6.4 ±1.1	6.2 ±1.0	2.3* ±0.3	5.8 ±0.8
3/11	Att. Inh.	2.6 ±0.8	0.6 ±0.5	2.5 ±0.8	2.4 ±0.7	1.0 ±0.5	2.8 ±0.8	-	-	-
2/11	none	4.5	2.1	4.9	2.9	1.5	2.5	4.8	2.4	5.9

(ii) The effects of CP-93,129

Number of cells	Effect	CON	CP	REC
		spikes s^{-1}		
31/41	excitation	1.9±0.5	3.8±0.8**	-
28/41	excitation	1.9±0.5	3.9±0.8**	1.9±0.4
5/41	inhibition	3.2±1.1	0.8±0.4*	3.4±0.9
5/41	none	4.4±1.3	4.6±1.3	4.5±1.4

Table 4.3 The effects of ionophoretic administration of sumatriptan and CP-93,129 on the vagal afferent-evoked and cardiopulmonary afferent-evoked activation of NTS neurones

Numbers reflect mean firing rates of neurones in spikes (20 sweeps)$^{-1}$ and spikes (burst)$^{-1}$

(i) The effects of ionophoretic administration of sumatriptan on the vagal-evoked activity of NTS neurones

(ii) The effects of ionophoretic administration of sumatriptan on the vagal-evoked activity of NTS neurones

(iii) The effects of ionophoretic administration of sumatriptan on the cardiopulmonary afferent-evoked activity of NTS neurones

(iv) The effects of ionophoretic administration of CP-93,129 on the cardiopulmonary afferent-evoked activity of NTS neurones

CON-baseline evoked activity, SUM-evoked activity in the presence of sumatriptan, CP-evoked activity in the presence of CP-93,129, none-evoked activity unaffected, REC-recovery of baseline evoked activity

** $P<0.01$ compared to CON

(i) The effect of sumatriptan on vagal-evoked activity

Number of cells	Effect	CON	SUM	REC
		spikes (20 sweeps)$^{-1}$		
20/23	inhibition	31.7 ±3.1	24.2** ±2.5	-
12/23	inhibition	31.7 ±4.4	25.1** ±3.8	28.1 ±3.8
2/23	none	32.6	31.6	31.2
1/23	excitation	14.3	18.3	-

(ii) The effect of CP-93,129 on vagal-evoked activity

Number of cells	Effect	CON	CP	REC
		spikes (20 sweeps)$^{-1}$		
22/25	excitation	27.6 ±4.3	36.7** ±4.6	-
15/25	excitation	24.3 ±3.8	33.0** ±3.8	29.2 ±3.6
3/25	none	29.4 ±8.8	29.3 ±8.5	29.0 ±7.3

(iii) The effect of sumatriptan on cardiopulmonary afferent-evoked activity

Number of cells	Effect	CON	SUM	REC
		spikes (burst)$^{-1}$		
2/3	attenuated	48	28	-
1/3	none	47	48	-

(iv) The effect of CP-93,129 on cardiopulmonary afferent-evoked activity

Number of cells	Effect	CON	CP	REC
		spikes (burst)$^{-1}$		
3/5	potentiated	4.7±1.3	16±4.0	4.7±2.0
2/5	none	25	26	-
1/5	none	43	47	48

Table 4.4 The effects of ionophoretic administration of ketanserin, GR55562B, saline at pH 4 and the CP-93,129 vehicle ascorbic acid on the activity of NTS neurones

Numbers reflect mean firing rates of neurones in spikes s^{-1}

(i) The effects of ionophoretic administration of ketanserin on the activity of NTS neurones

(ii) The effects of ionophoretic administration of GR55562B on the activity of NTS neurones

(iii) The effects of ionophoretic administration of saline at pH 4 on the activity of NTS neurones

(iv) The effects of ionophoretic administration of the CP-93,129 vehicle ascorbic acid on the activity of NTS neurones

CON-baseline activity, KET-activity in the presence of ketanserin, GR5-in the presence of GR55562B, SAL pH 4-in the presence of saline at pH 4, CP VEH-in the presence of CP-93,129 vehicle, none-activity unaffected, REC-recovery of baseline activity

** $P<0.01$ compared to CON

(i) The effects of ketanserin

Number of cells	Effect	CON	KET	REC
		spikes s^{-1}		
7/14	inhibition	4.8±1.8	2.5±0.8	-
2/14	inhibition	2.8	1.6	2.0
6/14	none	4.8±1.3	4.7±1.5	-
4/14	none	3.5±1.2	3.1±1.0	2.5±0.3
1/14	excitation	0.3	1.5	0.1

(ii) The effects of GR55562B

Number of cells	Effect	CON	GR5	REC
		spikes s^{-1}		
20/32	inhibition	4.7±1.0	2.6±0.5**	-
19/32	inhibition	4.9±1.0	2.7±0.6**	4.1±0.8
12/32	none	4.8±1.2	4.3±0.9	-
9/32	none	3.8±0.8	3.6±0.7	3.6±0.6

(iii) The effects of saline at pH 4

Number of cells	Effect	CON	SAL pH 4	REC
		spikes s^{-1}		
11/12	none	8.2±2.8	8.2±2.8	9.0±3.1
1/12	excitation	3.9	4.9	5.3

(iv) The effects of the CP-93,129 vehicle, ascorbic acid

Number of cells	Effect	CON	CP VEH	REC
		spikes s^{-1}		
5/6	none	2.1±0.8	2.2±0.8	2.1±0.8
1/6	inhibition	1.9	0.8	1.7

4.3 Discussion

Main findings

The present studies focused on the effects of ionophoretic administration of 5-$HT_{1B/1D}$ receptor ligands on the ongoing or DLH-evoked activity of NTS neurones. The 5-$HT_{1B/1D}$ receptor agonist sumatriptan decreased this baseline activity in the majority of NTS neurones and this reduction was attenuated in almost every case by the 5-$HT_{2A/1D}$ receptor antagonist ketanserin. In contrast, the sumatriptan-mediated reduction in baseline activity was potentiated in the presence of the h5-HT_{1B} receptor antagonist GR55562B in the majority of cases. This implies that 5-HT_{1B} and 5-HT_{1D} receptors have opposing actions on NTS neurones, an implication further supported by the observation that ionophoretic administration of the 5-HT_{1B} receptor agonist CP-93,129 excited the majority of cells. The role of 5-HT_{1B} and 5-HT_{1D} receptors was further confirmed when sumatriptan attenuated, and CP-93,129 potentiated, the vagal and cardiopulmonary afferent-evoked activation of the majority of neurones.

Selectivity of 5-$HT_{1B/1D}$ receptor ligands used

Sumatriptan

Sumatriptan, a compound selective for the 5-$HT_{1B/1D}$ receptor binding sites, has been shown to be effective in the acute treatment of migraine (Ferrari & Saxena, 1993). However, sumatriptan also has affinity for 5-HT_{1F} and, to a small extent, 5-HT_{1A} receptors binding sites (pK_i's 7.6 and 6.1 respectively; Hoyer, 1991) and it has been shown to produce some effects that were sensitive to blockade by the 5-HT_{1A} receptor antagonist WAY-100635 in the rat (Pagniez *et al.*, 1998). Furthermore, previous studies (Wang *et al.*, 1997) have shown that ionophoresis of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT does modulate the activity of NTS neurones, therefore, at high currents of ejection, activation of 5-HT_{1A} receptors by ionophoretic administration of sumatriptan cannot be ruled out. The activation of 5-HT_{1F} receptors also remains a possibility although there is no evidence as to how this may modulate NTS neuronal activity. However, the affinity of sumatriptan at 5-HT_2 receptors has been shown to be very low (Schoeffter & Hoyer, 1989). In addition, there

are differences between the affinity of this ligand for human 5-HT_{1B} compared to rat 5-HT_{1B} binding sites, due to their different pharmacological profiles caused by a single amino acid modification (see section 1.5.1; Oksenberg *et al.*, 1992). However, although sumatriptan has a 10 fold higher affinity for the human 5-HT_{1B} receptor the K_i value for the rat 5-HT_{1B} receptor is still in the nanomolar range (K_i 465±8 nM; Oksenberg *et al.*, 1992). Therefore, activation of both 5-HT_{1D} receptors and, possibly to a lesser extent, 5-HT_{1B} receptors during ionophoresis of sumatriptan seems likely, although without the use of selective antagonists it is not possible to distinguish between the two receptor subtypes. In addition, an ionophoresis study has previously described an inhibitory action for sumatriptan on the vagal afferent-evoked activity of NTS neurones (Wang *et al.*, 1998b).

Ketanserin

Ketanserin, a 5-HT_2 receptor antagonist which has been in clinical use (see Barrett, 1992), has 70 fold selectivity for the 5-HT_{2A} receptors over other 5-HT_2 receptor subtypes and is also a potent α_1-adrenoceptor antagonist. However, the key characteristic in relation to the present experiments is its high selectivity for the 5-HT_{1D} receptor (pK_i 7.1; Hoyer *et al.*, 1991) over the r5-HT_{1B} receptor (pK_i 5.7; Hoyer *et al.*, 1991). The use of ketanserin as an effective 5-HT_{1D} receptor antagonist has been exploited before e.g. Gallacher & Ramage (1996) who demonstrated that activation of central 5-HT_{1D} receptors can cause hypotension. In addition, ketanserin has been used in ionophoresis studies in the NTS (Sévoz-Couche *et al.*, 2000b) where it was found to attenuate the modulation of NTS neuronal activity by the 5-HT_2 receptor agonist DOI. However, their study did not report any actions of ketanserin itself, at the currents used (10-20 nA), on the ongoing or DLH-evoked activity of NTS neurones.

GR55562B

There appears to be a certain level of confusion surrounding the actions and/or selectivity of the 5-HT_1 receptor antagonist GR55562B. Initial literature has reported the use of GR55562 as a silent antagonist at both 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor binding sites, providing pK_i values of 7.3 and 6.3 for 5-$HT_{1D\beta}$ and

5-$HT_{1D\alpha}$ receptors respectively (Walsh *et al.*, 1995). However, more recent evidence, provided after the revised nomenclature for 5-HT_{1B} and 5-HT_{1D} receptors (see section 1.5.1), has defined GR55562 as a neutral antagonist of h5-HT_{1B} receptors (Lamothe *et al.*, 1997). Due to the similar pharmacological profiles of the r5-HT_{1B} and h5-HT_{1B} receptors it might be expected that GR55562 might also have higher affinity for the r5-HT_{1B} over the 5-HT_{1D} receptor. Indeed, in further recent studies in rat GR55562 has been found to attenuate effects mediated by the selective 5-HT_{1B} receptor agonist CP-93,129, and this work was carried out in rat striatal synaptosomes (Sarhan *et al.*, 1999), the mesenteric arteries of deoxycorticosterone acetate salt rats (Banes & Watts, 2001) and in the accumbal shell or core of rats (Przegalinski *et al.*, 2002). Thus, it seems likely that this compound can antagonise the responses of selective r5-HT_{1B} receptor activation, and may therefore be a useful tool in the discrimination of 5-HT_{1D} and r5-HT_{1B} receptor functions in the rat.

CP-93,129

CP-93,129 is a selective 5-HT_{1B} receptor agonist with >100 fold selectivity over 5-HT_{1A}, h5-HT_{1B} and 5-HT_2 receptors (Koe *et al.*, 1992). It has been widely used in the characterisation of this receptor including those studies described above, and a recent study using ionophoresis has described an excitatory action for this compound in the NTS of anaesthetized rats (Wang *et al.*, 1998b).

5-$HT_{1B/1D}$ receptor actions in the NTS

The findings of the present experiments support those found previously (Wang *et al.*, 1998b) in describing an inhibitory action for the 5-$HT_{1B/1D}$ receptor ligand sumatriptan. This study further characterises this response using the ligands ketanserin and GR55562B.

Generally, in the relatively large population of NTS neurones examined, sumatriptan caused an inhibition in the ongoing or DLH-evoked activity. Only 2 neurones were excited by sumatriptan suggesting that the receptor(s) activated by sumatriptan has a predominant inhibitory action. The 5-$HT_{2A/1D}$ receptor antagonist ketanserin attenuated this inhibitory response and, since ketanserin has high affinity for the 5-HT_{1D} receptor and sumatriptan has very low affinity for

the 5-HT_{2A} receptor, this implicates the 5-HT_{1D} receptor as mediating at least a large part of the inhibitory effect of sumatriptan.

Before looking further at the response to sumatriptan it is important to highlight the observation that the selective 5-HT_{1B} receptor agonist CP-93,129 excited the majority of cells, thus suggesting an excitatory action for 5-HT_{1B} receptors on recorded neurones. This observation may explain the potentiation of the inhibitory response to sumatriptan evoked by the 5-HT_{1B} antagonist GR55562B i.e. this compound is blocking the excitatory actions of 5-HT_{1B} receptor activation evoked by sumatriptan, leading to a greater level of inhibition from the sole 5-HT_{1D} receptor activation.

Further evidence for sumatriptan activating both 5-HT_{1B} as well as 5-HT_{1D} receptors comes from the observation that, in some neurones, ionophoresis of sumatriptan caused an excitation during the blockade of 5-HT_{1D} receptors with ketanserin, consistent with the activation of 5-HT_{1B} receptors. The fact that this did not occur in all neurones may be a result of either the lack of 5-HT_{1B} receptors or their distant location, or even by a possible inhibitory action for some 5-HT_{1B} receptors. This potential inhibitory action was supported by the inhibitory response of a number of neurones to CP-93,129, the group data of which indicated that this was a statistically significant result. Further support may also come from the observation that the inhibitory response evoked by sumatriptan was attenuated by GR55562B in a small number of cases i.e. sumatriptan is activating both 5-HT_{1D} and 5-HT_{1B} receptors, with both receptors mediating inhibition, and the subsequent administration of GR55562B attenuates the 5-HT_{1B}-mediated inhibition lowering the compound inhibition evoked by sumatriptan. It is also worth discussing that in some experiments the sumatriptan-evoked reduction in the activity of neurones was not completely attenuated by ketanserin. This may be, as mentioned above, due to the simultaneous activation of 5-HT_{1B} receptors by sumatriptan, although the possible, but unlikely activation of 5-HT_{1F} and even 5-HT_{1A} receptors cannot be ruled out either. It is also likely that the antagonism of the 5-HT_{1D} receptors by ketanserin is not complete due to insufficient drug delivery and there is some residual activation of 5-HT_{1D} receptors by sumatriptan.

In relation to 5-HT_{1B} receptors, ionophoresis of GR55562B alone caused an inhibition in over half of the neurones tested; this may reveal a tonic, ongoing activation of 5-HT_{1B} receptors in the NTS of these anaesthetized rats. However, this response to GR55562 was not observed in all neurones and was also unstable in some neurones which may indicate further non-specific actions of this compound, i.e. partial agonist effects at other receptor binding sites etc. The instabilities seen in the response of some neurones to ionophoretic administration of GR55562B took two forms. Either a reduction in the amplitude of the ongoing neuronal spikes during the inhibitory effect evoked by GR55562B administration or in some cases the GR55562B-evoked reduction in neuronal activity was intermittent and of much shorter duration than the period of ionophoretic administration. These effects could possibly be explained by a local anaesthetic effect of GR55562B previously reported after ionophoretic administration of drugs at high currents (>30 nA) in a study examining the effects of 5-HT_3 receptor ligands (Ashby *et al.*, 1991). Unfortunately, due to these effects the compound GR55562B was not examined on the excitatory responses evoked by CP-93,129 and therefore the GR55562B-mediated potentiation of the inhibitory response evoked by sumatriptan may also have been caused by such a non-specific action.

In contrast to the findings of a previous study (Sévoz-Couche *et al.*, 2000b), these experiments show that ketanserin did affect the ongoing or DLH-evoked activity of half of all neurones tested in these experiments. Despite this decrease in activity being >20% in 7 neurones the group data did not indicate that this was a statistically significant effect, a result likely to have been caused by the high variation in firing rates seen in this group of neurones. Therefore, it would seem looking at the response of these 7 neurones that there is some effect of ketanserin on the activity of NTS neurones, which may indicate a tonic activation of 5-HT_{1D} receptors. However, the responses seen are the opposite to what might be expected and so may be mediated either by 5-HT_{2A} receptors, which ketanserin has higher affinity for, or as in the case of GR55562B they may be via non-specific actions of this compound.

5-$HT_{1B/1D}$ receptors and identified inputs

Sumatriptan was also found to attenuate the excitatory unmyelinated vagal afferent input to NTS neurones suggesting that 5-HT_{1D} receptors activation may decrease vagal afferent transmission to NTS cells. Whether this is via a direct effect of sumatriptan on the vagal afferent terminals of NTS neurones or merely via a lowering of the excitability of the postsynaptic neurones, via 5-HT_{1D} receptors located on the cell body, was not determined in these experiments. These data do support the findings of a previous study (Wang *et al.*, 1998b), and are further confirmed by studies on the effect of sumatriptan on the excitation of NTS neurones evoked by right atrial PBG administration i.e. activation of cardiopulmonary afferents.

This excitatory response to cardiopulmonary afferent activation was found to be consistently stable between injections (see chapter 3), and was therefore used to examine the effects of ionophoretic ligand administration. This response was attenuated by sumatriptan in the small number of neurones tested, indicating that activation of 5-HT_{1D} receptors is certainly capable of suppressing afferent input to these neurones, although, as mentioned above, this may not be mediated by a direct afferent terminal modulation. In order to confirm the activation of 5-HT_{1D} receptors on vagal or cardiopulmonary afferent terminals it would be necessary to examine the effects of a silent antagonist on the cardiopulmonary afferent-evoked response of neurones, this was not tested in these experiments. Specifically, ketanserin was not used due to its inability to distinguish between 5-HT_{1D} and 5-HT_{2A} mediated effects on this input. However, with reference to figure 4.10 the attenuation of the cardiopulmonary afferent activation of this neurone by sumatriptan is greater, proportionally, than the sumatriptan-evoked attenuation of the ongoing activity. This suggests that there may be a significant population of 5-HT_{1D} receptors on the cardiopulmonary afferent terminals of this neurone, and that these cardiopulmonary afferents are not supplying a continuous excitatory input to these neurones, thus the ongoing activity is relatively unaffected by sumatriptan. Once more though it is not possible to tell if there is a tonic activation of 5-HT_{1D} receptors during cardiopulmonary or vagal afferent

activation. Furthermore, the ability of sumatriptan to modulate the cardiopulmonary afferent activation was only examined in a small number of NTS neurones.

The effects of CP-93,129 were also examined on the vagal afferent- and cardiopulmonary afferent-evoked responses of NTS neurones and unsurprisingly both of these excitatory input responses were potentiated by this ligand. This confirms the excitatory actions of this ligand, and therefore the likely excitatory effects of 5-HT_{1B} receptor activation. However, in these neurones no inhibitory responses were evoked by ionophoresis of CP-93,129 in comparison to observations of the effects of CP-93,129 on the ongoing or DLH-evoked activity of neurones, some of which were inhibited. This may be due to the smaller number of neurones tested. In addition, as mentioned above, although 5-HT_{1B} receptor activation is capable of potentiating vagal afferent input whether these receptors are located on vagal or cardiopulmonary afferent terminals, or whether they are merely increasing the excitability of neurones via activation of 5-HT_{1B} receptors on the cell body cannot be determined from the present experiments. The compound GR55562B was not used to further examine the role of 5-HT_{1B} receptors in the vagal and cardiopulmonary afferent activation of neurones due to the ability of this compound to reduce the excitability of neurones itself. Thus, further conclusion on the specific location or function of 5-HT_{1B} receptors cannot be made.

Further comments

Another important point in relation to the above data is that there was no obvious difference in the responses of neurones to 5-$HT_{1B/1D}$ receptor ligands based on their location. Neurones were recorded from caudal regions of the NTS within 0.5 mm rostral or caudal to obex, and within 0.5 mm lateral of midline on the side ipsilateral to vagal stimulation. Neurones were identified by their receiving a vagal afferent input, and the majority of neurones were correlated to cardiovascular and/or respiratory variables (for further detail see chapter 3). There was also no clear difference between the responses of neurones to 5-$HT_{1B/1D}$ receptor ligands and their sensitivity to cardiopulmonary afferent activation and/or level of correlation to autonomic variables. With

respect to cardiovascular roles for these receptor subtypes there is some evidence that activation of central 5-HT_{1B} receptors increase whilst 5-HT_{1D} receptors decrease blood pressure in the anaesthetized rat, and that this response is largely due to activation of these receptors in hindbrain areas (Gallacher & Ramage, 1996). A differential effect for 5-HT_{1B} and 5-HT_{1D} receptors is obviously supported by the findings of the present experiments, however, despite the closely correlated activity of these neurones to cardiovascular and respiratory variables, no conclusions can be made as to any specific vascular function of individual neurones. In addition, i.c. administration of sumatriptan has been shown to attenuate the reflex activation of cardiac vagal preganglionic neurones (CVPNs; Dando *et al.*, 1996), and this may also be supported in the present study since sumatriptan was found to attenuate the excitatory input to NTS neurones evoked by cardiopulmonary afferent stimulation and a proportion of this excitation might well be directed to CVPNs.

The ongoing discharge rate of ~50% of neurones was nil and therefore, as mentioned, ionophoretic administration of DLH was used to raise the activity of these neurones in order to examine the inhibitory effects of some ligands. However, since raising the baseline activity did not appear to have any qualitative effect on the 5-$HT_{1B/1D}$ receptor ligand-mediated effects neurones were not separated on the basis of their neuronal firing rates. With respect to this last point it is worth noting that these neurones were recorded from anaesthetized animals, therefore the level of neuronal activity is unlikely to reflect that occurring in a conscious animal. Indeed, the ongoing firing rate of neurones can be suppressed during recording by the supplementary addition of anaesthetic, in this case pentobarbitone sodium (see chapter 3).

One remaining question is the effects of altering the pH of solutions of compounds, necessary for their ionophoresis, on the activity or excitability of neurones. Vehicle controls of saline at pH 4 did not affect the ongoing or DLH-evoked activity of the large majority of neurones tested, confirming that the effects of solutions containing receptor ligands are indeed mediated via these ligands and that neurones are unaffected by mildly acidic solutions. In addition, the 5-HT_{1B} receptor agonist CP-93,129 was initially dissolved in 1% ascorbic

acid and then adjusted to pH 4, once more the control solution did not affect the ongoing activity of the majority of neurones. However, a mild inhibition was seen in the activity of a single NTS neurone, although ionophoresis of CP-93,129 to the same neurone caused excitation. This may provide an explanation for the inhibitory effect of CP-93,129 on the baseline activity of a small but significant number of NTS neurones i.e. these effects are mediated via the vehicle, 1% ascorbic acid, rather than an active effect of the 5-HT_{1B} receptor ligand.

Summary

In conclusion, 5-HT_{1D} receptors mediate an inhibitory effect, whilst 5-HT_{1B} receptors mediate an excitatory effect within the NTS of anaesthetized rats. Activation of these receptors can modulate vagal and cardiopulmonary afferent input to these neurones, although whether these receptors are activated during afferent stimulation remains to be determined. Further discussion of these data, with reference to the potential origin of these serotoninergic inputs is contained within chapter 6: discussion.

Chapter 5 5-HT_3 receptor modulation of NTS neuronal activity

5.1 Introduction

The nucleus of the tractus solitarius (NTS) is a region described as having one of the richest densities of 5-HT_3 receptors in the brain (Leslie *et al.*, 1994). Studies carried out using microinjection methods have demonstrated that the administration of 5-HT_3 receptor agonists into the region of the NTS can increase both blood pressure and sympathetic outflow (Merahi *et al.*, 1992; Nosjean *et al.*, 1995).

More recent investigations have demonstrated that microinjection of the selective 5-HT_3 receptor agonist 1-(*m*-chlorophenyl)-biguanide into the NTS attenuated the bradycardic component of the cardiopulmonary reflex in both awake and urethane-anaesthetized rats (Sevoz *et al.*, 1996; Leal *et al.*, 2001). In contrast, other research has demonstrated that, in contrast to activation, blockade of 5-HT_3 receptors using the selective antagonist granisetron, attenuated the bradycardia evoked by cardiopulmonary afferent activation (Dando *et al.*, 1995; Pires *et al.*, 1998). These latter experiments were performed in urethane-anaesthetised rabbits (Dando *et al.*, 1995) and rats (Pires *et al.*, 1998) and drugs were administered intracisternally (i.c.).

The limitations of microinjection and i.c. injection as methods of accurately targeting specific nuclei within the dorsal brainstem may partially explain these conflicting data. There are large populations of 5-HT_3 receptors in the dorsal vagal motor nucleus (DVN) and the area postrema, both directly adjacent to the NTS which may contribute to the modulations of cardiopulmonary reflex that have been described. Indeed, in the DVN, neurones have been shown to be

activated by cardiopulmonary afferent activation (Jones *et al.*, 1998) and by 5-HT_3 receptor activation at the neuronal level (Wang *et al.*, 1996). More accurate methods of studying 5-HT_3 receptors action within the NTS may provide further evidence of their role in cardiopulmonary reflex and other functions.

Extracellular recordings, in combination with ionophoresis techniques, have revealed that the selective 5-HT_3 receptor agonist phenylbiguanide (PBG) excites the large majority of NTS neurones (Wang *et al.*, 1997). These neurones were identified as receiving vagal afferent input, and studies focused solely on the effects that selective ligand administration had on the baseline activity of neurones.

Aims:

- Experiments were carried out to further examine the role of 5-HT_3 receptors in the NTS at the neuronal level. Firstly, confirming the predominant excitatory effect that activation of 5-HT_3 receptors has on NTS neuronal activity and secondly examining any role that these receptors may have on the vagal afferent-evoked and cardiopulmonary afferent-evoked activation of NTS neurones in the anaesthetized rat.

5.2 Results

5.2.1 Effects of 5-HT_3 receptor ligands on neuronal activity

The effect of ionophoretic administration of 5-HT_3 receptor ligands and their vehicles on the activity of NTS neurones was tested in a total population of 127 cells. A proportion of these neurones showed little or no ongoing activity therefore in some cases it was necessary to raise the activity of these cells using ionophoretic application of the excitatory amino acid DL-homocysteic acid (DLH) to examine the effects of these 5-HT receptor ligands. Excitation or inhibition of the activity of each neurone was classified by a change in this baseline firing rate of greater than 20%. Recording of the baseline activity, post-ligand administration, was not achieved in all neurones. However, mean firing rates (spikes s^{-1}) for both the full populations tested and the proportions with a recorded post-ligand response are given.

Phenylbiguanide

Ionophoretic administration of the 5-HT_3 receptor agonist phenylbiguanide (PBG) excited 96 of 106 NTS neurones with a mean increase of 129% in baseline firing rates (figure 5.1). The post-PBG activity of 92 of these neurones was recorded and complete recovery was observed (table 5.1). Of the remaining 10 neurones, 9 were unaffected by PBG, whilst the baseline activity of one neurone was inhibited by greater than 20% (table 5.1).

Phenylbiguanide in the presence of granisetron

The predominant excitatory response evoked by ionophoretic administration of PBG was examined during ionophoretic administration of the selective 5-HT_3 receptor antagonist granisetron (see table 5.1; figures 5.2, 5.3 and 5.5). In 17 NTS neurones tested PBG evoked a mean increase in baseline activity of 106% and this was reduced to a 27% increase during the simultaneous administration of granisetron. In 4 of these 17 neurones the post-granisetron PBG response was recorded and a complete recovery of the control PBG excitation was observed (figure 5.3). In addition, in 3 of these 4 neurones the recovery PBG

response was bigger in magnitude than the control PBG response, although this increase was not found to be significant.

The effect of granisetron on the baseline activity of NTS neurones was also examined (table 5.2). In 11 of 18 neurones granisetron did not affect the baseline firing rate (figure 5.5). Of the 7 remaining neurones 4 were excited, whilst 3 were inhibited by granisetron administration, although the group data did not indicate that the inhibitory response was statistically significant (figure 5.5B).

Phenylbiguanide in the presence of ondansetron

A second 5-HT_3 receptor antagonist was tested on the excitation evoked by ionophoretic administration of PBG. Ondansetron was chosen due to its use in other studies examining 5-HT_3 receptor-mediated effects in the brainstem. The response to PBG was tested with ondansetron in just one NTS neurone and a large attenuation in the PBG-evoked excitation was observed, recovery was achieved (figure 5.4; table 5.1).

In addition, studies showed that ondansetron did not affect the baseline activity of 3 out of 4 NTS neurones, although the activity of the remaining neurone was increased by greater than 20% (table 5.2).

Figure 5.1 The effect of ionophoretic administration of phenylbiguanide on the activity of NTS neurones in the anaesthetized rat

A Excitation in the activity of an NTS neurone evoked by the ionophoretic administration of phenylbiguanide (PBG; during the bars and at the current stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. From left to right: baseline activity (■; CON), activity evoked by ionophoretic administration of PBG (3-320 nA; □; PBG). 96 out of 106 neurones were excited by the ionophoretic administration of PBG, with the post-PBG activity recorded in 92. Of the remaining 10 neurones one was inhibited, and 9 were unaffected by PBG.

** $P<0.01$

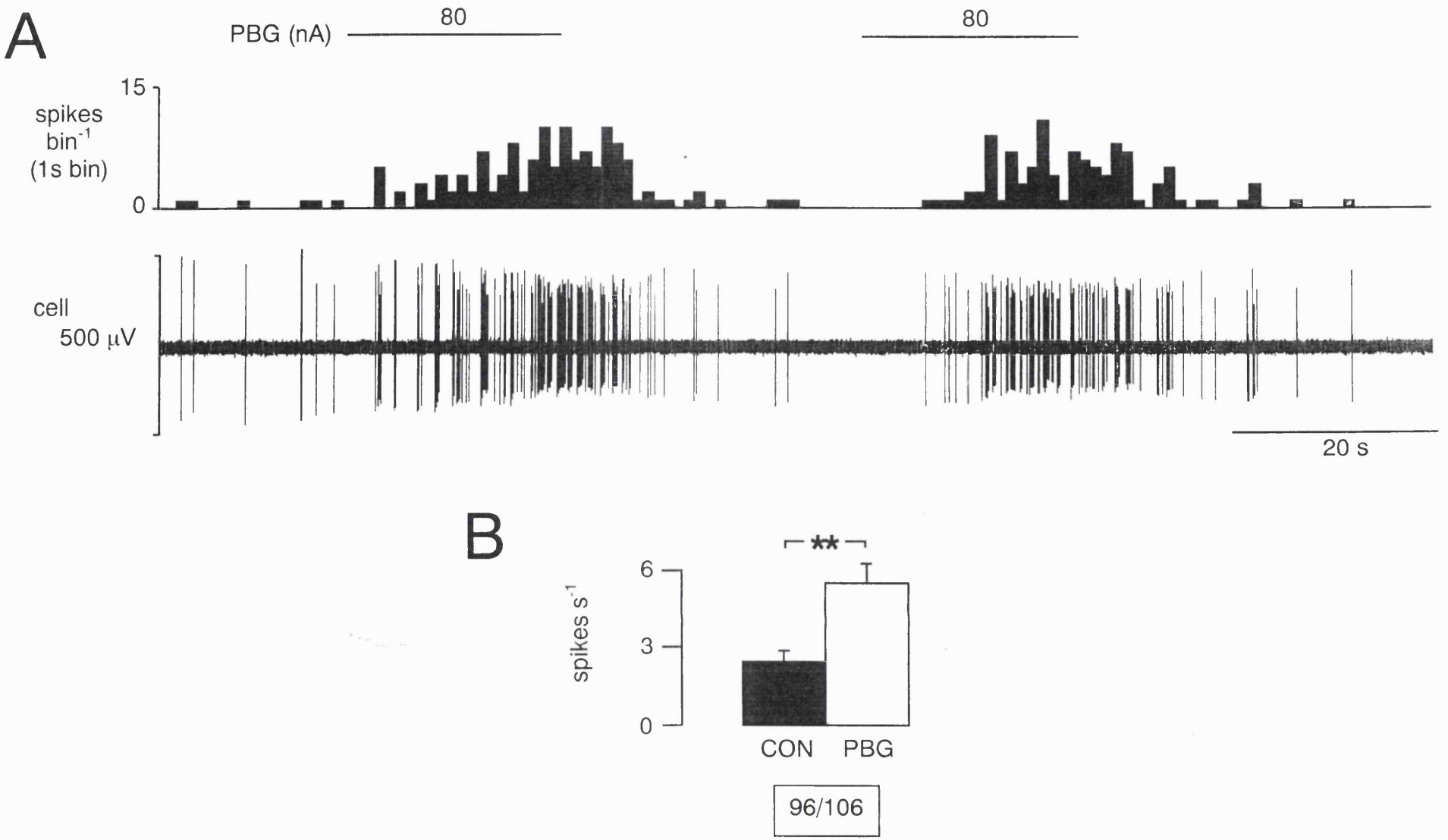

A
PBG (nA)
80
80
15
spikes
bin-1
(1s bin)
0
cell
500 μV
20 s
B
**
6
3
0
spikes s-1
CON
PBG
96/106

Figure 5.2 The effect of ionophoretic administration of granisetron on the excitatory effect evoked by ionophoretic administration of phenylbiguanide in the anaesthetized rat I

A The response of an NTS neurone to the ionophoretic administration of phenylbiguanide (PBG) before and during the ionophoretic administration of granisetron (during the bars and at the currents stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. From left to right: activity evoked by ionophoretic administration of PBG (5-120 nA; ☐; PBG), activity evoked by PBG in the presence of granisetron (5-60 nA; ▤; PBG + gran), baseline activity (■; CON) and recovery of baseline activity (▨; REC). In 17 out of 17 neurones, the control response to PBG was attenuated by granisetron, with the baseline firing rates of 15 of these neurones shown. The post-granisetron PBG response was recorded in 4 of these 17 neurones (see figure 5.3).

* $P<0.05$

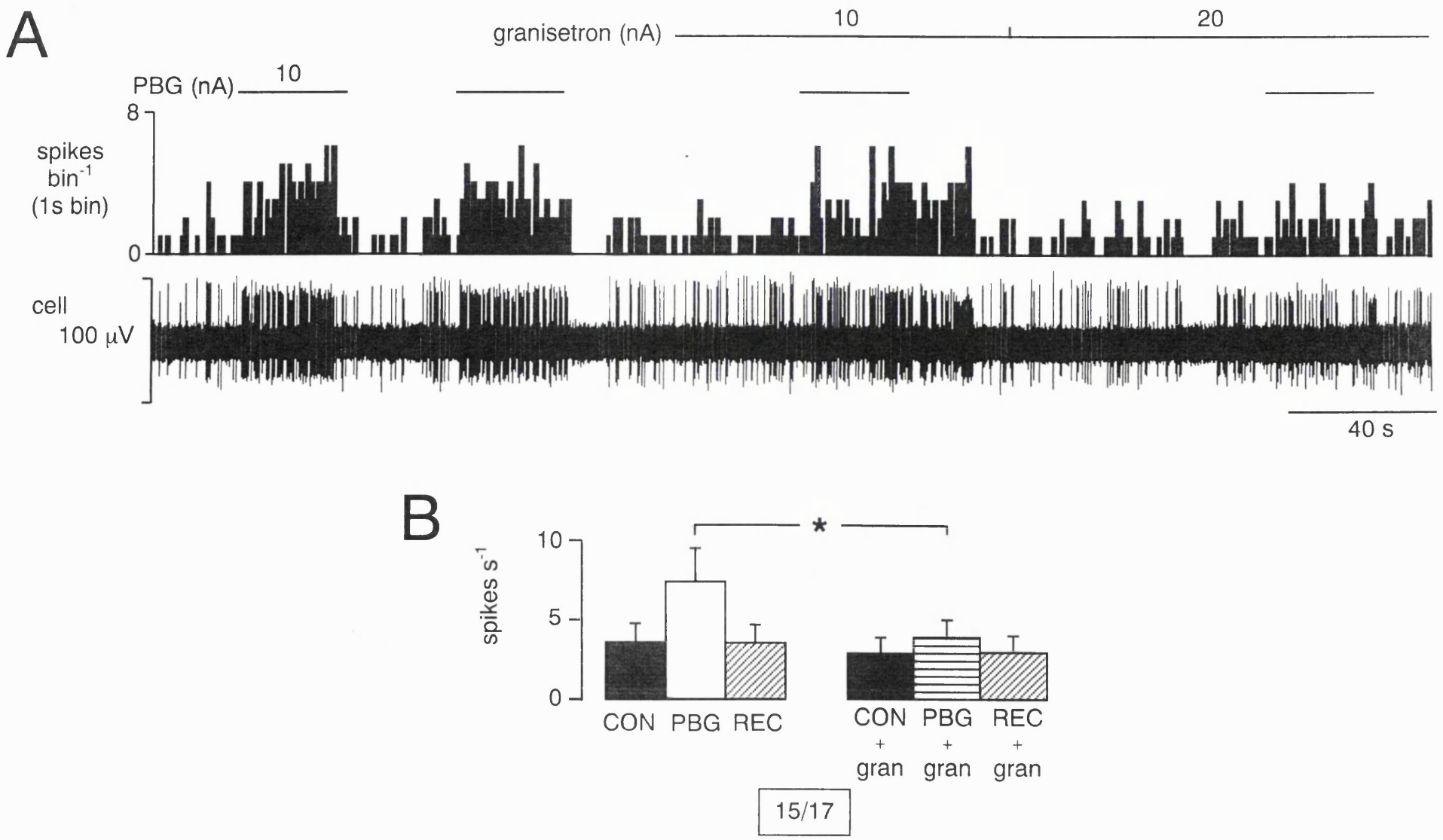

A
granisetron (nA)
10
20
PBG (nA)
10
8
spikes
bin-1
(1s bin)
0
cell
100 μV
40 s
B
spikes s-1
10
5
0
*
CON
PBG
REC
CON + gran
PBG + gran
REC + gran
15/17

Figure 5.3 The effect of ionophoretic administration of granisetron on the excitatory effect evoked by ionophoretic administration of phenylbiguanide in the anaesthetized rat II

A The response of an NTS neurone to the ionophoretic administration of phenylbiguanide (PBG; during the bars and at the current stated) before, during and after the ionophoretic administration of granisetron. The trace shows a rate histogram of neuronal activity (spikes bin^{-1}).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. From left to right: baseline activity (■; CON), activity evoked by ionophoretic administration of PBG (10-120 nA; □; PBG), recovery of baseline activity (▨; REC) and activity evoked by PBG in the presence of granisetron (5-60 nA; ▤; PBG + gran). In a population of 17 neurones (see figure 5.2) the post-granisetron PBG response was recorded in 4.

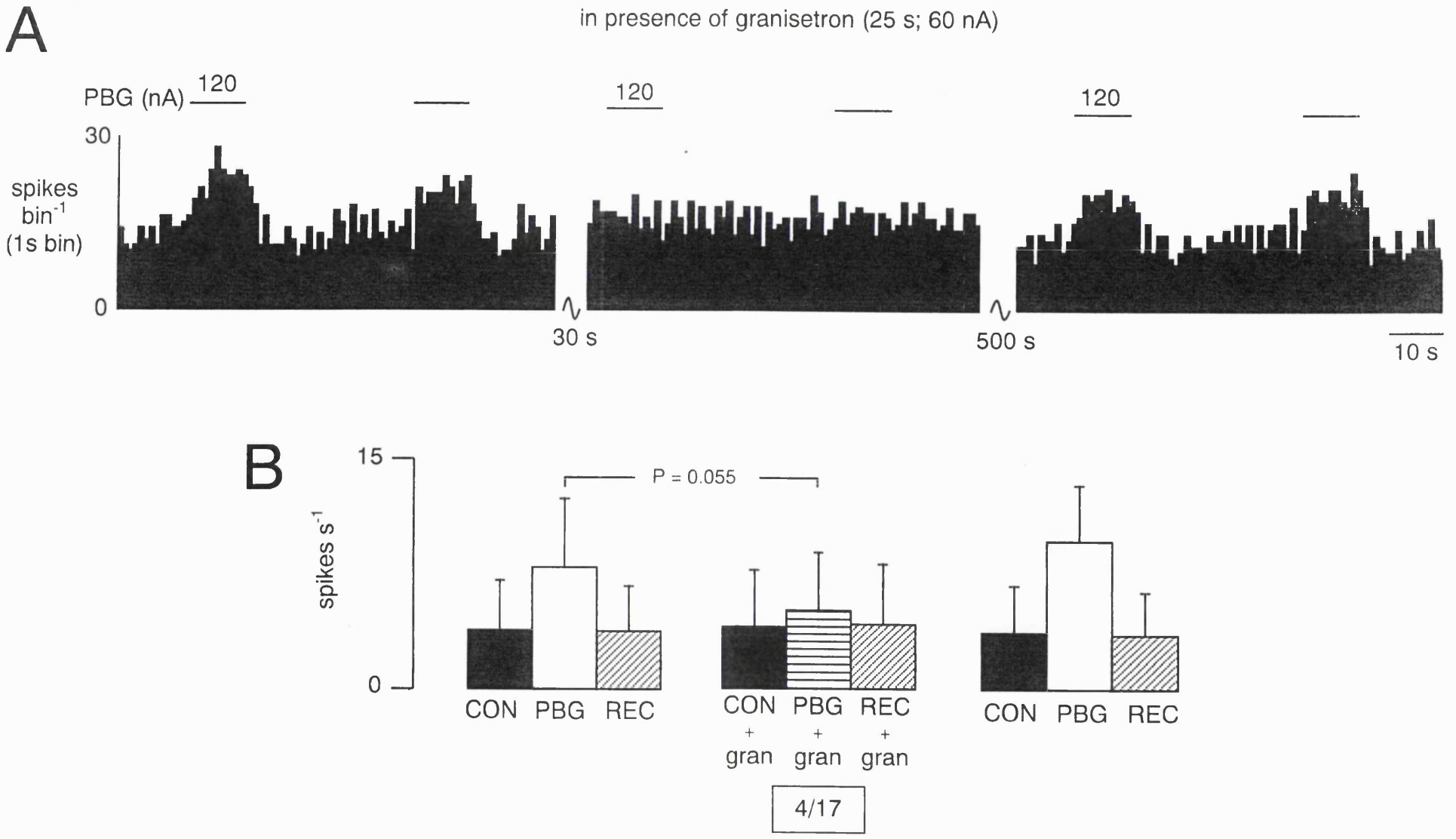

A
in presence of granisetron (25 s; 60 nA)
PBG (nA)
120
120
120
30
spikes bin-1 (1s bin)
0
30 s
500 s
10 s
B
15
spikes s-1
0
P = 0.055
CON PBG REC
CON + gran PBG + gran REC + gran
CON PBG REC
4/17

Figure 5.4 The effect of ionophoretic administration of ondansetron on the excitatory effect evoked by ionophoretic administration of phenylbiguanide in the anaesthetized rat

The response of an NTS neurone to the ionophoretic administration of phenylbiguanide (PBG; during the bars and at the current stated) before, during and after the ionophoretic administration of ondansetron. From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

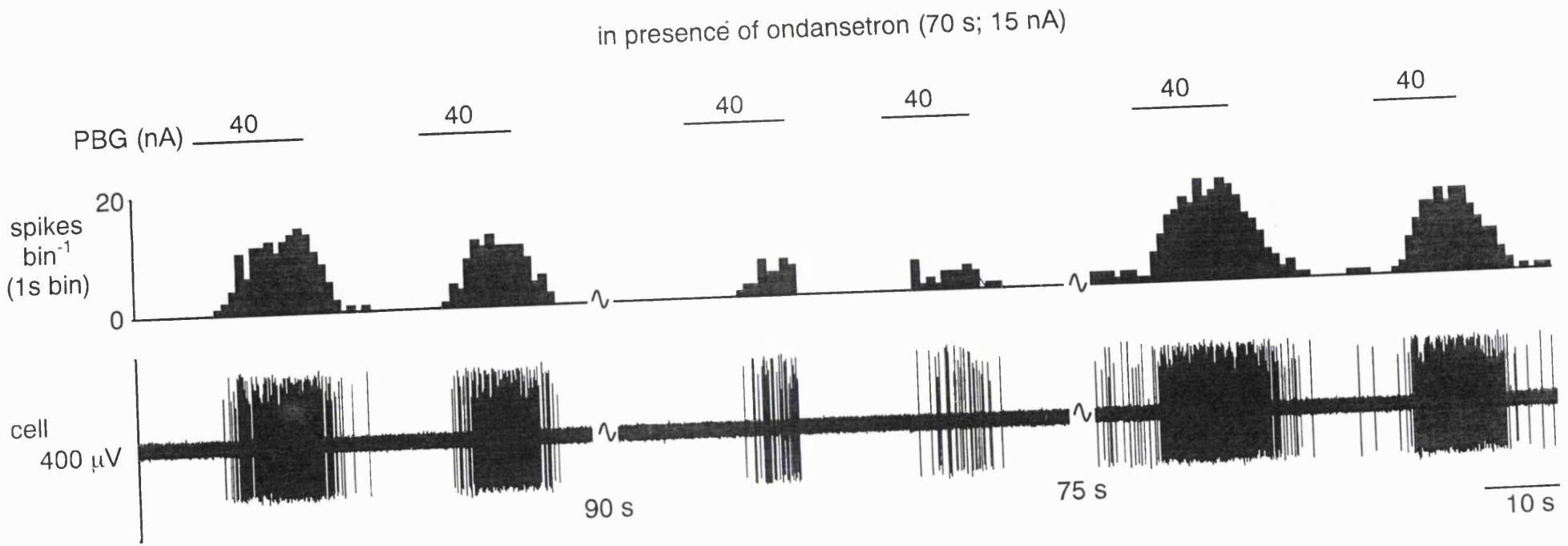

in presence of ondansetron (70 s; 15 nA)
PBG (nA)
40
40
40
40
40
40
spikes bin-1 (1s bin)
20
0
cell
400 μV
90 s
75 s
10 s

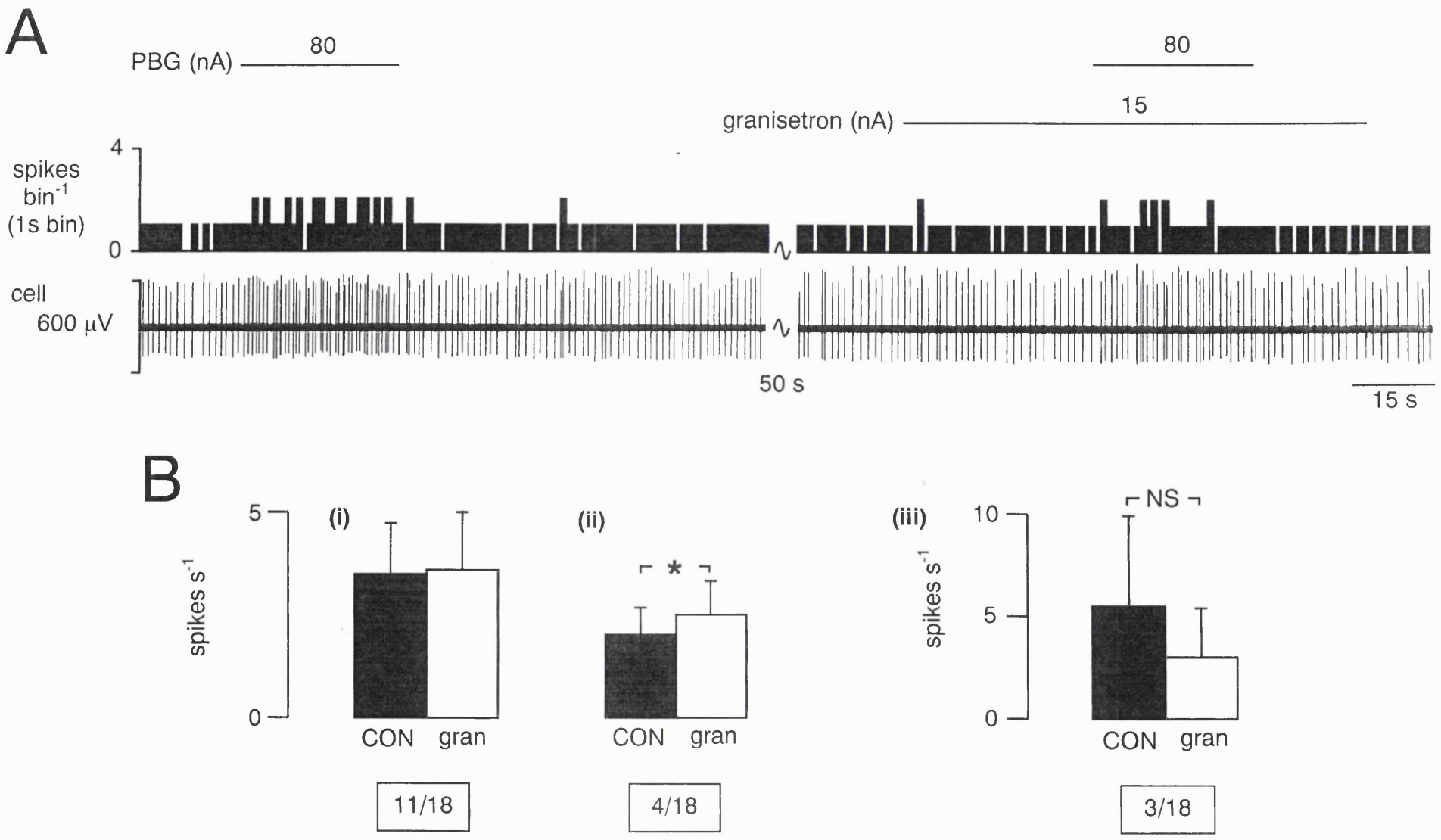
Figure 5.5 The effect of ionophoretic administration of granisetron on the activity of NTS neurones in the anaesthetized rat

A The activity of an NTS neurone, and its response to phenylbiguanide, prior to (left panel) and during (right panel) the ionophoretic administration of granisetron (during the bar and at the current stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. **(i)**, **(ii)** & **(iii)** From left to right: baseline activity (■; CON), activity evoked by ionophoretic administration of granisetron (5-60 nA; □; gran). In 11 of 18 neurones the baseline activity was unaffected by the ionophoretic administration of granisetron **(i)**. Of the remaining 7 neurones, 4 were excited **(ii)** whilst 3 were inhibited **(iii)** by granisetron. The response of neurones in which the post-granisetron baseline activity was recorded can be seen in table 5.1.

* $P<0.05$

NS - not significant


A
PBG (nA)
80
80
granisetron (nA)
15
spikes bin-1 (1s bin)
4
0
cell
600 μV
50 s
15 s
B
spikes s-1
5
0
(i)
CON
gran
11/18
(ii)
*
CON
gran
4/18
(iii)
NS
10
5
0
spikes s-1
CON
gran
3/18

5.2.2 5-HT_3 receptor ligands and identified inputs

The effects of the 5-HT_3 receptor ligands PBG and granisetron were examined on the vagal afferent-evoked and cardiopulmonary afferent-evoked activation of NTS neurones (see table 5.3).

Vagal afferent activation of neurones was measured as the total number of spikes evoked by vagus nerve stimulation, including both short and long latency spikes over a time period of a minimum of 20 sweeps of nerve stimulation. The mean response of neurones was subsequently calculated per 20 sweeps of stimulation. In addition, cardiopulmonary afferent activation was measured as the total number of spikes in the burst of neuronal activity evoked by right atrial administration of phenylbiguanide. Responses prior to, during, and where possible, after the ionophoretic administration of each receptor ligand were then compared.

Vagal-afferent-evoked activation

Ionophoretic administration of PBG potentiated vagal afferent-evoked activity by a mean of 72% in 13 out of 17 neurones (figure 5.6). The post-PBG response was recorded in 11 of these neurones and partial recovery of control evoked activity was observed. The vagal afferent-evoked activity of the remaining 4 neurones was unaffected by ionophoretic PBG administration.

Cardiopulmonary afferent-evoked activation

Ionophoretic administration of PBG also potentiated the cardiopulmonary afferent-evoked activation in 2 of 3 NTS neurones by a mean of 160% (figures 5.7). The activation of the third neurone was unaffected by PBG administration.

The effect of ionophoretic administration of granisetron on the activation of NTS neurones evoked by cardiopulmonary afferent stimulation was examined in 10 neurones (figure 5.8). This activation was significantly attenuated by a mean of 48% in 9 of the 10 neurones, an example of which is shown in figure 5.8. In 5

of these 9 neurones, recording of the reflex post-granisetron administration was achieved and complete or partial recovery of the control response was observed in 4 of these 5 neurones, whilst the post-granisetron response of the fifth neurone remained attenuated.

The cardiopulmonary afferent-evoked activation of the remaining neurone was increased by greater than 20% in the presence of granisetron, although the post-granisetron response was not recorded.

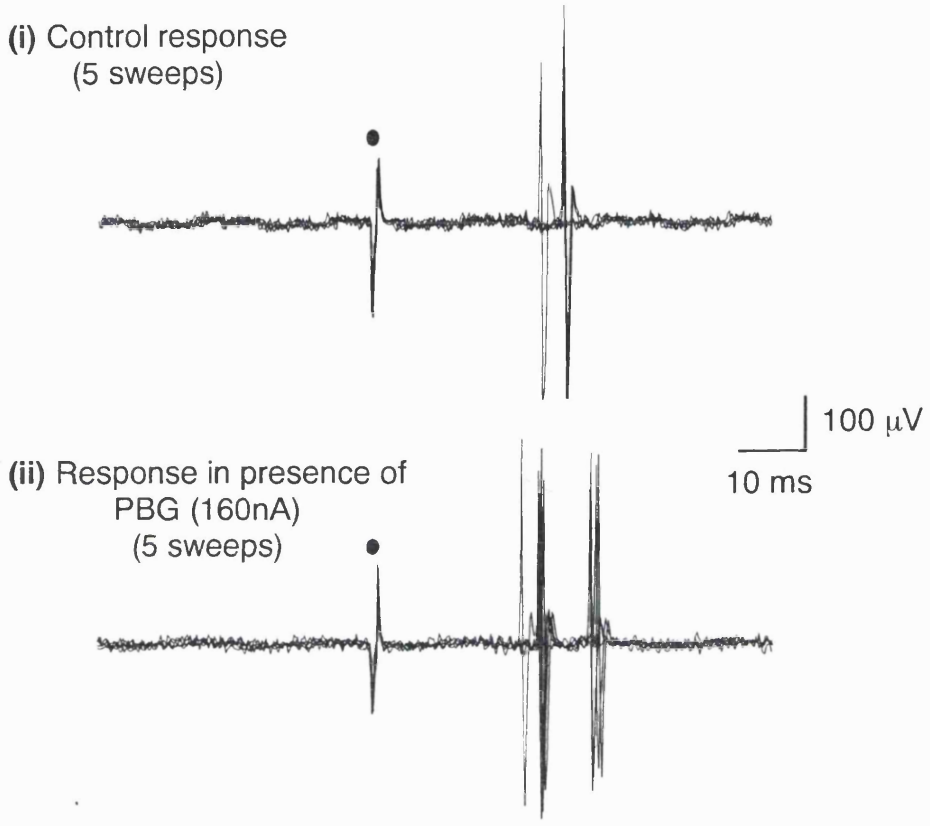
Figure 5.6 Potentiation of the vagal afferent-evoked activation of NTS neurones by ionophoretic administration of phenylbiguanide in the anaesthetized rat

A Potentiation of the vagal afferent-evoked activity of an NTS neurone by the ionophoretic administration of phenylbiguanide (PBG; at the current stated). From the top, traces show the raw extracellular recording of neuronal activity (5 sweeps; μV) and before **(i)** and during **(ii)** the administration of PBG. ● - stimulus artefact.

B Histogram of the mean data of vagal afferent-evoked activity with vertical bars representing s.e.mean. From left to right: control evoked activity (mean response per 20 sweeps; ■; CON) and evoked activity during the ionophoretic administration of PBG (20-160 nA; □; PBG). Of 17 neurones, the total evoked activity of 13 was potentiated by the ionophoretic administration of PBG. The post-PBG activity was recorded in 11 of these neurones. The total evoked response of the remaining 4 neurones was unaffected by PBG.

** $P<0.01$

A


(i) Control response
(5 sweeps)
100 μV
10 ms
(ii) Response in presence of
PBG (160nA)
(5 sweeps)

B

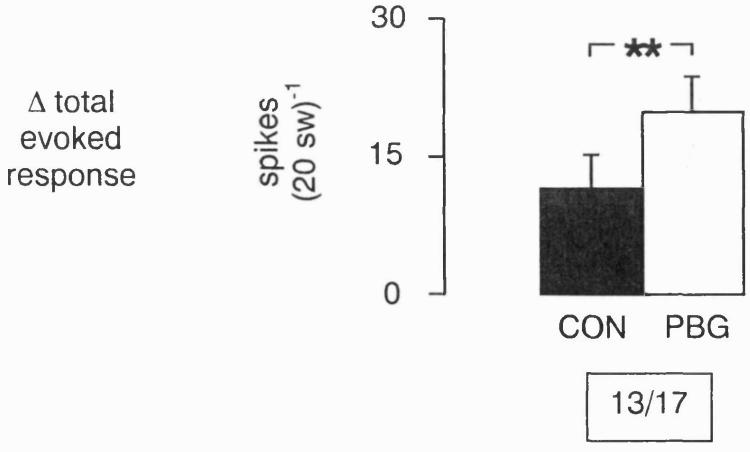

Δ total
evoked
response
spikes
(20 sw)-1
30
15
0
**
CON
PBG
13/17

Figure 5.7 Potentiation of the cardiopulmonary afferent-evoked activation of NTS neurones by ionophoretic administration of phenylbiguanide in the anaesthetized rat

The response of an NTS neurone to activation of cardiopulmonary afferents by right atrial administration of phenylbiguanide (PBG) prior to (left panel) and during (right panel) the ionophoretic administration of PBG. From the top, traces show blood pressure (BP; mmHg), a continuous rate histogram of neuronal activity (spikes bin^{-1}) and the raw recording of neuronal activity (μV). The administration of PBG is highlighted by a solid horizontal line (▬; PBG - 24 μg kg^{-1}, 40 μl). Circled numbers highlight the number of spikes in the corresponding burst of activity. Of 3 neurones, the cardiopulmonary afferent-evoked response of 2 was potentiated in the presence of PBG (80-160 nA), whilst the response of the third neurone was unaffected.

NB. Background activity is vagal-evoked activity (0.7 Hz), and is unaffected by ionophoretic PBG administration in this neurone, in addition cell identity between panels is confirmed by the expanded sections at the bottom of the page.

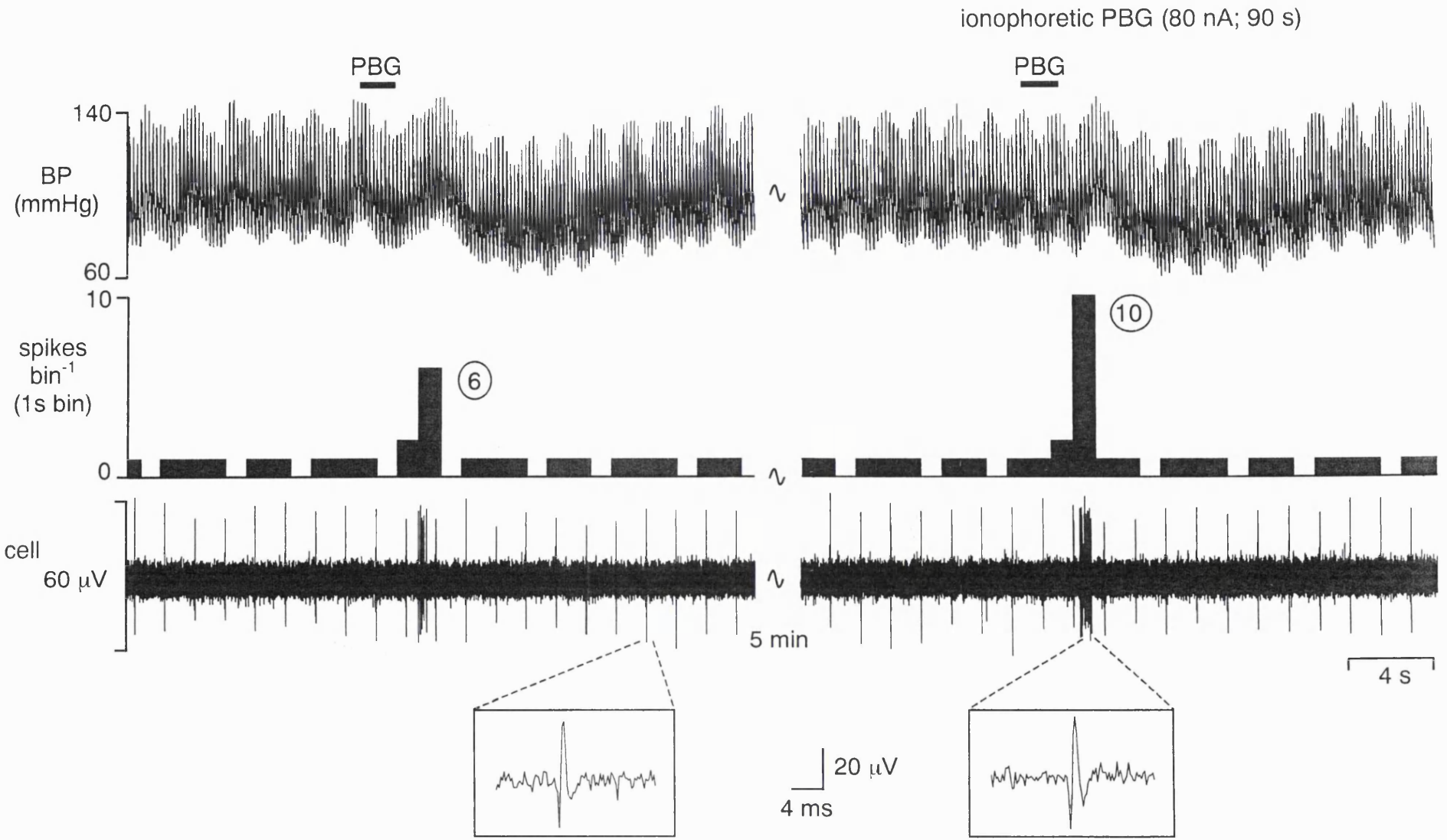
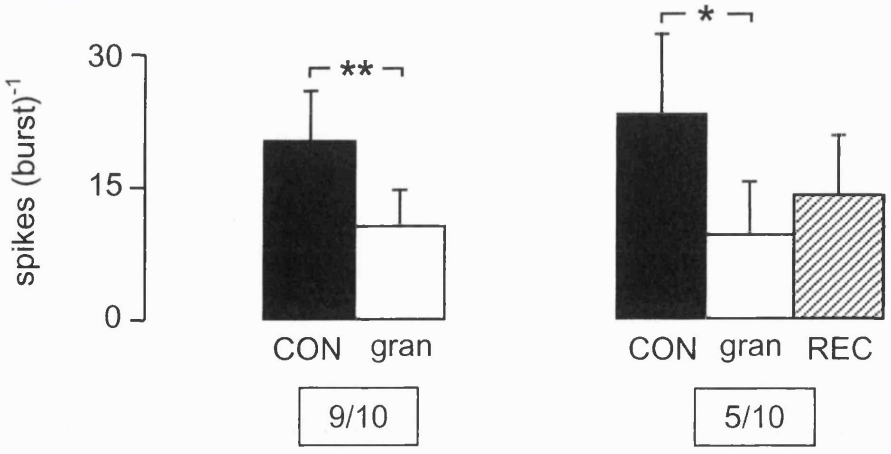

ionophoretic PBG (80 nA; 90 s)
PBG
PBG
140
BP
(mmHg)
60
10
spikes
bin-1
(1s bin)
0
6
10
cell
60 μV
5 min
4 s
20 μV
4 ms

Figure 5.8 Attenuation of the cardiopulmonary afferent-evoked activation of NTS neurones by ionophoretic administration of granisetron in the anaesthetized rat

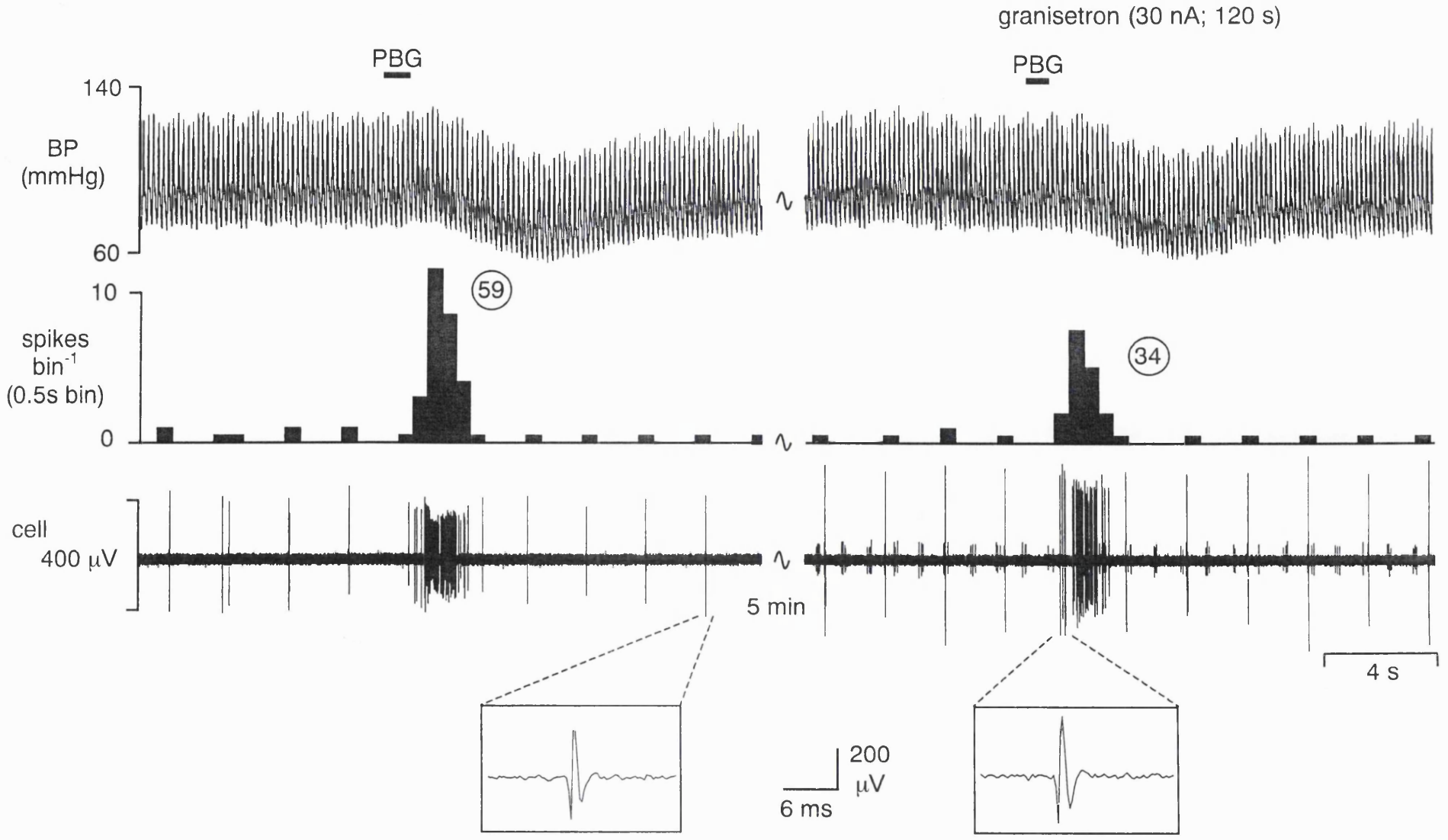
The response of an NTS neurone to activation of cardiopulmonary afferents by intra-atrial administration of phenylbiguanide (PBG) prior to (left panel) and during (right panel) the ionophoretic administration of granisetron. From the top, traces show blood pressure (BP; mmHg), a continuous rate histogram of neuronal activity (spikes bin^{-1}) and the raw recording of neuronal activity (μV). The administration of PBG is highlighted by a solid horizontal line (▬; PBG - 24 μg kg^{-1}, 40 μl). Circled numbers highlight the number of spikes in the corresponding burst of activity. Of 10 neurones ionophoretic administration of granisetron attenuated the cardiopulmonary afferent-evoked activation of 9 (20-80 nA), with the post-granisetron response recorded in 5 of these neurones. The response of the remaining neurone was potentiated by granisetron.

NB. Background activity is vagal-evoked activity (0.5 Hz) and is unaffected by granisetron, in addition cell identity between panels is confirmed by the expanded sections.

Group data:



CON - control cardiopulmonary afferent-evoked response
gran - response in the presence of granisetron
REC - post-granisetron response
** - P<0.01 * - P<0.05


granisetron (30 nA; 120 s)
PBG
PBG
140
BP
(mmHg)
60
10
spikes
bin-1
(0.5s bin)
0
59
34
cell
400 μV
5 min
4 s
200
μV
6 ms

5.2.3 Vehicle and other controls of neuronal activity

DL-homocysteic acid in the presence of granisetron

Ionophoretic administration of the excitatory amino acid DL-homocysteic acid (DLH) was used in experiments to raise the baseline activity of quiescent or hyperpolarised neurones. This excitatory response of neurones to the ionophoretic administration of DLH was examined in the presence of granisetron (table 5.4), acting as a control for the attenuation by granisetron of the excitation evoked by ionophoretic administration of PBG. In 4 out of 5 neurones the DLH-evoked increase in baseline activity was unaffected by the ionophoretic administration of granisetron (figures 5.9B and 5.10). In the example shown in figure 5.9A the DLH-evoked response is only slightly attenuated by a dose of granisetron that completely inhibited the excitation evoked by ionophoretic administration of PBG. Ionophoretic administration of granisetron did attenuate the DLH-evoked excitation of the fifth neurone by 46%, although, in the same neurone a bigger attenuation (76%) of the excitation evoked by ionophoretic administration of PBG was observed.

Saline at pH 10

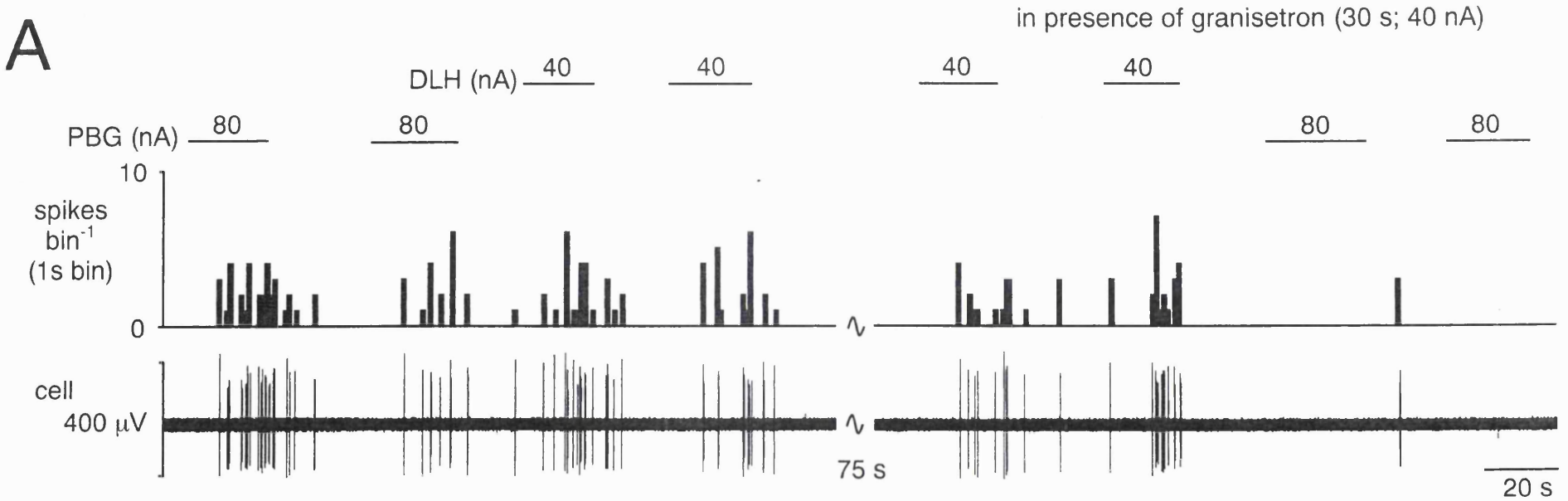
Granisetron and ondansetron were both dissolved in saline adjusted to pH 4, for the vehicle controls for these drugs refer to chapter 4, section 4.2.3. However, PBG was dissolved in saline and adjusted to pH 10, therefore the effects of saline at pH 10 was examined on the activity of NTS neurones (table 5.4). The baseline activity of 2 out of 3 neurones was unaffected by the ionophoretic administration of pH 10 saline (figure 5.11B). However, the activity of the third neurone was increased by 88% by pH 10 saline, although ionophoretic administration of PBG to the same neurone raised activity by 300% (figure 5.11A)

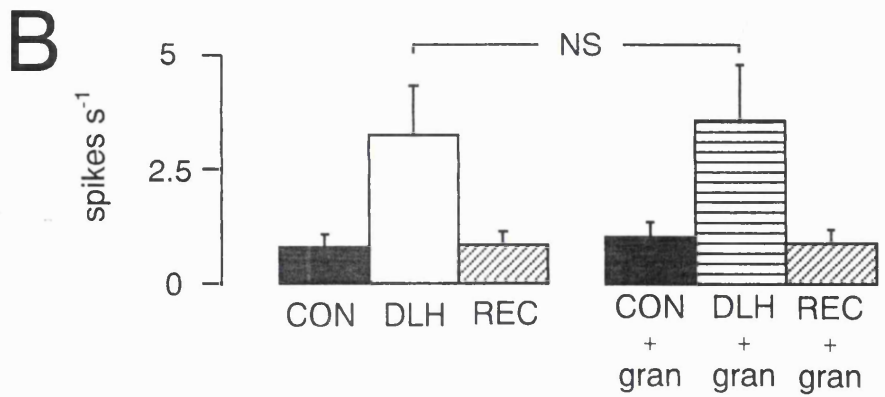
Figure 5.9 The effect of ionophoretic administration of granisetron on the excitatory effect evoked by ionophoretic administration of DL-homocysteic acid in the anaesthetized rat I

A The response of an NTS neurone to the ionophoretic administration of DL-homocysteic acid and phenylbiguanide (DLH and PBG respectively; during the bars and at the current stated) before and during the ionophoretic administration of granisetron. From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. From left to right: activity evoked by ionophoretic administration of DLH (20-40 nA; □; DLH), activity evoked by DLH in the presence of granisetron (15-80 nA; ▤; DLH + gran), baseline activity (■; CON) and recovery of baseline activity (▨; REC). In 5 neurones, the control response to DLH was unaffected by granisetron in 4 with the post-granisetron DLH response recorded in one of these neurones (see figure 5.10). The response to DLH of the remaining neurone was attenuated in the presence of granisetron.

NS - not significant


A
in presence of granisetron (30 s; 40 nA)
DLH (nA)
40
40
40
40
PBG (nA)
80
80
80
80
10
spikes
bin-1
(1s bin)
0
cell
400 μV
75 s
20 s


B
NS
5
2.5
0
spikes s-1
CON
DLH
REC
CON + gran
DLH + gran
REC + gran

4/5

Figure 5.10 The effect of ionophoretic administration of granisetron on the excitatory effect evoked by ionophoretic administration of DL-homocysteic acid in the anaesthetized rat II

The response of an NTS neurone to the ionophoretic administration of DL-homocysteic acid (DLH) before, during and after the ionophoretic administration of granisetron (during the bars and at the currents stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

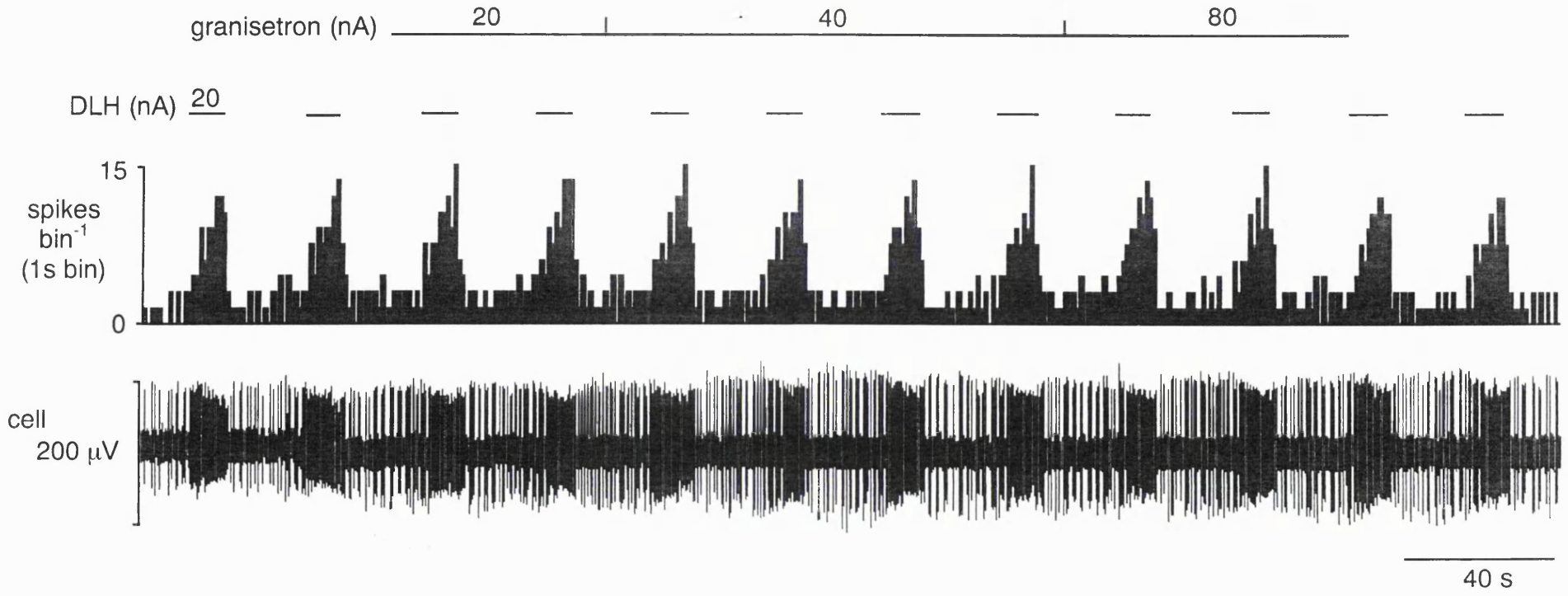

granisetron (nA)
20
40
80
DLH (nA) 20
15
spikes
bin-1
(1s bin)
0
cell
200 μV
40 s

Figure 5.11 The effect of ionophoretic administration of saline at pH 10 on the activity of NTS neurones in the anaesthetized rat

A The response of a single NTS neurone to the ionophoretic administration of pH 10 saline and phenylbiguanide (PBG; during the bars and at the currents stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity. From left to right: baseline activity (■; CON), activity evoked by ionophoretic administration of saline at pH 10 (40-120 nA; □; pH 10 SAL) and recovery of baseline activity (▨; REC). Of 3 neurones, 2 were unaffected by the ionophoretic administration of saline at pH 10 saline whilst one neurone was excited (shown in **A**).

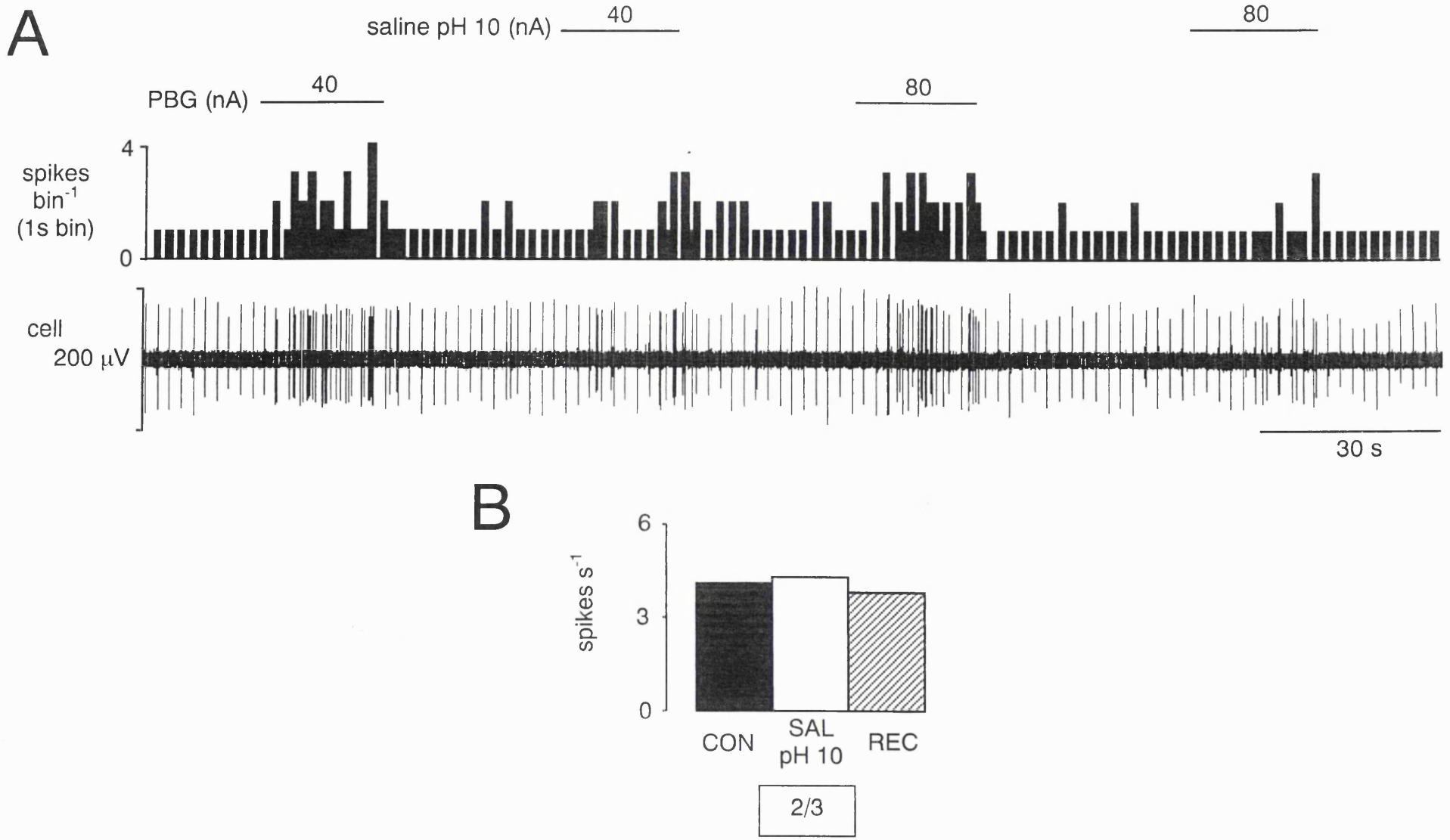

A
saline pH 10 (nA)
40
80
PBG (nA)
40
80
spikes bin-1 (1s bin)
4
0
cell
200 μV
30 s
B
spikes s-1
6
3
0
CON
SAL pH 10
REC
2/3

Table 5.1 The effect of ionophoretic administration of phenylbiguanide, in addition to the effects of ionophoretic administration of granisetron and ondansetron on the excitation evoked by phenylbiguanide, on the activity of NTS neurones

Numbers reflect mean firing rates of neurones in spikes s^{-1}

(i) The effects of ionophoretic administration of phenylbiguanide on the activity of NTS neurones

(ii) The effects of ionophoretic administration of phenylbiguanide before, during, and after the ionophoretic administration of granisetron, on the activity of NTS neurones

(iii) The effects of ionophoretic administration of phenylbiguanide before, during, and after the ionophoretic administration of ondansetron, on the activity of NTS neurones

CON-baseline activity, PBG-activity in the presence of phenylbiguanide, REC-recovery of baseline activity, + gran-in the presence of granisetron, + onda-in the presence of ondansetron, Att. Exc.-attenuated excitation, none-activity unaffected.

* $P<0.05$ ** $P<0.01$ compared to CON

† $P<0.05$ compared to PBG

‡ $P=0.055$ compared to PBG

(i) The effects of phenylbiguanide

Number of cells	Effect	CON	PBG	REC
		spikes s^{-1}		
96/106	excitation	2.4±0.4	5.5±0.8**	-
92/106	excitation	2.4±0.5	5.5±0.8**	2.5±0.5
9/106	none	3.7±1.0	4.0±1.1	3.5±1.0
1/106	inhibition	1.5	0.7	3.1

(ii) The effects of phenylbiguanide in the presence of granisetron

Number of cells	Effect	CON	PBG	REC	CON +gran	PBG +gran	REC +gran	CON	PBG	REC
		spikes s^{-1}								
17/17	Att. Exc.	3.4 ±1.1	7.0** ±1.9	-	3.0 ±0.9	3.8† ±1.1	-	-	-	-
15/17	Att. Exc.	3.5 ±1.2	7.4** ±2.1	3.5 ±1.2	3.0 ±1.1	4.1† ±1.2	3.1 ±1.1	-	-	-
4/17	Att. Exc.	3.8 ±3.2	7.9 ±4.5	3.8 ±3.0	4.0 ±3.7	5.0‡ ±3.8	4.2 ±3.9	3.6 ±3.1	9.6* ±3.6	3.5 ±2.8

(iii) The effects of phenylbiguanide in the presence of ondansetron

Number of cells	Effect	CON	PBG	REC	CON +onda	PBG +onda	REC +onda	CON	PBG	REC
		spikes s^{-1}								
1/1	Att. Exc.	0.0	5.6	0.0	0.0	1.5	0.0	0.0	8.5	0.0

Table 5.2 The effects of ionophoretic administration of granisetron and ondansetron on the activity of NTS neurones

Numbers reflect mean firing rates of neurones in spikes s^{-1}

(i) The effects of ionophoretic administration of granisetron on the activity of NTS neurones

(ii) The effects of ionophoretic administration of ondansetron on the activity of NTS neurones

CON-baseline activity, gran-activity in the presence of granisetron, onda-in the presence of ondansetron, REC-recovery of baseline activity, none-activity unaffected.

* $P<0.05$ compared to CON

(i) The effects of granisetron

Number of cells	Effect	CON	gran	REC
		spikes s^{-1}		
11/18	none	3.5±1.2	3.6±1.4	-
7/18	none	3.2±1.8	3.4±2.1	3.4±2.2
4/18	excitation	2.0±0.7	2.5±0.8*	-
2/18	excitation	2.2	2.8	2.8
3/18	inhibition	5.5±4.4	3.0±2.4	-
2/18	inhibition	7.5	4.0	4.3

(ii) The effects of ondansetron

Number of cells	Effect	CON	onda	REC
		spikes s^{1}		
3/4	none	1.3±1.3	1.4±1.3	1.9±1.9
1/4	excitation	2.1	6.0	2.5

Table 5.3 The effects of ionophoretic administration of phenylbiguanide and granisetron on the vagal afferent-evoked and cardiopulmonary afferent-evoked activation of NTS neurones

Numbers reflect mean firing rates of neurones in spikes (20 sweeps)$^{-1}$ and spikes (burst)$^{-1}$

(i) The effects of ionophoretic administration of phenylbiguanide on the vagal-evoked activity of NTS neurones

(ii) The effects of ionophoretic administration of phenylbiguanide on the cardiopulmonary afferent-evoked activity of NTS neurones

(iii) The effects of ionophoretic administration of granisetron on the cardiopulmonary afferent-evoked activity of NTS neurones

CON-baseline evoked activity, PBG-evoked activity in the presence of phenylbiguanide, gran-evoked activity in the presence of granisetron, none-evoked activity unaffected, REC-recovery of baseline evoked activity

* $P<0.05$ ** $P<0.01$ compared to CON

(i) The effect of phenylbiguanide on vagal-evoked activity

Number of cells	Effect	CON	PBG	REC
		spikes (20 sweeps)$^{-1}$		
13/17	potentiated	11.5 ±3.7	19.8** ±3.9	-
11/17	potentiated	12.6 ±4.3	20.4** ±4.6	14.2 ±4.9
4/17	none	22.2 ±1.5	21.9 ±1.3	-
3/17	none	21.2 ±1.6	20.8 ±1.1	17.3 ±2.7

(ii) The effect of phenylbiguanide on cardiopulmonary afferent-evoked activity

Number of cells	Effect	CON	PBG	REC
		spikes (burst)$^{-1}$		
2/3	potentiated	2.5	6.5	2.5
1/3	none	116	110	93

(iii) The effect of granisetron on cardiopulmonary afferent-evoked activity

Number of cells	Effect	CON	gran	REC
		spikes (burst)$^{-1}$		
9/10	attenuated	20.2 ±5.7	10.6** ±4.1	-
5/10	attenuated	23.1 ±9.1	9.5* ±6.0	14.0 ±6.7
1/10	potentiated	16	21	-

Table 5.4 The effect of ionophoretic administration of granisetron on the excitation evoked by ionophoretic administration of phenylbiguanide, in addition to the effect of ionophoretic administration of saline at pH 10, on the activity of NTS neurones

Numbers reflect mean firing rates of neurones in spikes s^{-1}

(i) The effects of ionophoretic administration of DL-homocysteic acid before, during, and after the ionophoretic administration of granisetron, on the activity of NTS neurones

(ii) The effects of ionophoretic administration of saline at pH 10 on the activity of NTS neurones

CON-baseline activity, DLH-activity in the presence of DL-homocysteic acid, +gran-in the presence of granisetron, SAL pH 10-in the presence of saline at pH 10, REC-recovery of baseline activity, Att.Exc.-attenuated excitation, none-activity unaffected

* $P<0.05$ ** $P<0.01$ compared to CON

(i) The effects of DL-homocysteic acid in the presence of granisetron

Number of cells	Effect	CON	DLH	REC	CON +gran	DLH +gran	REC +gran	CON	DLH	REC
		spikes s^{-1}								
4/5	none	0.8 ±0.3	3.3 ±1.1	0.8 ±0.3	1.0 ±0.3	3.5 ±1.2	0.9 ±0.3	-	-	-
1/5	none	1	5.5	1.1	1.6	5.9	1.3	1.2	5.4	1.2
1/5	Att. Exc.	0	7.6	0	0	4.1	0	-	-	-

(ii) The effects of saline at pH 10

Number of cells	Effect	CON	SAL pH 10	REC
		spikes s^{-1}		
2/3	none	4.1	4.3	3.8
1/3	excitation	0.6	1.1	0.7

5.3 Discussion

Main findings

The present experiments focused on the effects of ionophoretic administration of 5-HT_3 receptor ligands on the activity of NTS neurones in the anaesthetized rat. The selective 5-HT_3 receptor agonist phenylbiguanide (PBG) increased the ongoing or DLH-evoked activity of the vast majority of NTS neurones recorded, and this excitation was attenuated by ionophoretic administration of the selective 5-HT_3 receptor antagonists granisetron and ondansetron. In addition, in the majority of neurones the cardiopulmonary afferent-evoked activation of neurones was potentiated by PBG and attenuated by granisetron.

Selectivity of 5-HT_3 receptor ligands used

Phenylbiguanide (PBG)

The selective 5-HT_3 receptor agonist PBG has been widely used in research into the actions of both central and peripheral 5-HT_3 receptors (see Hoyer, 1991). It has been used throughout this study to activate peripheral 5-HT_3 receptors in the pulmonary circulation, in order to evoke the cardiopulmonary reflex. This well known effect of 5-HT_3 receptor activation has been used in the characterisation of novel 5-HT_3 receptor antagonists (see Leslie *et al.*, 1994). PBG has high affinity at the 5-HT_3 receptor binding site (K_i -141 nM), although it may act as a partial agonist at this receptor and has also been shown to block dopamine uptake (see Hoyer, 1994; for further details see section 1.5.3).

Granisetron and ondansetron

Both granisetron and ondansetron are highly potent and selective 5-HT_3 receptor antagonists (Butler *et al.*, 1988; Sanger & Nelson, 1989). They have both been in clinical use as anti-emetic agents, particularly in the prevention of the emetic side effects of cancer chemotherapy and radiotherapy (see Hoyer, 1994).

5-HT_3 receptor actions in the NTS

The reported excitatory response of NTS neurones to ionophoretic administration of the 5-HT_3 receptor agonist PBG in the present experiments supports data described previously (Wang *et al.*, 1997). This excitation was deemed to occur in response to PBG since ionophoretic administration of its vehicle had no effect on the activity of the majority of neurones. This excitatory effect was confirmed as being mediated by 5-HT_3 receptors since it was attenuated by the selective 5-HT_3 receptor antagonists granisetron and ondansetron. Granisetron attenuated the PBG-evoked excitation in all neurones tested but did not alter excitations evoked by the excitatory amino acid DLH. In the single neurone in which the DLH-evoked excitation was attenuated by granisetron, the PBG-evoked excitation was reduced to a much greater extent. Ondansetron also attenuated the PBG-evoked excitation of neurones, demonstrating that the attenuation of this response by granisetron could be repeated with other selective 5-HT_3 receptor antagonists. Thus, in conclusion, the excitatory effect evoked by PBG within the NTS is likely to be due to the activation of 5-HT_3 receptors.

These experiments also demonstrate that a high proportion of NTS neurones were sensitive to activation of 5-HT_3 receptors (>90%), as well as showing that the response is almost completely excitatory. Only a single neurone of 106 responded with inhibition. This suggests that at least in the areas recorded (discussed below) there are large numbers of 5-HT_3 receptors and their predominant action is excitatory.

Interestingly, the excitation evoked by ionophoretic PBG administration was not completely blocked by administration of either granisetron or ondansetron in the majority of neurones. This may indicate that PBG is mediating an excitatory effect via activation of other receptor populations or, it may suggest that, at the low currents of granisetron used, only partial blockade of 5-HT_3 receptor was achieved due to insufficient drug delivery. Unfortunately this cannot be determined in these experiments, although it can be said that the large majority of the excitation evoked by PBG is mediated by 5-HT_3 receptor activation.

As described in chapter 3, NTS neurones were identified by their receiving vagal afferent input. This vagal afferent input was split into two groups: neurones receiving input with a jitter >5 ms and those receiving an input <5 ms which may reflect the synaptic location of these neurones within the NTS (see chapter 3). However, there was no differentiation between these two groups based on their response to 5-HT_3 receptor ligand administration, with a similar proportion of neurones with a vagal afferent input of jitter >5 ms excited by PBG administration compared with the entire population of NTS neurones used in these studies (see chapter 3). The majority of neurones tested had a jitter of <5 ms which would indicate that these neurones are more likely lower or middle order NTS neurones. In addition, most neurones had activity correlated to cardiovascular or respiratory variables suggesting some role in autonomic control. They were recorded in caudal areas of the NTS <0.5 mm lateral to midline, an area known to have a high degree of unmyelinated chemo/pulmonary/bronchial receptor afferent termination (see section 1.3.1). In support of this the majority of neurones were found to be sensitive to cardiopulmonary afferent activation evoked by right atrial administration of PBG and a proportion of this response is likely to result directly from the activation of afferents in the pulmonary circulation (see chapter 3: discussion).

These experiments have also demonstrated that activation of 5-HT_3 receptors potentiated both the vagal afferent- and cardiopulmonary afferent-evoked excitation of the majority of NTS neurones, suggesting that these excitatory receptors are capable of facilitating sensory afferent input. However, ionophoretic administration of the selective 5-HT_3 receptor antagonist granisetron did not affect the ongoing or DLH-evoked activity of the majority of NTS neurones, although small excitatory and inhibitory responses were seen in a small number of neurones. Thus, it would seem that in resting conditions in these anaesthetized rats there is no significant background activation of 5-HT_3 receptors, although it is important to remember that the level of activity of neurones recorded in this preparation is unlikely to mirror that occurring in conscious animals.

Subsequent experiments demonstrated that ionophoretic administration of granisetron, at currents which did not affect the ongoing activity of neurones, attenuated the excitation of neurones evoked by cardiopulmonary afferent activation. This suggests that cardiopulmonary afferent transmission to these NTS neurones involves the activation of 5-HT_3 receptors. The involvement of 5-HT_3 receptors in the cardiopulmonary reflex has been demonstrated before in a number of different studies (see Leal *et al.*, 2001; Dando *et al.*, 1995; Pires *et al.*, 1998) although there are inconsistencies between some of the data (see section 1.6.4, 5.1 & below). The findings of the present experiments are consistent with those of Dando *et al* (1995) and Pires *et al* (1998) in the description of an attenuation of vagal reflex-evoked responses with the 5-HT_3 receptor antagonist granisetron. However, Leal *et al* (2001) have reported that activation, in contrast to blockade, of 5-HT_3 receptors in the NTS attenuates the cardiac component of the von Bezold-Jarisch reflex. These inconsistencies are further discussed below.

The present experiments describe a population of neurones located in caudomedial regions of the NTS, the majority of which were excited by activation of cardiopulmonary afferents and this involved activation of 5-HT_3 receptors. One relevant question is: are these low order input neurones receiving monosynaptic projections from vagal afferents or are they higher order neurones receiving polysynaptic vagal afferent input? Most of the neurones receives an unmyelinated vagal afferent input with jitter <5 ms, suggesting that they are lower order neurones. Indeed, some of the recorded neurones had a jitter of only 1 ms, which may indicate that they are likely to be receiving monosynaptic vagal afferent input. Therefore, the total population is likely to reflect a host of different neurones in this area of the NTS, from input neurones to output neurones. However, there were no differences in the effect of 5-HT_3 receptor blockade on the cardiopulmonary afferent-evoked activation of neurones based on the likely synaptic location of neurones through the NTS. Furthermore, this still might not provide further information on the function of 5-HT_3 receptors in the NTS since there are both monosynaptic (Luiten *et al.*, 1987; Blinder *et al.*, 1998) and likely polysynaptic pathways from the NTS to autonomic preganglionic cell groups, whilst other neurones may project to

higher central nuclei, such as the parabrachial nucleus etc. (see section 1.2). Thus, without chemical or electrical stimulation of some/all of these brain nuclei in order to determine the target of individual NTS neurones, the likely function of these neurones and therefore the role of 5-HT_3 receptors in these functions cannot be determined. In addition, not all of the cardiopulmonary reflex-evoked excitation of neurones is necessarily mediated via the activation of afferents in the cardiac or pulmonary circulation (see chapter 3: discussion) and therefore the attenuation of this response by granisetron may reflect a role for 5-HT_3 receptors in a non-cardiopulmonary component of the neuronal response. However, this is unlikely since the granisetron-evoked suppression of this neuronal response was not different based on the latency of the response i.e. granisetron also attenuated neuronal responses occurring within a short latency post-reflex activation, and this short latency would indicate a component mediated via the pulmonary and/or cardiac circulation.

As mentioned briefly, another group has reported that activation of 5-HT_3 receptors in the NTS inhibits the bradycardic component of the von Bezold-Jarisch reflex, the baroreceptor reflex and the chemoreflex in both anaesthetized and conscious rats (see Leal *et al.*, 2001). Those studies used microinjections to apply 5-HT_3 receptor ligands to the NTS and, specifically, suggested that the inhibitory neuromodulation of parasympathetic cardiac activity is mediated via 5-HT_3 receptors located in the lateral commissural NTS. Their proposed mechanism is that the activation of these 5-HT_3 receptors releases glutamate which subsequently activates GABAergic interneurones and that these inhibitory neurones are responsible for the attenuation seen in the bradycardia evoked by the von Bezold-Jarisch reflex (see Leal *et al.*, 2001). In support of this they have shown that activation of $GABA_A$ receptors also attenuated the von Bezold-Jarisch bradycardia (Callera *et al.*, 1999), and the $GABA_A$ antagonist bicuculline blocked the cardioinhibitory effects of 5-HT_3 receptor activation (Sévoz *et al.*, 1996). In terms of the response to activation of 5-HT_3 receptors alone, this mechanism would fit the findings of the present experiments i.e. 5-HT_3 receptors are excitatory and these NTS neurones could be projecting onto GABAergic interneurones in more lateral areas. However, the majority of neurones recorded in these experiments are also excited by

cardiopulmonary afferent activation, which would also subsequently activate these GABAergic interneurones preventing the production of a cardiopulmonary afferent-evoked bradycardia! One possible way round this might be the observation that some neurones are inhibited by cardiopulmonary afferent activation (see chapter 3) and in these neurones 5-HT_3 receptors still mediate an excitation. However, only a small proportion of all neurones tested with cardiopulmonary afferent activation responded with inhibition (17%; see chapter 3). Furthermore, the inhibitory cardiopulmonary afferent-evoked response was not tested in the presence of a 5-HT_3 receptor antagonist, so it is not possible to identify the role of 5-HT_3 receptors in this inhibition. It is possible that the proportion of inhibitory responding neurones does not reflect the pattern of neuronal response to cardiopulmonary afferent activation in other areas of the NTS, although anatomical studies have described medial and commissural areas as opposed to more lateral areas as having the highest density of unmyelinated pulmonary receptor afferent termination (Kalia & Mesulam, 1980). One other possible explanation for the inconsistencies between some of the findings for 5-HT_3 receptors is in the method of drug application, with all the studies described above used either i.c. injection or microinjection methods of administration. This may have resulted in the diffusion of drugs to other adjacent brainstem nuclei such as the area postrema or dorsal vagal motor nucleus (DVN) known to have high numbers of 5-HT_3 receptors (see Leslie *et al.*, 1994) which might be mediating some of the effects seen.

Another important point in relation to the understanding of 5-HT_3 receptor action within the NTS is the report of a pressor response upon microinjection of the 5-HT_3 receptor agonist 2-methyl-5-HT into the NTS of conscious rats (Leal *et al.*, 2001), an effect mirrored by the microinjection of glutamate into the NTS of conscious rats (Machado & Bonagamba, 1992). As described in section 1.3.3 glutamate is thought to be the primary neurotransmitter in the NTS (Talman *et al.*, 1980; Leone & Gordon, 1989), mediating as opposed to modulating synaptic transmission through this nucleus. In addition, there is thought to be a link between 5-HT acting at 5-HT_3 receptors and glutamate receptor function in the NTS (Ashworth-Preece *et al.*, 1995), an interaction already confirmed in the adjacent DVN, where, at least a proportion of 5-HT_3 receptors are located on

glutamatergic terminals facilitating the release of glutamate (Wang *et al.*, 1998a). If there is a similar interaction occurring in the NTS, at least in medial areas, then this would also not be compatible with the proposed mechanism of all NTS located 5-HT_3 receptors mediating a cardio-inhibitory function i.e. glutamate is unlikely to be involved in suppressing the bradycardia as it is primarily involved in the production of it. However, once more there could be two populations of 5-HT_3 receptors located in the NTS: a population in the medial NTS closely connected with and potentially modulating glutamate release, which are activated by cardiopulmonary, and possibly other cardiovascular or respiratory afferent stimulation (see chapter 3); and a second population in more lateral areas which, when activated, stimulate a group of GABAergic interneurones which subsequently suppress the parasympathetic cardio-inhibitory effects of cardiopulmonary-, baroreceptor and chemo-reflex activation. This second population may underlie a protective effect in the case of extremely strong or multiple reflex activations, preventing dramatic reductions in heart rate. This explanation would fit the majority of proposed mechanisms to date, although it should be pointed out that there is no direct evidence for two populations of the 5-HT_3 receptor mediating separate effects.

Added support for a population of 5-HT_3 receptors closely involved in afferent modulation in the NTS comes from their reported location on vagal sensory afferents in the NTS (Pratt & Bowery, 1989). As mentioned, these afferents would be expected to release glutamate and this therefore provides further evidence that at least some 5-HT_3 receptors are facilitating glutamate transmission and thus vagal afferent transmission through the NTS.

Summary

In conclusion, 5-HT_3 receptors mediate an excitatory action in caudomedial aspects of the NTS and are activated by cardiopulmonary afferent transmission through these regions. This action may or may not involve the activation of glutamate and/or GABA receptors. Further discussion of these data, with reference to the potential origin of these serotoninergic inputs is contained within chapter 6: discussion.

Chapter 6 Interactions between 5-HT_3 and NMDA receptors in the NTS

6.1 Introduction

Many studies have focused on the likely synaptic location of 5-HT_3 receptors in the brainstem, mainly dealing with more dorsally located nuclei such as the dorsal vagal motor nucleus (DVN) and NTS (Pratt & Bowery, 1989; Merahi *et al.*, 1992; Glaum *et al.*, 1992; Leslie *et al.*, 1994; Ashworth-Preece *et al.*, 1995; Albert *et al.*, 1996; Wang *et al.*, 1998a).

In the NTS there is now anatomical evidence for populations of 5-HT_3 receptors located on both afferent terminals arriving into the nucleus, and also on the soma and dendrites of NTS neurones themselves (see Leslie *et al.*, 1994; Pratt & Bowery, 1989). In addition, nodose ganglionectomy has been shown to reduce the numbers of 5-HT_3 receptors in the NTS, providing evidence for a population of these receptors on the presynaptic terminals of vagal afferents (Merahi *et al.*, 1992).

Furthermore, studies have revealed likely interactions between 5-HT, acting at 5-HT_3 receptors, and other neurotransmitters within the NTS (Glaum *et al.*, 1992; Ashworth-Preece *et al.*, 1995 ; Bonagamba *et al.*, 2000;). One such interaction between 5-HT and GABA was described in a patch clamp study, indicating the presence of populations of 5-HT_3 receptors, located on both the presynaptic terminals and soma/dendrites of GABAergic NTS neurones (Glaum *et al.*, 1992). Whilst another group, using microinjection techniques *in vivo*, has proposed a $GABA_A$ receptor-dependent mechanism for 5-HT_3 receptor-mediated effects (Bonagamba *et al.*, 2000).

Interactions between 5-HT and glutamate have also been described, with a microdialysis study demonstrating the ability of phenylbiguanide to increase glutamate release in the NTS, a mechanism confirmed to be mediated by 5-HT_3 receptors after its blockade with the selective antagonist ondansetron (Ashworth-Preece *et al.*, 1995). Evidence currently implicates glutamate as the primary neurotransmitter in the NTS, with other, secondary, neurotransmitters such as 5-HT able to modulate, but not mediate, neurotransmission in this nucleus (see Lawrence & Jarrott, 1996). Therefore, the findings of Merahi *et al.* (1992) which described 5-HT_3 receptors located on vagal afferent terminals may also implicate interactions between glutamate and 5-HT in the NTS. Indeed, studies in the DVN are consistent with the presence of 5-HT_3 receptors located on glutamate-releasing presynaptic terminals and mediating excitatory effects on DVN neurones via NMDA and AMPA receptors (Wang *et al.*, 1998a).

Aims:

- Experiments were carried out to investigate interactions between 5-HT_3 receptors and NMDA receptors in the NTS at the neuronal level in the anaesthetized rat. In addition, the role of NMDA receptors in the cardiopulmonary afferent-evoked activation of NTS neurones was investigated.

6.2 Results

6.2.1 Effects of glutamate receptor ligands on neuronal activity

The effect of ionophoretic application of glutamate receptor ligands and a 5-HT_3 receptor agonist on the activity of NTS neurones was tested in a total population of 69 cells. A proportion of these neurones showed little or no ongoing activity therefore in some cases it was necessary to raise the activity of these cells using ionophoretic application of either AMPA or NMDA to examine the effects of some ligands. Excitation or inhibition in the activity of each neurone was classified by a change in this baseline firing rate of greater than 20%. Recording of the baseline response, post-ligand administration, was not achieved in all neurones. However, mean firing rates (spikes s^{-1}) for both the full populations tested and the proportions with a recorded post-ligand response are given.

NMDA and AMPA

Ionophoretic administration of NMDA excited all 51 NTS neurones tested, raising baseline activity by a mean of 500%, whilst ionophoretic administration of AMPA also excited all 44 neurones tested, increasing mean baseline activity by 492% (figure 6.1; table 6.1).

NMDA in the presence of AP-5

The effects of ionophoretic administration of the NMDA receptor antagonist AP-5 on the NMDA-evoked excitation was examined (table 6.1). In 15 of 18 neurones AP-5 attenuated the NMDA-evoked excitation (figures 6.2, 6.6 and 6.7). In these 15 neurones NMDA increased mean baseline activity by 663% which was reduced to an excitation of 300% in the presence of AP-5. In addition, the post-AP-5 NMDA response was recorded in 13 neurones and the mean data indicated a partial recovery of the initial NMDA excitation, although in some neurones complete recovery was observed (figure 6.2A). AP-5 was unable to affect the NMDA-evoked excitation of the remaining 3 neurones at the currents tested.

The effect of AP-5 on the baseline activity of NTS neurones was also examined (table 6.2). Figure 6.7A shows how the baseline activity of a single neurone is unaffected by AP-5 although it is able to attenuate NMDA-evoked excitation at the same current. Indeed AP-5 did not affect the baseline activity in 10 of 20 neurones tested (figure 6.7B). Of the 10 remaining neurones 5 were excited, whilst 5 were inhibited by AP-5 administration and although the group data did not indicate that these responses were statistically significant (figure 6.7B), the post-AP-5 baseline activity recorded 8 of these 10 neurones did return to control levels.

AMPA in the presence of AP-5

To confirm selective antagonism of the NMDA response, ionophoretic administration of AP-5 was also examined on the excitation evoked by AMPA (table 6.1). In 6 of 12 neurones the excitatory response to AMPA was unaffected by AP-5 (figure 6.3), with the post-AP-5 AMPA response recorded in 5 of these neurones. However, in 5 of the 12 neurones the AMPA-mediated increase in baseline activity was significantly potentiated in the presence of AP-5 (figures 6.3B and 6.6A). The post AP-5 AMPA response recorded in 2 of these neurones did indicate a partial recovery of the initial AMPA response. In only one neurone was the AMPA response attenuated in the presence of AP-5.

In some cases the effects of NMDA and AMPA in the presence of AP-5 were examined on the same neurone. Figure 6.4 shows an attenuation of the excitatory response to NMDA during AP-5 administration, whilst the excitatory response to AMPA remains unchanged over the same period.

Phenylbiguanide in the presence of AP-5

Associations between the actions of 5-HT_3 and NMDA receptors were studied by examining any modulation of the previously described (chapter 5) excitatory effect of phenylbiguanide (PBG) by the NMDA receptor antagonist AP-5 (table 6.2). In 6 of 7 neurones the excitation evoked by ionophoretic PBG

administration was attenuated in the presence of AP-5 (figure 8.9). This attenuation was significant at a P level of 0.1 (P value - 0.078).

The baseline firing rate of one of the 6 neurones was much higher (37 spikes s^{-1} compared to an average of 2 spikes s^{-1}) and so was excluded from the group data presented in figure 6.5B. The post-AP-5 PBG response was recorded in 5 of these 6 neurones and a partial recovery of the initial PBG response was observed (figure 8.9). In addition, figure 6.5A shows an attenuation in the PBG-evoked excitation of one of these neurones by AP-5, whilst in the same neurone the excitation evoked by AMPA was unaffected by AP-5. The PBG response of the remaining neurone of the 7 was unaffected in the presence of AP-5 (figure 8.9).

Figure 6.6 shows an attenuation of PBG-evoked excitation, an attenuation of NMDA-evoked excitation and a potentiation of AMPA-evoked excitation in the presence of AP-5 in the same NTS neurone. Once more, this confirms that the responses seen are not specific to individual groups of neurone tested with each drug, and can therefore be reproduced for all drugs on the same neurone.

Figure 6.1 The effect of ionophoretic administration of AMPA and NMDA on the activity of NTS neurones in the anaesthetized rat

A Excitation of the ongoing activity of an NTS neurone evoked by the ionophoretic administration of NMDA and AMPA (during the bars & at the currents stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. **(i)** & **(ii)** From left to right: baseline activity (■; CON), activity evoked by ionophoretic administration of NMDA (20-240 nA; □) or AMPA (5-120 nA; □) and recovery of baseline activity (▨; REC). **(i)** 51 of 51 neurones were excited by the ionophoretic administration of NMDA. **(ii)** 44 of 44 neurones were excited by the ionophoretic administration of AMPA.

** $P<0.01$

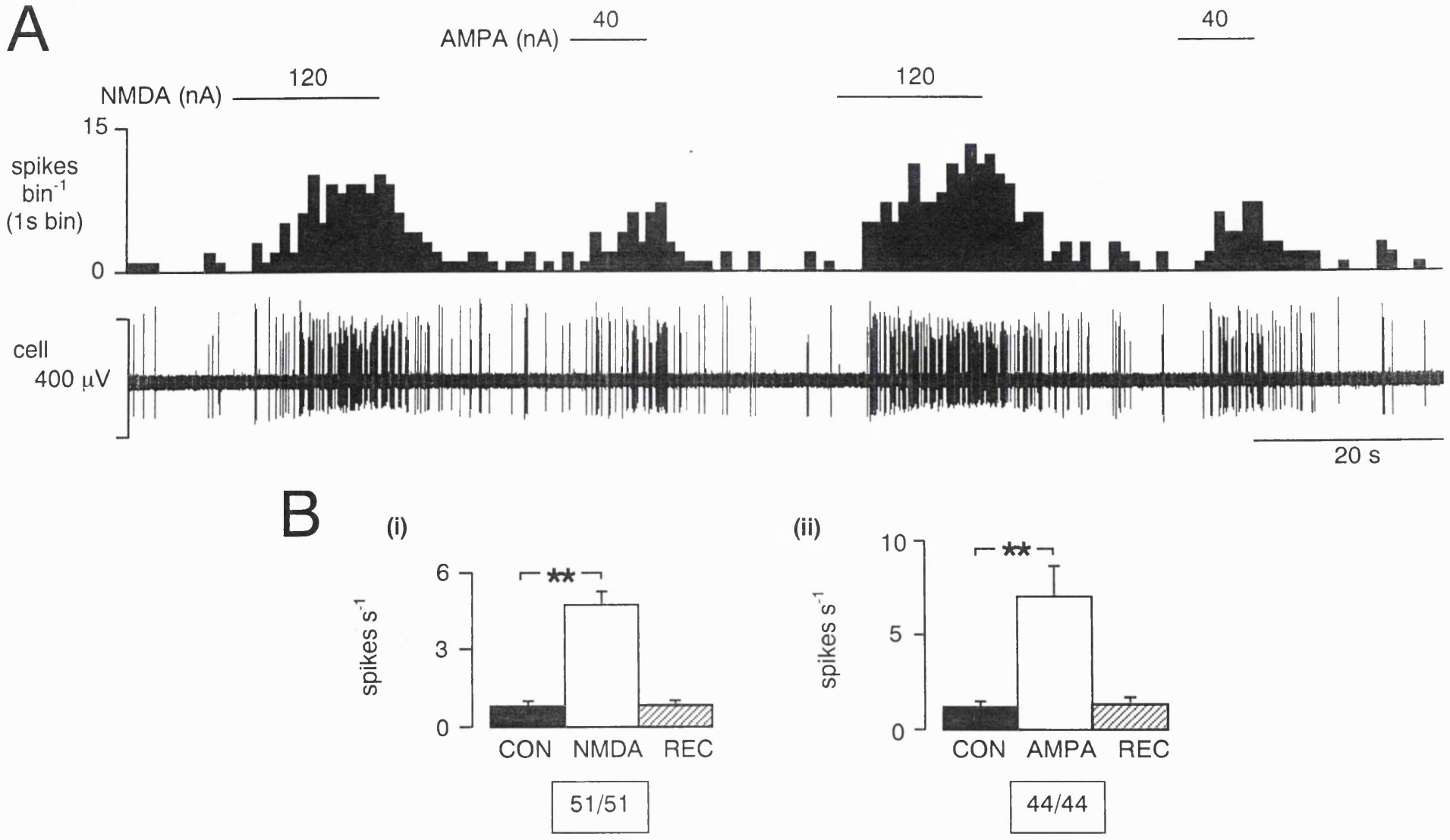

A
AMPA (nA)
40
40
NMDA (nA)
120
120
15
spikes
bin-1
(1s bin)
0
cell
400 μV
20 s
B
(i)
spikes s-1
6
3
0
**
CON
NMDA
REC
51/51
(ii)
spikes s-1
10
5
0
**
CON
AMPA
REC
44/44

Figure 6.2 The effect of ionophoretic administration of AP-5 on the excitation evoked by ionophoretic administration of NMDA in the anaesthetized rat

A The response of an NTS neurone to the ionophoretic administration of NMDA before, during and after the ionophoretic administration of AP-5 (during the bars and at the currents stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. From left to right: activity evoked by ionophoretic administration of NMDA (20-240 nA; ☐; NMD), activity evoked by NMDA in the presence of AP-5 (3-240 nA; ▤; NMD + AP5), baseline activity (■; CON) and recovery of baseline activity (▨; REC). The control response to NMDA was attenuated by AP-5 in 15 of 18 neurones. The post-AP-5 NMDA response was recorded in 13 of these neurones. In the remaining 3 neurones the response to NMDA was unaffected in the presence of AP-5.

** $P<0.01$

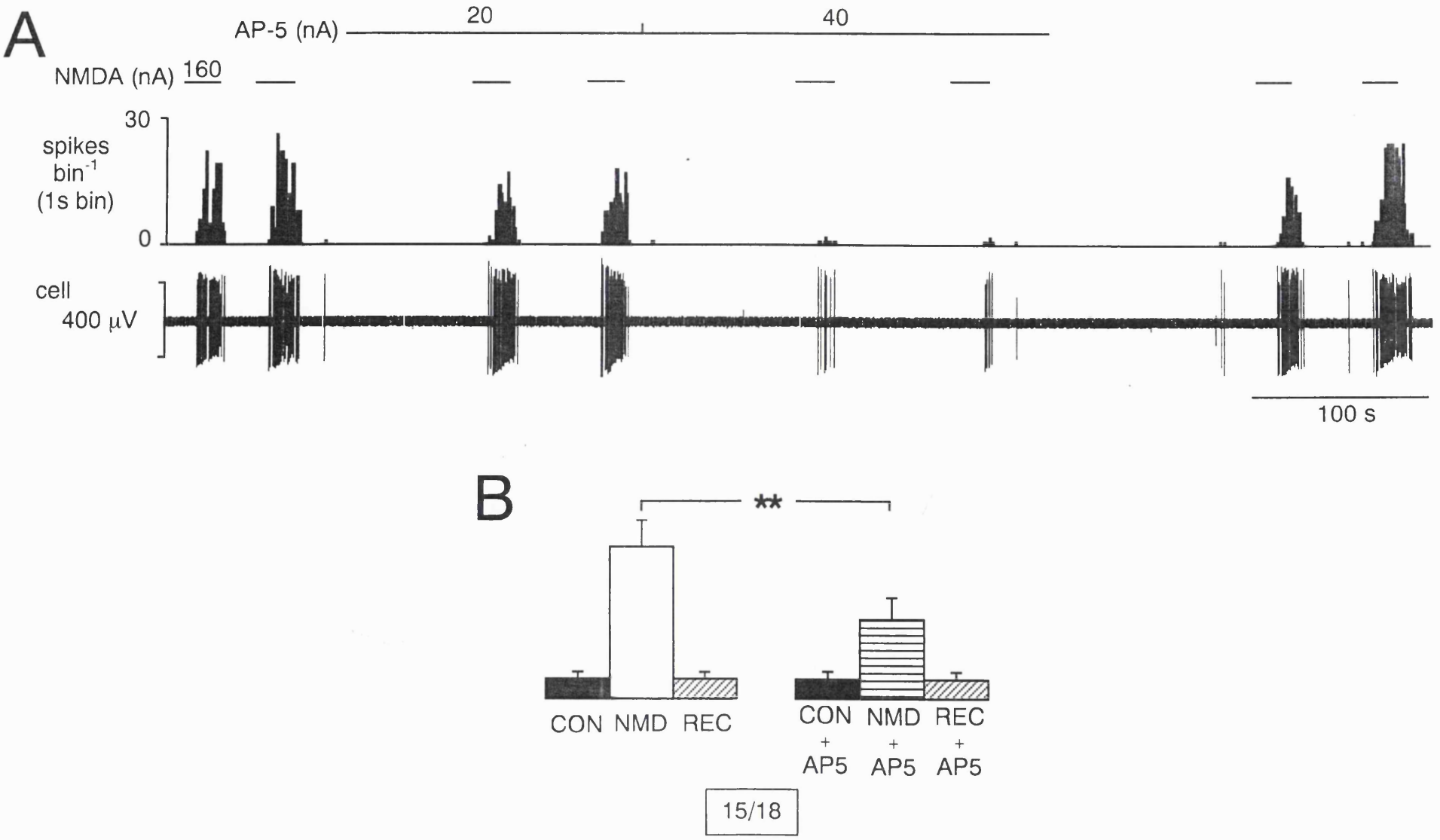

A
AP-5 (nA)
20
40
NMDA (nA) 160
30
spikes
bin-1
(1s bin)
0
cell
400 μV
100 s
B
**
CON NMD REC
CON + AP5
NMD + AP5
REC + AP5
15/18

Figure 6.3 The effect of ionophoretic administration of AP-5 on the excitation evoked by ionophoretic administration of AMPA in the anaesthetized rat

A The response of an NTS neurone to the ionophoretic administration of AMPA before, during and after the ionophoretic administration of AP-5 (during the bars and at the currents stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. **(i)** & **(ii)** From left to right: activity evoked by ionophoretic administration of AMPA (5-120 nA; □; AMP), activity evoked by AMPA in the presence of AP-5 (3-80 nA; ▤; AMP + AP5), baseline activity (■; CON) and recovery of baseline activity (▨; REC). In 12 neurones, the control excitatory response to AMPA was unaffected in 6 **(i)**, whilst the excitation was potentiated in 5 neurones **(ii)**. The post-AP-5 AMPA response was recorded in 5 and 2 neurones respectively whilst in the remaining neurone the excitatory response to AMPA was attenuated in the presence of AP-5.

** $P<0.05$

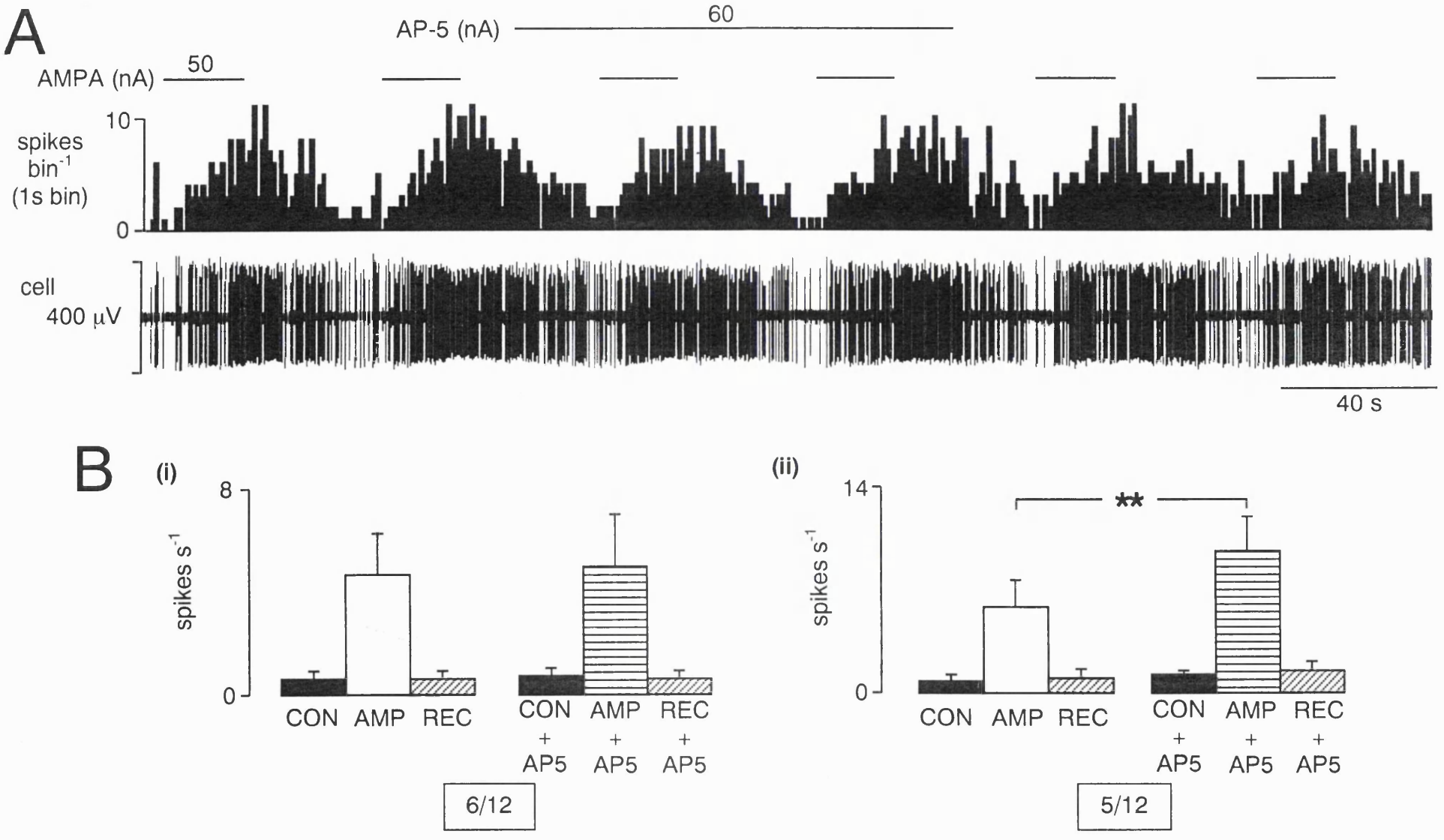

A
AP-5 (nA)
60
AMPA (nA)
50
spikes bin-1 (1s bin)
10
0
cell
400 μV
40 s
B
(i)
8
0
spikes s-1
CON AMP REC
CON + AP5
AMP + AP5
REC + AP5
6/12
(ii)
14
0
spikes s-1
**
CON AMP REC
CON + AP5
AMP + AP5
REC + AP5
5/12

Figure 6.4 The effect of ionophoretic administration of AP-5 on the excitatory response evoked by ionophoretic administration of NMDA and AMPA, on the activity of the same NTS neurone in the anaesthetized rat

Traces show the response of an NTS neurone to the ionophoretic administration of AMPA and NMDA before, during and after the ionophoretic administration of AP-5 (during the respective bars and at the currents stated). From the top, traces shown are a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

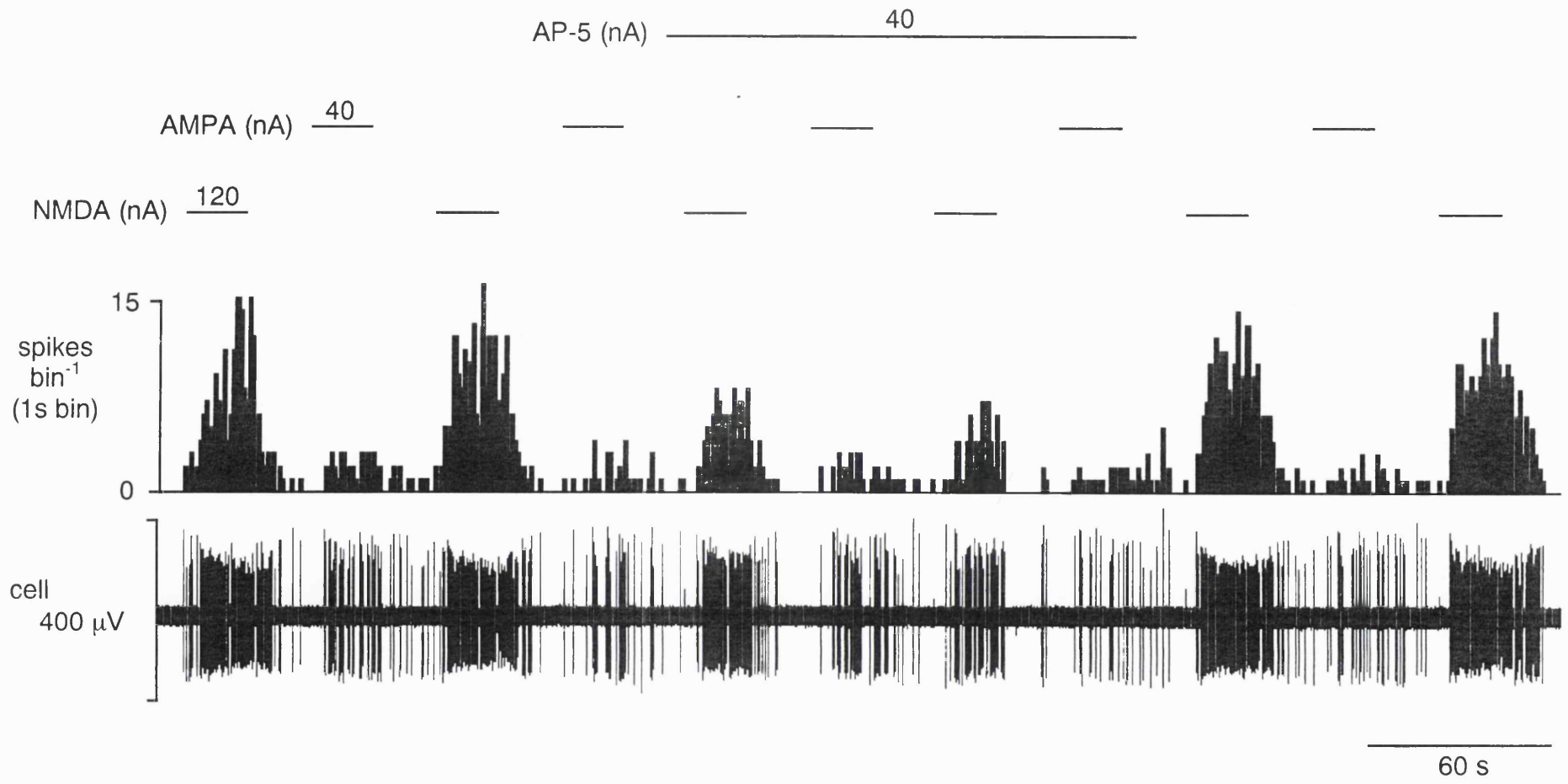

AP-5 (nA)
40
AMPA (nA)
40
NMDA (nA)
120
15
spikes
bin-1
(1s bin)
0
cell
400 μV
60 s

Figure 6.5 The effect of ionophoretic administration of AP-5 on the excitation evoked by ionophoretic administration of phenylbiguanide in the anaesthetized rat

A The response of an NTS neurone to the ionophoretic administration of phenylbiguanide (PBG) before, during and after the ionophoretic administration of AP-5 (during the bars and at the currents stated). Also shown is the response of this neurone to the ionophoretic administration of AMPA before and during the ionophoretic administration of AP-5 (during the bars and at the currents stated. From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. From left to right: activity evoked by ionophoretic administration of PBG (20-160 nA; ☐; PBG), activity evoked by PBG in the presence of AP-5 (40-120 nA; ▤; PBG + AP5), baseline activity (■; CON) and recovery of baseline activity (▨; REC). In 7 neurones the control response to PBG was attenuated by AP-5 in 6, with the baseline firing rates of 5 of these neurones shown. The post-AP-5 PBG response was recorded in 5 of these neurones. The response to PBG of the remaining neurone was unaffected in the presence of AP-5.

NS - not significant

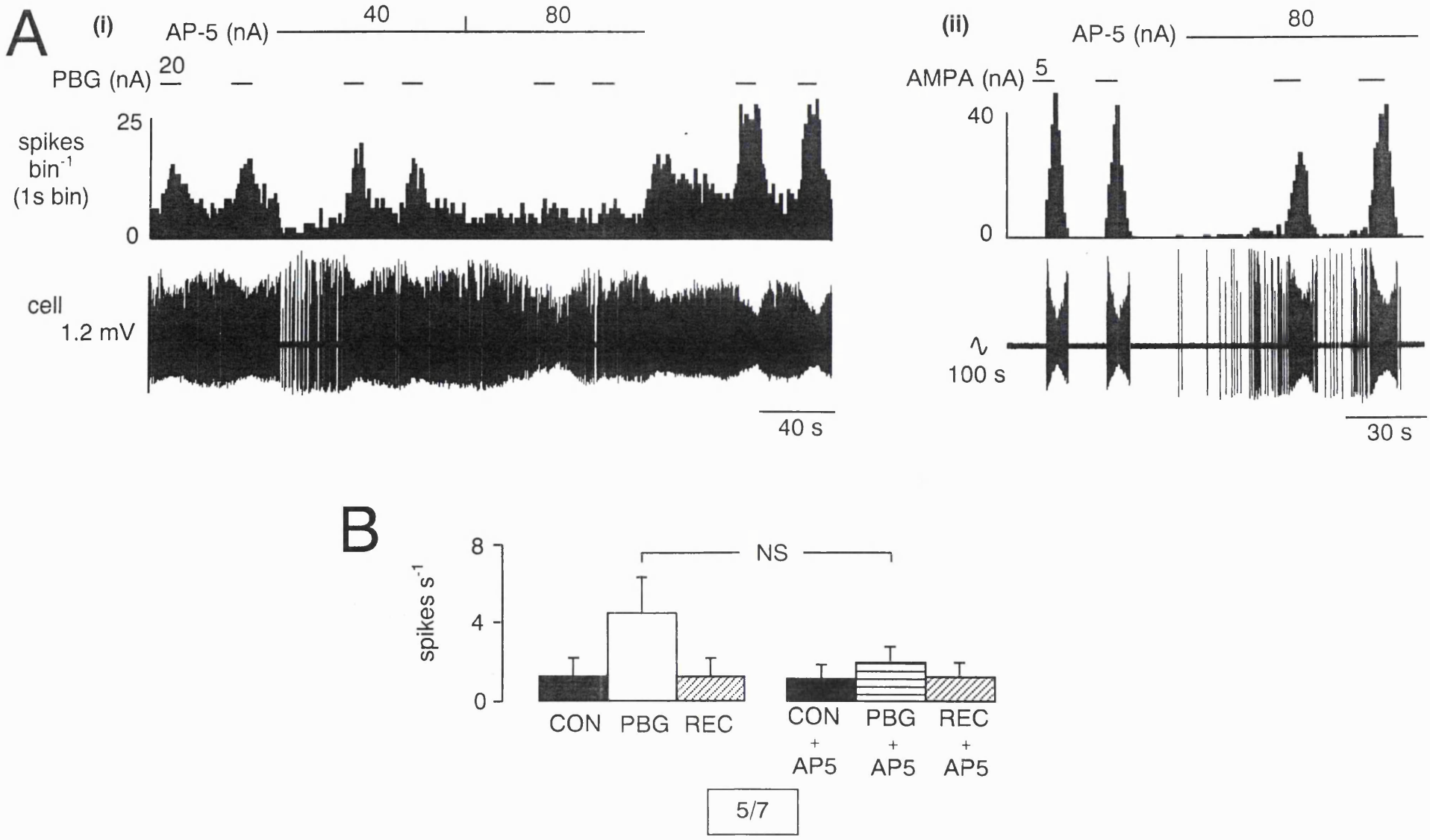

A
(i)
AP-5 (nA)
40
80
PBG (nA)
20
25
spikes bin^{-1} (1s bin)
0
cell
1.2 mV
40 s
(ii)
AP-5 (nA)
80
AMPA (nA)
5
40
0
100 s
30 s
B
spikes s^{-1}
8
4
0
NS
CON
PBG
REC
CON + AP5
PBG + AP5
REC + AP5
5/7

Figure 6.6 The effect of ionophoretic administration of AP-5 on the excitatory response evoked by ionophoretic administration of phenylbiguanide, AMPA and NMDA, on the activity of the same NTS neurone in the anaesthetized rat

A The response of an NTS neurone to the ionophoretic administration of phenylbiguanide (PBG) and AMPA before, during and after the ionophoretic administration of AP-5 (during the respective bars and at the currents stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B The response of the same NTS neurone as shown in **A**, at a time 60 s later, to the ionophoretic administration of NMDA before, during and after the ionophoretic administration of AP-5 (during the respective bars and at the currents stated). From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

NB. The activity of a second, unidentified, background neurone can be seen in the raw neuronal recording, although the activity of this neurone is not included in the rate histogram.

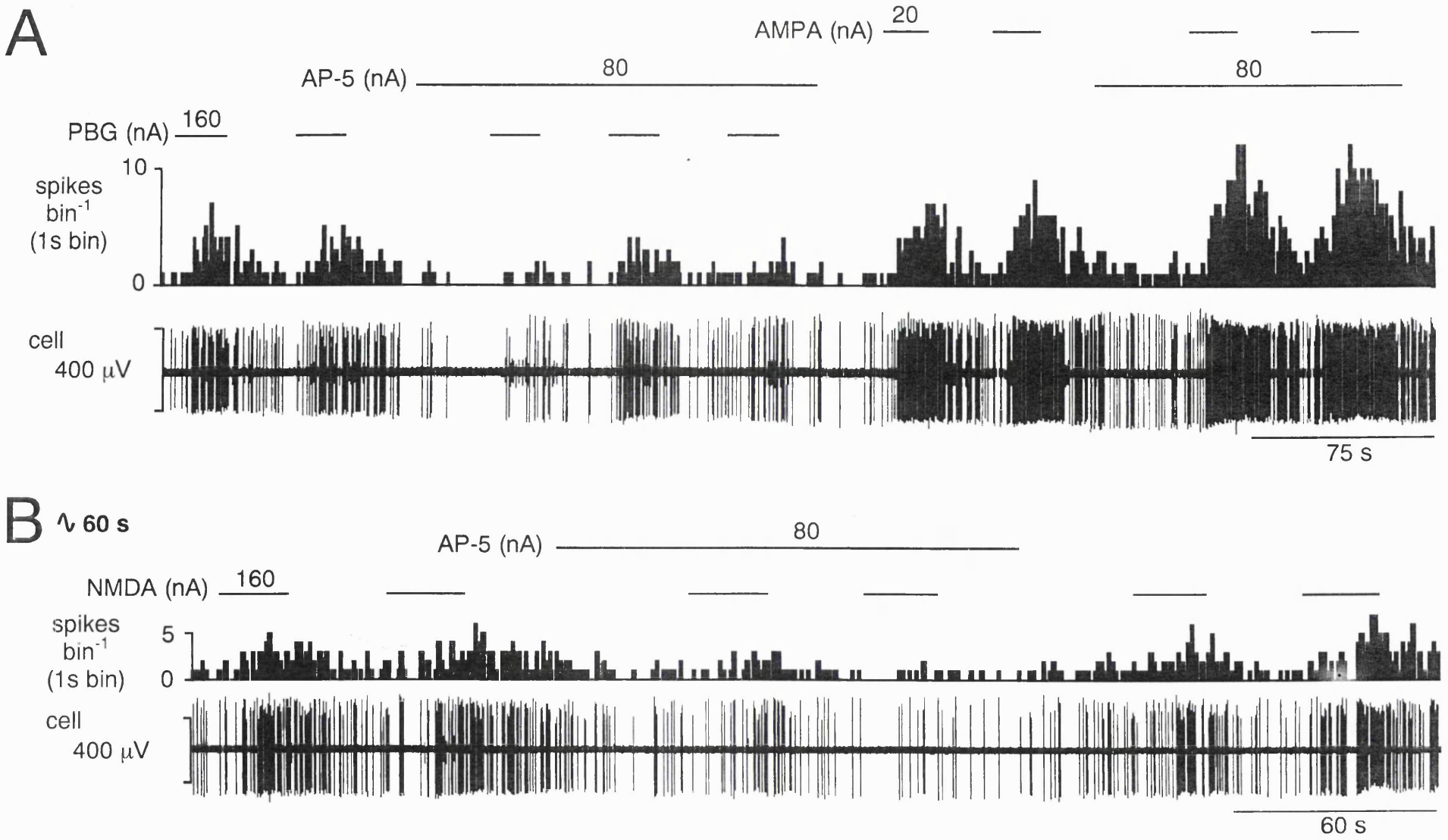
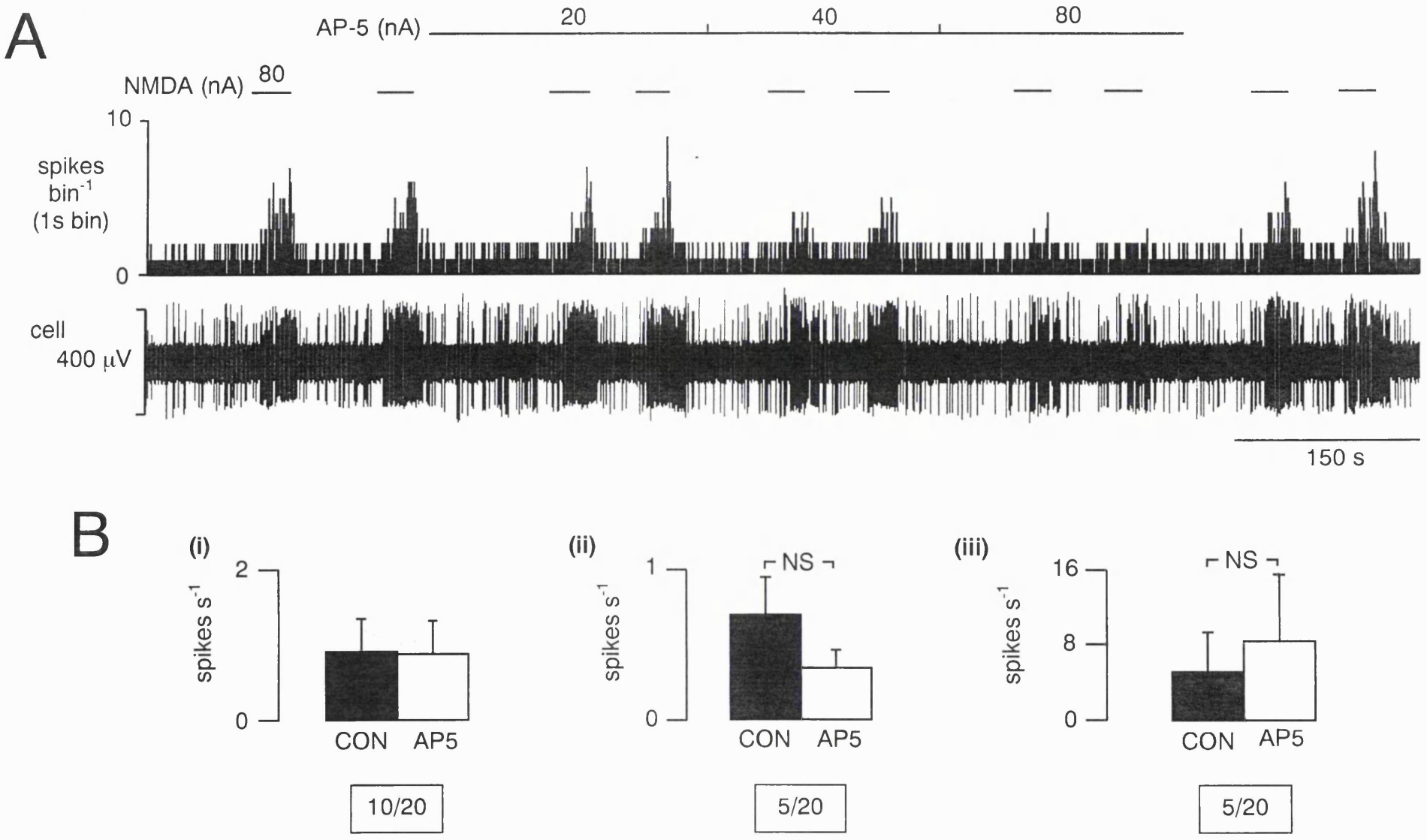

A
AMPA (nA) 20
AP-5 (nA) 80
80
PBG (nA) 160
10
spikes bin-1 (1s bin)
0
cell
400 μV
75 s
B
∿ 60 s
AP-5 (nA) 80
NMDA (nA) 160
spikes bin-1 (1s bin)
5
0
cell
400 μV
60 s

Figure 6.7 The effect of ionophoretic administration of AP-5 on the activity of NTS neurones in the anaesthetized rat

A The response of an NTS neurone to the ionophoretic administration of AP-5 (during the bar & at the currents stated), in addition to the current dependent attenuation of the response to NMDA (during the bars and at the current stated) by AP-5. From the top, traces show a rate histogram (spikes bin^{-1}) and the raw extracellular recording of neuronal activity (cell).

B Histograms of the mean data of NTS neuronal activity with vertical bars representing s.e.mean. **(i)**, **(ii)** & **(iii)** From left to right: baseline activity (■; CON) and activity evoked by ionophoretic administration of AP-5 (3-80 nA; □; AP5). 10 of 20 neurones were unaffected by AP-5 **(i)**, of which post-AP-5 activity was recorded in 9. A further 5 neurones were inhibited by AP-5 **(ii)**, of which the post-AP-5 response was recorded in 4, whilst the remaining 5 neurones were excited by AP-5 **(iii)**, with the post-AP-5 response also recorded in 4 of these neurones.


A
AP-5 (nA)
20
40
80
NMDA (nA)
80
10
spikes
bin-1
(1s bin)
0
cell
400 μV
150 s
B
(i)
2
spikes s-1
0
CON
AP5
10/20
(ii)
1
NS
spikes s-1
0
CON
AP5
5/20
(iii)
16
NS
8
spikes s-1
0
CON
AP5
5/20

6.2.2 Effects of AP-5 on cardiopulmonary afferent input

The effect of ionophoretic administration of AP-5 on the cardiopulmonary afferent-evoked activation of NTS neurones was examined (table 6.2).

Cardiopulmonary afferent activation of NTS neurones was measured as the total number of spikes in the burst of neuronal activity evoked by right atrial administration of phenylbiguanide. Responses were then compared prior to, during, and where possible, after the ionophoretic administration of AP-5.

AP-5 attenuated the cardiopulmonary afferent-evoked activation of 3 of 5 NTS neurones by a mean of 58% (figures 6.8). The post-AP-5 response was recorded in 2 neurones, an example of which is shown in figure 6.8, and partial recovery of the initial response was observed. The activation of the remaining 2 neurones was unaffected by AP-5 administration.

Figure 6.8 Attenuation of the cardiopulmonary afferent-evoked activation of NTS neurones by ionophoretic administration of AP-5

The response of an NTS neurone to activation of cardiopulmonary afferents by intra-atrial administration of phenylbiguanide (PBG) before (left panel), during (middle panel) and after (right panel) the ionophoretic administration of AP-5. From the top, traces show blood pressure (BP; mmHg), a continuous rate histogram of neuronal activity (spikes bin^{-1}) and the raw recording of neuronal activity (μV). The administration of PBG is highlighted by a solid horizontal line (━ ; PBG - 12 μg kg^{-1}, 20 μl). Circled numbers highlight the number of spikes in the corresponding burst of activity. In 5 neurones, the cardiopulmonary afferent-evoked response of 3 was attenuated in the presence of AP-5 (80-120 nA). The post-AP-5 response was recorded in 2 of these neurones, whilst the cardiopulmonary afferent-evoked response of the remaining 2 neurones was unaffected by AP-5.

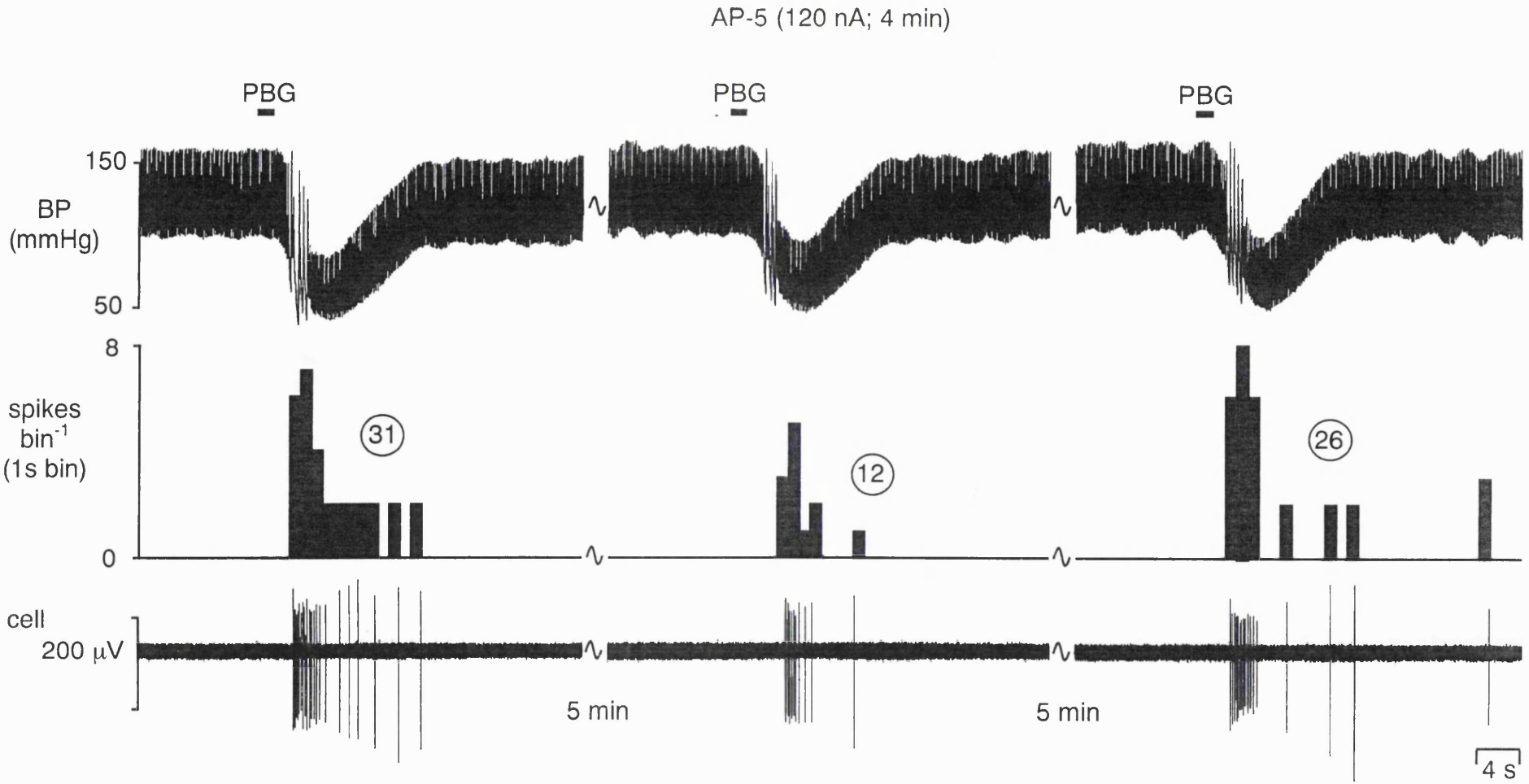

AP-5 (120 nA; 4 min)
PBG
PBG
PBG
150
BP
(mmHg)
50
8
spikes
bin-1
(1s bin)
0
31
12
26
cell
200 μV
5 min
5 min
4 s

Table 6.1 The effect of ionophoretic administration of NMDA and AMPA, in addition to the effects of ionophoretic administration of AP-5 on the excitation evoked by these drugs, on the activity of NTS neurones

Numbers reflect mean firing rates of neurones in spikes s^{-1}

(i) The effects of ionophoretic administration of NMDA on the activity of NTS neurones

(ii) The effects of ionophoretic administration of AMPA on the activity of NTS neurones

(iii) The effects of ionophoretic administration of NMDA before, during, and after the ionophoretic administration of AP-5, on the activity of NTS neurones

(iv) The effects of ionophoretic administration of AMPA before, during, and after the ionophoretic administration of AP-5, on the activity of NTS neurones

CON-baseline activity, NMD-activity in the presence of NMDA, AMP-activity in the presence of AMPA, REC-recovery of baseline activity, + AP5-in the presence of AP-5, Att. Exc.-attenuated excitation, Pot. Exc.-potentiated excitation, none-activity unaffected.

* $P<0.05$ ** $P<0.01$ compared to CON

† $P<0.05$ †† $P<0.01$ compared to NMD/AMP

(i) The effects of NMDA

Number of cells	Effect	CON	NMD	REC
		spikes s^{-1}		
51/51	excitation	0.8±0.2	4.8±0.5**	0.8±0.2

(ii) The effects of AMPA

Number of cells	Effect	CON	AMP	REC
		spikes s^{-1}		
44/44	excitation	1.2±0.3	7.1±1.6**	1.3±0.4

(iii) The effects of NMDA in the presence of AP-5

Number of cells	Effect	CON	NMD	REC	CON +AP5	NMD +AP5	REC +AP5	CON	NMD	REC
		spikes s^{-1}								
15/18	Att. Exc.	0.8 ±0.3	6.1** ±1.1	0.8 ±0.3	0.8 ±0.3	3.2**†† ±0.9	0.8 ±0.3	-	-	-
13/18	Att. Exc.	0.7 ±0.3	5.7** ±1.2	0.7 ±0.3	0.6 ±0.3	2.4*†† ±0.9	0.7 ±0.3	0.6 ±0.2	4.9** ±1.2	0.6 ±0.3
3/18	none	0.5 ±0.1	3.7** ±0.3	0.4 ±0.1	0.3 ±0.1	3.5* ±0.6	0.4 ±0.2	-	-	-
1/18	none	0.3	3.1	0.3	0.3	2.9	0.5	0.7	2.4	0.6

(iv) The effects of AMPA in the presence of AP-5

Number of cells	Effect	CON	AMP	REC	CON +AP5	AMP +AP5	REC +AP5	CON	AMP	REC
		spikes s^{-1}								
6/12	None	0.6 ±0.3	4.7* ±1.6	0.6 ±0.3	0.7 ±0.3	5.0 ±2.0	0.6 ±0.3	-	-	-
5/12	None	0.8 ±0.3	3.4* ±1.0	0.7 ±0.4	0.6 ±0.4	3.2* ±0.9	0.6 ±0.4	0.9 ±0.4	3.4** ±0.8	0.8 ±0.4
5/12	Pot. Exc.	0.8 ±0.4	5.9* ±1.9	1.0 ±0.6	1.2 ±0.3	9.8*†† ±2.3	1.5 ±0.6	-	-	-
2/12	Pot. Exc.	0.1	5.8	0.1	0.6	8.9	0.4	0.4	7.9	0.2
1/12	Att. Exc.	0.2	2.9	0.3	0.8	1.7	0.4	0.6	1.5	0.6

Table 6.2 The effect of ionophoretic administration of AP-5 on the excitation evoked by ionophoretic administration of phenylbiguanide, in addition to the effects of ionophoretic administration of AP-5 on the baseline and cardiopulmonary afferent activity of NTS neurones

Numbers reflect mean firing rates of neurones in spikes s^{-1} and spikes $(burst)^{-1}$

(i) The effects of ionophoretic administration of PBG before, during, and after the ionophoretic administration of AP-5, on the activity of NTS neurones

(ii) The effects of ionophoretic administration of AP-5 on the activity of NTS neurones

(iii) The effects of ionophoretic administration of AP-5 on the cardiopulmonary afferent-evoked activity of NTS neurones

CON-baseline/evoked activity, AP5- activity in the presence of AP-5, PBG-activity in the presence of PBG, REC-recovery of baseline/evoked activity, Att.Exc.-attenuated excitation, none-activity unaffected.

* - the mean activity of neurones shown in figure 6.5B

(i) The effect of phenylbiguanide in the presence of AP-5

No.'s	Effect	CON	PBG	REC	CON +AP5	PBG +AP5	REC +AP5	CON	NMD	REC
		spikes s^{-1}								
6/7	Att. Exc.	6.4 ±5.2	12.8 ±8.5	6.7 ±5.6	7.5 ±6.5	8.7 ±6.8	7.8 ±6.6	-	-	-
* 5/7	Att. Exc.	1.2 ±0.9	4.4 ±1.8	1.2 ±0.9	1.1 ±0.7	1.9 ±0.8	1.2 ±0.7	-	-	- *
5/7	Att. Exc.	7.5 ±6.2	14.8 ±10.1	8.0 ±6.7	9.0 ±7.7	10.2 ±8.1	9.2 ±7.9	6.8 ±4.9	12.4 ±7.9	6.7 ±5.0
1/7	none	0.3	8.6	0.2	0.3	9.3	0.4	1.7	13.7	1.2

(ii) The effects of AP-5

Number of cells	Effect	CON	AP5	REC
		spikes s^{-1}		
10/20	none	0.9±0.4	0.9±0.4	-
9/20	none	0.9±0.5	0.9±0.5	1.3±0.8
5/20	excitation	5.0±4.2	8.3±7.1	-
4/20	excitation	5.7±5.4	9.6±9.0	5.6±5.3
5/20	inhibition	0.7±0.3	0.3±0.1	-
4/20	inhibition	0.8±0.3	0.4±0.1	0.8±0.3

(iii) The effects of AP-5 on cardiopulmonary afferent-evoked activity

Number of cells	Effect	CON	AP5	REC
		spikes $(burst)^{-1}$		
3/5	attenuated	45±12	19±4	-
2/5	attenuated	51	16	26
2/5	none	17	17	21

6.3 Discussion

Main findings

The present experiments examined interactions between 5-HT, acting at 5-HT_3 receptors, and glutamate, acting at NMDA receptors. The glutamate receptor ligands AMPA and NMDA and the 5-HT_3 receptor agonist PBG all had excitatory effects on NTS neurones. Ionophoretic administration of the NMDA receptor antagonist AP-5 attenuated the excitation evoked by ionophoretic administration of NMDA and PBG but did not affect, or potentiated the excitation evoked by ionophoretic administration of AMPA in the majority of NTS neurones. In addition, AP-5 attenuated the cardiopulmonary afferent-evoked activation of the majority of NTS neurones examined.

Receptor ligands used

The selective 5-HT_3 receptor agonist PBG was used to activate 5-HT_3 receptors (see Hoyer, 1991; chapter 5), whilst AMPA receptors were activated by AMPA. In addition, NMDA receptors were activated by NMDA and blockade of these receptors was achieved using the selective NMDA receptor antagonist AP-5 (Evans *et al.*, 1982). All these compounds are highly selective and have high affinity for their respective binding sites.

5-HT_3 and NMDA receptors in the NTS

Activation of 5-HT_3 receptors using PBG produces an almost exclusively excitatory effect on the ongoing activity of neurones in caudomedial areas of the NTS (also see chapter 5). This response is thought to result from the activation of 5-HT_3 receptors as the selective 5-HT_3 receptor antagonists granisetron and ondansetron attenuated this excitation (see chapter 5). The present experiments also demonstrated that both NMDA and AMPA excited all NTS neurones tested, indicating that glutamate, acting at both NMDA and AMPA receptors, also has a predominant excitatory action within these regions of the NTS. Confirmation of the NMDA-evoked excitation as being mediated via the activation of NMDA receptors was achieved after AP-5, an NMDA receptor

antagonist, attenuated this excitation in the large majority of neurones. AP-5 also attenuated the excitation evoked by PBG in the majority of neurones, indicating that a proportion of the excitation evoked by 5-HT_3 receptor stimulation is mediated via the activation of NMDA receptors. The AMPA-evoked excitation was unaffected by AP-5 in half of all neurones tested and potentiated in the majority of the remaining neurones.

Unfortunately, the group data did not indicate that the attenuation by AP-5 of the PBG-evoked excitation was statistically significant at a P level of 0.05, since the absolute P value lies just outside this at 0.078. This is a limitation of this method of statistical analysis which is most likely explained by the high variations in firing rates seen in this particular group of neurones. However, detailed analysis reveals that the PBG-evoked excitation was attenuated by >20% in the presence of AP-5 in almost all neurones tested and moreover this excitation recovered almost completely to control levels after AP-5 administration was stopped. Therefore, despite the lack of complete statistical support it seems likely that the 5-HT_3 receptor-mediated excitation of these NTS neurones is partially blocked in the presence of an NMDA receptor antagonist. Further support that this attenuation is not just a random event, or even a non-specific effect of AP-5, is provided by the evidence that AP-5 did not affect, or potentiated, a control excitation evoked by AMPA i.e. AP-5 is not simply suppressing the excitability of neurones. The mechanism by which AP-5 potentiates the excitatory effects of AMPA is unclear, although it may be mediated via an increased availability of glutamate at AMPA receptors in the presence of NMDA receptor blockade.

The excitation evoked by PBG was not completely attenuated in the presence of AP-5 and from the present data it is not possible to determine if this indicates that there is an AMPA component of 5-HT_3 receptor activation, or if it suggests that there are significant numbers of postsynaptic 5-HT_3 receptors located on the soma of these NTS neurones. In addition, it is worth pointing out that the selective antagonist granisetron did not fully attenuate the PBG-evoked excitation either (see chapter 5) so another possible explanation is insufficient drug delivery via ionophoresis. This attenuation of the PBG-evoked excitation

of neurones by AP-5 has previously been shown in the dorsal vagal motor nucleus (DVN; Wang *et al.*, 1998), with these authors demonstrating that this excitation was also sensitive to attenuation with the AMPA receptor antagonist DNQX and the neurotransmitter release inhibitors Mg^{2+} and Cd^{2+}. However, the compound Mg^{2+} was not used in the present studies due its ability to interfere with the postsynaptic 5-HT_3 receptor channel at low concentrations (Peters, Hales & Lambert, 1988). Furthermore, in the NTS an interaction between the role of 5-HT, acting at 5-HT_3 receptors, and glutamate has previously been reported (Ashworth-Preece *et al.*, 1995), and the findings of the present experiments would support this.

5-HT_3 receptors have previously been shown to modulate synaptic activity (Glaum *et al.*, 1992) and be located on vagal afferent terminals in the NTS (Pratt & Bowery, 1989; Leslie *et al.*, 1990). In reference to a presynaptic terminal location of 5-HT_3 receptors Glaum *et al* (1992) have shown that 5-HT_3 receptor agonists increased the frequency and amplitude of spontaneous postsynaptic potentials (PSPs) in NTS neurones *in vitro*, whilst histological studies have identified 5-HT_3 receptors located on specifically vagal afferent terminals (Leslie *et al.*, 1990). This is partially supported in the present studies since neurones are identified by their receiving vagal afferent input and there is evidence for a proportion of these neurones to be 2nd order NTS neurones receiving monosynaptic vagal afferent input (see chapter 3). However, this is only a small proportion of the population and there was no difference based on the effect of AP-5 on the PBG-evoked response of neurones and their possible synaptic location within the NTS.

It is likely that these NTS neurones are receiving glutamatergic fibre afferent input since the majority of neurones are activated by cardiopulmonary afferent stimulation and studies have shown that glutamate is vital in the transmission of cardio-respiratory reflex information (see Lawrence & Jarrott, 1996). In fact, the activation of NTS neurones by cardiopulmonary C-fibre afferent stimulation has previously been shown to be attenuated by ionophoresis of glutamate receptor antagonists in the NTS (Wilson, Zhang & Bonham, 1996). However, that study demonstrated that the activation of NTS neurones by right atrial administration

of PBG was largely mediated via non-NMDA receptors since the non-NMDA antagonist NBQX significantly attenuated this activation but AP-5 did not. In the present studies AP-5 did attenuate the cardiopulmonary reflex activation of neurones, and although this attenuation partially recovered the group data once more indicated that this was not a statistically significant result. Therefore, in the present experiments there does appear to be a component of the reflex activation of neurones mediated by NMDA receptors although this cannot be wholly determined due to the limited numbers of neurones in this group. In addition, as mentioned above a number of the neurones recorded in the present experiments may well be 2^{nd} order NTS neurones receiving monosynaptic vagal afferent input (see chapter 3), and this observation, together with the findings of a histological study demonstrating the presence of glutamate-containing vagal afferent terminals in the NTS (Saha *et al.*, 1995), provides further confirmation that at least some of these neurones receive glutamatergic primary afferent input.

The present experiments have also shown that the majority of recorded NTS neurones are excited by stimulation of cardiopulmonary afferents (see chapter 3) and a proportion of this excitation involves the activation of 5-HT_3 receptors (chapter 5) and, since there is evidence that glutamate is the primary neurotransmitter of cardiopulmonary information in the NTS (see Lawrence & Jarrot, 1996; Wilson *et al.*, 1996; Vardhan *et al.*, 1993), this implies that 5-HT, acting at 5-HT_3 receptors, facilitates the glutamatergic cardiopulmonary input to these NTS neurones. However, the possibility that these receptors are located on the soma of intrinsic glutamate-containing neurones antecedent to the recorded NTS neurones cannot be ruled out.

Another recent study has also identified an interaction between 5-HT and glutamate in the NTS, describing how microinjection of a 5-HT_3 receptor agonist into the NTS blocks the bradycardic response to NMDA microinjection in the NTS (Bonagamba *et al.*, 2000). They hypothesise that these 5-HT_3 receptors are located on glutamatergic presynaptic terminals mediating non-cardiovascular vagal afferent input to GABAergic interneurones in the NTS, and these GABAergic neurones suppress, via $GABA_A$ receptors, the parasympathetic component of vagal reflexes as it is transmitted through other

neurones of the NTS (see Leal *et al.*, 2001). This hypothesis would only fit the results of the current experiments if all recorded neurones were GABAergic and non-cardio-respiratory related. However, these neurones have activity related to cardio-respiratory variables, are activated by cardiopulmonary afferent stimulation and some are even likely to be 2nd order NTS neurones receiving monosynaptic vagal afferent input (see chapter 3) and therefore the results are perhaps a little surprising. Indeed, even if all neurones were GABAergic there is still the observation that the majority are activated by both cardiopulmonary afferent stimulation and by 5-HT_3 receptor stimulation negating the possibility of activation of 5-HT_3 receptors, in these neurones at least, mediating a decrease in any component of cardiopulmonary reflex. How the findings of the present study and those of the above mentioned study (Bonagamba *et al.*, 2000) are integrated remains to be determined, although as described in chapter 5 there is the distinct possibility that there are two populations of 5-HT_3 receptors, some located in caudomedial areas directly modulating vagal afferent input via the facilitation of glutamate release and some in more lateral areas also facilitating glutamate release, but to GABAergic interneurones which negatively modulate vagal afferent input. It should be remembered though that it is pure speculation to indicate the presence of two functionally opposed populations of 5-HT_3 receptors.

Origin and stimulus of 5-HT release

There are a number of different possibilities as to the origin of these 5-HT-containing neurones. The NTS has been shown to contain 5-HT immunoreactive fibres (Steinbusch, 1981; Sykes *et al.*, 1994) which make synaptic contact with NTS cells (Maley & Elde, 1982). As demonstrated in the present experiments some of these fibres are likely to be targetting cells expressing 5-HT_3, 5-HT_{1B} and 5-HT_{1D} receptors, although the possibility that 5-HT_{1B} and 5-HT_{1D} receptors are functionally dormant cannot be ruled out. This serotonergic innervation of the NTS has been reported to originate from both peripheral (Nosjean *et al.*, 1990; Sykes *et al.*, 1994) and central (Schaffer *et al.*, 1988) sites, and possibly from within the NTS itself (Calza *et al.*, 1985). There is also the question as to what causes the 5-HT release that is subsequently activating 5-HT_3 receptors during cardiopulmonary afferent stimulation. One

possibility is that 5-HT containing neurones, located in sensory afferent ganglia such as the nodose ganglia, are activated alongside glutamatergic primary afferent neurones also located there. This afferent information is then transmitted simultaneously into the NTS with 5-HT, via the activation 5-HT_3 (and possibly 5-$HT_{1B/1D}$) receptors, modulating the excitability of glutamatergic terminals or directly affecting NTS neuronal activity. Another possibility is that afferent input is transmitted to other central nuclei (particularly nuclei rich in 5-HT such as the caudal raphe nuclei), which subsequently sends 5-HT fibres to the NTS, some terminating on glutamatergic terminals, modulating the excitability of afferent information arriving there. There may also be local 5-HT circuits within the NTS which, in different physiological or pathological states can modulate the excitability of neurones. Which of these possibilities is actually occurring cannot be determined from the present experiments, although since some of the NTS neurones were likely to be 2^{nd} order NTS neurones receiving monosynaptic afferent input there is a good chance that a proportion of this serotonergic input is arising from peripheral sources. The complexity of the circuitry within the NTS is such that without stimulation of multiple peripheral and central sites simultaneously the exact function and nature of the serotonergic system in this nuclei remains unclear.

Summary

In conclusion, the excitatory response of NTS neurones to 5-HT_3 receptor activation appears to be mediated in part via the activation of NMDA receptors, this excitation may or may not involve the activation of AMPA receptors. In addition, these neurones appear to be glutamatergic, transmitting a proportion of evoked-cardiopulmonary afferent information via the activation of NMDA receptors. In combination with the findings of other studies these data imply that in caudomedial regions of the NTS cardiorespiratory afferent information is transmitted via glutamate and in these anaesthetized rats a proportion of this glutamate release is mediated via the activation of 5-HT_3 receptors. Unfortunately the origin of these serotonergic inputs cannot be determined from the present experiments.

Chapter 7 General Discussion

7.1 Conclusions

- In cats, stimulation of pulmonary C-fibres does excite cardiac vagal preganglionic neurones with B-fibre axons located within or ventrolateral to the nucleus ambiguus, although how the bradycardia of the pulmonary chemoreflex escapes respiratory modulation remains to be determined. In rats, activation of cardiopulmonary afferents excite the majority of neurones located in caudomedial aspects of the NTS and a proportion of this excitation is likely to result from the activation of afferents in the pulmonary circulation. In addition, a high proportion of these NTS neurones have activity related to cardio-respiratory variables.

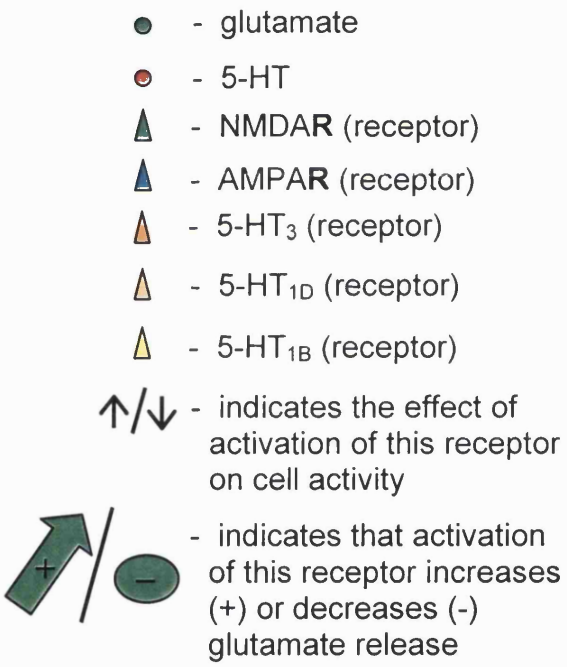
- 5-HT_{1B} and 5-HT_{1D} receptors have opposing actions in caudomedial aspects of the NTS, with 5-HT_{1B} receptors mediating an excitatory action capable of increasing the response of NTS neurones to vagal and cardiopulmonary afferent stimulation whilst activation of 5-HT_{1D} receptors decreases neuronal activity, including activity evoked by vagal and cardiopulmonary afferent stimulation (see figure 7.1).

- 5-HT_3 receptors mediate an excitatory effect in caudomedial regions of the NTS studies and this excitation is capable of augmenting both vagal and cardiopulmonary afferent input. In addition, a proportion of the excitation of neurones evoked by cardiopulmonary afferent stimulation involves the activation of 5-HT_3 receptors indicating that these receptors are involved in the transmission of cardiopulmonary reflex information through the NTS (see figure 7.1).

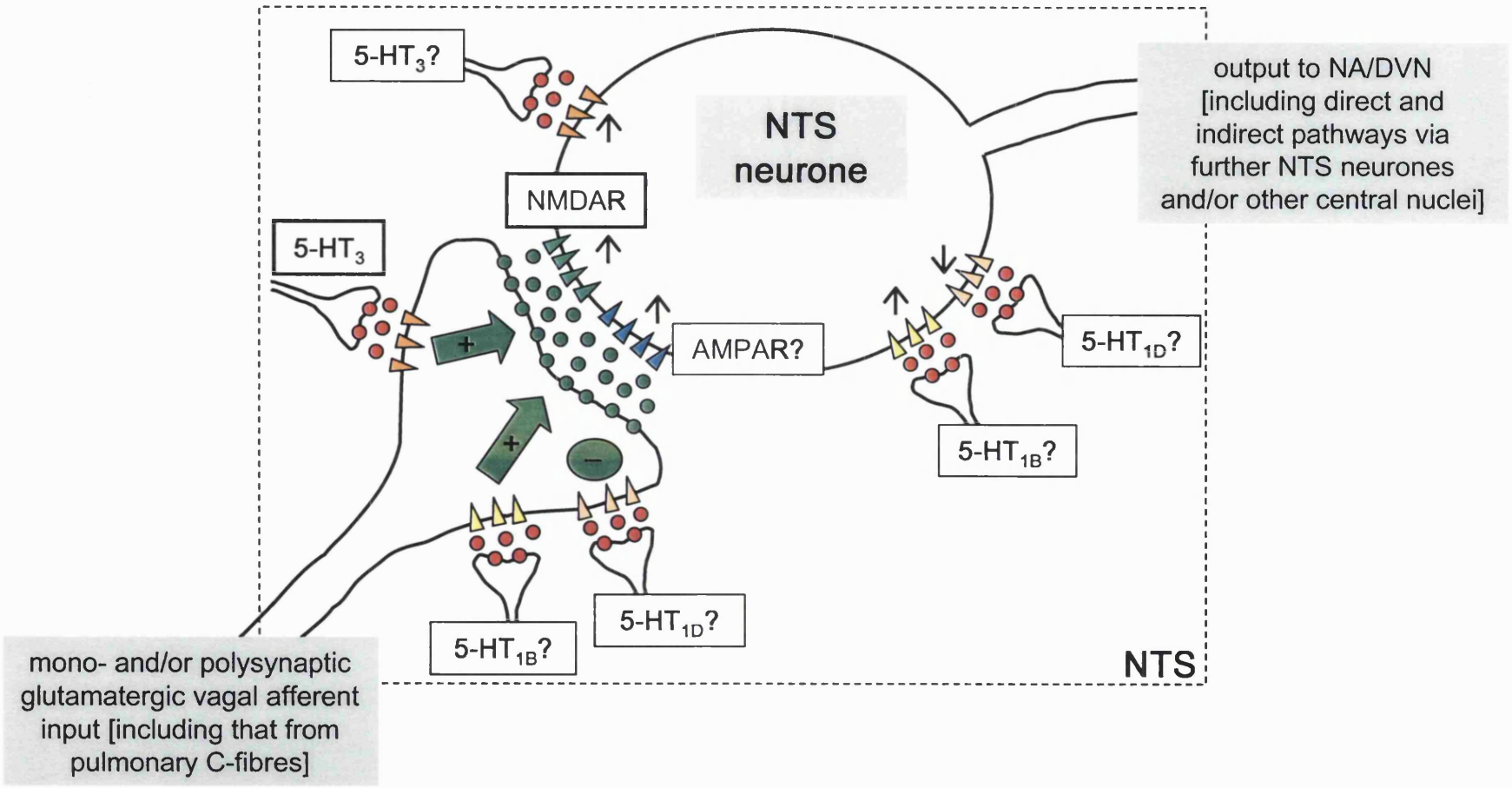
- Furthermore, 5-HT_3 receptors exert a proportion of this excitatory effect on these NTS neurones via the activation of NMDA receptors and the present experiments also demonstrate that the transmission of cardiopulmonary reflex information through this area of the NTS is mediated in part by the activation of NMDA receptors (see figure 7.1).

Figure 7.1 Summary of 5-HT_{1B}, 5-HT_{1D} and 5-HT_3 receptor function and location in the NTS from the findings of the present experiments in the anaesthetized rat

A schematic illustration of the organisation of glutamatergic and serotonergic inputs to neurones receiving cardiopulmonary afferent input in caudomedial aspects of the NTS that summarises the pharmacological findings of this thesis. Question marks are included to indicate the location and/or function of receptors that are likely but cannot be wholly determined from the findings of the present experiments.



NB. For the purposes of this figure each proposed 5-HT receptor subtype location receives an individual serotonergic input, this may or may not be the case.


5-HT3?
NTS neurone
output to NA/DVN [including direct and indirect pathways via further NTS neurones and/or other central nuclei]
NMDAR
5-HT3
AMPAR?
5-HT1D?
5-HT1B?
+
+
−
5-HT1B?
5-HT1D?
NTS
mono- and/or polysynaptic glutamatergic vagal afferent input [including that from pulmonary C-fibres]

7.2 Limitations

There are a number of factors important in the interpretation of the experimental results of this study. These are discussed below:-

Ionophoresis

Ionophoresis is the process of administering a drug by application of an electrical current to a solution containing the drug, usually via a glass micropipette. It has been used in different forms for over 30 years particularly in examinations of the skin, where substances can be ejected to stimulate sweat glands in the collection of sweat. This technique received widespread use in the diagnosis of cystic fibrosis.

In respect of this study the advantage of ionophoresis is that you can examine the effects of very small amounts of drug, with reference to the effects on single unit activity in the central nervous system. Some of the recordings in this study were achieved using multibarrel glass microelectrodes which utilised one of the barrels for the recording of neurones. However, one disadvantage of this electrode was that in order to ionophorese compounds the tip of the multibarrel had to be relatively big, otherwise individual barrels could block preventing drug release, and this larger size made neuronal recording difficult, particularly neurones of the NTS which are relatively small, due to the low resistance of the electrode tip. Therefore, the development of the compound or 'piggyback' glass microelectrode (see methods; Wang *et al.*, 1998) made recordings and ionophoresis a lot easier since a single electrode could be used in the recording of neurones at a chosen resistance, and the multibarrel could be made of a size best suited to ionophoresis. This provided better neuronal recordings via a much bigger signal to noise ratio, which was vital in experiments examining the activity of neurones during large blood pressure changes.

Ionophoresis requires drugs to be ionised in order for current ejection to occur, unfortunately this restricted the number of drugs that could be used in the present studies. In addition, drugs that were unable to be dissolved in saline were not used since the majority of solvents, e.g. DMSO, were found to have significant effects on the activity of neurones.

Another limitation of ionophoresis is that the current administered, in the present experiment between 0 and 320 nA, does not accurately denote the amount of drug being ejected. As mentioned, each electrode will have a different resistance based on the diameter and shape of the tip and this affects the amount of current required to eject drugs and thus the amount of drug released, as does the affinity and concentration of the drug being administered. However, small currents (<40 nA) are likely to reflect the ejection of lower quantities of drug compared to higher currents (>120 nA) between experiments, although as mentioned this cannot be correlated to a dose of drug administered i.v. etc. Local anaesthetic effects induced by the ionophoretic administration of specifically 5-HT_3 receptor ligands at high currents of ionophoretic ejection have also been reported (>30 nA; Ashby *et al.*, 1991). These suppressive effects on neuronal activity were characterised by attenuation in the amplitude of the neuronal spikes. In the majority of the current experiments antagonists were used to confirm the effects of each agonist as being mediated by the specific receptor and, although the compound GR55562 did cause a reduction in activity and amplitude, this was only seen in a small number of cases (see chapter 4), therefore the presence of these effects cannot be ruled out altogether but seems unlikely. In addition, in some experiments antagonists were only applied at low currents (<40 nA) in order to reduce the chance of local anaesthetic effects occurring and also to reduce the chance of the antagonist effecting neuronal activity itself, although in some cases this may have resulted in insufficient drug delivery and therefore only partial antagonism of the specific receptor.

Finally, it is not possible to verify that the effects of drug ionophoresis are a result of the effects of that drug on the specific cell being recorded. Although it can be assumed that the cell being recorded is in close proximity to the tip of the recording electrode, the possibility that the drug effects are mediated via the stimulation of neighbouring cells that are connected to, and subsequently affect the activity of, the recorded cell cannot be ruled out. In this respect it was noted that the speed of response to ionophoretic administration of drugs varied, not only between the drugs used but also between electrodes and/or experiments. This is most likely due to the variation in the electrode size/shape, and therefore

resistance, as described above although it is also important to consider which receptors are being targeted and their individual mechanism of action i.e. activation of 5-HT_3 receptors, which are ligand gated ion channels, is likely to affect the activity of neurones sooner than 5-HT_{1B} receptor activation, since these receptors mediate their effects via a 2^{nd} messenger system.

Despite these limitations ionophoresis remains a unique tool in examining the roles of receptors in the central nervous system. It allows examination of the effects of a number of different drugs on the same neurone and it is also possible to examine the effects of withdrawal after ionophoretic ejection of each drug is terminated. In addition, the excitability of neurones can be varied by altering the administration of an excitatory ligand, such as the NMDA agonist DL-homocysteic acid (DLH; used in the present experiments).

Analysis of ionophoretic results

As detailed in the methods, the effects of a drug on the activity of NTS neurones was described as excitatory or inhibitory if this activity changed by more than 20% during drug administration, as has been done previously (Wang *et al.*, 1996). The value 20% was chosen due to the general stability in the firing rates of neurones recorded in the NTS and, although it must be remembered that this is a descriptive method of analysis, it can be seen from the figures contained within this thesis that the effects of ionophoretic administration of drugs on the activity of neurones is clear. Indeed, some neurones may be affected by drug administration but not included in excitatory/inhibitory groups if this effect is less than 20%, which is a limitation in choosing this value. However, the use of 20%, as opposed to a lower value, lowers the likelihood of random changes in neuronal activity during drug administration being included into excitatory or inhibitory groups.

Areas of the brainstem targeted

In the cat, neurones of the nucleus ambiguus were initially targeted based on their stereotaxic co-ordinates. Once at the appropriate depth the activation of an antidromic response to stimulation of the cardiac branch of the vagus, and their known respiratory modulation (McAllen & Spyer, 1976, 1978a,b; Gilbey *et*

al., 1984) confirmed identification. Localisation was demonstrated via the ionophoretic ejection of Pontamine Sky Blue to recording sites. In contrast, caudomedial aspects of the NTS were specifically targeted for a number of reasons. Caudal areas were chosen due to the known high density of vagal afferent termination there, as opposed to more rostral areas, which the glossopharyngeal nerve primarily supplies (Cottle, 1964), whilst medial areas have been described as receiving unmyelinated chemo-, bronchial and pulmonary receptor afferent termination (see Loewy & Burton, 1979; Kalia & Mesulam, 1980; Jordan & Spyer, 1986). A more recent study has also described how pulmonary afferent fibres, from primarily terminal areas of the lungs such as the alveoli, project to both dorsal and ventral aspects of the medial NTS at a level around obex (Xie *et al.*, 1998). Furthermore, another study has described the sensitivity of neurones in caudomedial areas of the NTS to the activation of afferents in the pulmonary circulation (Wilson *et al.*, 1996). This study directly compared right atrial injections of PBG with left ventricular injections of the same substance, and found that the neuronal response to left ventricular PBG were much smaller, confirming that a large proportion of the neuronal response was mediated via the activation of pulmonary afferents. Therefore, in the present experiments, in order to identify a high proportion of neurones that are likely to modulated by right atrial injections of PBG and are likely to have vagal afferent input the area targeted was within 0.5 mm rostral or caudal to the obex and <0.5mm lateral to midline.

Peripheral 5-HT_3 receptor activation

As described in the methods section, stimulation of cardiopulmonary afferents in both rats and cats with the 5-HT_3 receptor agonist PBG was carried out with an inter-reflex interval of a minimum of 5 min. There was no observed tachyphylaxis on any of the peripheral responses to this stimulus, which is in contrast to the findings of other studies (Pires & Ramage, 1990; Whalen *et al.*, 2000). Whalen *et al* (2000) have described how all of the systemic responses to PBG or 2-methyl-5-HT progressively diminished upon repeated injection in conscious rats although the dose (100 $\mu g\ kg^{-1}$) and method of administration (i.v.) differ compared to the present experiments. In addition, they propose that the tachyphylaxis is mediated via the desensitization of 5-HT_3 receptors on cardiopulmonary afferents rather than down-regulation of the central or efferent

components of the reflex. However, in the present experiments the use of sub-maximal doses of PBG in both rats and cats (24-32 μg kg^{-1}) via direct right atrial administration appears to avoid peripheral desensitization of 5-HT_3 receptors.

7.3 Clinical aspects

One important observation in the demonstration of a role for 5-HT in pathological states has been in the description of increased brainstem serotonin metabolism in the development of hypertension (Koulu *et al.*, 1986). Another study has also shown that the depressor and bradycardic responses to microinjection of small amounts of serotonin into the NTS are significantly bigger in spontaneously hypertensive rats (SHR rats; Tsukamoto *et al.*, 2000). This study also suggested that 5-HT_2 receptors may become tonically active in SHR rats, since the 5-HT_2 antagonist sarpogrelate produced a pressor response upon bilateral microinjection into the NTS. However, another study, although supporting an altered role for 5-HT_2 receptors in SHR rats, has demonstrated that the function of 5-HT_3 receptors is unchanged since the response to microinjection of PBG into the NTS was unchanged in the SHR rats (Merahi & Laguzzi, 1995). However, this study did not examine the effects of a 5-HT_3 receptor antagonist in SHR rats and so the possibility remains that the 5-HT_3 receptor subtype may also play a role in these hypertensive animals, as may 5-$HT_{1B/1D}$ receptor subtypes. It would seem likely that this is the case since activation of these 5-HT receptor subtypes in the NTS evokes such strong effects on neuronal activity in the present experiments; effects which are likely to be enhanced in a model of increased serotonin sensitivity, as the hypertensive model appears to be. Further examination of the roles of 5-HT receptors in states of hypertension may be crucial in the development of new treatments for this condition; indeed the 5-HT_{2A} receptor antagonist ketanserin has already been used to reduce blood pressure in hypertension (Barrett, 1992).

Some of the pharmacological findings of the present studies may also implicate the serotonergic system in the NTS as being involved in the pathology of certain disorders, such as migraine in the case of 5-$HT_{1B/1D}$ receptors. These are briefly discussed below:

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

Levels of 5-HT have been shown to decrease during migraine along with an associated carotid vasodilation and the last decade has seen the emergence of a group of 5-$HT_{1B/1D/1F}$ agonists, known as the 'triptans' (e.g. sumatriptan), in the treatment of this condition. In support of the current vascular and neurogenic theories of migraine the triptans produce selective vasoconstriction (via 5-HT_{1B} receptors) and presynaptic inhibition of the trigeminovascular inflammatory responses implicated in migraine (via 5-HT_{1D}/5-ht_{1F} receptors; see Villalon *et al.*, 2002). In addition, the binding sites of sumatriptan in post-mortem human brain have revealed extensive levels of binding in the NTS, which may reflect the anti-emetic and analgesic effects found in the clinical use of sumatriptan (Pascual *et al.*, 1996), whilst there is also evidence for increased activity in the NTS during experimental trigeminal pain (Frese *et al.*, 2003). Taken together, these observations, and the findings of the present experiments describing the strong and opposing effects of 5-HT_{1B} and 5-HT_{1D} activation on the activity of NTS neurones, may well indicate a role for these NTS located 5-HT_1 receptors in the pathology of migraine, or certainly in the treatment of this condition with one of the triptans. Although this cannot be determined from the present study, it may be of interest to examine if the role of these receptors is also found in NTS neurones receiving identified trigeminal afferent input.

5-HT_3 receptors

The 5-HT_3 receptor antagonist granisetron was first described as having anti-emetic properties 15 years ago (Carmichael *et al.*, 1988), leading to the widespread use of this, and other 5-HT_3 receptor antagonists, in the treatment of nausea and vomiting. 5-HT_3 receptors residing in the NTS and the adjacent brainstem structure the area postrema have been described as one site mediating at least a proportion of these anti-emetic actions (Higgins *et al.*, 1989). In addition, the caudomedial areas of the NTS examined in the present experiments are known to have a high degree of gastrointestinal (GI) vagal afferent termination (Kalia & Mesulam, 1980), thus, the findings of the present experiments support these data in the demonstration of a strong action for 5-HT_3 receptors in this region. However, the role of these neurones in emesis or GI function cannot be determined although neurones were identified by their

receiving at least cervical vagal afferent input. In addition, right atrial injection of PBG activated the majority of neurones and 5-HT_3 receptors have been demonstrated to mediate a component of this excitation (see chapter 5). This PBG-evoked response has been demonstrated to be mediated via the vagus nerve (see chapter 3), and a proportion of this excitation may be mediated via the activation of 5-HT_3 receptors in the GI tract since left ventricular PBG injection has been shown to evoke a smaller, but significant excitation in caudomedially located NTS neurones (Wilson *et al.*, 1996). Therefore, the present experiments may provide further *in vivo* support that blockade of 5-HT_3 receptors in the NTS may play a role in the anti-emetic effects of 5-HT_3 receptor antagonists.

7.4 Future studies

There are a number of experiments which would be useful in further interpretation of the findings of the present experiments. Initially, regarding the role of 5-HT in the NTS some of the groups of data would benefit from higher numbers as this would increase the statistical significance, allowing clearer statements to be made about the findings. In addition, since the NTS is so diverse in terms of the numbers of functionally and pharmacologically different populations of neurones, increased numbers in every group would still provide a clearer picture of the roles of 5-HT receptor subtypes in the NTS. In this regard the observation that small numbers of neurones in certain groups caused opposing effects to the majority, e.g. sumatriptan, although causing inhibition in the majority of neurones, also caused excitation in a small number of cells, may hold some significance in a larger population of neurones. Further support for this theory comes from the observation that **all** neurones were excited by both NMDA and AMPA i.e. there was only a single effect caused by these receptor ligands in population numbers of a similar size. However, the fact that certain 5-HT receptor ligands did not have exclusive effects may also be explained by their lower affinity for their respective binding sites and also by the ability of some compounds to have affinities at more than one receptor known to be present in the NTS e.g. ketanserin has high affinity for both 5-HT_{1D} and 5-HT_{2A} receptors. It would also be beneficial to examine the effects of an AMPA antagonist on the excitation evoked by 5-HT_3 receptor activation in the NTS to

confirm if this excitation involves the stimulation of AMPA, as well as NMDA, receptors.

Further functional characterisation of individual NTS neurones would also create a clearer understanding of the interaction between receptor subtypes and identified inputs, i.e. animals could be further instrumented, e.g. a cannula placed in the lingual artery for the activation of chemoreceptor afferents in the carotid sinus, to identify if neurones received, in this case, chemoreceptor input. It would also be beneficial to examine a more substantial area of the NTS to confirm if the roles of 5-HT receptor subtypes seen in the areas examined so far are consistent throughout the nucleus. In addition, this may help to explain some of the inconsistencies in the understanding of 5-HT_3 receptors in this nucleus. Another potentially beneficial experiment would be to carry out the present experiments in a working heart-brainstem preparation of the rat (WHBP; Paton, 1996) in order to demonstrate whether these pharmacological results are mirrored, or different, in an unanaesthetized animal. Indeed, the physiological observation that the majority of neurones are excited by pulmonary C-fibre stimulation evoked by right atrial PBG administration has previously been demonstrated in a WHBP of the mouse (Paton, 1998).

Electrical and/or chemical stimulation of other central serotonergic sites, such as the raphe nuclei, may also help to identify the origin of the serotonergic input to these NTS neurones, or at least confirm that it comes from a peripheral afferent source such as the nodose ganglion or from within the NTS itself. The continued development of new, more selective, receptor ligands will also aid the characterisation of 5-HT receptor function in the NTS, although as mentioned there are the limitations regarding the solubility of recently developed compounds as regards ionophoretic ejection. For example, a novel 5-HT_{1B} receptor antagonist may confirm the actions of GR55562B, and therefore further confirm that this ligand is able to bind to, and antagonise, r5-HT_{1B} receptors; unfortunately the relatively new 5-HT_{1B} receptor antagonist SB224289 (Davidson & Stamford, 1995) does not readily dissolve in saline adjusted to pH 4 and so cannot be ejected via ionophoresis.

The use of other recording techniques may help provide support for the findings of the present experiments, and may also reveal more accurately the specific actions of 5-$HT_{1B/1D/3}$ receptor ligands on the excitability of neurones with particular reference to intracellular recordings confirming the location of 5-HT_3 receptors on glutamatergic terminals, as has been done before in the dorsal vagal motor nucleus (see Wang *et al.*, 1998a). Patch clamp recording from brain slices could also help to characterise some of these effects since receptor ligands could be diffused across the whole preparation allowing the use of some of more selective, recently developed, drugs.

REFERENCES

ADAIR, J.R., HAMILTON, B.L., SCAPPATICCI, K.A., HELKE, C.J. & GILLIS, R.A. (1977). Cardiovascular responses to electrical stimulation of the medullary raphe area of the cat. *Brain Res.*, **128,** 141-145.

AGARWAL, S.K. & CALARESU, F.R. (1992). Electrical stimulation of nucleus tractus solitarius excites vagal preganglionic cardiomotor neurons of the nucleus ambiguus in rats. *Brain Res.*, **574,** 320-324.

AGARWAL, S.K., GELSEMA, A.J. & CALARESU, F.R. (1990). Inhibition of rostral VLM by baroreceptor activation is relayed through caudal VLM. *Am. J. Physiol.*, **258,** R1271-R1278.

AGOSTINI, E., CHINNOCK, J.E., DALY, M. DE B. & MURRAY, J.E. (1957). Functional and histological studies of the vagus nerve and its branches to the heart, lungs and abdominal viscera in the cat. *J. Physiol.*, **135,** 182-205.

AICHER, S.A., SARAVAY, R.H., CRAVO, S., JESKE, I., MORRISON, S.F., REIS, D.J. & MILNER, T.A. (1996). Monosynaptic pathways from the nucleus tractus solitarii to C1 adrenergic neurons in the rostral ventrolateral medulla: comparison with input from caudal ventrolateral medulla. *J.Comp. Neurol.*, **373,** 62-75.

ALBERT, A.P., SPYER, K.M. & BROOKS, P.A. (1996). The effect of 5-HT and selective 5-HT receptor agonists and antagonists on rat dorsal vagal preganglionic neurones in vitro. *Br. J. Pharmacol.*, **119,** 519-526.

ALEXANDER, R.S. (1946). Tonic and reflex functions of medullary sympathetic centers. *J. Neurophysiol.*, **9,** 205-217.

ALLCHIN, R.E., BATTEN, T.F.C., McWILLIAM, P.N. & VAUGHAN, P.F.T. (1994). Electrical stimulation of the vagus increases extracellular glutamate recovered from the nucleus tractus solitarius of the cat by *in vivo* microdialysis, *Exp. Physiol.*, **79,** 265-268.

AMIN, A.H., CRAWFORD, T.B.B. & GADDUM, J.H. (1954). The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. *J. Physiol.*, **126,** 596-618.

ANDERSON, I.K., MARTIN, G.R. & RAMAGE, A.G. (1992). Evidence that i.c.v. administration of 5-HT causes sympathoexcitation through activation of 5-HT_{1A} receptors and vasopressin release through activation of 5-$HT_{2/1C}$ receptors in anaesthetized rats. *Br. J. Pharmacol.*, **107,** 1020-1028.

ANDERSON, I.K., MARTIN, G.R. & RAMAGE, A.G. (1995). Evidence that activation of 5-HT_2 receptors in the forebrain of anaesthetized cats causes sympathoexcitation. *Br. J. Pharmacol.*, **116,** 1751-1756.

ANDRESEN, M.C. & YANG, M. (1990). Non-NMDA receptors mediate sensory afferent synaptic transmission in medial nucleus tractus solitarius. *Am. J. Physiol.*, **259,** H1307-H1311.

ASHBY, JR, C.R., MINABE, Y., EDWARDS, E. & WANG, R.Y. (1991). 5-HT_3-like receptors in the rat medial prefrontal cortex: an electrophysiological study. *Brain Res.*, **550,** 181-191.

ASHWORTH-PREECE, M.A., JARROTT, B. & LAWRENCE, A.J. (1995). 5-Hydroxytryptamine$_3$ receptor modulation of excitatory amino acid release in the rat nucleus tractus solitarius. *Neurosci. Lett.*, **191,** 75-78.

AZMITIA, E.C. (1978). The serotonin-producing neurons of the midbrain median and dorsal raphe nuclei. In *Handbook of Psychopharmacology, Chemical Pathways in the Brain.* ed. Iversen, L.L., Iversen, S.D. & Snyder, S.H. pp. 233-314. New York: Plenum Press.

AZMITIA, E.C. & SEGAL, M. (1978). An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.*, **179,** 641-668.

BALLANTYNE, D., JORDAN, D., MEESMAN, M., RICHTER, D.W. & SPYER, K.M. (1986). Presynaptic depolarization in myelinated vagal afferent fibres terminating in the nucleus of the tractus solitarius in the cat. *Pflugers Arch.*, **406,** 12-19.

BALLANTYNE, D., JORDAN, D., SPYER, K.M. & WOOD, L.M. (1988). Synaptic rhythm of caudal medullary expiratory neurones during stimulation of the hypothalamic defence area of the cat. *J. Physiol.*, **405,** 527-546

BANES, A.K.L. & WATTS, S.W. (2001). Enhanced contraction to 5-hydroxytryptamine is not due to "unmasking" of 5-hydroxytryptamine$_{1B}$ receptors in the mesenteric artery of the deoxycorticosterone acetate-salt rat. *Hypertension*, **38,** 891-895.

BARILLOT, J.-C. (1970). Depolarisation présynaptique des fibres sensitives vagales et laryngées. *J. Physiol.(PARIS)*, **62,** 273-294.

BARNES, N.M. & SHARP, T. (1999). A review of central 5-HT receptors and their function. *Neuropharmacology*, **38,** 1083-1152.

BARRACO, R.A. & JANUSZ, C.J. (1988). Microinjections of isoproterenol into the nucleus tractus solitarius modulates respiration. *Med. Sci. Res.*, **16,** 977-979.

BARRETT, R.J. (1992). Therapeutic potential of 5-HT$_2$ antagonists. *Drug News and Perspectives*, **5,** 453-460

BASBAUM, A.I. & FIELDS, H.L. (1984). Endogenous pain control system. Brain stem spinal pathways and endorphin circuitry. *Ann. Rev. Neurosci.*, **7,** 309-338.

BAXTER, G., KENNETT, G., BLANEY, F. & BLACKBURN, T. (1995). 5-HT_2 receptor subtypes: a family reunited? *Trends Pharmacol. Sci.*, **16,** 105-110.

BENNETT, J.A., GOODCHILD, C.S., KIDD, C. & McWILLIAM, P.N. (1985). Neurones in the brain stem of the cat excited by vagal afferent fibres from the heart and lungs. *J. Physiol.*, **369,** 1-15.

BIAGGIONI, I., WHETSELL, W.O., JOBE, J. & NADEAU, J.H. (1994). Baroreflex failure in a patient with central nervous system lesions involving the nucleus tractus solitarii. *Hypertension*, **23,** 491-495.

BISSERBE, J.C., PATEL, J. & MARANGOS, P.J. (1985). Autoradiographic localization of adenosine uptake sites in rat brain using [^{3}H]nitrobenzylthioinosine. *J. Neurosci.*, **5,** 544-550.

BLINDER, K.J., GATTI, P.J., JOHNSON, T.A., LAUENSTEIN, J-M., COLEMAN, W.P., GRAY, A.L. & MASSARI, J. (1998). Ultrastructural circuitry of cardiorespiratory reflexes: there is a monosynaptic path between the nucleus of the solitary tract and vagal preganglionic motoneurons controlling atrioventricular conduction in the cat. *Brain Res.*, **785,** 143-157.

BOBKER, D.H. & WILLIAMS J.T. (1989). Serotonin agonists inhibit synaptic potentials in the rat locus ceruleus in vitro via 5-hydroxytryptamine$_{1A}$ and 5-hydroxytryptamine$_{1B}$ receptors. *J. Pharmacol. Exp. Ther.*, **250,** 37-43.

BOCKAERT, J., ANSANAY, H. & WAEBER, C., SEBBEN, N., FAGNI, L. & DUMUIS, A. (1994). 5-HT_4 receptors: potential therapeutic implications in neurology and psychiatry. *CNS drugs*, **1,** 6-15.

BONAGAMBA, L.G.H., SÉVOZ-COUCHE, C., N'DIAYE, A., UYGUN-LOUVET, K., CALLERA, J.-C., MACHADO, B.H., HAMON, M. & LAGUZZI, R. (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. *Neuropharmacol.*, **39,** 2336-2345.

BONHAM, A.C., COLES, S.K. & McCRIMMON, D.R., (1993). Pulmonary stretch receptor afferents activate excitatory amino acid receptors in the nucleus tractus solitarii in rats. *J. Physiol.*, **464,** 725-745.

BOSCAN, P. & PATON, J.F. (2002). Nociceptive afferents selectively modulate the cardiac component of the peripheral chemoreceptor reflex via actions within the solitary tract nucleus. *Neuroscience*, **110,** 319-328.

BOURSON, A., BORRONI, E., AUSTIN, R.H., MONSMA Jnr, F.J. 7 SLEIGHT, A.J. (1995). Determination of the role of 5-HT_6 receptors in the rat brain: a study using antisense oligonucleotides. *J. Pharmacol. Exp. Ther.*, **274,** 173-180.

BRADLEY, P.B., ENGEL, G., FENUIK, W., FOZARD, J.R., HUMPHREY, P.P., MIDDLEMISS, D.N., MYLECHARANE, E.J., RICHARDSON, B.P. & SAXENA, P.R. (1986). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacol.*, **25,** 563-576.

BRATTSTROM, A., DE JONG, W. & DE WIED, D. (1988). Vasopressin microinjections into the nucleus tractus solitarii decrease heart rate and blood pressure in anaesthetised rats. *J. Hypertens.*, **6,** S521-S524.

BRODIE, B.B. & SHORE, P.A. (1957). A concept for the role of serotonin and noradrenaline as chemical mediators in the brain. *Ann. N. Y. Acad. Sci.*, **66,** 631-642.

BRODY, M.J. & JOHNSON, A.K. (1980). Role of the anteroventral third ventricle region in fluid and electrolyte balance, arterial pressure regulation and hypertension. In *Frontiers in Neuroendocrinology*. ed. Martini, L. & Ganong, W.F. pp. 249-292. New York: Raven.

BROPHY, S., FORD, T.W., CAREY, M. & JONES, J.F. (1999). Activity of aortic chemoreceptors in the anaesthetized rat. *J. Physiol.*, **514,** 821-828.

BRUINVELS, A.T., LANDWEHRMEYER, B., GUSTAFSON, E.L., DURKIN, M.M., MENGOD, G., BRANCHEK, T.A., HOYER, D. & PALACIOS, J.M. (1994). Localization of 5-HT_{1B}, 5-$HT_{1D\square}$, 5-HT_{1E} and 5-HT_{1F}, receptor messenger RNA in rodent and primate brain. *Neuropharmacol.*, **33,** 367-386.

BRUINVELS, A.T., PALACIOS, J.M. & HOYER, D. (1993). Autoradiographic characterisation and localisation of 5-HT_{1D} compared to 5-HT_{1B} binding sites in rat brain. *Naunyn-Schmiedberg's Arch. Pharmacol.*, **347,** 569-582.

BURNET, P.W.J., EASTWOOD, S.L., LACEY, K. & HARRISON, P.J. (1995). The distribution of 5-HT_{1A} and 5-HT_{2A} receptor mRNA in human brain. *Brain Res.*, **676,** 157-168.

BUTLER, A., HILL, J.M., IRELAND, S.J., JORDAN, C.C. & TYERS, M.B. (1988). Pharmacological properties of GR38032F, a novel antagonist at 5-HT_3 receptors. *Br. J. Pharmacol.*, **94,** 397-412.

CALLERA, J.C., BONAGAMBA, L.G.H., SÉVOZ, C., LAGUZZI, R. & MACHADO, B.H. (1997a). Cardiovascular effects of microinjection of low doses of serotonin into the NTS of unanaesthetized rats. *Am. J. Physiol.*, **272,** R1135-R1142.

CALLERA, J.C., SÉVOZ, C., LAGUZZI, R. & MACHADO, B.H. (1997b). Microinjection of a serotonin3 receptor agonist into the NTS of unanaesthetized rats inhibits the bradycardia evoked by activation of the baro- and chemoreflexes. *J. Autonom. Nerv. Syst.*, **63,** 127-136.

CALLERA, J.C., BONAGAMBA, L.G.H., NOSJEAN, A., LAGUZZI, R. & MACHADO, B.H. (1999). Activation of $GABA_A$ but not $GABA_B$ receptors in the NTS blocked bradycardia of chemoreflex in awake rats. *Am. J. Physiol.*, **276,** H1902-H1910.

CALZA, L., GIARDINO, L., GRIMALDI, R., RIGOLI, M., STEINBUSCH, H.W.M. & TIENGO, M. (1985). Presence of 5-HT-positive neurons in the medial nuclei of the solitary tract. *Brain Res.*, **347,** 135-139.

CAMPAGNOLE-SANTOS, M.J., DIZ, D.I. & FERRARIO, C.M. (1988). Baroreceptor reflex modulation by angiotensin II at the nucleus tractus solitarii. *Hypertension*, **11,** I167-I171.

CARMICHAEL, J., CANTWELL, B.M.J., EDWARDS, C.M., RAPEPORT, W.G. & HARRIS, A.L. (1988). The serotonin type 3 receptor antagonist BRL 43694 and nausea and vomiting induced by cisplatin. BMJ, **297,** 110-111.

CARRIVE, P., BANDLER, R. & DAMPNEY, R.A.L. (1989). Viscerotopic control of regional vascular beds by discrete groups of neurones within the midbrain peraqueductal gray. *Brain Res.*, **493,** 385-390.

CARRIVE, P. & BANDLER, R. (1991). Viscerotopic organisation of neurons subserving hypotensive reactions within the midbrain periaqueductal grey. A correlative functional and anatomical study. *Brain Res.*, **541,** 205-215.

CARSON, M.J., THOMAS, E.A., DANIELSON, P.E. & SUTCLIFFE, J.G. (1995). The 5-HT5A 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.

CASSELL, M.D., ROBERTS, L. & TALMAN, W.T. (1992). Glycine-containing terminals in the rat dorsal vagal complex. *Neuroscience*, **50,** 970.

CASTO, R. & PHILLIPS, M.I. (1986). Angiotensin II attenuates baroreflexes at nucleus tractus solitarius of rats. *Am. J. Physiol.*, **250,** R193-R198.

CATELLI, J.M., GIAKAS, W.J. & SVED, A.J. (1987). GABAergic mechanisms in nucleus tractus solitarius alter blood pressure and vasopressin release. *Brain Res.*, **403,** 279-289.

CECHETTO, D.F. & CALARESU, F.R. (1983). Response of single units in the amygdala to stimulation of buffer nerves in cat. *Am. J. Physiol.*, **244,** R646-R651.

CECHETTO, D.F. & SAPER, C.B. (1987). Evidence for a viscerotopic sensory representation in the cortex and thalamus in the rat. *J. Comp. Neurol.*, **262,** 27-45.

CHAMBERLIN, N.L. & SAPER, C.B. (1992). Topographic organisation of cardiovascular responses to electrical and glutamate microstimulation of the parabrachial nucleus of the rat. *J. Comp. Neurol.*, **326,** 245-262.

CHAN, J.Y.H., SHIH, C.-D. & ABD CAHN, S.H.H. (1993). Participation of endogenous neuropeptide Y in the suppression of baroreceptor reflex response by locus coeruleus in the rat. *Reg. Pep.*, **48,** 293-300.

CHIBA, T. & DOBA, N. (1975). The synaptic structure of catecholaminergic axon varicosities in the dorso-medial portion of the nucleus tractus solitarius of the cat: possible roles in the regulation of cardiovascular reflexes. *Brain Res.*, **84,** 31-46.

CHIANCA, JR. D.A. & MACHADO, B.H. (1996). Microinjection of NMDA antagonist into the NTS of conscious rats blocks the Bezold-Jarisch reflex. *Brain Res.*, **718,** 185-188.

CHIBA, T. & DOBA, N. (1976). Catechoaminergic axo-axonic synapses in the nucleus of the tractus solitarius (Pars commissuralis) of the cat: possible relation to presynaptic regulation of baroreceptor reflexes. *Brain Res.*, **102,** 255-265.

CIRIELLO, J., HRYCYSHYN, A.W. & CALARESU, F.R. (1981). Horseradish peroxidase study of brain stem projections of carotid sinus and aortic depressor nerves in the cat. *J. Auton. Nerv. Syst.*, **4,** 43-61.

COLERIDGE, J.C.G. & COLERIDGE, H.M. (1979). Chemoreflex regulation of the heart. In *Handbook of Physiology,* section 2, *The Cardiovascular System,* vol. 1, *The Heart,* pp. 653-676. American Physiological Society, Bethesda, MD, USA.

COLERIDGE, H.M. & COLERIDGE, J.C.G. (1980). Cardiovascular afferents involved in regulation of peripheral vessels. *Ann. Rev. Physiol.*, **42,** 413-427.

COLERIDGE, H.M., COLERIDGE, J.C.G. & JORDAN, D. (1991). Integration of ventilatory and cardiovascular control systems. In *The Lung.* ed. Crystal, R.G. & West, J. B. pp. 1405-1418. New York: Scientific Foundations, Raven Press.

COLOMBARI, E., BONAGAMBA, L.G.H. & MACHADO, B.H. (1994). Mechanisms of pressor and bradycardic responses to L-glutamate microinjected into the NTS of conscious rats. *Am. J. Physiol.*, **266,** R730-R738.

CONSOLO, S., ARNABOLDI, S., GIORGI, S., RUSSI, G. & LADINSKY, H. (1994). 5-HT_4 receptor stimulation facilitates acetylcholine release in rat frontal cortex. *Neuroreport*, **5,** 1230-1232.

CONSOLO, S., ARNABOLDI, S., RAMPONI, S., NANNINI, L., LADINSKY, H. & BALDI, G. (1996). Endogenous serotonin facilitates in vivo acetylcholine release in rat frontal cortex through 5-HT_{1B} receptors. *J. Pharmacol. Exp. Ther.*, **277,** 823-830.

COOTE, J.H. (1990). The central antihypertensive action of 5-hydroxytryptamine: The location of site of action. In *Cardiovascular Pharmacology of 5-Hydroxytryptamine; Prospective Therapeutic Applications.* ed. Saxena, P.R., Wallis, D.I., Wouters, W. & Bevan, P. pp. 259-270. Dordrecht: Kluwer Academic publishers.

COOTE, J.H. (1995). Cardiovascular function of the paraventricular nucleus of the hypothalamus. *Biol. Signals*, **4,** 142-149.

COTTLE, M.K. (1964). Degeneration studies of primary afferents of IXth and Xth cranial nerves in the cat. *J. Comp. Neurol.,* **122,** 329-343.

CRISCIONE, L., REIS, D.J. & TALMAN, W.T. (1983). Cholinergic mechanisms in the nucleus tractus solitarii and cardiovascular regulation in the rat. *Eur. J. Pharmacol.*, **88,** 47-55.

DAHLSTROM, A. & FUXE, K. (1964). Evidence for the existence of monoamine containing neurones in the central nervous system. I. Demonstration of monoamines in cell bodies of brain stem neurones. *Acta Physiol. Scand.*, **62,** (suppl. 232), 1-55.

DALE, N., GOURINE, A.V., LLAUDET, E., BULMER, D., THOMAS, T. & SPYER, K.M. (2002). Rapid adenosine release in the nucleus tractus solitarii during defence response in rats: real-time measurement *in vivo. J. Physiol.*, **544,** 149-160.

DALY, M. DE B. (1997). *Peripheral Arterial Chemoreceptors and Respiratory-Cardiovascular Integration.* Oxford: Clarendon Press.

DALY, M. DE B. (1991). Some reflex cardioinhibitory responses in the cat and their modulation by central inspiratory neuronal activity. *J. Physiol.*, **439,** 559-577.

DALY, M. DE B., JORDAN, D. & SPYER, K.M. (1992). Modification of respiratory activities during stimulation of carotid chemoreceptors, arterial baroreceptors and pulmonary C fibre afferents in the anaesthetized cat. *J. Physiol.*, **446,** 466P.

DALY, M. DE B. & 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.

DALY, M. DE B. & KIRKMAN, E. (1989). Differential modulation by pulmonary stretch afferents of some reflex cardioinhibitory responses in the cat. *J. Physiol.*, **417,** 323-341.

DALY, M. DE B., WARD, J. & WOOD, L.M. (1987). The peripheral chemoreceptors and cardiovascular-respiratory integration. In *Neurobiology of the Cardio-Respiratory System.* ed. Taylor. E.W. pp. 342-368. Manchester: Manchester University Press.

DAMPNEY, R.A.L. (1994a). Functional organisation of central pathways regulating the cardiovascular system. *Phys Rev.*, **74,** 323-364.

DAMPNEY, R.A.L. (1994b). The subretrofacial vasomotor nucleus; anatomical, chemical and pharmacological properties and role in cardiovascular regulation. *Prog. Neurobiol.*, **42,** 197-227.

DAMPNEY, R.A.L., CZACHURSKI, J., DEMBOWSKY, K., GOODCHILD, A.K. & SELLER, H. (1987). Afferent connections and spinal projections of the vasopressor region in the rostral ventrolateral medulla of the cat. *J. Auton. Nerv. Syst.*, **20,** 73-86.

DAMPNEY, R.A.L., GOODCHILD, A.K., ROBERTSON, L.G. & MONTGOMERY, W. (1982). Role of ventrolateral medulla in vasomotor regulation: a correlative anatomical and physiological study. *Brain Res.*, **249,** 223-235.

DANDO, S.B., JORDAN, D. & RAMAGE, A.G. (1995). The role of central 5-HT_3 receptors in the modulation of the response to upper airway stimulation in the anaesthetized rabbit. *J.Physiol.*, **489,** 156P.

DANDO, S.B., JORDAN, S. & RAMAGE, A.G. (1996). Central 5-HT_{1A} and 5-HT_{1D} receptors have opposing roles in the reflex activation of cardiac vagal motoneurones by upper airway stimulation in anaesthetized rabbits. *Br. J. Pharmacol.*, **117,** 48P.

DANDO, S.B., SKINNER, M.R., JORDAN, D. & RAMAGE, A.G. (1998). Modulation of the vagal bradycardia evoked by stimulation of upper airway receptors by central 5-HT_1 receptors in anaesthetized rabbits. *Br. J. Pharmacol.*, **125,** 409-417.

DASHWOOD, M.R., GILBEY, M.P., JORDAN, D. & RAMAGE, A.G. (1988). Autoradiographic localisation of 5-HT_{1A} binding sites in the brainstem of the cat. *Br. J. Pharmacol.*, **94,** 386P.

DAVIDSON, C. & STAMFORD, J.A. (1995). Evidence that 5-hydroxytryotamine release in rat dorsal raphe nucleus is controlled by 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} autoreceptors. *Br. J. Pharmacol.*, **114,** 1107-1109.

DEDEOGLU, A. & FISHER, L.A. (1991). Central nervous actions of serotonin and a serotonin$_{1A}$ receptor agonist: cardiovascular excitation at low doses, *J. Pharmacol. Exp. Ther.*, **257,** 425-432.

DIETRICH, W.D., LOWRY, O.H. & LOEWY, A.D. (1982). The distribution of glutamate, GABA and aspartate in the nucleus tractus solitarius of the cat. *Brain Res.*, **237,** 254-260.

DIMICCO, J.A., GALE, K., HAMILTON, B. & GILLIS, R.A. (1979). GABA receptor control of parasympathetic outflow to heart: Characterization and brainstem localization. *Science*, **204,** 1106-1109.

DONOGHUE, S., FOX, R.E., KIDD, C. & KOLEY, B.N. (1981). The distribution in the cat brain stem of neurones activated by vagal non-myelinated fibres from the heart and lungs. *Quart. J. Exp. Physiol.*, **66,** 391-404.

DONOGHUE, S., GARCIA, M., JORDAN, D. & SPYER, K.M. (1982). Identification and brain-stem projections of aortic baroreceptor afferent neurones in nodose ganglia of cats and rabbits. *J. Physiol.*, **322,** 337-352.

DONOGHUE, S., FELDER, R.B., JORDAN, D. & SPYER, K.M. (1984). The central projections of carotid baroreceptors and chemoreceptors in the cat: a neurophysiological study. *J. Physiol.*, **347,** 397-409.

DOUCET, E., POHL, M., FATTACCINI, C.M., ADRIEN, J., MESTIKAWY, S.E. & HAMON, M. (1995). In situ hybridisation evidence for the synthesis of 5-HT_{1B} receptor in serotoninergic neurons of anterior raphe nuclei in the rat brain. *Synapse*, **19,** 18-28.

DOURISH, C.T., HUTSON, C.T. & CURZON, G. (1986). Putative anxiolytics 8-OH-DPAT, buspirone and TVX Q7821 are agonists at 5-HT_{1A} autoreceptors in the raphe nuclei. *Trends Pharmacol. Sci.*, **7,** 212-214.

DOYLE, M.W. & ANDRESEN, M.C. (2001). Reliability of monosynaptic sensory transmission in brain stem neurons *in vitro. J. Neurophysiol.*, **85,** 2213-2223.

DRETELER, G.H., WOUTERS, W. & SAXENA, P.R. (1990). Comparison of the cardiovascular effects of the 5-HT_{1A} receptor agonist flesinoxan with that of 8-OH-DPAT in the rat. *Eur. J. Pharmacol.*, **180,** 339-349.

DRETELER, G.H., WOUTERS, W., SAXENA, P.R. & RAMAGE, A.G. (1991). Pressor effects following microinjection of 5-HT_{1A} receptor agonists into the raphe obscurus of the anaesthetised rat. *Br. J. Pharmacol.*, **102,** 317-322.

DUXON, M.S., FLANIGAN, T.P., REAVLEY, A.C., BAXTER, G.S., BLACKBURN, T.P. & FONE, K.C. (1997a). Evidence for the expression of the 5-hydroxytryptamine$_{2B}$ receptor protein in the rat central nervous system. *Neuroscience*, **76,** 323-329.

DUXON, M.S., KENNETT, G.A., LIGHTOWLER, S., BLACKBURN, T.P. & FONE, K.C. (1997b). Activation of 5-HT_{2B} receptors in the medial amygdala causes anxiolysis in the social interaction test in the rat. *Neuropharmacol.*, **36,** 601-608.

EDWARDS, E. & PATON, J.F.R. (1999). 5-HT_4 receptors in nucleus tractus solitarii attenuate cardiopulmonary reflex in anaesthetized rats. *Am. J. Physiol.*, **46,** H1914-H1923.

EGLEN, R.M., JASPER, J.R., CHANG, D.J. & MARTIN, G.R. (1997). The 5-HT_7 receptor: orphan found. *Trends Pharmacol. Sci.*, **18,** 104-107.

ERGENE, E., DUNBAR, J.C., O'LEARY, D.S. & BARRACO, R.A. (1994). Activation of P2-purinoceptors in the nucleus tractus solitarius mediate depressor responses. *Neurosci. Lett.*, **174,** 188-192.

ERLANDER, M.G., LOVENBERG., BARON, B.M., DE LECEA, L., DANIELSON, P.E., RACKE, M., SLONE, A.L., SIEGEL, B.W., FOYE, P.E., CANNON, K., et al, (1993). Two members of a distinct subfamily of 5-hydroxytryptamine receptors differentially expressed in rat brain. *Proc. Natl. Acad. Sci.*, **90,** 3452-3456.

EVANS, R.H., FRANCIS, A.A., JONES, A.W., SMITH, D.A. & WATKINS, J.C. (1982). The effects of a series of omega-phosphonic alpha-carboxylic amino acids on electrically evoked and excitant amino acid-induced responses in isolated spinal cord preparations. *Br. J. Pharmacol.*, **75,** 65-75.

FELDMAN, P.D. & FELDER, R.B. (1989). α-Adrenergic influences on neuronal responses to visceral afferent input in the nucleus tractus solitarius. *Neuropharmacol.*, **28,** 1081-1087.

FELDMAN, P.D. & GALIANO, F.J. (1995). Cardiovascular effects of serotonin in the nucleus of the solitary tract. *Am .J. Physiol.*, **269,** R48-R56.

FERRARI, M.D. & SAXENA, P.R. (1993). Clinical and experimental effects of sumatriptan in humans. *Trends Pharmacol. Sci.*, **14,** 129-133.

FEUERSTEIN, T.J., HURING, H., VAN VELTHOVEN, V., LUCKING, C.H. & LANDWEHRMEYER, G.B. (1996). 5-HT_{1D}-like receptors inhibit the release of endogenously formed[^{3}H]GABA in human, but not in rabbit, neocortex. *Neurosci. Lett.*, **209,** 210-214.

FIOR, D.R., HEDLUND, P.B. & FUXE, K. (1993). Autoradiographic evidence for a bradykinin angiotensin II receptor-receptor interaction in the rat brain. *Neurosci. Lett.*, **163,** 58-62.

FLETCHER, A., BILL, D.J., BILL, S.J., CLIFFE, I.A., DOVER, G.M., FORSTER, E.A., HASKINS, J.T., JONES, D., MANSELL, H.L. & REILLY, Y. (1993). WAY 100135: a novel, selective antagonist at presynaptic and postsynaptic 5-HT_{1A} receptors. *Eur. J. Pharmacol.*, **237,** 283-291.

FLETCHER, A., FORSTER, E.A., BILL, D.J., BROWN, G., CLIFFE, I.A., HARTLEY, J.E., JONES, D.E., McLENACHAN, A., STANHOPE, K.J., CRITCHLEY, D.J., CHILDS, K.J., MIDDLEFELL, V.C., LANFUMEY, L., CORRADETTI, R., LAPORTE, A.M., GOZLAN, H., HAMON, M. & DOURISH, C.T. (1996). Electrophysilogical, biochemical, neurohormonal and behavioural studies with WAY 100635, a potent, selective and silent 5-HT_{1A} receptor antagonist. *Behav. Brain Res.*, **73,** 337-353.

FOLEY, J.O. & DUBOIS, F.S. (1934). An experimental study of the rootlets of the vagus nerve in the cat. *J. Comp. Neurol.*, **60,** 137-159.

FORD, T.W., BENNETT, J.A., KIDD, C. & McWILLIAM, P.N. (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.

FOREMAN, R.D. & BLAIR, R.W. (1988). Central organization of sympathetic cardiovascular responses to pain. *Ann. Rev. Physiol.*, **50,** 607-622.

FORNAL, C.A., METZLER, C.W., GALLEGOS, R.A., VEASEY, S.C., McCREARY, A.C. & JACOBS, B.L. (1996). WAY-100635, a potent and selective 5-hydroxytrptamine$_{1A}$ antagonist, increases serotonergic neuronal activity in behaving cats: comparison with (S)-WAY-100135. *J. Pharmacol. Exp. Ther.*, **278,** 752-762.

FORTIN, G. & CHAMPAGNAT, J. (1993). Spontaneous synaptic activities in rat nucleus tractus solitarius neurons *in vitro*: evidence for re-excitatory processing. *Brain Res.*, **630,** 125-135.

FOZARD, J.R. (1984). MDL 72222: a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. *Naunyn-Schmeidberg's Arch. Pharmacol.*, **326,** 36-44.

FOZARD, J.R., MIR, A.K. & MIDDLEMISS, D.N. (1987). Cardiovascular response to 8-hydroxy-2-(di-n-propylamin)tetralin (8-OH-DPAT) in the rat: site of action and pharmacological analysis. *J. Cardiovasc. Pharmacol.*, **9,** 328-347.

FRESE, A., EVERS, S. & MAY, A. (2003). Autonomic activation in experimental trigeminal pain. *Cephalalgia*, **23,** 67-68.

FULWILER, C.E. & SAPER, C.B. (1984). Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. *Brain Res. Rev.*, **7,** 229-259.

GALLACHER, M. & RAMAGE, A.G. (1996). Evidence which indicates that the activation of central 5-HT$_{1D\alpha}$ can cause hypotension in anaesthetized rats. *Br. J. Pharmacol.*, **83,** 391P.

GAO, X., PHILLIPS, P.A., WIDDOP, R.E., TRINDER, D., JARROTT, B. & JOHNSTON, C.I. (1992). Presence of functional vasopressin V_1 receptors in rate vagal afferent neurons. *Neurosci. Lett.*, **145,** 79-82.

GE, J. & BARNES, N.M. (1996). 5-HT_4 receptor-mediated modulation of 5-HT release in the rat hippocampus in vivo. *Br. J. Pharmacol.*, **117,** 1475-1480.

GILBEY, M.P., COOTE, J.H., FLEETWOODWALKER, S. & PETERSON, D.F. (1982). The influence of the paraventriculo-spinal pathway, and oxytocin and vasopressin on sympathetic preganaglionic neurones. *Brain Res.*, **251,** 283-290.

GILBEY, M.P., JORDAN, D., RICHTER, D.W. & SPYER, K.M. (1984). Synaptic mechanisms involved in the inspiratory modulation of vagal cardio-inhibitory neurones in the cat. *J. Physiol.*, **365,** 65-78.

GILLES, R.A., HELKE, C.J., HAMILTON, B.L., NORMAN, B.L. & JACOBWITZ, D.M. (1980). Evidence that substance P is a neurotransmitter of baro- and chemoreceptor afferents in nucleus tractus solitarius. *Brain Res.*, **181,** 476-481.

GILLIS, R.A., HILL, K., KIRBY, J.S., QUEST, J.A., HAMOSH, P., NORMAN, W.P. & KELLER, K.J. (1989). Effect of activation of central nervous system serotonin 1A receptors on cardiorespiratory function. *J. Pharmacol. Exp. Ther.*, **248,** 851-857.

GLATZL, J., STERNINI, C., ROBIN, C., ZITTEL, T.T., WONG, H., REEVE, J.R. & RAYBOULD, H.E. (2002). Expression of 5-HT_3 receptors in the rat gastrointestinal tract. *Gastroenterol.*, **123,** 217-226.

GLAUM, S.R., BROOKS, P.A., SPYER, K.M. & MILLER, R.J. (1992). 5-Hydroxytrptamine-3 receptors modulate synaptic activity in the rat nucleus tractus solitarius in vitro. *Brain Res.*, **589,** 62-68.

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.

GRADIN, K., PETTERSSON, A., HEDNER, T., & PERSSON B. (1985). Acute administration of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), a selective 5-HT-receptor agonist, causes a biphasic blood pressure response and a bradycardia in the normotensive Sprague-Dawley and in the spontaneously hypertensive rat. *J. Neural. Transm.*, **62,** 305-319.

GRANATA, A.R. & WOODRUFF, G.N. (1982). Dopaminergic mechanisms in the nucleus tractus solitarius and effects on blood pressure. *Brain Res. Bull.*, **8,** 483-488.

DE GROAT, W.C., FRASER, M.O., YOSHIYAMA, M., SMERIN, S., TAI, C., CHANCELLOR, M.B., YOSHIMURA, N. & ROPPOLO, J.R. (2001). Neural control of the urethra. *Scand. J. Urol. Nephrol. Suppl.*, **207,** 35-43:106-125.

GUYENET, P.G. (1990). Role of the ventral medulla oblongata in blood pressure regulation. In *Central Regulation of Autonomic Functions.* ed. Loewy, A.D. & Spyer, K.M. pp. 45-167. Oxford: Oxford University Press.

GUYENET, P.G. & YOUNG, B.S. (1987). Projections of nucleus paragigantocellularis lateralis to locus coeruleus and other structures in rat. *Brain Res.*, **406,** 171-184.

HAINSWORTH, R. (1991) Reflexes from the heart. *Physiol. Rev.*, **71,** 617-658.

HALL, M.E., MILEY, F.B. & STEWART, J.M. (1989). Cardiovascular effects of substance P peptides in the nucleus of the solitary tract. *Brain Res.*, **497,** 280-290.

HAMBLIN, M.W., MCGUFFIN, R.W., METCALF, M.A., et al. (1992). Distinct 5-HT_{1B} and 5-HT_{1D} receptors in rat: structural and pharmacological comparison of the two cloned receptors. *Mol. Cell. Neurosci.*, **3,** 578-587.

HARFSTRAND, A., FUXE, K., TERENUIS, L. & KALIA, M. (1987). Neuropeptide-Y immunoreactive perikarya and nerve terminals in the rat medulla oblongata: relationship to cytoarchitecture and catecholaminergic cell groups. *J. Comp. Neurol.*, **260,** 20-35.

HARTIG, P.R., HOYER, D., HUMPHREY, P.P.A. & MARTIN, G.R. (1996). Alignment of receptor nomenclature with the human genome: classification of 5-HT_{1B} and 5-HT_{1D} receptor subtypes. *Trends Pharmacol. Sci.*, **17,** 103-105.

HEALY, D.P., RETTIG, R., NGUYEN, T. & PRINTZ, M.P. (1989). Quantitative autoradiography of angiotensin II receptors in the rat solitary-vagal area: effects of nodose ganglionectomy or sinoaortic denervation. *Brain Res.*, **489,** 1-12.

HEGARTY, A.A. & FELDER, R.B. (1993). Antagonism of vasopressin V_1 receptors in the nucleus tractus solitarius attenuates baroreflex responses to aortic depressor nerve stimulation. *Soc. Neurosci. Abstr.*, **19,** 133.7

HEURING, R.E. & PEROUTKA, S.J. (1987). Characterization of a novel [^{3}H]-5-hydroxytryptamine binding site subtype in bovine brain membranes. *J. Neurosci.*, **7,** 894-903.

HIGGINS, G.A., KILPATRICK, G.J., BUNCE, K.T., JONES, B.J. & TYERS, M.B. (1989). 5-HT_3 receptor antagonists injected into the area postrema inhibit cisplatin-induced emesis in the ferret. *Br. J. Pharmacol.*, **97,** 247-255.

HINES, T., TONEY, G.M. & MIFFLIN, S.W. (1994). Responses of neurons in the nucleus tractus solitarius to stimulation of heart and lung receptors in the rat. *Circ. Res.*, **74,** 1189-1196.

HJORTH, S., CARLSSON, A., LINDBERG, P., et al. (1982). 8-Hydroxy-2-(di-*n*-propylamino)tetralin, 8-OH-DPAT, a potent and selective simplifies ergot congener with central 5-HT receptor stimulating activity. *J. Neural. Transm.*, **55,** 169-188.

HJORTH, S. & TAO, R. (1991). The putative 5-HT_{1B} receptor agonist suppresses rat hippocampal 5-HT release in vivo: comparison with RU 24969. *Eur. J. Pharmacol.*, **209,** 249-252.

HOFF, E.C., KELL, J.F. & CARROLL, M.N. (1963). Effects of cortical stimulation and lesions on cardiovascular function. *Physiol. Rev.*, **43,** 68-114.

HOYER, D. (1991). The 5-HT receptor family: ligands, distribution and receptor-effector coupling. In: Rodgers, R.J. & Cooper, S.J. (eds). *5-HT_{1A} agonists, 5-HT_3 antagonists and benzodiazepines: Their comparative behavioural pharmacology*, John Wiley and Sons Ltd: U.K. pp. 35-37.

HOYER, D., CLARK, D.E., FOZARD, J.R., HARTIG, P.R., MARTIN, G.R., MYLECHARANE, E.J., SAXENA, P.R. & HUMPHREY, P.P. (1994). International union of pharmacological classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.*, **46,** 157-193.

HOYER, D., PAZOS, A., PROBST, A. & PALACIOS, J.M. (1986). Serotonin receptors in the human brain. I. Characterisation and autoradiographic localisation of 5-HT_{1A} recognition sites, apparent absence of 5-HT_{1B} recognition sites. *Brain Res.*, **376,** 85-96.

HURLEY, K.M., HERBERT, H., MOGA, M.M. & SAPER, C.B. (1991). Efferent projections of the infralimbic cortex of the rat. *J. Comp. Neurol.*, **308,** 249-276.

INGRAM, W.R. & DAWKINS, E.A. (1945). The intramedullary course of afferent fibres of the vagus in the cat. *J. Comp. Neurol.*, **82,** 157-168.

IOVINO, M. & STEARDO, L. (1985). Thirst and vasopressin secretion following central administration of angiontensin II in rats with lesions of the septal area and subfornical organ. *Neuroscience*, **15,** 61-67.

ITOH, H., ALPER, R.H. & BUNANG, R.D. (1992). Baroreflex changes produced by serotoninergic or catecholaminergic lesions in the rat nucleus tractus solitarius. *J. Pharmacol. Exp. Ther.*, **261,** 225-233.

JOHNSON, S.W., MERCURI, N.B. & NORTH, R.A. (1992). 5-hydroxytryptamine$_{1B}$ receptors block the GABA$_B$ synaptic potential in rat dopamine neurons. *J. Neurosci.*, **12,** 2000-2006.

JONES, J.F.X. (1993). The central control of the pulmonary chemoreflex. PhD Thesis, University of London.

JONES, J.F.X., MARTIN, G.R. & RAMAGE, A.G. (1995). Evidence that 5-HT$_{1D}$ receptors mediate inhibition of sympathetic ganglionic transmission in anaesthetized cats. *Br. J. Pharmacol.*, **116,** 1715-1717.

JONES, J.F.X., WANG, Y. & JORDAN, D. (1995). Heart rate responses to selective stimulation of cardiac vagal C fibres in anaesthetized cats, rats and rabbits. *J. Physiol.*, **489,** 203-214.

JONES, J.F.X., WANG, Y. & JORDAN, D. (1998). Activity of C fibre cardiac vagal efferents in anaesthetized cats and rats. *J. Physiol.*, **507,** 869-880.

JORDAN, D. (1995). CNS integration of cardiovascular regulation. In *Studies in Physiology.* ed. Jordan, D. & Marshall, J. pp. 1-14. London: Portland Press.

JORDAN, D. & SPYER, K.M. (1979). Studies on the excitability of sinus nerve afferent terminals. *J. Physiol.*, **297,** 123-134.

JORDAN, D. & SPYER, K.M. (1986). Brainstem integration of cardiovascular and pulmonary afferent activity. *Prog. Brain Res.*, **67,** 295-314.

JORDAN, D., SPYER, K.M., WITHINGTON-WRAY, D.J. & WOOD, L.M. (1986). Histochemical and electrophysiological identification of cardiac and pulmonary vagal preganglionic in the cat. *J. Physiol.*, **372,** 87P.

KAADA, B.R. (1960). Cingulate, posterior orbital, anterior insular and temporal pole cortex. In *Handbook of Physiology, Section 1: Neurophysiology,* Vol II. ed. Field, J., Magoun, H.W. & Hall, V.E. pp. 345-372. Washington DC: American Physiological Society.

KALIA, M., FUXE, K., HOKFELT, T., JOHANSSON, D., LANG, R., GANTEN, D., CUELLO, C. & TERENIUS, L. (1984). Distribution of neuropeptide immunoreactive nerve terminals within the subnuclei of the nucleus tractus solitarius of the rat. *J. Comp. Neurol.*, **222,** 409-444.

KALIA, M. & MESULAM, M. (1980). Brain stem projections of sensory and motor components of the vagus complex in the cat. *J. Comp. Neurol.*, **193,** 435-508.

KALIA, M. & RICHTER, D.W. (1985). Morphology of physiologically identified slowly adapting lung stretch receptor afferents stained with intra-axonal horseradish peroxidase in the nucleus of the tractus solitarius of the cat. II. An ultrastructural analysis. *J. Comp. Neurol.*, **241,** 521-535.

KAPP, B.S., GALLAGHER, M., UNDERWOOD, M.D., McNALL, C.L. & WHITEHORN, D. (1982). Cardiovascular responses elicited by electrical stimulation of the amygdala central nucleus in the rabbit. *Brain Res.*, **234,** 251-262.

KATAFUCHI, T., OOMURA, Y. & KUROSAWA, M. (1988). Effects of chemical stimulation of paraventricular nucleus on adrenal and renal nerve activity in rats. *Neurosci. Letts.*, **86,** 195-200.

KAUMANN, A.J., FRENKEN, M., POSIVAL, H. & BROWN, A.M. (1994). Variable participation of 5-HT_1-like receptors and 5-HT_2 receptors in serotonin-induced contraction of human isolated coronary arteries. *Circulation*, **90,** 1141-1153.

KENNETT, G.A. (1999). Serotonin receptors and their function, *Tocris supplement*, 1-12.

KENNETT, G.A., (1993). 5-HT_{1C} receptors and their therapeutic relevance. *Curr. Opin. Invest Drugs*, **2,** 317.

KENNETT, G.A., AINSWORTH, K., TRAIL, B. & BLACKBURN, T.P. (1997). BW 723C86, a 5-HT_{2B} receptor agonist, causes hyperphagia and reduced grooming in rats. *Neuropharmacol.*, **36,** 233-239.

KENNETT, G.A., BRIGHT, F., TRAIL, B., BLACKBURN, T.P. & SANGER, G.J. (1997). Anxiolytic-like actions of the selective 5-HT_4 receptor antagonists SB 204070A and SB 207266A in rats. *Neuropharmacol.*, **36,** 707-712.

KENNETT, G.A., WOOD, M.D., BRIGHT, F., TRAIL, B., RILEY, G., HOLLAND, V., AVENELL, K.Y., STEAN, T., UPTON, N., BROMIDGE, S., FORBES, I.T., BROWN, A.M., MIDDLEMISS, D.N. & BLACKBURN, T.P. (1997). SB242084, a selective and brain penetrant 5-HT_{2C} receptor antagonist. *Neuropharmacol.*, **36,** 609-620.

KING, K.A. & HOLTMAN, J.R. (1989). Characterization of the effects of activation of ventral medullary serotonin receptor subtypes on cardiovascular activity and respiratory motor outflow to the diaphragm and larynx. *J. Pharmacol. Exp. Ther.*, **252,** 665-674.

KILPATRICK, G.J., BUTLER, A., BURRIDGE, J. & OXFORD, A.W. (1990). 1-(m-chlorophenyl)-biguanide, a potent high affinity 5-HT3 receptor agonist. *Eur. J. Pharmacol.*, **182,** 193-197.

KNOWLES, I.D. & RAMAGE, A.G. (1998). The pressor response evoked by activation of forebrain 5-HT_{2A} receptors involves the activation of central AT_1 receptors in anaesthetized rats. *Br. J. Pharmacol.*, **123,** 96P.

KNOWLES, I.D. & RAMAGE, A.G. (1999). Evidence for a role for central 5-HT_{2B} as well as 5-HT_{2A} receptors in cardiovascular regulation in anaesthetized rats. *Br. J. Pharmacol.*, **128,** 530-542.

KNOWLES, I.D. & RAMAGE, A.G. (2000). Evidence that activation of central 5-HT_{2B} receptors causes renal sympathoexcitation in anaesthetized rats. *Br. J. Pharmacol.*, **129,** 177-183.

KNUEPFER, M.M., GEBHART, G.F. & BRODY, M.J. (1985). Effects of baroreceptor activation on single unit activity of the anteroventral third ventricle region of the rat. *Neurosci. Lett.*, **56,** 79-85.

KOE, B.K., NIELSEN, J.A., MACOR, J.E. & HEYM, J. (1992). Biochemical and behavioural studies of the 5-HT_{1B} receptor agonist, CP-94,253. *Drug Dev. Res.*, **26,** 241-250.

KOHEN, R., METCALF, M.A., KHAN, N., DRUCK, T., HUEBNER, K., LACHOWICZ, J.E., MELTZER, H.Y., SIBLEY, D.R., ROTH, B.L. & HAMBLIN, M.W. (1996). Cloning, characterization and chromosomal localization of a human 5-HT_6 serotonin receptor. *J. Neurochem.*, **66,** 47-56.

KOSHIYA, N. & GUYENET, P.G. (1996). NTS neurons with carotid chemoreceptor inputs arborize in the rostral ventrolateral medulla. *Am. J. Physiol.*, **270,** R1273-R1278.

KOULU, M., SAAVEDRA, J.M., NIWA, M., SCHEININ, M. & LINNOILA, M. (1986). Association between increased serotonin metabolism in rat brainstem nuclei and development of spontaneous hypertension. *Brain Res.*, **371,** 177-181.

KRAYER, O. (1961). The history of the Bezold-Jarisch effect. *Naunyn-Schmeidbergs Arch. Exp. Pathol. Pharmakol.*, **240,** 361-368.

KUBIN, L. & DAVIES, R.O. (1985). Antidromic mapping of the brainstem projections of pulmonary rapidly adapting receptor neurons in the cat. In *Neurogenesis of Central Respiratory Rhythm.* ed. Bianchi, A.L. & Denavit-Saubie, M. pp. 262-265. Lancaster: MTP Press Ltd.

KUBIN, L., KIMURA, H. & DAVIES, R.O. (1991). The medullary projections of afferent bronchopulmonary C fibres in the cat as shown by antidromic mapping. *J. Physiol.*, **435,** 207-228.

KUHN, D.M., WOLF, R.A. & LOVENBERG, W. (1980). Review of the role of the central serotonergic system in blood pressure regulation. *Hypertension*, **2,** 243-255.

KWIAT, G.C. & BASBAUM, A.I. (1990). Organization of tyrosine hydroxylase-immunoreactive and serotonin-immunoreactive brainstem neurons with axon collaterals to the periaqueductal gray and the spinal cord in the rat. *Brain Res.*, **528,** 83-94.

LAGUZZI, R., REIS, D.J. & TALMAN, W.T. (1984). Modulation of cardiovascular and electrocortical activity through serotonergic mechanisms in the nucleus tractus solitarius of the rat. *Brain Res.*, **304,** 321-328.

LAMOTHE, M., PAUWELS, P.J., BELLIARD, K., SCHAMBEL, P. & HALAZY, S. (1997). Differentiation between partial agonists and neutral 5-HT_{1B} antagonists by chemical modulation of 3-[3-(N,N-dimethylamino)propyl]-4-hydroxy-N-[4-pyridin-4-yl)phenyl]benzamide (GR-55562). *J. Med. Chem.*, **40,** 3542-3550.

LAUBIE, M., DROUILLAT, M., DABIER, H., CHERIQUI, 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, A. & JARROTT, B. (1996). Neurochemical Modulation of cardiovascular control in the nucleus tractus solitarius, *Prog. Neurobiol.*, **48,** 21-53.

LAWRENCE, A.J., KRSTEW, E. & JARROTT, B. (1995). Functional dopamine D_2 receptors on rat vagal afferent neurons. *Br. J. Pharmacol.*, **114,** 1329-1334.

LEAL, D.M., CALLERA, J.C., BONAGAMBA, L.G.H., NOSJEAN, A., LAGUZZI, R. & MACHADO, B.H. (2001) Microinjection of a 5-HT_3 receptor agonist into the NTS of awake rats inhibits the bradycardic response to activation of the von Bezold-Jarisch reflex. *Brain Res. Bull.*, **54,** 7-11.

LEE, T.M., KUO, J.S. & CHAI, C.Y. (1972). Central integrating mechanism of the Bezold-Jarisch and baroreceptor reflexes. *Am. J. Physiol.*, **222,** 713-720.

LEONE, C. & GORDON, F.J. (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-HT_{1E}) in human brain : interaction with a GTP-binding protein. *J. Neurochem.*, **53,** 465-471.

LESLIE, R.A., REYNOLDS, D.J.M., ANDREWS, P.L.R., GRAHAME-SMITH, D.G., DAVIS, C.J. & HARVEY, J.M. (1990). Evidence for presynaptic 5-Hydroxytryptamine$_3$ recognition sites on vagal afferent terminals in the brainstem of the ferret. *Neuroscience*, **38,** 667-673.

LESLIE, R.A., REYNOLDS, D.J.M. & NEWBERRY, N.R. (1994). Localization of 5-HT_3 receptors. In *5-Hydroxytryptamine-$_3$ receptor antagonists.* ed. King, F.D., Jones, B.J. & Sanger, G.J. pp. 79-96. Ann Arbor, MI: CRC Press Inc.

LEXCHIN, J.L., CUDE-SIMPSON, K.D. & STANCER, H.C. (1977). Brain and blood indole metabolites after peripheral administration of ^{14}C-5-HT in rat. *Neurochem. Res.*, **2,** 39-50.

LI, J., SMITH, S.A. & MITCHELL, J.H. (2002). The arterial baroreflex attenuates the pressor response to exercise by increasing nitric oxide formation within the nucleus tractus solitarius. *Proceedings of the Experimental Biology 2002 meeting*, 131.4.

LOEWY, A.D., MARSON, L., PARKINSON, D., PERRY, M.A. & SAWYER, W.B. (1986). Descending noradrenergic pathways involved in the A5 depressor response. *Brain Res.*, **386,** 313-324.

LOEWY, A.D. & 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,** 421-450.

LOEWY, A.D. & NEIL, J.J. (1981). The role of descending monoaminergic systems in central control of blood pressure. *Fed. Proc.*, **40,** 2778-2785.

LOVICK, T.A. (1992). Inhibitory modulation of the cardiovascular defence response by the ventrolateral periaqueductal grey matter in rats. *Exp. Brain Res.*, **89,** 133-139.

LOVICK, T.A. & COOTE, J.H. (1988). Electrophysiological properties of paraventriculo-spinal neurones in the rat. *Brain Res.*, **454,** 123-130.

LUITEN, P.G.M., TER HORST, G.J. & STEFFANS, A.B. (1987). The hypothalamus, intrinsic connections and outflow pathways to the endocrine system in relation to the control of feeding and metabolism. *Prog. Neurobiol.*, **28,** 1-54.

LUITEN, P.G.M., TER HORST, G.J., KARST, H. & STEFFENS, A.B. (1985). The course of paraventricular hypothalamic efferents to autonomic structures in medulla and spinal cord. *Brain Res.*, **329,** 374-378.

MA, S., ABBOUD, F.M. & FELDER, R.B. (1995). Effects of L-arginine-derived nitric oxide synthesis on neuronal activity in nucleus tractus solitarius. *Am. J. Physiol.*, **268,** R487-R491.

MACHADO, B.H. & BONAGAMBA, L.G.H. (1992). Microinjection of L-glutamate into the nucleus tractus solitarii increases arterial pressure in conscious rats. *Brain Res.*, **576,** 131-138.

MACHADO, B.H. & BONAGAMBA, L.G.H. (1992). Microinjection of S-nitrosocysteine into the nucleus tractus solitarii of conscious rats decreases arterial pressure but L-glutamate does not. *Eur. J. Pharmacol.*, **221,** 179-182.

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.

MALEY, B.E. & SEYBOLD, V.S. (1993). Distribution of [^{3}H]quincyclidinyl benzilate, [^{3}H]nicotine, and [^{3}H]alpha-bungarotoxin binding sites in the nucleus tractus solitarii of the cat. *J. Comp. Neurol.*, **327,** 197-204.

MANAKER, S. & VERDERAME, H.M. (1990). Organization of serotonin 1A and 1B receptors in the nucleus of the solitary tract. *J. Comp. Neurol.*, **301,** 535-553.

MANDAL, A.K., KELLAR, K.J., NORMAN, W.P. & GILLIS, R.A. (1990). Stimulation of serotonin$_2$ receptors in the ventrolateral medulla of the cat results in non-uniform increases in sympathetic outflow. *Circ. Res.*, **67,** 1267-1280.

MARK, A.L. (1983). The Bezold-Jarisch reflex revisited: clinical implications of inhibitory reflexes originating in the heart. *J. Am. Coll. Cardiol.*, **1,** 90-102.

MARSHALL, J.M. (1994). Peripheral chemoreceptors and cardiovascular regulation. *Physiol. Rev.*, **74,** 543-594

MARWOOD, J.F. & STOKES, G.S. (1984). Serotonin (5-HT) and its antagonists: involvement in the cardiovascular system. *Clin. Exp. Pharmacol. Physiol.*, **11,** 439-455.

MATSUGUCHI, H., SHARABI, F.M., GORDON, F.J., JOHNSON, A.K. & SCMID, P.G. (1982). Blood pressure and heart rate responses to microinjection of vasopressin into the nucleus tractus solitarius region of the rat. *Neuropharmacol.*, **21,** 687-693.

MATTHES, H., BOSCHERT, U., AMLIAKY, N., GRAILHE, R., PLASSAT, J.L., MUSCATELLI, F., MATTEI, M.G. & HEN, R. (1993). Mouse 5-hydroxytryptamine$_{5A}$ and 5-hydroxytryptamine$_{5B}$ receptors define a new family of serotonin receptors: cloning, functional expression, and chromosomal localization. *Mol. Pharmacol.*, **43,** 313-319.

MAURA, G. & RAITERI, M. (1996). Serotonin 5-HT$_{1D}$ and 5-HT$_{1A}$ receptors respectively mediate inhibition of glutamate release and inhibition of cyclic GMP production in rat cerebellum in vitro. *J. Neurochem.*, **66,** 203-209.

McALLEN, R.M. & SPYER, K.M. (1976). The location of cardiac vagal preganglionic neurones in the medulla of the cat. *J. Physiol.*, **258,** 187-204.

McALLEN, R.M. & SPYER, K.M. (1978a). Two types of vagal preganglionic motoneurones projecting to the heart and lungs. *J. Physiol.*, **282,** 353-364.

McALLEN, R.M. & SPYER, K.M. (1978b). The baroreceptor input to cardiac vagal motoneurones. *J. Physiol.*, **282,** 365-374.

McCALL, R.B. & CLEMENT, M.E. (1994). Role of serotonin$_{1A}$ and serotonin$_2$ receptors in the central regulation of the cardiovascular system. *Pharmacol. Rev.*, **46,** R1044-R1049.

McCALL, R.B. & HARRIS, L.T. (1987). Sympathetic alterations after midline medullary raphe lesions. *Am. J. Physiol.*, **253,** R91-R100.

MEELEY, M.P., UNDERWOOD, M.D., TALMAN, W.T. & REIS, D.J. (1989). Content and *in vitro* release of endogenous amino acids in the area of the nucleus of the solitary tract of the rat. *J. Neurochem.*, **53,** 1807-1817.

MERAHI, N. & LAGUZZI, R. (1995). Cardiovascular effects of 5-HT_2 and 5-HT_3 receptor stimulation in the nucleus tractus solitarius of spontaneously hypertensive rats. *Brain Res.*, **669,** 130-134.

MERAHI, N., ORER, H.S., LAPORTE, A.M., GOZLAN, H., HAMON, M. & LAGUZZI, R. (1992). Baroreceptor reflex inhibition induced by the stimulation of serotonin$_3$ receptors in the nucleus tractus solitarius of the rat. *Neuroscience*, **46,** 91-100.

MIDDLEMISS, D.N., BLAKEBOROUGH, L. & LEATHER, S.R. (1977). Direct evidence for an interaction of β-adrenergic blockers with the 5-HT receptor. *Nature*, **267,** 289-290.

MIDDLEMISS, D.N. & HUTSON, P.H. (1990). The 5-HT_{1B} receptors. *Ann. N.Y. Acad. Sci.*, **600,** 132-147.

MIFFLIN, S.W. (1993). Inhibition of chemoreceptor inputs to nucleus of tractus solitarius neurons during baroreceptor stimulation. *Am. J. Physiol.*, **265,** R14-20.

MIFFLIN, S.W. (1996) Convergent carotid sinus nerve and superior laryngeal nerve afferent inputs to neurons in the NTS. *Am. J. Physiol.*, **271,** R870-R880.

MIFFLIN, S.W. & FELDER, R.B. (1990). Synaptic mechanisms regulating cardiovascular afferent inputs to solitary tract nucleus. *Am. J. Physiol.*, **259,** H653-661.

MIFFLIN, S.W., SPYER, K.M. & WITHINGTON-WRAY, D.J. (1988). Baroreceptor inputs to the nucleus tractus solitarius in the cat: modulation by the hypothalamus. *J. Physiol.*, **399,** 369-387

MIQUEL, M.C., DOUCET, E., BONI, C., ELMESTIKAWY, S., MATTHIESSEN, L., DAVAL, G., VERGE, D. & HAMON, M. (1991). Central serotonin$_{1A}$ receptors: respective distributions of encoding mRNA, receptor protein and binding sites by in situ hybridisation histochemistry, radioimmunohistochemistry and autoradiographic mapping in the rat brain. *Neurochem. Int.*, **19,** 453-465.

MIQUEL, M.C., DOUCET, M.C., RIAD, M., ADRIEN, J., VERGE, D. & HAMON, M. (1992). Effect of the selective lesion of serotoninergic neurons on the regional distribution of 5-HT$_{1A}$ receptor mRNA in the rat brain. *Brain Res.*, **14,** 357-362.

MORALES, M., BATTENBERG, E. & BLOOM, F.E. (1998). Distribution of neurons expressing immunoreactivity for the 5-HT$_3$ receptor subtype in the rat brain and spinal cord. *J. Comp. Neurol.*, **385,** 385-401.

MORALES, M. & BLOOM, F.E. (1997). The 5-HT$_3$ receptor is present in different subpopulations of GABAergic neurons in the rat telencephalon. *J. Neurosci.*, **17,** 3157-3167.

MOSQUEDA-GARCIA, R., TSENG, C.-J., APPALSAMY, M. & ROBERTSON, D. (1989). Modulatory effects of adenosine on baroreflex activation in the brain-stem of normotensive rats. *Eur. J. Pharmacol.*, **174,** 119-122.

NEIL, J.J. & LOEWY, A.D. (1982). Decreases in blood pressure in response to L-glutamate microinjections into the A5 catecholamine cell group. *Brain Res.*, **241,** 271-278.

NELSON, D.R. & THOMAS, D.R. (1989). [^{3}H]-BRL 43694 (Granisetron), a specific ligand for 5-HT$_3$ binding sites in rat brain cortical membranes. *Biochem. Pharmacol.*, **38,** 1693-1695.

NORGREN, R. (1976). Taste pathways to hypothalamus and amygdala. *J. Comp. Neurol.*, **166,** 17-30.

NOSAKA, S., YAMAMOTO, T. & YASUNAGA, K. (1979). Localization of vagal cardioinhibitory preganglionic neurons within rat brain stem. *J. Comp. Neurol.*, **186,** 79-92.

NOSJEAN, A., COMPOINT, C., BUISSERET-DELMAS, C., ORER, H.S., MERAHI, N., PUIZILLOUT, J.-J. & LAGUZZI, R. (1990). Serotonergic projections from the nodose ganglia to the nucleus tractus solitarius: an immunohistochemical and double labelling study in the rat. *Neurosci. Lett.*, **114,** 22-26.

NOSJEAN, A., FRANC, B. & LAGUZZI, R. (1995). Increased sympathetic nerve discharge without alteration in the sympathetic baroreflex response by serotonin$_3$ receptor stimulation in the nucleus tractus solitarius of the rat. *Neurosci. Lett.*, **186,** 41-46.

OKADA, M. & BUNAG, R.D. (1994). Selective enhancement in SHR of hypotension and bradycardia caused by NTS-injected serotonin. *Am. J. Physiol.*, **266,** R599-R605.

OKSENBERG, D., MARSTERS, S.A., O'DOWD, B.F., JIN, H., HAVLIK, S., PEROUTKA, S.J. & ASHKENAZI, A. (1992). A single amino-acid difference confers major pharmacological variation between human and rodent 5-HT$_{1B}$ receptors. *Nature*, **360,** 161-163.

ORTELLS, M.O. & LUNT, G.G. (1995). Evolutionary history of the ligand-gated ion-channel superfamily of receptors. *Trends Neurosci.*, **18,** 121-127.

PAGNIEZ, F., VALENTIN, J-F., VIEU, S., COLPAERT, F.C. & JOHN, G.W. (1998). Pharmacological analysis of the haemodynamic effects of 5-HT$_{1B/D}$ receptor agonists in the normotensive rat. *Br. J. Pharmacol.*, **123,** 205-214.

PAINTAL, A.S. (1995). Some recent advances in studies on J receptors. *Adv. Exp. Med. Biol.*, **381,** 15-25.

PALACIOS, J.M., WAEBER, C., MENGOD, G., et al. (1991). Autoradiography of 5-HT receptors: a critical appraisal. *Neurochem. Int.*, **18,** 17-25.

PARKER, R.M.C., BENTLEY, K.R. & BARNES, K.R. (1996). Allosteric modulation of 5-HT_3 receptors: focus on alcohols and anaesthetic agents. *Trends Pharmacol. Sci.*, **17,** 95-99.

PASCUAL, J., DEL ARCO, C., ROMON, T., DEL OLMO, E., CASTRO, E. & PAZOS, A. (1996). Autoradiographic distribution of [3H]sumatriptan binding sites in post-mortem human brain. *Cephalalgia*, **16,** 317-322.

PATEL, S., ROBERTS, J., MOORMAN, J. & REAVILL, C. (1995). Localization of serotonin-$_4$ receptors in the striatonigral pathway in the rat brain. *Neuroscience*, **69,** 1159-1167.

PATEL, K.P. & SCHMID, P.G. (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, J.F.R. (1996). A working heart-brainstem preparation of the mouse. *J. Neurosci. Methods*, **65,** 63-68.

PATON, J.F.R. (1998). Pattern of cardiorespiratory afferent convergence to solitary tract neurons driven by pulmonary vagal C-fiber stimulation in the mouse. *J. Neurophysiol.*, **79,** 2365-2373.

PATON, J.F.R. & KASPAROV, S. (1999). Differential effects of angiotensin II on cardiorespiratory reflexes mediated by nucleus tractus solitarii – a microinjection study in rat. *J. Physiol.*, **521,** 213-225.

PATON, J.F.R., LI, Y.W. & KASPAROV, S. (1999). Reflex response and convergence of pharyngoesophageal and peripheral chemoreceptors in the nucleus of the solitary tract. *Neuroscience*, **93,** 143-154.

PATON, J.F.R., DEUCHARS, J., AHMAD, W., WONG, L-F., 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, P.J. & COLPAERT, F.C. (1995). The 5-HT_{1D} receptor antagonist GR 127,935 is an agonist at cloned human 5-$HT_{1D\square}$ receptor sites. *Neuropharmacol.*, **34,** 235-237.

PAUWELS, P.J. & COLPAERT, F.C. (1996). Selective antagonism of human 5-HT_{1D} and 5-HT_{1B} receptor-mediated responses in stably transfected C6-glial cells by ketanserin and GR 127935. *Eur. J. Pharmacol.*, **300,** 141-145.

PAZOS, A. & PALACIOS, J.M. (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain, I, Serotonin-1 receptors. *Brain Res.*, **346,** 205-230.

PERGOLA, P.E., SVED, A.F., VOOGT, J.L. & ALPER, R.H. (1993). Effect of serotonin on vasopressin release: A comparison to corticosterone, prolactin and renin. *Neuroendocrinol.*, **57,** 550-558.

PETERS, J.A., HALES, T.G. & LAMBERT, J.J. (1988). Divalent cations modulate 5-HT_3 receptor-induced currents in N1E-115 neuroblastoma cells. *Eur. J. Pharmacol.*, **151,** 491-495.

PETROV, T., KRIKOFF, T.L. & JHAMANDAS, J.H. (1992). The hypothalamic paraventricular and lateral parabrachial nuclei receive collaterals from raphe nucleus neurons-A combined double retrograde and immunocytochemical study. *J.Comp.Neurol.*, **318,** 18-26.

PINEYRO, G., CASTANON, N., HEN, R. & BLIER, P. (1995). Regulation of [^{3}H]5-HT release in raphe, frontal cortex and hippocampus of 5-HT_{1B} knock-out mice. *NeuroReport*, **7,** 353-359.

PIRES, J.G.P., SILVA, S.R., RAMAGE, A.G. & FUTURO-NETO, H.A. (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 anaesthetized rat. *Brain Res.*, **791,** 229-234.

PIRES, J.G. & RAMAGE, A.G. (1990). Evidence suggesting that the 5-HT_2 antagonist ICI 169,369 activates vagal afferents and in addition has a central hypotensive action in anaesthetized rats. *J. Auton. Pharmacol.*, **10,** 345-351.

POTTER, E.K. (1981). Inspiratory inhibition of vagal responses to baroreceptor and chemoreceptor stimuli in the dog. *J. Physiol.*, **316,** 177-190.

PONTIERI, V., VENEZUELA, M.K., SCAVONE, C. & MICHELINI, L.C. (1998). Role of endogenous nitric oxide in the nucleus tractus solitarii on baroreflex control of heart rate in spontaneously hypertensive rats. *J. Hypertens.*, **16,** 1993-1999.

PRATT, G.D. & BOWERY, N.G. (1989). The 5-HT_3 receptor ligand [^{3}H]BRL 43694 binds to presynaptic sites in the nucleus tractus solitarius of the rat. *Neuropharmacol.*, **28,** 1367-1376.

PRZEGALINSKI, E., SIWANOWICZ, J. PAPLA, I. & FILIP, M. (2002). Effects of 5-HT_{1B} receptor ligands microinjected into the accumbal shell or core on the sensitization to cocaine in rats. *Eur. Neuropsychopharmacol.*, **12,** 387-396.

RAMAGE, A.G. (2000) Central 5-HT_{1A} receptors and vagal tone to the airways. *Trends Pharmacol. Sci.*, **21,** 201-202.

RAMAGE, A.G. (2001) Central cardiovascular regulation and 5-hydroxytryptamine receptors. *Brain Res. Bull.*, **56,** 425-439.

RAMAGE, A.G., WOUTERS, W. & BEVAN, P. (1988). Evidence that the novel antihypertensive agent , flesinoxan causes differential sympathoinhibition and also increases vagal tone by a central action. *Eur. J. Pharmacol.*, **151,** 373-374.

RAMAGE, A.G. & DALY, M. DE B. (1998). The central action of the 5-HT_2 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, A.G. & MIRTSOU- FIDANI, V. (1995). Examination of the cardiovascular effects of WAY-100802, a selective 5-HT_{1A} antagonist in anaesthetized cats. *Br. J. Pharmacol.*, **116,** 289P.

RAMAGE, A.G., SHEPHEARD, S.L., JORDAN, D. & KOSS, M.C. (1993). Can the 5-$HT_{2/1C}$ agonist DOI cause differential sympatho-excitation in nerves supplying the heart in anaesthetized cats? *J. Auton. Nerv. Syst.*, **42,** 53-62.

RAPPORT, M.M., GREEN, A.A. & PAGE, I.H. (1948). Serum vasoconstrictor (serotonin) IV. Isolation and characterisation. *J. Biol. Chem.*, **176,** 1243-1251.

REID, I.A. (1992). Interactions between ANG II, sympathetic nervous system, and baroreceptor reflexes in regulation of blood pressure. *Am. J. Physiol.*, **262,** E763-E778.

RICARDO, J.A. & KOH, E.T. (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, B.P., ENGEL, G., DONATSCH, P. & STADLER, P.A. (1985). Identification of 5-hydroxytryptamine M-receptor subtypes and their specific blockade by a new class of drugs. *Nature*, **316,** 126-131.

ROBERTS, C., PRICE, G.W., GASTER, L., JONES, B.J., MIDDLEMISS, D.H. & ROUTLEDGE, C. (1997). Importance of h5-HT_{1B} receptor selectivity for 5-HT terminal autoreceptor activity: an in vivo microdialysis study in the freely moving guinea pig. *Neuropharmacol.*, **36,** 549-557.

ROSS,C.A., RUGGIERO, D.A. & REIS, D.J. (1985). Projections from the nucleus tractus solitarii to the rostral ventrolateral medulla. *J. Comp. Neurol.*, **242,** 511-534.

RUDOMIN, P. (1967). Presynaptic inhibition induced by vagal afferent volleys. *J. Neurophysiol.*, **30,** 964-981.

RUGGIERO, D.A., MRAOVITCH, S., GRANATA, A.R., ANWAR, M. & REIS, D.J. (1987). A role of insular cortex in cardiovascular function. *J. Comp. Neurol.*, **257,** 189-207.

SAHA, S., BATTEN, T.F.C. & McWILLIAM, P.N. (1995). Glutamate-immunoreactivity in identified vagal afferent terminals of the cat: A study combining horseradish peroxidase tracing and electron microscopic immunogold staining. *Exp. Physiol.*, **80,** 193-202.

SANGER, G.J. & NELSON, D.R. (1989). Selective and functional 5-hydroxytryptamine$_3$ receptor antagonism by BRL 43694 (granisetron). *Eur. J. Pharmacol.*, **159,** 113-124.

SAPER, C. (1995) Central autonomic system. In: The Rat Nervous System. 2nd Ed. ed. Paxinos, G. pp. 107-135. Academic Press.

SAPER, C.B. (1982) Reciprocal parabrachial-cortical projections in the rat. *Brain Res.*, **242,** 33-40.

SAPER, C.B. & LEVISOHN, D. (1983). Afferent connections of the median preoptic nucleus in the rat: Anatomical evidence for a cardiovascular integrative mechanism in the anteroventral third ventricular (AV3V) region. *Brain Res.*, **288,** 21-31.

SAPER, C.B. & LOEWY, A.D. (1980). Efferent connections of the parabrachial nucleus in the rat. *Brain Res.* **197,** 291-317.

SARHAN, H., CLOEZ-TAYARANI, I., MASSOT, O., FILLION, M-P. & FILLION, G. (1999). 5-HT_{1B} receptors modulate release of [^{3}H]dopamine from rat striatal synaptosomes. *Naunyn-Schmeidberg's Arch. Pharmacol.*, **359,** 40-47.

SAWCHENKO, P.E. & SWANSON, L.W. (1982). The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. *Brain. Res. Rev.*, **4,** 275-326.

SCHAFFER, N., KESSLER, J.P., BOSLER, O. & JEAN, A. (1988). Central serotonergic projections to the nucleus tractus solitarii: Evidence from a double labelling study in the rat. *Neuroscience*, **26,** 951-958.

SCHLICKER, E., FINK, K., MOLDERINGS, G.J., PRICE, G.W., DUCKWORTH, M., GASTER, L., MIDDLEMISS, D.N., ZENTNER, J., LIKUNGU, J. & GOTHERT, M. (1997). Effects of selective 5-HT_{1B} (SB-216 641) and 5-HT_{1D} (BRL-15572) receptor ligands on guinea-pig and human 5-HT auto- and heteroreceptors. *Naunyn-Schmeidberg's Arch. Pharmacol.*, **356,** 321-327.

SCHOEFFTER, P. & HOYER, D. (1989). How selective is GR 43175? Interactions with 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} receptors. *Naunyn-Schmeidberg's Arch. Pharmacol.*, **340,** 135-138.

SCHREIBER, R., BROCCO, M., AUDINOT, V., GOBERT, A., VEIGA, S. & MILLAN, M.J. (1995). 1-(2,5-dimethoxy-4 iodophenyl)-2-aminopropane)-induced head-twitches in the rat are mediated by 5-hydroxytryptamine $(5\text{-HT})_{2A}$ receptors: modulation by novel 5-$HT_{2A/C}$ antagonists, D_1 antagonists and 5-HT_{1A} agonists. *J. Pharmacol. Exp. Ther.*, **273,** 101-112.

SCHREIHOFER, A.M. & GUYENET, P.G. (2002). The baroreflex and beyond: control of sympathetic vasomotor tone by GABAergic neurons in the ventrolateral medulla. *Clin. Exp. Pharmacol. Physiol.*, **29,** 514-521.

SCHWABER, J.S., KAPP, B.S., HIGGINS, G.A. & RAPP, P.R. (1982). Amygdaloid and basal forebrain direct connections with the nucleus of the solitary tract and the dorsal motor nucleus. *J. Neurosci.*, **2,** 1424-1438.

SEBBEN, M., ANSANAY, H., BOCKAERT, J. & DUMUIS, A. (1994). 5-HT_6 receptors positively coupled to adenylyl cyclase in striatal neurone in culture. *NeuroReport*, **5,** 2553-2557.

SÉVOZ, C., NOSJEAN, A., CALLERA, J.C., MACHADO, B.H., HAMON, M. & LAGUZZI, R. (1996). Stimulation of 5-HT_3 receptors in the NTS inhibits the cardiac Bezold-Jarisch reflex response. *Am. J. Physiol.*, **271,** H80-H87.

SÉVOZ-COUCHE, C., WANG, Y., RAMAGE, A.G. & JORDAN, D. (2000a). Modulation of neurones in the nucleus tractus solitarius (NTS) of the rat by 5-HT_2 receptors. *Neuropharmacol.*, **39,** 2006-2016.

SÉVOZ-COUCHE, C., SPYER, K.M. & JORDAN, D. (2000b). *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.

SHEPHEARD, S.L., JORDAN, D. & RAMAGE, A.G. (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.

SHEPHEARD, S., EDVINSSON, L., CUMBERBATCH, M., WILLIAMSON, D., MASON, G., WEBB, J., BOYCE, S., Hill, R., & Hargreaves R. (1999). Possible antimigraine mechanisms of action of the 5HT1F receptor agonist LY334370. *Cephalalgia*, **19,** 851-858.

SILVA-CARVALHO, L., PATON, J.F.R., ROCHA, I., GOLDSMITH, G.E. & SPYER, K.M. (1998). Convergence properties of solitary tract neurons responsive to cardiac receptor stimulation in the anaesthetized cat. *J. Neurophysiol.*, **79,** 2374-2382.

SKINGLE, M., BEATTIE, D.T., SCOPES, D.I., STARKEY, S.J., CONNOR, H.E., FENIUK, W. & TYERS, M.B. (1996). GR127935: a potent and selective 5-HT_{1D} receptor antagonist. *Behav. Brain Res.*, **73,** 157-161.

SORENSEN, S.M., KEHNE, J.H., FADAYEL, G.M., HUMPHREYS, T.M., KETTELER, H.J., SULLIVAN, C.K., TAYLOR, V.L. & SCHMIDT, C.J. (1993). Characterization of the 5-HT_2 receptor antagonist MDL 100 907 as a putative atypical antipsychotic: behavioural, electrophysiological and neurochemical studies. *J. Pharmacol. Exp. Ther.*, **266,** 684-691.

SPROUSE, J.S. & AGHAJANIAN, G.K. (1986). (-)-Propanolol blocks the inhibition of serotonergic dorsal raphe cell firing by 5-HT_{1A} selective agonists. *Eur. J. Pharmacol.*, **128,** 295-298.

SPYER, K.M. (1982) Central nervous integration of cardiovascular control. *J. Exp. Biol.*, **100,** 109-128.

SPYER, K.M. (1994). Central nervous mechanisms contributing to cardiovascular control. *J. Physiol.*, **474,** 1-19.

SPYER, K.M. & THOMAS, T. (2000). A role for adenosine in modulating cardio-respiratory responses: A mini-review. *Brain Res. Bull.*, **53,** 121-124.

STARKEY, S.J. & SKINGLE, M. (1994). 5-HT_{1D} as well as 5-HT_{1A} autoreceptors modulate 5-HT release in the guinea pig dorsal raphe nucleus. *Neuropharmacol.*, **33,** 393.

STEINBUSCH, H.W.M. (1981). Distribution of serotonin-immunoreactivity in the central nervous system of the rat – cell bodies and terminals. *Neuroscience*, **6,** 557-618.

STEINBUSCH, H.W.M. (1984). Serotonin-immunoreactive neurons and their projections in the CNS. In *Handbook of Chemical Neuroanatomy*, Vol. 3. ed. Bjorklund, A., Hokfelt, T. & Kuhar, M.J. pp. 131-207. Amsterdam: Elsevier.

STEINBUSCH, H.W.M., VERHOFSTAD, A.A.J. & JOOSTEN, H.W.J. (1978). Localization of serotonin in the central nervous system: description of a specific and sensitive technique and some applications. *Neuroscience*, **3,** 811-819.

STEWARD, L.J., GE, J., BENTLEY, K.R., BARBER, P.C., HOPE, A.G., LAMBERT, J.J., PETERS, J.A., BLACKBURN, T.P. & BARNES, N.M. (1995). Evidence that the atypical 5-HT_3 receptor ligand, [^{3}H]-BRL 46470, labels additional 5-HT_3 binding sites compared to [^{3}H]-granisetron. *Br. J. Pharmacol.*, **116,** 1781-1788.

STREET, L.J., BAKER, R., DAVEY, W.B., GUIBLIN, A.R., JELLEY, R.A., REEVE, A.J., ROUTLEDGE, H., STERNFELD, F., WATT, A.P., BEER, M.S. et al. (1995). Synthesis and serotonergic activity of N,N-dimethyl-2-[54-(1,2,4-triazol-1-ylmethyl)-1H-indol-3-yl]ethylamine and analogues: potent agonists for 5-HT_{1D} receptors. *J. Med. Chem.*, **38,** 1799-1810.

SUKAMOTO, T., YAMAMOTO, T., WATANABE, S. & UEKI, S. (1984). Cardiovascular responses to centrally administered serotonin in conscious normotensive and spontaneously hypertensive rats. *Eur. J. Pharmacol.*, **100,** 173-179.

SUZUKI, M., KURAMOCHI, T. & SUGA, T. (1993). GABA receptor subtypes involved in the neuronal mechanisms of baroreceptor reflex in the nucleus tractus solitarii of rabbits, *J. Auton. Nerv. Sys.*, **43,** 27-36.

SVED, A.F., TSUKAMOTO, K. & SCHREIHOFER, A.M. (1992). Stimulation of α_2-adrenergic receptors in nucleus tractus solitarius is required for the baroreceptor reflex. *Brain Res.*, **576,** 297-303.

SYKES, R.M., SPYER, K.M. & IZZO, P.N. (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.

SZABAT, E., SOINILA, S., HAPPOLA, O., LINNALA, A. & VIRTANEN, I. (1992). A new monoclonal antibody against the GABA-protein conjugate shows immunoreactivity in sensory neurons of the rat. *Neuroscience*, **47,** 409-420.

TALMAN, W.T. (1989). Kynurenic acid microinjected into the nucleus tractus solitarius of rat blocks arterial baroreflex but not responses to glutamate. *Neurosci. Lett.*, **102,** 247-252.

TALMAN, W.T., PERRONE, M.H. & REIS, D.J. (1980). Evidence for L-glutamate as the neurotransmitter of baroreceptor afferent nerve fibres. *Science*, **209,** 813-815.

TALMAN, W.T. & ROBERTSON, S.C. (1989). Glycine, like glutamate, microinjected into the nucleus tractus solitarii of rat decreases arterial pressure and heart rate. *Brain Res.*, **477,** 7-13.

TAYLOR, E.W., JORDAN, D. & COOTE, J.H. (1999). Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. *Physiol. Rev.*, **79,** 855-916.

THOREN, P. (1979). Role of cardiac vagal C-fibers in cardiovascular control. *Rev. Physiol. Biochem. Pharmacol.*, **86,** 1-94.

TIMMS, T.J. (1981) A study of the amygdaloid defence reaction showing the value of althesin anaesthesia in studies of the functions of the fore-brain in cats. *Pflugers Arch.*, **391,** 49-56.

TRILLAT, A.-C., MALAGIE, I., SCEARCE, K., PONS, D., ANMELLA, M.C., JACQUOT, C., HEN, R. & GARDIER, A.M. (1997). Regulation of serotonin release in the frontal cortex and ventral hippocampus of homozygous mice lacking 5-HT_{1B} receptors: in vivo microdialysis studies. *J. Neurochem.*, **69,** 2019-2025.

TSENG, C-J., 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.

TSUKAMOTO, K., SVED, A.F., ITO, S., KOMATSU, K. & KATMATSUSE, K. (2000). Enhanced serotonin-mediated responses in the nucleus tractus solitarius of spontaneously hypertensive rats. *Brain Res.*, **863,** 1-8.

VAN NEUTEN, J.M., JANSSEN, P.A., VAN BEEK, J., XHONNEUX, R., VERBEUREN, T.J. & VANHOUTTE, P.M. (1981). Vascular effects of ketanserin (R 41 468), a novel antagonist of 5-HT_2 serotonergic receptors. *J. Pharmacol. Exp. Ther.*, **218,** 217-230.

VANOLI, E., DEFERRARI, G.M., STRAMBA-BADIALE, M., HULL Jnr., S.S., FOREMAN, R.D. & SCHWARTZ, P.J. (1991). Vagal stimulation and prevention of sudden death in conscious dogs with a healed myocardial infarction. *Circ. Res.*, **68,** 1471-1481.

VARDHAN, A., KACHROO, A. & SAPRU, H.N. (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.

VEELKEN, R., SAWIN, L.L. & DIBONA, G.F. (1990). Epicardial serotonin receptors in circulatory control in conscious Sprague-Dawley rats. *Am. J. Physiol.*, **258,** H466-H472.

VERBERNE, A.J.M., BEART, P.M. & LOUIS, W.J. (1989). Excitatory amino acid receptors in the caudal ventrolateral medulla mediate a vagal cardiopulmonary reflex in the rat. *Exp. Brain Res.*, **78,** 185-192.

VERBERNE, A.J.M. & GUYENET, P.G. (1992). Medullary pathway of the Bezold-Jarisch reflex in the rat. *Am. J. Physiol.*, **263,** R1195-R1202.

VERBEUREN, T.J. (1989). Synthesis, storage, release and metabolism of 5-hydroxytryptamine in peripheral tissues. In *The Peripheral Actions of 5-Hydroxytryptamine.* ed. Fozard, J.R. pp. 1-25. Oxford: Oxford University Press.

VILLALON, C.M., CENTURION, D., VALDIVIA, L.F., DE VRIES, P. & SAXENA, P.R. (2002). An introduction to migraine: from ancient treatment to functional pharmacology and antimigraine therapy. *Proc. West Pharmacol. Soc.*, **45,** 199-210.

WAKI, H. KASPAROV, S., WONG, L-F., MURPHY, D., SHIMIZU, T. & PATON, J.F.R. (2003). Chronic inhibition of endothelial nitric oxide synthase activity in nucleus tractus solitarii enhances baroreceptor reflex in conscious rats. *J. Physiol.*, **546,** 233-242.

WALSH, D.M, BEATTIE, D.T. & CONNOR, H.E. (1995). The activity of 5-HT_{1D} receptor ligands at cloned human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors. *Eur. J. Pharmacol.*, **287,** 79-84.

WANG, Y. & RAMAGE, A.G. (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., JONES, J.F., RAMAGE, A.G. & JORDAN, D. (1995). Effects of 5-HT and 5-HT_{1A} receptor agonists and antagonists on dorsal vagal preganglionic neurones in anaesthetized rats: an ionophoretic study. *Br. J. Pharmacol.*, **116,** 2291-2297.

WANG, Y., RAMAGE, A.G. & JORDAN, D. (1996). Mediation by 5-HT_3 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, A.G. & JORDAN, D. (1997). *In vivo* effects of 5-Hydroxytryptamine receptor activation on rat nucleus tractus solitarius neurones excited by vagal afferent C-fibre afferents. *Neuropharmacol.*, **36,** 489-498.

WANG, Y., RAMAGE, A.G. & JORDAN, D. (1998a). Presynaptic 5-HT_3 receptors evoke an excitatory response in dorsal vagal preganglionic neurones in anaesthetized rats. *J. Physiol.*, **509.3,** 683-694.

WANG, Y., SÉVOZ-COUCHE, C., JORDAN, D. & RAMAGE, A.G. (1998b). Activation of 5-HT_{1B} and 5-HT_{1D} receptors modulates the excitatory vagal afferent input to nucleus tractus solitarius (NTS) neurones in anaesthetized rats. *J. Physiol.*, **513,** 78P.

WHALEN, E.J., JOHNSON, A.K. & LEWIS, S.J. (2000). Functional evidence for the rapid desensitization of 5-HT_3 receptors on vagal afferents mediating the Bezold-Jarisch reflex. *Brain Res.*, **873,** 302-305.

WILSON, C.G., ZHANG, Z. & BONHAM, A.C. (1996). Non-NMDA receptors transmit cardiopulmonary C-fibre input in nucleus tractus solitarii in rats. *J. Physiol.*, **496,** 773-785.

XIE, Q., MIKI, T., SATO, K., ITOH, M. & TAKEUCHI, Y. (1998). Evaluation of pulmonary afferent fibers in the nucleus tractus solitarius: a horseradish peroxidase and c-fos like immunohistochemical study in the rat. *J. Auton. Nerv. Sys.*, **74,** 120-125.

YANG, Z. & COOTE, J.H. (1998). Influence of the hypothalamic paraventricular nucleus on cardiovascular neurones in the rostral ventrolateral medulla of the rat. *J. Physiol.*, **513,** 521-530.

YANG, Z. & COOTE, J.H. (1999). The influence of the paraventricular nucleus on baroreceptor dependent caudal ventrolateral medullary neurones of the rat. *Pflugers Arch.*, **438,** 47-52.

YASUI, Y., BREDER, C.D., SAPER, C.B. & CECHETTO, D.F. (1991). Autonomic responses and efferent pathways from the insular cortex in the rat. *J. Comp. Neurol.*, **303,** 355-374.

YU, X.J., WAEBER, C., CASTANON, N., SCEARCE, K., HEN, R., MACOR, J.E., CHAUVEAU, J., MOSKOWITZ, M.A. & CHAVEAU, J. (1996). 5-carboxamido-tryptamine, CP-122,288 and dihydroergotamine but not sumatriptan, CP-93,129 and serotonin-5-0-carboxymethyl-glycyl-tyrosinamide block dural plasma protein extravasation in knockout mice that lack 5-hydroxytryptamine$_{1B}$ receptors. *Mol. Pharmacol.*, **49,** 761-765.

ZANDBERG, P., DE JONG, W. & DE WIED, D. (1979). Effects of catecholamine-receptor stimulating agents on blood pressure after local application in the nucleus tractus solitarius of the medulla oblongata, *Eur. J. Pharmacol.*, **55,** 43-56.

ZHANG, X., ABDEL-RAHMAN, A.-R.A. & WOOLES, W.R. (1992). Vasopressin receptors in the area postrema differentially modulate baroreceptor responses in rats. *Eur. J. Pharmacol.*, **222,** 81-91.

ZHANG, W. & MIFFLIN, S.W. (1993). Excitatory amino acid receptors within NTS mediate arterial chemoreceptor reflexes in rats. *Am. J. Physiol.*, **265,** H770-H773.

ZIFA, E. & FILLION, G. (1992). 5-Hydroxytryptamine receptors. *Pharmacol. Rev.*, **44,** 401-458.

APPENDIX

Spike 2 script program for online heart rate derivation

```
File name: HeartBeatBoutique
'Author: Alan Donohoe
'Email: alandonohoe@rfc.ucl.ac.uk
'Description: Program detects blood pressure waveform peaks on-line to place
events in a memory buffer as beats/min, as a measurement of heart rate.
'Last changed: 13/7/00
'Basic Online Skeleton ('onSkel.s2s'), which enabled this script -
'written by Diarmid Campbell for CED September 1997.
var data%;                  'Handle of new data file
var sTime;          'The last time we looked at the idle routine
'**************VARIABLES THAT CAN BE SET TO INITIALISE DEFAULT
VALUES IN DIALOGUE BOX **********************
var readChan:=4;                'chan to get data from (eg: ECG trace)
var HCurs%:=0;                  'use HCursor(1) (1 = TRUE, 0 = FALSE)
var userval%:=1;                'use user's value (1 = TRUE, 0 = FALSE)
var membuf%:=1;                 'make new mem buffer (1 = TRUE, 0 =
FALSE)
var levelCross:=0.8;        'if own value of crossing is used this is the default
                            value of that level(eg: 1V)
var interval:=0.15;         'minimum interval b4 next peak
var dotz% := 2;                 'size of dots drawn
var lowY := 100;                'low range of Y axis of new mem buffer
var highY := 405;               'high range of Y axis of new mem buffer
*******************************************************************************************
var h% := 0;  'controls the choice whether to use HCursor(1) or own value. Used
in loop in 'idle()' func.
var newChan%;      'Memory Buffer that events are written to
var ChanGot;'chan to read from (either readChan (user def'd) or chan assoc'd
with HCursor(1)
var level:=0;  'level at which events are detected (set as either HCursor(1) or
levelCross)
var i%;                     'type of window active
i% := ViewKind();
if i%>0 then Message (" HeartBeatBoutique|     Not a Time View!     "); halt;
'checks that correct window (ie: time view) is active
UserValue(); 'prompts user for....(see below)...Proc UserValue()
var cancel%; 'exit control (ie: CANCEL BUTTON) of dialogue box
var label%;  'needed for label "If Not Using... " of dialogue box
DlgCreate ("HeartBeatBoutique");
DlgCheck(1,"Use Your Value (and Not Use HCursor)");
'choose either user defined level (entered above), or ...
DlgCheck(2,"Make New Memory Buffer");
DlgReal (3,"Min Interval btwn Peaks (Secs)",-100.0,300.0);
'..min interval btwn peaks
DlgLabel(4,"----------------If Not Using HCursor Level----------------");
DlgReal(5,"Channel to Read", 0, 100); 'chan to get data from (eg: ECG trace)
DlgReal (6,"Level Crossing (Y Axis Units)",-100.0,300.0); 'level to be crossed b4
peak is recorded range -100 to 300
cancel%:=DlgShow (userval%, membuf%, interval, label%, readChan,
levelCross); 'show dialog
```

```
if cancel% <1 then halt;              'if CANCEL pressed - QUIT          - controls
dialogue box
endif;
if userval% >0 then                          'if 'USE YOUR VALUE' box checked...
                level := levelCross;         '...use that value as the level        --
                                    sets up program to use user's value
ChanGot := readChan;                 'chan to read from =
else
level := (HCursor(1));               'use HCursor(1) as the level - sets up
program to use HCursor(1) h% := 1;
endif;
if membuf% >0 then                           'if membuf% box checked - then ...
newChan% := MemChan(3,0,0,0);        'make new mem buffer the chan written
to - sets up prog to make new buffer
ChanShow (newChan%);                              'make it visible
                                                  and have data written to it
var chan2%, cancel2%; ' user defined mem buffer, cancel of dialogue box.
DlgCreate ("HeartBeatBoutique");                                  'start new dialog
            - dialogue box to enter mem
DlgInteger(1, "Mem Buffer To Write To", 0, 200);'chan to write data to  buffer to
be written to cancel%:=DlgShow (chan2%); 'show dialog
newChan% :=        chan2%;
'- makes chan being written to
endif;
' chan that user defined
end;                          'End of 'UserValue()' proc.
ToolbarVisible(1);            'Make toolbar visible always
DoToolbar();                                      'Do the toolbar
proc DoToolbar()
ToolbarSet(0,"",Idle%);              'Call Idle%() whenever there is free time
ToolbarSet(1,"Quit",Quit%);                  'Set up toolbar buttons
ToolbarEnable(3,0);                               'Disable "Sample stop" button
Toolbar(" HeartBeatBoutique", 1023);              'Wait here until quit is pressed
func Quit%()                                      'If "Quit" is pressed
func Idle%()                         'The Idle routine is called when PC has time
eTime:=Maxtime();
if h%  > 0 then 'if "Use HCursor Level" box checked, then...
ChanGot := HCursorChan(1); 'makes sure that data is read from the chan with
the HCursor assc'd with it
MemImport(NewChan%,ChanGot,0.0,(MaxTime()),0,interval,level); 'see
'MemImport' function
DrawMode(NewChan%,12,dotz%);         'events drawn as inst. freq. beats/mi
YRange(newChan%,lowY, highY);        'sets the range of the Y axis of new
mem buffer
ToolBarVisible(1); 'make toolbar visible
sTime:=eTime;
return 1;                                         'Stay in toolbar
end;
```

Effect of pulmonary C-fibre afferent stimulation on cardiac vagal neurones in the nucleus ambiguus in anaesthetized cats

Yun Wang *, James F. X. Jones †, Ross D. Jeggo *, Michael de Burgh Daly ‡, David Jordan ‡ and Andrew G. Ramage *

*Departments of *Pharmacology and ‡Physiology, University College London, Royal Free Campus, Rowland Hill Street, Hampstead, London NW3 2PF, UK and †Department of Human Anatomy and Physiology, University College Dublin, Earlsfort Terrace, Dublin 2, Ireland*

(Received 16 February 2000; accepted after revision 7 April 2000)

1. It has been demonstrated previously that the vagal bradycardia evoked by activation of pulmonary C-fibres is not respiratory modulated. Experiments were carried out in α-chloralose anaesthetized cats to determine if these cardiac vagal preganglionic neurones (CVPNs) in the nucleus ambiguus (NA), which have respiratory modulated activity, can be activated when pulmonary C-fibre afferents are stimulated by right atrial injections of phenylbiguanide (PBG).

2. Eleven CVPNs with B-fibre axons in the right cardiac vagal branches were identified and found to be localized within or ventrolateral to the nucleus ambiguus. Ionophoretic application of a high current of DL-homocysteic acid (DLH) induced a vagally mediated bradycardia and hypotension in six of eight sites from which CVPNs were recorded.

3. The activity of B-fibre CVPNs, whether spontaneous ($n = 4$) or induced by ionophoresis of DLH ($n = 7$) was respiratory modulated, firing perferentially during post-inspiration and stage 2 expiration. This activity also correlated with the rising phase of the arterial blood pressure wave consistent with these CVPNs receiving an arterial baroreceptor input.

4. Right atrial injections of PBG excited nine of eleven CVPNs tested. In eight of these activated neurones the onset latency of the excitation was within the pulmonary circulation time, consistent with being activated only by pulmonary C-fibre afferents. In two neurones the PBG-evoked excitation still occurred when central inspiratory drive was inhibited, as indicated by the disappearance of phrenic nerve activity.

5. In conclusion, B-fibre respiratory modulated CVPNs can be activated following stimulation of pulmonary C-fibre afferents.

Most vagal cardiac reflexes are modulated by central respiratory drive such that cardiac slowing is greater during expiration than during inspiration. This is believed to be due to neural coupling between the brainstem respiratory system and the cardiovascular control system (see Taylor *et al.* 1999). This respiratory modulation has been considered to be due to inhibition of cardiac vagal preganglionic neurones in the nucleus ambiguus by central respiratory neurones (Gilbey *et al.* 1984) and disfacilitation of baroreceptor and chemoreceptor inputs by lung stretch afferents (Potter, 1981). These cardiac vagal preganglionic neurones have B-fibre axons which run in the cardiac branches of the vagus and receive an input from arterial baroreceptors (McAllen & Spyer, 1976, 1978*a*,*b*; Gilbey *et al.* 1984). However, the bradycardia evoked by injection of phenylbiguanide (PBG) into the right atrium, to activate pulmonary C-fibre afferents (Coleridge & Coleridge, 1984), is not influenced by either central respiratory drive or lung inflation (Daly & Kirkman, 1988, 1989; Daly, 1991; Daly *et al.* 1992). This led Daly (1991) to postulate that the pulmonary C-fibre-evoked bradycardia was mediated by a different group of cardiac vagal preganglionic neurones, possibly those with non-myelinated axons and located in the dorsal vagal nucleus (Donoghue *et al.* 1981; Jordan *et al.* 1986; Ford *et al.* 1990). Those neurones have been shown to be cardio-inhibitory in function (Jones *et al.* 1995) and to be activated synaptically by electrical stimulation of non-myelinated pulmonary vagal afferents (Bennett *et al.* 1985), but probably not by lung inflation (Jones *et al.* 1998). More recently, Jones *et al.* (1998) demonstrated in both cats and rats that these C-fibre cardiac vagal preganglionic neurones in the dorsal vagal nucleus lack respiratory modulation and are indeed excited by right atrial injection of PBG. However, such stimuli evoked only a short burst of excitation, which

was of a much shorter duration than that of the evoked bradycardia. Further, selective electrical stimulation of non-myelinated vagal efferents evoked a bradycardia in the cat of 11 beats min^{-1} (Jones *et al.* 1995) which is much smaller than that evoked by stimulating pulmonary C-fibre afferents, for instance activation of these afferents with PBG (6–15 $\mu g\ kg^{-1}$) injected in right atrium evoked a bradycardia of around 55 beats min^{-1} (Daly & Kirkman, 1988). Thus, it is difficult to explain how non-respiratory modulated C-fibre cardiac vagal preganglionic neurones in the dorsal vagal nucleus could be solely responsible for the vagal bradycardia evoked by pulmonary C-fibre afferent activation. Therefore the present study has been designed to determine whether respiratory modulated B-fibre cardiac vagal preganglionic neurones in the nucleus ambiguus are also activated by stimulation of pulmonary C-fibre nerve endings. A preliminary report of some of these observations has been published (Wang & Ramage, 1999).

METHODS

The experiments were carried out under the Animals (Scientific Procedures) Act, 1986 and at the end of the experiment the animals were killed by an overdose of anaesthetic and exsanguination.

General preparation

Experiments were carried out on seven adult cats (2·5–4·5 kg) of either sex, anaesthetized with a mixture of α-chloralose (70 mg kg^{-1}) and pentobarbitone sodium (6 mg kg^{-1}) injected I.V. Before and after neuromuscular blockade (see below), the level of anaesthesia was assessed by the absence of a withdrawal reflex and/or the cardiovascular response to paw-pinch and the stability of resting cardiovascular and respiratory variables and pupil size; if and when required, additional anaesthetic (α-chloralose, 10–15 mg kg^{-1}, I.V.) was administered.

Rectal temperature was monitored and maintained between 38–39 °C with a Harvard homeothermic blanket. When surgical anaesthesia was established, the brachial veins and arteries on both sides and one femoral vein were cannulated for administration or withdrawal of drugs/fluids and for recording blood pressure using a pressure transducer (Gould) connected to a Grass Model 7D Polygraph (Grass Medical Instruments, Quincy, MA, USA). The bladder was cannulated to prevent undue filling during the period of the experiment, avoiding reflex effects associated with bladder distension. A cervical tracheotomy was performed and the trachea cannulated just below the larynx. Tracheal pressure was monitored by a pressure transducer (Gould) connected to a side arm of the tracheal cannula. A silicone cannula, pre-filled with PBG (400 $\mu g\ ml^{-1}$), was advanced into the right atrium via the right external jugular vein. An ECG was recorded by leads attached to each of the forepaws of the animal from which heart rate was derived. The animals were placed in a stereotaxic frame and ventilated artificially (Harvard Ventilator model 551) with O_2-enriched air, maintaining a small positive end-expiratory pressure (1–2 cmH_2O). As soon as the ventilation had started, the animals were neuromuscularly blocked using vecuronium bromide (200 $\mu g\ kg^{-1}$, I.V.) and supplemented with an I.V. infusion of 480 $\mu g\ kg^{-1}\ h^{-1}$. This infusion (6 ml $kg^{-1}\ h^{-1}$) comprised 500 ml plasma substitute Gelofusine, 500 ml H_2O, 8·4 g $NaHCO_3$, 2 g glucose and 80 mg vecuronium bromide and was given to maintain blood volume, counteract the development of non-respiratory acidosis and maintain neuromuscular blockade. Arterial blood gas variables were measured using a Corning Blood Gas Analyser (Model 238). The blood gases and pH were regularly monitored and maintained at 100–180 mmHg P_{O_2}, 35–45 mmHg P_{CO_2}, and pH 7·3–7·4 by I.V. injection of sodium bicarbonate (1 M) and/or adjusting the volume and frequency of ventilation. In all experiments, animals were pretreated with the β_1-adrenoceptor antagonist atenolol (1 mg kg^{-1}, I.V.) to block sympathetic drive to the heart. Thus changes in heart rate could be presumed to be due to changes in activity in cardiac vagal efferents.

The right phrenic nerve was dissected from a dorsolateral approach, cut peripherally and desheathed. The cut central end of the nerve was placed on bipolar silver wire recording electrodes. Clamps applied to the vertebral spines at C7 and L2 or L3 were used to elevate and stabilize the animal. To expose the brainstem the nuchal muscles were removed, the occipital bone opened and the dura overlying the brainstem and cerebellum cut and reflected laterally. In some experiments the cerebellum was displaced rostrally with a small retractor to allow access to the region of the nucleus ambiguus.

Preparation of cardiac and pulmonary vagal branches

A thoracotomy was performed between the fourth and sixth ribs to gain access to the right cranial, caudal cardiac and pulmonary branches, as previously described (McAllen & Spyer, 1976). The intact cardiac and pulmonary branches and the vagus nerve between the cranio- and caudal cardiac branches were placed on fine silver wire (0·125 mm in diameter) bipolar electrodes with a 2 mm gap. The wires were insulated from one another with wax and sealed round the nerves with President light body dental polyvinyl-siloxane (Coltene UK Ltd, West Sussex, UK). These silver wires had been soldered onto insulated copper wires, which were secured to the thorax. The electrodes were connected to an isolated stimulator (DS2A, Digitimer Ltd, Welwyn Garden City) triggered by Digitimer D4030 Programmer. The vagal branches were left intact and typically, stimulation of the main cardiac branch (1 ms pulses at 100 μA, 50 Hz) evoked 'cardiac arrest' without change in tracheal pressure, whilst stimulation of the pulmonary branches evoked changes in tracheal pressure but not heart rate.

Single unit recording and identification of cardiac vagal preganglionic neurones

Extracellular recordings were made from neurones in the region of the nucleus ambiguus using 'piggy-back' electrodes which were assembled from a single glass recording electrode and a multi-barrelled glass electrode (Wang *et al.* 1998). The recording barrel contained 4 M sodium chloride. One of the barrels contained Pontamine Sky Blue dye (2% dissolved in 0·5 M sodium acetate) for automatic current balancing and marking the recording sites and the other barrel was filled with the glutamate receptor agonist DL-homocysteic acid (DLH, 100 mM, pH 8·5). Cardiac vagal preganglionic neurones were identified by their antidromic activation following electrical stimulation of the thoracic cardiac branches of the vagus (100–500 μA, 1 ms pulses, 0·2–1·0 Hz) as previously described (McAllen & Spyer, 1976, 1978*a*). The criteria used to determine antidromic activation were the constant latency of the evoked response and its collision with appropriately timed ongoing activity (Fig. 1*A*). The possibility of current spread from the cardiac branch to the whole vagus nerve was checked periodically during the experiment by the absence of antidromic activation to stimuli applied to the vagus nerve below the pulmonary nerve branching point. Pulmonary C-fibre afferents were stimulated by injection of a bolus of phenylbiguanide (PBG; 14–32 $\mu g\ kg^{-1}$ in 100–200 μl) into the right atrium. The minimum interval between two PBG injections was 5 min and the volume for a single injection

was restricted to less than 200 μl to avoid stimulation of receptors in the atrial wall by volume expansion.

Data capture and analysis

Neuronal activity, phrenic nerve activity and ECG were amplified (× 2000, × 20 000 and × 5000, respectively) and filtered (0·5–5 kHz; Neurolog, AC preamplifier NL104 and filter NL125; Neurolog System, Digitimer Ltd, Welwyn Garden City, UK). Phrenic nerve activity was then integrated using an EMG integrator (NL 703, Neurolog System). Arterial blood pressure (BP), heart rate (HR), tracheal pressure (TP), ECG, raw and integrated phrenic nerve activity and neuronal activity were displayed on a computer using a 1401 interface (1401 Plus, Cambridge Electronic Design (CED), Cambridge, UK) and Spike2 software (CED) and stored on videotape using a digital data recorder (VR100B, Instrutech Corp., Great Neck, NY, USA). Offline analysis of the recorded data (phrenic-, tracheal pressure-, and ECG-triggered correlations) was made using Spike2 software. Baseline values for mean arterial pressure (MAP) and HR were taken as the mean over 40 s before the administration of PBG. The maximal overall changes evoked by PBG were compared with baseline. The mean baseline neuronal firing rate was measured over 40 s. In addition, the mean number of spikes per burst and mean burst duration of the four respiratory cycles before PBG injection were taken as the control. The number of spikes and duration of the first burst after the PBG injection were compared with the control and if the changes in either burst number and/or duration were greater than 20%, this was considered to be excitation. This excitation was then re-analysed to determine if it occurred within 5 s; neuronal responses to PBG occurring within this latency can be taken as resulting from pulmonary C-fibre stimulation (5 s window, see Daly & Kirkman, 1988; Jones *et al.* 1998). Beyond this duration changes in activity could be attributed to activation by PBG of other afferents that are downstream of the pulmonary circulation (Daly & Kirkman, 1988). However, since the B-fibre cardiac vagal preganglionic neurones were firing in the post-inspiratory and stage 2 expiratory (PI–E2) phases of the respiratory cycle (Gilbey *et al.* 1984), it was difficult to analyse the mean change in firing rate after PBG injection, as in most cases the burst of firing after PBG injection overlapped the 5 s window. Therefore the 1st second of the PBG-evoked response, which fell within the 5 s window, was analysed and compared with the mean of the 1st second of the previous four bursts. All data are presented as means ± S.E.M. except where indicated, and all

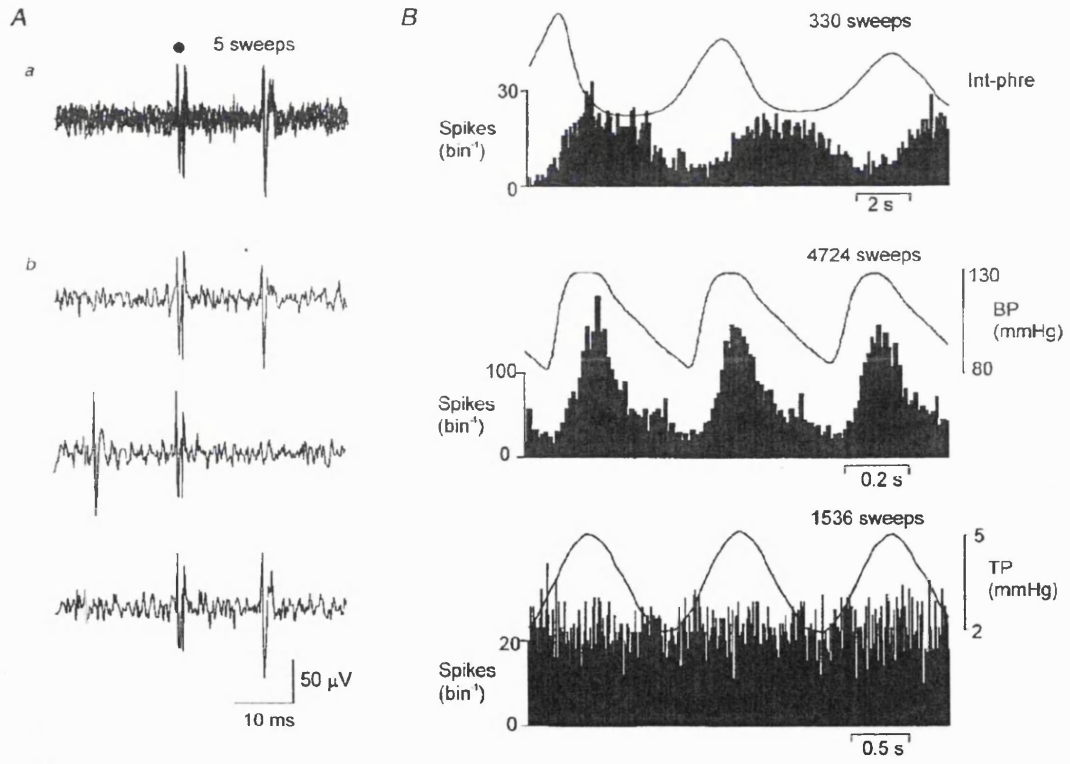


Figure 1. Identification of a B-fibre cardiac vagal preganglionic neurone in the nucleus ambiguus

A, traces showing a cardiac vagal preganglionic neurone antidromically activated (latency, 15 ms) by stimulating the right cardiac branch (200 μA, 1 ms, 0·5 Hz). *Aa*, five consecutive sweeps superimposed to show the constant latency of the evoked spike; *Ab*, three consecutive sweeps showing that the evoked spike (see top and bottom trace) was cancelled by the spontaneous spike (see middle trace). The ● indicates the stimulus artefacts. *B*, histograms of the activity (with DLH at 20 nA) of the same CVPN as in *A* triggered by integrated phrenic nerve activity (Int-phre; 50 ms bin width; top panel), the *R*-wave of the ECG (10 ms bin width; middle panel) and by tracheal pressure (10 ms bin width; lower panel). Above the histograms is an average of integrated phrenic activity, ECG triggered arterial blood pressure (BP) and the tracheal pressure (TP) wave, respectively. The number of sweeps on top of each panel refers to both the average and to the histogram.

comparisons of the means were made using Student's paired t test. Differences between means were taken as significant when $P < 0{\cdot}05$.

Localization of recording sites

Recording sites were marked by ionophoretic ejection of Pontamine Sky Blue. Following the experiments, brainstems were removed and fixed in 10% formal saline, and serial frozen sections (80 μm) were cut and stained with Neutral Red. The marked recording sites were visualized and displayed on standard sections of brainstem taken from the stereotaxic atlas of the cat (Berman, 1968, Fig. 2).

Drugs

Drugs were obtained from the following sources: α-chloralose, DL-homocysteic acid and atenolol from Sigma Aldrich Chemical Co., Poole, Dorset, UK; pentobarbitone sodium from Rhône Mérieux Ltd, Harlow, Essex, UK; Pontamine Sky Blue dye from BDH, Poole, Dorset, UK; Gelofusine from Braun Medical Ltd, Aylesbury, Bucks, UK; phenylbiguanide from Research Biochemicals, Semat Technical Ltd, St Albans, Herts, UK, and vecuronium bromide from Organon Technika Ltd, Cambridge, UK.

RESULTS

A total of 11 antidromically identified vagal preganglionic neurones with axons in the cardiac branches of the vagus nerve were recorded in this study. They had calculated axon conduction velocities within the B-fibre range (5·9–18·0 m s^{-1}, a mean of 11·4 ± 0·9 m s^{-1}). The recording sites of six of these were localized by pontamine injection and another two were recorded in very close proximity to a previously marked site. These sites were located within or ventrolateral to the nucleus ambiguus (Fig. 2). Based on the depth and rostro-caudal position of the recording electrode the other three B-fibre cardiac projecting neurones were considered also to be in this same region. Baseline values (means ± S.D.) were; mean arterial blood pressure 101 ± 5 mmHg; heart rate 160 ± 23 beats min^{-1}; tracheal pressure, inflation and deflation, 5·4 ± 2·5 and 2·1 ± 0·7 mmHg, respectively; P_{O_2} 141 ± 27 mmHg; P_{CO_2} 38 ± 10 mmHg and pH 7·32 ± 0·05.

B-fibre cardiac vagal preganglionic neurones

Ten of the B-fibre cardiac vagal preganglionic neurones had little or no ongoing spontaneous activity, whereas one neurone had an average firing rate of 6·3 spikes s^{-1}. The profile of the activity was analysed in detail in neurones either with ($n = 7$) or without ($n = 4$) DLH (10–120 nA) ionophoretically applied to induce or increase firing rate. This combined group had a mean firing rate of 2·0 ± 0·7 spikes s^{-1}. The activity in all 11 neurones showed a strong pulse-related rhythm (Fig. 1*B*). In addition, a component of this activity was correlated with central respiratory drive, being maximal during post-inspiration and stage 2 expiration (E2; Fig. 1*B*). Even during high discharge rates (up to 20 spikes s^{-1}) evoked by DLH application at high currents (60–120 nA) the activity in neurones remained respiratory modulated (Fig. 3*C*). This respiratory modulation of the activity was abolished in two neurones tested when inhibition of central inspiratory activity caused ongoing activity to become continuous (Fig. 4*B*). The ongoing activity of the majority of these neurones (8 out of 11) showed no obvious relationship to tracheal pressure (Fig. 1*B*), but in the other three neurones there was a correlation between neuronal activity and tracheal pressure, the maximal discharge occurring during the phase of lung deflation. In all the experiments phrenic nerve activity was locked to lung inflation.

At six out of the eight sites from which B-fibre cardiac vagal preganglionic neurones were recorded, ionophoretic application of DLH at a high current (30–160 nA) induced a

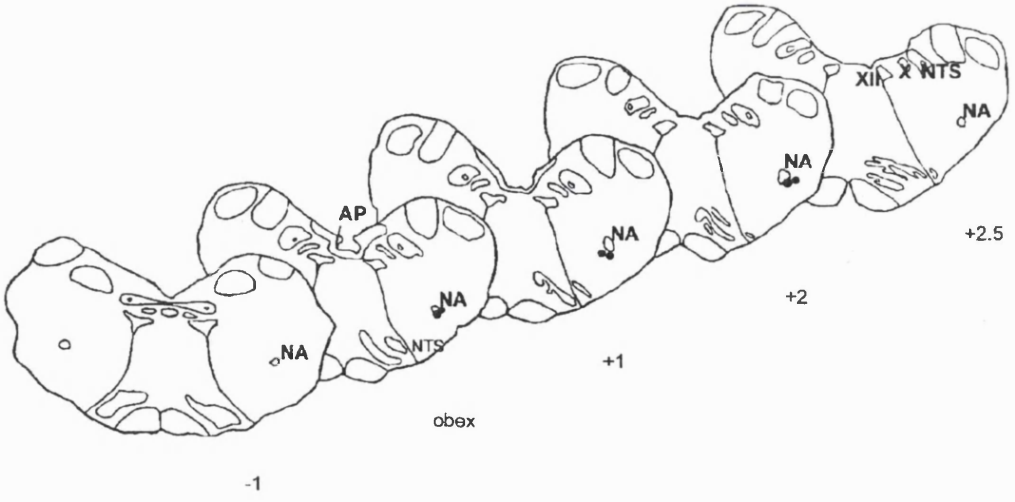


Figure 2. Pontamine Sky Blue marked locations of recordings from the medulla

The position of six B-fibre (●) cardiac vagal preganglionic neurones from which recordings were made are shown on five standard sections of the medulla taken from −1 to +2·5 mm caudal to rostral at the level of the obex. Abbreviations: NA, nucleus ambiguus; NTS, nucleus tractus solitarius; X, dorsal vagal nucleus; XII, hypoglossal nucleus; AP, area postrema.

significant bradycardia and hypotension. During ionophoresis of DLH, the heart rate decreased from 153 ± 6 to 139 ± 6 beats min^{-1} ($P < 0{\cdot}01$, $n = 6$) and the mean arterial blood pressure fell from 106 ± 4 to 90 ± 3 mmHg ($P < 0{\cdot}05$, $n = 6$). At three sites neuronal activity was monitored during and following these DLH applications. In all these cases the increase in firing rate was associated with a bradycardia and hypotension (Fig. 3).

Effect of right atrial injection of PBG on B-fibre cardiac vagal preganglionic neurones

Overall effect of PBG. PBG injections excited nine of the eleven B-fibre cardiac vagal preganglionic neurones. In these nine responding neurones the combined mean ongoing activity was 1·9 ± 0·7 spikes s^{-1} which comprised means of 2·0 ± 1·4 spikes s^{-1} ($n = 4$) for spontaneously firing neurones and 1·9 ± 0·8 spikes s^{-1} ($n = 5$) for those activated by DLH.

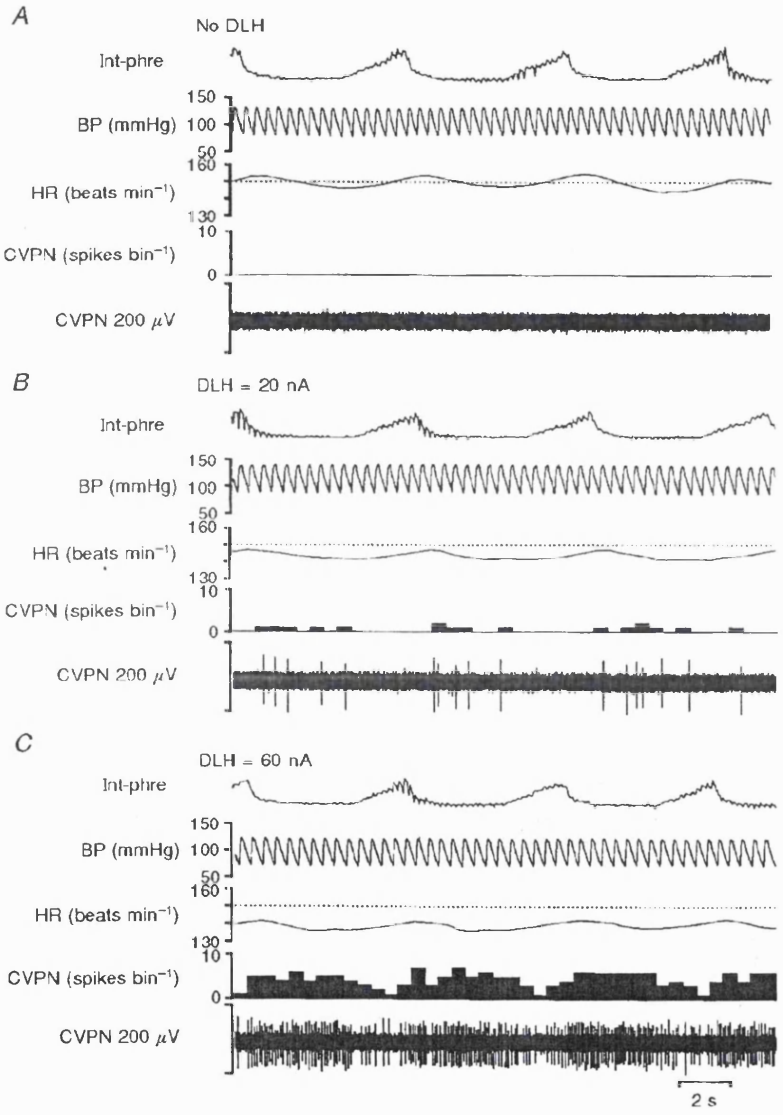


Figure 3. Traces showing the effects of ionophoretic application of DLH onto a CVPN with a B-fibre axon

Anaesthetized cat pretreated with atenolol (1 mg kg^{-1}, I.V.). Records from top downwards: Int-phre, integrated phrenic nerve activity; BP, arterial blood pressure (mmHg); HR, heart rate (beats min^{-1}) and rate histogram (0·5 s bin) and recording of the activity of a B-fibre CVPN. *A*, ongoing activity, no DLH; *B* and *C*, activity in the presence of 20 nA DLH and 60 nA DLH, respectively. Note the heart rate fell as CVPN discharge increased (the dotted lines on the heart rate traces represent the mean heart rate in control without DLH, 150 beats min^{-1}) and even at a high level of excitation the activity was still respiratory modulated (*C*).

Right atrial injection of PBG (14–32 μg kg^{-1} in 100–200 μl) increased both the number of spikes per burst from 11 ± 4 to 27 ± 6 ($P < 0{\cdot}01$, $n = 9$) and the burst duration from 1·9 ± 0·5 to 3·6 ± 0·8 ms ($P < 0{\cdot}01$, $n = 9$), respectively (Fig. 4*A*). In association with the increased neuronal activity, PBG also evoked a vagally mediated bradycardia of 69 ± 6 beats min^{-1} (heart rate fell from 159 ± 7 to 90 ± 6 beats min^{-1}, $P < 0{\cdot}001$, $n = 9$) and a reduction in arterial blood pressure of 22 ± 1 mmHg (mean arterial blood pressure decreased from 96 ± 5 to 74 ± 4 mmHg, $P < 0{\cdot}001$, $n = 9$). The latency for the evoked excitation in the cardiac vagal preganglionic neurones was 3·4 ± 0·3 s (range 1·8–4·5 s) which was significantly ($P < 0{\cdot}01$, $n = 9$) shorter than that of the latency for the evoked bradycardia (3·7 ± 0·3 s; range 2·5–4·5 s). Of the nine neurones, six were excited before the appearance of the bradycardia.

Effect of PBG within pulmonary circulation time. In order to establish that pulmonary C-fibre afferent-evoked responses in the cardiac vagal preganglionic neurones occurred within the pulmonary circulation time, the number of spikes in the 1st second of the evoked excitation within the 5 s window were analysed (see Methods). In eight out of nine neurones, PBG increased the number of spikes

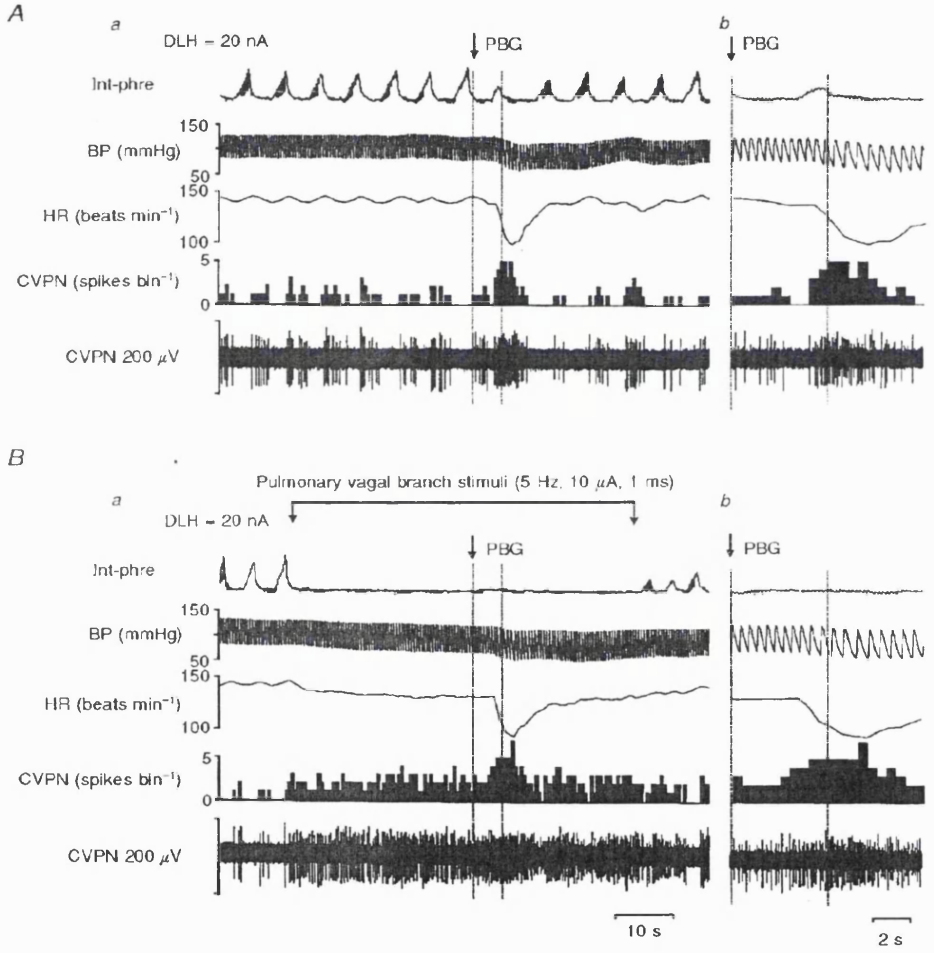


Figure 4. Traces comparing the effect of right atrial injections (at the point marked by arrow) of PBG (20 μg kg^{-1}) on a CVPN with a B-fibre axon in the presence (*A*) and absence (*B*) of central respiratory drive

Anaesthetized cat pretreated with atenolol (1 mg kg^{-1}, I.V.). Records from top downwards: Int-phre, integrated phrenic nerve activity; BP, arterial blood pressure (mmHg); HR, heart rate (beats min^{-1}) and CVPN rate histogram (0·5 s bin) and on-going activity. The 5 s window following the PBG injection (see Methods) is shown by the two vertical dotted lines. *Ab* and *Bb* are expanded traces after the PBG injections shown in *Aa* and *Ba*. Note in *B*: (1) low intensity electrical stimulation of the pulmonary vagal branch inhibits central respiratory drive, as indicated by the lack of phrenic nerve activity, and (2) the increase in neuronal activity during this stimulation is due to the inhibition of central respiratory drive.

within the 1st second of the burst from 4 ± 1 to 10 ± 1 ($P < 0{\cdot}01$, $n = 8$; Fig. 4*A*). The firing frequency in the 1st second in the one remaining neurone was not affected by PBG, although the burst discharge was increased and prolonged (data not illustrated). In all nine neurones the phrenic nerve activity was attenuated (Fig. 4*A*).

In one neurone, low intensity electrical stimulation of a pulmonary vagal branch (10 μA), was used to inhibit phrenic nerve activity. The firing pattern of this neurone changed from respiratory related bursts to a continuous firing pattern (Fig. 4*B*). Under these conditions PBG still caused excitation (Fig. 4*B*). Similarly, in a second neurone, PBG still evoked excitation of the cardiac vagal preganglionic neurone when phrenic nerve activity was inhibited by hyperventilation (data not shown).

Effect of electrical stimulation of cardiac and pulmonary vagal afferents on B-fibre cardiac vagal preganglionic neurones

Electrical stimulation of either thoracic cardiac or pulmonary vagal branches evoked an excitatory synaptic input in six out of the eight cardiac vagal preganglionic neurones activated by phenylbiguanide (Fig. 5*A* and *B*). Among these six neurones, three received inputs from both cardiac and pulmonary branches, one received an input from only the pulmonary branch and the other two received an input only from the cardiac vagal branch. These orthodromic vagal afferent synaptic inputs had longer onset latencies than the antidromic responses, i.e. onset latencies were between 125 and 230 ms for orthodromic excitation *versus* between 11 and 21 ms for the antidromic responses. There was only one exception, where activation of pulmonary vagal afferents evoked both a short (18 ms) and a long (180 ms) latency excitatory input (Fig. 5*A*).

DISCUSSION

Characteristics of cardiac vagal preganglionic neurones

In the present experiments recordings have been made from neurones located in or ventrolateral to the nucleus ambiguus that were antidromically activated following stimulation of one of the vagal cardiac branches. Their activity, either spontaneous or DLH evoked was primarily in the post-inspiratory and/or stage 2 expiratory phases of the respiratory cycle and was positively correlated to the arterial blood pressure wave. Thus these neurones have the same characteristics as those that have been recorded in this area previously (McAllen & Spyer, 1976, 1978*a*,*b*; Gilbey *et al.* 1984) and can be classified as cardiac vagal preganglionic neurones. However, the precise cardiac function of these neurones has not been determined directly. Although ionophoretic application of high currents of DLH at six of these recording sites caused a bradycardia, an atrial inotropic, dromotropic or coronary vasomotor function cannot be ruled out. As these animals were pretreated with the β-adrenoceptor antagonist atenolol this bradycardia can be attributed to an increase in vagal tone. Interestingly, in two neurones the changes in activity caused by DLH paralleled the accompanying changes in heart rate confirming a similar observation made by McAllen & Spyer (1978*a*).

Responses to stimulation of pulmonary C-fibre afferents

Nine of these eleven B-fibre cardiac vagal preganglionic neurones were activated by PBG injected into the right atrium indicating that they receive a synaptic input from cardiopulmonary C-fibres. It should be emphasized that right atrial injections of PBG, as well as activating

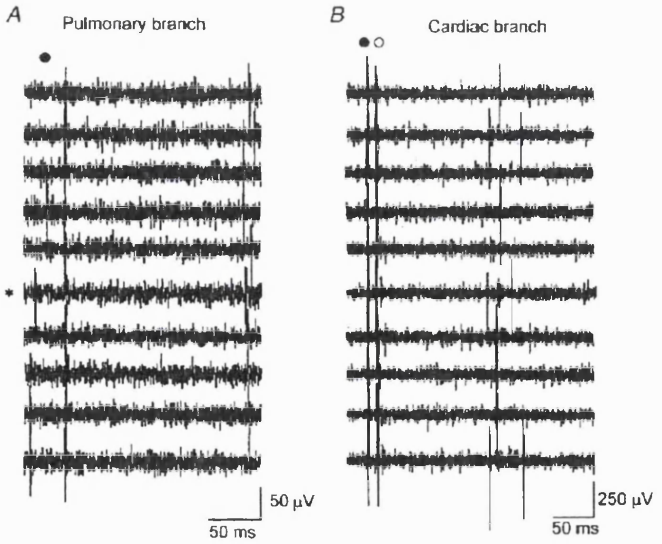


Figure 5. Synaptic input from vagal branches to two different CVPNs with B-fibre axons

Two sets of traces containing ten consecutive sweeps each showing the effect of stimulation (●) of the pulmonary branch (200 μA, 1 Hz, 1 ms) (*A*), which evoked both a short and a long latency excitatory input (Note: in the 6th trace (*) the spontaneous spike did not cancel the short latency input from the pulmonary nerve), and the cardiac branch (150 μA, 0·5 Hz, 1 ms) (*B*), which evoked a short latency antidromic spike (○) and a long latency synaptic input.

pulmonary C-fibres, may also activate afferents in the systemic circulation (see Coleridge & Coleridge, 1979). In the cat it has been demonstrated (Daly & Kirkman, 1988) that the onset latency, depending on the level of the cardiac output, for right atrial injections of PBG to evoke a bradycardia due to pulmonary C-fibre afferent stimulation alone ranges between 2 and 5 s. Thus, using a 5 s window for analysis in order to exclude any systemic effects of the PBG (see Methods), right atrial injection of this agent evoked an increase in activity in eight of these neurones, which could be considered to be due to pulmonary C-fibre activation alone. In addition, activity in six of these eight neurones was also elicited by orthodromic electrical stimulation of the cardiac and pulmonary vagal branches of the vagus, which would also indicate that they received an input from C-fibre afferents running in these branches. The failure to see excitation evoked by electrical stimulation in the other two neurones may be due to sampling, that is the afferents innervating these neurones may be running in cardiac and pulmonary branches other than those being stimulated.

As right atrial injections of PBG inhibited inspiration, the excitation of cardiac vagal preganglionic neurones by PBG could, at least in part, be an indirect disinhibition resulting from inhibition of central respiratory drive. However, in the present experiments activation of pulmonary C-fibre afferents still excited cardiac vagal preganglionic neurones during periods of central apnoea, as indicated by the disappearance of phrenic nerve activity, suggesting that the excitation is independent of the inhibition of central respiratory drive. In view of the finding that background activity of these neurones is respiratory modulated, i.e. the activity is reduced during the phase of inspiration, it might be anticipated that the PBG evoked activity in these neurones would also be respiratory modulated. However, in the present experiments this could not be established due to the variability in the size and occurrence of phrenic nerve activity during PBG-evoked excitation of the neurone within the 5 s window, and the short duration of the evoked excitation.

Pulmonary C-fibre activation and cardio-respiratory integration

The present results demonstrate that stimulation of pulmonary C-fibre afferents activates respiratory modulated cardiac vagal preganglionic neurones with B-fibre axons and that, at least part of this excitation, was not due to inhibition of central respiratory drive by these afferents. Thus this study, taken together with that of Jones *et al.* (1998) indicates that stimulation of pulmonary C-fibre endings with PBG activates simultaneously two groups of cardiac vagal preganglionic neurones: those with B-fibre axons located within or in the vicinity of the nucleus ambiguus and those with C-fibre axons located in the dorsal vagal nucleus. The question arises: how is it that the reflex vagal bradycardia resulting from stimulation of pulmonary C-fibres is not respiratory modulated (Daly & Kirkman, 1988, 1989; Daly 1991)? Since activity in cardiac vagal preganglionic neurones with B-fibre axons is known to be respiratory modulated (see Introduction; present experiments), it would be expected that pulmonary C-fibre evoked increases in activity would also be respiratory modulated, as are those evoked by baroreceptor and chemoreceptor inputs. However, since this was not tested in the present experiments, the excitation of the cardiac vagal preganglionic neurones may or may not be respiratory modulated. It is possible that respiratory modulation might be prevented if, in addition to exciting cardiac vagal preganglionic neurones, the pulmonary C-fibre afferents also prevented their respiratory modulation by inhibiting synaptic transmission of the inspiratory input to these neurones. However, it is unlikely that the high firing rate evoked in cardiac vagal preganglionic neurones by pulmonary C-fibre stimulation are overriding the respiratory modulation, since neurones activated with DLH to a similar level of activity as that caused by pulmonary C-fibre stimulation still showed respiratory modulation (compare Fig. 3*C* with Fig. 4*Ab*). A final possibility is that an interaction between activity in non-respiratory modulated cardiac vagal preganglionic neurones with C-fibre axons and that in respiratory modulated cardiac vagal preganglionic neurones with B-fibre axons occurs at the level of the cardiac ganglia and/or postganglionic nerve endings in the sino-atrial node (Jones, 1993; Jones *et al.* 1998). Clearly, this study demonstrates that respiratory modulated cardiac vagal preganglionic neurones with B-fibre projecting axons are excited by the activation of pulmonary C-fibre afferents. However, the precise mechanism by which the evoked bradycardia escapes respiratory modulation remains to be determined.

Bennett, J. A., Goodchild, C. S., Kidd, C. & McWilliam, P. N. (1985). Neurones in the brain stem of the cat excited by vagal afferent fibres from the heart and lungs. *Journal of Physiology* **369**, 1–15.

Berman, A. L. (1968). *The Brainstem of the Cat.* University of Wisconsin, Madison, WI, USA.

Coleridge, J. C. G. & Coleridge, H. M. (1979). Chemoreflex regulation of the heart. In *Handbook of Physiology*, section 2, *The Cardiovascular System*, vol. 1, *The Heart*, pp. 653–676. American Physiological Society, Bethesda, MD, USA.

Coleridge, J. C. G. & Coleridge, H. M. (1984). Afferent vagal C-fibre innervation of the lungs and airways and its function significance. *Reviews of Physiology, Biochemistry and Pharmacology* **99**, 1–110.

Daly, M. de B. (1991). Some reflex cardioinhibitory responses in the cat and their modulation by central inspiratory neuronal activity. *Journal of Physiology* **439**, 559–577.

Daly, M. de B., Jordan, D. & Spyer, K. M. (1992). Modification of respiratory activities during stimulation of carotid chemoreceptors, arterial baroreceptors and pulmonary C fibre afferents in the anaesthetized cat. *Journal of Physiology* **446**, 466*P*.

Daly, M. de B. & Kirkman, E. (1988). Cardiovascular responses to stimulation of pulmonary C fibres in the cat: their modulation by changes in respiration. *Journal of Physiology* **402**, 43–63.

Daly, M. de B. & Kirkman, E. (1989). Differential modulation by pulmonary stretch afferents of some reflex cardioinhibitory responses in the cat. *Journal of Physiology* **417**, 323–341.

Donoghue, S., Fox, R. E., Kidd, C. & Koley, B. N. (1981). The distribution in the cat brain stem of neurones activated by vagal non-myelinated fibres from the heart and lungs. *Quarterly Journal of Experimental Physiology* **66**, 391–404.

Ford, T. W., Bennett, J. A., Kidd, C. & McWilliam, P. N. (1990). Neurones in the dorsal motor vagal nucleus of the cat with non-myelinated axons projecting to the heart and lungs. *Experimental Physiology* **75**, 459–473.

Gilbey, M. P., Jordan, D., Richter, D. W. & Spyer, K. M. (1984). Synaptic mechanisms involved in the inspiratory modulation of vagal cardio-inhibitory neurones in the cat. *Journal of Physiology* **356**, 65–78.

Jones, J. F. X. (1993). The central control of the pulmonary chemoreflex. PhD Thesis, University of London.

Jones, J. F. X., Wang, Y. & Jordan, D. (1995). Heart rate responses to selective stimulation of cardiac vagal C fibres in anaesthetized cats, rats and rabbits. *Journal of Physiology* **489**, 203–214.

Jones, J. F. X., Wang, Y. & Jordan, D. (1998). Activity of C fibre cardiac vagal efferents in anaesthetized cats and rats. *Journal of Physiology* **507**, 869–880.

Jordan, D., Spyer, K. M., Withington-Wray, D. J. & Wood, L. M. (1986). Histochemical and electrophysiological identification of cardiac and pulmonary vagal preganglionic neurones in the cat. *Journal of Physiology* **372**, 87*P*.

McAllen, R. M. & Spyer, K. M. (1976). The location of cardiac vagal preganglionic motoneurones in the medulla of the cat. *Journal of Physiology* **258**, 187–204.

McAllen, R. M. & Spyer, K. M. (1978*a*). Two types of vagal preganglionic motoneurones projecting to the heart and lungs. *Journal of Physiology* **282**, 353–364.

McAllen, R. M. & Spyer, K. M. (1978*b*). The baroreceptor input to cardiac vagal motoneurones. *Journal of Physiology* **282**, 365–374.

Potter, E. K. (1981). Inspiratory inhibition of vagal responses to baroreceptor and chemoreceptor stimuli in the dog. *Journal of Physiology* **316**, 177–190.

Taylor, E. W., Jordan, D. & Coote, J. H. (1999). Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. *Physiological Reviews* **79**, 855–916.

Wang, Y. & Ramage, A. G. (1999). Activation of cardiac vagal preganglionic neurones in the nucleus ambiguus during cardiopulmonary reflex in anaesthetized cats. *Journal of Physiology* **518**, 26–27*P*.

Wang, Y., Ramage, A. G. & Jordan, D. (1998). Presynaptic 5-HT_3 receptors evoke an excitatory response in dorsal vagal preganglionic neurones in anaesthetized rats. *Journal of Physiology* **509**, 683–694.

Acknowledgements

The work is supported by the Wellcome Trust (Grant 050894/Z) and a Health Research Board (Ireland)/British Council travel grant. R.D.J is a British Heart Foundation PhD Student. We are also grateful for the technical assistance of Mr S. Wilkinson.

Corresponding author

Y. Wang: Department of Pharmacology, University College London, Royal Free Campus, Rowland Hill Street, London NW3 2PF, UK.

Email: yunwang@rfhsm.ac.uk