AN INVESTIGATION INTO THE ACTIONS OF 5-HT AS REVEALED BY WAY-100635 IN THE RAT DRN

By

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Declaration

The composition of this thesis and the research described therein is entirely my own work.

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Contents

Abstract

Intracellular recordings were made from presumptive serotonergic neurones in the *in vitro* slice preparation of the rat dorsal raphe nucleus confirming previous observations that the membrane hyperpolarisation evoked by bath-application of 5-HT is associated with a decrease in input resistance. The reversal level was approximately -90 mV, a value close to that predicted for a potassium-mediated event. The 5-HT-evoked hyperpolarisation was blocked by WAY-100635, a result consistent with the response being mediated by the 5-HT_{1A} receptor subtype. WAY-100635 alone was found to have no effect on membrane potential or input resistance in this study and in this sense is silent. In addition, WAY-100635 did not block the α_1-adrenoreceptor-mediated membrane depolarisation and increase in input resistance that follows bath-application of noradrenaline and phenylephrine.

The blockade of the 5-HT_{1A}-evoked hyperpolarisation with WAY-100635 revealed a 5-HT-evoked depolarisation associated with an increase in cell excitability. In the majority of cells tested there was no significant change in input resistance and I/V analysis failed to show a point of intersection in the range -120 mV to -60 mV. However, some cells did show a change in input resistance at approximately -50 mV. The depolarising response was observed in the presence of TTX showing it to be independent of propagated action potentials and thus unlikely to involve a transynaptic event.

In the presence of WAY-100635, the 5-HT_2 agonist DOI, evoked a depolarisation with similar properties to that evoked by 5-HT. The more selective 5-$HT_{2B/2C}$ agonist mCPP and the 5-HT_{1B} agonist CP 93129 had no effect in WAY-100635 treated cells. The broad spectrum 5-HT receptor antagonist methysergide blocked the 5-HT-evoked depolarisation in 6 of 8 cells as well as the depolarising action of DOI. The 5-HT_2 antagonist ketanserin completely blocked the 5-HT-evoked depolarisation, as did the 5-HT_{2A} selective antagonist MDL 100, 907. The 5-HT_{2C} selective antagonist, SB 200646, partially blocked the response. The 5-$HT_{1A/1B}$ antagonist pindolol did not

block the depolarisation. These findings suggest that a 5-HT_{2A} receptor subtype mediates the 5-HT-evoked depolarisation.

5-HT receptor mediated modulation of the action potential and the afterhyperpolarisation (AHP) were also investigated. Action potentials were overshooting, of long duration and sensitive to block by TTX. The repolarising phase of the action potential has a shoulder thought to be due to the activation of voltage-sensitive calcium channels. Bath-application of TEA resulted in an action potential of a much longer duration. Single action potentials were followed by an AHP that reversed at approximately -90 mV, consistent with a potassium-mediated event. Two components of the AHP were identified. TEA blocked a fast component that appears as a continuation of the repolarising phase of the action potential, while apamin blocked a slower component that normally decayed over 150 ms. These results indicate the involvement of TEA-sensitive potassium channels in the early component of the AHP and apamin-sensitive SK channels in the slower, late phase. Firing frequency and adaptation were also investigated. In control cells a long depolarising pulse generated a train of regularly spaced action potentials through the whole of the pulse with no apparent accommodation. Following bath-application of apamin a typical cell showed an increase in its firing rate and in addition displayed prominent accommodation. Bath-application of TEA also increased firing frequency and resulted in accommodation.

Bath-application of 5-HT led to a reduction in the shoulder of the action potential and a decrease in the duration of the AHP. Additionally, an increase in spike frequency was observed in response to a long depolarising current pulse. It has been reported that 5-HT_{1A} receptors mediate an inhibition of voltage-sensitive calcium channels. This supports the theory that 5-HT_{1A} receptor activation inhibits the influx of calcium during the action potential thereby leading to the inhibition of the calcium dependent phase of the AHP. In agreement with this WAY-100635 blocked the 5-HT-evoked increase in spike frequency.

In summary, 5-HT has an important role in the regulation of cell excitation in the DRN. Excitability is predominantly regulated by a 5-HT_{1A}-mediated hyperpolarisation, which inhibits cell firing, and the 5-HT_{1A}-mediated inhibition of voltage-sensitive calcium channels, which have a role in the regulation of spike width and the AHP. Significantly this study has shown that the hyperpolarisation is blocked by the 5-HT_{1A} antagonist WAY-100635. Cell excitability is also regulated by a 5-HT-evoked depolarisation. This is mediated by the 5-HT_{2A} receptor indicating a direct functional role for non-5-HT_{1A} receptor subtypes in the DRN. The absence of any significant resistance change associated with this depolarisation could indicate that in the DRN 5-HT_{2A} receptors either modulate separate potassium and sodium conductances or activate non-selective ion channels.

Glossary

AC	adenylyl cylcase
aCSF	artificial cerebrospinal fluid
AHP	afterhyperpolarisation
BK	large conductance calcium-activated potassium channels
cAMP	cyclic adenosine monophosphate
cDNA	complementary deoxynucleic acid
CNS	central nervous system
5-CT	5-carboxamidotryptamine
DAG	diacyl glycerol
DCC	discontinuous current clamp
DNA	deoxynucleic acid
DOM	1-(2, 5-dimethyoxy-4-methylphenyl)-2-aminopropane
DP-5-CT	dipropyl-5-carboxamidotryptamine
DR	dorsal raphe
DRN	dorsal raphe nucleus
EPSP	excitatory postsynaptic potential
GABA	γ-aminobutyric acid
G-protein	guanine nucleotide-binding protein
GTI	5-*O*-carboxamidomethylglycyl[^{125}I]tyrosinamidetryptamine
5, 7-DHT	5, 7-dihydroxtryptamine
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptmine
5-HTP	5-hydroxytryptophan
HVA	high-threshold calcium channels
IP_3	inositol trisphosphate
IPSP	inhibitory postsynaptic potential
IUPHAR	International Union of Pharmacology
LSD	lysergic acid diethylamide
LVA	low-threshold calcium channels
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
mRNA	messenger ribonucleic acid
NA	noradrenaline
NAD^+	nicotinamide adenine dinucleotide
NAN-190	1-(2-methoxyphenyl)-4-[4-(2-phthalimmido)butyl]piperzine HBr
NMDA	*N*-methyl-D-aspartate
8-OH-DPAT	8-hydroxy-2(di-*n*-propylamino)tetraline
p-CPA	p-chlorophenylalanine
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PSP	postsynaptic potential
REM	rapid eye movement
SK	small conductance calcium-activated potassium channels
SSRI	serotonin selective reuptake inhibitor

Compounds

Apamin	18 amino acid peptide blocker of Ca^{2+}-activated K^+ channels isolated from *Apis mellifera* honey bee venom.
CP 93129	3-(1, 2, 5, 6-tetrahydropyrid-4-yl)pyrrolo[3, 2-b]pyrid-5-one
DOI	1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane
5-HT	5-hydroxytryptamine
Ketanserin	3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl] ethyl]-2,4(1H,3H)-quinazolinedione
mCPP	1-(3-chlorophenyl)piperazine
MDL 100, 907	[*R*-(+)-α-(2, 3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol]
Methysergide	[8{BETA}(*S*)]-9,10-didehydro-*N*- [1-(hydroxymethyl)propyl]-1 6-dimethyl-ergoline-8-carboxamide
Noradrenaline	*L*-Noradrenaline
Phenylephrine	(*R*)-3-hydroxy- {ALPHA}-[(methylamino) methyl]-benzenemethanol
Pindolol	1-(1H-indol-4-yloxy)-3-[(1-methylethyl) amino]-2-propanol
SB 200646	*N*-(1-methyl-5-indolyl)-*N*-(3-pyridyl urea hydrochloride)
TEA	tetraethylammonium
TTX	tetrodotoxin
WAY-100635	(*N*- [2-[4(2-methoxyphenyl)-1-piperazinyl-1-piperazinyl]-*N*-2-pyridinyl)cyclohexanecarbonate

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CHAPTER 1
Introduction

General

Serotonin or 5-hydroxytryptamine (5-HT) is an important chemical messenger in the mammalian brain. It has proven to be involved in a number of different roles in the central nervous system (CNS) and continues to hold important functional secrets. It has been linked to many physiological and behavioural functions including aggression, appetite, cognition, endocrine function, motor function and sleep. Many drugs are thought to act through serotonergic mechanisms, including selective serotonin uptake inhibitors (SSRI's) used to treat depression and atypical antipsychotics. This multitude of different functions is possible due to the molecular diversity of the serotonin receptor and the anatomy of the serotonergic system.

5-HT production is centred in the raphe nuclei, which are clusters of cell bodies found in the brainstem. From the raphe nuclei projections are sent down the spinal column and up to high centres within the brain, such as the hippocampus and cortex. This makes the methods of control of the neuronal firing and so 5-HT release important mechanisms for study. The dorsal raphe nucleus (DRN) region generally has a constant tonic firing pattern and each action potential is accompanied by the release of 5-HT. The rate of cell firing can shift up or down depending upon the chemical environment of the cell that varies with different behavioural states. For example, during rapid eye movement (REM) sleep the DRN region is quiet. Receptor diversity allows 5-HT to have both excitatory and inhibitory actions even on the same brain region and same type of cell. One early example of this was found during intracellular studies of cortical pyramidal cells where 5-HT can be inhibitory via binding to one receptor subtype and excitatory via binding to another receptor subtype. This type of contradictory message has been found in a number of brain regions including the pyramidal cells of the hippocampus and the dorsal root

ganglion. Thus serotonin can act to both increase and decrease excitability in a single cell within a network of cells. Serotonin appears to have a role in modulating cell excitability throughout the CNS and in this way co-ordinates the activity of neurones and networks within a given behavioural state. In the DRN 5-HT predominantly causes a membrane hyperpolarisation via the 5-HT_{1A} receptor subtype. Investigations into a functional role for non-5-HT_{1A} receptor subtypes have been hindered by the lack of silent 5-HT_{1A} antagonists. The use of the newly developed compound WAY-100635, a selective 5-HT_{1A} antagonist, allows the search for a multiplicity of actions for 5-HT in the DRN.

Research Aims

Since it was first realised that the DRN was the largest site of serotonin producing cells in the CNS the physiological importance of the DRN has been investigated using a number of different techniques. Investigations using pharmacological, biophysical and molecular techniques have led to a greater understanding of the role of the DRN in the CNS. Biophysical investigations were limited at first due to the problems of electrode impalement stability. The *in vivo* preparation (Aghajanian and VanderMaelen 1982a) has largely been restricted to extracellullar investigations of the pharmacological alterations of unit activity of neurones within the DRN (Aghajanian et al 1970; Bramwell and Goyne 1973; Mosko and Jacobs 1977). With the introduction of the *in vitro* slice preparation (Li and McIlwain 1957) it became possible to study specific brain regions using both extracellular and intracellular electrodes. Some brain regions were found to lend themselves easily to this technique, but it was not until Crunelli et al (1983) that the first stable intracellular recordings were obtained from neurones in the DRN slice. The *in vitro* DRN slice preparation has now become a useful tool for studying the properties of neurones in this nucleus.

The actions of 5-HT in the DRN are mediated by the 5-HT_{1A} receptor so use of the silent 5-HT_{1A} antagonist WAY-100635 allows the opportunity to more completely study the actions of 5-HT in the DRN by selective blockade of the 5-HT_{1A} receptor.

These 5-HT-mediated actions include the effects of 5-HT on the action potential in the DRN and any functional role for non-5-HT_{1A} receptor mediated 5-HT effects. This programme of research set out to investigate the following issues:

1) The characterisation of the 5-HT_{1A}-evoked membrane hyperpolarisation *in vitro* in the hippocampal and DRN brain slice using intracellular electrophysiology.
2) An investigation into the effects, if any, of the 5-HT_{1A} antagonist WAY-100635 on the properties of hippocampal and DRN neurones.
3) The study of the mechanisms and pharmacology of non-5-HT_{1A} mediated actions of 5-HT in neurones of the DRN following pre-application of WAY-100635.

Neuronal excitability in the DRN is regulated by a number of mechanisms including the pacemaker potential, which in turn is made up of a number of different conductances, and the relative position of the membrane potential to the firing threshold. 5-HT is known to inhibit neuronal excitability indirectly via the 5-HT_{1A}-evoked hyperpolarisation. A more direct role for the regulation of firing by 5-HT should also be investigated. For instance, does 5-HT released by a neurone with a single action potential have a local feedback effect on that neurone's firing pattern. This was investigated with the following studies:

1) The characterisation of the conductances involved in the pacemaker potential of the DRN neurone with particular attention paid to spike width, and the AHP.
2) The observation of the actions of 5-HT on the spike width, AHP and the overall firing pattern of DRN neurones.

This chapter reviews the DRN and its anatomy and physiology. The introduction then goes on to discuss the role of serotonin in the brain and its discovery and production. Particular attention is paid to the actions of 5-HT released within the DRN and there is reference to the 5-HT receptor subtypes found in the DRN. Finally,

the introduction reviews the properties and pharmacological classification of each of the 5-HT receptor subtypes.

The Dorsal Raphe Nuclei

The DRN is one of the clusters of serotonergic cell bodies that make up the raphe nuclei. Dahlstrom and Fuxe first reported in 1965, using histochemical techniques, that 5-HT containing neurones were present in the CNS. Serotonergic neurones have now been found in many brain regions and several methods have been used to characterise these cells. Histochemical studies show the largest cluster of 5-HT cells is found in the brainstem and midbrain. These cells can be designated as nine distinct cell groups, B1-B9, where each cell group corresponds generally to a different area of innervation (Figure 1.0). For example, the medullary nuclei send projections mainly in the caudal direction, where they seem to have roles in the regulation of nocioceptive pathways within the spinal cord and motor control (Bowker et al 1983, 1987). The present study concentrated on the serotonergic neurones of the DRN (B7).

Anatomy

The largest group of 5-HT-containing cells in the brain is found in the DRN, however, there are also non-serotonergic neurones present in the DRN. Thanks to the use of a number of techniques, including autoradiography (Taber-Pierce et al 1976, Bobillier et al 1976) and immunocytochemistry (Steinbusch 1981, Steindler et al 1983) the efferent projections of the DRN have been mapped to a number of higher brain centres. Fibres arising from the DRN have fine axons with irregularly spaced granular or fusiform varicosities that branch frequently (Kosofsky and Molliver 1987; Mulligen and Tork 1988). The DRN innervates areas within the midbrain and forebrain, especially the thalamus, amygdala, cerebral cortex, septum and lateral habenula. The DRN has also been found to innervate more caudal structures such as the locus coeruleus and the pontine and reticular nuclei (Taber-Pierce et al 1976). It may be that these connections regulate the reciprocal firing rates of raphe and reticular neurones during the sleep cycle.

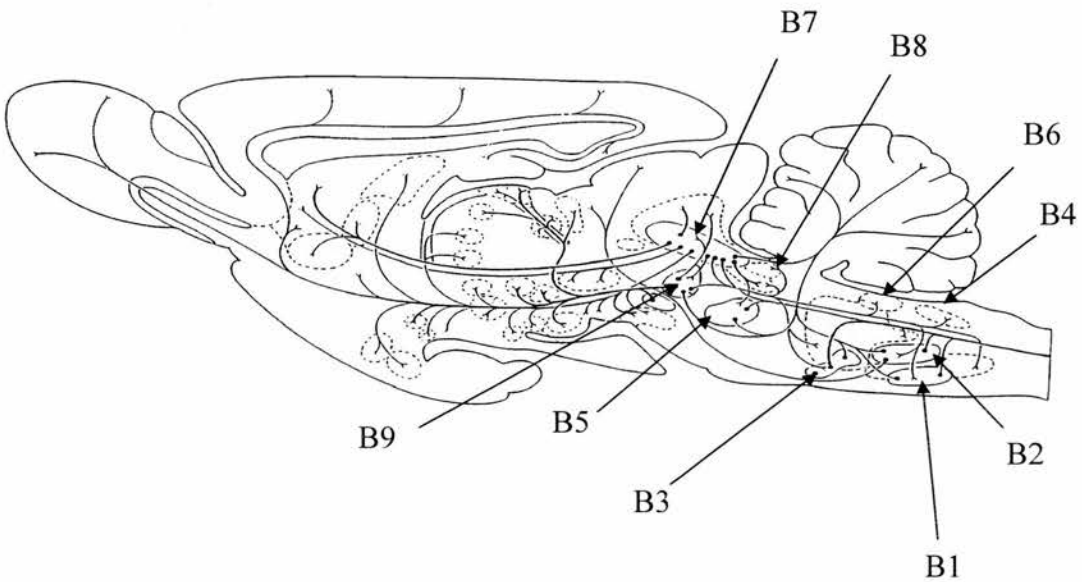


Figure 1.0. Schematic illustration of the major 5-HT pathways in the rat brain. The cell groups B1-B9 represent those described by Dahlstrom & Fuxe (1964) and correspond to the raphe nuclei (B1 – nucleus raphe pallidus; B2 – nucleus raphe obscurus; B3 – nucleus raphe magnus; B4 – nucleus raphe obscurus, dorso lateral part; B5 – median raphe nucleus caudal, part; B6 – dorsal raphe nucleus, caudal part; B7 – dorsal raphe nucleus rostral, part; B8 – caudal linear nucleus and nucleus raphe pontis; B9 – nucleus raphe pontis and supralemniscal region). Projections form B1 – B3 descend to innervate the spinal cord, whilst B5 – B9 innervate the forebrain. (Adapted from Cooper et al., The Biochemical Basis of Neuropharmacology 1982).

Physiology

Much of the work increasing the understanding of the physiology of the DRN has been carried out by observing single unit activity of the brain serotonergic neurones of conscious freely moving cats. This allows the neuronal activity of the DRN to be followed over long periods of time, from hours to weeks, in an animal capable of living a normal life. The raphe firing rate has been widely observed to vary with sleep and is almost completely suppressed during REM sleep in the cat (Adrien and Lanfumey 1986, Trulson 1985). This shows that the raphe firing is related to the state of the organism. During periods of wakefulness the raphe has a slow and regular rhythmic discharge (2-10 spikes/s). The rate of discharge decreases with drowsiness and again with each stage of progressively deeper sleep, such as slow-wave sleep and paradoxical sleep. These observations suggest that the DRN has a role in regulating an animal's level of general arousal. The depression of the raphe firing may be controlled by the level of activity in the brain structures mediating CNS motor control. REM sleep is associated with centrally induced atonia leading to a decrease in input to the raphe from the centres for motor control and therefore a decrease in raphe activity.

The regulation of the spontaneous firing activity of the DRN is in part down to the intrinsic nature of the DRN neurone and in part due to the influence of transmitter released from inside or outside the DRN. Noradrenaline release increases the activity of the DRN via the α_1-adrenoceptor (Baraban and Aghajanian 1980a). The DRN also regulates itself via the release of 5-HT, which takes the form of auto-inhibition by decreasing firing activity (Aghajanian and VanderMaelen 1982). Activation of the auto-inhibition mechanism has a number of physiological and behavioural effects. These effects include changes in locomotive behaviour (Hillegaart 1990; Higgins and Elliot 1991), a decrease in body temperature (Higgins et al 1988; Hillegaart 1991) and blood pressure (Connor and Higgins 1990; Valenta and Singer 1990) and an increase in food consumption (Bendotti and Samanin 1986; Hutson et al 1986). This implies that the DRN has more roles than simply regulating arousal. The regulation

of the spontaneous activity is an important mechanism for study and will be discussed further in later chapters.

Serotonin

Discovery

Serotonin was first described as a naturally occurring compound in the blood where it was thought to be an endogenous serum factor causing vasoconstriction (Stevens and Lee 1884; Brodie 1900). The compound was purified from serum and characterised as 5-hydroxytryptamine (5-HT) (Rapport et al 1948, Rapport 1949). Another substance, named enteramine, was found in the enterochromaffin cells of the gut where it was characterised as causing smooth muscle contraction (Vialli and Erspamer 1933). When enteramine was later purified it was found to be identical to 5-HT (Erspamer and Asero 1952).

The presence of 5-HT in the mammalian brain was established following the development of a 5-HT sensitive bioassay (Twarog and Page 1953). This bioassay was based on the observation that 5-HT excited the heart in some molluscs (Erspamer and Ghiretti 1951) and takes the form of an isolated heart from *Venus mercenaria*. Once 5-HT could be extracted from mammalian tissues and quantified it was realised that 5-HT was distributed heterogeneously throughout the canine brain (Amin et al 1954) making it apparent that it may act as a chemical messenger (Woolley and Shaw 1954a).

With the elucidation of the chemical structure of 5-HT it became obvious that a number of other compounds had structural similarities. These included alkaloids, such as the ergot derivatives, methysergide and lysergic acid diethylamide (LSD), which were observed *in vivo* to antagonise the contractile effects of 5-HT on vascular smooth muscle (Shaw and Woolley 1953). Several of these compounds had been observed to have actions on brain function and behaviour in man where they can cause "mental aberrations"; these included hallucinations and a transitory change in mental state resembling schizophrenia (Woolley and Shaw 1954a). As a consequence

of these observations it was proposed that 5-HT may be involved in the regulation of a normal mental state and that disruption of its actions lead to abnormal mental states such as schizophrenia (Woolley and Shaw 1954a).

Biosynthesis

5-HT is synthesised from the precursor amino acid tryptophan by enzymatic action. Tryptophan is found in the diet but this is largely metabolised in the gut before reaching the bloodstream. In the first stage of the synthesis of 5-HT tryptophan is converted to 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase by hydroxylation of its indole ring (Joh et al 1975). This process is found in chromaffin cells and neurones, but not in platelets. 5-HTP is then decarboxylated by the enzyme L-amino acid decarboxylase to give 5-HT (Clark et al 1954).

Storage and Release

Prior to release 5-HT is stored in vesicles at the nerve terminal with its specific 5-HT binding protein (Tamir and Gershon 1979). Release into the synaptic cleft is stimulus-evoked and mediated by calcium-dependent exocytosis (Elks et al 1979). Once stimulated 5-HT is then rapidly removed from the synaptic cleft by an active uptake mechanism (Shaskan and Snyder 1970) which takes the form of a Na^+-dependent transporter protein (Blakely et al 1991).

Degradation

Following uptake into the cell 5-HT is degraded by a process of oxidative deamination resulting in an aldehyde, this is catalysed by the enzyme monoamine oxidase (MAO) (Weissbach et al 1961) found on the outer membrane of the synaptic terminal mitochondria (Schnaitman and Greenawalt 1968). This is followed by oxidation to 5-hydroxyindoleacetic acid (5-HIAA) by an NAD^+-sensitive aldehyde dehydrogenase (Duncan and Sourkes 1974). 5-HIAA is then removed from the body by excretion in the urine and is a marker for 5-HT production in the body.

Serotonin and the DRN

To evaluate the role of 5-HT release on DRN excitability, animals were pre-treated with the 5-HT synthesis inhibitor p-chlorophenlyalanine (p-CPA) (Koe and Wiessman 1966). When administered alone p-CPA results in a depletion of brain 5-HT, but does not affect the DRN firing rate (Aghajanian et al 1970). Treatment with monoamine oxidase inhibitor's (MAOI's), such as paragyline, were also looked at. This resulted in an increase in extracellular 5-HT and a decrease in firing in the DRN. This suggests extracellular accumulation of 5-HT sets off a negative feedback loop on 5-HT containing cells. Pre-treatment with p-CPA prevents the inhibitory effects of MAOI's. This suggested the theory that 5-HT release has an autoregulatory role in DRN firing regulation.

Regulation of Firing in the DRN

Early histochemical studies showed the presence of noradrenergic inputs into the DRN (Loizou 1969, Roizen and Jacobowitz 1976). Later studies using electron microscopic radiography gave a more detailed picture and showed noradrenergic inputs terminating on 5-HT cells with the formation of synaptic machinery (Baraban and Aghajanian 1981). Baraban and Aghajanian (1980a) also found that noradrenaline increases the firing rate of serotonergic neurones in the DRN which could indicate that noradrenaline increases 5-HT release in the DRN.

Retrograde labelling showed that the DRN received innervation from the substantia nigra, medial preoptic nucleus and the lateral habenula (Aghajanian and Wang 1977). Stimulation of the substantia nigra was shown to depress the frequency of DRN firing activity (Stern et al 1979a). Stern et al also showed that single unit activity in the DRN was suppressed following stimulation of the lateral habenula. Stimulation of the lateral habenula also resulted in a reduction in the release of labelled [^{3}H] 5-HT from the substantia nigra and caudate nucleus. Local application of picrotoxin (a GABA antagonist) in the DRN antagonised the inhibition of 5-HT release (Reisine et al

1982). This was suggestive of a negative feedback loop regulating DRN firing activity involving one or more afferent inputs.

The feedback loop was thought to be a local effect following the work of Haigler and Aghajanian (1974). In this study they showed that the application of 5-HT and LSD to the DRN could still cause a decrease in baseline firing rates following transection of the brain between the diencephalon and the mesencephelon. This did not eliminate the brainstem as a site of the feedback mechanism. Work performed with the aromatic amino acid decarboxylase inhibitor R04-4602 did. This compound leads to the depletion of 5-HT in the cells of the DRN. Pre-treatment with R04-4602 was found to increase accumulation of 5-HTP in the perykarya of the DRN cell bodies and its projections (Carlsson and Lindqvist 1970). Gallager and Aghajanian (1976) showed that a reduction of DRN firing was seen following L-tryptophan treatment. This led to the idea that the local release of 5-HT in the extracellular fluid inhibited DRN firing. Thus adding support to the observations that 5-HT and LSD could inhibit firing following R04-4602 treatment, and that 5-HT was released following DRN depolarisation with potassium chloride (Henry et al 1982). The most direct route for the feedback loop was thought to be via 5-HT axon collaterals. Antidromic stimulation of the DRN was shown to cause a period of post-stimulus inhibition in the amygdala (Wang and Aghajanian 1977). This inhibition is still present following transection of the frontal afferent pathways (Wang and Aghajanian 1977) and following the destruction of noradrenergic pathways by 6-hydroxydopamine. It is lost, however, following the destruction of 5-HT pathways by 5, 7-DHT, confirming that local release of 5-HT suppressed DRN firing is probably due to the interaction of the transmitter with a receptor. In the DRN the 5-HT_{1A} receptor is now thought to act as a somatodendritic autoreceptor, which acts to control the release of 5-HT onto postsynaptic sites by inhibiting cell firing.

The Somatodendritic Autoreceptor

Serotonergic neurones of the DRN are inhibited by bath-application of 5-HT leading to the 5-HT_{1A} receptor, which mediates this inhibition, being termed the

somatodendritic autoreceptor. Activation of the somatodendritic autoreceptor was found to trigger a number of changes in the cell including a reduction of 5-HT synthesis and release and electrical activity (de Montigny and Blier 1992). As with all 5-HT_{1A} receptors activation leads to an opening of K^+ channels resulting in a membrane hyperpolarisation and decrease in input resistance (Aghajanian and Lakoski 1984). Some differences have been observed in each brain region expressing the 5-HT_{1A} receptor in the ability of agonists and antagonists to effect these K^+ channels which could suggest the molecular organisation of the "5-HT_{1A}-G protein-K^+ channel" complex might show regional differences (Corradetti et al 1996). For example, differences in the nature of the coupled G-protein and the ratio of the 5-HT_{1A} receptor binding protein and it corresponding G-proteins might explain why some drugs act as full agonists in the DRN, but as partial agonists in other areas, e.g. the hippocampus (Hamon et al 1990). The 5-HT_{1A} receptor has been found to have different functions in different regions of the brain. In the DRN the 5-HT_{1A} receptor acts as a somatodendritic autoreceptor, but is a postsynaptic receptor in the hippocampus. There is some evidence to show that these 5-HT_{1A} receptor types are different. This followed from the work with pindolol and (-)-pindolol, β-adrenoceptor antagonists with high affinity for the 5-HT_{1A} receptor subtype. Some studies have reported, using electrophysiological techniques, that these compounds block the action of 5-HT at the 5-HT_{1A} autoreceptor but have no effect at the postsynaptic 5-HT_{1A} receptor (Artigas et al 1996; Romero et al 1996). However, there is also much conflicting data to suggest that pindolol shows no preference for 5-HT_{1A} receptors and that these different 5-HT_{1A} receptor types are, at the pharmacologically identifiable level, identical.

The Pacemaker Potential

DRN neurones show a repeating cycle of membrane potential changes that take place during the generation of spontaneous action potentials; this is the pacemaker potential (Burlhis and Aghajanian 1987). The pacemaker potential is made up of four repeating stages:

1) a rapid depolarisation and an all-or-nothing action potential;
2) the action potential repolarises and then overshoots to give a long lasting AHP of 5-20 mV that gradually returns to the resting membrane potential, taking approximately 200 ms;
3) the AHP leads to de-inactivation of I_A, which slows the return of the AHP to the baseline membrane potential;
4) the AHP decays so the cell returns to resting membrane values. The hyperpolarisation of the AHP de-inactivates a low threshold calcium spike (or pre-potential), a slow depolarising potential. This leads to the activation of sodium channels and the next action potential.

This regular firing pattern has been given the term pacemaker activity. Several stages, each mediated by different ion channel conductances, are involved in the generation of the pacemaker potential. Each of these stages may be mediated by external influences such as the presence of neurotransmitter in the extracellular fluid. The ion channel conductances involved in the pacemaker potential include:

Sodium - The membrane potential undergoes a rapid depolarisation due to the activation of a TTX-sensitive sodium conductance. This leads to the generation of an all-or-nothing action potential.

Calcium - A broad action potential is generated with an extended shoulder due to the activation of a high-threshold calcium conductance (Burlhis and Aghajanian 1987) allowing the influx of calcium into the cell. This slows the repolarisation of the cell until it is overcome by the activation of the voltage-activated potassium current. Both the high threshold calcium channels and low threshold calcium channels are voltage-activated. 5-HT may have a role in the regulation of the high threshold calcium conductance through the 5-HT_{1A} receptor.

Potassium - The cell repolarises and then overshoots the resting membrane potential to give an AHP. This is due to the activation of calcium-activated potassium

channels (VanderMaelen and Aghajanian 1982b; Aghajanian 1985) and regulates the interspike interval, the greater the AHP the greater the interspike interval and the slower the firing frequency. A third potassium conductance inhibits the cell's ability to generate an action potential. This is the voltage-gated transient outward current, I_A, and has a role in the regulation of the interspike interval. At the end of the action potential I_A channels are inactivated, however, they have been de-inactivated by the membrane hyperpolarisation of the AHP. As the cell depolarises towards the next action potential the I_A channels open. This I_A current almost cancels the spike stimulus current, delaying depolarisation. However, this is overcome, resulting in the small depolarisation due to the activation of low threshold T-type calcium channels so the cell reaches firing threshold and the generation of an action potential.

Excitation in the DRN

It is well established that the neurones of the DRN respond to exogenously applied 5-HT by a membrane hyperpolarisation and a decrease in input resistance (Aghajanian and VanderMaelen 1982b) and that it is mediated by the 5-HT_{1A} autoreceptor (Sprouse and Aghajanian 1987). Activation of this receptor leads to inhibition of cell firing and the action of 5-HT on this site is mimicked by the action potential coded release of endogenous 5-HT in the nucleus *in vivo* (Wang and Aghajanian 1977; Yoshimura et al 1985). However, it is also known that there are a number of receptors other than the 5-HT_{1A} receptor found in the DRN and that throughout the CNS a multiplicity of 5-HT-evoked actions can coexist within the same neurone.

In the DRN a membrane depolarisation follows bath-application of noradrenaline increasing the firing rate of neurones of the DRN (Baraban and Aghajanian 1980a). However, there are a number of examples of 5-HT-evoking a membrane depolarisation associated with an increase in cell excitability. There are many examples of 5-HT having both an inhibitory and excitatory action on neurones of the same cell population. In the medial pontine reticular formation 5-HT acts on 5-HT_1 and 5-HT_2 receptors by evoking a membrane hyperpolarisation and depolarisation respectively, due to opposing actions on different potassium conductances. This is

also seen in the nucleus prepositus hypoglossi and in the cerebrocortical neurones (Araneda and Andrade 1991; Davies et al 1987). In the hippocampus the 5-HT_{1A} receptor mediates the hyperpolarisation but the 5-HT-evoked depolarisation is mediated by 5-HT_4-like receptors (Andrade and Nicoll 1987, Araneda and Andrade 1991; Colino and Halliwell 1987), these effects are again due to opposing actions on different potassium conductances.

Electrophysiology of the DRN

An early study by Aghajanian and Haigler (1974) used extracellular recording techniques *in vivo* to look at firing rates in neurones of the DRN. They found DR cells to fire with a regular rate of 0.5-2 Hz., the action potentials had a positive-negative waveform and that firing was inhibited by administration of LSD. Later studies by Aghajanian and VanderMaelen (1982) used intracellular recording methods *in vivo* to characterise the membrane properties of the cells. The action potentials were of a long duration with amplitudes in the range 62-80 mV and the cells input resistances ranged from 30-70 MΩ. The action potential was followed by a large post spike hyperpolarisation (approximately 6 mV) and this together with a gradual interspike depolarisation was termed the "pacemaker potential", these were thought to be regulatory factors in determining raphe spike firing pattern.

Later studies performed *in vitro* by Crunelli et al (1983) made intracellular recordings showing a spontaneous rhythmic pattern made up of single action potentials occurring at 0.25-5 Hz. The action potential was followed by a long lasting AHP with the input resistance in the range 100-320 MΩ, higher than *in vivo*. Trulson et al (1982) showed that the raphe cell maintained its own rhythmic firing pattern indicating that the DR cells had an intrinsic pacemaker activity.

Serotonergic neurones in the DRN hyperpolarise following exposure to 5-HT due to binding with the 5-HT_{1A} receptor. This is accompanied by a decrease in cell input resistance and results in a decrease in cell firing and 5-HT release (Aghajanian and VanderMaelen 1982). This is due to the opening of potassium channels in the cell

membrane (Aghajanian and Lakoski 1984) which are inwardly rectifying (Williams et al 1988). Similar findings have been observed in the acutely isolated (Penington et al 1993b) and microcultured (Johnson 1994) DRN neurones, which also shows that autoreceptor inhibition is independent of any input in the DRN. These effects are mimicked by the 5-HT_{1A} agonists 8-OH-DPAT and ipsapirone where neurones also hyperpolarise and show a decrease in input resistance indicating the effect is due to binding with the 5-HT_{1A} receptor (Sprouse and Aghajanian 1987).

The 5-HT_{1A} receptor is also coupled to calcium channels. Whole cell recordings in acutely isolated DRN neurones have shown that 5-HT decreases high-threshold calcium currents (HVA) and that this is mimicked by 8-OH-DPAT (Penington and Kelly 1990). This calcium current is insensitive to L-type channel blockers but is partially sensitive to ϖ-conotoxin, an N-type channel blocker (Penington et al 1991).

5-HT Receptors in the DRN

The 5-HT_{1A}-evoked membrane hyperpolarisation was one of the first to be identified and characterised in the DRN. This characterisation was aided by the development of the selective 5-HT_{1A} agonist 8-OH-DPAT which, when bath-applied, mimicked the response of 5-HT (Sprouse and Aghajanian 1987). It is known that a number of 5-HT receptor subtypes exist in the DRN but the search for electrophysiologically identifiable responses mediated by non-5-HT_{1A} receptors has proven difficult. There are low levels of a number of 5-HT receptors including 5-HT_{1B}, 5-ht_{1F}, 5-HT_{2A}, 5-ht_{5B} and 5-HT_7 found in the DRN. 5-HT_{1B} receptor mRNA has been reported in the raphe nuclei (Voigt et al 1991). There are reports of 5-ht_{1F} receptor mRNA being concentrated in the DRN (Adham et al 1993b). The 5-HT_{2A} receptor has been found to mediate neuroexcitation in the raphe cell body (Roberts and Davies 1989). The 5-ht_{5B} receptor has been detected in the DRN (Erlander et al 1993). Autoradiography has revealed a low density of 5-HT_7 receptor sites in the DRN (Waeber and Moskowitz 1995). Although the levels of expression of these receptors is low it does not rule out the physiological significance of any functional effects of 5-HT binding. However, it may be that these receptors will only be electrophysiologically

observable following the blockade of the dominant and highly expressed 5-HT_{1A} receptor. The study of this has so far been difficult because of the lack of a truly silent and selective 5-HT_{1A} antagonist.

Classification of the 5-HT Receptor Family

5-HT exerts its neurotransmitter actions via specific interactions with cell surface receptors. Currently seven families of 5-HT receptors have been identified, made up of a total of fourteen different subtypes. Early work characterised each of the receptor subtypes using pharmacological tools. The receptors can be activated by 5-HT and a range of different compounds or agonists. Differences in receptor protein structure give each receptor different binding affinities for various compounds. This allows receptor classification by its binding profile, which also allows any 5-HT-evoked responses to be classified to a receptor once its pharmacological profile has been established. Gaddum and Picarelli (1957) were the first to differentiate between 5-HT binding sites in the gut. They proposed that there were two receptor subtypes, the M (morphine) and D (dibenzyline) receptors and named them after the compounds found to block their activation. The D receptor was found predominantly in smooth muscle and the M receptor was found more commonly on nerve ganglia and fibres. The M receptor is now classified as the 5-HT_3 receptor and the D receptor is classified the 5-HT_{2A} receptor.

Molecular cloning has allowed a new way to classify receptors based in part on the pharmacological characteristics but also on molecular structure and second messenger system (Hoyer et al 1994). The International Union of Pharmacology (IUPHAR) decided that the nomenclature system of the 5-HT receptors should be based on operational (pharmacological), transductional (functional) and structural characteristics. This included the 5-HT_{1D}, 5-ht_{1E} and 5-ht_{1F} receptors as part of the 5-HT_1 receptor family (Amlaiky et al 1992) and recognised the 5-HT_4 receptor as a separate receptor class (Dumis et al 1988a, b). 5-HT_1 became the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1E} and 5-ht_{1F} receptors. The 5-HT_{1C} receptor was renamed the 5-HT_{2C}

receptor and the 5-HT_2 family came to include the 5-HT_{2B} receptor (Humphrey et al 1993). New receptor subtypes have been discovered and termed 5-ht_5, 5-ht_6 and 5-HT_7 (Hoyer et al 1994; Martin and Humphrey 1994). Receptors can now be expressed in cell lines making them more easily classified (Figure 1.1). Table 1.0 shows a summary of the current classification of the serotonin receptor subtypes.

The 5-HT_1 Family

The 5-HT_1 receptor family is made up of five different receptor subtypes defined by their structural characteristics where each receptor subtype has a single protein structure varying in size from 374 to 421 amino acids with an overall sequence homology of 40% (Hoyer et al 1994). All five subtypes are transmembrane G-protein coupled receptors (via G_i and G_o). These receptors are all classified as negatively linked to adenylyl cyclase. They can only be selectively labelled to a limited extent in the brain using techniques such as autoradiography, but identification has proven difficult pharmacologically due to the lack of truly selective antagonists. The 5-ht_{1E} and 5-ht_{1F} receptors do not have functional correlates in native tissue and so retain a lower case appellation. Table 1.1 shows a summary of the ligands selective for the 5-HT_1 subtypes.

The 5-HT_{1A} Receptor

The 5-HT_{1A} receptor is commonly linked via a pertussis toxin-sensitive G-protein to the inhibition of adenylyl cyclase (Albert et al 1990; Fargin et al 1988, 1989,1991). There are also reports that the 5-HT_{1A} receptor is positively coupled to adenylyl cyclase resulting in cAMP accumulation (Shenker et al 1983, 1985, 1987) although it has been suggested that the 5-HT_7 subtype may mediate this response (Tsou et al 1994). There are also reports that 5-HT_{1A} receptor activation leads to an inhibition of the carbachol-stimulated accumulation of inositol phosphates (Claustre et al 1989). The 5-HT_{1A} receptor is made up of seven hydrophobic, transmembrane regions (each of approximately 25 amino acid residues) that are arranged as α-helices characteristic of all G-protein coupled receptors. Fargin et al (1988) (Figure 1.2) first described an amino acid sequence and arrangement for the 5-HT_{1A} receptor.

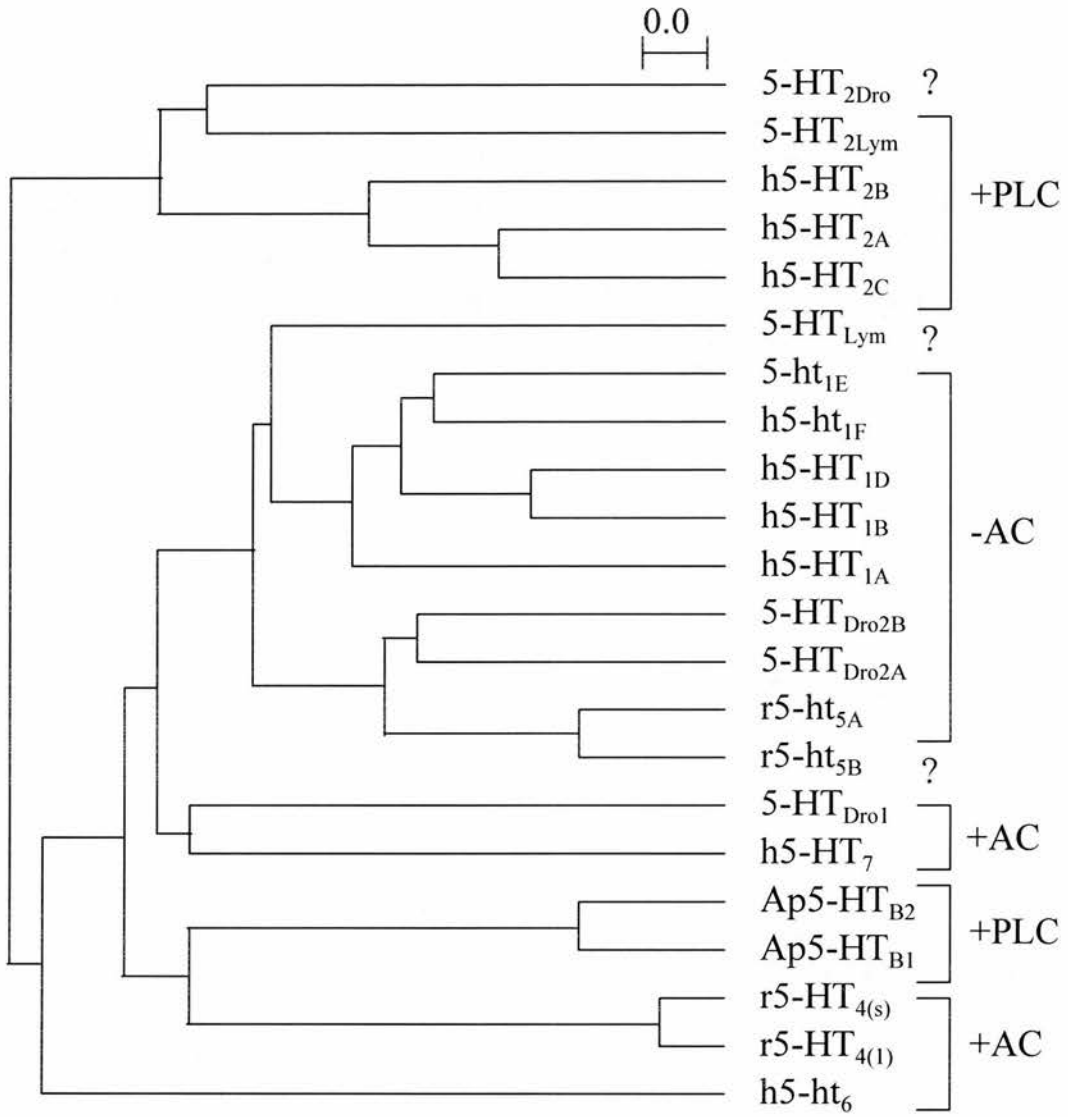


Figure 1.1. Dendrogram of vertebrate and invertebrate 5-HT receptors.
From the branches of the dendrogram its is possible to deduce the evolutionary age and relationship of the individual G-protein linked receptor subtype. The scale bar indicates the percentage divergence (4%) along each branch. The signal transduction mechanism of each receptor is also shown (+PLC – stimulation of phospholipase C; +AC and –AC – stimulation and inhibition of adenylyl cyclase, respectively; ? – signal transduction pathway unknown). h – human; r – rat; Dro – *Drosophila melanogaster*; Lym – *Lymnea stagnalis*; AP – *Aplysia californica*. Adapted from Gerhardt & van Heerikhuisen (1997).

Receptor	Subtype	Effector	Response (in native tissue)
5-HT$_1$	5-HT$_{1A}$	↓Adenylyl cyclase (G_i/G_o)	Neuronal hyperpolarisation
	5-HT$_{1B}$	↓Adenylyl cyclase (G_i/G_o)	Inhibition of 5-HT release
	5-HT$_{1D}$	↓Adenylyl cyclase (G_i/G_o)	Inhibition of 5-HT release
	5-ht$_{1E}$	↓Adenylyl cyclase (G_i/G_o)	Inhibition of adenylyl cyclase
	5-ht$_{1F}$	↓Adenylyl cyclase (G_i/G_o)	Inhibition of adenylyl cyclase
5-HT$_2$	5-HT$_{2A}$	↑Phospholipase C ($G_{q/11}$)	Neuronal excitation, vasoconstriction, bronchoconstriction, platelet aggregation
	5-HT$_{2B}$	↑Phospholipase C ($G_{q/11}$)	Muscle contraction in the stomach
	5-HT$_{2C}$	↑Phospholipase C ($G_{q/11}$)	↑Phosphoinositide turnover
5-HT$_3$		Ion Channel ($Na^+/K^+/Ca^+$)	Depolarisation
5-HT$_4$		↑Adenylyl cyclase (G_S)	Activation of acetylcholine release in gut, tachycardia, ↑cAMP and depolarisation
5-ht$_5$	5-ht$_{5A}$	↓Adenylyl cyclase (G_S)	**?**
	5-ht$_{5B}$	**?**	**?**
5-ht$_6$		↑Adenylyl cyclase (G_S)	**?**
5-HT$_7$		↑Adenylyl cyclase (G_S)	**?**

Table 1.0. Current classification of the serotonin receptor subtypes.
Adapted from Hoyer et al (1994).

Receptor Subtype	$5\text{-}HT_{1A}$	$5\text{-}HT_{1B}$ (rodent specific) and $5\text{-}HT_{1D}$ (guinea pig, pig, calf, monkey, human)	$5\text{-}ht_{1E}$	$5\text{-}ht_{1F}$
Distribution	Dorsal raphe, hippocampus, cortex.	Substantia nigra, basal ganglia, subiculum	Caudate putaman, amydala, frontal cortex, globus pallidus	Dorsal raphe, hippocampus, cortex
Agonist	8-OH-DPAT RU 24969 5-CT MDL 73005 (partial agonist)	Anpirotoline CP 93129 5-CT RU 24969 CGS 12066B Sumatriptan		LY 334370
Antagonist	NAN-190 Cyanopindolol Pindolol MM77 BMY 7378 WAY-100635	Isamoltane Cyanopindolol Pindolol GR 55562 SB 242289		

Table 1.1. Ligands selective for the $5\text{-}HT_1$ receptor subtype.
Adapted from Hoyer et al (1994).

Several agonists show selectivity for the 5-HT_{1A} subtype, e.g. 8-OH-DPAT, buspirone, isapirone (partial agonists), DP-5-CT (an agonist) and MDL 72832 (Richardson and Hoyer 1990). Developing a selective and silent antagonist has proven more difficult. Several antagonists have been characterised e.g. NAN-190, BMY 7378 and WAY-100135, but all have partial agonist activity at the 5-HT_{1A} receptor in the DRN. The only selective and high affinity silent antagonist available is WAY-100635 (Fletcher et al 1993)

Electrophysiology

Activation of the 5-HT_{1A} receptor is inhibitory, principally causing neurones to display membrane hyperpolarisation making cells less likely to fire an action potential. This is accompanied by a decrease in cell input resistance (Aghajanian and Lakoski 1984) due to the opening of inwardly rectifying potassium channels ($I_{K(IR)}$) (Williams et al 1988) and is independent of voltage changes. 5-HT_{1A} activation inhibits a number of calcium channel currents (N-, and L-type) in the DRN neurone cell bodies (Penington et al 1991). The inhibition is mediated directly through G-protein interaction and is also voltage and temperature sensitive (McAllister-Williams and Kelly 1995).

Distribution

The 5-HT_{1A} receptor is widely distributed in the CNS. It is found to a large extent in the hippocampus, septum, amygdala and the raphe nuclei, especially the DRN (Marcinkiewicz et al 1984; Radja et al 1991). The cell body in the DRN has been shown to express high levels of 5-HT_{1A} receptors (Verge et al 1985). Destruction of some brain areas using kainic acid results in a selective degeneration of pyramidal cells with a loss of the 5-HT_{1A} receptor binding sites (Hall et al 1985) suggesting cortical receptors are postsynaptic.

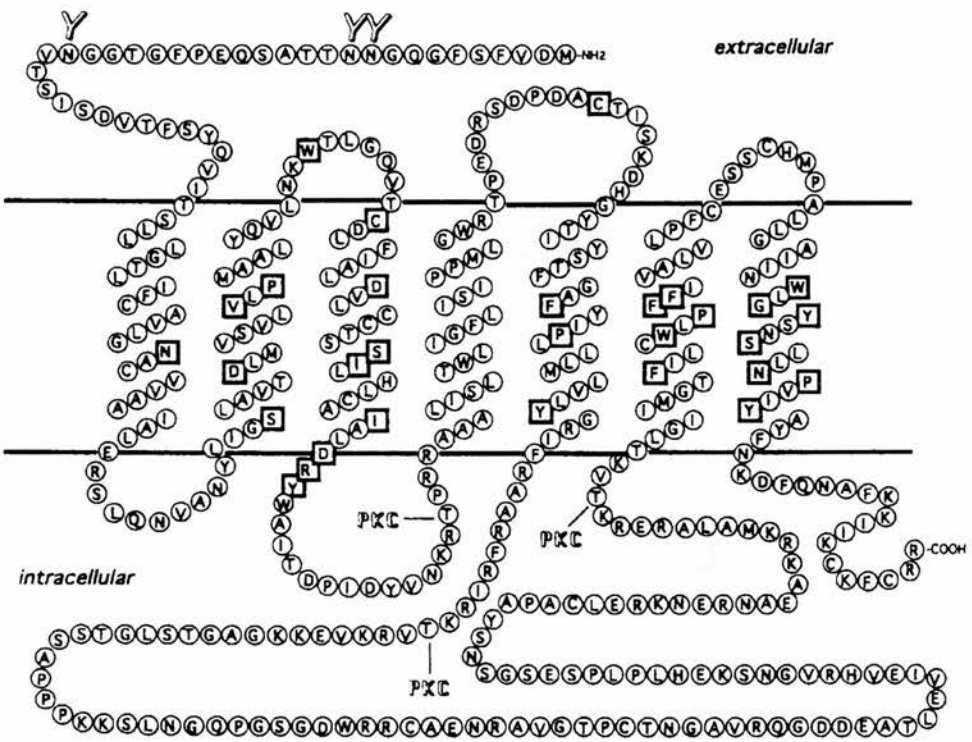


Figure 1.2. Diagram of transmembrane topology of the rat 5-HT$_{1A}$ receptor. Amino acid residues that are conserved in all known G-protein coupled 5-HT receptors are indicated by squares. Site directed mutagenesis studies have shown that several of these and other residues within the seven putative transmembrane segments contribute to ligand binding (for review Branchek 1993). Consensus sites for phosphorylation by protein kinase C (PKC) and *N*-glycosylation (Y) are marked. Adapted from Boess and Martin (1994).

Function

Many of the brain regions that express the 5-HT_{1A} receptor are thought to be involved in the modulation of mood and emotion and make up the limbic system. The predominance of 5-HT_{1A} receptors in the limbic system, the neocortex, hypothalamus and the substantia gelantinosa, suggests the actions that 5-HT and the 5-HT receptor ligands have on mood could be through this receptor (Iverson 1984). Its distribution is also suggestive of 5-HT having a role in the regulatory mechanisms of the hypothalamus and in the regulation of propioception and the integrative functions of the neocortex. Following activation of the 5-HT_{1A} receptor subtype, with low doses of 8-OH-DPAT, a behavioural syndrome is induced; this is characterised by flat body posturing, reciprocal forepaw treading and head weaving (Tricklebank 1985). In the DRN, an important site for the release of 5-HT, the 5-HT_{1A} receptor acts as an autoreceptor by inhibiting the further release of 5-HT.

Clinical Pharmacology

Agonists such as 8-OH-DPAT, gepirone, buspirone and ipsapirone have anxiolytic effects in animal models of anxiety (Traber and Glaser 1987). Blockade of the 5-HT_{1A} autoreceptor in rodents has little effect on extra-neuronal 5-HT levels alone, but does potentiate the increase observed following SSRI administration (Hoyer et al 1993). This may mean that the desensitisation of the 5-HT_{1A} autoreceptor can explain why SSRI's only raise the levels of 5-HT in the synaptic cleft following chronic administration (Hoyer et al 1993). Co-administration of SSRI's with the 5-HT_{1A} receptor antagonist pindolol is said to enhance therapeutic efficacy and shorten onset of the action of the SSRI (Blier and de Montigny 1994).

The 5-HT_{1B} and 5-HT_{1D} Receptors

The 5-HT_{1B} receptor was first described pharmacologically in the rat brain. However, a corresponding binding site could not be detected in the human brain by autoradiography with 5-HT_{1B} receptor ligands (Hoyer et al 1986a). Later, a pharmacologically distinct receptor class was discovered in non-rodents that was termed the 5-HT_{1D} receptor (Heuring and Peroutka 1987; Hoyer & Middlemiss 1989).

These two sites were noted to have the same distribution (Pazos and Palacios 1985; Waeber et al 1988a). Weinshank et al (1992) then demonstrated that the human 5-HT_{1D} receptor was made up of two subtypes, encoded by different genes but sharing 77% homology. These receptor subtypes were termed the 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors but were indistinguishable pharmacologically. However, it is now thought that the 5-$HT_{1D\beta}$ receptor is truly a species variant of the 5-HT_{1B} receptor and that the 5-$HT_{1D\alpha}$ has been renamed the 5-HT_{1D} receptor (Hartig et al 1996; Barnes and Sharp 1999).

The 5-HT_{1B} Receptor

The 5-HT_{1B} receptor is negatively coupled to adenylyl cyclase (Bouhelal et al 1988). There are few selective ligands for the 5-HT_{1B} receptor, however, there are some useful compounds available such as CP 93129 (Macor et al 1990), RU 24969 (Hamon et al 1986) and CGS 12066 (Schoeffter and Hoyer 1989b). There are also some antagonists for the 5-HT_{1B} receptor subtype including SE 242289 (Roberts et al 1997).

In the rat CNS the 5-HT_{1B} receptor is concentrated in the substantia nigra, globus pallidus, dorsal subiculum and superior colliculi (Segu et al 1991; Boulenquez et al 1992; Palacios et al 1992) and to a lesser extent in the raphe nucleus, striatum and hippocampus (Voigt et al 1991). Lesion experiments performed in the rat have shown that 5-HT_{1B} receptors may be localised on the terminals of the striatal intrinsic neurones, which innervate the substantia nigra pars reticulata. This was because destruction of caudate neurones resulted in a significant decrease in binding in the substantia nigra pars reticulata (Hamon et al 1990b). It was also known that lesions of the dopaminergic neurones in the substantia nigra pars compacta do not lead to a decrease in 5-HT_{1B} receptor binding sites. Taken together this points to the 5-HT_{1B} receptors being localised in the cells controlling activity of the basal ganglia, but not the dopaminergic cells. There is evidence that the 5-HT_{1B} receptor acts as an autoreceptor on the serotonergic neurone's nerve terminal. The 5-HT_{1B} receptor also acts as a terminal heteroreceptor in controlling the release of other neurotransmitters

such as acetylcholine and glutamate (Engel et al 1986; Middlemiss 1986; Limberger et al 1991; Maura and Raiteri 1986; Raiteri et al 1986.).

Functional correlates of the 5-HT_{1B} receptor subtype have been found in vascular tissue, but there has been little success in attributing any behavioural effects to the 5-HT_{1B} receptor. This is partly due to the lack of selective antagonists and partly due to the failure of the few antagonists available to penetrate the brain. The development of a gene knockout mouse lacking the 5-HT_{1B} receptor has had more success (Saudou et al 1994). The 5-HT_{1B} receptor knockout mouse is more aggressive than the wild type in the isolation induced aggression test. This is suggestive of the 5-HT_{1B} receptor being involved in the modulation of aggressive behaviour. Administration of the 5-HT_{1B} receptor agonist RU 24969 results in hyperlocomotion in the rat; this effect is antagonised by propanolol (Lucki 1992). The 5-HT_{1B} receptor seems to have a role in penile erection (Berendsen and Broekkamp 1987) and in hypophagia (Kennett and Curzon 1988a).

The 5-HT_{1D} Receptor

The activation of the 5-HT_{1D} receptor (formerly the 5-$HT_{D\alpha}$ receptor) was originally thought to lead to inhibition of the forskolin-stimulated adenylyl cyclase activity (Waeber et al 1989d) however it is now believed that the receptor detected in these studies was the species equivalent of the 5-HT_{1B} receptor. No second messenger has been conclusively attributed to the 5-HT_{1D} receptor expressed in native tissue. The drug profiles of the 5-HT_{1B} and the 5-HT_{1D} receptors are very similar. There have been a number of 5-$HT_{1B/1D}$ agonists characterised SKF 99101H, GR 46611 and L-694, 247 (Skingle et al 1993; Hagan et al 1995; Glennon et al 1996). There is also a 5-$HT_{1B/1D}$ receptor selective antagonist GR 127935 (Skingle et al 1993) and a 5-HT_{1D} selective compound (Price et al 1997).

The 5-HT_{1D} receptor subtype has been found in many non-rodent species including guinea pig, rabbit, dog, pig, calf and human (Heuring and Peroutka 1987; Waeber et al 1988a; Hoyer and Schoeffter 1988; Herrick-Davies and Titeler 1988; Beer et al

1992; Maura et al 1993). High densities of the 5-HT_{1D} receptor are found in the substantia nigra, basal ganglia and nigrostriatal pathway and low densities in the hippocampus, raphe and cortex (Waeber et al 1989a). The 5-HT_{1D} receptor subtype was first identified as mediating the inhibition of 5-HT release from cortical nerve terminals in the guinea pig (Middlemiss et al 1988) with similar findings in the pig and rabbit (Schlicker et al 1989; Limberger et al 1991). This is suggestive of the 5-HT terminal autoreceptor being of the 5-HT_{1D} receptor subtype in the pig, rabbit, guinea pig and human brain. The 5-HT_{1D} receptor functions as a heteroreceptor where 5-HT can inhibit release of glutamate from non-serotonergic cells in rat cerebellar synaptosomes and acetycholine from guinea pig hippocampal synaptosomes (Raiteri et al 1986; Harel-Dupas et al 1991). It is now thought that there are only low levels of the 5-HT_{1D} receptor found in the brain among higher levels of the 5-HT_{1B} receptor so drugs that can distinguish between the two receptors must be used to conclusively attribute any responses to the 5-HT_{1D} receptor.

The 5-ht_{1E} Receptor

The 5-ht_{1E} receptor is negatively coupled to adenylyl cyclase and is linked to a G-protein. There are no known selective agonists or antagonists. The 5-ht_{1E} receptor was first identified in man as a [^{3}H]-5-HT binding site in homogenates of the frontal cortex after blocking the 5-HT_{1A} and 5-HT_{1D} receptors with 5-CT (Leonhardt et al 1989). Human brain binding studies have reported the 5-ht_{1E} subtype (representing up to 60% of 5-HT_1 binding) to be concentrated in the caudate putaman with lower levels in the amygdala, frontal cortex and globus pallidus (Hoyer et al 1993). The function of the 5-ht_{1E} receptor is currently unknown.

The 5-ht_{1F} Receptor

The 5-ht_{1F} receptor is closely related to the 5-ht_{1E} receptor with 70% sequence homology. It mediates the inhibition of forskolin-stimulated adenylyl cyclase activity (Amlaiky et al 1992). There is one selective agonist, LY 334370 (Adham et al 1996), which is claimed to block the effects of trigeminal stimulation suggesting 5-ht_{1F} may have a role in neurogenic inflammation and migraine (Phebus et al 1996). There are

no selective antagonists. The function of the 5-ht_{1F} receptor is not yet clear but its distribution suggests it may act as an autorecepetor (Adham et al 1993b). From looking at the distribution of 5-ht_{1F} mRNA it seems to be concentrated in the dorsal raphe, hippocampus and cortex of the rat and also, to a lesser extent, in the striatum, thalamus and hypothalmus of the mouse (Adham et al 1993b; Amlaiky et al 1992).

The 5-HT_2 Family

The 5-HT_2 receptor family consists of three different receptor subtypes. Each of the subtypes has been cloned and found to be a G-protein-linked single protein molecule, which is of a similar size and sequence homology. Receptor activation leads to an increase in phosphoinositide metabolism leading to the production of ionsitol phosphates and DAG and increasing intracellular calcium. The 5-HT_{2A} receptor has, until recently, been called the 5-HT_2 receptor. 5-HT_{2C} was originally classified as part of the 5-HT_1 group when it was termed the 5-HT_{1C} receptor, but has now been reclassified due to its close relationship to the 5-HT_{2A} receptor following cloning and sequencing. The 5-HT_{2B} receptor has only recently been cloned and identified. Table 1.2 shows a summary of the ligands selective for the 5-HT_2 receptor subtype.

The 5-HT_{2A} Receptor

The first cloned cDNA sequence encoding for the complete 5-HT_{2A} receptor was taken from a rat brain library (Pritchett et al 1988). It was isolated by its similarity to the rat 5-HT_{2C} receptor gene with which it has 70% homology in its protein sequence. The 5-HT_{2A} receptor has been observed in a number of brain regions, including the rat cortex, aortic smooth muscle and human platelets (Conn and Sanders-Bush 1984, 1985; Roth et al 1984; De Chaffoy et al 1985; Doyle et al 1986). The receptors are coupled to phospholipase C, ionsitol phospholipid hydrolysis and Ca^{2+} mobilisation.

There are a large number of agonists, most, however have little 5-$HT_{2A/B/C}$ selectivity and have not yet been fully characterised. These include compounds such as LSD, DOM and DOI (all of which have hallucinogenic properties). There is still no ideal selective antagonist. The most selective antagonist for the 5-HT_{2A} receptor is

Receptor Subtype	5-HT$_{2A}$ (5-HT$_2$)	5-HT$_{2B}$	5-HT$_{2C}$ (5-HT$_{1C}$)
Distribution	Claustrum, olfactory tubercle, dorsal raphe, cortex	amgydala, septum, hypothalmus, cerebellum	Choroid plexus, globus pallidus, substantia nigra
Agonist	α-Me-5-HT DOI DOM LSD	α-Me-5-HT BW 723C86 DOI mCPP	α-Me-5-HT MK 212 mCPP DOI
Antagonist	Ketanserin Ritanserin Spiperone MDL 11, 939 MDL 100, 907 Mesulergine	Rauwolscine SB 206553 SB 204741 LY 266097	SB 206553 RS 102221 SB 221284 SB 200646 LY 53857

Table 1.2. Ligands selective for the 5-HT$_2$ receptor subtype.
Adapted from Hoyer et al (1994).

ketanserin, with 70-fold selectivity over the 5-$HT_{2B/C}$ subtypes. Spiperone is also useful with its 80-fold lower affinity for the 5-HT_{1A} subtype over the 5-HT_{2A} receptor and 100-fold selectivity for the 5-HT_{2A} receptor over the 5-$HT_{2B/2C}$ receptor. The more recently developed compound MDL 100, 907 may be more useful with its 100-fold selectivity for the 5-HT_{2A} receptor over the 5-HT_{2C} receptor.

The 5-HT_{2A} receptor is widely distributed in the periphery (Bradley et al 1986a). It mediates a number of effects including the contractile response in many smooth muscle preparations, inducing the contractile response in bronchial, uterine and urinary smooth muscle and makes up part of the contractile response to 5-HT in the guinea pig ileum. It also has a role in platelet aggregation and in capillary permeability. It is found in the CNS where it has been implicated in a number of behavioural effects in the rodent (head twitch and wet-dog shake). At the cellular level the 5-HT_{2A} receptor mediates neuronal depolarisation (rat facial motorneurone, rat spinal neurone and cat preganglionic sympathetic neurones). Neurones possessing the 5-HT_{2A} receptor are found in some areas of the cortex (Pazos and Palacios 1985, Pazos et al, 1987b).

In the neocortex, for example, it is found in laminae I and IV in the rat and laminae III and V in the human. Binding sites are also found in the claustrum, which is connected to the visual system, the limbic system especially the olfactory nuclei and parts of the basal ganglia. It is found to a lesser extent in the caudate-putamen and DRN (Pazos et al 1984). Cortical [^{3}H] ketanserin binding sites have been seen to decrease in the senile dementia of Alzheimer's disease paralleling the loss of intrinsic somatostatin-containing cells in the cortex.

The 5-HT_{2A} receptor is now thought to have a wide range of functions within the CNS especially in neuroendocrine systems. It mediates neuroexcitation in guinea pig cortical pyramidal neurones (Davies et al 1987), in the rat raphe cell body (Roberts and Davies 1989), and the rat nucleus accumbens neurones (North and Uchimura

1989). The discriminative stimuli and learning behaviour properties of LSD and DOM seem to be mediated by the 5-HT_{2A} receptor (this may also involve the α_1-adrenoceptor) (Tricklebank 1985, 1987). It plays a part in the 5-HT mediated inhibition of the release of glutamate from the rat cerebellum (Maura et al 1988). It has many neuroendocrine effects including regulation of the release of β-endorphin, corticosterone and luteinizing hormone in the rat and prolactin release in the rhesus monkey (Koenig et al 1987; Lenahan et al 1987; Heninger et al 1987). The 5-HT induced release of adrenaline from the adrenal medulla also appears to be mediated through the 5-HT_{2A} receptor (Humphrey and Feniuk 1987).

The 5-HT_{2B} Receptor

The 5-HT_{2B} receptor was first identified following the screening of a genomic DNA library (Foguet et al 1992b). It was described as a G-protein-coupled receptor that was similar to both 5-HT_{2A} and 5-HT_{2C} (62% and 65% homology respectively). The 5-HT_{2B} receptor has been cloned in the mouse, rat and human (Loric et al 1992; Kursar et al 1992; Schmuck et al 1994). It is linked to phospholipase C activation (Foguet et al 1992b).

Agonists available for the 5-HT_{2B} receptor include mCPP (a partial agonist) (Kennett et al 1988a, b). Other full agonists are α-methyl 5-HT, 5-methoxytryptamine (which is 25 and 400-fold more selective over the $5HT_{2A}$ and $5HT_{2C}$ receptors) and BW 723C86 (100 and 10-fold more selective over the 5-HT_{2A} and 5-HT_{2C} receptors respectively). Many *in vitro* studies have used the α_2-adrenergic receptor antagonists yohimbime and rauwolscine which have high affinity for the 5-HT_{2B} receptor and low affinity for 5-HT_{2A} and 5-HT_{2C} receptor sites. More recently the compounds SB 200646 and SB 206553 have been shown to be antagonists 100-fold more selective for 5-$HT_{2B/2C}$ receptors over 5-HT_{2A} receptors (Forbes et al 1993).

The 5-HT_{2B} receptor has been found in the rat stomach, especially the fundus strip where it mediates contraction (Baxter et al 1995). It is also in the gut, heart, kidney and lung (Foguet et al 1992b; Duxon et al 1997). It is involved in endothelium-dependent relaxation of the rat and cat jugular veins and the pig pulmonary artery following nitric oxide release (Ellis et al 1995). There is also the suggestion of a role in development following the observation that receptor mRNA is expressed in high amounts in the foetal small and large intestine at E13-16, after which levels decline (Fiorica-Howells and Gershon 1995). The 5-HT_{2B} receptor is found to some extent in the brain in particular the amygdala, septum, hypothalmus and cerebellum. It has also been suggested that the 5-HT_{2B} receptor has a role in the precipitation of migraine (Kalkman 1994). Finally, 5-HT_{2B} agonists have been shown to have some anxiolytic properties (Kennett et al 1996).

The 5-HT_{2C} Receptor

The 5-HT_{2C} receptor gene contains introns that allow for postranscriptional modification and so different receptor variants, although none have yet been reported physiologically (Burns et al 1997). There are seven functional correlates presently recognised that cannot be distinguished by pharmacological profiles. When expressed in cell lines each isoform has different second messenger affinity. Receptor activation leads to an increase in phospholipase C activation and inositol phosphate accumulation in common with the other 5-HT_2 receptors.

A commonly used agonist is mCPP, this is a partial agonist with 10-fold selectivity for 5-HT_{2C} over 5-HT_{2B} receptors (Kennett 1993). A more selective compound developed recently is the agonist MK 212, which is 25-fold more selective for the 5-HT_{2C} receptor over the 5-HT_{2B} receptor (Kennett 1993). Most 5-HT_2 receptor antagonists are non-selective for the 5-HT_{2C} receptor, but the more modern compounds are better. The antagonists SB 200646 and SB 206553 are 50 and 100-fold more selective for 5-HT_{2C} receptors over 5-HT_{2B} receptors (Kennett et al 1994). The highly selective compound SB 242084 also has a high affinity for 5-HT_{2C} receptors and is 100-fold selective over other receptors (Kennett et al 1994). RS

102221, which is similarly potent at 5-HT_{2C} receptors (Bonhaus et al 1997), should also help in elucidating the role of this receptor subtype.

Autoradiographic studies using [^{3}H] LSD, [^{3}H] 5-HT and [^{3}H] mesulergine have identified high densities for 5-HT_{2C} receptors in the choroid plexus (Meibach et al 1980; Pazos et al 1984). There are 5-HT_{2C} receptor rich sites in the epithelial cells of the choroid plexus (Yagaloff and Hartig 1985) and on the cerebral ventricles where it may regulate the composition and volume of cerebral spinal fluid (Pazos et al 1984). It is found to a lesser extent in the limbic system and brain regions involved with motor behaviour (Pazos and Palacios 1985). 5-HT_{2C} receptor sites are common in the human basal forebrain, in particular the globus pallidus and substantia nigra (Pazos et al 1987a). 5-HT_{2C} receptor transcripts are common in the rat olfactory nucleus, cingulate cortex, lateral habenula and subthalamic nucleus (Mengod et al 1990a).

5-HT_{2C} receptors are thought to have a role in various functions including locomotion, feeding and anorexia nervosa, cerebrospinal fluid production, adrenocorticotrophic release, migraine, obsessive compulsive disorders and anxiety (Kennett and Curzon 1988b; Kennett et al 1989; Brewerton et al 1988; Fozard and Gray 1989; Curzon and Kennett 1990; Lucki 1992). These findings, based on animal models and human volunteers using non-selective compounds such as mCPP, TFMPP and MK 212, may be inconclusive. Other 5-HT_{2C} receptor mediated effects are in penile erection, in the mediation of the loss of behaviours involved in social interaction, in the suppression of the consumption of hypertonic saline and the suppression of periaqueductal grey-induced aversion (Kalkman and Fozard 1991b; Koek et al 1992). The mutant gene knockout mouse which lacks the 5-HT_{2C} receptor is overweight compared to the wild type due to a lack of control of feeding behaviour (Tecott et al 1995). It is also prone to spontaneous seizures leading to death and has a lower threshold for metrazol-induced seizures.

The 5-HT_3 Receptor

Gaddum and Picarelli (1957) first described the 5-HT_3 receptor when they characterised the M receptor in the guinea pig ileum. Uniquely, the 5-HT_3 receptor is a ligand-gated cation channel (Derkach et al 1989). Three observations against the involvement of a G-protein are firstly, that the time course of electrophysiological changes are very rapid. Secondly, responses to 5-HT can be recorded for long periods of time from buffer-irrigated membrane patches (Derkach et al 1989) and finally, that neither the 5-HT_3-evoked currents nor the binding characteristics of radioligands are affected by treatment with G-protein activators or inhibitors (Derkach et al 1989; Kilpatrick et al 1987).

The number of 5-HT_3 agonists available is limited. The more widely used are 2-methyl-5-hydroxytryptamine, phenylbiguanide and chlorophenylbiguanide; the latter having the highest affinity (Fozard 1990; Kilpatrick et al 1990a; Tadipatri et al 1992). Unfortunately, all these compounds are partial agonists making them less functionally active than the high affinity would suggest (Ireland and Tyers 1987; Kilpatrick et al 1990a; Sepulveda et al 1991). There are many highly potent and selective 5-HT_3 receptor antagonists available including ondansteron, MDL 72222; granisetron and BRL 46470 (Butler et al 1988; Fozard 1984a; Sanger and Nelson 1989; Kilpatrick et al 1990a).

5-HT_3 receptors are found in neurones of both the central (Yakel and Jackson 1988; Waeber et al 1988b) and the peripheral (Fozard 1984a; Wallis 1989) nervous systems and a number of neuronally derived cell lines, such as NIE-115, NCB-20, NG 108-15 and NI8 cells (Peters and Lambert 1989; Peters et al 1991). The highest density of receptors is found in the discrete nuclei of the lower brainstem, including the dorsal vagal complex and spinal trigeminal nucleus, the area postrema, the nucleus tractus soliarius and the substantia gelatinosa (Hamon et al 1989; Pratt et al 1990). In lower densities the 5-HT_3 receptor is found in the cortex and the limbic system, including the hippocampus and amygdala (Kilpatrick et al 1987; Waeber et al 1988b; Palacios et al 1991).

5-HT_3 receptor activation triggers a rapid depolarisation due to a transient inward current generated by the opening of a cation-selective channel (Peters et al 1991). The response is fast to desensitise and resensitises (Yakel et al 1991). The depolarising action of 5-HT_3 activation is due to the movement of Na^+ and K^+ ions through the cell membrane (Peters et al 1991). The cellular depolarisation results in an increase in cystolic Ca^{2+} ion concentration due to Ca^{2+} ion influx, which can then change the activity of the cell. Table 1.3 is a summary of the ligands selective for the 5-HT_3 and 5-HT_4 receptor.

The 5-HT_4 Receptor

The 5-HT_4 receptor was first identified as a receptor that stimulated the activity of adenylyl cyclase in mouse colliculi and guinea pig hippocampal membranes (Dumis et al 1988a,b). It has been cloned from a rat brain cDNA library and can exist as two C-terminal splice variants, one of 407 amino acids termed the 5-HT_{4L} (long) receptor and the other with 387 amino acids the 5-HT_{4S} (short) receptor (Gerald et al 1995). It is linked to adenylyl cyclases via the G_S G-protein, which leads to the accumulation of cAMP.

Early studies used the benzamides for their 5-HT_4 agonist activity (Table 1.3). These include renzapride, cisapride and zacopride (Bockaert et al 1992). Later the benzimidazolones were found with higher affinities, these include BIMU8 and BIMU1 (Bockaert et al 1992). However, all these compounds also have high affinities for the 5-HT_3 receptor. There are several selective and potent antagonists at the 5-HT_4 receptor. These are GR 113808 (3000-fold more selective for 5-HT_4 over 5-HT_3), RS 23597-190 (125-fold more selective over 5-HT_3), SB 207266 (3000-fold more selective) and SB 204070 (5000-fold more selective) (Grossman et al 1993a,b; Bockaert et al 1992; Wardle et al 1993).

The 5-HT_4 receptor has been found in a wide variety of tissues both in the periphery and the CNS. Centrally, 5-HT_4 receptors appear to be located on the nerve cell where they mediate inhibition of voltage-activated potassium channels via stimulation of

Receptor	5-HT_3	5-HT_4
Distribution	Dorsal vagal nerve, solitary tract nerve, trigeminal nerve, area postrema, spinal cord, limbic system.	Colliculus, hippocampus, basal ganglia
Agonist	m-Chlorophenylbiguanide RS 56812 2-Methyl-5-hydroxytryptamine Phenylbiguanide	RS 67333 RS 67506 Renzapride
Antagonist	MDL 72222 Granisetron Ondansetron Tropisetron BRL 46470A GR 65630	RS 23597-190 RS 39604 SB 203186 GR 113808 SB 204070 LY 297582

Table 1.3. Ligands selective for the 5-HT_3 and 5-HT_4 receptor.
Adapted from Hoyer et al (1994).

cAMP (Fagni et al 1992). In the pyramidal cells of the rat hippocampus the 5-HT_4 receptor mediates a depolarisation with an increase in input resistance due to the closure of potassium channels, it also mediates a decrease in the calcium-evoked potassium conductance of the afterhyperpolarisation (Chaput et al 1990). This increases neuronal excitation and may have a role in the regulation of neurotransmitter release and enhance synaptic transmission (Fagni et al 1992; Bockaert et al 1992). The areas of highest density for 5-HT_4 receptor in the brain are the structures of the limbic region, including the olfactory tubercles, nucleus accumbens, corpus striatum, globus pallidus and substantia nigra. It has also been found in the hippocampus and cortex (Grossman et al 1993b; Brown et al 1993; Monferini et al 1993).

The 5-ht_5 Family

The 5-ht_5 receptor family is made up of two receptor subtypes. The two receptors have been cloned and exhibit 88% homology for each other, but are not closely related to any other 5-HT receptor families (Plassat et al 1992; Matthes et al 1993; Erlander et al 1993). They were termed the 5-ht_{5A} and 5-ht_{5B} receptors and retain a lower case appellation due to the lack of any functional responses, however their mRNA's have been found in man (Grailhe et al 1995).

A few non-selective compounds have been found to have high binding affinities for the 5-ht_5 receptors. These include 5-carboxyamidotryptamine, LSD and ergotamine. Little is known about the localisation, but mRNA has been found in the cortex, hippocampus, habenula, olfactory bulb and the granular layer of the cerebellum (Plassat et al 1992). Studies using 5-ht_{5A} receptor antibodies have identified receptor expression on glial cells expressing the cloned 5-ht_{5A} receptor and have found the receptor to be negatively coupled to adenylyl cyclase via the G_S G-protein (Carson et al 1995). The 5-ht_{5A} receptor may act as a terminal autoreceptor in the mouse frontal cortex (Pineyro et al 1995). Little is known about the 5-ht_{5B} receptor.

The 5-ht$_6$ Receptor

The 5-ht$_6$ receptor has been identified as a G-protein coupled receptor positively linked to adenylyl cyclase (Monsma et al 1993; Ruat et al 1993a). The 5-HT$_1$ agonists 5-CT and 8-OH-DPAT have low affinity for the 5-ht$_6$ receptor. Pharmacologically, the antagonist methiothepin has the highest affinity for the receptor. Other non-selective antagonists also have some affinity for the receptor; these include metergoline, mianserin and ritanserin. Many antipsychotic and antidepressant drugs have a high affinity for the 5-ht$_6$ receptor (e.g. clozapine, amitriptyline and loxepine) where they act as antagonists. Little is known about the distribution of the 5-ht$_6$ receptor. However, immunocytochemistry has shown that in the hippocampus and striatum 5-HT$_6$ receptor-like immunoreactivity was associated with dendritic processes synapsing with unlabelled axon terminals (Gerald et al 1997). Also mRNA coding for the 5-ht$_6$ receptor has been located in the brain at many sites including the striatum, olfactory tubercle, cerebral cortex and hippocampus. There is no evidence for the presence of the 5-ht$_6$ receptor in the periphery.

The 5-HT$_7$ Receptor

The 5-HT$_7$ receptor has been cloned from the rat, mouse, guinea pig and human (Bard et al 1993; Lovenberg et al 1993a; Plassat et al 1993; Ruat et al 1993b; Meyerhof et al 1993; Shen et al 1993). The 5-HT$_7$ receptor gene codes for a protein with 448, 404 and 435 amino acids (Ruat et al 1993b; Shen et al 1993; Lovenberg et al 1993b), where the differences are due to the presence of introns in the coding region. It is positively linked to adenylyl cyclase via the G_S G-protein. There are no selective agonists or antagonists yet known. It has high affinity to the 5-HT$_1$ agonists 5-CT, 5-MeOT and 8-OH-DPAT and the 5-HT$_2$ agonists LSD and DOI. The antagonists ritanserin, metergoline, methysergide and mesulergine have a high affinity at the 5-HT$_7$ receptor.In the CNS 5-HT$_7$ may mediate phase advancement of neuronal activity in the suprachiasmatic nucleus and may control the circadian rhythm of rats. The 5-HT$_7$ receptor seems to be highly expressed in the rat hypothalamus and thalamus and other forebrain regions, and to a lesser extent, in the DRN and the CA3 region of the hippocampus (Lovenberg et al 1993a). 5-HT$_7$-like responses have been characterised

in many smooth muscle preparations, including the 5-HT-induced relaxation of the rabbit femoral vein, cat saphenous vein, cynomolgous monkey and dog jugular vein (Eglen et al 1997).

5-HT_{1A} Receptor Antagonism in the DRN

Early work with 5-HT, involving binding studies and electrophysiological and biochemical investigations, indicated that a number of compounds previously characterised as antagonists at other receptors also had 5-HT antagonist activity. This includes the dopamine antagonist spiperone (found to be antagonistic to 5-HT_1 and 5-HT_2 receptors) and a number of the β-adrenergic antagonists including propanolol, penbutolol and pindolol (found to act at 5-HT_{1A} receptors). More recently developed compounds, such as methiothepin, metergoline and methysergide, had high affinities at the 5-HT_{1A} receptor but also bound to a number of 5-HT and monoamine receptors. Later compounds, such as NAN-190 and WAY-100135, were more selective but were found to have partial agonist activity at the DRN 5-HT_{1A} site (Hodgkiss et al 1992; Fletcher et al 1993). A more recently developed compound is claimed to be both selective and silent, the phenylpiperazine WAY-100635 (Fletcher et al 1996). This compound has a high affinity for the 5-HT_{1A} receptor and is said to be selective over a number of 5-HT receptors, ion channels and re-uptake sites.

WAY-100635 – a silent 5-HT_{1A} antagonist

The lack of truly silent 5-HT_{1A} antagonists has hindered the search for electrophysiologically observable responses due to binding to non-5-HT_{1A} receptors subtypes found in the DRN. The most recently developed 5-HT_{1A} antagonist is WAY-100635 which has been reported as a selective 5-HT_{1A} antagonist (Fletcher et al 1996). WAY-100635 can displace specific binding of the 5-HT_{1A} radioligand [h-3]8-OH-DPAT to rat hippocampal membranes with a $pIC_{(50)}$ of 8.87 repressing a 100-fold selectivity relative to binding to other 5-HT receptor subtypes and major neurotransmitter receptors (Forster et al 1995). Corradetti et al (1996) have reported, using extracellular techniques, that WAY-100635 has no partial agonist activity at

the 5-HT_{1A} receptor in the DRN neurone. They also investigated the pyramidal cells of the CA1 region of the hippocampus intracellularly, where it has no partial agonist activity and blocks the 5-HT_{1A}-evoked hyperpolarisation. WAY-100635 has no partial agonist activity in many physiological and behavioural models including the isolated guinea pig ileum preparation and the 8-OH-DPAT induced behaviour syndrome (Forster et al 1995). WAY-100635 also antagonises the decrease in the amplitude of the NMDA receptor-mediated component of the EPSP's induced by 5-HT (Corradetti et al 1998) and blocks the 5-HT and 5-HT_{1A} agonist induced inhibition of cell firing in the DRN (Craven et al 1994; Fletcher et al 1993; Forster et al 1995). WAY-100635 application can lead to an increase in the firing rate of serotonergic cells of the DRN in the anaesthetised cat (Fornal et al 1994), increasing 5-HT release. WAY-100635 reverses the partial agonist activity of pindolol in the DRN, where pindolol inhibits cell firing, and in the frontal cortex, where pindolol reduced 5-HT release (Clifford et al 1998). WAY-100635 shows competitive antagonism with 8-OH-DPAT in the guinea pig DRN (Mundey et al 1996). This study also showed that WAY-100635 application *in vivo* can increase neuronal firing. Mundey et al concluded that this is probably because of the blockade of a 5-HT_{1A}-mediated inhibitor tone acting on serotonergic neurones resulting in an increase in 5-HT release and 5-HT_2 receptor-mediated effects. WAY-100635 has been widely used as an *in vivo* radioligand (Hume et al 1994) and has been shown to have heterogeneity of signal consistent with the known localisation of the 5-HT_{1A} receptor subtype.

Overview of Thesis

Chapter 2 will describe the experimental methods used to set up and conduct the experiments performed throughout this research programme. The chapter will also report the biophysical properties of the neurones studied in this thesis and compare each property with that reported in the literature.

Chapter 3 will cover the characterisation of the 5-HT_{1A} receptor-mediated hyperpolarisation in the DRN and hippocampus. It will then report the investigation into the blockade of the 5-HT_{1A} receptor-mediated hyperpolarisation with WAY-100635 in the DRN and hippocampus. Chapter 3 will go on to characterise the actions of WAY-100635 in the DRN and hippocampus. Finally, this chapter will report the additional actions of 5-HT in the DRN and hippocampus as revealed by WAY-100635.

Chapter 4 will report the pharmacological characterisation of the 5-HT-evoked depolarisation in the DRN using both agonists and antagonists.

Chapter 5 covers the characterisation of the action potential, AHP and firing pattern of the neurones of the DRN. This chapter then goes on to discuss the actions of 5-HT on the action potential, AHP and firing pattern of the neurones of the DRN.

Chapter 6 will discuss each issue of the thesis as highlighted in the previous chapters and will draw each point together to show the complex pattern of roles 5-HT has in the DRN.

CHAPTER 2

Methods

Introduction

This chapter will discuss the methodology used throughout the experimental programme. This will include information on the setting up of the DRN slice preparation and brain slice chamber. Reference will also be made to the storage and preparation of drugs, the basis of recording from neurones and the methods of data analysis. The chapter will also state the methods of visual identification with the microscope and electrophysiological methods used to identify serotonergic neurones in the DRN. The chapter will then go on to discuss the biophysical membrane properties as determined from the neurones used throughout the study and makes comparisons for each property with those described in the literature.

Experimental Techniques

DRN and Hippocampal Slice Preparation

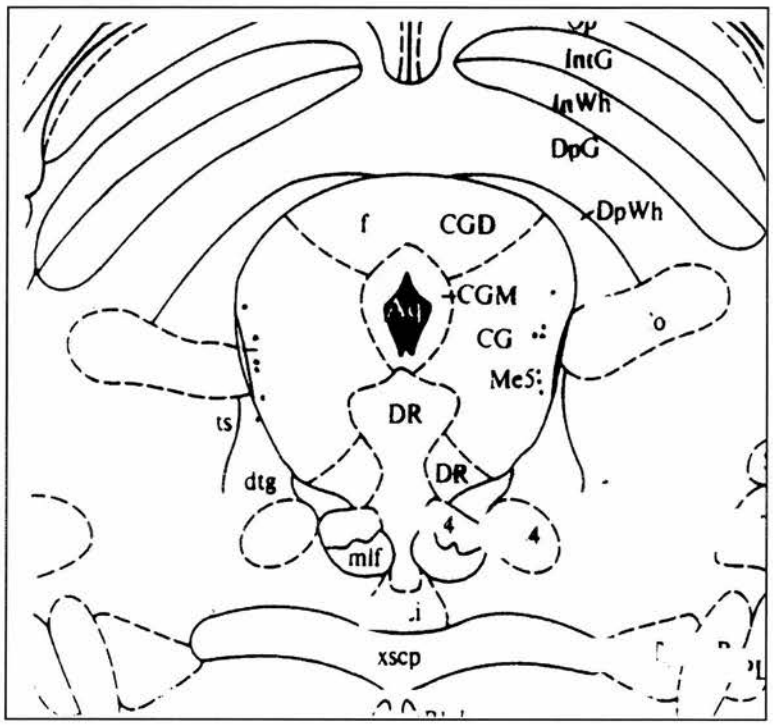
To prepare the DRN slice male Wistar rats (3-6 weeks old) were decapitated and the brain quickly removed and cooled to 4°C in oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (aCSF). The cortex was reflected back and two coronal cuts were made, one at the rostral end of the thalamus and the other at the rostral end of the cerebellum. Two parasagital cuts were then made between 3 and 4 mm from the midline to give a small block of tissue. This block of tissue was then transferred to the chuck of a vibrotome slicer (Campden Instruments) using a strip of filter paper (Whatman). The tissue block was fixed to the chuck with cyanoacrylate glue with the distal cut surface uppermost. The chuck was then transferred to the bath of the vibratome containing oxygenated aCSF at 4°C. The aCSF contained (in mM) NaCl 126, KCl 6.25, Mg SO_4 1, $CaCl_2$ 2.5, NaH_2PO_4 1.25, $NaHCO_3$ 26 and glucose 10. Transverse sections were cut dorso-ventrally to a thickness of 450 μm. Sections

where the cerebral aqueduct retained its integrity and the medial longitudinal fasiculus could be observed were selected for the recording chamber (Figure 2.0).

To prepare the hippocampal slice the brain was removed as described previously then the brain was hemisected and the rostral and caudal ends removed from each lobe. The lateral surface of the remaining tissue was then removed to give a flat surface. This block of tissue was then transferred to the chuck of a vibratome with the cut surface facing down. Each lobe was cut into brain slices to a thickness of 400 μm. The hippocampus was removed form the rest of the brain and the CA3 region cut off with a scalpel to prevent feedback from the CA3 region resulting in less excitable cells.

The selected slices were transferred to the recording chamber using a fine spatula. The slices were supported on a fine nylon mesh and superfused from below with aCSF, which had been warmed to 34°C and was flowing at approximately 0.8 ml per minute. Above the slices flowed a warmed (34°C) moist atmosphere of 95% O_2 and 5% CO_2, which had been bubbled through the water bath of the chamber and acted as the main supply of oxygen. The entire procedure, from decapitation to placement of the slice in the recording chamber, took from 5 to 9 minutes. The slice was allowed to recover and equilibrate in the aCSF for 1 hour prior to starting recording. The recording chamber was adapted from the Haas bath (Haas et al 1979) (Figure 2.1). The chamber is made up of a thermostatically controlled heated water bath arranged below the incubation chamber. Artificial CSF, which had been gassed with 95% O_2, 5% CO_2, is gravity fed via polypropylene tubing through the water bath and into the well of the incubation chamber. As the well overflows the fluid is channelled through the nylon mesh below the slice to a tissue wick that syphons the aCSF into a collecting funnel. An adjustable membrane flow regulator positioned between the aCSF reservoir and the input port of the recording chamber regulates the flow rate. The slice rests at the interface between the aCSF and a warmed humidified atmosphere of 95% O_2 and 5% CO_2. The gas mixture is gently bubbled through the heated water bath and enters the incubation chamber by a port situated at its head.

Dorsal



Ventral

Figure 2.0. Diagram of the DRN slice.
Schematic diagram of the mesencephalic area routinely taken for the DR slice and selected for the recording chamber.

Aq	Cerebral aqueduct
CG	Central grey
CGD	Central grey dorsalis
CGM	Central grey medialis
DpWh	Deep white layer superior coliculus
DR	Dorsal raphe
InG	Intermediate grey layer superior coliculus
InWh	Intermediate white layer superior coliculus
Me5	Mesencephalic trigeminal nerve coliculus
mlf	Median longitudinal fasiculus
xscp	Dessucation superior cerebrallar peduncle

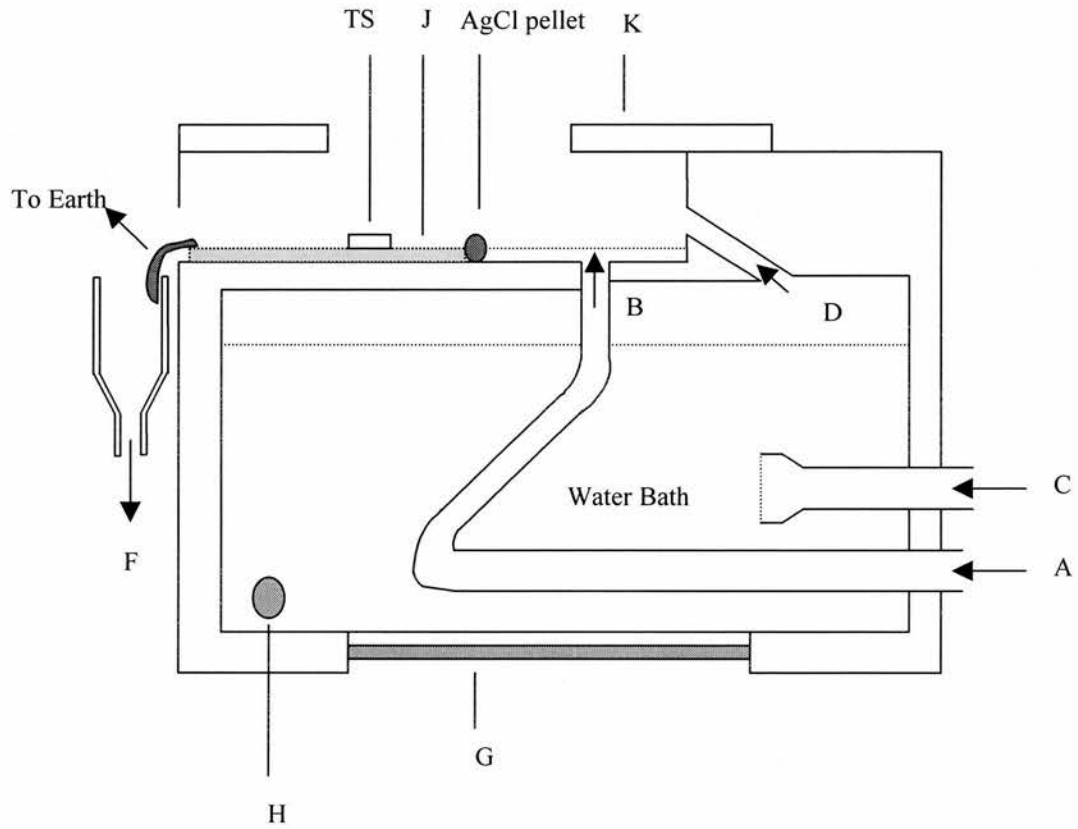


Figure 2.1. The Recording Chamber (modified from Haas et al 1979).
The tissue preparation (TS) was placed on the nylon netting (J) and perfused by oxygenated aCSF. The aCSF was introduced into the chamber at port A, it was warmed as it passed through the water bath and entered the incubation well at B. The water bath was heated with a thermopad (G) (Minco, Cavel Components Ltd. U.S.A.) the temperature of which was thermostatically regulated with reference to probe H. The aCSF flowed through the netting and underneath the tissue, and was siphoned into a collecting funnel (F) by a tissue wick. An O_2/CO_2 (95: 5 %) gas mixture was bubbled from a sintered glass tube (C) through warmed distilled water and entered the incubation chamber via port D. On entering the chamber the warmed, humidified gas was deflected across the preparation by a baffle (K). A hole in the baffle allowed access to the slice with microelectrodes and helped to prevent the slice from being dehydrated by exposure to dry air. Dotted lines represent fluid levels.

Drug Administration

Drugs were made up as concentrated stock solutions in distilled deionised water and frozen until needed. The drugs were thawed and diluted in oxygenated aCSF to the required concentration immediately prior to the start of drug administration. The diluted drug was then superfused under the slice by switching a stopcock from a reservoir containing control aCSF to a reservoir containing drug aCSF. Stock solutions were made up in mM concentrations with a dilution factor of 1 to 1000. See Table 2.0 for a summary of the compounds used in the research programme.

Visual Identification of Serotonergic Neurones

Serotonergic neurones tend to be arranged in four distinct areas in the DRN as labelled DR in Figure 2.0. The largest cluster of cells is located ventromedially within the isthmus of the DRN. Two smaller clusters are found either side of the midline and the smallest cluster is found in the midline in the most dorsal aspect of the DRN. The electrode was placed in the areas of high cell density and was moved slowly through the slice until a cell was impaled. Following impalement, the cell was first allowed to stabilise before a control application of 5-HT was given. One of the major characteristics of serotonergic cells in the DRN is a 5-HT-evoked hyperpolarisation and associated decrease in cell input resistance (Aghajanian and VanderMaelen 1982b). Following the identification of the membrane properties of a cell being recorded its response to bath-application of 5-HT was tested to confirm its serotonergic nature.

Intracellular Recording Technique

Intracellular electrodes were made from borosilicate glass capillary tubing (Kwick fil, GC 120-10F, Clark Electromedical Instruments) on a horizontal puller (P-97 Flaming Brown Micropipette Puller, Sutter Instruments Co.). The electrodes were filled with 2M KCl and had resistances in the range 50-100 MΩ. The tip of the electrode was placed on the surface of the slice in the area of the medial DRN under microscopic control (Wild M5 stereomicroscope). The electrode was then stepped through the slice in 2 μm steps using an Inchworm microdrive (Burleigh Instruments) until a cell was penetrated. Potentials were recorded with respect to a.

Substance	Source	Concentration
Apamin	Sigma	100 nM
CP 93129	Tocris	10 μM
DOI	RBI	10 μM
5-HT	Sigma	100 μM
Ketanserin	RBI	100 nM
mCPP	Tocris	100 μM
MDL 100, 907	Gift from Merrel Dow	100 μM
Methysergide	RBI	30 μM, 100 μM
Noradrenaline	RBI	10 μM
Phenylephrine	RBI	10 μM
Pindolol	Tocris	1 mM
SB 200646	Gift from Tom Blackburn to JSK	1 mM
TEA	Sigma	3 mM
TTX	Sigma	3 μM
WAY-100635	Gift from Wyeth to JSK	100 nM -100 μM

Table 2.0. Summary of the compounds used throughout this research.

silver/silver chloride pellet placed against the tissue paper wick in the outflow of the recording chamber. Recordings were made using a high input impedence DC preamplifier (Axoclamp 2A, Axon Instrument Inc.) with an active bridge facility to enable simultaneous current injection and voltage recording through a single microelectrode (Figure 2.2). The voltage response to a rectangular current pulse gives an indication of the resistance of the microelectrode according to Ohm's law:

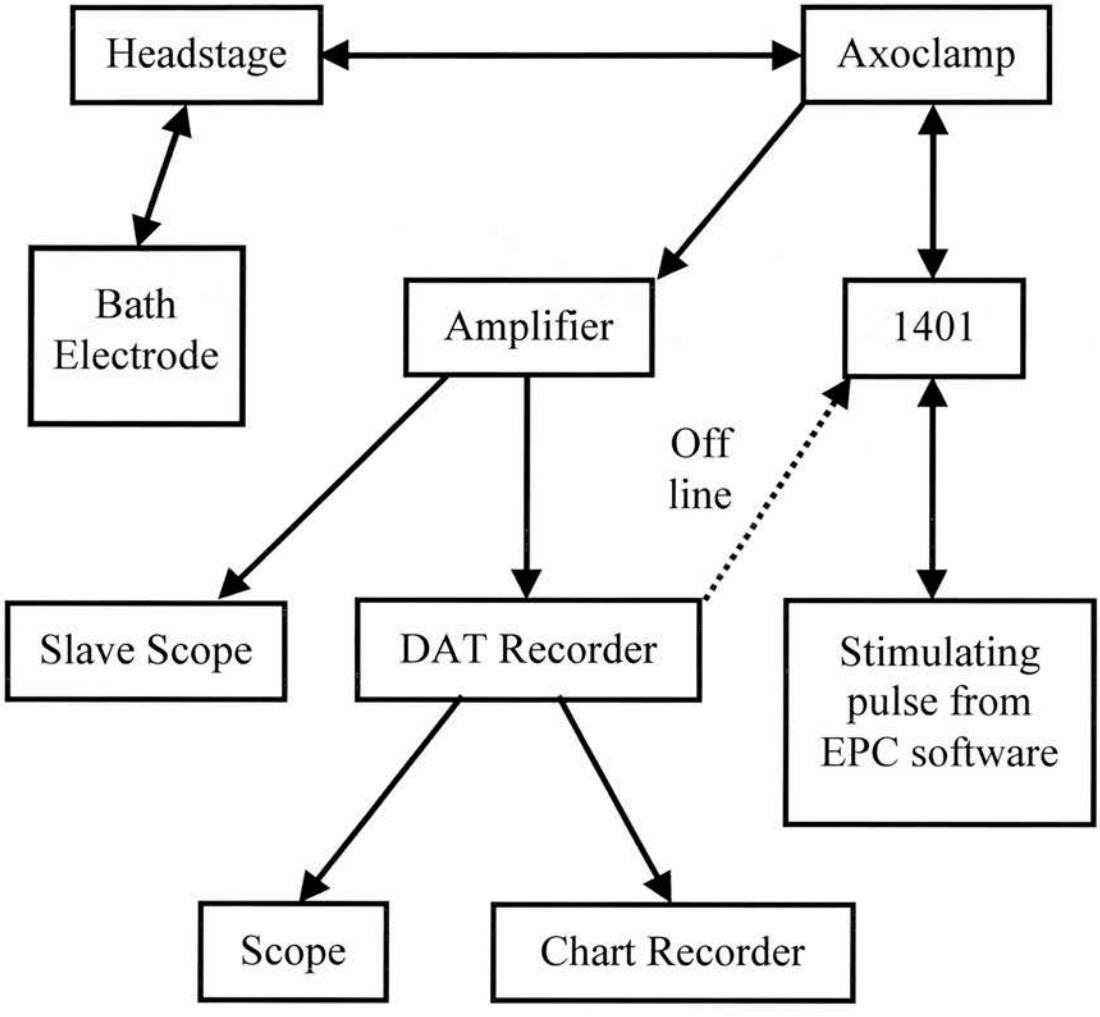


Figure 2.2. Electrical recording apparatus.
Electrotonic potentials were recorded with respect to a silver/silver chloride reference ground attached to a the outflow of the recording chamber. Signals were amplified using an Axoclamp 2A preamplifier via a 0.1 current gain headstage. Hyperpolarising and depolarising current steps were applied to the cell, which were generated via a stimulating pulse from the EPC software, reaching the preamplifier via a 1401 interface. The EPC software regulated the timing and duration of all current steps. The decay of the electrode discharging was continuously monitored on the slave scope in discontinuous current clamp (DCC). Voltage responses were amplified by a 1-20 variable gain, second stage amplifier before being recorded on a digital tape recorder. Waveforms were monitored on an oscilloscope (Gould) to ensure that no attenuation of the responses occurred during recording.

$$R_e = V_e/I_o$$

Where R_e = *electrode resistance*

V_e = *voltage drop recorded across the electrode*

I_o = *amplitude of the test current pulse*

The resistance of the microelectrode was compensated for using the active bridge mode. The microelectrode's resistance changed when resting against a cell as indicated by a voltage deflection in response to a repetitive (1 Hz.) current step. The capacitance neutraliser on the preamplifier compensated for the capacitance of the microelectrode. Over compensating for the microelectrode capacitance caused it to oscillate or "buzz" allowing the microelectrode to move into the cell. Recordings were made from cells showing a membrane potential of -55 mV or less on the voltage monitor. The absolute membrane potential was calculated when the electrode was withdrawn at the end of the experiment. If a residual voltage was seen on withdrawal it was subtracted from the measured cell voltage of each current step

Current Clamp Recordings

In current clamp mode the current injected into the cell is kept constant (clamped) and the voltage response of the cell is measured. The bridge balanced mode of the Axoclamp was used for all current clamp experiments. In this mode not only can the voltage of the microelectrode be sampled continuously, current can also be passed continuously along the electrode from a constant current source.

When current flow (I_o) is passed along the microelectrode there is an associated voltage drop (V_e) along it which is dependent on the microelectrode's resistance (R_e). By bridge balancing for the microelectrode resistance the voltage drop is removed from the potential measurement to give the cell's membrane potential. The capacitance currents of the microelectrode and the cell membrane were reduced by optimal negative capacity compensation. To set the negative capacity value the Axoclamp was set to discontinuous current clamp (DCC) mode where the microelectrode was used to pass current and record voltage. It has a 30% duty cycle

where current is injected for 30% of the time and voltage measured for 70% of the time. The voltage source, V_o, is in series with the resistors R_o (100 MΩ for the 0.1× headstage) and R_e acts as a current source when the switch S1 is closed (Figure 2.3).

Under steady state conditions the voltage measured at the amplifier (A1) output is the sum of $R_e.I_o$ and the membrane potential (V_m). In order to sample the true membrane voltage V_e must be eliminated from the voltage records. When the switch S1 is opened after 30% of the cycle period current flow stops and V_e decays to V_m. Closure of the switch S2 allows the new membrane potential to be sampled at A2, this is held until another sample of V_m is made. In this way the membrane potential can be measured independently of the voltage drop across the microelectrode. The time rise and decay of the voltage change observed on the voltage monitor is dependent on the capacitance of both the amplifier and the microelectrode, but this can be optimised by negative capacitance compensation. This involves the injection of current into the headstage to offset the current required to charge the capacitors. Over-compensation can be seen as an overshoot in the voltage response across the microelectrode and under compensation by a failure to return to 0 mV before the sample cycle repeat. Once the microelectrode has been properly compensated the Axoclamp is returned to bridge balance mode for the remainder of the experiment. The electrode resistance is now compensated for using bridge balance control.

The neuronal input resistance was indicated by the size of the steady state voltage deflection recorded in response to the injected hyperpolarising current steps. This is calculated from:

$$R_m = V_m/I_o$$

A more accurate measurement of resistance was obtained from the slope of the I/V relationship generated from voltage responses to a series of current steps of varying amplitudes. When during drug application the resting membrane potential was seen to change, a constant DC current of the opposite polarity was applied to the cell to manually clamp the cell to its resting membrane potential. This was used to

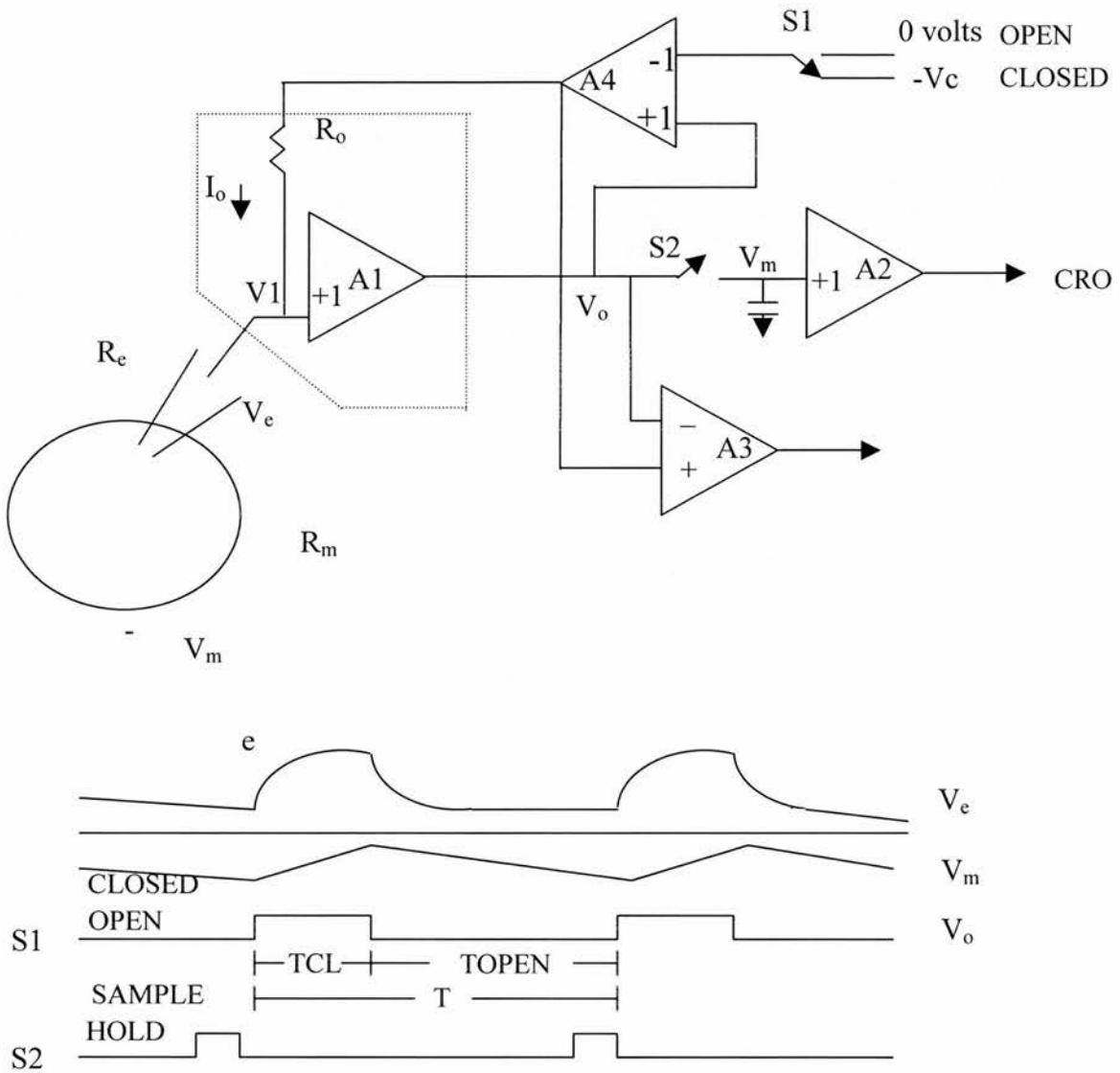


Figure 2.3. Circuit diagram for the axoclamp in discontinuous current clamp. Current I_o was injected for 30% of the cycle time and voltage measured for 70% of the cycle time. Due to the high input impedence of the preamplifier A1 the current was injected into the cell. During the voltage sampling phase switch S1 changes to the zero volts position, this allowed the voltage drop across the electrode to decay to zero as measured by the differential amplifier A3. Hence when the sample and the hold device, switch S2, capacitor and amplifier A2, measured the membrane potential this value was independent of the voltage drop across the microelectrode.

compensate for any voltage-dependent changes in the cell membrane so only the absolute affect of the drug would be examined.

Data Analysis

Data was collected and analysed using the CED Electrophysiology Package software. Data was played from the DAT recorder into the CED software, which generated traces of the current and voltage pulses. The voltage traces were first averaged by the CED software to give a series of traces. The current and voltage is measured at the same position in each trace. These values are then plotted by the CED software to generate the I/V plot. Least squares linear regression analysis is then performed on the I/V plot to calculate the input resistance. Reversal potentials where there is no net change between two I/V plots is the point at which the plots cross and can give an indication of the ion channel mediating the change. The membrane potential, input resistance and time constant was taken from each cell. The mean and standard deviation were calculated for each of the cell membrane parameters and have been written in the form mean ± standard deviation throughout the thesis, as calculated by the Microsoft Excel97 statistical analysis facility. Where data was tested for significance the t-test was used to a 95% confidence interval, as performed by the Microsoft Excel97 statistical analysis facility.

Biophysical Properties of Serotonergic Neurones

The initial experiments on the DRN performed *in vivo* used both intracellular and extracellular techniques to characterise the neurones of the DRN. The first study, using extracellular recording techniques *in vivo*, looked at firing rates and found DRN cells to fire with a regular rate of 0.5-2 Hz, the action potentials had a positive-negative waveform, and firing was inhibited by administration of LSD (Aghajanian and Haigler 1974). Later studies, using intracellular recording methods *in vivo*, characterised the membrane properties of the cells. The action potentials were of long duration with amplitudes in the range 62-80 mV and the cells input resistances ranged from 30-70 MΩ (Aghajanian and VanderMaelen 1982b). A large post spike hyperpolarisation (approximately 6 mV) was observed and this together with a

gradual interspike depolarisation was termed the "pacemaker potential", these were thought to be regulatory factors in determining raphe spike firing pattern.

Later it was shown that intracellular recordings could be obtained *in vitro* (Crunelli et al 1983). Again a spontaneous rhythmic pattern was observed made up of single action potentials occurring at 0.25-5 Hz. The action potential was followed by a long lasting AHP and the input resistance was found to be in the range 100-320 MΩ, higher than *in vivo*. Trulson et al (1982) also showed that the raphe cell maintained its own rhythmic firing pattern *in vitro* thus indicating that the DR cells have an intrinsic pacemaker activity. It was also observed that the neuronal population of the DR region was not homogenous and occasionally a second cell type was impaled (Crunelli et al 1983; VanderMaelen and Aghajanian 1983). These cells had a much faster firing rate (≥10 Hz), shorter duration action potentials (approximately 1 ms) and a short duration AHP, but were not responsive to 5-HT.

The remainder of this chapter will present the membrane properties of the neurones studied in this thesis and compare each property with those described in the literature (giving confirmation that the experimental technique used during the research is correct).

Passive Membrane Properties

Stable intracellular recordings were made from 102 dorsal raphe neurones. The neurones all had overshooting action potentials and resting membrane potentials ranging from -58 to -69 mV with a mean value of -63.5 ± 4.1 mV. Of those impaled 60% appeared spontaneously active initially, but only 5% remained spontaneously active during prolonged periods of recording.

Current-Voltage relationship

The most accurate calculation of the cell input resistance (R_m) is drawn from the slope of the current-voltage (I/V) relationship of the cell. This was carried out by injecting a series of rectangular hyperpolarising current steps of varying amplitudes into the cell and measuring the cell's voltage output. A typical example is shown in

Figure 2.4. The resting membrane potential was -64 mV and hyperpolarising current steps, with increments of 0.025 nA, were used to evoke electrotonic potentials (Figures 2.4A). The I/V relationship in the hyperpolarising direction was linear in the range -110 mV to -50 mV and in this cell showed an input resistance of 316.6 MΩ over this range (Figure 2.4B). Membrane voltage responses to hyperpolarising current commands showed a linear relationship. No inward rectification was observed in voltage deflections as negative as -130 mV. The current-voltage relationships in the linear portion of the curve from the population of neurones gave an apparent mean input resistance of 263.5 ± 58.3 MΩ, and ranged from 147.1 to 334.9 MΩ (n=102). The high input resistances of the DRN neurones are due to the small size of each individual neurone and allows cells to show a marked voltage response to even very small synaptic inputs. The cells had a linear I/V relationship in the hyperpolarising direction and did not show rectification in hyperpolarising current steps.

Time Constant

The membrane time course of the potential change (τ_m) resulting from rectangular current pulses was measured at the resting membrane potential using small hyperpolarising current pulses (approximately 0.05 nA). By using small hyperpolarising current pulses to determine τ_m alterations in the charging curve due to the activation of voltage-activated channels were minimised. In all cells studied the charging curve could be fitted by a single exponential curve. The exponential change in membrane potential had a time constant of 32.75 ± 8.6 ms (n=102).
The passive membrane properties of the DR neurones in the present study were in agreement with those reported by Crunelli et al (1983) who also worked with the *in vitro* slice preparation. The neuronal input resistance values reported by Crunelli et al were twice those found during the *in vivo* work of Aghajanian and VanderMaelen (1982). Aghajanian and VanderMaelen also found low input resistance values for DR neurones in the *in vitro* slice preparation. More recent studies performed *in vitro* agree with the input resistances from the present study, a typical example is from the work of Corradetti et al (1998) who report a high input resistance for DR neurones of 320 ± 33 MΩ. Another study (Craven et al 1997) reported an input resistance value

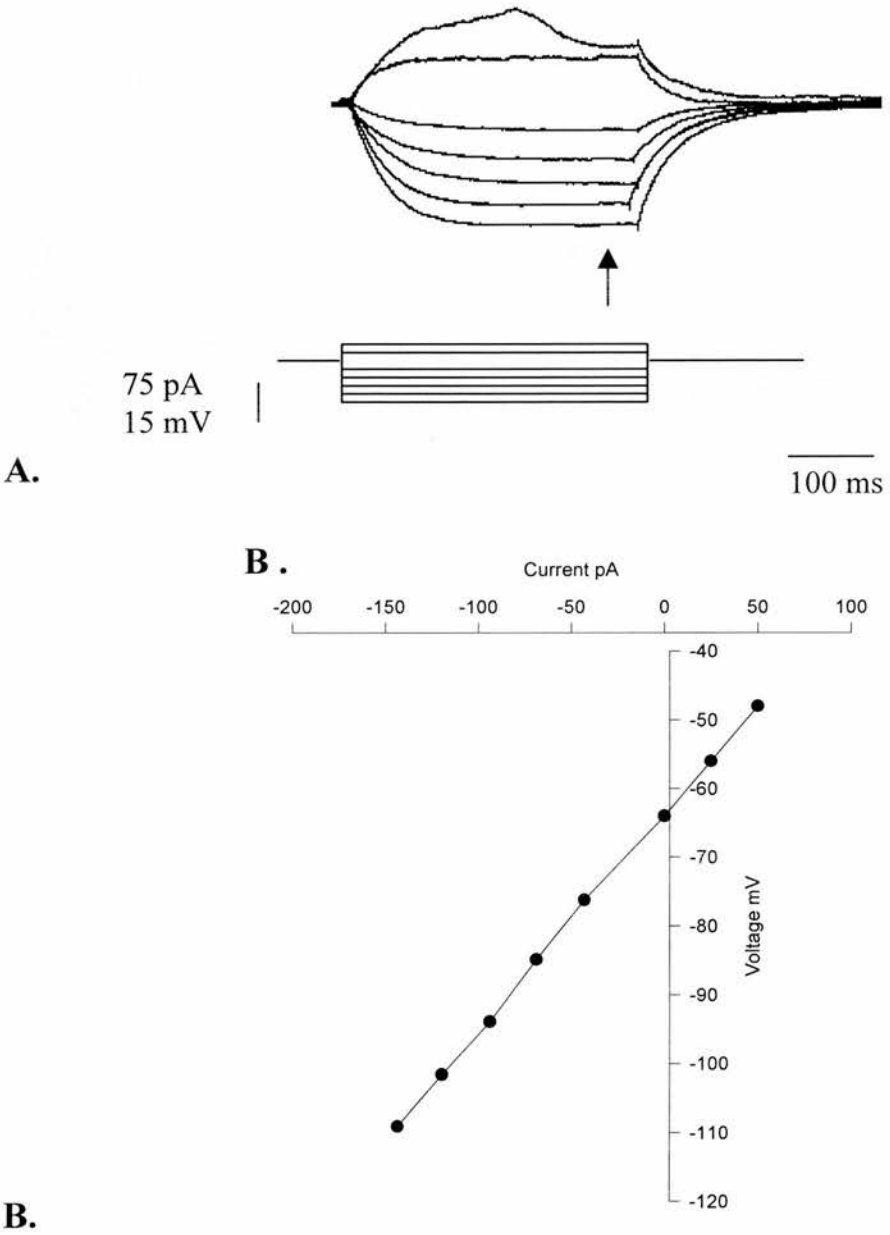


Figure 2.4. Typical electrophysiological characteristics of a DRN neurone.
A. Voltage traces (upper record) are recorded intracellularly from DR neurones in response to graded depolarising and hyperpolarising current steps (lower record). The resting membrane potential was -64 mV and hyperpolarising and depolarising current steps, at increments of 0.025 nA, were used to evoke electrotonic potentials. **B.** The I/V relationship of the traces displayed was linear and in this cell showed an input resistance of 316.6 MΩ (as measured by linear regression over the range -110 mV to -50 mV). No rectification was seen with voltage deflections in the hyperpolarising range to approximately -130 mV.

of 270 MΩ. The difference between the input resistance values reported from *in vitro* and *in vivo* investigations is probably accounted for in part by the removal of tonic synaptic inputs from areas of the brain that regulates the activity of the DR neurones. There is also a case for the discrepancy simply being due to the removal of axons and dendrites which occurs during slice preparation.

Synaptic Potentials

In a number of the cells studied (53 of 89 cells) spontaneous depolarising postsynaptic potentials (PSP's) could be observed with 2M KCl filled electrodes. The depolarising PSP's typically had a fast onset and showed a gradual decay back to the resting membrane potential. These depolarising PSP's were not observed with 3M K acetate filled electrodes (n=14), which would imply that they were reversed IPSP's due to the change in Cl^- driving force associated with KCl filled electrodes. TTX treatment abolished these depolarising PSP's implying they result from activity dependent transmitter release. These potentials can be inhibited by manually hyperpolarising the membrane potential. This spontaneous activity was often lost over time, but could be evoked by manually depolarising the membrane potential by injecting positive current through the recording electrode. A quiet cell could be driven to fire action potentials rhythmically in a similar manner to those seen spontaneously by the addition of the α_1-adrenoceptor agonist, phenylephrine, to the bath-aCSF. These observations are in agreement with those made by Baraban and Aghajanian (1980a) who showed that α_1-adrenoceptor antagonists suppress neuronal firing and Heym et al (1981) who described the effects of adrenergic drugs on unit activity in the freely moving cat.

Active Membrane Properties

Action Potential

A large number of neurones (60%) were seen to be spontaneously active with a rhythmical action potential firing rate of approximately 2 Hz. Manual hyperpolarisation of these cells inhibited action potential generation and did not reveal any excitatory synaptic events that may have accounted for the spontaneous activity. The generation of spontaneous action potentials is due to the inherent

membrane properties of the DRN neurone. Cell viability was in part determined by the ability of the neurone to produce an overshooting action potential. Cells unable to evoke an overshooting action potential were assumed to have suffered damage during the electrode impalement.

A typical action potential in a DRN cell consisted of a rapid overshooting rising phase where the time from spike onset to peak amplitude of 118 mV was 3 ms (Figure 2.5). The falling phase is broad and slow with a marked shoulder due to the activation of a calcium current. The repolarisation undershoots the resting membrane potential producing a transient AHP of 15 mV that persists for 100 ms before the cell returns to its resting potential.

Actions of TTX

TTX is a sodium channel blocker and can block the action potential in the DRN. In a typical cell a short (2 ms) depolarising pulse generated an overshooting action potential which reached a maximum of +40 mV after 2 ms (Figure 2.6A). Following the addition of TTX (3 μM) the same depolarising pulse no longer generated an action potential. This confirms the involvement of TTX-sensitive sodium channels in the first stage of the action potential.

TTX also causes a membrane hyperpolarisation accompanied with a slight increase in input resistance. In Figure 2.6B TTX (3 μM) evoked a hyperpolarisation from a resting membrane potential of -58 mV to -64 mV, with a slight increase in input resistance from 205.8 MΩ to 225.9 MΩ. This could be due to a direct action on the activity of sodium channels. It could also be due to the loss of tonic synaptic input following the abolition of the action potential. The reversal level is not indicative of any known ion channel. This implies it is unlikely that the change in membrane properties is mediated simply by modulation of an ion channel. It is more likely to be the result of either a combination of both possibilities or because of the blockade of synaptic activity involving multiple ionic mechanisms. The inhibition of a tonic inhibitory action on serotonergic cells in the DRN may in part account for the

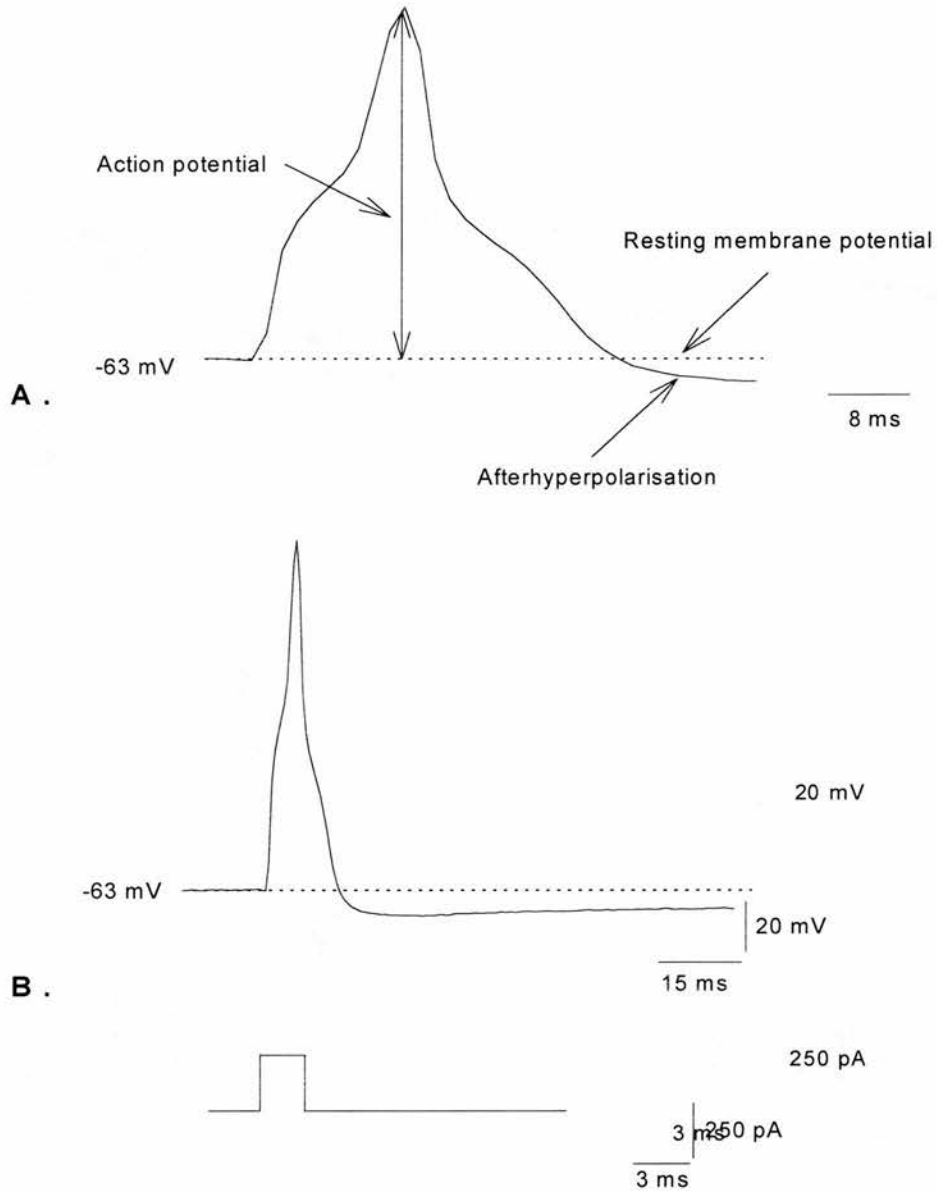


Figure 2.5. The action potential in a typical DRN neurone.
A. A typical action potential evoked following a short, 3 ms, positive current pulse, 250 pA, in a cell with resting membrane potential of -63 mV. The action potential consists of a rapid overshooting rising phase where the time from spike onset to peak amplitude of 118 mV is approximately 4 ms. The falling phase is broad and slow with a marked shoulder due to the activation of a calcium current. The repolarisation overshoots the resting membrane potential producing a transient AHP of 14 mV.
B. The same action potential on a slower time base shows that the AHP persists for 100 ms before the cell returns to it resting potential.

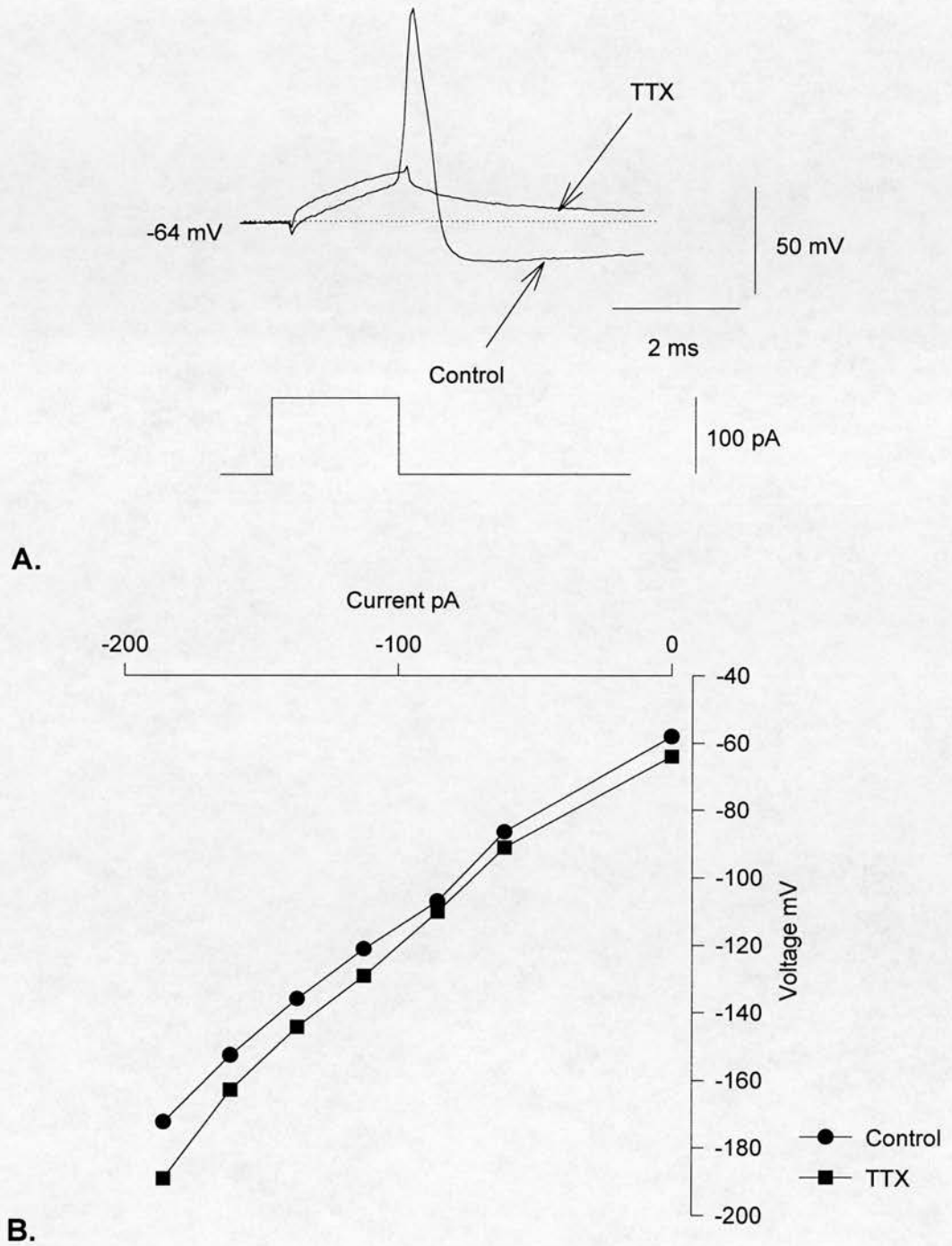


Figure 2.6. The action of TTX in a DRN neurone.
A. The cell can no longer generate an action potential following the injection of a short (2 ms) depolarising current step, 0.1nA, while in the presence of TTX (3 μM). **B.** I /V plot showing the action of TTX (3 μM). The cell hyperpolarises from a resting membrane potential of -58 mV to -64 mV, with a slight increase in input resistance from 205.8 MΩ to 225.9 MΩ.

changes seen with TTX. This again implies that the regulation of the activity of the DRN is due to a number of mechanisms.

These findings concur with those made in other intracellular studies performed *in vitro* in the DRN slice. The DRN neurone has been shown to have an action potential, generated following the activation of sodium channels, with a broad shoulder followed by a large AHP which decays slowly during the interspike interval (Crunelli et al 1983; VanderMaelen and Aghajanian 1982a).

Afterhyperpolarisation

Following the generation of an action potential the cell repolarises past its resting membrane potential level to give an AHP (Figure 2.6). From observing a number of cells the peak amplitude of the AHP was found to be 11.4 ± 2.3 mV (n=14).

Effect of membrane potential on the AHP

The ionic nature of the AHP was investigated by changing the membrane potential of the cell and looking for a reversal potential. This was performed by injecting a known positive current into the cell to change the membrane potential and measuring any effect on the size of the AHP. When at its resting membrane potential a cell generated a broad action potential followed by an AHP of 10 mV (Figure 2.7A). When the same cell was hyperpolarised to a membrane potential of -90 mV the AHP was no longer observed (Figure 2.7B). When the cell was further hyperpolarised to -100 mV the AHP inverted showing reversed polarity (Figure 2.7C). The AHP has been shown to reverse at -90 mV as would be expected for a potassium-mediated event suggesting the involvement of a potassium current. This observation was made in a further three cells where the AHP reversed at approximately -90mV in all cells tested (-89 mV ± 1.2 mV).

VanderMaelen and Aghajanian (1983) reported AHP's in the range 10-20 mV, lasting for 200-800 ms. A number of studies have shown that a calcium-activated potassium current underlies the AHP in the DRN, consistent with the AHP reversing approximately at the value of a potassium-mediated event (Crunelli et al 1983;

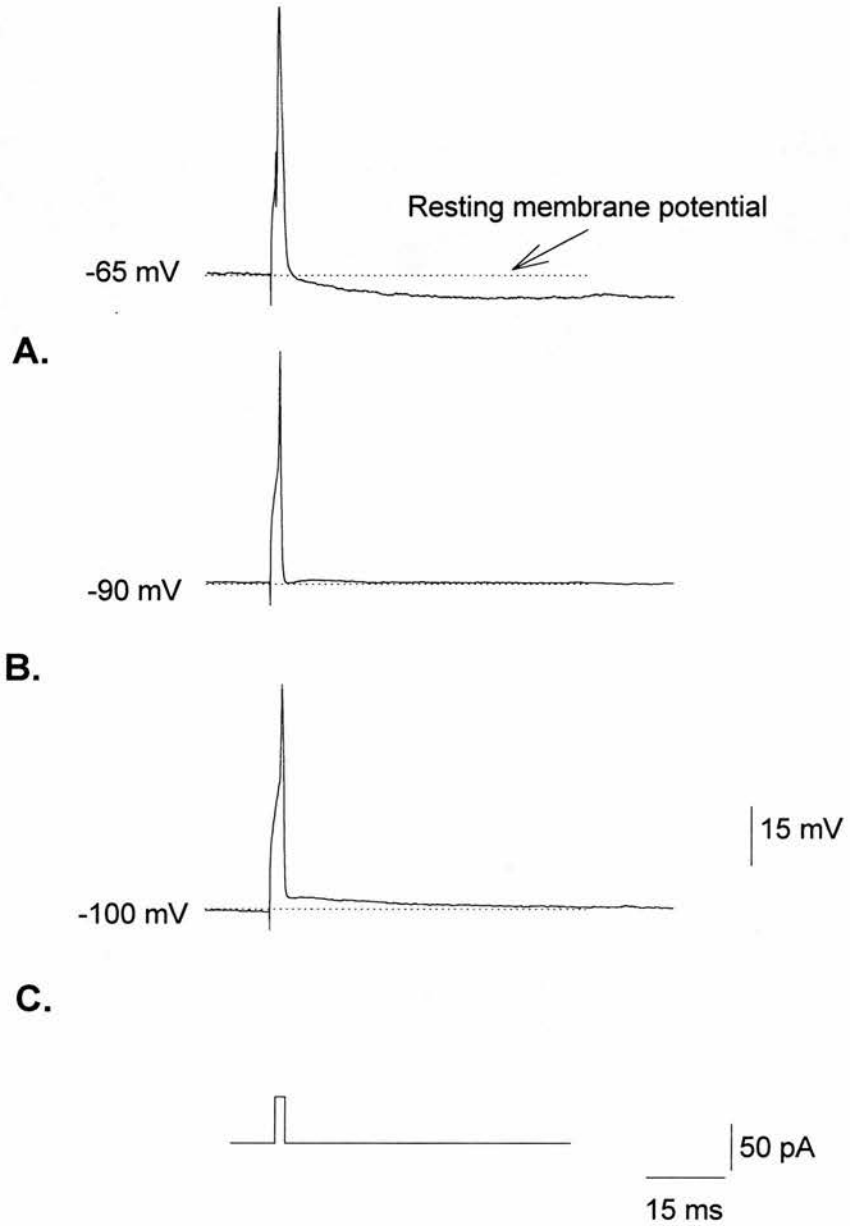


Figure 2.7. The AHP reverses at -90 mV.
Intracellular recording made from a typical cell showing an action potential generated by injecting a short (3 ms) positive current pulse of 50 pA.
A. When at its resting membrane potential, -65 mV, the cell generates a broad action potential followed by an AHP of 10 mV.
B. When the cell is hyperpolarised to a membrane potential of -90 mV the AHP is no longer observed.
C. The cell is further hyperpolarised to -100 mV and the AHP is now inverted.

VanderMaelen and Aghajanian 1982b; Aghajanian 1985). However, the AHP may be mediated by different potassium conductances. Evidence for multiple components to the AHP was first uncovered in the experiments on the hippocampus of Pennefather et al (1985), Fowler et al (1985) and Lancaster and Adams (1986). In the hippocampus for example, the AHP is made up of three components termed the fast, medium and slow components.

CHAPTER 3

Actions of WAY-100635

Introduction

The DRN contains the largest cluster of serotonergic neurones in the rat brain. However, the DRN also contains non-serotonergic neurones. This makes it important to be able to identify serotonergic neurones by their electrophysiological and pharmacological characteristics. The most commonly found 5-HT-evoked response that is electrophysiologically observable in the DRN is a membrane hyperpolarisation. This is associated with a decrease in cell input resistance, consistent with ion channel opening, and reverses at the predicted equilibrium potential for a potassium mediated event. This was first characterised *in vitro* by Aghajanian and VanderMaelen (1982b) who also discovered that iontophoretically applied 5-HT inhibited the cell firing induced by iontophoretically applied noradrenaline. The same result was obtained with the 5-HT agonist LSD.

The 5-HT-evoked hyperpolarisation was one of the first to be identified and characterised, this characterisation was aided by the development of the selective 5-HT_{1A} agonist 8-OH-DPAT. It is now thought that the 5-HT-evoked hyperpolarisation is mediated by the 5-HT_{1A} receptor (Sprouse and Aghajanian 1987). However, although there is known to be a number of 5-HT receptor subtypes reported in the DRN, the search for electrophysiological identifiable responses mediated by non-5-HT_{1A} receptors has proven difficult. This is because of the lack of a truly silent and selective 5-HT_{1A} antagonist. Early work with 5-HT involving binding studies and electrophysiological and biochemical investigations indicated that a number of compounds previously characterised as antagonists at other non-5-HT receptors also had 5-HT antagonist activity. A more recently developed compound is claimed to be

both selective and silent, the phenylpiperazine WAY-100635. Forster et al (1995) found that WAY-100635 blocked the inhibitory action of 8-OH-DPAT on DRN neuronal cell firing in the anaesthetised rat. In the hippocampus WAY-100635 has been found to prevent the 5-HT-evoked hyperpolarisation (Corradetti et al 1996) and has been found to increase DRN neuronal activity and 5-HT release in behaving cats (Fornal et al 1994) and in anaesthetised rats (Fletcher et al 1993).

This chapter will present data from both the DRN and the hippocampus characterising the 5-HT_{1A}-mediated hyperpolarisation, the actions of the 5-HT_{1A} antagonist WAY-100635 and the blockade of the 5-HT_{1A}-mediated hyperpolarisation by WAY-100635. This will allow comparison between the DRN and hippocampus where some studies have suggested differences in the 5-HT_{1A} receptor in the two brain regions (Artigas et al 1996). This chapter will then discuss the actions of 5-HT as revealed by the 5-HT_{1A} antagonist WAY-100635 in the *in vitro* slice preparation in the DRN neuronal cell.

Results

Study of WAY-100635 in the DRN

The 5-HT_{1A}-evoked hyperpolarisation in the DRN

All healthy DRN cells impaled responded to 5-HT superfusion with a membrane hyperpolarisation and an associated decrease in cell input resistance. In one cell the addition of 5-HT (100 μM) to the bath-aCSF evoked a membrane hyperpolarisation from a resting membrane potential of -58 mV to -73 mV with 5-HT, a peak amplitude of 15 mV (Figure 3.0A). This was accompanied by a decrease in the cell input resistance from a resting value of 354.9 MΩ to 114.9 MΩ (Figure 3.0B). On washout the cell recovered to a membrane potential of -58 mV and cell input resistance of 349.3 MΩ. Further analysis involving the generation of current-voltage (I/V) plots in the absence and presence of 5-HT showed a point of intersection at -87 mV. A value close to the predicted reversal potential for potassium mediated events as calculated from the Nernst equation, suggesting that the 5-HT-evoked hyperpolarisation was mediated by a potassium conductance.

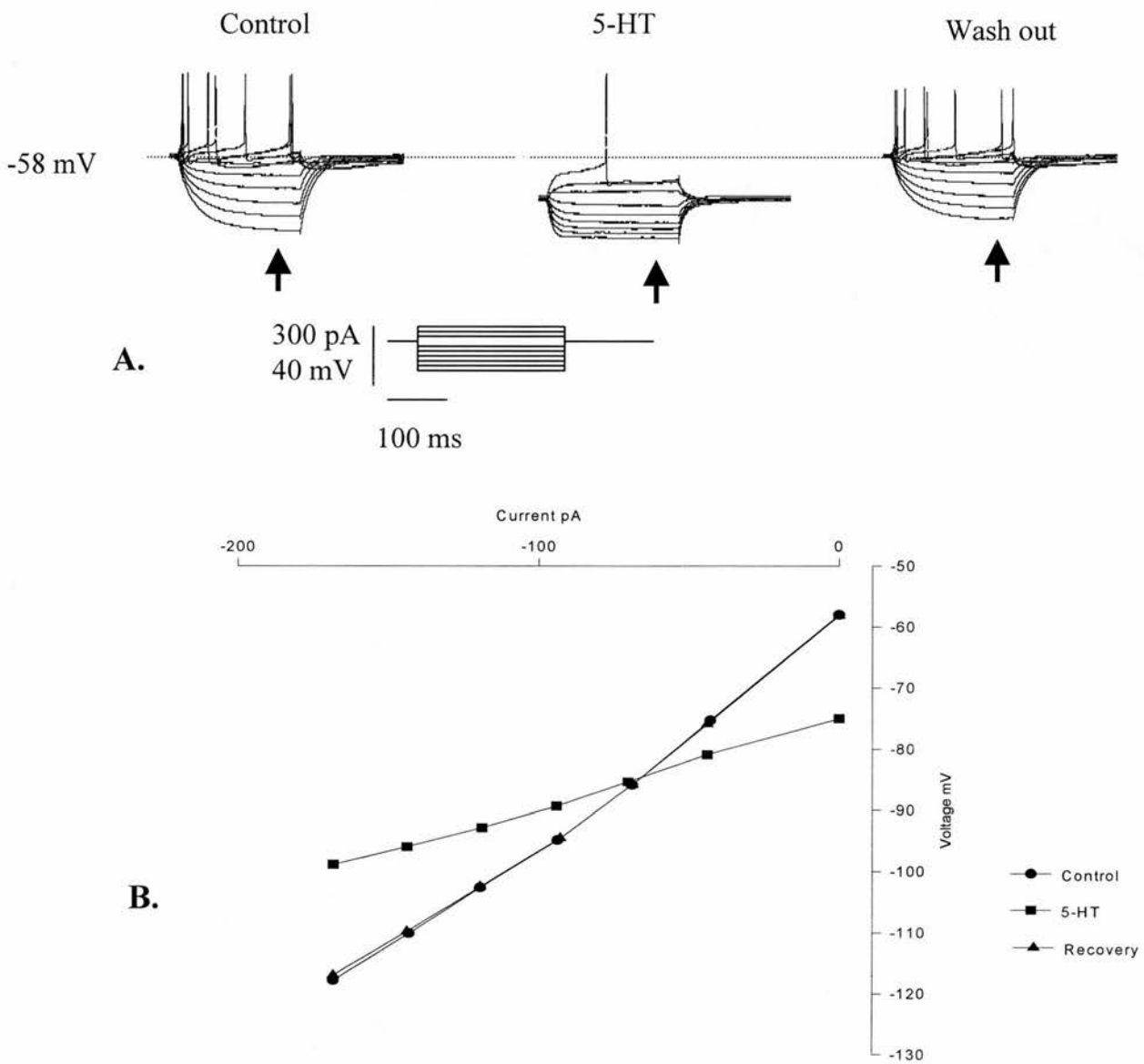


Figure 3.0. A 5-HT-evoked hyperpolarisation in the DRN.
A. Voltage traces of a cell before, during and after bath-application of 5-HT.
B. Current-voltage relationships were obtained by measuring at the point ↑ after injecting a series of depolarising and hyperpolarising current pulses in the cell. The cell had a resting membrane potential of -58 mV and an input resistance of 354.9 MΩ (circles). Following bath-application of 5-HT (100 μM) the cell hyperpolarised to -73 mV (squares). This was accompanied by a decrease in input resistance to a value of 114.9 MΩ. The control and 5-HT I/V relationships intersected at -87 mV. The 5-HT was washed out and the cell returned to its resting membrane potential of -58 mV and resting input resistance of 349.3 MΩ (triangles).

In 32 similar experiments 5-HT (100 μM) caused a 7.0 ± 2.3 mV hyperpolarisation from a resting value of -63.5 ± 4.1 mV to a peak of -70.5 ± 3.9 mV. This was accompanied with a reduction in cell input resistance from 263.5 ± 58.3 MΩ to 197.9 ± 52.5 MΩ. Statistical analysis using the t-test showed with a confidence interval of 95% that both the resting membrane potential and the input resistance are significantly altered by 5-HT. The reversal potential was observed to be -89 ± 1.3 mV, approximately the reversal potential for a potassium conductance.

Blockade by WAY-100635 in the DRN

The action of 5-HT on DRN neurones was investigated in the presence of WAY-100635. Prior to WAY-100635 application a typical cell, with a resting membrane potential of -63 mV and an input resistance of 354.5 MΩ, responded to bath-applied 5-HT (100 μM) with a control membrane hyperpolarisation (Figure 3.1A). In this case the cell hyperpolarised to -72 mV with an associated decrease in input resistance to 263.1 MΩ. Following bath-application of WAY-100635 (100 nM) for 15 minutes subsequent 5-HT (100 μM) applications did not evoke the 5-HT_{1A} receptor-mediated hyperpolarisation. The 5-HT_{1A}-evoked response was still blocked following WAY-100635 washout of up to one hour. Application of WAY-100635 (100 nM) for only 15 mins blocked the 5-HT (100 μM) evoked hyperpolarisation in a further 5 cells.

The selectivity of WAY-100635 in the DRN

Whether WAY-100635 had α_1-adrenorecptor antagonist activity was also investigated. WAY-100635 did not block the depolarisation associated with bath-applied phenylephrine and noradrenaline. In a typical cell where pre-treatment with WAY-100635 (100 nM) blocked the 5-HT_{1A}-mediated hyperpolarisation, bath-application of phenylephrine (10 μM) evoked a membrane depolarisation from a resting membrane potential of -64 mV to -59 mV, a change of approximately 5 mV (Figure 3.2A). This was accompanied by an increase in cell input resistance from 147.1 MΩ to 173.4 MΩ. The I/V plots reversed at approximately -94 mV a value consistent with the event being mediated by a potassium channel. Similarly in a different cell, which had demonstrated a blockade of the 5-HT_{1A}-mediated

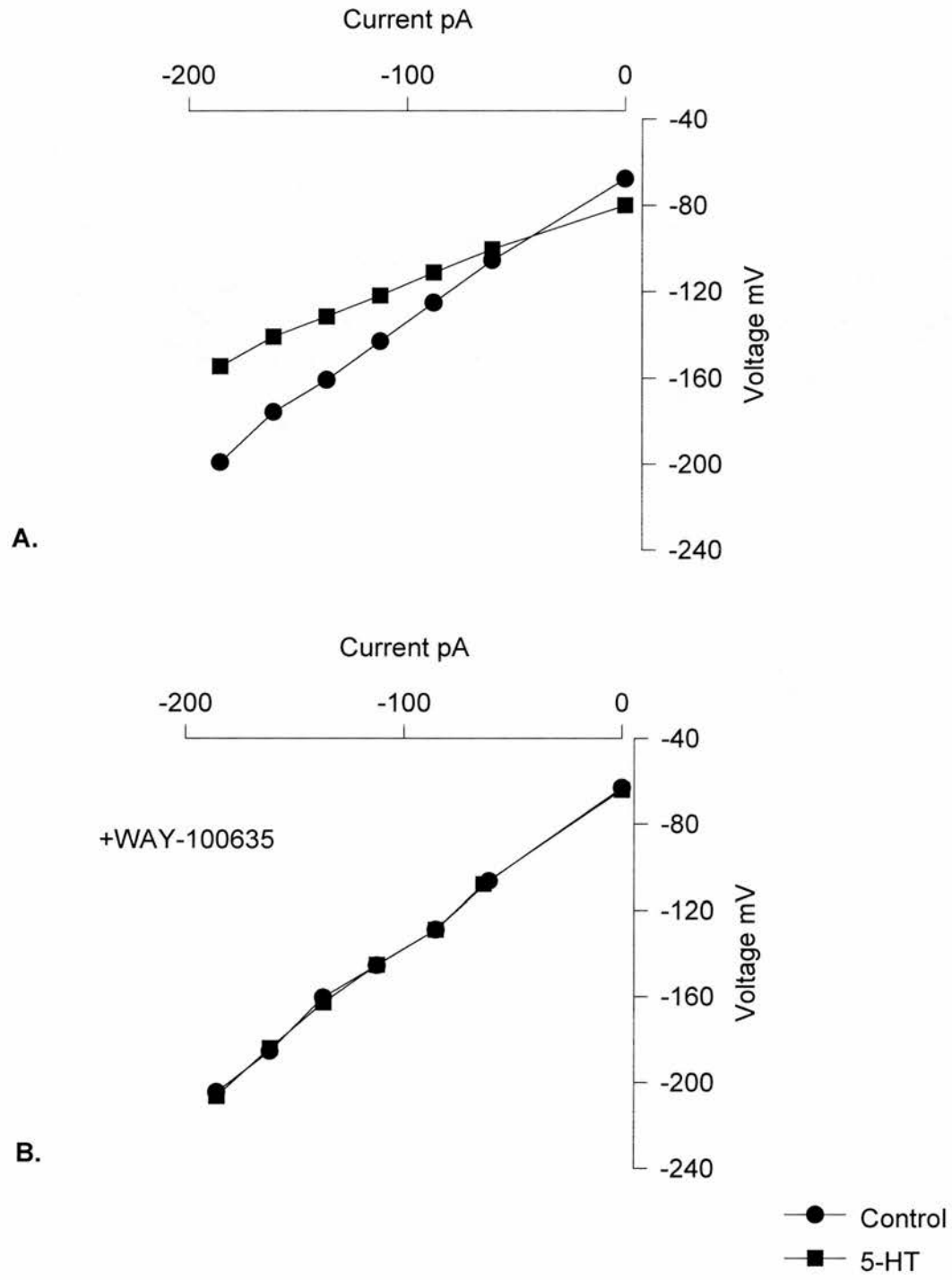


Figure 3.1. WAY-100635 blocked the 5-HT-evoked hyperpolarisation in the DRN.

A. I/V plots obtained from a DRN neurone before (circles) and after (squares) bath-application of 5-HT (100 μM). The DRN neurone hyperpolarised from a resting membrane potential of -63 mV to -72 mV, a change of approximately 9 mV. This was accompanied by a decrease in cell input resistance from 354.5 MΩ to 263.1 MΩ.
B. Application of WAY-100635 (100 nM) for 15 mins completely abolished the hyperpolarisation, where the cell had a membrane potential of -64 mV and cell input resistance of 384.4 MΩ with 5-HT (100 μM).

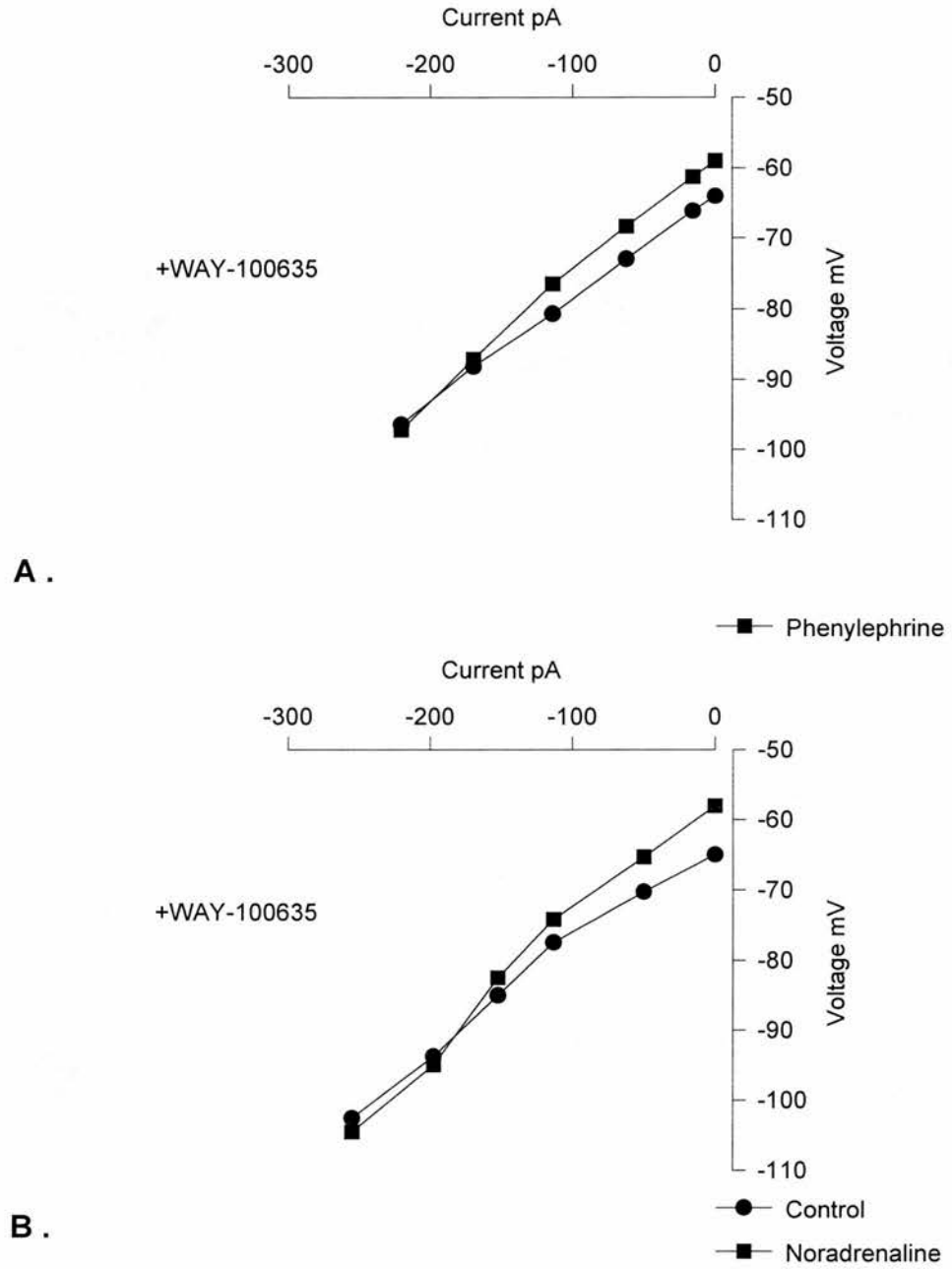


Figure 3.2. WAY-100635 does not act at the α_1-adrenoreceptor in the DRN.
A. I/V plots obtained from a DR neurone pre-applied with WAY-100635 before (circles) and after (squares) bath-application of phenylephrine (10 μM). The DRN neurone depolarised from a resting membrane potential of -64 mV to -59 mV, a change of approximately 5 mV. This was accompanied by an increase in cell input resistance from 147.1 MΩ to 173.4 MΩ. The I/V plots reversed at approximately -94 mV.
B. I/V plots obtained from a DR neurone pre-applied with WAY-100635 before (circles) and after (squares) bath-application of noradrenaline (10 μM). The DR neurone depolarised from a resting membrane potential of -65 mV to -58 mV, a change of approximately 7 mV. This was accompanied by an increase in cell input resistance from 147.4 MΩ to 182.6 MΩ. The I/V plots reversed at approximately -90 mV.

hyperpolarisation following continued exposure to WAY-100635 (100 nM), bath-application of noradrenaline (10 μM) evoked a membrane depolarisation from a resting membrane potential of -65 mV to -58 mV, a change of approximately 7 mV (Figure 3.2B). The noradrenaline-evoked depolarisation was accompanied by an increase in cell input resistance from 147.4 MΩ to 182.6 MΩ. The I/V plots reversed at approximately -90 mV, again close to that for a potassium-mediated event. Both phenylephrine and noradrenaline evoke a membrane depolarisation in the DRN via the α_1-adrenorecptor so if WAY-100635 does not block the depolarisation it may not have antagonist activity at the α_1-adrenorecptor.

WAY-100635 is silent in the DRN

WAY-100635 (100nM) was applied for up to 45 minutes and was found to have no effect on resting membrane potential or input resistance (n=35). Prior to WAY-100635 application the membrane potential was -63 ± 3.5 mV, with an input resistance of 263.1 ± 80.2 MΩ. Following WAY-100635 (100 nM) application the membrane potential stayed constant at -63 ± 3.1 mV, as did the input resistance at 258.9 ± 79.2 MΩ within the first 45 minutes of WAY-100635 application. Statistical analysis using t-tests show that the membrane potential and input resistance stay the same with application of WAY-100635 for up to 45 minutes. This suggests WAY-100635 has no partial agonist activity.

However, there were some examples of WAY-100635 having a slight effect on the resting membrane potential and input resistance following long term exposure. Following bath-application of WAY-100635 (100 nM) for long time periods (up to 90 mins) some cells did show a slight depolarisation (n=8 of 35). This is illustrated in a typical cell in Figure 3.3. The cell demonstrates a slight depolarisation in membrane potential from a resting membrane potential of -66 mV to -63 mV, there was also a slight increase in input resistance from 290.2 MΩ to 310.5 MΩ. This depolarising membrane drift may represent WAY-100635 having some ion channel blocking effect that can only be observed following a long application. It seems unlikely that the membrane depolarisation is due to an agonist action on the 5-HT_{1A}

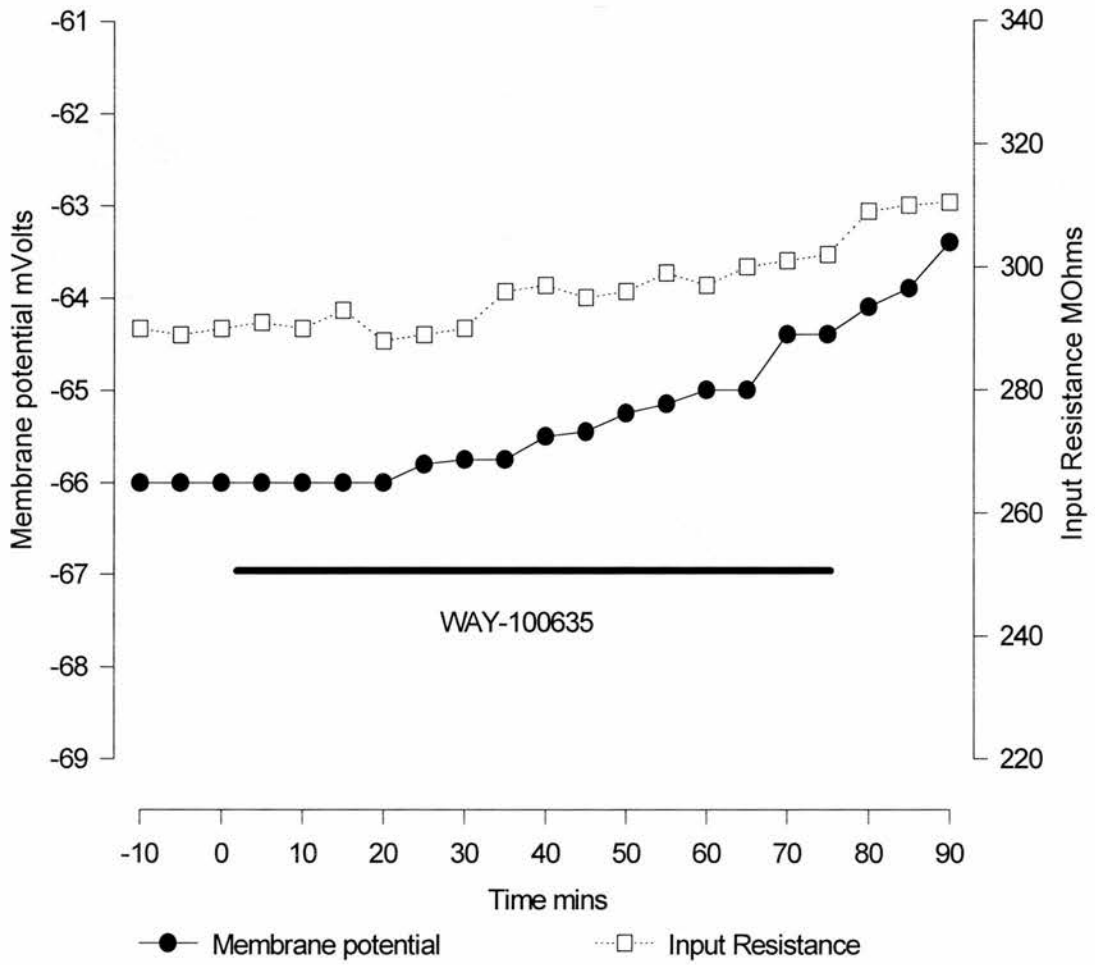


Figure 3.3. Long term application of WAY-100635 in the DRN.
A plot showing the change in membrane potential (closed circles) and input resistance (open circles) during a prolonged application of WAY-100635 (100 nM). Membrane potential and input resistance were measured every 5 mins. Following bath-application of WAY-100635 (solid bar) the resting membrane potential was observed to slowly drift in the depolarising direction from an initial value of -66 mV to -63 mV after 90 mins. The input resistance also increased slightly from a value of 290.2 MΩ to 310.5 MΩ.

receptor as this would be observed as a hyperpolarisation. Also the concentration of WAY-100635 in the chamber is high enough to block the 5-HT-evoked hyperpolarisation after 15 mins exposure, so it seems unlikely that WAY-100635 would take over one hour to show agonist activity.

Additional actions of 5-HT revealed by WAY-100635

A 5-HT-evoked depolarisation in the DRN

In a cell illustrated in Figure 3.4 a short application of WAY-100635 (15 mins, 100 nM) blocked the 5-HT_{1A}-mediated hyperpolarisation (Figure 3.4A). This 5-HT-evoked depolarisation was observed in a further 28 cells (n=29 of 35). However a long application of WAY-100635 (35 mins, 100 nM) revealed a 5-HT (100 μM) evoked depolarisation (Figure 3.4B) showing that 5-HT can have multiple actions in the DRN.

A typical example of the 5-HT-evoked depolarisation is shown in Figure 3.5 which shows the voltage responses to injected current pulses in a typical neurone, resulting in the generation of a series of voltage deflections or steps. The resting membrane potential was indicated in the upper part of the step and the height of the step gives an indication of cell input resistance. Figure 3.5A shows a 5-HT_{1A}-evoked hyperpolarisation. The resting membrane potential was -63 mV. The membrane potential can be seen to move in a hyperpolarising direction with 5-HT (100 μM) and reaches -71 mV, a change of 8 mV. The cell input resistance decreases following the application of 5-HT. A more accurate measurement of the input resistance can be obtained by measuring the voltage responses to a series of graded hyperpolarising current steps and can be displayed as I/V plots. Figure 3.5B shows a 5-HT-evoked depolarisation in the same cell following exposure to WAY-100635 (100 nM, 35 mins). The resting membrane potential is -64 mV. The membrane potential can be seen to move in a depolarising direction with 5-HT (100 μM) and reaches -60 mV, a change of 4 mV. The cell input resistance decreases slightly following the application of 5-HT.

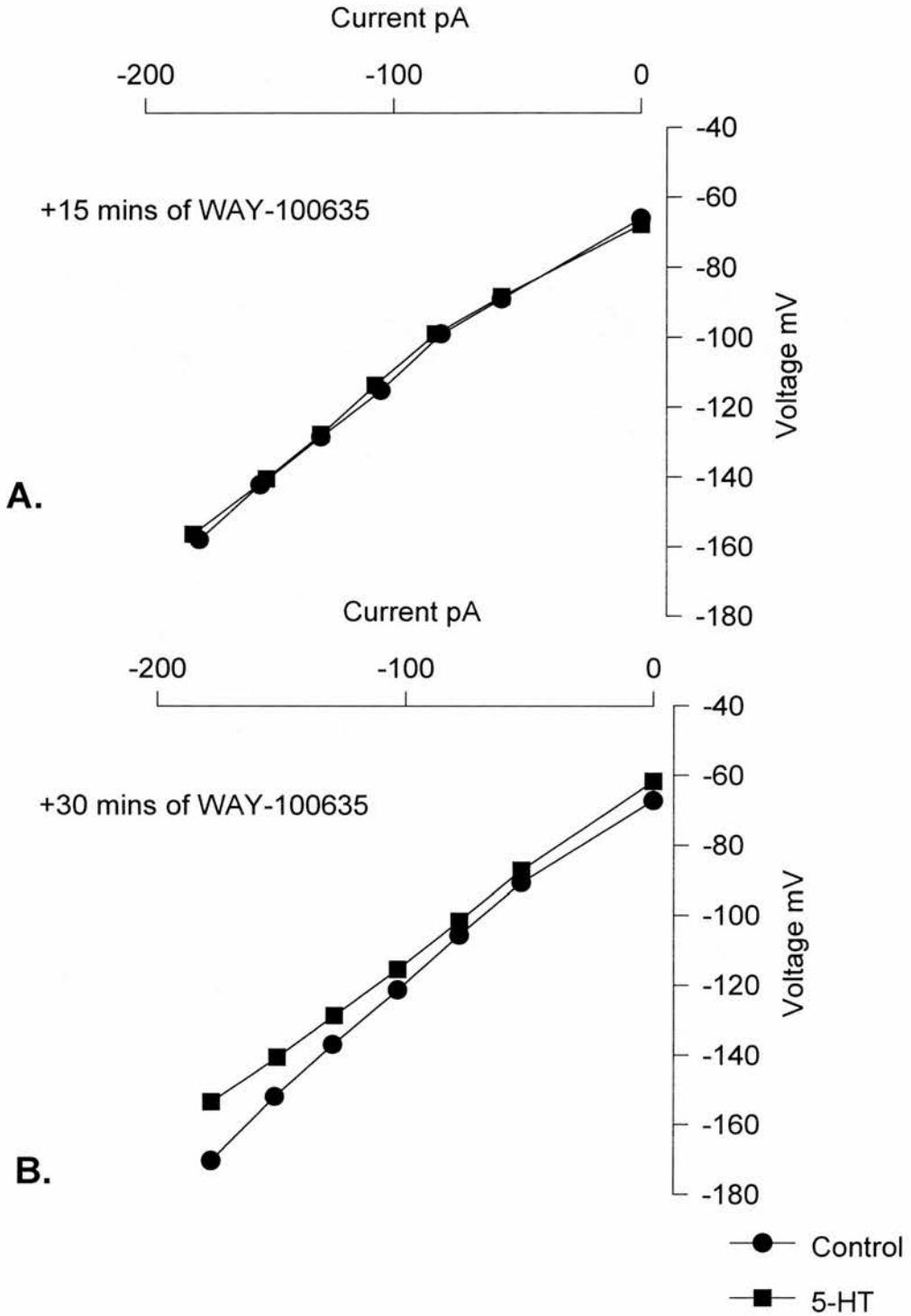


Figure 3.4. A 5-HT-evoked depolarisation with WAY-100635 in the DRN.
A. Following a short application of WAY-100635 (100 nM) for 15 mins the 5-HT (100 μM) evoked hyperpolarisation is blocked.
B. Following a long application of WAY-100635 (100 nM) for 30 mins a 5-HT (100 μM) evoked depolarisation was observed. The cell depolarised from -67 mV to -61 mV; this was accompanied by a decrease in input resistance from 289.6 MΩ to 257.7 MΩ.

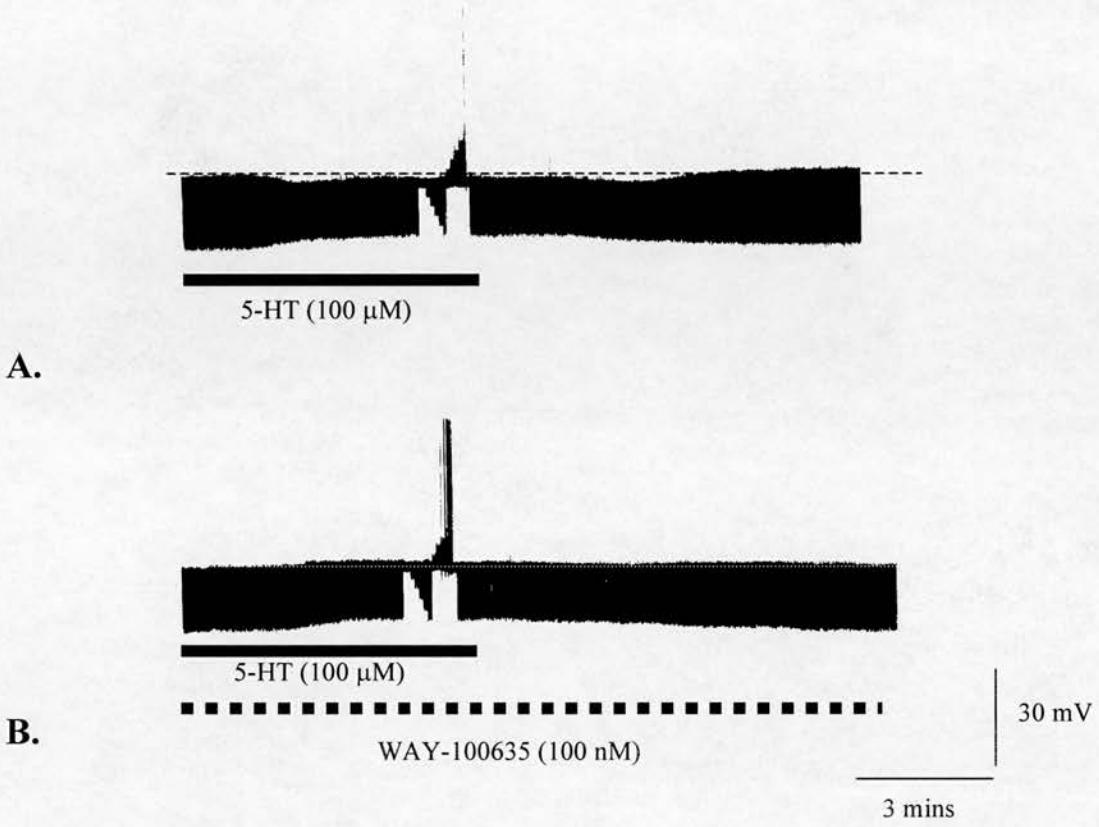


Figure 3.5. Chart recording of the two 5-HT-evoked actions.
Current pulses of 250 pA and 500 ms duration were injected into a neurone every second and the voltage responses were recorded intracellularly.
A. The chart recording showed a 5-HT_{1A}-evoked hyperpolarisation. The resting membrane potential was -63 mV. The membrane potential moved in a hyperpolarising direction when 5-HT (100 μM) was added to the bath of aCSF (solid bar) and reaches -71 mV, a change of 8 mV. The voltage response decreased in height in the presence of 5-HT indicating a decrease in cell input resistance. A more accurate measurement of the input resistance was obtained by measuring the voltage responses to a series of graded hyperpolarising and depolarising current steps. The series of voltage steps can be displayed in I/V plots for analysis.
B. The chart recording showed a 5-HT-evoked depolarisation in a cell pre-applied with WAY-100635 (100 nM) (dashed bar). The resting membrane potential was -64 mV. The membrane potential moved in a depolarising direction with 5-HT (100 μM) (solid bar) and reached -60 mV, a change of 4 mV. The cell input resistance decreased slightly following the application of 5-HT.

I/V plots from another DRN neurone are displayed in Figure 3.6 that shows the action of bath-applied 5-HT before and after application of WAY-100635. In control conditions the neurone exhibited a 5-HT (100 μM) evoked hyperpolarisation from a resting membrane potential of -64 mV to -75 mV, a change of 11 mV (Figure 3.6A). This was accompanied by a decrease in cell input resistance from 334.9 MΩ to 157.4 MΩ. The I/V plots intersected at -87 mV. Following bath-application of WAY-100635 (100 nM) the neurone displayed a 5-HT (100 μM) evoked depolarisation from a resting membrane potential of -65 mV to -60 mV, a change of approximately 5 mV (Figure 3.6B). This was accompanied by a decrease in cell input resistance from 308.5 MΩ to 272.2 MΩ, measured over the linear portion of the I/V plot in the range -90 mV to -120 mV. The I/V plots did not intersect over the measured range. In addition, many cells showing the 5-HT (100 μM) evoked depolarisation was associated had an increase cell excitability as seen by an increase in firing rate (n=15 of 29).

Using all the cases where WAY-100635 (100 nM) uncovered a slow 5-HT (100 μM) evoked depolarisation the mean depolarisation was 4 mV ± 1.2 mV, from a resting value of -64 ± 2.7 mV to -60 ± 2.3 mV with 5-HT (n=29 of 35). Statistical analysis using the t-test showed with a confidence interval of 95% that the resting membrane potential change is significantly different from the membrane potential with 5-HT. Overall, the depolarisation was accompanied by a slight decrease in cell input resistance from 261.6 MΩ ± 58.9 MΩ to 241.4 MΩ ± 50.2 MΩ, also further I/V plot analysis showed the 5-HT response did not reverse in the range -100 mV to -70 mV (n=29). However, I/V analysis revealed three distinct populations of cells. In one cell population the input resistance before (250.1 ± 10.5 MΩ) and after 5-HT (100 μM) (238.2 ± 9.3 MΩ) showed no change (n=9 of 29). In some cells the input resistance increased from 248.3 ± 37.9 MΩ before 5-HT (100 μM) to 272.8 ± 39.1 MΩ in the presence of 5-HT (100 μM) (n=14 of 29). A small number of cells showed a slight decrease in input resistance of 273.5 MΩ ± 56.6 MΩ to 238.6 MΩ ± 47.6 MΩ with

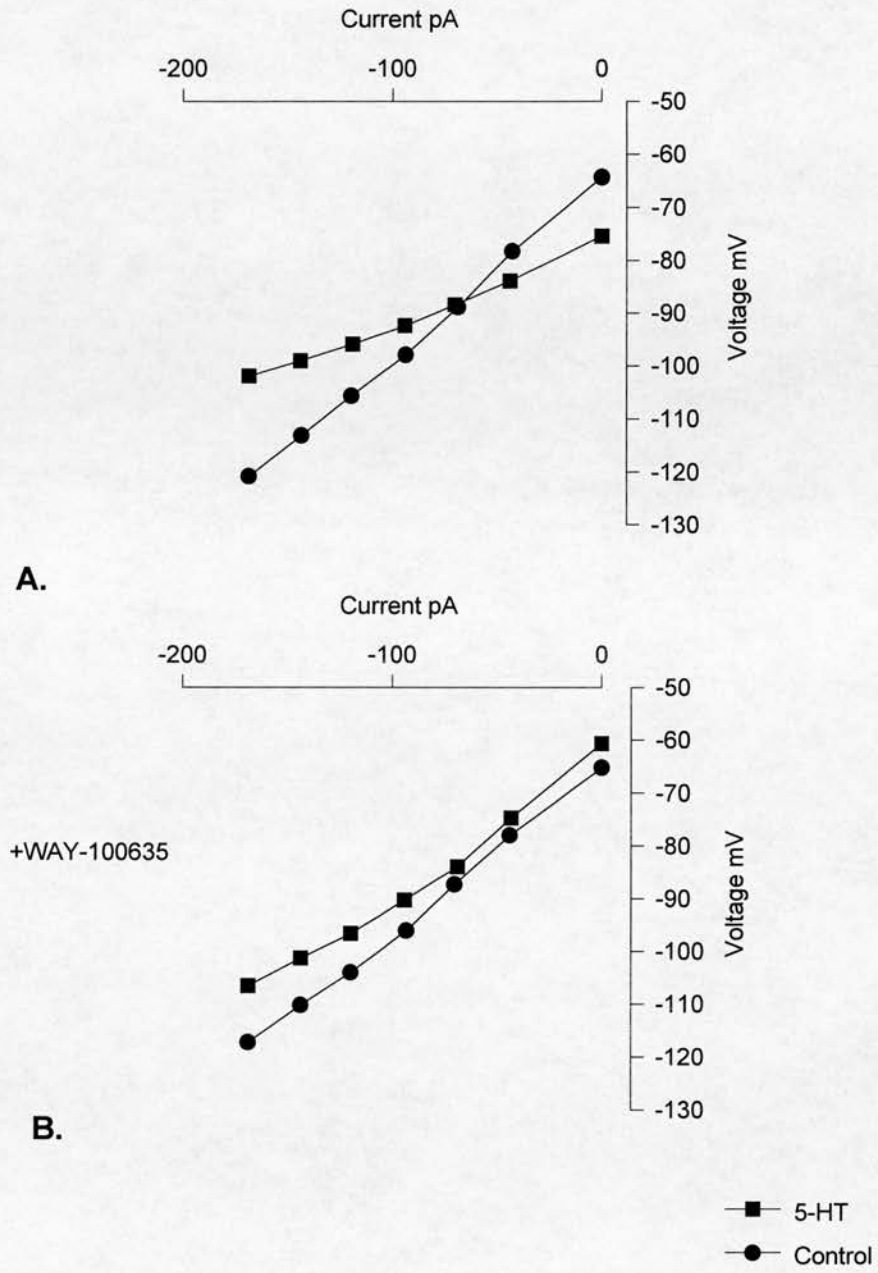


Figure 3.6. 5-HT evoked a depolarisation with WAY-100635 in the DRN.
A. I/V plots obtained from a DRN neurone before (circles) and after (squares) bath-application of 5-HT (100 μM). The DRN neurone hyperpolarised from a resting membrane potential of -64 mV (circles) to -75 mV. This was accompanied by a decrease in cell input resistance from 334.9 MΩ to 157.4 MΩ. The I/V plots intersected at -87 mV.
B. I/V plots obtained from the same DR neurone before (circles) and after (squares) bath-application of 5-HT (100 μM) while in the presence of WAY-100635 (100 nM). The DR neurone depolarised from a resting membrane potential of -65 mV to -60 mV. This was accompanied by a decrease in cell input resistance from 308.5 MΩ to 272.2 MΩ, measured over the linear portion of the I/V plot in the range -90 mV to -120 mV. The I/V plots did not intersect over the measured range.

5-HT (100 μM) (n=16 of 29). In cells showing a clear point of intersection I/V plots showed a reversal potential of -47 ± 1.9 mV (n=16 of 29).

Actions of the channel blocker TTX in the DRN

The importance of synaptic activity in the generation of the 5-HT-evoked depolarisation was investigated using the sodium channel blocker TTX, which prevents initiation of sodium action potentials. The depolarisation persisted in the presence of TTX (3 μM, n=2). One cell treated with WAY-100635 (100 nM) demonstrated a depolarisation due to bath-application of 5-HT (100 μM) from a resting membrane potential of -64 mV to -59 mV, a change of approximately 5 mV (Figure 3.7A). This was accompanied by a decrease in cell input resistance from 344.1 MΩ to 303.9 MΩ. In the presence of TTX (3 μM) the cell depolarised following an application of 5-HT (100 μM) from -65 mV to -61 mV, this was accompanied by a change in input resistance from 330.8 MΩ to 307.4 MΩ (Figure 3.7B). This indicates that the 5-HT-evoked depolarisation is independent of synaptic activity.

A non-5-HT_{1A}-evoked hyperpolarisation in the DRN

Another example of a 5-HT-evoked membrane change, in this case a 5-HT-evoked hyperpolarisation, was observed in a small number of cells following blockade of the 5-HT_{1A}-mediated hyperpolarisation. In one cell a small 5-HT (100 μM) evoked hyperpolarisation (3mV) was revealed in the presence of WAY-100635 (100 nM) and methysergide (100 μM). This DRN neurone which had been treated with WAY-100635 (100 nM) depolarised with 5-HT (100 μM) from a resting membrane potential of -60 mV to -56 mV, a change of approximately 4 mV (Figure 3.8A). This was accompanied by a slight increase in cell input resistance from 163.9 MΩ to 169.9 MΩ. While in the presence of methysergide (100 μM) and WAY-100635 the same cell showed a 5-HT-evoked hyperpolarisation from -60 mV to -63 mV, this was accompanied by a decrease in input resistance from 174.9 MΩ to 140.6 MΩ (Figure 3.8B).

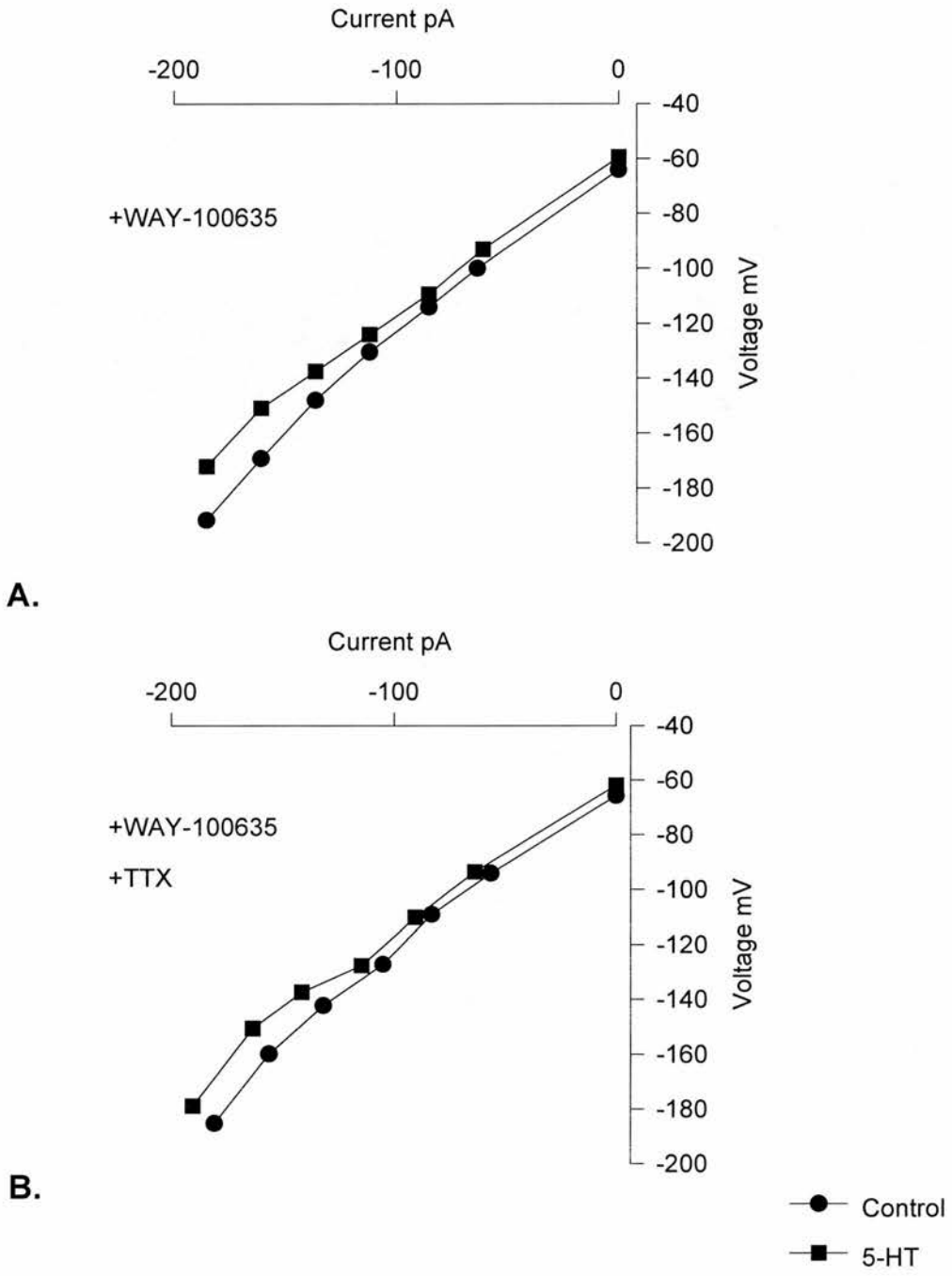


Figure 3.7. 5-HT evoked a depolarisation in the presence of TTX in the DRN.
A. I/V plots obtained from a DRN neurone before (circles) and after (squares) bath-application of 5-HT (100 μM). The DRN neurone depolarised from a resting membrane potential of -64 mV to -59 mV. This was accompanied by a decrease in input resistance from 344.1 MΩ to 303.9 MΩ.
B. In the presence of TTX (3 μM) the cell depolarised following an application of 5-HT from -65 mV to -61 mV. This was accompanied by a change in input resistance from 330.8 MΩ to 307.4 MΩ.

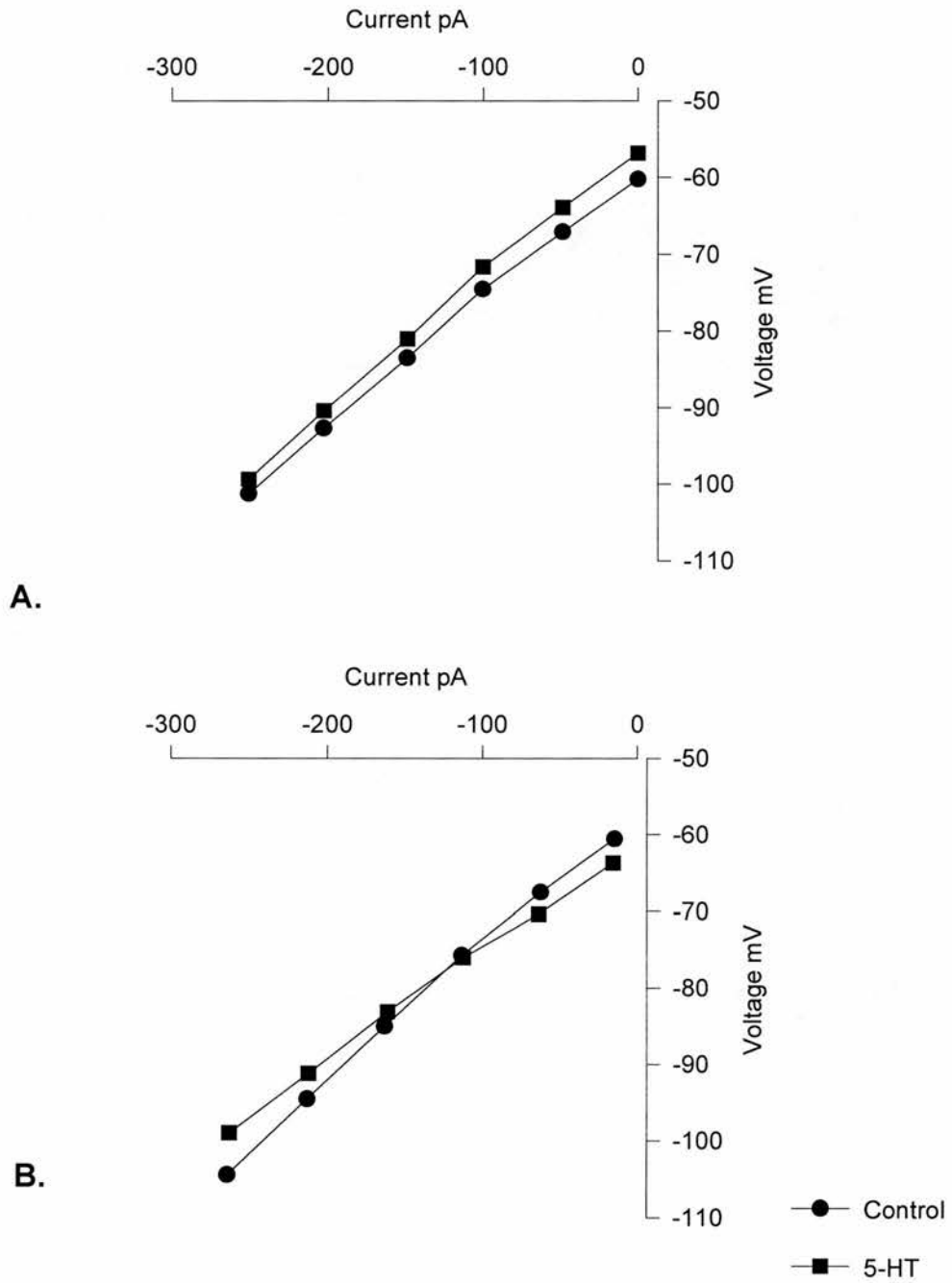


Figure 3.8. 5-HT-evoked hyperpolarisation with WAY-100635 and methysergide in the DRN.

A. I/V plots obtained from a DRN neurone before (circles) and after (squares) bath-application of 5-HT (100 μM). The DR neurone depolarised from a resting membrane potential of -60 mV to -56 mV, a change of approximately 4 mV. This was accompanied by a slight increase in cell input resistance from 163.9 MΩ to 169.9 MΩ.

B. While in the presence of methysergide bath-application of 5-HT (100 μM) evoked a membrane hyperpolarisation, from a resting membrane potential of -60 mV to -63 mV with 5-HT, there was also a decrease in cell input resistance from 174.9 MΩ to 140.6 MΩ.

A 5-HT evoked membrane hyperpolarisation was observed in three cells following the bath-application of 5-HT while in the presence of mCPP, a 5-$HT_{2B/2C}$ agonist that also has some 5-HT_2 antagonist activity, and WAY-100635. A typical cell that had demonstrated a 5-HT (100 μM) depolarisation with WAY-100635 (100 nM) (Figure 3.9A). Then in the continued presence of WAY-100635 (100 nM) with mCPP (100 μM) bath-application of 5-HT (100 μM) evoked a membrane hyperpolarisation from a resting membrane potential of -64 mV to -76 mV with 5-HT (Figure 3.9B). There was also an associated decrease in cell input resistance from 343.1 MΩ to 174.6 MΩ.

Study of WAY-100635 in the hippocampus

A study was also made in the hippocampus, a brain region where 5-HT_{1A} antagonists can have different activity to that in the DRN (Artigas et al 1994). First the passive and active membrane properties were established and then the 5-HT_{1A}-mediated hyperpolarisation characterised. WAY-100635 was applied to test its ability to block the 5-HT_{1A}-mediated hyperpolarisation and for any agonist activity.

Passive and active properties of the hippocampal neurone

Data was obtained from ten stable intracellular recordings from pyramidal cells of the CA1 area of the hippocampus. The neurones all had overshooting action potentials and showed resting membrane potentials ranging from -58 mV to -69 mV with a mean value of -63.2 ± 2.8 mV (n=10). Membrane voltage responses to hyperpolarising current commands showed a linear relationship (Figure 3.10). No inward rectification was observed in voltage deflections as negative as -100 mV. The current-voltage relationships in the linear portion of the curve gave an apparent input resistance of 154.6 ± 35.4 MΩ, and ranged from 127.3 MΩ to 203.6 MΩ.

The 5-HT_{1A}-mediated hyperpolarisation in the hippocampus

Data was obtained from a typical pyramidal neurone of the CA1 region of the hippocampus before and after bath-application of 5-HT (100 μM).

The pyramidal neurone hyperpolarised from a resting membrane potential of -63 mV to -69 mV, a change of approximately 6 mV with 5-HT (100 μM) (Figure 3.10A).

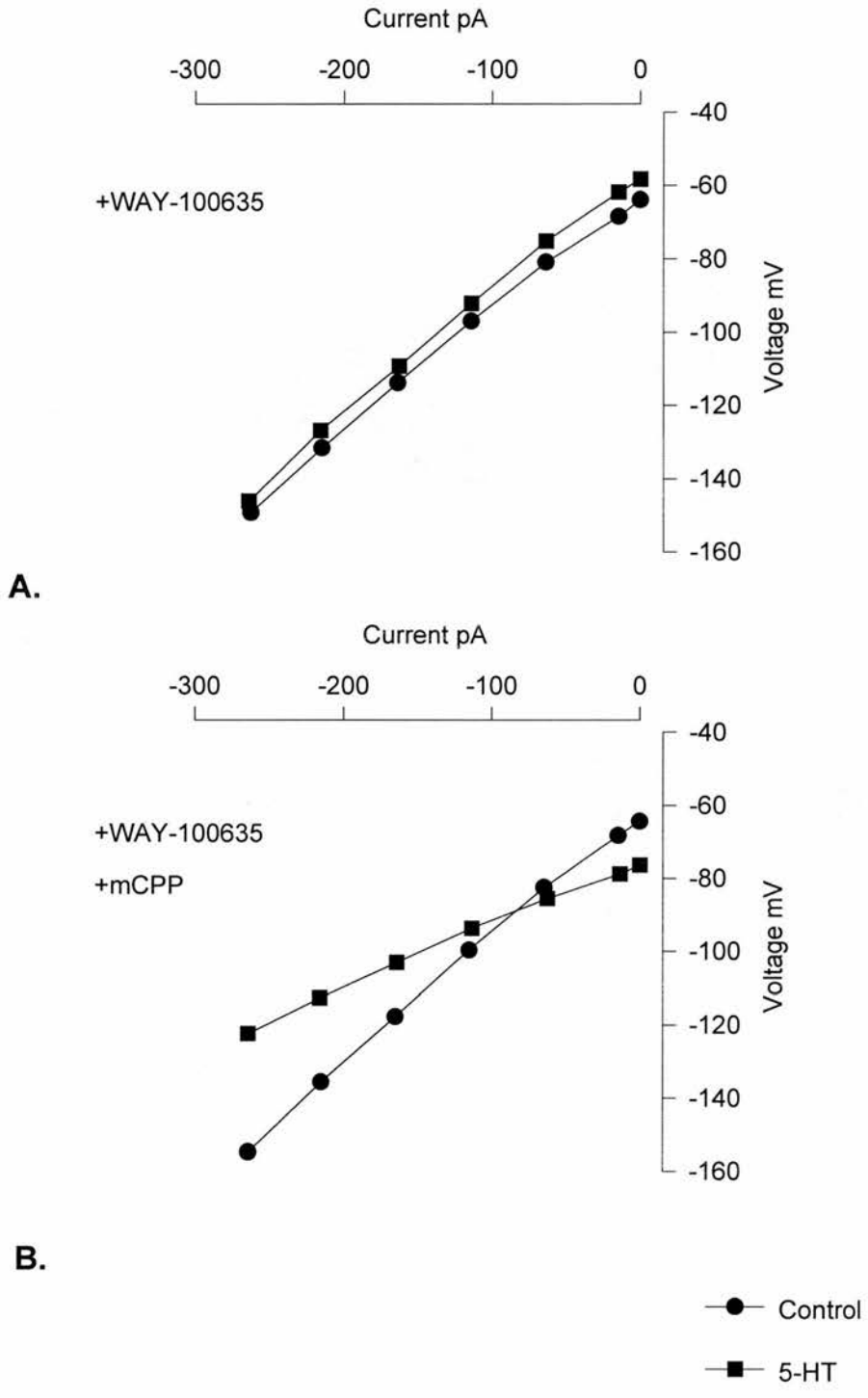


Figure 3.9. 5-HT evoked hyperpolarisation with WAY-100635 and mCPP in the DRN.

A. In the presence of WAY-100635 (100 nM) the cell underwent a 5-HT-evoked depolarisation from -63 mV to -58 mV. This was accompanied by a change in input resistance from 325.1 MΩ to 333.1 MΩ.

B. In the presence of mCPP (100 μM) the cell hyperpolarised following bath-application of 5-HT from -64 mV to -76 mV. This was accompanied by a change in input resistance from 343.1 MΩ to 174.6 MΩ.

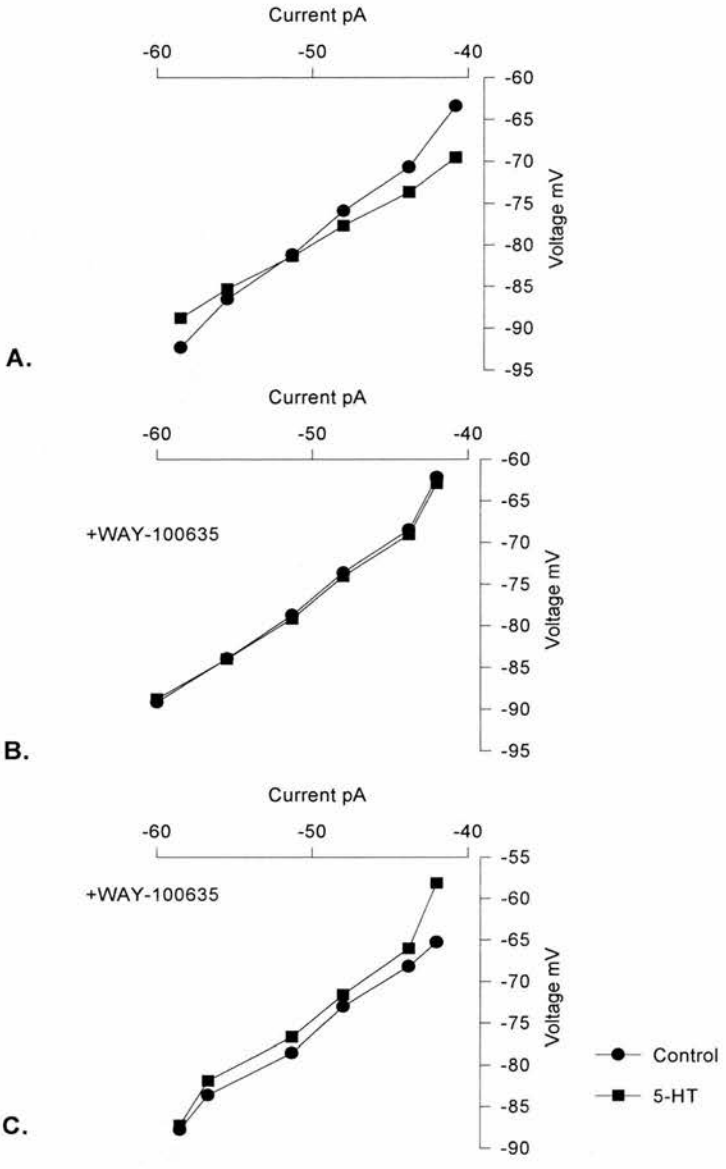


Figure 3.10. 5-HT-evoked two responses in the hippocampus.
I/V plots obtained from a pyramidal neurone of the CA1 region of the hippocampus. **A**. Before (circles) and during (squares) bath-application of 5-HT (100 μM). The pyramidal neurone hyperpolarised from a resting membrane potential of -63 mV (circles) to -69 mV with a decrease in cell input resistance from 163.2 MΩ to 108.9 MΩ. The I/V plots intersected at -81 mV. 5-HT washout returned the cell to resting values of -62 mV and 150.1 MΩ.
B. Before (circles) and after (squares) bath-application of WAY-100635 (100 μM). The neurone slightly depolarised from a resting membrane potential of -63 mV to -62 mV. There was a slight decrease in input resistance from 150.1 MΩ to 144.1 MΩ, measured over the linear portion of the plot in the range -70 mV to -90 mV.
C. Before (circles) and after (squares) bath-application of 5-HT (100 μM) while in the presence of WAY-100635. The neurone depolarised from a resting membrane potential of -65 mV to -58 mV, a change of approximately 7 mV, with an increase in cell input resistance from 136.5 MΩ to 176.6 MΩ, over the linear portion of the plot and showed a point of intersection at 87 mV. 5-HT washout returned the cell to its resting values of -65 mV and 139.4 MΩ.

This was accompanied by a decrease in cell input resistance from 163.2 MΩ to 108.9 MΩ. The I/V plots intersected at -81 mV. Following 5-HT washout the cell returned to its resting values of -62 mV and 150.1 MΩ. Pooled data showed a 5-HT (100 μM) evoked a hyperpolarisation from a resting membrane potential of -63.2 ± 2.8 mV to a peak of -73.1 ± 3.9 mV (n=10). This was accompanied by an input resistance decrease from 154.6 ± 35.4 MΩ to 98.7 ± 35.6 MΩ. The mean reversal potential was found to be -84 ± 3.2 mV, indicating the involvement of a potassium conductance.

WAY-100635 in the hippocampus

Following bath-application of 5-HT (100 μM) while in the presence of WAY-100635 (100 nM), six of eight cells showed a complete block of the 5-HT (100 μM) evoked hyperpolarisation. The group of six cells had a resting membrane potential of -61.3 ± 1.5 mV and input resistance of 147.3 ± 23.2 MΩ. WAY-100635 was applied to hippocampal at a range of concentrations, namely 100 μM, 10 μM and 100 nM Following pre-treatment with WAY-100635 at all the concentrations tested bath-application of 5-HT (100 μM) caused no change in the membrane potential at -62.3 ± 1.6 mV and in input resistance at 147.8 ± 20.8 MΩ.

WAY-100635 did not have any partial agonist activity; the same neurone as above showed a slight change from its resting membrane potential of -62 mV following a bath-application of WAY-100635 (100 nM) (Figure 3.10B). There was a slight change from a resting input resistance of 150.1 MΩ to 144.1 MΩ; this was measured over the linear portion of the I/V plot in the range -70 mV to -90 mV. WAY-100635 (100 nM) was found to have no partial agonist activity in seven cells when applied for up to 20 minutes. There was little change in the membrane potential from the resting membrane potential of -63.3 ± 1.6 mV to -61.5 ± 1.8 mV following the application of WAY-100635 at any of the drug concentrations. This was also true of the input resistance, with a control value of 156.3 ± 35.2 MΩ and 158.1 ± 24.9 MΩ when exposed to WAY-100635.

A 5-HT-evoked depolarisation in the hippocampus

In two of eight WAY-100635 (100 nM) treated cells there was a depolarisation following bath-application of 5-HT (100 μM). In one of these cells the membrane showed depolarisation from a resting membrane potential of -65 mV to -58 mV following the bath-application of 5-HT (100 μM), a change of 7 mV (Figure 3.10C). This was accompanied by an increase in cell input resistance from 136.5 MΩ to 176.6 MΩ, measured over the linear portion of the I/V plot in the range -70 mV to -85 mV. Control and 5-HT I/V plots intersected at -87 mV. Following 5-HT washout the cell returned to its resting values of -65 mV and 139.4 MΩ. When the data from both experiments was pooled there was a 5-HT (100 μM) evoked depolarisation in membrane potential from -64.5 ± 2.1 mV to -58.9 ± 1.8 mV with an accompanying increase in input resistance from 159.2 ± 50.5 MΩ to 201.6 ± 63.9 MΩ. The 5-HT-evoked depolarisation was accompanied by an increase in cell excitability observed as an increase in the firing rate of the neurones. The increase in input resistance indicates a closure of ion channels in the cell membrane. The point of response reversal (approximately -90 mV) indicates the involvement of potassium channels. This agrees with the work of Colino and Halliwell (1987) who concluded that a 5-HT-evoked depolarisation in the CA1 region of the hippocampus was due to a decrease in potassium conductance.

Summary

5-HT_{1A}-evoked hyperpolarisation in the DRN

The present study has found that the principal action of bath-application of 5-HT to the serotonergic neurones of the DRN is inhibitory. This inhibitory action of 5-HT is due to an evoked membrane hyperpolarisation, making the cell less likely to fire an action potential. This action is associated with a decrease in input resistance. Analysis of I/V plots showed that the response reversed at around -90 mV, the value expected for potassium mediated events and in agreement with other studies where the hyperpolarisation has been shown to be dependent on external potassium concentration (Aghajanian and Lakoski 1984). This indicates that the 5-HT-evoked hyperpolarisation is due to the opening of a potassium channel.

WAY-100635 in the DRN

The present study has shown, in agreement with current literature, that WAY-100635 blocks 5-HT_{1A}-mediated events in the DRN (Fletcher et al 1996). WAY-100635, following a 15 minute exposure, was found to block the 5-HT-evoked hyperpolarisation in all cells tested.

WAY-100635 Selectivity in the DRN

WAY-100635 did not block the depolarisation associated with bath-applied phenylephrine and noradrenaline suggesting that WAY-100635 has no α_1-adrenoceptor antagonistic activity. In both cases the membrane α_1-adrenorecptor – mediated depolarisation was accompanied with an increase in input resistance indicating the closure of ion channels in the cell membrane. Also in each case reversal values could be extrapolated and were both found to be approximately -90 mV, close to that predicted for potassium-mediated events. This is indicative of a membrane depolarisation that is mediated only by the closure of potassium channels.

Is WAY-100635 truly silent in the DRN?

When applied to the DRN neurone for up to 45 minutes WAY-100635 was found to have no effect on the resting membrane potential or input resistance indicating that it has no agonist activity at the 5-HT_{1A} receptor. This is in contrast to a number of compounds previously claimed to be "silent" antagonists, where partial agonist properties have been subsequently revealed (Meller et al 1990). However, while under the influence of WAY-100635 for longer time periods some cells did tend to show a membrane potential drift in a depolarising direction, leading to an increase in firing rate. This may be due to the blockade of the tonic inhibition exerted by endogenously released 5-HT in the DRN (Craven et al 1994; Forster et al 1995), or may be due to an excitatory action of endogenously released 5-HT. Alternatively, it could be that WAY-100635 is acting by blocking ion channels involved in maintaining the resting membrane potential.

A 5-HT-evoked depolarisation in the DRN

Blockade of the 5-HT_{1A}-evoked response with a long application (30-45 mins) of WAY-100635 revealed a slow 5-HT-evoked membrane depolarisation. The 5-HT-evoked depolarisation appears to be a separate phenomenon to the membrane depolarisation observed with the possible blockade of tonic inhibition with WAY-100635. The depolarisation closely follows the application of 5-HT and reverses with 5-HT wash out, making it appear that the application of 5-HT evokes the depolarisation.

The 5-HT-evoked depolarisation was observed in a large number of cells (n=29 of 35). In some cells the depolarisation was associated with a decrease in input resistance when under the influence of 5-HT. In some cells there appeared to be no net change in input resistance. I/V analysis showed that in some cells the 5-HT-evoked depolarisation reversed at approximately -50 mV (n=8) a value not associated with the potassium channel. The 5-HT-evoked hyperpolarisation in these cells reversed at approximately -90 mV, as consistent with a potassium-mediated event. The 5-HT-evoked depolarisation revealed by 5-HT_{1A} blockade in the DRN does not seem to be mediated by the closure of potassium channels. There is no reversal in the range from -120 mV to -70 mV and no significant increase in input resistance, so the 5-HT-evoked depolarisation it is not solely due to the closure of potassium channels and so is unlike the depolarisation that follows the application of phenylephrine and noradrenaline.

TTX and the 5-HT-evoked depolarisation in the DRN

The 5-HT-evoked depolarisation could be demonstrated in the presence of TTX, a known sodium channel blocker. This indicates that the response is independent of the action potential dependent synaptic events involved in networks and so must be postsynaptic. 5-HT binding to the target cell changes the activity of that cell by causing a membrane depolarisation leading to an increase in neuronal excitability. This data points to the 5-HT-evoked depolarisation not being dependent on network connections. This means that 5-HT can evoke a depolarisation in serotonergic cells independent of inputs from other neurones.

Non-5-HT_{1A}-evoked hyperpolarisation in the DRN

In the DRN there was not only a 5-HT-evoked depolarisation revealed with WAY-100635 but also a 5-HT-evoked hyperpolarisation in the presence of the compounds mCPP and methysergide. The continued application of WAY-100635 together with methysergide in one cell and mCPP in three cells revealed a non-5-HT_{1A}-evoked membrane hyperpolarisation. This hyperpolarisation was associated with a decrease in input resistance, indicating the opening of ion channels. The response was observed to reverse at approximately -90 mV, indicating the involvement of potassium channels. The hyperpolarisation was also associated with a decrease in excitability. This observation could reflect the electrophysiological activity of one of the other 5-HT receptors described in the DRN.

5-HT_{1A}-evoked hyperpolarisation in the hippocampus

The present study has shown that 5-HT-evokes a hyperpolarisation associated with a decrease in input resistance and I/V analysis showed the response to reverse at approximately the value that would be expected for a potassium mediated event. The decrease in input resistance also indicates the opening of channels. This is typical of the 5-HT_{1A}-mediated hyperpolarisation well described in the hippocampus (Andrade and Nicoll 1987).

WAY-100635 in the hippocampus

WAY-100635 was shown to block the 5-HT_{1A}-mediated hyperpolarisation. This is also consistent with that observed in the present study in the DRN. Also, WAY-100635 blocked the 5-HT_{1A}-mediated hyperpolarisation at the same concentration (100 nM) and with the same exposure time (15 mins) in the DRN and the hippocampus. As with other studies WAY-100635 was found to have no partial agonist activity in the hippocampus (Fletcher et al 1996).

5-HT-evoked depolarisation in the hippocampus

Following the blockade of the 5-HT_{1A}-mediated hyperpolarisation by WAY-100635 a 5-HT-evoked depolarisation was revealed. The 5-HT-evoked depolarisation was

associated with an increase in input resistance and I/V analysis showed a reversal value in the region of that expected for a potassium mediated event. This has been previously reported in the hippocampus and is thought to be due to the closure of potassium channels. The characteristics of the hippocampal 5-HT-evoked depolarisation appear to be different to that observed in the DRN. The I/V data suggests the hippocampal 5-HT-evoked depolarisation is due to the closure of potassium channels, however, the I/V data from the DRN is not consistent with this mechanism.

CHAPTER 4

A 5-HT-evoked depolarisation in the DRN

Introduction

Serotonergic neurones of the DRN respond to exogenously applied 5-HT by a membrane hyperpolarisation and a decrease in input resistance (Aghajanian and VanderMaelen 1982b) this is mediated by the 5-HT_{1A} autoreceptor (Sprouse and Aghajanian 1987) the activation of which leads to the inhibition of cell firing. However, there are known to be a number of 5-HT receptors other than the 5-HT_{1A} receptor found in the DRN. It is also known that throughout the CNS a multiplicity of 5-HT-evoked actions can coexist within the same neurone. For example, in the medial pontine reticular formation 5-HT acts on 5-HT_1 and 5-HT_2 receptors evoking a membrane hyperpolarisation and depolarisation respectively, due to opposing actions on different potassium conductances (Stevens et al 1992). 5-HT-evoked effects with similar characteristics have also been observed in the nucleus prepositus hypoglossi (Bobker and Williams 1995) and the cerebrocortical neurones (Araneda and Andrade 1991; Davies et al 1987). In the hippocampus the 5-HT_{1A} receptor mediates the hyperpolarisation but the depolarisation is mediated by 5-HT_4-like receptors (Andrade and Nicoll 1987; Andrade and Chaput 1991), these effects are due to opposing actions on the same potassium conductance. The present study has used the 5-HT_{1A} antagonist WAY-100635 to look for similar multiple actions of 5-HT coexisting within the DRN. This chapter will present evidence for the receptor classification of the 5-HT-evoked depolarisation in the DRN.

Results

A 5-HT-evoked depolarisation in the DRN

Following bath-application of 5-HT (100 μM) a typical cell hyperpolarised from a resting membrane potential of -61 mV to -73 mV, a change of 12 mV (Figure 4.0 Ai, Bi). This was accompanied by a decrease in input resistance from a resting value of 292.2 MΩ to 121.7 MΩ with 5-HT. The control and 5-HT I/V relationships intersected at -88 mV. Following pre-application of WAY-100635 (100 nM), bath-application of 5-HT (100 μM) evoked a membrane depolarisation from a resting value of -61 mV to -57 mV (Figure 4.0 Aii, Bii). This was accompanied by a slight decrease in input resistance from 298.2 MΩ to 292.7 MΩ. Following the identification of the 5-HT-evoked depolarisation a pharmacological characterisation was performed.

A Pharmacological Characterisation

To characterise the receptor involved in the 5-HT-evoked depolarisation in the DRN the effects of different agonists and antagonists were investigated in the presence of WAY-100635.

DOI mimics the depolarising action of 5-HT

The 5-HT_2 receptor subtypes are commonly associated with 5-HT-evoked depolarisation and so made a sensible starting point for investigation. The 5-HT_2 agonist DOI (10 μM) evoked a depolarisation with similar properties to that of the 5-HT-evoked depolarisation. In a typical WAY-100635 (100 nM) treated cell, 5-HT (100 μM) evoked a depolarisation from a resting membrane potential of -63 mV to -59 mV, a decrease of 4 mV (Figure 4.1Ai, Bi). This was accompanied by a decrease in cell input resistance from 235.1 MΩ to 222.7 MΩ. In the same neurone a subsequent application of DOI (10 μM) depolarised the cell from -63 mV to -59 mV, which was accompanied by a slight decrease in input resistance from 233.2 MΩ to 225.9 MΩ (Figure 4.1 Aii, Bii). Pooled data gave an average DOI (10 μM) evoked depolarisation from a resting membrane potential of -62.4 ± 2.4

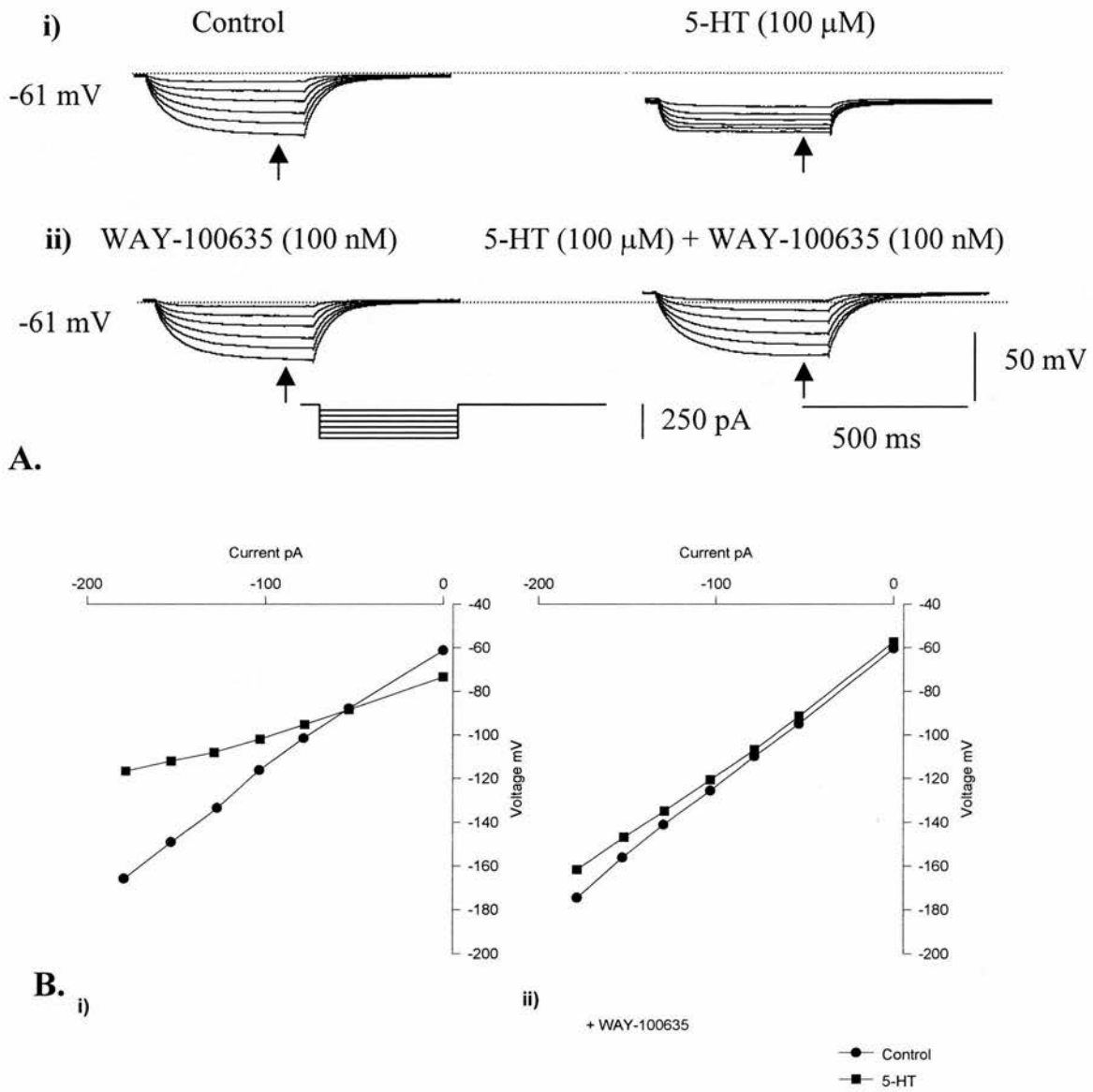


Figure 4.0. Different actions of 5-HT in the DRN.

A. Voltage traces made in response to injected current pulses of 500 ms and increments of 50 pA of a cell before and after the application of 5-HT (100 μM).

i) 5-HT (100 μM) evoked a hyperpolarisation.

ii) 5-HT (100 μM) evoked a depolarisation followingWAY-100635 (100 nM) treated.

B. Current-voltage relationships were obtained by measuring at the point ↑ after injecting a series of depolarising and hyperpolarising current pulses into the cell. The cell had a resting membrane potential of -61 mV and an input resistance of 292.2 MΩ.

i) Following bath-application of 5-HT (100 μM) the cell hyperpolarised to -73 mV. This was accompanied by a decrease in input resistance to 121.7 MΩ. The control and 5-HT I/V relationships intersected at -88 mV.

ii) Following pre-application of WAY-100635 (100nM), bath-application of 5-HT evoked a membrane depolarisation to -57 mV. This was accompanied by a slight change in input resistance from 298.2 MΩ to 292.7 MΩ.

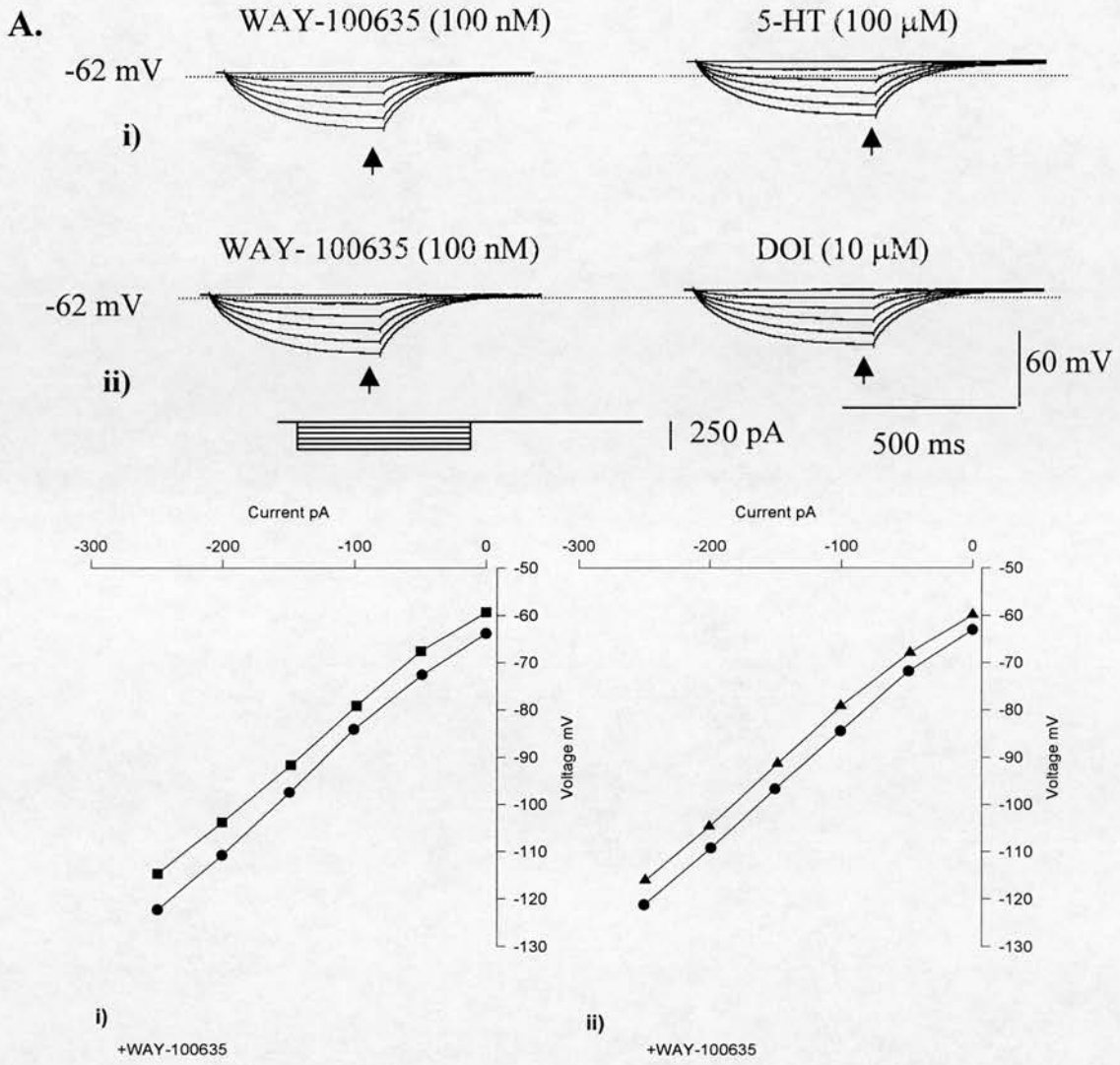


Figure 4.1. DOI evoked a depolarisation with WAY-100635 in the DRN.
A. Voltage traces of cells in response to injected current pulses of 500 ms duration and increments of 50 pA.
i) A depolarisation with 5-HT (100 μM).
ii) A depolarisation with DOI (10 μM).
B. Current-voltage relationships were obtained by measuring at the point ↑ after injecting a series of depolarising and hyperpolarising current pulses in the cell. I/V plots obtained from a DRN neurone before (circles) and after (squares) bath-application of 5-HT (100 μM).
i) The DR neurone depolarised from a resting membrane potential of -63 mV to -59 mV. This was accompanied by a decrease in cell input resistance from 235.1 MΩ to 222.7 MΩ.
ii) I/V plots obtained from the same DR neurone before (circles) and after (triangles) bath-application of DOI (10 μM) while in the presence of WAY-100635. The DRN neurone depolarised from -63 mV to -59 mV following an application of DOI (10 μM). This was accompanied by a decrease in input resistance from 233.2 MΩ to 225.9 MΩ.

mV to -59.1 ± 3.2 mV, which was accompanied by a decrease in input resistance from the resting level of 267.3 ± 10.3 MΩ to 241.2 ± 15.2 MΩ (n=3). Statistical analysis with the t-test showed that DOI caused a significant membrane depolarisation to a confidence interval of 95%.

mCPP did not evoke a depolarisation

The 5-$HT_{2B/2C}$ partial agonist mCPP (100 μM) did not evoke any response in WAY-100635 (100 nM) treated cells at the concentration tested. In a typical neurone pre-treated with WAY-100635 (100 nM) bath-application of 5-HT (100 μM) evoked a depolarisation from a resting membrane potential of -60 mV to -56 mV, a change of 4 mV (Figure 4.2A). This was accompanied by a slight increase in cell input resistance from 165.8 MΩ to 172.7 MΩ. Application of mCPP (100 μM) to the same cell in the presence of WAY-100635 (100nM) did not evoke a change in membrane potential from its resting value. There was, however, a slight decrease in cell input resistance from 167.8 MΩ to 157.2 MΩ (Figure 4.2B). Averaged data for a group of cells, that demonstrated a 5-HT (100 μM) evoked depolarisation with WAY-100635 (100 nM) treatment, showed no change with mCPP (100 μM). At rest the neurones had a mean membrane potential of -60.1 ± 2.1 mV and -61.5 ± 1.9 mV while in the presence of mCPP (n=3, 100 μM).

CP 93129 did not evoke a depolarisation

The 5-HT_{1B} agonist CP 93129 was administered to a cell with a resting membrane potential of -64 mV and an input resistance of 344.2 MΩ in the presence of WAY-100635 (100 nM). The cell showed a 5-HT (100 μM) evoked depolarisation in the presence of WAY-100635 (100 nM) to -59 mV and 303.9 MΩ (Figure 4.3A). Following bath-application of CP 93129 (10 μM) the cell showed no change with a membrane potential of -63 mV and an input resistance of 344.4 MΩ (Figure 4.3B). Pooled data (n=3) also showed CP 93129 had no effect on resting membrane potential from -61.1 ± 3.1 mV at rest to -62.2 ± 1.1 mV with CP 93129 (100 μM).

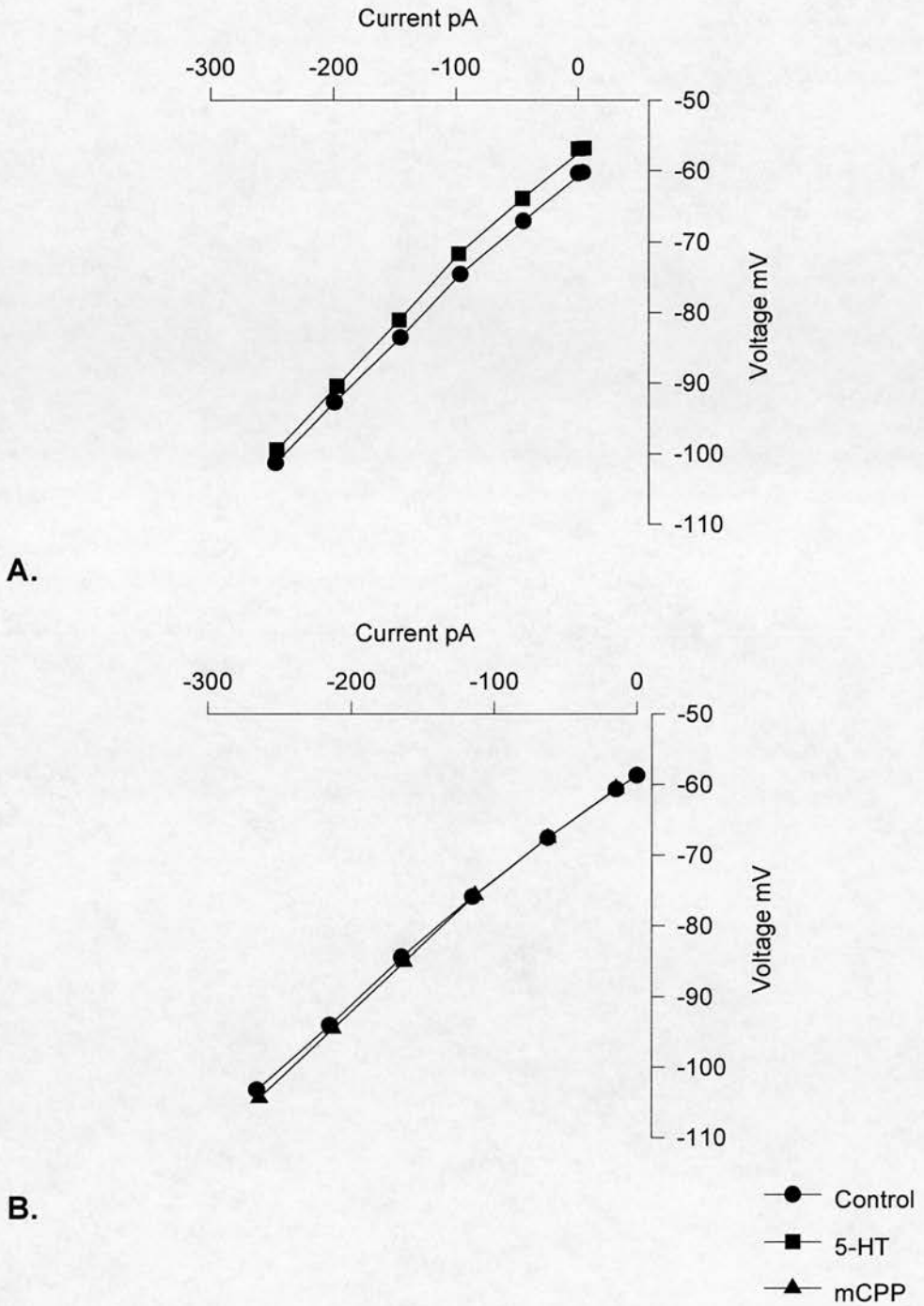


Figure 4.2. mCPP with WAY-100635 did not change a DRN neurone.
A. I/V plots obtained from a DRN neurone before (circles) and after (squares) bath-application of 5-HT (100 μM). The DRN neurone depolarised from a resting membrane potential of -60 mV to -56 mV, a change of approximately 4 mV. This was accompanied by an increase in cell input resistance from 165.8 MΩ to 172.7 MΩ.
B. I/V plots obtained from the same DRN neurone before (circles) and after (squares) bath-application of mCPP (100 μM) while in the presence of WAY-100635. The DRN neurone did not change its membrane potential, -58 mV at rest and -58 mV with mCPP. There was, however, a slight decrease in cell input resistance from 167.8 MΩ to 157.2 MΩ.

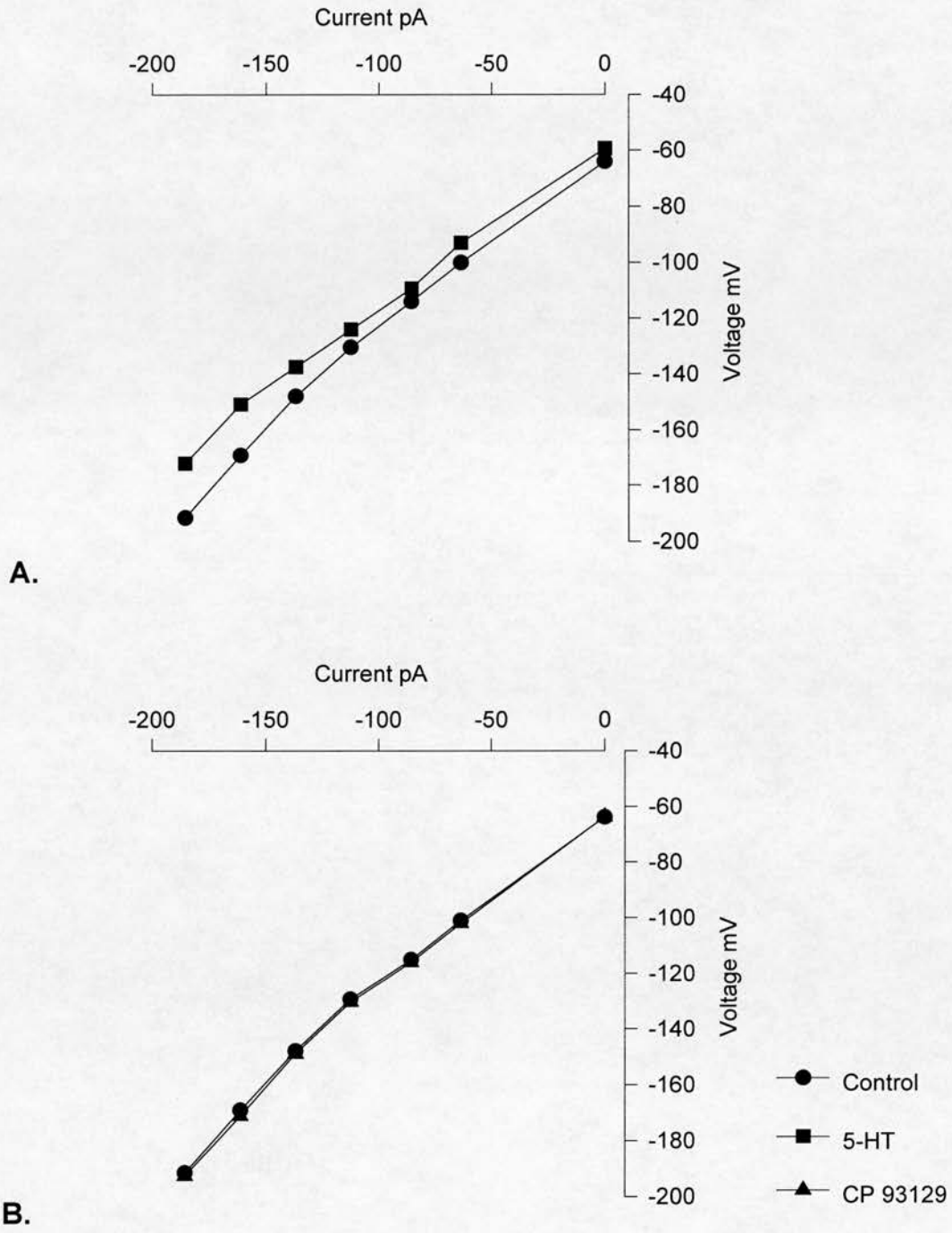


Figure 4.3. CP 93129 with WAY-100635 did not change a DRN neurone.
A. I/V plots from a WAY-100635 treated cell (100 nM) with a resting membrane potential of -64 mV and an input resistance of 344.2 MΩ (circles) shows a 5-HT (100 μM) evoked depolarisation to -59 mV and input resistance of 303.2 MΩ (squares).
B. In the same cell in the continued presence of WAY-100635, bath-application of CP 93129 (10 μM) the cell, showed no change with a membrane potential of -63 mV and an input resistance of 344.4 MΩ (squares).

Methysergide blocked the 5-HT-evoked depolarisation

To further characterise the receptor involved in the 5-HT-evoked depolarisation antagonists were used to block the response. Application of the broad spectrum 5-HT antagonist methysergide (100 μM) blocked the depolarisation in five cells confirming the serotonergic nature of the depolarisation. In a typical cell exposed to WAY-100635 (100 nM) 5-HT (100 μM) evoked a depolarisation from a resting membrane potential of -65 mV to -61 mV, a change of approximately 4 mV (Figure 4.4A). This was accompanied by a decrease in cell input resistance from 323.4 MΩ to 264.4 MΩ. Following application of methysergide (100 μM) to the same WAY-100635 (100 nM) treated cell the depolarisation was blocked (Figure 4.4B).

Bath-application of methysergide (n=8 of 8) (100 μM) had no effect on membrane potentials from -64 ± 2.6 mV at rest to -65 ± 1.9 mV with methysergide, a slight input resistance increase was observed from resting a value of 313.1 ± 1.6 MΩ to 320.1 ± 2.6 MΩ. Prior to methysergide application 5-HT (100 μM) evoked a depolarisation in the presence of WAY-100635 (100 nM) from a resting value of -63.6 ± 2.2 mV to -59.9 ± 1.6 mV during 5-HT (100 μM) application (n=8 of 8), a significant depolarisation when statistically analysed with the t-test. There was an associated input resistance decrease from 279.6 ± 61.7 MΩ to 234.6 ± 52.7 MΩ. Pooled data from methysergide (100 μM) treated cells showed a blockade of the effects of 5-HT (100 μM) with a slight membrane potential change from -63.6 ± 0.6 mV at rest to -62.9 ± 1.4 mV with 5-HT. The associated input resistance change was also blocked, from 300.8 ± 88.1 MΩ at rest to 296.6 ± 84.5 MΩ with 5-HT (n=6 of 8). Statistical analysis showed, to a confidence interval of 95%, that 5-HT no longer significantly changed the membrane potential in cells treated with methysergide.

The membrane potential and input resistance changes during the course of a typical experiment are displayed in Figure 4.5. This figure first shows the action of bath-application of 5-HT (100 μM) with the characteristic membrane hyperpolarisation and associated decrease in input resistance of the 5-HT_{1A}-evoked hyperpolarisation.

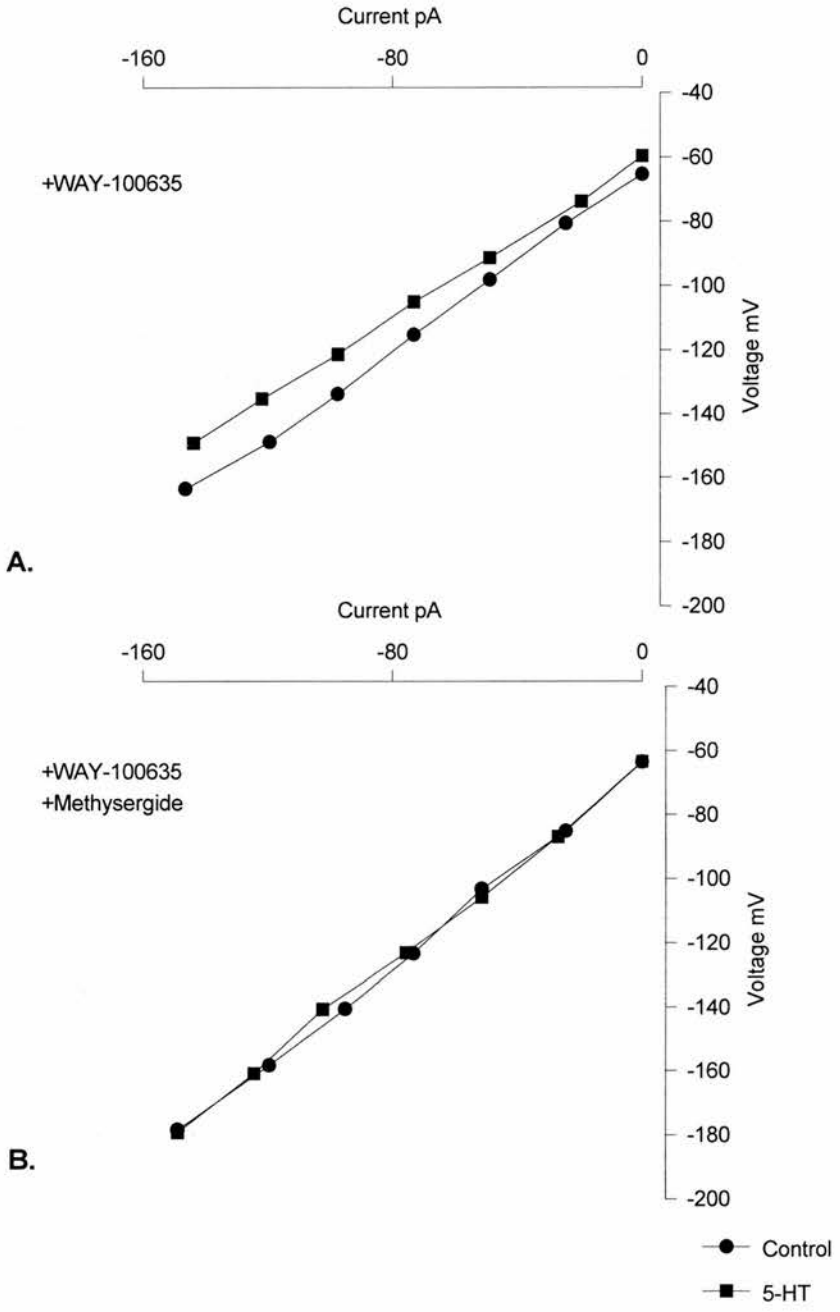


Figure 4.4. Methysergide blocked the 5-HT-evoked depolarisation in the DRN.
A. I/V plots obtained from a DR neurone before (circles) and after (squares) bath-application of 5-HT (100 μM). The DR neurone depolarised from a resting membrane potential of -65 mV to -61 mV, a change of approximately 4 mV. This was accompanied by a decrease in cell input resistance from 323.4 MΩ to 264.4 MΩ.
B. Application of methysergide (100 μM) while in the presence of WAY-100635 completely abolished the depolarisation, from a resting membrane potential, -63 mV at rest and -63 mV with 5-HT, there was also with no change in cell input resistance from 385.9 MΩ to 398.3 MΩ.

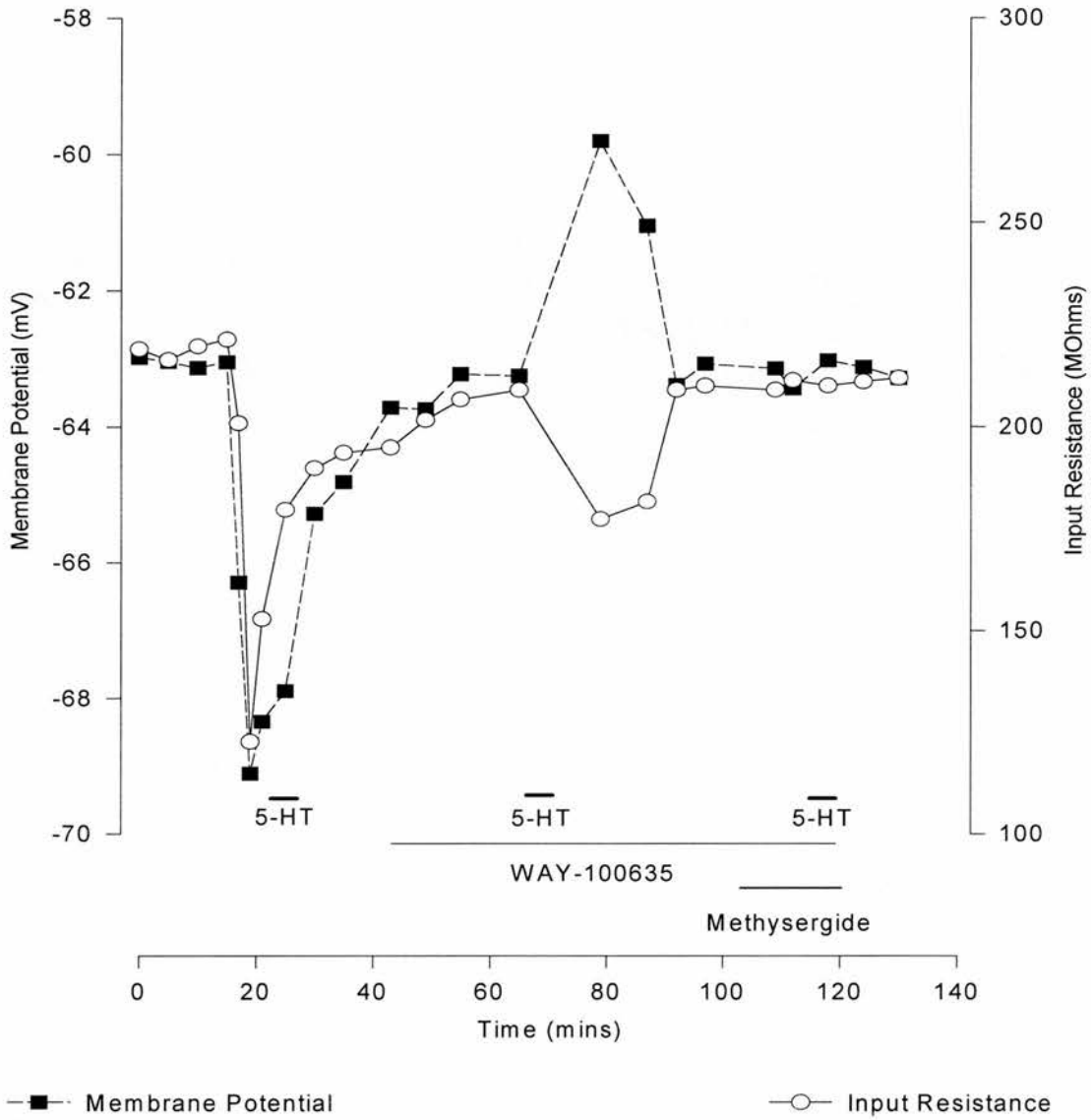


Figure 4.5. Changes in a DRN neurone with three 5-HT applications.
A typical cell showed a demonstrated a 5-HT_{1A}-mediated hyperpolarisation with bath-application of 5-HT (100 μM), it hyperpolarised from -63 mV to -69 mV, with an associated decrease in input resistance, from 219.9 MΩ to 122.2 MΩ. The cell recovered to resting values following 5-HT washout. The addition of WAY-100635 (100 nM) to the bath aCSF had no effect on membrane potential and input resistance. A subsequent application of 5-HT (100 μM) while in the presence of WAY-100635 (100 nM) no longer evoked a hyperpolarisation due to the blockade of the 5-HT_{1A} receptor by WAY-100635. The blockade with WAY-100635 revealed a 5-HT (100 μM) evoked a membrane depolarisation, from -63 mV to -59 mV, with a decrease in input resistance, from 209.6 MΩ to 177.4 MΩ. Methysergide (100 μM) was then bath-applied to the cell and was found to have no intrinsic activity in WAY-100635 (100 nM) treated cells. While in the presence of WAY-100635 (100 nM) and methysergide (100 μM) bath-application of 5-HT (100 μM) had no effect on the membrane potential, from -63 mV at rest to -63 mV with 5-HT. This was also observed in the input resistance from a value of 209.1 MΩ at rest to 210.6 MΩ with 5-HT. Methysergide blocked the 5-HT-evoked depolarisation.

The neurone changed in membrane potential from -63 mV to -69 mV and in input resistance from 219.9 MΩ to 122.2 MΩ while in the presence of 5-HT (100 μM) and showed recovery values with 5-HT wash out. The addition of WAY-100635 (100 nM) to the bath-aCSF is shown to have no effect on membrane potential and input resistance. A subsequent bath-application of 5-HT (100 μM), in the continued presence of WAY-100635 (100 nM), no longer showed the 5-HT_{1A}- evoked hyperpolarisation due to the blockade of the 5-HT_{1A} receptor by WAY-100635. The blockade with WAY-100635 (100 nM) revealed a 5-HT (100 μM) evoked membrane depolarisation, from -63 mV to -59 mV, and decrease in input resistance, from 209.6 MΩ to 177.4 MΩ. The analysis of the I/V data showed the 5-HT-evoked depolarisation did not reverse in the range -120 mV to -60 mV (for an example see Figure 4.0B). Methysergide (100 μM) was then bath-applied to the cell and was found to have no intrinsic activity in WAY-100635 (100 nM) treated cells. While in the presence of WAY-100635 (100 nM) and methysergide (100 μM) an application of 5-HT (100 μM) had no effect on the membrane potential. Methysergide blocked the 5-HT-evoked depolarisation.

Methysergide, however, did not block the depolarisation in another two cells at lower drug concentrations. In a typical cell from this group a control application of 5-HT (100 μM) evoked a depolarisation in the presence of WAY-100635 (100 nM) from a resting membrane potential of -61 mV to -58 mV, a change of approximately 3 mV (Figure 4.6A). This was accompanied by a decrease in cell input resistance from 306.4 MΩ to 280.3 MΩ. Following the application of methysergide (30 μM) in the continued presence of WAY-100635 (100 nM) 5-HT (100 μM) could still evoke a depolarisation from -64 mV to -60 mV; this was accompanied by a decrease in input resistance from 306.4 MΩ to 280.3 MΩ (Figure 4.6B).

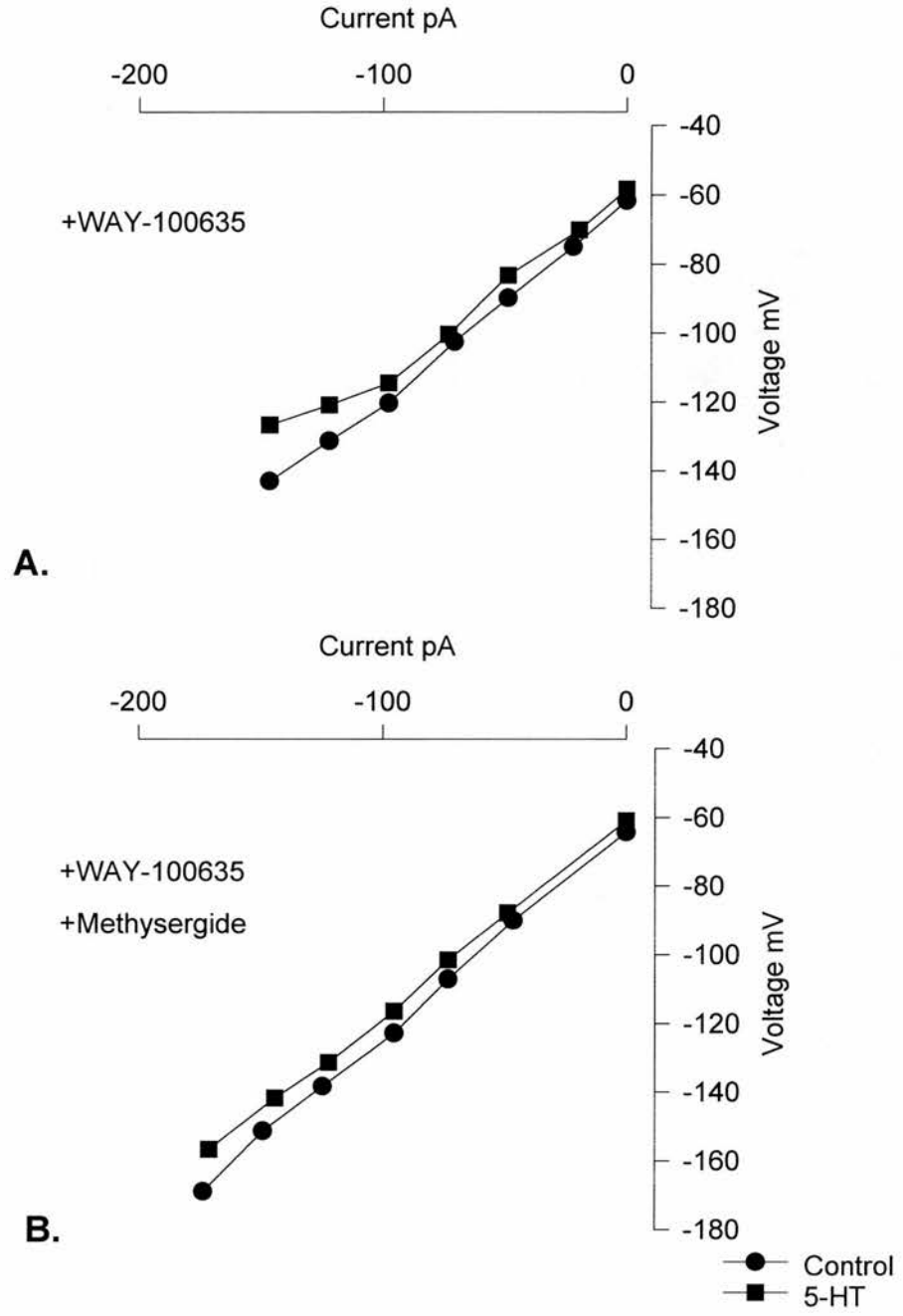


Figure 4.6. Methysergide did not block the 5-HT-evoked depolarisation at lower concentrations.

A. I/V plots obtained from a DR neurone before (circles) and after (squares) bath-application of 5-HT (100 μM). The DR neurone depolarised from a resting membrane potential of -61 mV to -58 mV, a change of approximately 3 mV. This was accompanied by a decrease in cell input resistance from 306.4 MΩ to 280.3 MΩ.
B. Following bath-application of methysergide (30 μM) 5-HT could still evoke a depolarisation from -64 mV to -60 mV; this was accompanied by a decrease in input resistance from 306.4 MΩ to 280.3 MΩ.

Methysergide blocked the DOI-evoked depolarisation

Methysergide also blocked the depolarising action of DOI (n=2). A cell that had already shown a 5-HT (100 μM) evoked depolarisation in the presence of WAY-100635 (100 nM) was treated with a bath-application of DOI (10 μM) and a depolarisation was evoked, this was performed in the continued presence of WAY-100635 (100 nM). The neurone depolarised from a resting membrane potential of -68 mV to -63 mV with DOI, a change of approximately 5 mV. This was accompanied by a decrease in cell input resistance from 196.2 MΩ to 128.1 MΩ (Figure 4.7A). Application of methysergide (100 μM) completely abolished the DOI (10 μM) evoked depolarisation, with the membrane potential remaining unchanged at -67 mV during DOI (10 μM) application in the continued presence of WAY-100635 (100 nM). There was also little change in cell input resistance from 184.4 MΩ to 192.1 MΩ (Figure 4.7B).

Ketanserin blocked the 5-HT-evoked depolarisation

Application of the non-specific 5-HT_2 receptor antagonist ketanserin (100 nM, n=3) blocked the 5-HT-evoked depolarisation. A WAY-100635 (100 nM) treated cell from this group depolarised in response to control application of 5-HT (100 μM) from a resting membrane potential of -68 mV to -64 mV, a change of approximately 4 mV. This was accompanied by a decrease in cell input resistance from 257.5 MΩ to 246.6 MΩ (Figure 4.8A). In the same cell the 5-HT (100 μM) evoked depolarisation was blocked following the addition of ketanserin (100 nM) to the bath-aCSF (Figure 4.8B). Figure 4.8B also shows that the I/V plots of the 5-HT-evoked depolarisation reverse at approximately -50 mV. Pooled data showed that in the presence of WAY-100635 (100 nM) the cells depolarised on average from -67 ± 1.1 mV to -64 ± 2.1 mV and showed an input resistance change with 5-HT (100 μM) from 260.1 ± 36.5 MΩ to 245.3 ± 23.9 MΩ (n=3). The 5-HT-evoked depolarisation was blocked completely by ketanserin (100 nM) from resting values of -66 ± 2.1 mV and 255.1 ± 14.0 MΩ to -65 ± 2.4 mV and 258.1 ± 22.3 MΩ with 5-HT.

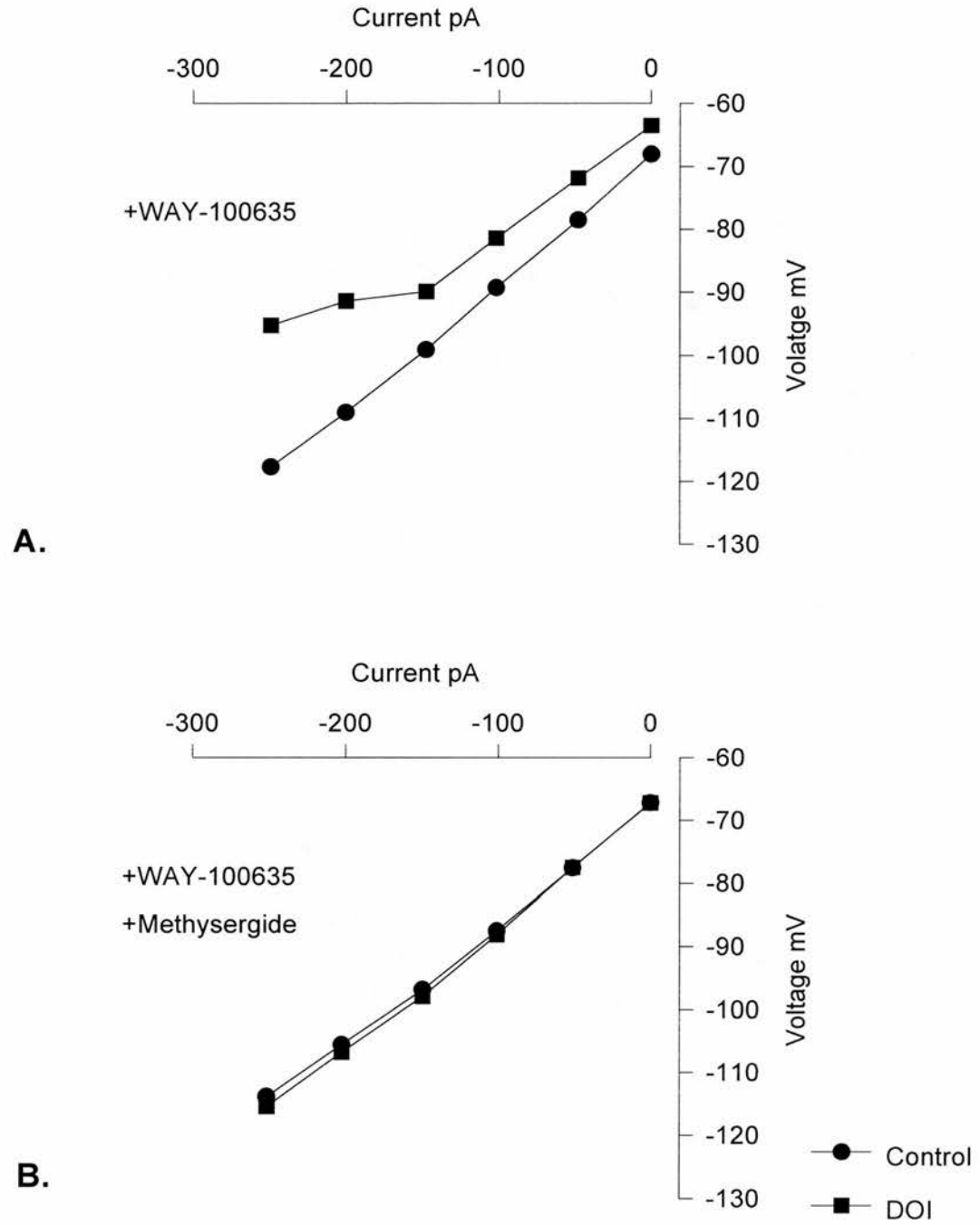


Figure 4.7. Methysergide blocked the DOI-evoked depolarisation in the DRN.
A. I/V plots obtained from a DR neurone before (circles) and after (squares) bath-application of DOI (10 μM). The DR neurone depolarised from a resting membrane potential of -68 mV to -63 mV, a change of approximately 5 mV. This was accompanied by a decrease in cell input resistance from 196.2 MΩ to 128.1 MΩ.
B. Application of methysergide (100 μM) completely abolished the depolarisation, with the membrane potential staying constant at -67 mV before and during DOI (10 μM) application, there was also little change in cell input resistance from 184.4 MΩ to 192.1 MΩ.

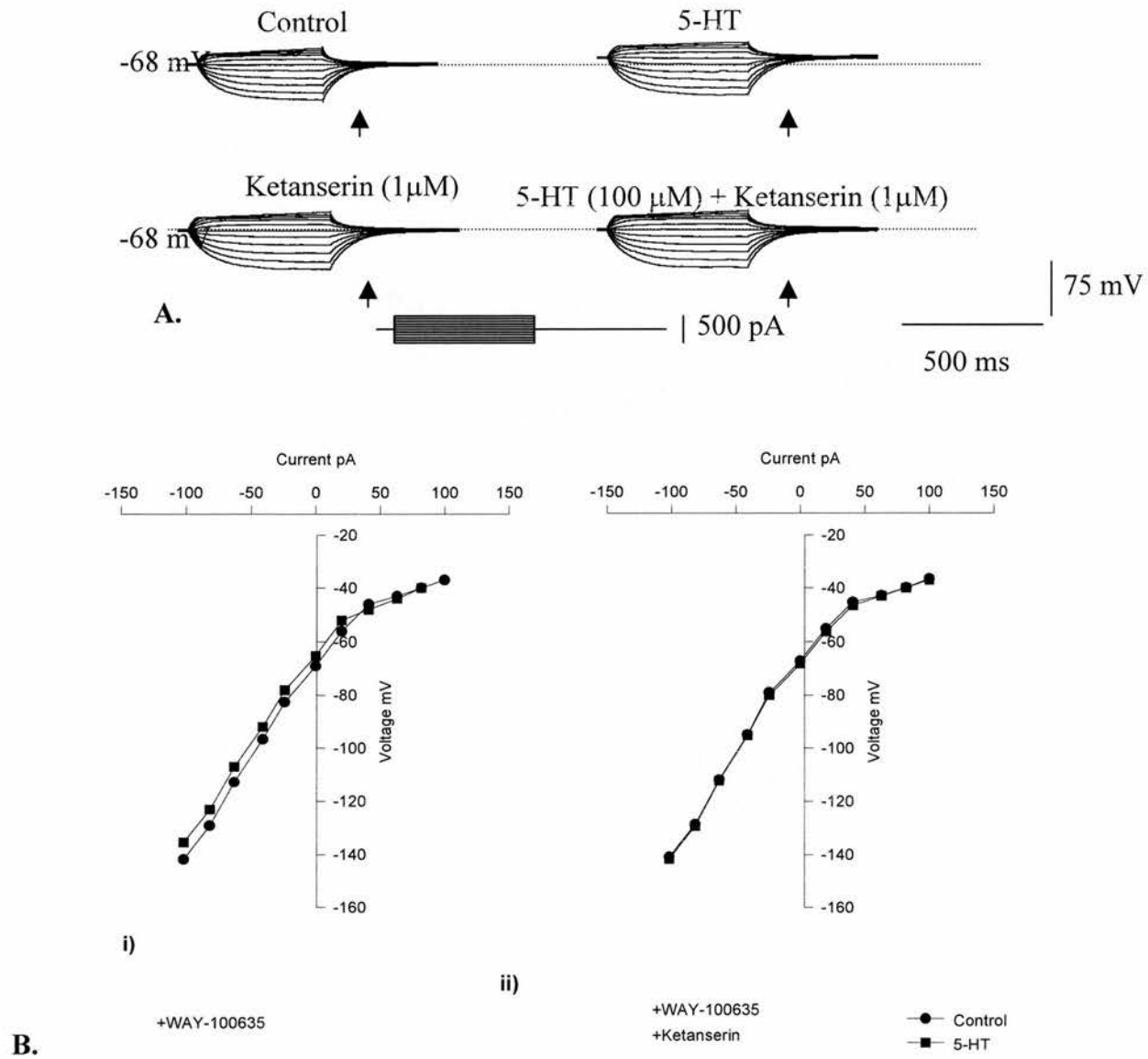


Figure 4.8. Ketanserin blocked the 5-HT-evoked depolarisation in a DRN cell.
A. Voltage traces in response to injected current pulses of 500 ms and increments of 25 pA inWAY-100635 treated cells (100 nM).
i) A 5-HT (100 μM) evoked depolarisation.
ii) Ketanserin (100 nM) blocked the 5-HT (100 μM) evoked depolarisation.
B. Current-voltage relationships were obtained by measuring at the point ↑ after injecting a series of depolarising and hyperpolarising current pulses in the cell. The cell had a resting membrane potential of -69 mV and an input resistance of 257.5 MΩ.
i) I/V plots obtained from a DR neurone before (circles) and after (squares) bath-application of 5-HT (100 μM). The DR neurone depolarised to -63 mV. This was accompanied by a decrease in cell input resistance to 246.6 MΩ.
ii) The 5-HT-evoked depolarisation was then blocked following the addition of the 5-HT_2 antagonist ketanserin (100 nM) to the bath-aCSF.

SB 200646 partially blocked the 5-HT-evoked depolarisation

The 5-$HT_{2B/2C}$ receptor antagonist SB 200646 (1 mM, n=3) partially blocked the 5-HT-evoked depolarisation. This group of cells is typified by the WAY-100635 (100 nM) treated neurone illustrated in Figure 4.9 which shows a control depolarisation with 5-HT (100 μM) from a resting membrane potential of -65 mV to -62 mV, a change of 3 mV. This was accompanied by a decrease in cell input resistance from 152.1 MΩ to 134.3 MΩ. Following the application of SB 200646 (1 mM) 5-HT (100 μM) evoked a reduced depolarisation from -64 mV to -62 mV, a change of 2 mV, with a change in input resistance from 156.3 MΩ to 136.2 MΩ. Pooled data for a group of WAY-100635 (100 nM) treated cells showing a 5-HT (100 μM) evoked depolarisation from -63 ± 2.9 mV to -59 ± 1.9 mV, with an input resistance decrease from 165.1 ± 32.7 MΩ to 152 ± 42.6 MΩ (n=3). In the same cells exposed to SB 200646 (1 mM) in the continued presence of WAY-100635 (100 nM) a membrane potential depolarisation was observed with 5-HT (100 μM) from -63 ± 4.1 mV to -60 ±1.9 mV, with an associated input resistance decrease from 159 ± 38.8 MΩ to 142 ± 22.1 MΩ. This may show that SB 200646 can partially block the 5-HT-evoked depolarisation at the concentration tested.

MDL, 100 907 completely blocked the 5-HT-evoked depolarisation

The 5-HT_{2A} antagonist MDL 100, 907 completely blocked the 5-HT-evoked depolarisation (100 μM, n=3). A typical neurone from this group following pre-treatment with WAY-100635 (100 nM) showed a control depolarisation with 5-HT (100 μM) from a resting membrane potential of -66 mV to -62 mV, a change of 4 mV (Figure 4.10A). This was accompanied by a decrease in cell input resistance from 210.1 MΩ to 201.2 MΩ. Following bath-application of MDL 100, 907 (100 μM) while still in the presence of WAY-100635 (100 nM) a subsequent application of 5-HT (100 μM) did not evoke a depolarisation. The cell membrane potential remained unchanged at -66 mV with 5-HT. The input resistance also showed little change following incubation with MDL 100, 907, from 219.5 MΩ at rest to 222.9 MΩ with 5-HT (100 μM) (Figure 4.10A). This is a total block of the 5-HT-evoked

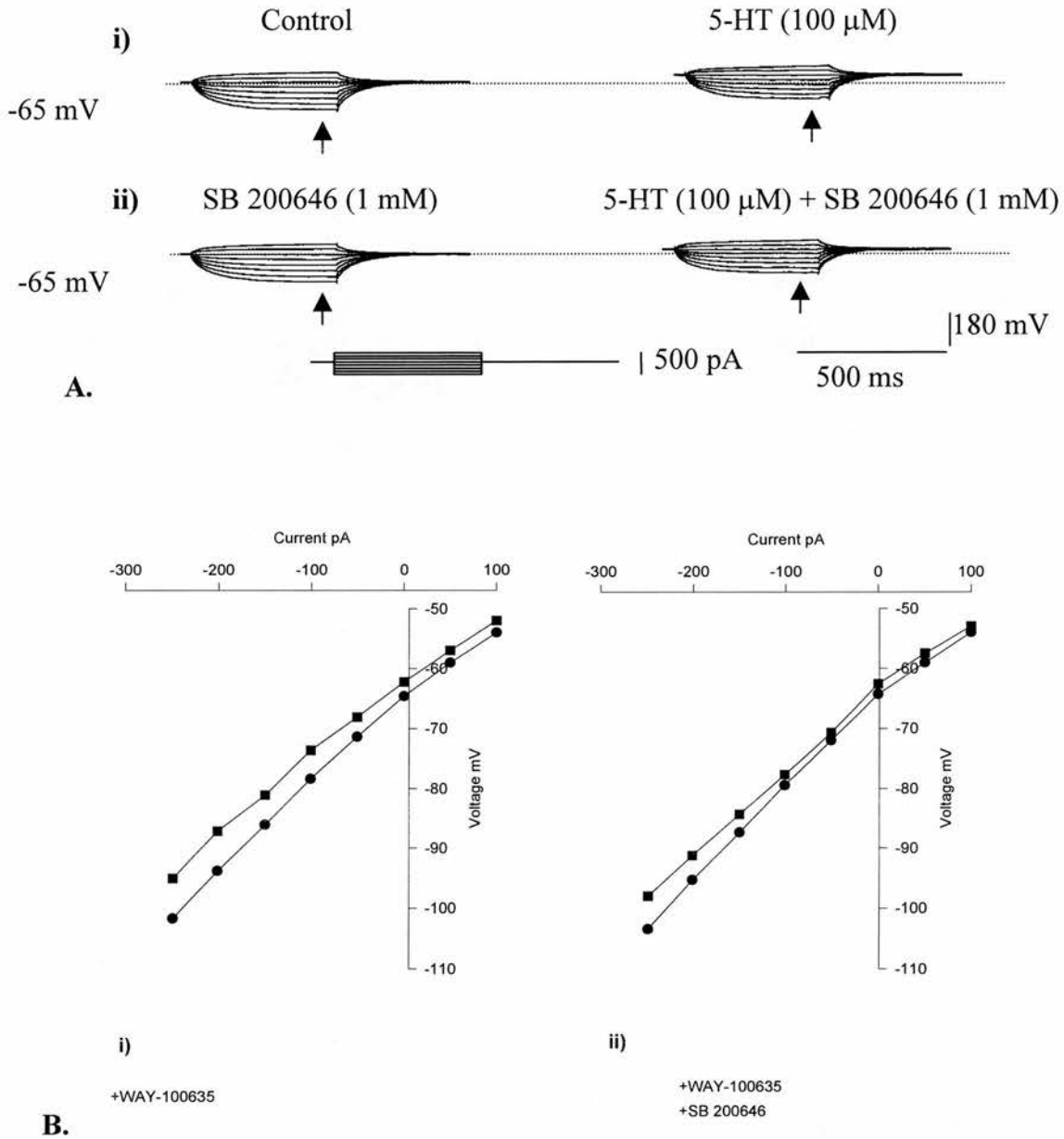


Figure 4.9. SB 200646 partially blocked the 5-HT-evoked depolarisation in a DRN cell.

A. Voltage traces in response to injected current pulses of 500 ms duration and increments of 50 pA in WAY-100635 (100 nM) treated cells.

i) A 5-HT (100 μM) evoked depolarisation.

ii) SB 200646 (1 mM) partially blocked the 5-HT (100 μM) evoked depolarisation.

B. Current-voltage relationships were obtained by measuring at point ↑ after injecting a series of depolarising and hyperpolarising current pulses into the cell. The cell had a resting membrane potential of -65 mV and resting input resistance of 153.4 MΩ.

i) I/V plots obtained from a DR neurone before (circles) and after (squares) bath-application of 5-HT (100 μM) show a depolarisation to -62 mV, accompanied by a decrease in cell input resistance to 131.8 MΩ.

ii) Following bath-application of SB 200646 (1 mM) 5-HT (100 μM) evoked a much reduced depolarisation to -62 mV, with a change in input resistance to 142.4 MΩ.

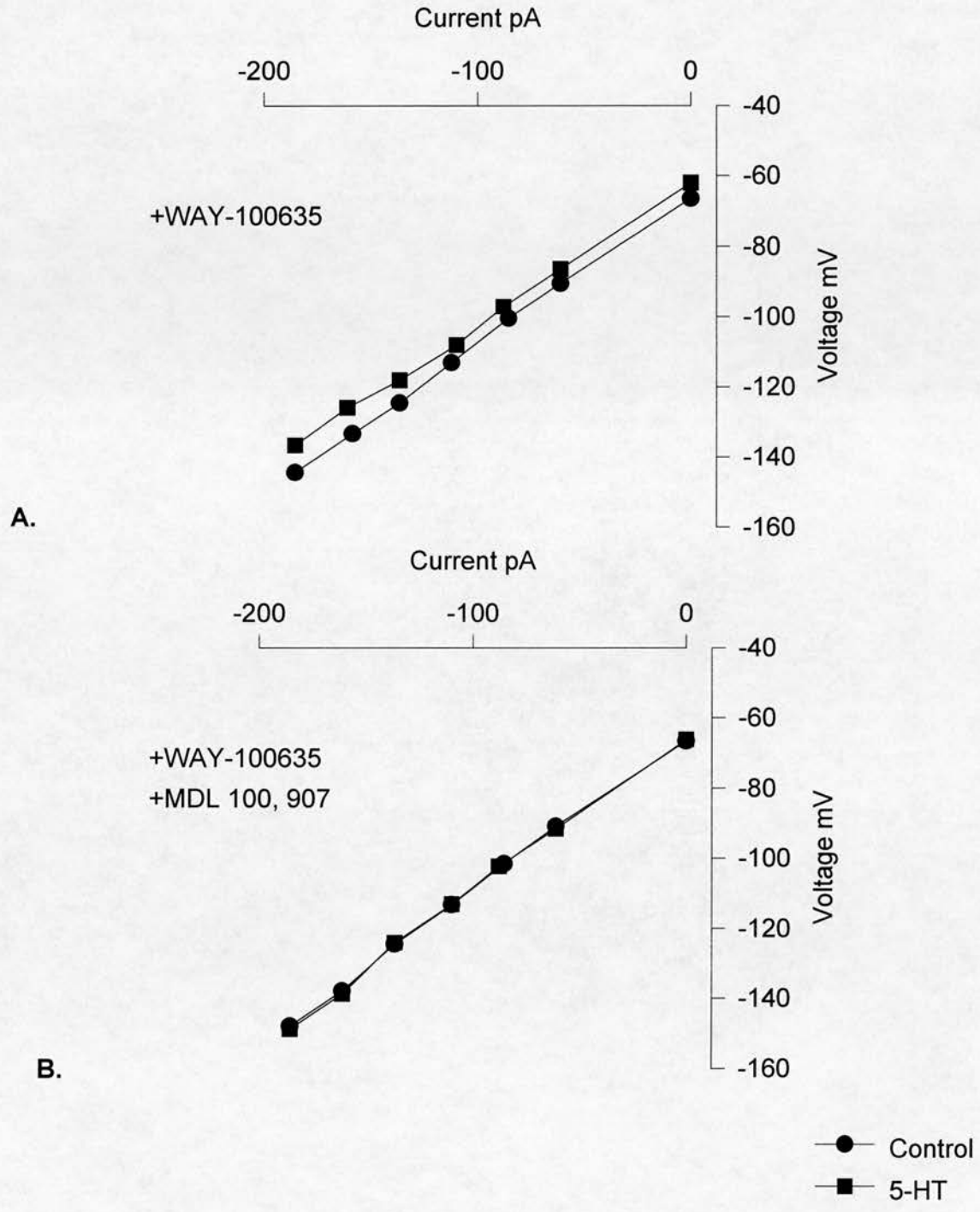


Figure 4.10. MDL 100, 907 blocked the 5-HT-evoked depolarisation in the DRN. **A.** I/V plots obtained from a DR neurone before (circles) and after (squares) bath-application of 5-HT (100 μM). The DR neurone depolarised from a resting membrane potential of -66 mV to -62 mV, a change of approximately 4 mV. This was accompanied by a decrease in cell input resistance from 210.1 MΩ to 201.2 MΩ. **B.** Application of MDL 100, 907 (100 μM) while in the presence of WAY-100635 completely abolished the depolarisation, from a membrane potential of -66 mV at rest and -66 mV with 5-HT (100 μM), there was also little change in cell input resistance from 219.5 MΩ to 222.9 MΩ.

depolarisation and is indicative of an effect mediated by the 5-HT_{2A} receptor. This was repeated in a further two cells with similar effects. Pooled data showed that with WAY-100635 (100 nM) cells depolarised in response to 5-HT (100 μM) from -65 ± 2.1 mV to -61 ± 1.2 mV and showed an input resistance change from 220.1 ± 26.3 MΩ at rest to 203.2 ± 29.1 MΩ with 5-HT. The 5-HT-evoked depolarisation was blocked completely by MDL 100, 907 (100 μM) from resting values of -65 ± 1.9 mV and 225.1 ± 22.9 MΩ to -64 ± 31.5 mV and 218.1 ± 28.1 MΩ with 5-HT (100 μM).

Pindolol did not block the 5-HT-evoked depolarisation

The 5-$HT_{1A/1B}$ antagonist pindolol (1 mM) failed to block the depolarisation (n=3) at the concentration tested. A typical neurone pre-treated with WAY-100635 (100 nM) depolarised with 5-HT (100 μM) from a resting membrane potential of -62 mV to -58 mV, a change of approximately 4 mV (Figure 4.11A). This was accompanied by a decrease in cell input resistance from 250.1 MΩ to 235.2 MΩ. Pooled showed that in the presence of WAY-100635 (100 nM) and pindolol (1 mM) the cell still depolarised following an application of 5-HT from -63 mV to -59 mV (Figure 4.11B). This was also accompanied by a decrease in input resistance from 239.1 MΩ to 226.5 MΩ. This was repeated in a further two cells with similar effects. In the presence of WAY-100635 (100 nM) the cells depolarised in response to 5-HT (100 μM) from -63 ± 1.1 mV to -59 ± 2.0 mV and showed an input resistance change from 249.1 ± 22.3 MΩ to 229.2 ± 41.5 MΩ with 5-HT. The 5-HT (100 μM) evoked depolarisation was not blocked by pindolol (1 mM) with the group showing a depolarisation from resting values of -62 ± 2.3 mV and 255.1 ± 34.1 MΩ to -62 ± 2.5 mV and 249.2 ± 36.4 MΩ with 5-HT.

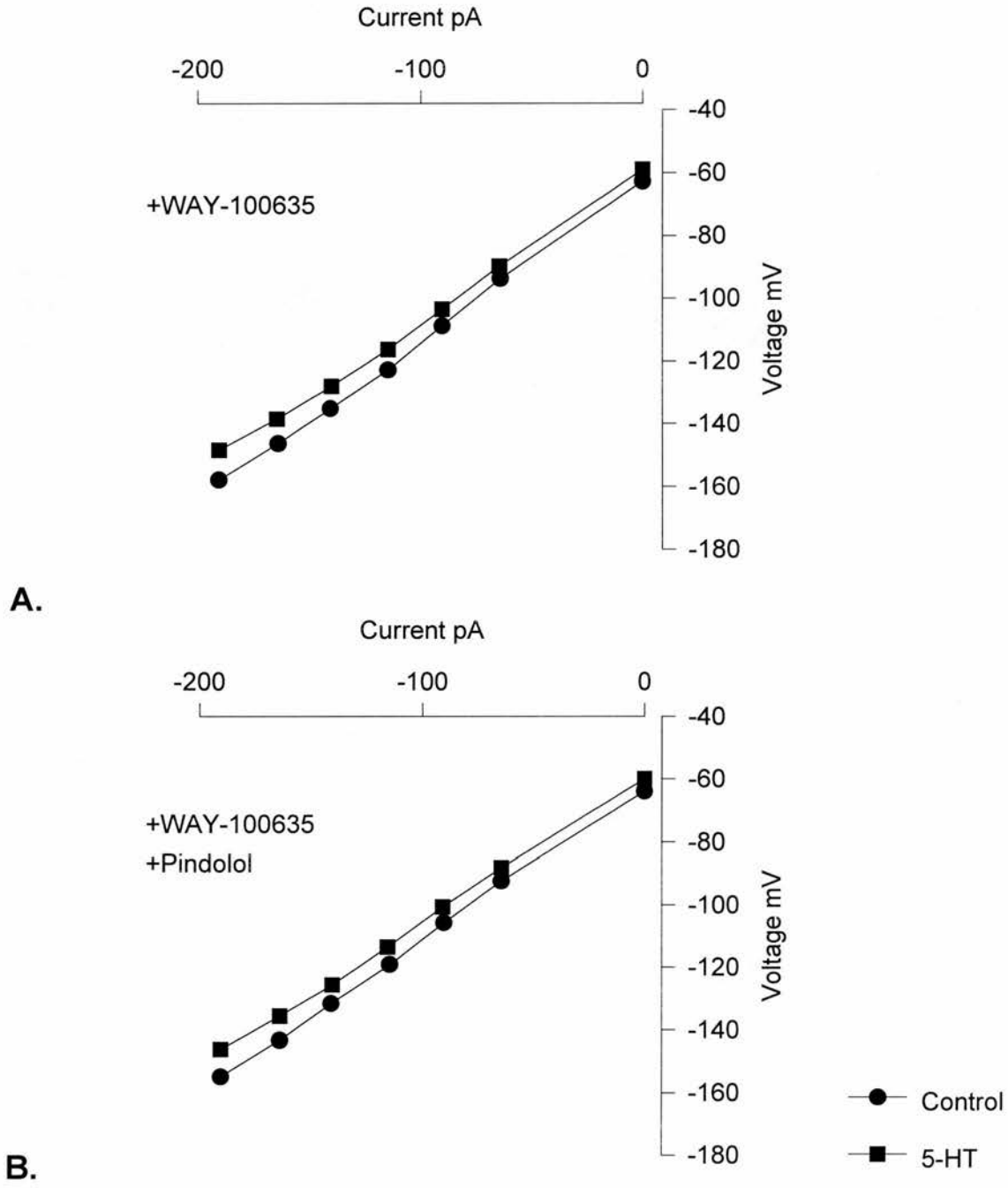


Figure 4.11. Pindolol did not block the 5-HT evoked depolarisation in the DRN.
A. I/V plots obtained from a DR neurone before (circles) and after (squares) bath-application of 5-HT (100 μM). The DR neurone depolarised from a resting membrane potential of -62 mV to -58 mV, a change of approximately 4 mV. This was accompanied by a decrease in cell input resistance from 250.1 MΩ to 235.2 MΩ.
B. In the presence of pindolol (1 mM) the cell still depolarised following an application of 5-HT (100 μM) from -63 mV to -59 mV. This was accompanied by a decrease in input resistance from 239.1 MΩ to 226.5 MΩ.

Summary

To discover which of the many 5-HT receptors was mediating the 5-HT-evoked depolarisation a number of agonists (Table 4.0) and antagonists (Table 4.1) were used. The agonists and antagonists were applied to WAY-100635 treated cells.

Agonists

Following the bath-application of DOI, selective for 5-HT_2 receptors, a membrane depolarisation was observed with the same characteristics as the 5-HT-evoked depolarisation. This points to the involvement of the 5-HT_2 receptor subtypes in the 5-HT-evoked depolarisation. Bath-application of mCPP, selective for the 5-$HT_{2B/2C}$ receptors, did not have any effect on membrane potential or input resistance in WAY-100635 treated cells at the concentration tested. This not only indicates that the 5-$HT_{2B/2C}$ receptor subtypes do not mediate the 5-HT-evoked depolarisation, but also that there is unlikely to be any electrophysiologically observable 5-$HT_{2B/2C}$ receptor mediated events in the DRN under the present experimental conditions.

Agonist	Site of Action	Effect on WAY-100635 treated cells
DOI (10 μM, n=3)	5-HT_2	Mimicked depolarisation
mCPP (100 μM, n=3)	5-$HT_{2B/2C}$	None
CP 93129 (10 μM, n=3)	5-HT_{1B}	None

Table 4.0. Actions of 5-HT agonists on the 5-HT-evoked depolarisation.

Following the bath-application of CP 93129, selective for 5-HT_{1B} receptors, to WAY-100635 treated cells there was no change to the membrane potential or input resistance. This rules out the 5-HT_{1B} receptor subtype as mediating the 5-HT-evoked depolarisation. This also indicates that any 5-HT_{1B} receptors in the DRN (Voigt et al 1991) have no electrophysiologically observable response under these conditions. The 5-HT_{1B} receptor subtype has been shown to have autoreceptor properties (Limberger et al 1991) and would be predicted to cause a membrane

hyperpolarisation. This study has found that the 5-HT_{1B} agonist used has no effect on the excitability of serotonergic neurones in the DRN at the concentration tested. The data suggests the involvement of 5-HT_2 receptors in the mediation of the 5-HT-evoked depolarisation in the DRN. It also indicates that the 5-HT_{2A} receptor is most likely to be involved as the lack of action of mCPP rules out the 5-$HT_{2B/2C}$ receptor subtypes. Confirmation of the involvement of 5-HT_2 receptors in the 5-HT-evoked depolarisation could only be made using antagonists.

Antagonists

The broad spectrum 5-HT antagonist methysergide was found to have no effect on the membrane potential or input resistance. Pre-application of methysergide to a number of cells showing the 5-HT-evoked depolarisation blocked the 5-HT-evoked depolarisation (n=6 of 8). This suggests that the response is mediated by a 5-HT receptor. Pre-application of methysergide also blocked the action of DOI confirming that methysergide has antagonistic actions at the 5-HT_2 receptor subtype. Methysergide at lower concentrations did however fail to block the depolarisation in another two cells. In one cell, while under the influence of WAY-100635 and methysergide, a hyperpolarisation was seen with 5-HT. This is a 5-HT-evoked hyperpolarisation not mediated by the 5-HT_{1A} receptor subtype providing further evidence that other receptor subtypes found in the DRN are physiologically active.

Antagonist	**Site of Action**	**Effect on Depolarisation**
Methysergide (100 μM, n=8)	5-$HT_{broad\ spectrum}$	Abolished
Ketanserin (100 nM, n=3)	5-HT_2	Abolished
MDL 100, 907 (100 μM, n=3)	5-HT_{2A}	Abolished
mCPP (100 μM, n=3)	5-$HT_{2B/2C}$	No effect
SB 200646 (1 mM, n=3)	5-$HT_{2B/2C}$	Possible partial blockade
Pindolol (1mM, n=3)	5-$HT_{1A/1B}$	No effect

Table 4.1. Actions of 5-HT antagonists on the 5-HT-evoked depolarisation.

The 5-HT_2 receptor antagonist ketanserin has been shown to have binding sites within the DRN (Pazos and Palacios 1985). Ketanserin was found to have no effect on the membrane potential and input resistance of DRN cells following treatment with WAY-100635. Ketanserin blocked the 5-HT-evoked depolarisation, this indicates that 5-HT_2 receptors are involved in the 5-HT-evoked depolarisation. This also confirms that some of the ketanserin binding sites observed in the DRN are due to binding to 5-HT_2 receptor subtypes. When applied to WAY-100635 treated cells the 5-$HT_{2B/2C}$ receptor antagonist SB 200646 was found to have no effect on membrane potential or input resistance. SB 200646 possibly showed a partial blocked the 5-HT-evoked depolarisation at the concentration tested. The cells showed a slight change in membrane potential, but still showed a decrease in input resistance. This could be evidence that more than one receptor is involved in mediating the 5-HT-evoked depolarisation, or it could be that SB 200646 is acting at a limited number of 5-HT_{2A} receptors. Bath-application of the 5-HT_{2A} antagonist MDL 100, 907 had no effect on membrane potential or input resistance and so has no agonist activity on WAY-100635 treated cells. Pre-application with MDL 100, 907 resulted in blockade of the 5-HT-evoked depolarisation pointing to the probable involvement of the 5-HT_{2A} receptor subtype. Finally the 5-$HT_{1A/1B}$ antagonist pindolol was used and did not block the 5-HT-evoked depolarisation at the concentration tested. This strongly suggests that the 5-HT_{2A} receptor subtype mediates the 5-HT-evoked depolarisation in the DRN.

CHAPTER 5

Regulation of firing in the DRN neurone

Introduction

The firing pattern of neurones in the DRN is governed by a number of different membrane properties and ion conductances. Burlhis and Aghajanian (1987) first described the pacemaker potential as an intrinsic mechanism that allows a neurone to follow a repeating cycle of membrane changes resulting in a regular firing pattern. Each stage of the pacemaker potential is independently open to influence. Further to direct changes to the intrinsic conductances of each stage of the pacemaker potential, the pacemaker potential can only operate within tight membrane potential ranges. For example, when the membrane in hyperpolarised by the presence of 5-HT the first and initiating stage of the pacemaker potential is inoperative and firing is switched off. Likewise, when the membrane in depolarised by the presence of noradrenaline only a slight membrane change is needed to trigger an action potential and this increases the rate of neuronal firing.

This chapter will investigate the conductances involved in regulating the firing pattern of neurones in the DRN. This will be carried using the potassium channel blockers tetraethylammonium (TEA) and apamin. TEA is a non-specific potassium channel blocker at, for example, the delayed rectifier channel, the large conductance calcium-activated potassium channel and voltage-sensitive potassium channels (Stanfield 1983). Apamin, a toxin taken from honey bee venom, selectively blocks small conductance calcium-activated potassium channels (Hugues et al 1982). These compounds have a profound effect on neuronal firing, such as delaying repolarisation following the action potential and blocking the AHP (Scroggs and Anderson 1990; Yarom et al 1985). The role of 5-HT in regulating the firing properties of DRN neurones will also be investigated to discover if the 5-HT released with each action potential can feedback to modulate further firing and 5-HT release.

Results

Actions of TEA on the action potential and the AHP

A short (3 ms) depolarising current step (0.25 nA) was injected into a typical cell generating an action potential overshooting to +38 mV, which was 5 ms in length at its broadest point, followed by an AHP of 9 mV (Figure 5.0A). Following the application of TEA (3 mM) the cell generated an action potential overshooting by +35 mV, which was much longer in duration, 50 ms, took longer to repolarise and reduced the amplitude of the AHP to 5 mV (Figure 5.0B). It seems likely that TEA is acting by blocking potassium conductances involved in action potential repolarisation and the generation of the AHP. The extended action potential allows more calcium to enter the cell. This extra calcium does not appear to affect the AHP. This may be due to TEA blocking the fast component of the AHP, which governs the peak amplitude. Also shown in Figure 5.0B the slow AHP was shorter in duration in the presence of TEA from 150 ms to 20 ms. This was observed in a further two cells.

Action of apamin on the AHP

To investigate which of the many potassium channels was involved in the AHP apamin was applied to the cell. In a typical cell at its resting membrane potential a short (5 ms) depolarising pulse (0.2 nA) was injected and an all-or-nothing action potential was evoked, followed by an AHP with a peak of 10 mV after approximately 5 ms (Figure 5.1). Following bath-application of apamin (100 nM) there was a long lasting inhibition of the AHP. The AHP only reached 8 mV and was much shorter in duration; this did not recover after extensive apamin washout. This indicated that the duration of the AHP in the DRN cell is determined by the activity of an apamin-sensitive conductance suggesting that small conductance calcium-activated potassium (SK) channels mediate this late phase. These SK channels are activated either as calcium enters the cell or is released from internal stores during the action potential. The amount of calcium that enters the cell could depend on the duration of the spike and if so the AHP would end when most of the extra calcium had been removed. A similar blockade of the late component of the AHP was observed in a further two cells. The blockade of the late, apamin-sensitive

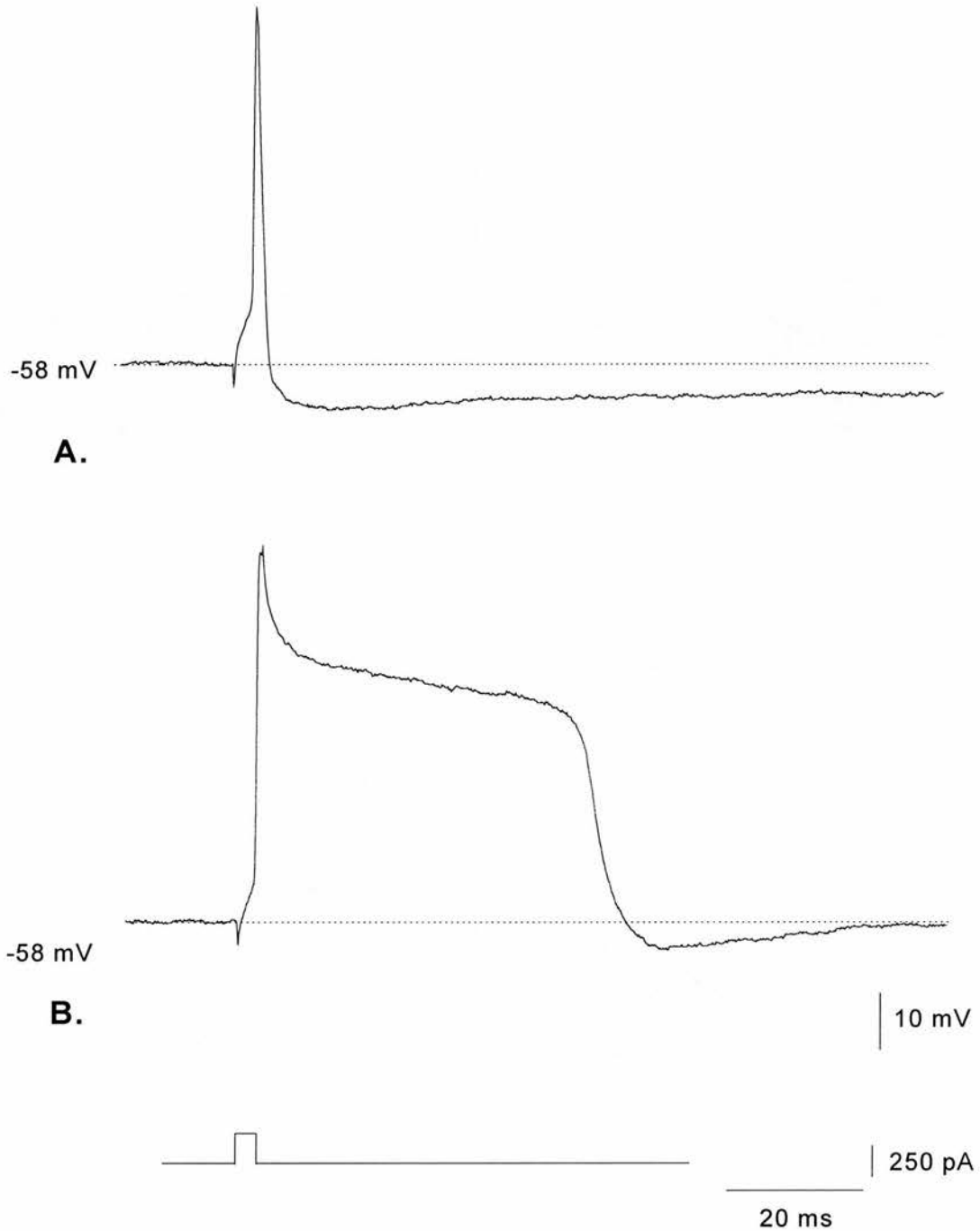


Figure 5.0. Action of TEA on the action potential and AHP in the DRN.
A. A short (3 ms) depolarising current step (0.25 nA) was injected into a cell generating an overshooting action potential of +38 mV which was 3 ms in duration and was followed by an AHP of 9 mV.
B. Following the application of TEA (3 mM) the cell generated an overshooting action potential of +35 mV, which was much longer in duration, 50 ms, took much longer to repolarise and has a reduced AHP of 5 mV following injection of the same current step. The membrane potential was clamped to the resting membrane potential by the manual injection of current prior to injection of the depolarising pulse.

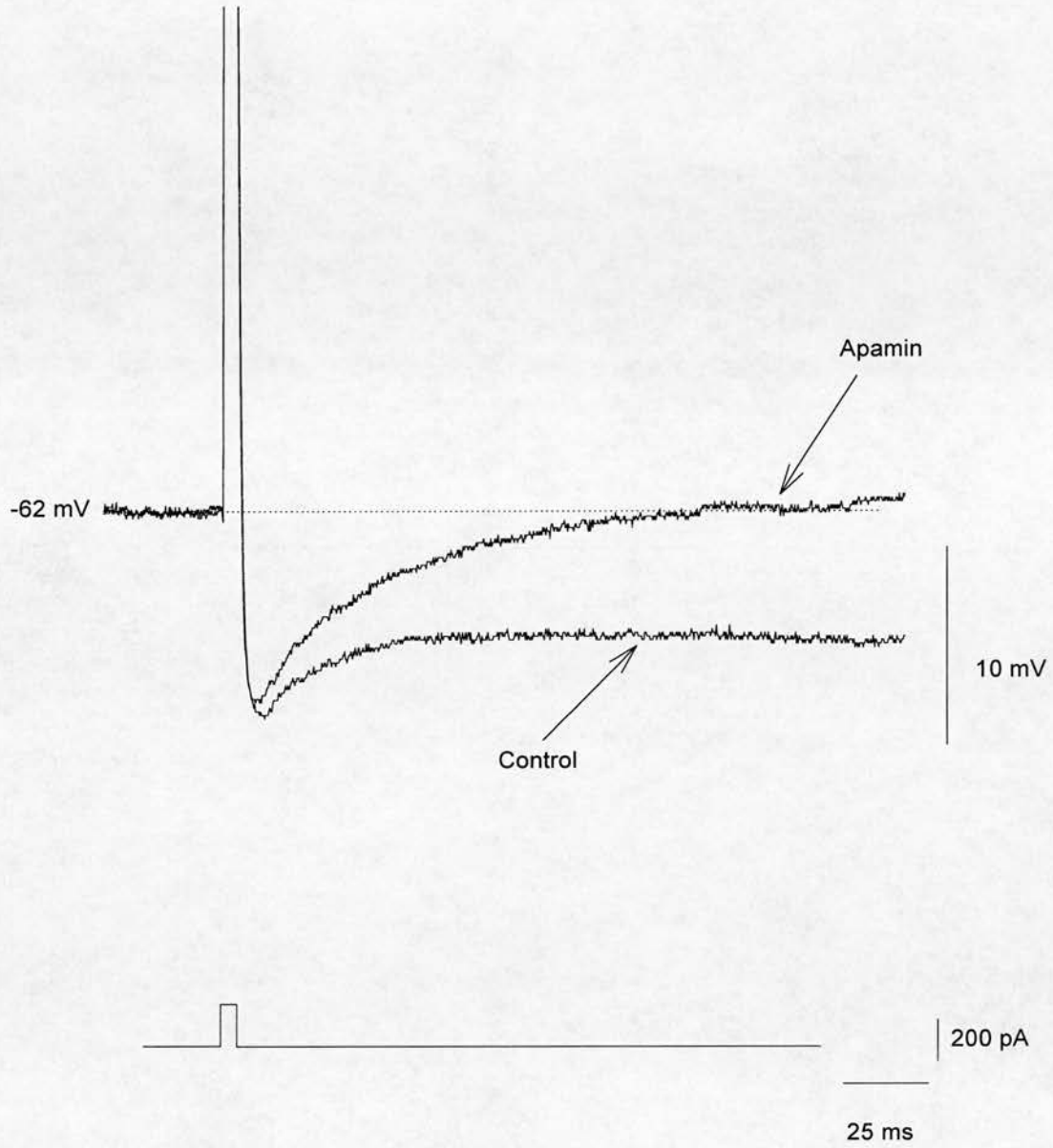


Figure 5.1. Action of apamin on the AHP in the DRN.
When at a resting membrane potential of -62 mV a short (5 ms) depolarising pulse (0.2 nA) was injected and an all-or-nothing action potential was evoked. This was followed by an AHP. The AHP reached a peak of 14 mV after approximately 5 ms. Following bath-application of apamin (100 nM) there was a long lasting inhibition of the AHP. The AHP only reached 11 mV in the presence of apamin and had a shorter duration; this did not recover after extensive apamin washout. The membrane potential was clamped to the resting membrane potential by the manual injection of current.

component of the AHP, termed the slow AHP, highlights the remaining early, TEA-sensitive component, termed the fast AHP.

Action of TEA on spike firing

The role of the TEA-sensitive conductance of the action potential and AHP in the regulation of cell firing was investigated. In one neurone at its resting membrane potential a long (1000 ms) depolarising current pulse (0.2 nA) evoked three regular, all-or-nothing action potentials followed by an AHP of 5 mV (Figure 5.2A). Following bath-application of TEA (3 mM) there was an increase in spike firing frequency to five spikes where each successive spike was shorter in duration and the cell showed adaptation (Figure 5.2B). Adaptation was characterised by a gradually slowing spike frequency until the cell could no longer reach its firing threshold and firing was switched off (Hille 1992). The AHP had a much longer duration possibly due to increased calcium influx. The AHP following a single spike does not appear to be the same as an AHP following a train of spikes; this is highlighted in the lack of blockade by TEA of the early component of the AHP following a train of spikes.

Action of apamin on spike firing

The ability of the DRN cell to show spike frequency adaptation was investigated using apamin. DRN cells showed a regular firing pattern following the injection of a long depolarising current step. In a typical cell at its resting membrane potential of -63 mV a long (1000 ms) depolarising pulse (0.2 nA) evoked a series of 9 regular, all-or-nothing action potentials forming a train of spikes along the whole length of the pulse, followed by an AHP of 12 mV (Figure 5.3A).

Following bath-application of apamin (100 nM) there was a long lasting inhibition of this regular firing pattern, any change in membrane potential was compensated for by manual injection of current to return the cell to the resting membrane potential. There was an increase in firing rate with apamin; the cell fired twenty-two high frequency action potentials in response to the same amplitude current pulse when under the influence of apamin and the cell showed a gradually increasing interspike interval or

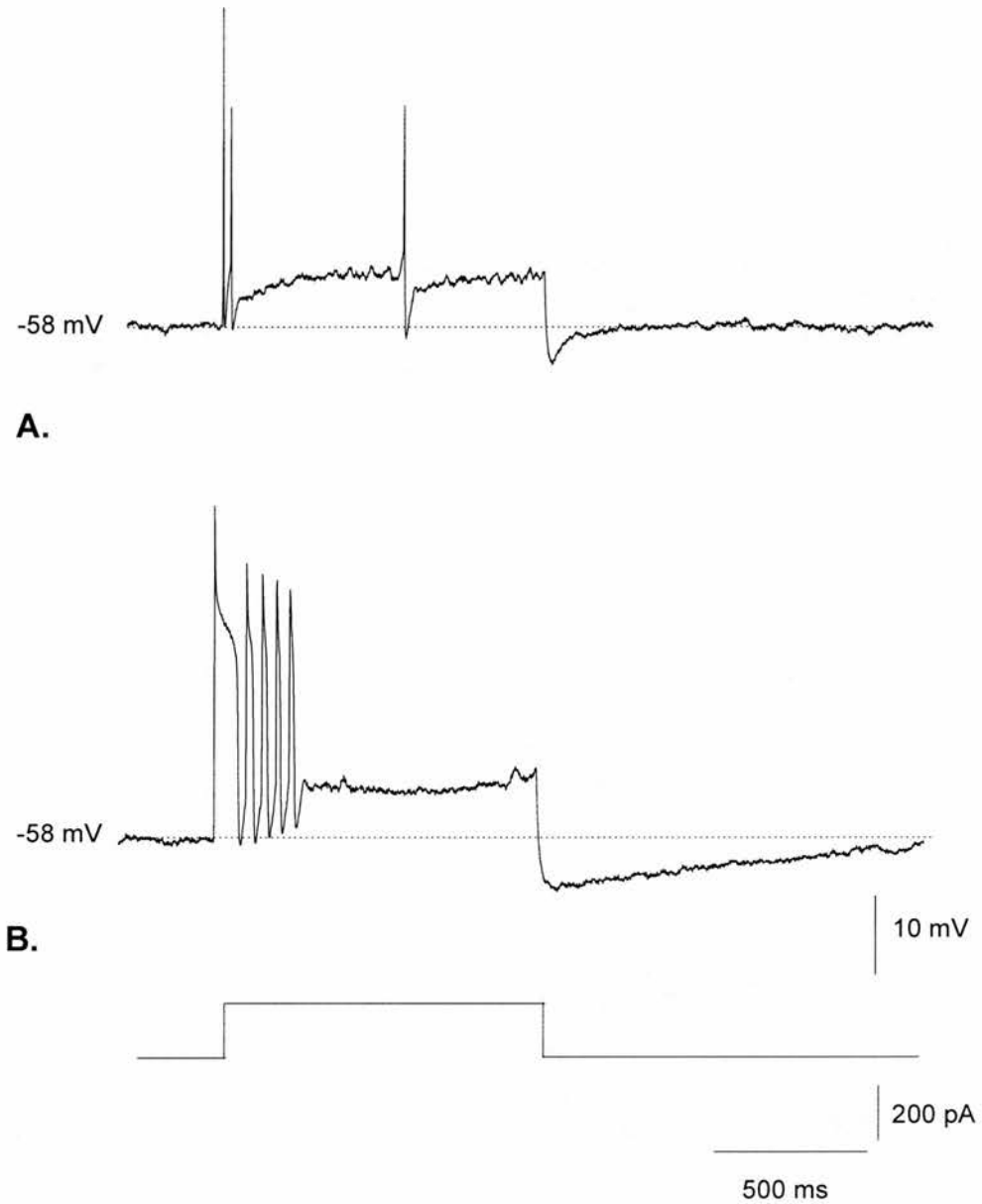


Figure 5.2. Action of TEA on the firing pattern in the DRN.
A. When at a resting membrane potential of -58 mV a long (1000 ms) depolarising pulse (0.2 nA) evoked a series of regular, all-or-nothing action potentials followed by an AHP of 5mV.
B. Following bath-application of TEA (3 mM) there was an increase in spike firing frequency with a long lasting inhibition of spike accommodation. The action potential was followed an AHP of 7 mV, with a longer lasting late component. This did not recover after extensive washout. The membrane potential was clamped to the resting membrane potential by the manual injection of current.

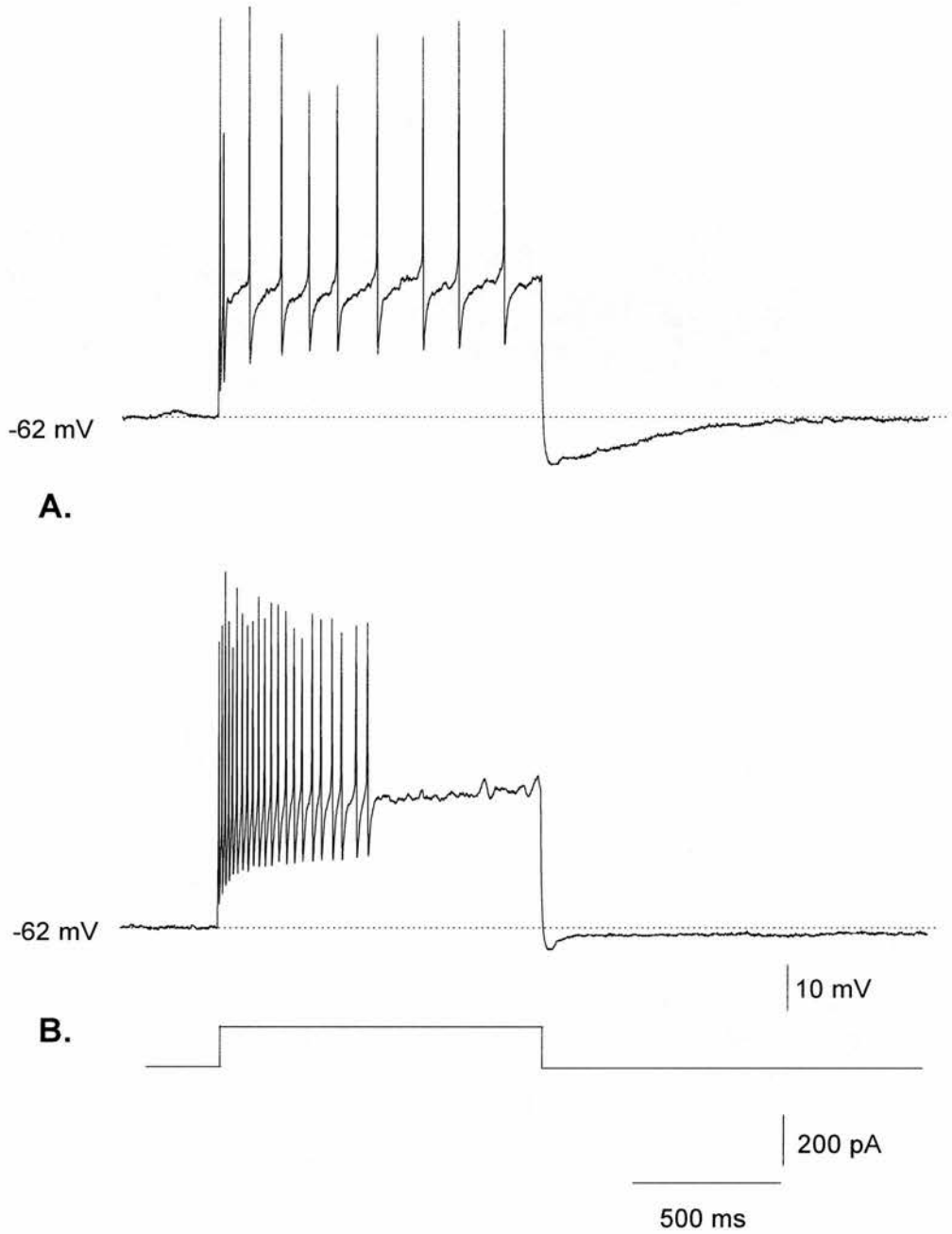


Figure 5.3. Action of apamin on spike firing in the DRN.
A. When at a resting membrane potential of -63 mV a long (1000 ms) depolarising pulse (0.2 nA) evoked a series of regular, all-or-nothing action potentials followed by an AHP of 12 mV.
B. Following bath-application of apamin (100 nM) there was a long lasting inhibition of spike accommodation and an increase in firing rate. The AHP was reduced to 4 mV in the presence of apamin; this did not recover after extensive apamin washout. The membrane potential was clamped to the resting membrane potential by the manual injection of current.

firing accommodation. There was also an overall decrease in spike height throughout the spike train and the cell showed adaptation (Figure 5.3B). The AHP was reduced to 7 mV despite the increase in the number of spikes; this did not recover after extensive apamin washout. The duration of the AHP is completely inhibited by apamin, this may indicate that the AHP following a train of spikes is completely made up of the activity of SK channels. When under the influence of apamin the cell showed spike adaptation pointing to the involvement of the SK channels in the generation of the spike and the regulation of spike frequency.

Action of TEA on membrane properties

Examination of any change in membrane potential and cell input resistance gave a guide to what conductances were involved in the action TEA has on a cell. In a typical experiment the cell had a control membrane potential of -65 mV and an input resistance of 276.5 MΩ. Following bath-application of TEA (3 mM) the cell retained its resting membrane potential of -65 mV, but showed an increase in input resistance to 362.9 MΩ (Figure 5.4A). This showed the cell was undergoing channel blockade under the influence of TEA. TEA was found to increase input resistance in a further three cells from 281.1 ± 10.2 MΩ to 351 ± 15.2 MΩ.

Action of apamin on membrane properties

Examination of any change in membrane potential and cell input resistance gave a guide to what conductances are involved in the action of apamin on a cell. In a typical experiment the cell had a control membrane potential -66 mV and an input resistance of 260.1 MΩ (Figure 5.4B). Following bath-application of apamin (100 nM) the cell showed a membrane depolarisation to -62 mV, with an associated increase in input resistance to 332.8 MΩ and a reversal value of -90 mV. Thus apamin blocks channels that are open at the resting membrane potential. Similar changes were observed in another two cells with an average membrane depolarisation from -67 ± 3.9 mV to -63.3 ± 2.6 mV, an average increase in input resistance from 278.5 ± 10.4 MΩ to 321 ± 8.9 MΩ and an average reversal value of -91 ± 1.9 mV, close to that expected for a potassium-mediated event.

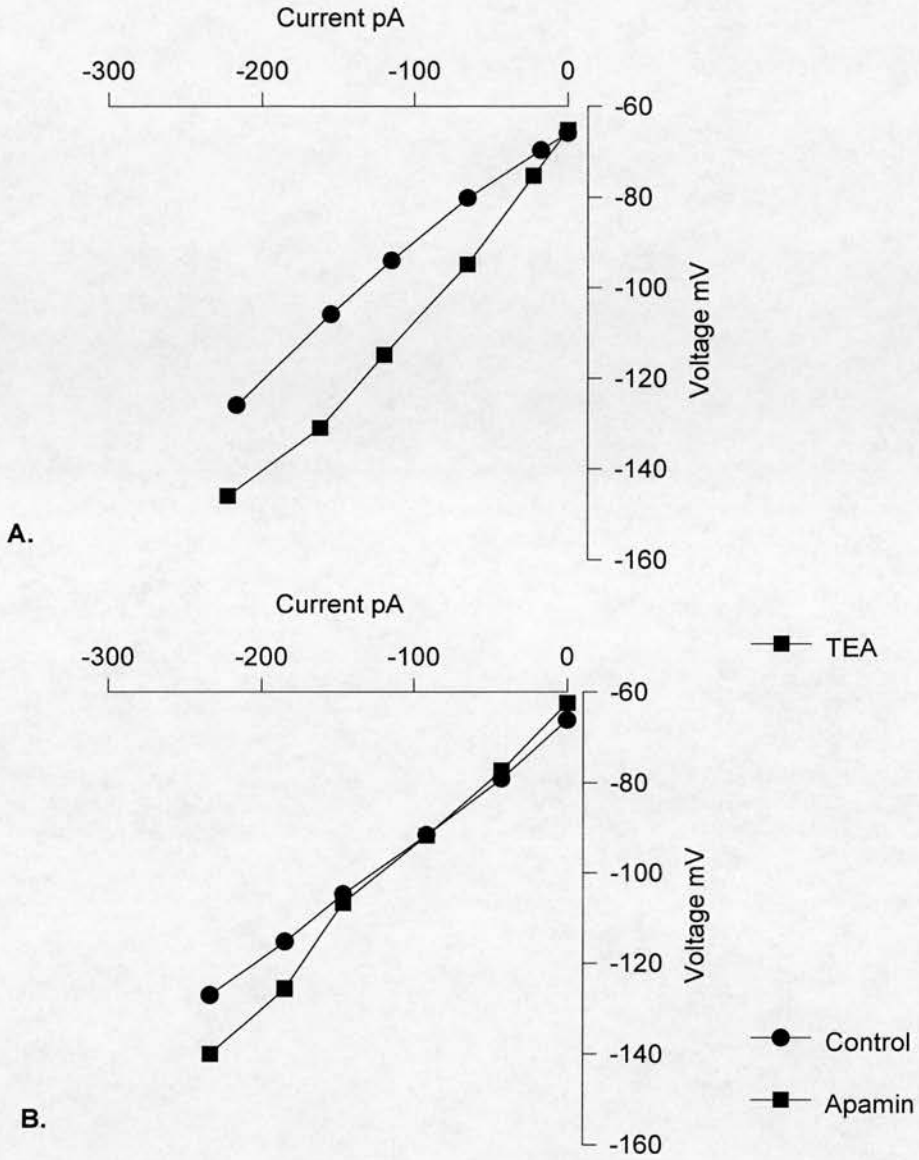


Figure 5.4. Action of TEA and apamin on membrane properties in the DRN.
A. Following bath-application of TEA (3 mM) a cell with a resting membrane potential of -65 mV and an input resistance of 276.5 MΩ (circles) retained its resting membrane potential of -65 mV, but showed an increase in input resistance to 362.9 MΩ (squares).
B. Following bath-application of apamin (100 nM) a cell with a resting membrane potential of -66 mV and an input resistance of 260.1 MΩ (circles) depolarised to -62 mV, with an associated increase in input resistance to 332.8 MΩ (squares). The I/V plots reversed at -90 mV.

Action of 5-HT on spike width

In typical cell at a resting membrane potential of -66 mV a short (3 ms) depolarising pulse (0.1 nA) evoked an all-or-nothing action potential followed by an AHP. Following bath-application of 5-HT (100 μM) the spike width was reduced from 4.0 ms to 3.5 ms, measured at its broadest point. The spike height was slightly reduced. The decreased spike width may be due to a reduction in the amount of calcium entering the cell leading to a smaller AHP (Figure 5.5A). Bath-application of WAY-100635 (100 nM) reduced the spike width in a similar way to 5-HT, from 4.3 ms to 4.0 ms at its broadest point (Figure 5.5B). Following the application of WAY-100635, 5-HT continued to have a slight effect on spike width suggesting that 5-HT may modulate spike width through non-5-HT_{1A} receptors (Figure 5.5C). Similar affects on the spike width were observed in a further eight cells where on average 5-HT reduced the spike width from 4.0 ± 0.2 ms to 3.4 ± 0.1 ms (n=9 of 15).

Action of 5-HT on the AHP

5-HT is known to inhibit a calcium channel (Penington et al 1991), the role this channel has on the action potential and AHP was investigated. The 5-HT_{1A} receptor is negatively coupled to calcium influx, where the application of 5-HT reduces calcium conductance (Penington et al 1991). By blocking the 5-HT_{1A}-mediated effect using the 5-HT_{1A} antagonist WAY-100635 the actions of 5-HT on the calcium current could be investigated indirectly by examining AHP amplitude and duration. In a neurone at its resting membrane potential a short (5 ms) depolarising pulse (0.2 nA) evoked an all-or-nothing action potential followed by an AHP (Figure 5.6A). As the membrane potential hyperpolarisation and decrease in input resistance evoked by 5-HT can affect the size of the AHP, the cell was manually held at the resting level by applying a depolarising current to the cell. The AHP reached a peak of 9 mV after approximately 5 ms. Following bath-application of 5-HT (100 μM) the peak of the AHP reduced to 7 mV. The duration of the AHP is reduced under the influence of 5-HT so that the AHP returned to the resting membrane potential more quickly. Following an incubation in WAY-100635 (100 nM) the AHP reached the same peak amplitude, 9 mV, but the duration was reduced (Figure 5.6B). Following the

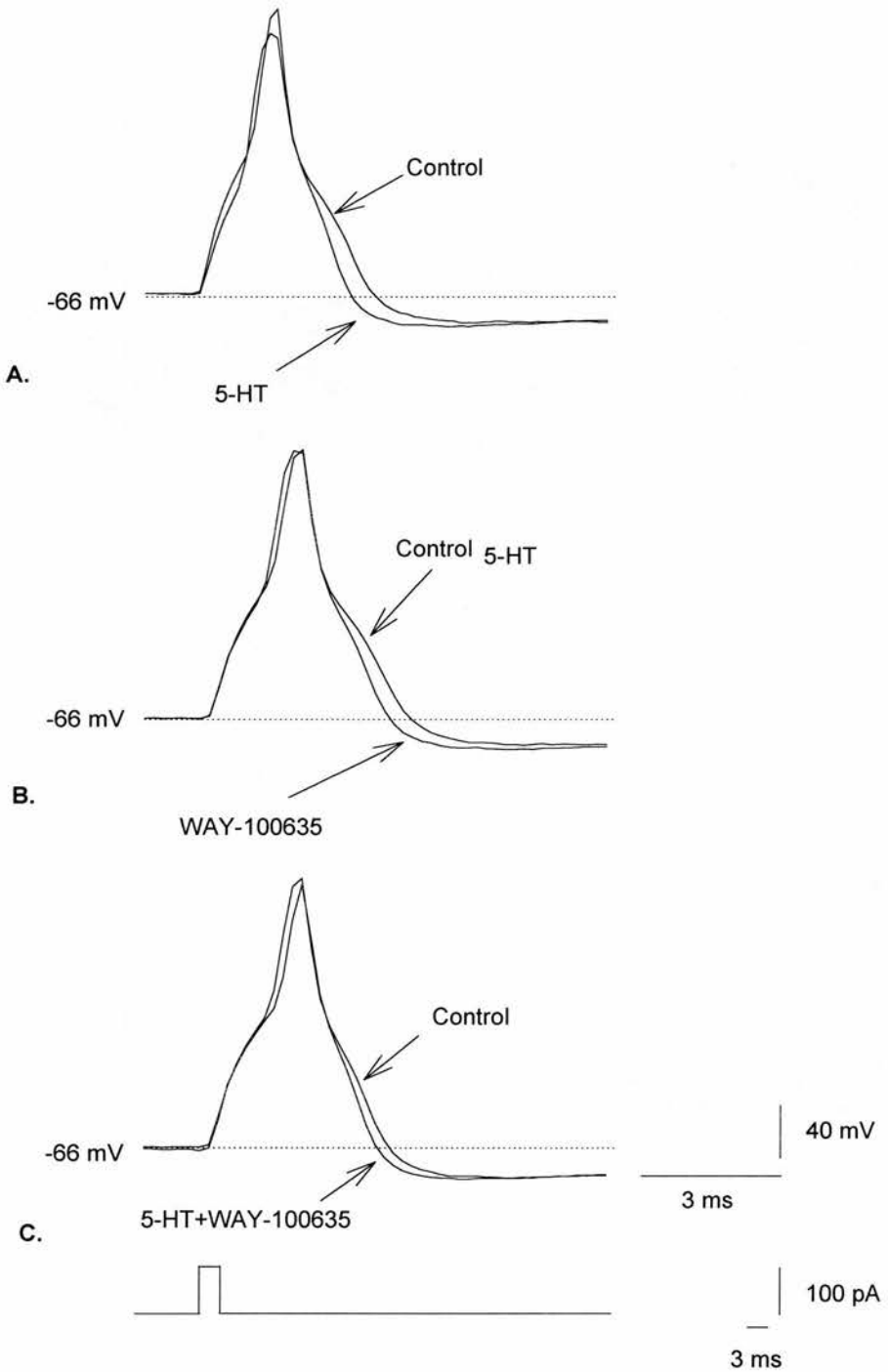


Figure 5.5. Action of 5-HT on spike width in the DRN.

A. Superimposed traces in the same cell before and during an application of 5-HT. At a resting membrane potential of -66 mV a short (3 ms) depolarising pulse (0.1 nA) evoked an all-or-nothing action potential followed by an AHP. Following bath-application of 5-HT (100 μM) the spike width was reduced from 4.0 ms to 3.5 ms when measured at it broadest point. This resulted in a decrease in the AHP. The spike height was slightly reduced.

B. Bath-application of WAY-100635 (100 nM) reduced the spike width from 4.3 ms to 4.0 ms.

C. Following the application of WAY-100635 5-HT continues to have a slight effect on the spike, the width was reduced from 4.3 ms to 4.1 ms. The membrane potential was clamped to the resting membrane potential by the manual injection of current.

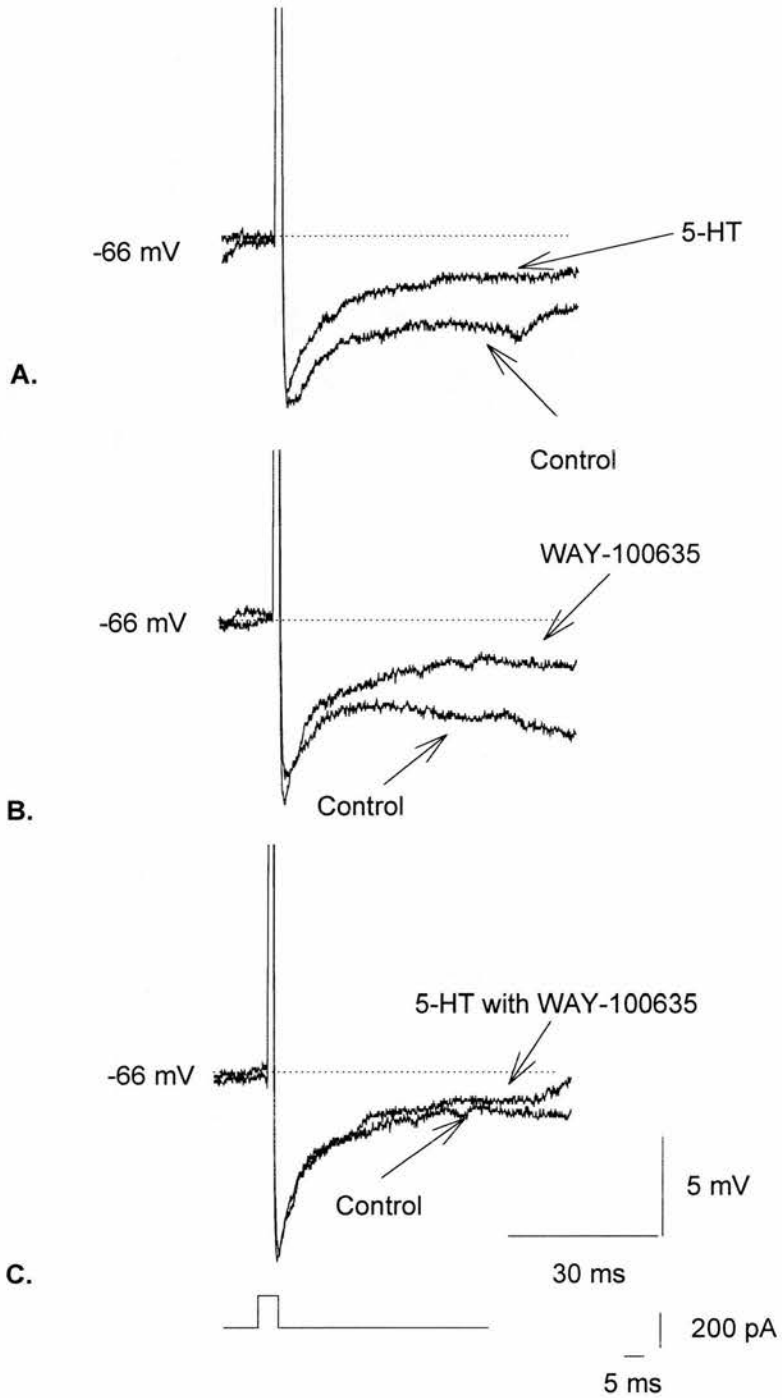


Figure 5.6. Action of 5-HT on the AHP in the DRN.
A. At a resting membrane potential of -66 mV a short (5 ms) depolarising pulse (0.2 nA) evoked an all-or-nothing action potential followed by an AHP. The AHP reached a peak of 9 mV after approximately 5 ms. Following bath-application of 5-HT (100 μM) there was an inhibition of the AHP, it reached a peak of 7 mV. The AHP decreased more quickly under the influence of 5-HT.
B. In the same cell bath-application of WAY-100635 (100 nM) the AHP was similar in size, it reached a peak of 9 mV, but the AHP did reduce more quickly.
C. Following pre-application of WAY-100635 (100 nM) bath-application of 5-HT (100 μM) only slightly reduced the duration of the AHP. The membrane potential was clamped to the resting membrane potential by the manual injection of current.

application of WAY-100635 (100 nM) bath-application of 5-HT (100 μM) no longer inhibited the AHP (Figure 5.6C). This may indicate the involvement of the 5-HT_{1A} receptor and the calcium conductance modulated by 5-HT_{1A} in the regulation of cell firing via its inhibition of the AHP. However, 5-HT may be acting directly on potassium channels. The blockade of the 5-HT-evoked reduction of the duration of the AHP was observed in a further five cells (n=6 of 15).

Action of 5-HT on spike frequency

5-HT also has a role in spike firing frequency regulation that may be the consequence of the actions of 5-HT on the action potential and the AHP. In a typical cell at rest a long (1000 ms) depolarising pulse (0.1 nA) evoked a series of six regular, all-or-nothing action potentials followed by an AHP (Figure 5.7A). Following bath-application of 5-HT (100 μM) there was an inhibition of the AHP from 11 mV to 5 mV where the duration of the AHP was reduced (Figure 5.7B). There was also an increase in the spike-firing rate; the cell now fired seven action potentials. In the presence of WAY-100635 (100 nM) the AHP was still small at 6 mV, following an application of 5-HT, but there was recovery of the spike-firing rate, the cell fired five action potentials (Figure 5.7C). A similar 5-HT-induced increase in spike firing rate was observed in a further 4 cells and may indicate the involvement of the 5-HT_{1A} receptor in regulating spike frequency.

Action of CP 93129 on the AHP

The 5-HT_{1B} agonist CP 93129 (10 μM) was tested on WAY-100635 (100 nM) treated cells that had already demonstrated a 5-HT modulated decrease in the AHP. In one cell at a resting membrane potential of -64 mV a short (3 ms) depolarising pulse (0.5 nA) evoked an all-or-nothing action potential followed by an AHP of 15 mV (Figure 5.8A). Bath-application of 5-HT (100 μM) resulted in an AHP with the same peak, but a reduced duration. In the same cell bath-application of CP 93129 (10 μM) did not change the peak of the AHP, but the duration of the AHP was slightly reduced (Figure 5.8B). CP 93129 did not change the spike width or height of the AHP in three DRN neurones.

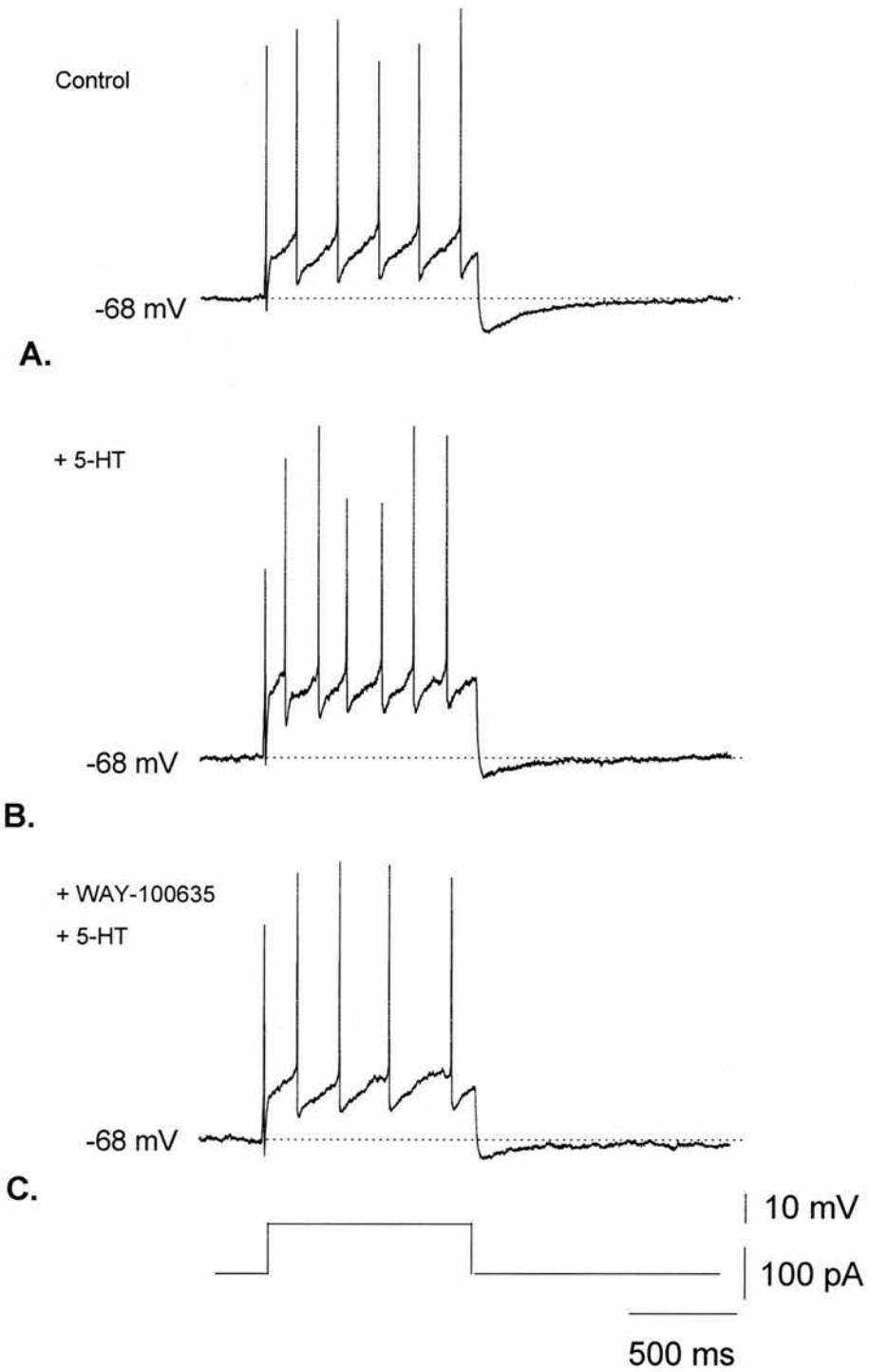


Figure 5.7. Action of 5-HT on spike frequency in the DRN.
A. When at a resting membrane potential of -68 mV a long (1000 ms) depolarising pulse (0.1 nA) evoked a series of regular, all-or-nothing action potentials followed by an AHP of 11 mV.
B. Following bath-application of 5-HT (100 μM) there was a slight inhibition of the AHP to 5 mV where the AHP duration was reduced. There was also an increase in the spike-firing rate.
C. In the presence of WAY-100635 (100 nM) the AHP was still small, 6 mV, following an application of 5-HT (100 μM), but the spike-firing rate was restored. The membrane potential was clamped to the resting membrane potential by the manual injection of current.

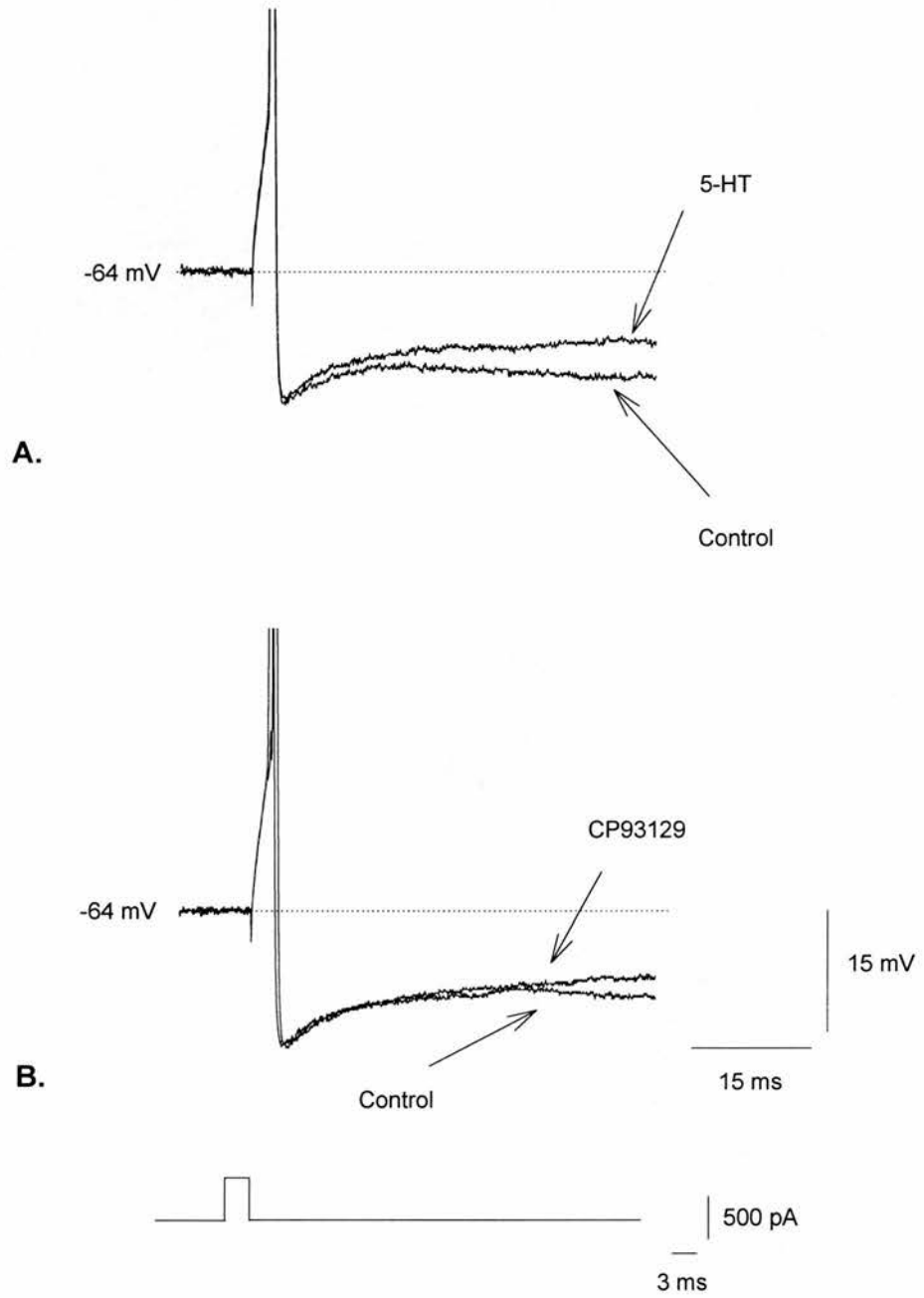


Figure 5.8. CP 93129 does not change the AHP in the DRN.
A. At a resting membrane potential of -64 mV a short (3 ms) depolarising pulse (0.5 nA) evoked an all-or-nothing action potential followed by an AHP of 15 mV. Bath-application of 5-HT (100 μM) resulted in an AHP with the same peak, but a reduced duration.
B. In the same cell bath-application of CP 93129 (10 μM) did not change the peak of the AHP, but the duration of the AHP was only slightly reduced. The membrane potential was clamped to the resting membrane potential by the manual injection of current.

Summary

The Afterhyperpolarisation

The present study has shown that the DRN exhibits evidence of two independent conductances being involved in the generation and maintenance of the AHP. The AHP shows a reversal level of approximately -90 mV; the predicted reversal value of potassium mediated events. The first conductance is made up of a fast TEA-sensitive conductance activated during the action potential and is involved in the cell repolarisation and controls the size of the peak of the AHP. The second conductance is a later slow conductance that governs how quickly the cell returns to the resting membrane potential. Bath-application of apamin to the cell resulted in the AHP being shorter duration; therefore SK channels are responsible for maintaining the duration of the AHP in the DRN. The SK channels are activated following the increase in calcium concentration during the action potential.

Firing Frequency, Accommodation and Adaptation

DRN neurones typically fire single action potentials in a regular pattern. DRN neurones also do not typically show spike accommodation, adaptation or burst firing; properties that might be consistent with pacemaker activity. Following the application of TEA the firing rate of the neurone increased and the cell showed adaptation where the AHP gradually slows the spike frequency until the train of spikes is halted. This shows that the TEA-sensitive conductance is important in regulating spike frequency.

Following the addition of apamin, DRN cells showed an increase in firing frequency, firing accommodation and adaptation. Cells fired a rapid, high frequency train of action potentials where each spike is consistently smaller in amplitude with a gradually increasing interspike interval. This is firing accommodation. The cells also show adaptation. The pattern of firing now resembles burst firing. There is also a reduction in the AHP. This shows that SK channels are involved in preventing the DRN cell showing a burst firing pattern and allowing the cell to show pacemaker activity. This indicates that not only do DRN neurones possess the conductances

needed to maintain pacemaker activity it also shows that these conductances can be altered by ion channel activation. It may be that these ion channels can be switched on and off to change the firing pattern of the neurone. Therefore, neurones of the DRN may be able to switch from firing either a single regular action potential, which is most commonly observed, to firing a burst, which may give a cell certain advantages such as the ability to release extra quantities of 5-HT or change the pattern of 5-HT release.

5-HT-evoked Spike Narrowing

Following the application of 5-HT the duration of the action potential was reduced. This may be the result of the inhibition of the calcium component of the action potential. 5-HT is known to modulate a calcium channel via activation of the 5-HT_{1A} receptor (Penington and Kelly 1990) where the application of 5-HT reduces the calcium current. WAY-100635 itself reduced the spike width and may be acting as a partial agonist. Alternatively WAY-100635 could be acting directly by blocking potassium or calcium channels. If WAY-100635 does have 5-HT_{1A} agonist properties it is impossible to know if WAY-100635 is blocking 5-HT-evoked spike narrowing, because the spike is already reduced in duration by the action of WAY-100635. The work of Nooney and Kelly (1998) has shown that WAY-100635 antagonises the 5-HT-evoked modulation of calcium channels in the isolated channel preparation. This may mean either that the 5-HT-evoked spike narrowing is due to modulation of calcium channels not linked to the 5-HT_{1A} receptor or that 5-HT is acting at other ion channels, such as potassium ion channels, activated during the action potential. This may also be true of the action of WAY-100635 on the duration of the action potential.

The AHP and 5-HT

Following bath-application of 5-HT the peak of the AHP is similar, however the duration of the AHP is reduced. This effect is seen in conjunction with the 5-HT-evoked spike, but may not be linked. As SK channels are calcium-sensitive it may be that if less calcium enters the cell then less channels will be activated. If so the

calcium channel mediated by the 5-HT_{1A} receptor may have a role in the generation of the AHP. However, 5-HT may also be acting directly on the apamin-sensitive SK channels that generate the later component of the AHP. Penington et al (1991) have shown that 5-HT-evoked modulation of calcium channels is linked to the activation of an outward calcium-sensitive potassium current.

WAY-100635 was also found to reduce the AHP. Again this is an example of WAY-100635 showing agonist activity and may be related to the effect WAY-100635 was having on spike width or could be due to the modulation of apamin-sensitive SK channels. Due to the action of WAY-100635 it was impossible to discover if the 5-HT_{1A} receptor subtype mediated the 5-HT-evoked reduction in the AHP.

Firing frequency and 5-HT

Bath-application of 5-HT may have increased the firing rate of the cell, resulting in more action potentials being generated by the same size current step. Firing frequency is regulated in a number of ways. It is partly regulated by the AHP. The AHP delays return to the resting membrane potential and in turn the threshold potential. 5-HT increases the rate of decay of the AHP. Following the addition of WAY-100635 to the bath-aCSF, 5-HT application no longer changed the firing rate, but the AHP was still small. This indicates that the 5-HT_{1A} receptors may in some way modulate the firing rate of DRN neurones. However, the AHP following a train of spikes seems to be unaffected by the 5-HT_{1A} receptor activation. This means that the calcium influx due to the calcium channels modulated by the 5-HT_{1A} receptor have either only a minor or no role in the calcium influx that occurs during a train of action potentials. The SK channels appear to have a role in the regulation of firing frequency so 5-HT could be acting directly on SK channels to increase firing rate. However, the work with apamin revealed a very distinctive firing pattern that was not observed with 5-HT. This may indicate that 5-HT is changing the firing rate of neurones by modulating calcium entry and so the AHP.

CHAPTER 6

Discussion

This chapter will review some of the points raised in the result chapters. Firstly, there will be discussion of the 5-HT_{1A}-mediated hyperpolarisation and its blockade by WAY-100635 and the actions of WAY-100635 in the DRN and hippocampus. Secondly, the chapter will review and discuss the actions of 5-HT as revealed by WAY-100635. This section pays particular attention to the 5-HT-evoked depolarisation revealed in the DRN following the application of WAY-100635, including data on the ionic mechanism and receptor classification. Thirdly, this chapter will review the ionic mechanisms that control the firing pattern of DRN neurones and the actions of 5-HT on this firing pattern.

Actions of 5-HT on the DRN neurone

The present study has shown that 5-HT can regulate cell firing in the DRN by manipulating the membrane potential. In agreement with other reports (Aghajanian and VanderMaelen 1982) 5-HT evokes an inhibitory response via activation of 5-HT_{1A} receptors. This study tested the new 5-HT_{1A} selective antagonist WAY-100635 on this well characterised response.

The 5-HT_{1A}-evoked hyperpolarisation

The principal action of bath-application of 5-HT on neurones of the DRN is inhibitory. This inhibitory action is the result of an evoked membrane hyperpolarisation making the cell less likely to fire an action potential by holding its membrane potential at a level below the firing threshold membrane potential. The action is associated with a decrease in input resistance. I/V analysis showed that the

response reversed at around -90 mV, indicative of the 5-HT-evoked hyperpolarisation following the opening of potassium channels.

The receptor mediating the 5-HT-evoked membrane hyperpolarisation has been termed the somatodendritic autoreceptor. Somatodendritic refers to the receptors being located on the soma and dendrites on serotonergic cells. The term autoreceptor derives from the fact that serotonergic cells of the DRN release 5-HT into the extracellular space and when the concentration of this released 5-HT has reached a certain level further 5-HT release is inhibited by the membrane hyperpolarisation, making the cell less excitable. Early work showed that LSD inhibited DRN cell firing in a similar way to 5-HT (Aghajanian et al 1968). Using the brain slice preparation it was shown that the ionic basis of the response to 5-HT was potassium mediated and involved the opening of potassium channels (Aghajanian and Lakoski 1984). The potassium channels involved were further characterised and found to be inwardly rectifying (Williams et al 1988). This 5-HT-mediated opening of inwardly rectifying potassium channels has also been characterised in acutely isolated (Penington et al 1993a) and individually microcultured (Johnson 1994) DRN neurones. Further to this, Penington et al (1993b) showed that the 5-HT induced increase in the potassium current results from a greater probability of opening of unitary resting potassium channel activity. The data of the present study is consistent with these previously reported results.

The inhibitory 5-HT_{1A} receptor mediated hyperpolarisation has been observed in a number of neuronal cell types and many different areas of the brain. The neurones of the CA1 region of the hippocampus show a 5-HT-evoked, dose-dependent membrane hyperpolarisation, which is associated with a decrease in input resistance as a result of the opening of inwardly rectifying potassium channels (Andrade and Nicoll 1987), characteristics in common with the observations in the DRN. These characteristics are also true of the 5-HT_{1A} receptor mediated hyperpolarisation in the medial pontine nucleus (Stevens et al 1992), the rat association cortex (Araneda and Andrade 1991), the lateral septal neurones (Joels et al 1987), the neurones of the midbrain periaqueductel grey region (Behbehani et al 1993) and in rat ventromedial

hypothalamic neurones (Newberry 1992). Roberts and Straughan (1967) observed *in vivo* that 5-HT inhibits firing in cortical neurones. This inhibition has also been observed in the somatosensory cortex (Davies et al 1987) and the cerebral cortex (Bradshaw et al 1983).

It is now widely accepted that the 5-HT_{1A} receptor subtype mediated this inhibitory effect. However, this was assumed from the use of 5-HT_{1A} agonists, such as 8-OH-DPAT or non-selective antagonists, such as spiperone. Used *in vivo* the 5-HT_{1A} agonist 8-OH-DPAT potently inhibits cell firing in a dose dependent manner (Pan et al 1993; Sprouse and Aghajanian 1987). In the brain slice 8-OH-DPAT produced a membrane hyperpolarisation and a decrease in input resistance (Sprouse and Aghajanian 1987) mimicking the action of 5-HT. However, the lack of compounds with purely antagonist activity has prevented the characterisation of this action with antagonists. In this study the recently developed 5-HT_{1A} antagonist WAY-100635 was tested by its ability to block the 5-HT-evoked hyperpolarisation.

WAY-100635 in the DRN and the hippocampus

WAY-100635 has been reported as a selective 5-HT_{1A} antagonist (Fletcher et al 1996) in the rat brain, with the binding characteristics of a 5-HT_{1A} radioligand in the rat CNS (Khawaja et al 1995). It is reported as having no agonist activity at the 5-HT_{1A} receptor in the DRN neurone, when studied extracellularly (Corradetti et al 1996), or the pyramidal cells of the CA1 region of the hippocampus. Studies performed *in vivo* showed WAY-100635 to have no partial agonist activity in many physiological and behavioural models including the isolated guinea pig ileum preparation and the 8-OH-DPAT induced behaviour syndrome (Forster et al 1995). WAY-100635 can block the 5-HT_{1A}-evoked membrane hyperpolarisation observed in the CA1 region of the hippocampus where WAY-100635 can also block the 5-HT induced decrease in excitatory neurotransmission (Corradetti et al 1996). WAY-100635 antagonises the decrease in the amplitude of the NMDA receptor-mediated component of the EPSP's induced by 5-HT (Corradetti et al 1998). It also blocks the 5-HT and 5-HT_{1A} agonist induced inhibition of cell firing in the DRN (Craven et al

1994; Fletcher et al 1993; Forster et al 1995). WAY-100635 application performed *in vivo* leads to an increase in the firing rate of serotonergic cells of the DRN in the anaesthetised cat (Fornal et al 1994), increasing 5-HT release. WAY-100635 reverses the partial agonist activity of pindolol in the DRN, where pindolol inhibits cell firing, and in the frontal cortex, where pindolol reduces 5-HT release (Clifford et al 1998). WAY-100635 shows competitive antagonism with 8-OH-DPAT in the guinea pig DRN (Mundey et al 1996). This study also showed that WAY-100635 application *in vivo* can increase neuronal firing. Mundey et al (1996) concluded that this is probably because of the blockade of a 5-HT_{1A}-mediated inhibitor tone acting on serotonergic neurones resulting in an increase in 5-HT release and 5-HT_2 receptor-mediated effects. WAY-100635 has been widely used as an *in vivo* radioligand and has been shown to have heterogeneity of signal consistent with the known localisation of the 5-HT_{1A} receptor subtype (Hume et al 1994).

The present study agreed with the findings that WAY-100635 has no action on membrane potential or input resistance and blocks 5-HT_{1A}-mediated events. When applied to the DRN neurone for up to 30 minutes WAY-100635 was found to have no effect on resting membrane potential or input resistance indicating that it has no agonist activity at the 5-HT_{1A} receptor. This is in contrast to a number of compounds previously claimed to have purely antagonist activity. For example, NAN-190 has been found to have partial agonist activity in the DRN (Hodgkiss et al 1992). This study showed that application of NAN-190 alone frequently caused a membrane hyperpolarisation associated with a decrease in input resistance. BMY 7378, SDZ-216-525 and WAY-100135 also have partial agonist activity (Fletcher et al 1993). The problem of partial agonist activity in the DRN may be the result of the DRN neurones having a large receptor reserve for the 5-HT_{1A} receptor (Meller et al 1990). Compounds that have previously been described as 5-HT_{1A} receptor antagonists have been found to be either non-selective or partial agonists or a combination of both. Spiperone is a 5-HT_{1A} antagonist (Schoeffter and Hoyer 1990) but is non-selective, for example it has activity at the 5-HT_2 receptor subtypes (Leysen et al 1981). A number of β-adrenoceptor antagonists, such as propanolol and pindolol, have 5-HT_{1A} antagonistic activity (Tricklebank 1985; Sprouse and Aghajanian 1987) but are also

5-HT_{1B} receptor antagonists. The more recently developed compounds are more selective but cannot be described as "silent". The compounds BMY 7378 (Yocca et al 1987) and NAN-190 (Glennon et al 1988) block many of the reported 5-HT_{1A}-evoked responses (Yocca et al 1987). However, they have also been described as partial agonists at the 5-HT_{1A} receptor (VanderMaelen et al 1987; Hodgkiss et al 1992). The recent compound SDZ 216-525 was originally described as "silent" and has antagonist activity at a number of known 5-HT_{1A}-evoked responses (Hoyer et al 1992; Boddeke et al 1992). However, this too has been found to have partial agonist activity at the 5-HT_{1A} autoreceptor (Sharp et al 1997) where it can inhibit cell firing. The compound WAY-100135 (Fletcher et al 1993; Lanfumey et al 1993b) has 5-HT_{1A} antagonist activity, but also has 5-HT_{1A} partial agonist actions, for example, it can induce an inhibition in cell firing in the DRN (Fletcher et al 1993). WAY-100635 is the latest candidate for a silent 5-HT_{1A} antagonist and all studies so far have shown much evidence to support this.

However, in the present study, while under the long term influence of WAY-100635 some cells showed a slight membrane potential drift in a depolarising direction, leading to an increase in firing rate. This depolarisation is not consistent with partial agonist activity, which would be observed as a hyperpolarisation. The depolarisation following long term application of WAY-100635 may be a consequence of the blockade of the tonic inhibition exerted by 5-HT in the DRN (Craven et al 1994; Forster et al 1995), or may result from an excitatory action of endogenously released 5-HT. Another possibility is that WAY-100635 is blocking the potassium channels involved in maintaining the resting membrane potential in the DRN neurone. If this were true then the depolarising membrane potential drift would be associated with an increase in input resistance and indeed a slight increase in input resistance is observed in some cells. To investigate if WAY-100635 is acting by blocking a potassium current then this may be investigated by performing a voltage clamp experiment to follow the activity of the potassium current maintaining the membrane potential.

The membrane potential changes more commonly observed in the DRN following long-term recordings are the result of the loss of the excitatory action of noradrenaline (Baraban and Aghajanian 1980a). Noradrenaline acts at the α_1-adrenoceptor to drive cell firing. In the brain slice preparation noradrenaline is washed away leading to a loss of firing and a drift in membrane potential in the hyperpolarising direction. This observation suggests that the depolarising effect of WAY-100635 may be an active phenomenon.

WAY-100635 was also applied to pyramidal neurones of the CA1 region of the hippocampus. WAY-100635 had no partial agonist activity in cells in the hippocampus as has been previously reported by Fletcher et al (1995). Therefore WAY-100635 is silent at the postsynaptic 5-HT_{1A} receptor. WAY-100635 was also shown to block the 5-HT_{1A}-evoked membrane hyperpolarisation in the neurones of the hippocampus, again in agreement with the previous study of Fletcher et al (1995). Blockade of the 5-HT_{1A}-evoked hyperpolarisation revealed a 5-HT-evoked depolarisation. This was associated with an increase in input resistance and therefore assumed to be the depolarisation described by Andrade and Nicoll (1987) later characterised as being mediated by the 5-HT_4 receptor (Andrade and Chaput 1991).

WAY-100635 blocks the actions of 5-HT at the 5-HT_{1A} receptor in both the hippocampus and DRN. This may indicate that the somatodendritic 5-HT_{1A} autoreceptor in the DRN and the postsynaptic 5-HT_{1A} receptor in the hippocampus are, pharmacologically at least, identical. It has been hypothesised that these receptors are pharmacologically different. This follows from the work with (+/-)-pindolol and (-)-pindolol, β-adrenoceptor antagonists with high affinity for the 5-HT_{1A} receptor subtype. Some groups have reported, using electrophysiological techniques, that these compounds can block the action of 5-HT at the somatodendritic 5-HT_{1A} autoreceptor while having no effect at the postsynaptic 5-HT_{1A} receptor (Artigas et al 1994; Romero et al 1996). However, there is also much conflicting data to suggest that pindolol shows no preference for 5-HT_{1A} receptors. There are reports of pindolol blocking hormonal and behavioural responses to

postsynaptic 5-HT_{1A} receptor stimulation (Scott et al 1994; Ybema et al 1994; Levy et al 1995). A more recent study has shown electrophysiologically that (-)-pindolol does indeed block the 5-HT_{1A}-mediated hyperpolarisation in the CA1 region of the hippocampus (Corradetti et al 1998). This is an example of (-)-pindolol acting at postsynaptic 5-HT_{1A} receptors. The present study adds supports the identical pharmacological nature of somatodendritic autoreceptors and postsynaptic 5-HT_{1A} receptors by showing that WAY-100635 can block the 5-HT_{1A}-mediated hyperpolarisation in both the hippocampus and the DRN at the same concentration while showing no partial agonist activity in the hippocampus. WAY-100635 is highly selective at the 5-HT_{1A} receptor subtype and so could be assumed to identify any pharmacological differences between any proposed different 5-HT_{1A} receptor types. This does not rule out differences in the way that 5-HT_{1A} receptors couple to second messenger systems, which could account for the many reported differences between the postsynaptic and autoreceptors of the hippocampus and DRN

In addition, WAY-100635 did not block the α_1-adrenoceptor-mediated depolarisation evoked by the application of noradrenaline and phenylephrine. This indicates that WAY-100635 does not block actions mediated by the α_1-adrenoceptor. The α_1-adrenoceptor-mediated depolarisation, as has been widely reported (Baraban and Aghajanian 1980a), is associated with an increase in input resistance and when analysed using I/V plots shows a reversal at a value approximately that expected for a potassium mediated event. The α_1-adrenoceptor-mediated depolarisation is due to the closure of potassium channels.

In the DRN, long-term application of WAY-100635 revealed a 5-HT-evoked depolarisation. This 5-HT-evoked depolarisation could only be observed in the presence of WAY-100635 and did not have a consistent input resistance increase more commonly associated with membrane depolarisations, such as that described above for noradrenaline and phenylephrine. A 5-HT-evoked hyperpolarisation was observed in WAY-100635 treated cells also exposed to methysergide and mCPP. This hyperpolarisation is associated with a decrease in input resistance and reverses

at approximately the value expected for a potassium event. These are two examples of 5-HT having actions hidden by the dominant effect of the 5-HT_{1A} receptor, showing that 5-HT can have a multiplicity of actions in the DRN once the 5-HT_{1A} receptor is blocked.

A 5-HT-evoked depolarisation was also observed in WAY-100635 treated cells in the hippocampus. This depolarisation was associated with an increase in input resistance and reversed at approximately the value expected for a potassium-mediated event. These are characteristics are not consistent with the 5-HT-evoked depolarisation observed in the DRN which does not show a consistent input resistance increase and does not reverse at the value expected for a potassium mediated event.

In summary this study has shown that WAY-100635 does not appear to have partial agonist activity at the somatodendritic autoreceptor in the DRN or the postsynaptic receptor in the hippocampus. However, long-term application of WAY-100635 in the DRN does appear to have some effect on the resting membrane potential. WAY-100635 can block the 5-HT_{1A}-mediated hyperpolarisation in the hippocampus and DRN. WAY-100635 also reveals a 5-HT-evoked depolarisation in the hippocampus and DRN.

A 5-HT-evoked depolarisation

Following the discovery of a 5-HT-evoked depolarisation the 5-HT receptor subtype mediating the depolarisation was investigated. The observation of a 5-HT-evoked depolarising response associated with an increase in cell firing implies that 5-HT also has a role in increasing cell excitability as well as the more traditional role of inhibition. This section will discuss possible ionic mechanisms underlying the 5-HT-evoked membrane depolarisation.

Characteristics of the 5-HT-evoked depolarisation

The 5-HT-evoked depolarisation was observed in the majority of cells. In most cells demonstrating the 5-HT-evoked depolarisation there was no net change in input resistance. In a sub-population of cells the depolarisation was associated with a slight decrease in input resistance and in a smaller population of cells there was a slight increase in input resistance. In most cells showing a change in input resistance I/V analysis revealed that the depolarising response did not show a reversal level in the range -120 to -60 mV. However, in a few of those cells I/V plots obtained during the 5-HT-evoked depolarisation in control conditions reversed at approximately -50 mV (n=5). This may point to the depolarising response being made up of a change in a number of conductances with different reversal values. If this is so then the relative dominance of any component in generating the response may vary in different cells, resulting in the variation in overall resistance observed. The response could include a component due to the closure of potassium channels, which would make the reversal value move towards that expected for a potassium-mediated event. Given that in some cases the 5-HT-evoked depolarisation is associated with a decrease in input resistance the other component may be due to the opening of another channel. This second component would have a positive reversal value and could be made up of either a non-voltage activated sodium channel, for example, or possibly a non-selective cation channel. Also, the voltage responses to injected depolarising current pulses in the cells of the DRN commonly showed rectification. This rectification could be observed as a non-linearity in the input resistance. The I/V plots of the 5-HT-evoked depolarisation against a control show rectification and reversal in the

region of -45 mV, which is inconsistent with the 5-HT-evoked depolarisation being due only to the closure of potassium channels.

The 5-HT-evoked depolarisation was only observed following pre-treatment with WAY-100635. The inability to observe any non-5-HT_{1A} mediated events in the absence of WAY-100635 could be because of 5-HT released within the slice acting at the 5-HT_{1A} receptors masking any other 5-HT-evoked actions. A tonic effect of 5-HT as it accumulates in the *in vitro* slice could artificially hold a neurone at a hyperpolarised membrane potential. If this were so then the application of WAY-100635 would lead to a membrane depolarisation, as the tonic action of 5-HT is blocked by the 5-HT_{1A} receptor antagonist. This depolarisation could either appear as a gradual membrane drift in the depolarising direction or as a more pronounced membrane change due to the action of endogenously released 5-HT on the receptor subtype mediating the 5-HT-evoked depolarisation. A slow drift in the membrane potential was observed in a number of cells while in the presence of WAY-100635, consistent with the first theory and could be indicative of endogenous 5-HT acting on neurones in the *in vitro* slice preparation to keep the membrane potential at an artificially hyperpolarised level and inhibiting cell firing. A drift in the membrane potential could also be due to the blockade of potassium channels involved in holding the cell at its resting membrane potential. The observation of membrane changes during the recording of cells adds to the problems associated with working in the *in vitro* brain slice. As this shows it is difficult to completely maintain the properties of neurones in the *in vivo* brain slice environment.

The 5-HT-evoked depolarisation could be demonstrated in the presence of TTX, known to block the sodium channel involved in generating the action potential. This implies that the response is independent of the action potential dependent transynaptic events involved in networks. 5-HT must be acting postsynaptically, where 5-HT binding to the target cell changes the activity of that cell by causing a membrane depolarisation leading to an increase in excitability.

The first reported 5-HT-evoked excitation was observed by Roberts and Straughan (1967). They looked at the excitatory effects of 5-HT on cells of the cerebral cortex in the anaesthetised cat. There are now many reported 5-HT-evoked excitations or membrane depolarisations. These responses have been associated with a number of ionic mechanisms and receptor subtypes. In the motorneurone a number of studies have reported 5-HT-evoked membrane depolarisation. Intracellular studies *in vitro*, with the facial motorneurone, have shown that 5-HT induces a slow, sub-threshold depolarisation associated with an increase in input resistance, indicating a decrease in resting potassium conductance (Larkman et al 1989). This has also been seen in the medullary repiratory neurones (Lalley et al 1995) and spinal motorneurones (White and Fung 1989; Yamazki et al 1992). A 5-HT-evoked depolarisation due to the closure of potassium channels has also been seen in neurones of the medial pontine reticular (Stevens et al 1992), of the substantia nigra (Rick et al 1995) and of the olivary nucleus (Sugihara et al 1995). The neurones of the nucleus accumbens depolarise and are induced to fire action potentials by the application of 5-HT in association with an increase in input resistance due to the reduction of an inwardly rectifying potassium conductance (North and Uchimura 1989). GABAergic neurones of the nucleus reticularis thalami and medial septal nucleus depolarise in response to 5-HT due to the decrease in a potassium conductance (McCormick and Wang 1991; Alreja 1996). There are also reports of 5-HT evoking a depolarisation due to a decrease in a potassium conductance in areas of the cortex, for example, in the cerebral cortex (Araneda and Andrade 1991; Davies et al 1987) and in the pyramidal cells of the piriform cortex (Sheldon and Aghajanian 1990). In the prefrontal cortex the application of 5-HT evokes a delayed membrane depolarisation, there is also the appearance of a slow depolarising afterpotential following an action potential (Araneda and Andrade 1991), it is not associated with an increase in input resistance. However, this response undergoes desensitisation upon repeated agonist administration and the ionic nature of this has so far been difficult to elucidate. The ionic mechanism underlying these responses are inconsistent with the results of this study, where the depolarisation is associated with either no or small changes in input resistance.

5-HT has also been shown to depolarise a number of central neurones by augmentation of a hyperpolarisation-activated, time-dependent, non-selective and inwardly rectifying cation current, termed I_h (Bobker and Williams 1989; Pape and McCormick 1989). There is little evidence for the DRN neurone having the I_h current. One of the characteristics of the I_h current is a depolarising "sag" observed in hyperpolarising current steps where the cell attempts to repolarise to the resting membrane potential before the end of the negative current pulse. In the DRN hyperpolarising current steps up to -120 mV show no evidence of this repolarising sag. This indicates that the 5-HT-evoked depolarisation is unlikely to be due to the activation of the I_h channels. The action of 5-HT in the facial motorneurone is an example of 5-HT acting at more than one ion channel. Larkman and Kelly (1992) showed that 5-HT can have a dual effect by depolarising facial motorneurones by a combination of mechanisms. This depolarisation is in part due to decrease of a potassium conductance and the enhancement of I_h. This shows that 5-HT not only modulates non-selective ion channels, but can also activate a combination of channels to evoke a membrane depolarisation.

The 5-HT_3 receptor also mediates depolarising responses. Activation of this receptor has a very different mechanism to that of the other 5-HT-evoked depolarisations and is due to the direct activation of a cation channel. Evidence for an electrophysiologically observable response due to 5-HT interacting with the 5-HT_3 receptor was first observed in cultured mouse hippocampal and striatal neurones. It was characterised as a membrane depolarisation with a rapid onset, which is also rapid to desensitise (Yakel and Jackson 1988; Yakel et al 1990, 1991). These features are typical of ligand-gated ion channels and not receptors coupled to a G-protein. 5-HT_3 mediated depolarisations have also been observed in the brain slice preparation. In the lateral nucleus of the amygdala 5-HT_3 mediated fast-excitatory synaptic responses follow focal electrical stimulation and can only be demonstrated when glutamate receptors are blocked (Sugita et al 1992), this response also shows rapid desensitisation. In the hippocampal slice 5-HT can increase spontaneous GABAergic IPSP's through a 5-HT_3 mediated receptor excitation of inhibitory neurones (Ropert 1988; Ropert and Guy 1991), this response also shows fast desensitisation. Most 5-

HT_3 mediated events have so far been characterised as fast to inactivate, but there has been a 5-HT_3 mediated depolarisation observed in neurones of the dorsal root ganglion that is fast in onset but non-inactivating (Todorovic and Anderson 1990). The features of rapid onset and rapid desensitisation are not consistent with that of the 5-HT-evoked depolarisation in DRN neurones, which is slow to activate and slow to reverse. These features are more consistent with a mechanism involving a G-protein coupled receptor and probably rules out the 5-HT_3 receptor as the receptor mediating the 5-HT-evoked depolarisation in the DRN.

From the study of hippocampal pyramidal neurones discussed in this thesis, blockade of the 5-HT_{1A}-mediated hyperpolarisation with WAY-100635 revealed a 5-HT-evoked membrane depolarisation. This was associated with an increase in input resistance. This observation agrees with the work of Andrade and Nicoll (1987) and Colino and Halliwell (1987) who describe a 5-HT-evoked depolarisation associated with an increase in cell input resistance, the result of a decrease in a potassium conductance, this has more recently been reported by Corradetti et al (1996).

The 5-HT-evoked depolarisation revealed by the blockade of the 5-HT_{1A} receptor subtype in the DRN does not seem to be mediated solely by the closure of potassium channels. Craven et al (1997) described a 5-HT-evoked depolarisation in the guinea pig DRN. They reported that the response was in part associated with a slight increase in input resistance. Voltage-clamp experiments revealed a 5-HT-evoked inward current with a decrease in conductance. However, they also found that the response did not reverse in the range normally associated with potassium mediated events and concluded that the response must also involve another current. In the present study an increase in resistance was observed in some cells, but I/V analysis did not show a point of intersection in the range normally associated with potassium mediated events, where reversal was found it was in a more positive direction. This suggests the involvement of another current. A similar voltage-clamp study could be carried out to investigate the currents involved in the 5-HT-evoked depolarisation in the rat DRN.

The mechanism of action of this 5-HT-evoked depolarisation is therefore different to that typically associated with 5-HT, where the effect is solely mediated by a reduction in a potassium conductance or an increase in I_h. The input resistance data from the present study indicates that the depolarising response could be made up of a number of different components that can vary in importance with each cell. This accounts for some cells showing a change in input resistance and others not. Where cells did show a change in input resistance the response reversed at a level significantly more positive than that predicted for a potassium mediated event. This implies the response could be made up by an ionic conductance change in potassium channels and a change in an ion channel conductance with a positive reversal value such as sodium ions. Also given the small size of the response only a slight change in the conductance of a sodium or non-selective ion channel would be needed to mediate the response. The data reported in the guinea pig DRN points to the 5-HT-evoked depolarising response being mediated by more than one conductance. Craven et al (1998) conclude that the 5-HT-evoked depolarisation observed in the guinea pig DRN partly results from a closure of potassium channels. These works also concluded that the 5-HT-evoked depolarisation is probably also due to an increase in another conductance that is due to the opening of a cation channel that is either selective for sodium ions or non-selective for sodium or potassium ions.

There are a number of examples of neurotransmitters modulating a voltage-independent non-selective cation conductance. In rat locus coeruleus neurones muscarine can activate voltage-independent and voltage-dependent non-selective cation conductances, this together with a decrease in potassium conductance results in an increase in excitability (Shen and North 1992). In bullfrog ganglion cells a combined potassium conductance decrease and cation conductance increase has been suggested to account for the slow excitatory effects of muscarine (Kuba and Koketsu 1978; Tsuji and Kuba 1988). This kind of mixed conductance change is also thought to underlie the excitatory effects of substance P on guinea pig inferior ganglion cells (Dun and Minota 1981). The same mechanism has also been reported to account for the actions of 5-HT, substance P, muscarine, vasoactive intestinal polypeptide, forskolin and slow excitatory synaptic transmission in guinea pig submucosal

neurones (Shen and Surprenant 1993). Many slow depolarising effects have been associated with neurotransmitters and a number are thought to be mediated by a combination of ionic mechanisms, these include the action of neurotensin on tegmental neurones (Jiang et al 1994) and carbachol on rat hippocampal neurones (Colino and Halliwell 1993).

There is a well characterised membrane depolarisation described in the DRN neurone. Noradrenaline and the α_1-adrenoceptor agonist phenylephrine have been described as evoking a membrane depolarisation accompanied by an increase in cell excitability (VanderMaelen and Aghajanian 1983). Data from the present study shows the phenylephrine-evoked membrane depolarisation is associated with an increase in input resistance consistent with previously reported data (Baraban and Aghajanian 1980a). I/V analysis shows the depolarisation reversed at approximately -90 mV indicating that the response is due to the closure of potassium channels.

It may be that this 5-HT-evoked depolarising response in the DRN is only in part the result of the closure of potassium channels. This could be investigated using voltage-clamp conditions, which would allow the measurement of the currents underlying the 5-HT-evoked depolarisation. The opening of sodium or non-selective channels may also contribute to the response. These two components possibly make up the 5-HT-evoked depolarisation to a different degree in each example. There are many examples of neurotransmitters evoking an increase in excitability due to this type of mechanism.

A study performed in rostral ventromedial medulla neurones by Hwang and Dun (1999) investigated a 5-HT-evoked depolarisation with similar properties to those discussed in this thesis. They described a mechanism involving the combination of a potassium conductance decrease and a caesium-insensitive, non-selective cation conductance increase to account for the variable conductance associated with the 5-HT-evoked depolarisation. This reported mechanism could account for the varying conductances associated with the 5-HT-evoked depolarisation discussed in this thesis. However, Hwang and Dun had not determined whether a single class of 5-

HT_2 receptor mediates the 5-HT-evoked depolarisation by coupling to different ion channels or if each channel is linked to different subclasses of 5-HT_2 receptor.

Pharmacology of the 5-HT-evoked depolarisation

There are many reported examples of 5-HT_2 receptors mediating depolarising responses and there is also evidence of 5-HT_2 receptors being found in the DRN (Leysen et al 1982; Davies et al 1988). There are no examples of 5-HT_2 agonists having any action on control DRN neurones. This study found 5-HT hyperpolarised neurones in every case and only showed the depolarising response following pre-treatment with WAY-100635. This may be explained by the lack of selectivity of the agonists available for the 5-HT receptor subtypes. Even though DOI, for example, has pEC_{50} values for 5-HT_{2A} receptors of 7.6 and for 5-HT_{2C} receptors of 7.0, it still shows some binding to the 5-HT_{1A} receptor. As the 5-HT_{1A}-evoked hyperpolarisation appears to be dominant over the 5-HT-evoked depolarisation then even some binding at the 5-HT_{1A} receptor may result in the membrane hyperpolarisation. This is also compounded by the action of endogenously released 5-HT acting on 5-HT_{1A} receptors masking any other 5-HT-evoked membrane changes.

Action of agonists

The 5-HT_2 agonist DOI was found to mimic the 5-HT-evoked depolarisation when applied to WAY-100635 treated neurones showing that the 5-HT_2 receptor may be involved in the depolarisation. DOI has been used extensively in behavioural studies to investigate the role of the 5-HT_2 receptor subtypes in anxiety (for review see Griebel 1995). DOI has similar affinities for each of the 5-HT_2 receptor subtypes showing little selectivity, but has been used to selectively identify the 5-HT_{2A} mediated component of the inhibition of glutamate release from the rat cerebellum, a 5-HT_1-like receptors make up the other component of this effect (Maura et al 1988). Due to the lack of DOI selectivity this data does not distinguish between the three known 5-HT_2 receptors, but does show they are involved.

Bath-application of mCPP (pEC_{50} = 6.9), a partial agonist more selective for 5-HT_{2C} receptors (Groteweil et al 1994) did not have any effect on membrane potential or input resistance on WAY-100635 treated cells in the DRN. mCPP is known to induce hypophagia and hypolocomotion (Curzon and Kennett 1990) via its 5-HT_{2C} activity. The 5-HT_{2C} receptor activation is also thought to account for the anxiogenic and panic precipitating properties induced by mCPP in behavioural studies (Trail et al 1995). In rodents mCPP can cause hypoactivity, hypophagia, oral dyskinesia, penile erection and hyperthermia. In the DRN neurone the lack of membrane changes following the application of mCPP indicates that not only is the 5-HT-evoked depolarisation not mediated by the 5-HT_{2C} receptor subtype, but also that the 5-HT_{2C} receptor may not mediate electrophysiologically observable events in the DRN. However, only one agonist has been used at only one drug concentration so this may be inconclusive.

Agonist	**Site of Action**	**Effect on WAY-100635 Treated Neurone**	**Conclusion**
DOI	5-HT_2	Mimicked depolarisation	5-HT_2 mediated.
mCPP	5-$HT_{2B/C}$	No effect	Not mediated by 5-$HT_{2B/C}$
CP 93129	5-HT_{1B}	No effect	Not mediated by 5-HT_{1B}

Table 6.0 Summary of the results derived from 5-HT agonists.

The 5-HT_{1B} agonist CP 93129 (pEC_{50} = 7.8) had no action on membrane potential or input resistance. CP 93129 has been shown to cause a reduction in 5-HT release from the hippocampus in anaesthetised rats (Hjorth and Tao 1991). The present study indicates that any 5-HT_{1B} receptors present in the DRN do not mediate direct electrophysiologically observable responses in serotonergic neurones in the brain slice preparation. The 5-HT_{1B} receptor subtype has been shown to have autoreceptor properties (Limberger et al 1991). A report in the caudal raphe nucleus describes a

mechanism of autoinhibition of neuronal activity by 5-HT_{1B} receptor-mediated inhibition of glutamatergic inputs, where the 5-HT_{1B} receptor is acting via a presynaptic inhibitory effect (Li and Bayliss 1998). The present study has found that the 5-HT_{1B} receptors have no direct effect on the excitability of serotonergic neurones in the DRN.

This agonist derived data suggests the involvement of the 5-HT_2 receptors in the generation of the 5-HT-evoked depolarisation in the DRN. It also indicates that the 5-HT_{2A} receptor is more likely to be involved, as the lack of action of mCPP rules out the 5-HT_{2C} receptor.

Action of Antagonists

The broad spectrum 5-HT antagonist methysergide was applied to a number of WAY-100635 treated cells and found to have no effect on the membrane potential or input resistance. Methysergide has been reported as having agonist activity at some receptor subtypes and acts as an antagonist at a wide range of 5-HT receptor subtypes. It is also reported as having α_1-adrenergic receptor affinity where it acts as an antagonist. Pre-application of methysergide to cells showing the 5-HT-evoked depolarisation blocked the 5-HT-evoked depolarisation in six of eight cells. This confirms the response is mediated by a 5-HT receptor. Pre-application of methysergide also blocked the action of DOI confirming methysergide has antagonistic actions at the 5-HT_2 receptor subtypes in the DRN. Methysergide did not block the depolarisation in another three cells at lower drug concentrations. Methysergide has been characterised as a partial agonist in a number of preparations. For example, in the dog and rabbit saphenous vein methysergide evokes contraction. Methysergide also binds to the 5-ht_5 receptor and 5-HT_7 receptor subtypes. 5-ht_{5B} receptors have been detected in the DRN (Erlander et al 1993) and may have a role in involvement of the DRN in depression, anxiety and feeding (Plassat et al 1992; Wilkinson and Dourish 1991). Activation of this receptor could account for the inability to observe the blockade of the 5-HT-evoked depolarisation due to the activation of a hyperpolarisation mediated by the 5-ht_{5B} receptor.

Ketanserin, the well characterised 5-HT_2 antagonist, has been shown to have a number of binding sites within the DRN and cannot be used conclusively to distinguish between the 5-HT_{2A} and 5-HT_{2C} receptor subtypes (Pazos and Palacios 1985). Ketanserin was found to have no effect on the membrane potential and input resistance of cells of the DRN following treatment with WAY-100635. Ketanserin has been found to inhibit firing in the DRN by observing extracellular measurements of the single unit activity in the anaesthetised rat (Lakoski and Aghajanian 1985). This was attributed to ketanserin antagonising the effects of noradrenaline at the α_1-adrenergic receptor, well known to drive firing in the DRN. Ketanserin was also noted to neither attenuate nor potentiate any responses to 5-HT. This was attributed to the low number of 5-HT_2 binding sites in the DRN. Ketanserin blocked the 5-HT-evoked depolarisation, confirming the result indicated by the DOI experiments that 5-HT_2 receptors are involved. This also confirms that some of the ketanserin binding sites in the DRN are the result of binding to the 5-HT_2 receptor subtypes.

SB 200646 (ED_{50} > 10 at the 5-HT_{2A} receptor), selective for the 5-$HT_{2B/2C}$ receptor subtypes over the 5-HT_{2A} receptor subtype (Schreiber et al 1994), was applied to WAY-100635 treated cells and had no effect on membrane potential or input resistance. This compound may be partially blocking the 5-HT-evoked depolarisation. WAY-100635 and SB 200646 treated cells showed a slight change in membrane potential and a decrease in input resistance in response to the application of 5-HT. This could mean that the 5-HT-evoked depolarisation is the result of 5-HT interacting with a number of 5-HT_2 receptor subtypes. If this were true then the remaining response would be the result of the 5-HT_{2A} receptor portion of the 5-HT-evoked depolarisation. However, the partial blockade could also be the result of SB 200646 acting at a small number of 5-HT_{2A} receptors resulting in an incomplete block of the 5-HT-evoked depolarisation. The results observed with mCPP, where application does not cause a membrane potential change, point to the second theory.

The 5-HT_{2A} selective compound MDL 100, 907 (ED_{50} = 0.0006 at the 5-HT_{2A} receptor) had no action on WAY-100635 treated cells (Schreiber et al 1994). MDL

100, 907 completely blocked the 5-HT-evoked depolarisation. This points to the involvement of the 5-HT_{2A} receptor subtype. MDL 100, 907 has been recently characterised as an antagonist selective for the 5-HT_{2A} receptor, it is 300-fold more selective for the 5-HT_{2A} receptor over the 5-HT_{2C} receptor subtype. It has been found to block the 5-HT-evoked excitation of interneurones in the piriform nucleus when looking at the firing rate in the extracellular *in vitro* preparation (Marek and Aghajanian 1994).

Antagonist	Site of Action	Effect on Depolarisation	Conclusion
Methysergide	5-$HT_{broad\ spectrum}$	Blockade	5-HT mediated.
Ketanserin	5-HT_2	Blockade	5-HT_2 mediated
MDL 100, 907	5-HT_{2A}	Blockade	5-HT_{2A} mediated
SB200646	5-$HT_{2B/2C}$	Partial Blockade	Not 5-$HT_{2B/2C}$ mediated
Pindolol	5-$HT_{1A/1B}$	No effect	Not 5-HT_{1B} mediated

Table 6.1 Summary of the results derived from antagonists.

Finally the 5-$HT_{1A/1B}$ antagonist pindolol did not affect the membrane potential of WAY-100635 treated cells. It also did not block the 5-HT-evoked depolarisation. This rules out the 5-HT_{1B} receptor being the receptor mediating the 5-HT-evoked depolarisation. The antagonist data has suggested that the 5-HT-evoked depolarisation is mediated by the 5-HT_{2A} receptor subtype (Table 6.1).

At first sight the concentration of agonists and antagonists used throughout this study may seem high and hence be thought to show little receptor selectivity. However, this does not take into account the problems associated with the use of the interface chamber in supporting the viability of the brain slice for electrophysiology. Although the drug concentration in the chamber can accurately reach the desired level it is

much more difficult to predicate the drug concentration reaching the cell under study. It can be assumed that the concentration at the cell membrane of a drug is lower than that in the chamber due to the difficulty in diffusing through the brain slice. Using high drug concentrations ensures that the drug is reaching the cell membrane and so will have the predicated effect.

Using the isolated DR cell preparation Nooney and Kelly (1998) found that pre-application of WAY-100635 blocked the actions of 5-HT at the 5-HT_{1A} receptor. However, further 5-HT-evoked responses were not observed in the isolated cell. It can be surmised from this that the 5-HT_{2A} receptor mediating the response seen in DRN slices may be located on the cell dendrites. The process of tissue trituration involved in preparing the isolated cell tends to result in dendrite damage or removal from the cell body. A remote receptor site could also account for the small size of the response because of the effect of electrotonic distance. If the site of action of the receptor is remote from the site of electrode impalement at the cell body electrotonic decay will lead to a misleadingly smaller response being recorded. The membrane change at the point of 5-HT_{2A} action may be larger than the membrane change around the electrode at the cell body. The receptor being sited remotely from the electrode could also account for the lack of significant data on cell input resistance changes. It could be that in some cells the electrode is too remote from the site of the 5-HT effect to detect any input resistance changes whereas in some cases the electrode is closer and can pick up some resistance change. However, the membrane depolarisation is associated with an increase in cell excitability and so has the power to change the cell's activity. Also, if the depolarisation is being underestimated due to the electrotonic distance then it would probably trigger an action potential, which does not, although there is an increase in excitability. Alternatively, there is a low density of 5-HT_{2A} receptor sites in the DRN, which could account for the small voltage change of the membrane depolarisation.

The problems with the current clamp method limit the information that can be drawn form this experimental procedure. The problem of space clamp is the result of a neurone having long dendrites and axons. The electrode can only inject enough

current to clamp the cell body and the area of dendrites and axons immediately surrounding it. Therefore the complete cell membrane cannot be completely clamped throughout the neurone and its processes. This can affect the voltage response of the neurone where the receptor site and the site of membrane change are remote from the electrode. This would mean that the input resistance might not be a true reflection of the membrane activity at that remote site.

The 5-HT-evoked depolarisation appears to be mediated by the 5-HT_{2A} receptor. The size of the response has made it difficult to explore the molecular mechanism underlying the cellular effects signalled by activation of the 5-HT_{2A} receptor and the 5-HT-evoked depolarisation. If the 5-HT-evoked depolarisation is the result of changes in two ion channels as has been discussed, then the 5-HT_{2A} receptor must be linked to multiple ion channels. The 5-HT_{2A} receptor is a G_q-coupled receptor and there are a number of different receptors that show similar effects upon activation. For example, in the association and prefrontal cortex muscarinic and α_1-adrenergic receptors show a robust membrane depolarisation on chronic agonist exposure (Andrade 1991; Araneda and Andrade 1991). The ionic mechanisms underlying these responses have been more thoroughly investigated and the resulting information could be extrapolated for other G_q-coupled receptors. In these examples the membrane depolarisation resulted from the activation of non-selective cationic channels (Haj-Dahmane and Andrade 1996). This is consistent with the characteristics of the 5-HT-evoked depolarisation in the present study and it seems likely that a similar or even identical mechanism could mediate this 5-HT_{2A}-evoked depolarisation in the DRN.

The study of cortical neurones by Szabadi et al (1977) reported that the probability of observing an excitatory or inhibitory response to 5-HT was related to the firing rate of the neurone. A greater number of cells are excited by 5-HT when the cell has a low resting firing rate. In the DRN slice, cells with a high resting membrane potential showed a small inhibitory response to application of 5-HT. Likewise, with the 5-HT-evoked depolarisation, where the cell has a hyperpolarised resting membrane potential, it exhibited a larger membrane depolarisation.

There are a number of examples of 5-HT having opposing responses in many different regions of the brain. For example, in the medial pontine reticular formation 5-HT acts on 5-HT_1 and 5-HT_2 receptors by evoking a membrane hyperpolarisation and depolarisation respectively. This is due to opposing actions on different potassium conductances. These opposing responses are observed on separate populations of neurones where each cell is only able to show one response and blockade of one response does not reveal the other (Stevens et al 1992). Similarly in the nucleus prepositus hypoglossi and the rat association cortex 5-HT-evokes a hyperpolarisation, mediated by the 5-HT_{1A} receptor, and a depolarisation, mediated by the 5-HT_2 receptor (Bobker and Williams 1995; Araneda and Andrade 1991). These opposite effects are due to the manipulation of the different potassium conductances. Roberts et al (1967) observed that 5-HT could increase cell firing by an excitatory response or decrease cell firing in an inhibitory response. This has also been observed in cortical neurones where all neurones are capable of showing both hyperpolarising and depolarising responses to 5-HT (Davies et al 1987). The 5-HT-evoked depolarisation was the most commonly observed. The responses could be observed on all cells and blockade of one with an antagonist revealed the other. In the hippocampus the 5-HT_{1A} receptor mediates a hyperpolarisation with an associated decrease in input resistance and an inhibition of cell firing. Following blockade of the hyperpolarising response it is possible to observe the depolarisation, which is associated with an increase in resistance and is mediated by 5-HT_4-like receptors (Colino and Halliwell 1987). These effects are again due to opposing actions on different potassium conductances. The 5-HT_4 receptor also acts to reduce the AHP by modulating the calcium-activated potassium channels. The presence of opposing 5-HT-evoked responses in the brain either on all neurones within a brain region or separate populations of neurones within the same region is gradually becoming more commonly described. With the development of more selective antagonists the incidence of a multiplicity of 5-HT-evoked responses mediated by many 5-HT receptor subtypes in the same brain region will become widespread. The DRN now has two 5-HT-evoked actions, hyperpolarising and depolarising.

There are reports of behavioural evidence for a functional interaction between 5-HT_{1A} and 5-HT_2 receptors. A number of 5-HT_2 antagonists, ritanserin, ICI 170, 809 and ketanserin have been observed to enhance 5-HT_{1A} agonist-induced behaviour through blockade of an inhibitory 5-HT_2 receptor that regulates or is coupled to 5-HT_{1A} receptor-mediated function (Backus et al 1990). This could be explained by endogenous 5-HT exerting an inhibitory response on 5-HT_{1A} receptor function via 5-HT_2 receptors and that blockade of these receptors releases 5-HT_{1A} receptors from this inhibitory influence. There is also electrophysiological evidence of the functional relationship between 5-HT_{1A} and 5-HT_2 receptors (Lakoski and Aghajanian 1985). Therefore, it is not unusual for 5-HT to have opposing actions in the same cell population. A functional reason for a 5-HT-evoked depolarisation could be to counteract the inhibitory action of the 5-HT_{1A}-mediated hyperpolarisation and so fine tune the membrane potential to control which synaptic events the DRN neurone can respond to. This allows a neurone to integrate incoming synaptic inputs and only transmit the true synaptic input.

Ionic mechanism of the 5-HT-evoked depolarisation

The 5-HT_{2A}-evoked depolarisation was extensively investigated pharmacologically to determine the receptor mediating the response. However, the ionic mechanism underlying the response is still in doubt. Investigations performed so far indicate that the response has variable changes in input resistance. Whereas the majority of neurones showing the 5-HT_{2A}-evoked depolarisation showed a slight decrease in input resistance some showed no input resistance change and a small group of cells showed slight increases in input resistance. In the cells showing an input resistance change the response was observed to reverse in the region of -50 mV. Two possible theories to explain these observations are:

1) The 5-HT_{2A}-evoked depolarisation is due to the activation of a non-selective ion channel that allows the flow of sodium ions and the closure of a potassium channel;

2) The receptors mediating the depolarisation are found remotely from the cell body, on the dendrite for example, so that the electrode recording the membrane changes does not detect the complete response. In this case the remote site of the receptor will result in the recording electrode only detecting a small voltage change and would also indicate that the electrode is not detecting the true input resistance changes associated with the response.

The best way to determine which of these possible theories is correct is to perform a series of ion substitute experiments where the external ion concentrations of those thought to be involved in the response are manipulated. In this case the important ions are potassium and sodium. One way to perform the potassium substitute experiment is to increase the external levels to observe if this has any effect on the response. With sodium there is a large concentration of sodium ions found in the aCSF, therefore reducing the ion concentration would be the best way to observe any change to the 5-HT-evoked response.

A 5-HT-evoked hyperpolarisation

The 5-HT-evoked depolarising response was in some cases replaced by a 5-HT-evoked hyperpolarisation. Firstly, in a cell treated with WAY-100635 and methysergide where subsequent applications of 5-HT evoked a membrane hyperpolarisation. Secondly, in cells treated with WAY-100635 and mCPP, applications of 5-HT could evoke a membrane hyperpolarisation. This membrane hyperpolarisation was associated with a decrease in membrane potential and I/V analysis found that the response reversed at around -90 mV. This 5-HT-evoked hyperpolarisation could be explained in two ways, either by an incomplete block of the 5-HT_{1A} response, or it could be due to 5-HT interacting with another receptor subtype. WAY-100635 has a high potency at the 5-HT_{1A} receptor subtype and the inability to observe the return of the 5-HT_{1A} response following wash out of WAY-100635 makes the possibility of an incomplete blockade of 5-HT_{1A} receptors seem unlikely. It seems more probable that the response is due to an as yet uncharacterised

receptor. There are a number of candidates for the receptor mediating the non-5-HT_{1A}-mediated hyperpolarisation.

There have been reports of the presence of mRNA for the 5-HT_{1B} receptor in the DRN (Voigt et al 1991). Many studies have established that terminal receptors of the rat cortex are of the 5-HT_{1B} receptor subtype. There is also a significant correlation between the affinity of a drug's potency for the rat autoreceptor and its affinity for the 5-HT_{1B} receptor (Middlemiss et al 1984, 1985, 1986; Limberger et al 1991). A recent study has found that 5-HT_{1B} receptors modulate excitatory glutamatergic inputs into the caudal raphe via a presynaptic inhibitory effect (Li and Bayliss 1998). This study recorded from EPSP's in caudal raphe neurones following local electrical stimulation in the neonatal rat brainstem slice. They stimulated one neuronal cell and measured the resulting EPSP in a neighbouring neurone. This was later shown to be serotonergic using immounohistochemical staining of tryptophan hydroxylase. It is not certain if the cell targeted in stimulation is the source of the synaptic inputs and may mean that the 5-HT_{1B} receptor is found on interneurones rather than serotonergic neurones. Results from a study performed by Sprouse and Aghajanian (1987) showed that the 5-HT_{1B} agonist CP 93129 did not have any activity in the DRN. Results from the present study showed that CP 93129 had no activity in WAY-100635 treated cells. This seems to imply that the 5-HT-evoked hyperpolarisation observed in WAY-100635 treated cells is not mediated by the 5-HT_{1B} receptor.

The 5-HT_2, 5-HT_3 and 5-HT_4 receptor subtypes are more commonly associated with depolarising responses and are unlikely to mediate a 5-HT-evoked hyperpolarisation. The 5-ht_5 receptor subtypes have been difficult to characterise and study due to the lack of selective agonists and antagonists. 5-ht_{5A} may act as a terminal autoreceptor in the mouse frontal cortex (Pineyro et al 1995), but little as yet is known about 5-ht_{5B}. 5-ht_{5B} receptors have, however, been detected in the DRN (Erlander et al 1993). The 5-ht_{5A} receptors are negatively coupled to adenylyl cyclase via the G_i G-protein, this is in common with the 5-HT_1 receptor subtypes which are also negatively coupled to adenylyl cyclase, but by the G_i and G_o G-proteins. This makes the 5-ht_{5A} and the 5-

ht_{5B} receptor subtypes good candidates for mediating the 5-HT-evoked hyperpolarisation.

The 5-ht_6 receptor subtype has so far proven difficult to study, it is however positively coupled to adenylyl cyclase via the G_S G-protein making it unlikely to mediate a hyperpolarising response.
The 5-HT_7 receptor seems to be highly expressed in the rat hypothalamus and thalamus and other forebrain regions and to a lesser extent, brain regions including the dorsal raphe nucleus and the CA3 region of the hippocampus (Lovenberg et al 1993a). It is positively coupled to adenylyl cyclase via the G_S G-protein. The 5-HT_7 receptor has been associated with a number of excitatory responses. For example, a 5-HT-like response has been characterised in many smooth muscle preparations, including the 5-HT-induced relaxation of the rabbit femoral vein, cat saphenous vein, cynomolgous monkey and dog jugular vein (Eglen et al 1997). This makes it unlikely that the 5-HT_7 receptor subtype would mediate an inhibitory response in the DRN.

This may indicate that the new 5-HT-evoked hyperpolarisation is mediated by one of the 5-ht_5 receptor subtypes. The response was only observed in a small number of experiments and the lack of useful agonists and antagonists makes it difficult to conclusively characterise the nature of the receptor mediating the hyperpolarisation. Also it may be that the 5-HT-evoked hyperpolarisation is a transynaptic event, due to inputs from the local network of neurones. This can be investigated using compounds that block synaptic transmission, such as TTX, a sodium channel blocker, and bicculine and picrotoxin which block GABAergic transmission from interneurones.

Regulation of the firing pattern in the DRN neurone

5-HT can modulate the excitability of a cell by hyperpolarising or depolarising its membrane potential. 5-HT can also more directly modulate excitability by changing the firing pattern of a cell. This is brought about by the regulation of the action potential and AHP and in turn the firing frequency. This section will discuss the ionic mechanisms that regulate the spike, AHP and firing frequency and will then go onto discuss the role of 5-HT in the regulation of firing in the DRN.

The duration of the action potential

The generation of the action potential in the DRN is a well described phenomenon and involves a number of different conductances. In the DRN the action potential is the result of the activation of sodium channels, which cause a membrane depolarisation. The present study blocked the action potential with TTX, a known sodium channel blocker. This rapid depolarisation then activates a TEA-sensitive current, which causes membrane repolarisation. This is most probably due to the activity of voltage-activated potassium channels. These potassium channels would be activated with the sodium channels, but open more slowly so their effects are only dominant once the sodium channels inactivate. In the DRN the duration of the action potential can be simplified to the balance between two different ion channel conductances. The balance is between a TEA-sensitive potassium current and a voltage-activated calcium channel (Penington et al 1991). Bath-application of TEA greatly increases the duration of the action potential. This is the result of the blockade of channels that produce cell repolarisation following the action potential. This allows an extension of the calcium shoulder of the action potential.

Channel types that may contribute to cell repolarisation and the early part of the AHP include voltage-activated potassium channels and large conductance calcium-activated potassium channels, termed BK channels. TEA blocks many of the known potassium channels and it is only possible to differentiate between each channel type by gating characteristics. Voltage-activated potassium channels are a likely candidate for cell repolarisation as BK channels need high levels of calcium for activation,

which would only happen during the action potential. However, a role of the BK channel in repolarisation has been previously described in vertebrate sympathetic neurones (Adams et al 1982). The channels that mediate repolarisation allow the efflux of potassium ions lowering cystolic potassium levels making the cell more negative, this effect may contribute to the repolarising phase of the action potential. Blockade of a voltage-sensitive potassium current with TEA would be observed as an extension of the calcium dominated phase of the action potential slowing repolarisation. TEA has been observed to increase action potential duration in a number of neuronal cell types. In rat supraoptic neurones TEA increases action potential duration as it does in the acutely dissociated chick ciliary-ganglion neurones and rat locus coeruleus neurones (Kirkpatrick and Bourque 1996; Dryer et al 1991; Osmanovic and Shefner 1992).

Spike Narrowing

Narrowing of the action potential can result either from an increase in the activation of the potassium currents involved in repolarisation, from a reduction in the amount of calcium entering the cell, or a combination of both. Bath-application of TEA extended the action potential allowing more calcium to enter the cell. However, the AHP was also blocked by TEA despite the extra calcium entry.

Actions of 5-HT on the spike

In normal aCSF this study found that bath-application of 5-HT resulted in a reduction of the duration of the action potential. The narrowing may be because of a reduction of the calcium component of the action potential. 5-HT is known to inhibit calcium channels in isolated DRN neurones via activation of the 5-HT_{1A} receptor (Penington and Kelly 1990). It could be that spike narrowing is the result of reduced calcium entry. It could also be because of an increase in the activity of the TEA-sensitive potassium current which repolarises the cell. Similar actions of 5-HT have been reported in two other neuronal cell types. Ropert (1988) noted that 5-HT reduced the duration of the calcium spike in pyramidal cells of the CA1 region of the hippocampus. Scroggs and Anderson (1990) observed a 5-HT_{1A} receptor mediated

narrowing of the action potential in the bullfrog dorsal root ganglion. Both these studies concluded that this effect is the result of a 5-HT-evoked modulation of the calcium component of the action potential. Although the studies looked at the enhanced calcium component of a spike generated in the presence of TEA, the large calcium shoulder observed in the cells of the DRN allows a valid comparison with the present study.

The present study also showed that in the presence of WAY-100635, a 5-HT_{1A} antagonist, the effects of 5-HT on spike width were reduced. This is consistent with the theory of spike narrowing being due to a reduction in calcium channel activation during the action potential. However, it was also observed that WAY-100635 itself reduced spike width, although to a lesser extent that 5-HT. Several possibilities exist to explain the actions of WAY-100635; it may be acting at either calcium channels directly or via the 5-HT_{1A} receptor. WAY-100635 could be acting at some other channel including the potassium current involved in repolarising the membrane potential. If WAY-100635 is acting at the 5-HT_{1A} receptor to give spike narrowing then it is acting as a partial agonist. If this is so then WAY-100635 is not a truly "silent" 5-HT_{1A} antagonist. However, Nooney and Kelly (1998) have shown in the isolated cell that WAY-100635 has no effect on calcium channels in the DRN. This contradicts the possibility that WAY-100635 can act as a partial agonist at the 5-HT_{1A} receptor mediated modulation of calcium channels. It thus seems unlikely that WAY-100635 is acting at the 5-HT_{1A} receptor and that WAY-100635 may be causing spike narrowing by a different mechanism to 5-HT-evoked spike narrowing.

Regulation of the AHP

The generation of the rhythmical single action potential firing pattern characteristic of the DRN involves a number of intrinsic conductances. This includes the currents governing spike width, those involved in generating the AHP and those controlling AHP duration. The AHP is made up of two conductances; first the conductance generated by a TEA-sensitive potassium current that governs the amplitude of the AHP. The duration of the AHP is governed by an apamin-sensitive current, this

probably represents the activity of SK channels. Evidence for multiple conductance involvement in AHP's in other neuronal cell types was first uncovered in the experiments of Pennefather et al (1985), Fowler et al (1985) and Lancaster and Adams (1986). The present study has shown that in the DRN the AHP following a single action potential is made up of a fast and slow component.

The fast AHP

The fast AHP governs the amplitude of the AHP and is created as the membrane undershoots the resting potential due to continued activation of the TEA-sensitive potassium current. There may also be a contribution by the calcium-activated potassium channels termed BK. The fast AHP is blocked by TEA where bath-application blocks the voltage-activated potassium channels that also bring about repolarisation. This fast AHP may also be due to the action of BK channels such as in the hippocampus where the current responsible for the rapid AHP following spike generation has been termed I_C (Brown and Griffith 1983). The channel is a calcium-activated potassium channel, with low sensitivity to calcium; it controls the peak size of the AHP and is inactivated following repolarisation.

The slow AHP

The slow AHP is made up of an apamin-sensitive, calcium-activated potassium current. Apamin is known to block subtypes of the SK channels (Hugues et al 1982) suggesting that SK channels are responsible for maintaining the duration of the AHP in the DRN. The SK channel is activated following the influx of calcium during the action potential. The calcium source of the SK channel activation is as yet unclear and may be the result of release from internal calcium stores or the calcium influx evoked during the action potential. The release of calcium from internal stores can be triggered by the calcium influx and so it may be difficult to separate the two events. 5-HT modulates a calcium channel via activation of the 5-HT_{1A} receptor (Penington and Kelly 1990) where the application of 5-HT reduces the calcium current. Thus less calcium entering a cell during an action potential will activate fewer SK channels leading to a short duration AHP. The role that calcium, which enters the cell via the channels modulated by activation of the 5-HT_{1A} receptors, has in the

generation of the AHP was investigated. Modulation of this channel may account for the spike narrowing discussed earlier.

The AHP following a train of spikes

The present study has shown that TEA only blocks the fast AHP following a single spike, but does not inhibit the AHP following a train of spikes, indeed this AHP is increased by TEA, probably due to the increase spike duration and the increase in calcium entry. In contrast, only apamin block the slow AHP following a single spike, but completely blocks the AHP following a train of spikes. This may indicate that the AHP following a train of spikes has only one component and is completely due to the activity of apamin-sensitive SK channels.

Action of 5-HT on the AHP

Following bath-application of 5-HT the peak of the AHP is unchanged, however the duration is decreased so that the cell returned to its resting membrane potential more quickly. This effect was observed in-conjunction with the 5-HT_{1A}-mediated spike narrowing. The SK channels underlying the membrane potential of the late part of the AHP are calcium activated, if less calcium enters the cell during the action potential fewer SK channels will be activated. This observation may indicate that the calcium channel inhibited by the 5-HT_{1A} receptor has a role in the generation of the AHP. In the DRN high-threshold calcium channels (HVA) are activated during the action potential and allow the influx of calcium (Marrion and Tavalia 1998). 5-HT has been found to modulate HVA channels via the 5-HT_{1A} receptor in the DRN where the current amplitude is reduced and the current activation is slowed by 5-HT (Penington and Kelly 1990). As a HVA channel lengthens the action potential and 5-HT reduces it, these channels may, in part at least, be the same channels. The apamin-sensitive component of the action potential is very calcium sensitive, so that only low concentrations of calcium are needed for SK activation. The first part of the AHP is largely voltage-activated and is much less dependent on calcium for its activation; this would account for the AHP peak amplitude being unaffected even though there is less calcium entering the cell.

Alternatively, 5-HT could be acting directly on the apamin-sensitive SK channels that generate the later component of the AHP. If this were so then 5-HT-evoked spike narrowing would be unconnected to the reduction in the duration of the AHP. WAY-100635 was also found to reduce the AHP, but to a lesser extent than 5-HT. Again this could be an example of WAY-100635 showing agonist activity at the 5-HT_{1A} receptor making the observation connected to the effect WAY-100635 has on spike width. The action of WAY-100635 may also be due to the direct blockade of apamin-sensitive SK channels and is not associated with WAY-100635-evoked spike narrowing.

5-HT has been reported as having actions on the AHP in other brain regions. In the hippocampus 5-HT evokes a 5-HT_4 receptor mediated reduction in the AHP, this is brought about by an inhibition of SK channels (Chaput et al 1990). This reduced AHP increases neuronal excitation and may have a role in the regulation of neurotransmitter release and synaptic transmission (Fagni et al 1992).

The calcium source for the maintenance of the AHP

The duration of the AHP is maintained by a calcium-sensitive potassium channel, but the source of the calcium is as yet unclear in the DRN. The neurones of the DRN have been observed to have a number of calcium channels namely HVA channels which can be inhibited by 5-HT, and LVA channels which are unaffected by 5-HT. Calcium channels modulate the intracellular calcium concentration.

Noradrenaline has been reported to augment the AHP due to the mobilisation of internal calcium stores (Pan et al 1993). Therefore, neurones of the DRN do possess intracellular calcium stores that can be triggered to release calcium by neurotransmitter binding. These intracellular stores are commonly found in the endoplasmic reticulum and are coupled to the IP_3 signal that opens calcium-release channels. Calcium release can be evoked by second messenger activation, but can also be observed following an increase in the cystolic calcium concentration through increased calcium entry across the cell membrane. There is also evidence that 5-HT

can increase intracellular calcium and the accumulation of cAMP (Ebersole et al 1993) in the cultured vascular smooth muscle cells derived from the basilar artery. This group found that 5-HT-evokes an increase in intracellular calcium, firstly because of intracellular release of calcium from internal stores and secondly a sustained influx of extracellular calcium resulting in muscular contraction. This appears to be mediated by a 5-HT_1-like receptor other than the 5-HT_{1A} receptor. The observations that 5-HT can inhibit the AHP is in contrast to the work with noradrenaline in the DRN. Application of noradrenaline or the α_1-agonist, phenylephrine, not only caused a membrane depolarisation, but also augmented the duration of the AHP so that the cell took longer to return to its membrane potential (Freedman and Aghajanian 1987). Both these effects appear to be due to enhancement of the apamin-sensitive component of the AHP. The α_1-adrenoceptors are positively coupled to phosphoinositide turnover (Brown et al 1984) which can lead to a mobilisation of internal calcium stores (Pan et al 1994). This results in the increase of the concentration of intracellular calcium, which may lead to an enhancement of the apamin-sensitive, calcium activated SK channels. This may serve to regulate the activity of neurones during noradrenaline-evoked depolarisation; this is also mediated by the α_1-adrenoceptor (Yoshimura et al 1985).

The neurotransmitter noradrenaline, through the activation of the α_1-adrenoceptor, has been shown to modulate a number of potassium conductances in the DRN (Pan et al 1993). Noradrenaline acting via α_1-adrenoceptors increased the size of the fast potassium-current, I_C. Noradrenaline can also evoke a membrane depolarisation associated with an increase in input resistance due to the closure of potassium channels. This results in an increase in cell firing due to a membrane depolarisation, but also an inhibition of cell firing frequency due to the increase in size and duration of the AHP. Pan et al (1993) concluded that the manipulation of the AHP by noradrenaline might serve to regulate cell activity during the membrane depolarisation.

5-HT has the opposite effect to noradrenaline, its causes a membrane hyperpolarisation and decreases the duration of the AHP by increasing the rate of decay. This is probably due to the modulation of the calcium channel mediated by 5-HT_{1A} receptors. However, the 5-HT modulation of the SK channels directly cannot be ruled out. This may serve to regulate the activity of neurones during 5-HT_{1A}-evoked hyperpolarisation. These opposing actions may be important in maintaining the rhythmical firing pattern of a cell showing pacemaker activity allowing it to keep its regular firing pattern.

Firing frequency

The AHP can regulate firing frequency by delaying the return to the threshold membrane potential, the AHP is modulated by 5-HT and so can modulate firing frequency. Further to this, 5-HT can modulate firing frequency in other ways. Bath-application of 5-HT increased the number of action potentials fired during a long depolarising current step and decreased the AHP. It may be that WAY-100635 can block the 5-HT-evoked increase in firing rate. However, the AHP was reduced by 5-HT application. This indicates that the activation of the 5-HT_{1A} receptors modulates the firing rate of DR neurones. However, the AHP created following a train of spikes seems to be unaffected by the 5-HT_{1A} receptor blockade. There is other evidence for the AHP following a train of action potentials being different from that following a single spike. This is in contrast to the AHP that follows a single action potential, which decays more quickly in the presence of 5-HT, the effect is blocked by pre-application of WAY-100635. This could mean that the calcium channels modulated by the 5-HT_{1A} receptor have only a minor or no role in the maintenance of the AHP following a train of action potentials. In conclusion, 5-HT can increase the firing frequency of a train of action potentials by a 5-HT_{1A} mediated action which may be independent of the 5-HT-evoked spike narrowing and AHP reduction observed in the single spike situation.

Burst firing

DRN neurones do not typically show spike accommodation, adaptation or burst firing, all properties consistent with a neurone that shows pacemaker activity. Following bath-application of TEA the firing rate of the neurone increased and the cell showed adaptation. This shows that a TEA-sensitive potassium current is important in regulating spike frequency.

Apamin-sensitive SK channels have been identified as important in the regular, single spike firing pattern characteristic of the pacemaker activity observed in the cells of the DRN. Blockade of the SK channels with apamin changed the cell-firing pattern. A DRN neurone normally responds to a long depolarising current step with a series of regularly spaced action potentials. Following exposure to apamin, DRN neurones fired a high frequency train of action potentials that resembled a burst. The train of action potentials had a number of characteristics. Spike height was reduced in each successive spike. There was also a progressive increase in the interspike interval. This is accommodation and does not normally occur in the absence of apamin. DRN neurones also showed adaptation, the cessation of firing during the injected current pulse, in the presence of apamin. This is characteristic of a burst firing pattern. A small number of DRN neurones show burst firing and the SK channels may act as the switch between the two states. When the SK channels are active a cell fires a regular firing pattern, when blocked the cells fires action potentials in a burst. Burst firing may be an important mechanism for increasing the amount of 5-HT released, as burst firing is an efficient mechanism to release large amounts of transmitter.

The ability to show firing accommodation and adaptation was also investigated with TEA. Following the application of TEA cells showed an increase in cell firing frequency and firing adaptation, but not accommodation. The late phase of the AHP was also inhibited so that the cell returned to its resting membrane potential more quickly. This is in contrast to the observations seen in the single spike. This may

indicate that the mechanisms involved in generating the AHP following a single spike are different to those following a train of spikes.

The interspike period allows the cell time to remove sodium ions, which flood into the cell during the action potential, and the transport of potassium ions into the cell to recover the resting ion concentrations. This period also allows time for the sodium channel to recover from inactivation. This allows the cell to show a repetitive-firing pattern for long periods of time without rundown, which is also important for pacemaker activity.

In cells of cultured skeletal muscle a long lasting AHP allows prolonged and regular repetitive discharge to a constant depolarising current by clamping the membrane to negative potentials between each action potential, this the allows sodium channels enough time to recover from inactivation (Barrett et al 1981). By blocking the opening of SK channels with apamin to give an AHP with a reduced duration the DRN neurone was observed to no longer demonstrate pacemaker-like activity. The apamin treated neurone could not generate its typical train of regularly firing action potentials in response to a long depolarising current step. The DRN neurones fire a burst of high frequency action potentials and then became silent. The control of repetitive discharge of spikes has also been described *in vitro* in bullfrog sympathetic ganglion cells (Pennefather et al 1985), in guinea pig neurones of the olfactory cortex (Constanti and Simm 1987), in rat hippocampal neurones (Lancaster and Nicoll 1987) and in the neurones of the sensorimotor cortex of the cat (Schwindt et al 1988). This is similar to that observed in the DRN where the SK channels are blocked with apamin. This indicates that SK channels are involved in regulating firing frequency in the DRN as in other neuronal cell types.

There is evidence for a low level of burst firing within the DRN although the single regular spike associated with pacemaker activity is more commonly observed. An electrophysiological study performed *in vivo* by Wang and Aghajanian (1982) using the extracellular technique found evidence for spike trains in the anaesthetised rat. They found that these trains were made up of pairs of spikes with a short time

interval. They assumed this was the result of two closely positioned cells firing in synchrony. More recent work, also performed in the anaesthetised rat, suggests this evidence may be due to one cell firing a burst of two action potentials (Hajos et al 1995). They noticed that a sub-population of cells fired two (sometimes three or four) spikes in quick succession and that each subsequent spike was smaller in amplitude. They also found that any cell firing trains of spikes did not change to firing single spike or vice versa. This resembles the cell when under the influence of apamin indicating that the blockade of apamin-sensitive SK channels may act as a switch between a cell with a single and regular action potential firing pattern to a cell with a burst firing pattern. This study has also shown that 5-HT can modulate the SK channel and so may be part of the switching mechanism.

The importance of the burst firing pattern, the interburst interval and the excitation-coupled release of neuropeptide has been investigated in the isolated rat neural lobe (Cazalis et al 1985). They found that the interspike intervals in a burst and the duration of silent periods between bursts are both important determinants of the effectiveness of a burst pattern in promoting neuropeptide, in this case vasopressin, release. Cells firing spikes with an interburst interval released more vasopressin than when firing a similarly sized burst of spikes with no interburst interval. This means that the amount of neurotransmitter or hormone released from a cell can be determined in a number of ways and not just from the number of spikes. The regular firing of a single action potential gives a tonic release of transmitter from DRN neurone. Burst firing and the pattern of burst firing may be a mechanism for releasing extra 5-HT or altering the pattern of 5-HT release. Cazalis et al (1985) also showed that most neuropeptide is released in the early part of the burst. This corresponds to the evidence of burst firing in the DRN where each successive action potential is shorter in amplitude.

Functional aspects of 5-HT's complexity of actions

5-HT has now been demonstrated to have a range of effects in the DR neurone:

- A 5-HT_{1A}-mediated membrane hyperpolarisation (Aghajanian and VanderMaelen 1982);
- A 5-HT_{1A}-mediated inhibition of calcium currents (Penington and Kelly 1990);
- A 5-HT_{2A}-mediated membrane depolarisation;
- A 5-HT-evoked inhibition of the AHP;
- A 5-HT-evoked enhancement of firing frequency.

However the physiological function of this range of 5-HT-evoked actions is unclear. A study by Andrade and Nicoll (1987) first highlighted the complex series of actions of 5-HT in the pyramidal neurone of the CA1 region of the hippocampus. They noticed 5-HT had multiple effects including a 5-HT_{1A}-mediated hyperpolarisation leading to a 5-HT-evoked depolarisation, both due to activity at different potassium channels and a 5-HT-evoked blockade of the AHP. The blockade of the AHP by 5-HT reduced spike frequency adaptation and counteracted the inhibitory action of the 5-HT_{1A}-mediated hyperpolarisation. From these observations Andrade and Nicoll came to believe that 5-HT can influence the activity of a neurone in subtle ways leading to a pattern of activity that is both intensity and time dependent. 5-HT may be acting to control the way pyramidal neurones integrate incoming synaptic inputs. In other words the multiple actions of 5-HT allow pyramidal neurones to pass on only significant synaptic inputs.

In a more recent study by Bayliss et al (1997) the effects of 5-HT on caudal raphe neurones was investigated paying particular attention to calcium channels and the AHP. Their data indicated that 5-HT can inhibit N- and P/Q-type calcium currents mediated by the 5-HT_{1A} receptor via pertussis-sensitive G-proteins. They also showed that 5-HT could cause a decrease in the spike AHP and an enhancement of the repetitive firing response to injected current pulses. They concluded that the physiological significance of the decrease in the AHP and increase in the firing

response to current inputs evoked by 5-HT would be to enhance the effects of brief supra-threshold synaptic inputs. They also thought that this effect coupled with the 5-HT_{1A}-evoked hyperpolarisation, which would gate out smaller synaptic inputs by moving the cell away from the threshold potential, would be to enhance strong synaptic inputs and inhibit weak synaptic inputs onto the caudal raphe neurone. In effect, the actions of 5-HT on the caudal raphe neurone would be to increase the signal-to-noise ratio of the synaptic input-firing frequency transfer function. The observations of the actions of 5-HT in the caudal raphe neurone are similar to those observed in the DRN neurone. The DRN neurone shows a 5-HT_{1A}-mediated membrane hyperpolarisation and inhibition of calcium currents. There is 5-HT_{2A}-evoked depolarisation in the DRN neurone. 5-HT can also decrease the duration of the AHP and increase firing frequency. It maybe that the 5-HT_{1A}-mediations of 5-HT are not to simply inhibit cell firing, but are indeed to filter out background synaptic inputs which should generate postsynaptic events. The 5-HT_{2A}-mediated depolarisation may be acting to enhance the intermediate synaptic event. Overall, the combined actions of 5-HT ensure to control which synaptic events the DRN neurones can respond to, so that that a neurone can only respond to true synaptic inputs.

Appendix A

The receptor binding profile of WAY-100635. Competitive studies were performed to calculate the K_i values which were displayed as means ± S. E. of at least two experiments.

Receptor	**Affinity values (K_i, nM) for WAY-100635**
5-HT_{1A}	0.24 ± 0.01
α_1	45 ± 3
α_2	> 1000
β	> 1000
D_{2A}	79 ± 5
D_3	67 ± 1
5-HT_{2A}	1100 ± 150
Muscarinic	> 1000
Nicotinic	> 10000
D_1	> 1000
Histamine H_1	> 10000
Histamine H_2	> 10000
$GABA_A$	> 10000
NMDA	> 10000
AMPA	> 10000
Benzodiazepine	> 10000
Galanin	> 2600

Adapted from Johansson et al (1987).

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