THE ROLE OF
5-HYDROXYTRYPTAMINE RECEPTORS
IN THE CONTROL OF MICTURITION

By

Katharine Emma Read

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Abstract

Central 5-HT containing neurones, via activation of 5-HT$_{1A}$ receptors, play an important role in the facilitation of parasympathetic outflow to the bladder. However, there are no reports on the role of 5-HT$_7$ receptors in the reflex control of micturition, even though many non-selective 5-HT receptor ligands have been shown to bind to 5-HT$_7$ receptors, and have both excitatory and inhibitory effects on micturition. Using selective 5-HT$_7$ receptor antagonists SB-269970 and SB-656104, this work demonstrates an important physiological role for supraspinal, but not spinal, 5-HT$_7$ receptors in the control of micturition in the urethane anaesthetized rat. Blocking supraspinal 5-HT$_7$ receptors, micturition is inhibited, with an increased volume and pressure required in the bladder to initiate micturition, but no significant effects on urethral function. In addition these data show that central administration of the 5-HT$_2C$ receptor antagonist mesulergine, which binds with high affinity to the 5-HT$_7$ receptor, also inhibits the micturition reflex, but only at a high dose. However, these data do not support a role for the 5-HT$_2C$ receptor in the control of micturition, as demonstrated using the selective 5-HT$_2C$ receptor antagonist SB-243213.

There are no selective agonists for the 5-HT$_7$ receptor. However, 5-CT can be used to activate 5-HT$_7$ receptors in mice and guinea pigs to induce hypothermia, which can be inhibited by selective 5-HT$_7$ receptor antagonist SB-269970, but not by WAY-100635 or GR127935. Therefore, central administration of 5-CT was used to facilitate micturition, via 5-HT$_7$ receptor activation, in the anaesthetized rat. These data demonstrate that facilitation of micturition was possible using 5-CT, but only in the presence of the selective 5-HT$_{1B/1D}$ receptor antagonist GR127935. Therefore these data further support a facilitatory role for the 5-HT$_7$ receptor, but also an inhibitory role for 5-HT$_{1B/1D}$ receptor in the control of micturition, which was further confirmed using selective 5-HT$_{1B/1D}$ receptor agonist sumatriptan and 5-HT$_{1B}$ receptor agonist CP93,129, which both inhibited micturition. Overall this thesis further characterises the functional role of 5-HT receptors in the control of micturition.
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Chapter One

Introduction
Chapter One: Introduction

1 Introduction

Urine storage and its intermittent elimination (micturition) is a highly complex function in both animals and humans. A combination of central and peripheral neuronal pathways control micturition and are dependent on the functional activity of two components - the bladder and the urethra, which together comprise the lower urinary tract (LUT). Micturition is an essential mechanism that helps to preserve homeostasis, allowing waste products, foreign compounds, excess water and ions to be excreted. In addition, many species also use urine for territorial demarcation or sexual attraction, which makes micturition an important behavioural process (Blok & Holstege, 1998). In humans, controlling micturition is a learned activity. For human infants micturition is initiated when the bladder fills and a threshold is reached, occurring regardless of time or place, as an involuntary reflex. As children get older, micturition needs to be more controlled for both health and social reasons. Therefore, children are trained into controlling micturition and it becomes a reflex initiated by the cerebral cortex (see de Groat et al., 1993). This makes micturition a fairly unique process as few visceral structures, such as the bladder and urethra, are under voluntary control. The LUT is also unique as its function is totally dependent on the central nervous system control, where other visceral structures such as the heart or gastrointestinal tract can maintain some function even after extrinsic denervation. There are numerous neurotransmitters involved in the complex control of the LUT and these monoamines, amino acids and peptides provide clinical targets for treatment of LUT disorders both peripherally and centrally. This thesis will concentrate on one neurotransmitter 5-Hydroxytryptamine (5-HT), and the role that it plays in the central control of micturition.
1.1 The lower urinary tract

There are two functional units of the LUT, the bladder, which acts as a reservoir for urine, and the outlet to the bladder, the bladder neck, plus the smooth and striated muscles of the urethra. In rat, and other species, the bladder lies in the pelvic cradle ventral to the rectum (and also to uterus, cervix and vagina in the female). The kidneys feed urine to the lower region of the bladder, via the ureters, which enter the bladder on the dorsal wall. Below the vesico-ureteric junction the bladder narrows to form the bladder neck, which in turn extends as a tubular structure called the urethra, through which urine is expelled. The function of the bladder is to store urine until a suitable disposal time is available. The role of the bladder neck and the smooth and striated muscles of the urethra is to provide a controllable ‘stopper’ to the bladder to maintain continence when necessary and to allow free passage of urine during voiding (see Gosling & Dixon, 1987; see Elbadawi, 1987).

1.1.1 The bladder

There are two distinct regions of the bladder, the detrusor and the trigone. In most species the bladder body or detrusor is composed of numerous smooth muscle bundles that are interwoven and running in a variety of directions, creating a strong expandable meshwork of smooth muscle (Gosling & Dixon, 1975). There are no discrete layers of muscle as such, but in the outer muscle coat the bundles tend to run longitudinally compared to deeper within the muscle, particularly in the cat bladder (Gosling & Dixon, 1975). Muscle bundles are heterogeneous in size and made up of smooth muscle cells closely packed with connective tissue, microfilaments, blood vessels (capillaries), small elastic fibres and collagen fibrils, which are also in abundance beneath the epithelium.
and in the serosal layers of the bladder (Gabella & Uvelius, 1990). This structural arrangement is ideal for reducing the size of the bladder upon contraction of the intramural muscle coat (see Gosling, 1979) to void urine out efficiently, and also allow the bladder to have increased distensibility upon filling (Gabella & Uvelius, 1990). The two distinct regions of the bladder, the detrusor and the trigone are formed from different embryonic cell types and have different characteristics and functions (see Shafik, 1998). The trigone is a triangular area of the posterior bladder wall extending between the vesical orifices of the ureters and the internal urethral meatus. One part of the trigone, previously termed the deep trigonal muscle, is no different morphologically than the detrusor itself (see Gosling, 1979). The superficial trigone is morphologically distinct from the detrusor, comprising a thin layer of smaller muscle bundles, of which the component muscle cells are without acetylycholinesterase (see Gosling, 1979) and are situated internal to the deep trigonal area. This thin muscular area extends to the proximal urethra in both sexes and also between the vesico-uteric junctions, where the ureters join the bladder. Although the trigone is now considered to be unimportant in control of micturition (see Shafik, 1998), some investigators think that it has significant control of the vesical orifices of the ureters, preventing vesico-ureteral reflux during micturition (Elbadawi, 1982). However, this is still under debate as there is evidence that during voiding a ‘vesicoureteral junction reflex’ is evoked, and it is this that is responsible for preventing vesico-ureteral reflux and efflux (Shafik, 1996).

1.1.2 The urethra and bladder neck

In the bladder neck and proximal urethra there is a change in the arrangement and properties of the smooth muscle compared to the body of the bladder. The muscle cells
are arranged in small groups, not bundles, and are separated by amounts of connective tissue (see Gosling & Dixon, 1975). In the human male bladder neck a complete collar of circular smooth muscle extends distally, totally surrounding the proximal urethra. In the female bladder neck the circular smooth muscle is not as prominent but the smooth muscle is comprised of small muscle bundles that run obliquely or in a longitudinal course within the urethral wall (see Gosling, 1979). Interestingly, this area of circular smooth muscle in the bladder neck and proximal urethra has been the subject of much debate and is thought to act as an internal urethral sphincter, although anatomically it is not a distinct structure (Krane & Olsen, 1973). Indeed, Kleeman (1970) showed that blocking the nerve supply of the striated muscle of the external urethral sphincter, or using striated muscle relaxants did not cause urinary incontinence, where the internal sphincter was intact, in both sexes.

In humans, the gender differences between urethral structures are marked, mainly due to the dual function of the penis as a reproductive organ. In the male, urethral divisions can be made between the bladder neck, the prostatic region, the membranous region, and the bulbar and penile sections. The bladder neck, prostatic and membranous regions are the only areas significantly involved in urinary continence (see Brading, 1999). Smooth muscle (circular and longitudinal) extends up to the end of the membranous urethra. The female urethra is shorter than the male and less varying in structure. The entire length of the female urethra consists of smooth muscle made up of both circular and longitudinal types. The inner most layer of the urethra is the mucosa and this is surrounded by a thick layer of submucosa, rich in vasculature (Huisman, 1983). The longitudinal smooth muscle layer surrounding the submucosa is continuous with the inner longitudinal coat of the bladder, and forms the greatest part of the
urethral wall. This is then further surrounded by a thin layer of circular smooth muscle (see Gosling, 1985).

1.1.3 The external urethral sphincter

The rhabdosphincter or external urethral sphincter (EUS) is a well-developed skeletal or striated muscle structure that encircles the inner urethral layers. In both sexes this striated muscle is distinct from the periurethral skeletal muscle of the pelvic floor (see Brading, 1999). Turner-Warwick (1975) showed that in males the EUS could maintain continence even when the internal sphincter has been damaged. In the human male the striated muscle extends from the base of the bladder and anterior aspect of the prostate, along the full length of the membranous urethra and is orientated circularly, according to Gosling (1979), to completely surround the smooth muscle to form the external urethral sphincter. This is now thought to be the case for young people, with older people having more of a horse shoe shape surrounding the smooth muscle, with the posterior deficiency filled with circular smooth muscle and elastic fibres (see Brading, 1999). Gosling (1979) functionally classified these striated fibres as ‘slow twitch’, with them being able to sustain prolonged periods of contraction and maintain the muscle tone required for continence. However, further evidence shows that the fibres are more heterogeneous (Benoit et al., 1988) and in human males the population consists of 65% fast twitch and 35% slow (Ho et al., 1997). This heterogeneity has also been found in the female EUS muscle fibre population with 13% being found to be fast twitch fibres (Ho et al., 1997).
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The female urethral striated muscle extends from the proximal urethra distally in the human, and also lies in a circular orientation around the urethral smooth muscle. The middle third of the human female EUS is thickest, although this varies between species (see Brading, 1999). The muscle is horse-shoe shaped at the proximal and distal areas of the urethra where its posterior portion fuses with the connective tissue of the anterior vaginal wall (see Gosling, 1985).

The pelvic floor should not be overlooked as an extrinsic structure that aids the LUT in some functions. The peri-urethral levator ani striated muscle – an important part of the pelvic floor musculature, is formed mainly of large diameter fibres, a number of which are ‘fast-twitch’ and are able to contract rapidly, but only over short periods of time. These muscle fibres are important in maintaining continence by contracting to cause increased urethral resistance during large, rapid increase in intra-abdominal pressure during activities such as coughing or sneezing (see Brading, 1999).

1.2 The mechanics of micturition

1.2.1 Urine storage

The urinary bladder, acts as a reservoir to store urine but its behaviour during filling is constrained by the requirements of normal kidney function, which is to keep intravesical bladder pressure low to ensure the low filtration pressure at the glomerulus, in the kidney (see Brading, 1997). The storage element of the micturition reflex is somewhat controversial and there are two main theories as to how the bladder is able to store urine in the way that it does. The myogenic theory proposes that the physical
properties of the bladder wall result in the bladder being able to accommodate increasing amounts of urine, independently of neural activity, whereas the neurogenic theory proposes that tone and accommodation of the bladder reflect neural inhibition or stimulation via sympathetic and parasympathetic neurons.

1.2.1.1 Myogenic theory

The bladder wall consists of collagen, elastin and smooth muscle as well as epithelium, nerves and blood vessels. During urine storage the bladder maintains a low intravesical pressure during filling up to its physiological capacity, and this adaptation to maintain a low pressure is referred to as accommodation or compliance (see Finkbeiner, 1999). The interaction between the passive elements making up the bladder wall, i.e. collagen, elastin and fibronectin and the active elements i.e. smooth muscle, are crucial in determining the compliance within the tissue. If visceral smooth muscle is stretched it initially exerts increased tension, which gradually decreases and may relax below the level before being stretched. This is a phenomenon called stress-relaxation, which allows the bladder to accommodate increasing volumes of urine without increasing the intravesical pressure (see Finkbeiner 1999). The connective tissue within the bladder is also important in the filling phase of the bladder as it is thought that functional bladder capacity is in part defined as that volume where connective tissue impedes any further smooth muscle stretch or accommodation (Baskin et al., 1993).

The inherent properties of the smooth muscle cells of the bladder are of importance when examining the storage function of the bladder. Bladder smooth muscle is somewhat different from other types of smooth muscle in the way the cells are coupled together. During filling the smooth muscle cell activity is not synchronous as each
smooth muscle cell is coupled to only a few of its neighbours, and extensive coupling between bundles does not exist. Smooth muscle can be categorized as unitary or multi-unit. Multi-unit smooth muscle is loosely arranged cellular units, without intracellular bridges, that need nerve impulses to initiate contraction. They are not sensitive to quick stretch, or spontaneously rhythmic and have rapid, discreet and localized contractions. Unitary smooth muscles are closely packed in a parallel manner, with low resistance bridges between cells. They are capable of spontaneously contracting, and have inter-fibre conduction; they are activated by quick stretch and can elicit rhythmic contractions even after extrinsic denervation. The bladder smooth muscle combines properties of both smooth muscle types, with morphology and response to stretch resembling unitary smooth muscle, but resembling multi-unit smooth muscle in terms of activation and control (see Finkbeiner, 1999).

All of these physical properties of the bladder in combination allow the bladder to accommodate increasing volumes of urine and maintain a low intravesical pressure. In fact, the response of the bladder to filling is unchanged following parasympathectomy, sympathectomy or complete denervation (Klevmark, 1974, 1977) indicating the key role these properties play in bladder accommodation to increasing amounts of urine. Compliance of the bladder tissue however can be affected by a number of factors. The rate at which the bladder is filled has been shown to affect the compliance of the bladder. Studies have shown that rapid filling or stretch of the bladder smooth muscle will cause a higher and more rapid increase in bladder pressure curve compared to slower filling (Coolsaet, 1985), which has implications for clinicians studying cystometrograms of their patients. The underlying tone of the smooth muscle also affects the overall compliance of the bladder tissue. The underlying tone of the smooth
muscle does not depend on intact neural innervation, although nerves can modify tone (Tang et al., 1955). Therefore any stretch of the smooth muscle in addition to the natural tone will be superimposed on the tonic contraction pattern of smooth muscle. Bladder smooth muscle cells are not electrically silent during storage, and show spontaneous action potentials, like pacemaker cells, as well as slower waves of depolarisation (Ursillo, 1961). Spontaneous detrusor smooth muscle activity, which is common in normal bladders, is thought to be an important target in bladder dysfunctions such as detrusor instability and urge incontinence, where the myogenic properties of the bladder have altered. Spontaneous activity can cause sustained or intermittent changes in bladder wall contractility, which may in turn cause dysfunction of the LUT.

1.2.1.2 Neurogenic theory

Animal studies have shown that the sympathetic drive to the bladder is tonically active during the bladder storage phase (de Groat & Lalley, 1972) and can inhibit bladder contractions and transmission in parasympathetic ganglia in the bladder wall of cats, as well as causing increasing urethral resistance (de Groat & Theobald, 1976). This allows the bladder to store increasing amounts of urine. Afferent vesical activity travelling in the pelvic nerves from the bladder carries information to the spinal cord regarding the level of bladder distension. If the bladder has below micturition threshold volumes of urine present, an intersegmental spinal reflex, triggered by this vesical activity in the pelvic nerves, inhibits bladder activity (de Groat & Lalley, 1972). Sympathetic drive to the bladder can be thought of as a negative feedback loop, in which distension of the bladder leads to the reflex suppression of bladder activity, thus allowing the bladder to accommodate more. De Groat & Saum (1972) showed that inhibition of bladder
activity is mediated by two distinct mechanisms; 1) a direct inhibition of bladder smooth muscle by activation of the β-adrenoceptors which predominate in the bladder body, and 2) an inhibition of transmission in parasympathetic ganglion cells mediated via α-adrenoceptors. It is widely regarded that both myogenic properties of the bladder itself and the neurogenic influences on the bladder work in combination to allow the bladder to accommodate increasing volumes of urine.

1.3 Innervation of the LUT

The LUT is innervated by the parasympathetic, sympathetic and somatic nervous systems, each contributing in a different way to the control of micturition (see Lincoln & Burnstock, 1993). The parasympathetic nerves supplying the bladder are carried in the pelvic nerve, the sympathetic nerves supplying the urethra are carried in the hypogastric nerves and the somatic nerves carrying the striated muscle innervation are carried in the pudendal nerves. These nerves arise from various places in the central nervous system and understanding the pattern of the LUT innervation can provide much information on the nature of functionality in the periphery and possible targets for treatments of LUT disorders.
Figure 1.1 Schematic representation of the innervation of the lower urinary tract. Modified from Lincoln & Burnstock (1993)
1.3.1 Parasympathetic nervous system

The pelvic nerves arise from the sacral parasympathetic nucleus (SPN) and using axonal tracing and electrophysiological techniques, parasympathetic preganglionic neurons have been localised in the SPN in the intermediolateral grey matter of the spinal cord in various species. There is slight variation in the exact segments of spinal cord from which the nerves arise, e.g. humans S2-S4 region, cats and monkeys S1-S3 region and rats L6-S1 region (see de Groat et al., 1993), but all neurons are cholinergic in nature. A prominent feature of the sacral preganglionic neurons is their extensive axon collaterals, which are thought to be important in integrating function in the spinal cord, especially in the case of micturition (de Groat, 1976). The parasympathetic preganglionic neurones terminate in the pelvic plexus, which lies very close to the target organs of the bladder and urethra. From there postganglionic fibres emerge, which structurally possess few or no dendrites (see Keast 1999), to innervate the LUT.

Interestingly, there is a particular viscerotopic spatial organisation of preganglionic neurones in the cat SPN. It has been shown that the dorsal and ventral cell groups that make up the SPN, innervate the colon (a more dorsal organ) while the ventral group innervate the bladder – a more ventral organ (de Groat et al., 1981, 1982). However, this type of anatomical division has not been found in the rat (Nadelhaft & Booth, 1984). Early work carried out by Gianunuzzi (1863) showed that electrical stimulation of sacral (pelvic) nerves caused a contraction of the dog bladder. The contractions elicited were more powerful than those due to stimulation of nerves arising from lumbar spinal segments...
(hypogastric nerves). Later work by Barrington (1915, 1931 & 1941) demonstrated the importance of the pelvic nerves in the control of micturition. He noted that sensory afferent activity arising from the bladder was carried in the pelvic nerves, as well showing that efferent parasympathetic activity, resulting in bladder contraction, was also carried in these nerves. The main excitatory input to the bladder is through parasympathetic innervation and the integrity of parasympathetic innervation is essential for the normal performance of micturition (see Langworthy et al., 1940). In addition, Barrington showed that stimulation of the pelvic nerves also caused marked relaxation to the urethra in cats and the efferent nerve activity to evoke that response was carried in the pelvic nerves urethra (Barrington, 1925, 1931). McGuire & Herlihy (1978) have since confirmed these early findings that parasympathetic nerve stimulation causes a relaxation in urethral pressure, and this is in part mediated via β-adrenoceptors, as the effects were blocked by propranolol. This provides one theory as to the mechanism behind the relaxation of the bladder neck and proximal urethra at the onset of voiding. There are also findings that indicate a role for cholinergic innervation causing contraction of the urethra, in the isolated cat LUT preparation (Persson & Andersson, 1976), although it is uncertain how this contributes to urine flow through the urethra. Therefore, the main role of the parasympathetic nervous system is in the voiding phase of micturition as it provides excitatory input to the bladder and has been shown to cause relaxation of the bladder neck and reduction in urethral pressure necessary for the passing of urine. Parasympathetic outflow is inhibited during the storage phase of micturition.
1.3.2 Pelvic plexus

The pelvic plexus is a bilateral ‘nerve relay station’ consisting of an interlacing network of neurons and embedded ganglia, which lies close to pelvic viscera, and differs considerably in size, complexity and location between sexes and species. The plexus is innervated by a collection of sympathetic and parasympathetic neurons arising in distinct regions of the spinal cord (Kuntz & Moseley, 1936). The hypogastric nerve carries sympathetic postganglionic innervation from the thoracolumbar spinal cord to the plexus and the pelvic nerve carries parasympathetic preganglionic fibres arising from the lower lumbar and sacral spinal cord segments to ganglion cells within the plexus (Dail, 1996). These two groups of nerves are identifiable and discrete and enter the ganglia separately, from where they send off numerous branches (see Keast, 1999).

The pelvic plexus is arranged in a similar manner in cat, dog, rabbit, macaque monkey and man, situated on the lateral aspect of the rectum. In the cat and dog the plexus is a dense structure compared to the rabbit and macaque, where the plexus occupies a larger area within the pelvis. Unsurprisingly, in man the plexus is the most complicated in structure and sits in a horseshoe shape surrounding the rectum (Wozniak & Skowronska, 1967). Rats and mice have the simplest anatomical arrangement of pelvic ganglia with a concentrate of ganglia rather than a broadly scattered arrangement (Langworthy, 1965). In male rats the plexus is a multilobulated structure with numerous nerve branches entering and leaving, innervating not only the LUT, but the sex organs and the rectum also. In female rats the pelvic plexus is a much finer and more dispersed structure than in the male (Langworthy 1965) and has significantly fewer cells compared to the male, 6000 versus
15,000 respectively (Greewnwood et al., 1985). In some species microscopic ganglia are scattered in the plexus and also found on the serosal surface of the LUT musculature itself e.g. guinea pigs (Crowe et al., 1986), cat (Dixon & Gosling, 1974) and man (Gilpin et al., 1983), and these are referred to as 'intramural' or vesical ganglia. However, intramural ganglia are not present in the rat bladder (Gabella & Uvelius, 1990). Instead, macroscopic collections of ganglion cells exist bilaterally and in the male rat each side of the pelvic plexus is marked by a single large ganglion called the major pelvic ganglion (MPG; Langworthy, 1965) with 'accessory ganglia' occurring around the ureter and prostatic end of the vas deferens (Keast et al., 1989). In female rats the collection of ganglion cells is called paracervical or 'Frankenhausers ganglion' (see Keast, 1999).

Unusually, the pelvic plexus contains both sympathetic and parasympathetic neurons (Kuntz & Mosely, 1936), and close interaction between the two nerve sets has been demonstrated in the vesical ganglia in cats (de Groat & Saum, 1972). Typically these nerve groups are not found in the same ganglion, which illustrates the importance of the role of the pelvic plexus in the co-ordination and integration of LUT function.

1.3.3 Sympathetic nervous system

Cell bodies of the sympathetic preganglionic neurons innervating the bladder and urethra are found in the intermediolateral nucleus of the spinal cord (Kuru, 1965), with nerves arising from T11 to L5, depending on species. In rats the sympathetic nerves arise from the L1-L2 region (Nadelhaft & Vera, 1991), whereas in cats they arise from L3-L5 region.
Most sympathetic preganglionic fibres pass to the inferior mesenteric ganglia (prevertebral ganglia) or the lumbosacral sympathetic chain ganglia (see de Groat 1993; see Fig 1.1). The hypogastric nerves arise from the inferior mesenteric ganglia and pass to the pelvic plexus from where neurons innervate the bladder and urethra. Some fibres from the sympathetic chain ganglia pass to the pelvic nerve and are carried in conjunction with parasympathetic preganglionic neurons to the pelvic plexus (de Groat & Booth, 1980). There is a collection of both pre- and post-ganglionic fibres in the hypogastric nerves; the degree to which type is present varies between species, with the cat having numerous postganglionic axons and the rat having majority preganglionic axons (Janig & McLachlan, 1987). Many of the sympathetic noradrenergic axons arising from the sympathetic chain ganglia have also been shown to pass through the pelvic ganglia but not terminate within it (Kuntz & Moseley, 1936).

The role of the sympathetic nervous system in the control of micturition has caused much controversy over the years as it has been shown that an intact sympathetic nervous system to the bladder is not essential for micturition to take place (de Groat et al., 1993). Indeed, Nordling (1983) showed that sympathectomy and operations that affect sympathetic ganglia rarely cause a lasting effect on micturition of a normal individual. In the anaesthetized dog, electrical stimulation of the hypogastric nerves (HGN) has been shown to cause an initial contraction of the bladder, followed by a relaxation (Andersson et al., 1990). In the cat, stimulation of the sympathetic nerves also caused a transient rise in intravesical pressure of the bladder followed by a fall to below control levels (de Groat & Saum, 1972). The precise role of the sympathetic drive to the detrusor muscle is not clear.
from these experiments and is further discussed in section 1.6.6. The role and importance of the sympathetic nervous system in control of the urethra is also not simple when considering experimental evidence from different species and gender. In the dog, hypogastric nerve stimulation causes an increase in intraurethral pressure (Imagawa et al., 1989). In the female rat, electrical stimulation of the hypogastric nerves has been shown to cause a reduction in urethral pressure (Kontani & Shiraoya, 2000) due to the release of nitric oxide. However, the opposing effect was seen upon electrical stimulation of the hypogastric nerves of male rats with contraction of the bladder neck and proximal urethra (see Lincoln & Burnstock, 1993). There are varying results produced between gender and species by hypogastric nerve stimulation and this will be influenced by the non-uniform sympathetic innervation of the bladder and urethra and receptor densities (α and β), as well as the various methodologies used by different investigators. Antagonists have also been used in an attempt further elucidate the situation and demonstrate the physiological role of the sympathetic nervous system in LUT control. Maggi et al., (1985) showed that blocking β-adrenoceptors in rats, had no significant effect on micturition threshold. However, prazosin an α₁-adrenoceptor antagonist, and yohimbine an α₂-adrenoceptor antagonist, reduced micturition threshold (Maggi & Meli, 1983) most likely by blocking the sympathetic drive to the urethral α-adrenoceptors. Therefore these results indicate that the main role of the sympathetic nervous system is the activation of α-adrenoceptors in the urethra. Although in cats, de Groat & Theobald (1976) also demonstrated that α-adrenoceptors actively mediate sympathetic inhibition, at the ganglionic level, illustrating an additional site at which the sympathetic nervous system can exert influence over micturition. In human males the bladder neck and pre-prostatic urethra are supplied with a
rich plexus of sympathetic noradrenergic nerve terminals. Functionally, sympathetic outflow results in the contraction of the smooth muscle wall of the bladder neck and urethra, via $\alpha_1$-adrenoceptor subtypes that predominate in this region, and also prevent the retrograde ejaculation of semen into the bladder (see Gosling, 1979). In contrast to the male, human female smooth muscle of the bladder neck and urethra is poorly innervated with noradrenergic nerves (see Gosling et al., 1977) and work by Thind (1995) has shown that systemic administration of noradrenaline has little effect on urethral pressure in healthy women. However, the $\alpha_1$-adrenoceptor antagonist prazosin does cause small but significant reductions in maximal urethral pressure when given systemically. In addition using reserpine, a ligand that depletes the sympathetic nerve terminal of noradrenaline, has shown to be a useful treatment in cases of urinary retention and the cause of stress incontinence when given as a treatment for high blood pressure (Kleeman, 1970). This illustrates that even though the female urethra has a much less dense sympathetic innervation compared to the human male, the sympathetic nervous system is important in maintaining continence. Overall the findings show that sympathetic outflow causes an increase in urethral resistance via activation of $\alpha_1$-adrenoceptors in bladder neck and urethra, where they predominate (see section 1.6.6). Sympathetic outflow to the LUT, and particularly the urethra, is predominantly active during the storage phase of micturition and helps to maintain continence by increasing urethral resistance via a continuous reflex loop within the spinal cord. Afferents relay information from the bladder and urethra to the spinal cord and, during urine storage, sympathetic activity causes increasing urethral resistance (vesicospinovesical reflex). When the bladder threshold is reached the sympathetic activity to the urethra is inhibited at the spinal cord level, and the urethra
relaxes to allow urine to flow. The precise role of the sympathetic nervous system in relation to the bladder, during micturition is not well understood and the innervation of the detrusor muscle is sparse (see Elbadawi, 1982). The sympathetic nervous system can inhibit or facilitate bladder contractility via effects on ganglionic transmission in the cat, as already mentioned. However, the predominance of β-adrenoceptors over α-adrenoceptors in the detrusor muscle and the precise role of these receptors and of the sympathetic innervation still remains unclear and is further discussed in section 1.6.6.

1.3.4 Somatic nervous system

The pudendal nerve supplies somatic innervation to the external urethral striated sphincter (EUS) and the periurethral muscles, with species variation in where the nerves arise from, but usually from S2 to S4 region of the spinal cord. The pudendal nerve carries axons of both afferent and efferent axons (Barrington, 1931; Kuru, 1965). A discrete region, termed Onuf’s nucleus, was identified using retrograde axonal tracing techniques, in monkeys (Roppolo et al., 1985), cats (Thor et al., 1989) and humans (Onufrowicz, 1899) illustrating that the motoneurone cell bodies of the pudendal nerve are centred together in the sacral ventral horn of the spinal cord. In rat spinal cord the motoneurone cell bodies controlling the EUS are contained in two separate nuclei in the dorsolateral and dorsomedial region in L5-L6 segments (McKenna & Nadelhaft, 1986). The role of the pudendal nerve and somatic nervous system is essentially that of storage as lesions of the pudendal nerve can result in stress incontinence (see de Groat et al., 1993). Motoneurones innervating the EUS and muscles of the pelvic floor exhibit a tonic activity that increases as the bladder fills (de
Groat & Booth, 1980). This reflex increase in pudendal firing also occurs during times of rapid increases of abdominal pressure such as coughing or laughing (see Brading, 1999), and in combination with sympathetic control of the smooth muscle of the urethra, can be described as the ‘guarding’ or ‘vesicospinovesical reflex’, which contributes to the maintenance of urinary continence.

As micturition is initiated supraspinally, the increase in afferent firing from tension receptors in the bladder passes via the spinal cord to supraspinal sites, when the micturition threshold is reached, and reverses the pattern of efferent outflow, producing firing in the sacral parasympathetic pathways and concurrent inhibition of sympathetic and somatic pathways. The motorneurones in Onuf’s nucleus are inhibited and the EUS relaxes, along with the bladder neck. The bladder then contracts and urine flows. Interestingly, in rats the EUS exhibits high frequency bursting during voiding, which is superimposed on the slow relaxation of the smooth muscles of the urethra. This is in contrast to humans where there is complete inhibition of sphincter activity (Kakizaki et al., 1997). There are different theories as to the reason for such phasic activity, it could function as a pump to enhance urine flow (Kakizaki et al., 1997), but the reason has yet to be elucidated.

1.3.5 Triple innervation theory of the EUS

Traditionally, it was believed that the mammalian EUS had a purely somatic innervation but the fact that the EUS could be closed at will, but not opened at will, did not fit with this theory (see Elbadawi & Schenk, 1974). Histochemical studies in the male cat have shown
that aside from the motor-end-plate, somatic innervation there is a clearly identifiable cholinergic neuroplexus that spreads and circles fibres in the rhabdosphincter (Elbadawi & Schenk, 1974). In addition, an adrenergic neuroplexus has also been identified in this region in the cat and in the male rat there is evidence of sympathetic input to the EUS (Elbadawi & Schenk, 1974; Watanabe & Yamamoto, 1979). Kakizaki et al., (1994) showed that in anaesthetised cats with transected pudendal nerves, electrical stimulation of the hypogastric nerve elicited electrical potentials in the EUS. The innervation of the EUS therefore may seem to conform more closely to the general pattern of LUT innervation, receiving somatic innervation with sympathetic and parasympathetic input superimposed on top. However, evidence presented by Wein et al., (1979) for an adrenergic supply to the EUS could not be demonstrated by histofluorescence studies in dogs and humans, suggesting there was little noradrenergic innervation present in this region. The main problem with clarifying the situation are the anatomical constraints when trying to identify such a precise location and boundary of the EUS, as there is naturally an intimate association between the smooth muscle innervation of the proximal urethra and the EUS. Therefore the theory remains equivocal.

1.4 Afferent innervation of the bladder and urethra

Sensory input from the bladder, urethra and EUS are essential for conscious, co-ordinated bladder control. Afferent nerves monitor the volume in the bladder and regulate the amplitude of bladder contraction (see Morrison et al., 1999). Afferent pathways innervating the LUT are carried in the pelvic, hypogastric and pudendal nerves and have
been identified projecting to discrete regions of the spinal cord such as SPN and Onuf’s nucleus. The position, at which LUT afferents enter the spinal cord, does vary between species, but in general there is a correlation between the level of entry and the level of the SPN in each species (Morrison, 1987). This is a most suitable arrangement as afferent information is passed to the SPN and from there it is passed onto various regions of the higher central nervous system that co-ordinate reciprocal bladder and urethral function (see section 1.5).

The most important afferents for the micturition process travel in the pelvic nerve, which contains small myelinated (Aδ) fibres that convey information from mechanoceptors in the bladder and urethral wall (Janig & Morrison, 1986) in a graded manner, to the spinal cord. The tension or mechanoceptors in the bladder wall have been shown to increase their activity in response to bladder distension and bladder contractions (Habler et al., 1990). Further, these afferents in cats that respond to passive changes in the volume of the bladder (Morrison et al., 1999) can also be activated by cold (Fall et al., 1990) or noxious stimulation (Habler et al., 1990). Sensory afferents carrying information from the bladder and somatic and sympathetic efferents innervating the urethral smooth and striated muscle constitute the vesicospinovesical storage reflex (see section 1.3 of this chapter).

C-(unmyelinated) fibre afferents also travel in the pelvic nerve and have been shown to mediate specialized functions such as signalling inflammation, as they generally do not respond to high levels of tension, and do not appear to be involved in ‘normal’ micturition in cats (Habler et al., 1990). Although, in rat a proportion of C-fibre afferents in the pelvic nerve have been found to be active during physiological distensions, indicating species
differences (Morrison et al., 1999). Chronic inflammation can lead to functional changes in C-fibre afferents, and these normally ‘silent’ fibres can be the cause of increased pain sensation (Yoshimura et al., 2002). De Groat et al., (1981) also postulated re-arrangement of the role of C-fibre afferents in the micturition reflex in paraplegic cats, becoming important in initiating micturition.

Urethral afferents also have a role in micturition, which was first postulated by Barrington (1931). Stimulating the urethra mechanically or with the flow of saline caused reflex contraction of the detrusor. Further evidence by Mahony et al., (1977) described the same urethro-detrusor reflexes, where flow in the urethra causes detrusor contractions, and consequently increased excitability of the micturition reflex. This afferent pathway is thought to be important in patients with stress incontinence, as detrusor contraction leading to leakage of urine into the proximal urethra could exacerbate increased excitability of the micturition reflex and incontinence (Jung et al., 1999).

1.5 Brain and micturition: supraspinal sites of importance

1.5.1 The pons

Barrington’s early work on cats (1915 & 1925) demonstrated that the micturition reflex involves a spinalbulbospinal reflex loop, which passes through the dorsal pontine tegmentum in the brainstem. Physiological and pharmacological experiments have provided substantial support for the view that the neuronal circuitry in the brainstem acts to
function as a simple ‘on-off switch’ circuit, which during storage inhibits bladder activity and promotes urethral closure, and during voiding reverses the pattern. Barrington showed that bilateral lesions in the pontine micturition centre (PMC) area of the pons resulted in urinary retention, depressed bladder activity and increased bladder capacity. This area is now known as Barrington’s nucleus (or PMC) and is crucial for normal micturition. The importance of this central innervation originating in the pons and the functional relationship, in terms of control over the LUT, becomes clear when examining the retrograde tracing studies in the cat. These show efferent projections coming from two bilateral regions in the pons in the brain and projecting to the sacral spinal cord. There is a direct excitatory monosynaptic projection from the medial region (M-region or PMC) of the pons to the intermediolateral cell column (IML) in the spinal cord, which houses the parasympathetic bladder innervation (Blok & Holstege, 1997). Electrical and chemical stimulation with excitatory amino acids, of the PMC in the rat (Sugaya et al., 1998) and cat (Mallory et al., 1991) produces detrusor contraction and an immediate decrease in urethral pressure and EUS-EMG. There is also a projection from a more lateral region (L-region) of the pontine formation to Onuf’s nucleus, which gives rise to the somatic innervation of the urethral sphincter (Holstege et al., 1986). The L-region of the pons is also called the ‘pontine storage centre’ (PSC) as it has been shown to directly excite the urethral sphincter motorneurones and pelvic floor musculature (Holstege et al., 1986). Furthermore, a bilateral lesion of this area induces urinary incontinence and bladder hyperactivity (Griffiths et al., 1990). In contrast, there is no direct projection from the PMC to Onuf’s nucleus in the spinal cord (Holstege et al., 1986), even though stimulation of the PMC elicits urethral relaxation. The evidence suggests that the projections from the PMC to the
dorsal grey commissure (DGC), where many local neurons projecting to Onuf’s nucleus are located (Nadelhaft & Vera, 1996), control urethral relaxations. In fact, direct stimulation of the DGC in the cat elicits sharp decreases in urethral pressure (Blok et al., 1998). Positron Emission Topography (PET) brain scans in the human pons confirm the idea of a ‘micturition centre’ and a ‘storage centre’, with data showing that in human the PMC (or M-region) becomes active during voiding and the PSC (or L-region) is activated when subjects were asked to void but unable, presumably contracting their sphincters involuntarily and suppressing voiding (Blok et al., 1998). Interestingly, no direct projections between the PMC and the PSC have been found in the cat, indicating that although they work closely together, micturition and continence are controlled by two separate descending functional systems, at the level of the sacral spinal cord (Blok & Holstege, 1999). In rats a PMC has been well defined but a discrete storage centre has yet to be elucidated, as in the cat and human (Griffiths, 2002). Yamao et al., (2001), however, have demonstrated evidence for a discrete organization of the laterodorsal pontine tegmental area in the rat, with specific regions in the control of the bladder alone and the EUS alone.

1.5.2 The periaqueductal grey (PAG)

The periaqueductal grey (PAG) refers to an area of the brain that surrounds the cerebral aqueduct, extends posteri tally to the level of the posterior commissure, rostrally to the level of the third nucleus and caudally to the dorsal tegmental nucleus. It is known that the PAG is involved in many autonomic functions including pain processing and analgesia, fear,
anxiety, central regulation of heart rate and particularly the integration of emotional aspects of cardiovascular regulation (see Behbehani, 1995). Another important role for the PAG is in the control of micturition. Gjone (1966) demonstrated that electrical stimulation of the PAG in the cat gave rise to bladder contractions. Later evidence from Matsuura et al., (1998) have shown that the synaptic transmission blocker cobalt chloride (CoCl₂) inhibits micturition when injected into the ventrolateral PAG. Divalent metals such as cobalt are used because they selectively block terminal calcium conductance, and in this case these experiments indicate a critical role for the PAG in micturition in the anaesthetized rat. In the rat, the PAG has also been shown to receive direct connections from the lumbosacral spinal cord (Ding et al., 1997) and project directly to the PMC (Ding et al., 1998). In fact, the PAG is the only caudal brainstem structure known to project specifically to the PMC and Blok et al., (1994) have postulated that the PAG serves as a relay centre in conveying information concerning bladder filling to the PMC, as there are very few projections from the sacral spinal cord to the PMC direct. Indeed, field potentials evoked by pelvic nerve stimulation have been shown to be of shorter latency in the PAG, when compared to those evoked in the PMC in anaesthetized rats (Kakizaki et al., 1998), demonstrating that the PAG is sent information regarding the bladder first.

1.5.3 Other brain areas important in the control of micturition

Other brain areas, although not essential for micturition, have been shown to influence the voluntary control of micturition in humans, in particular the cerebral cortex and areas of the diencephalon, such as the hypothalamus. Three main areas of the cerebral cortex are
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concerned with voluntary micturition, and these are the frontal lobe, the paracentral lobule and the cingulate gyrus. The frontal cortex and cingulate gyrus have connections with the hypothalamus and limbic systems but the paracentral lobule directly connects with sphincter pudendal motorneurones in the corticospinal tract (see Morrison, 1987).

Connections between the frontal cortex and the pre-optic/septal region of the hypothalamus and the paracentral lobule and the brain stem and spinal cored are crucial for voluntary control and becomes apparent when there is disruption of the pathways in patients with lesions in these regions, which cause disturbances of micturition (Nathan, 1976). Lesions of these regions of the cortex affect the sensory processing of information from the bladder with patients either failing to experience the desire to void, even though they can discern that micturition is imminent, or they can be completely unaware of the fullness of their bladders, and therefore surprised when they have passed urine without knowing. The cortical influence over the hypothalamus and limbic regions can therefore be considered important in integrating the sensory and motor aspects of micturition. Patients with lesions in the paracentral lobule of the cortex, which projects directly to urethral sphincter motor neurones, have extensive spasticity of the lower limbs and are unable to relax the pelvic floor and perineal muscles as well as their external sphincters hindering micturition (Nathan, 1976). In animal studies, Gjone and Setekleiv (1963) found that electrical stimulation of the cortex of the cat could give rise to bladder contractions or relaxations depending on location. Axonal tracing studies have shown that many cortical areas project to the PMC, as well as the hypothalamus and brain stem (Sugaya et al., 1987; see de Groat et al., 1993) and these may mediate the effects of electrical stimulation to the cortex.
The hypothalamus is situated in the ventral part of the diencephalon, forming the floor and part of the lateral wall of the third ventricle in the mid brain. It includes the pre-optic area, optic tract, optic chiasm, mamillary bodies, tuber cinereum, infundibulum and neurohypophysis. The hypothalamus may be divided into four regions: anterior, dorsalis, intermedia, and posterior; or into three longitudinal zones: periventricular zone, medial zone and lateral zone. The hypothalamic nuclei activate, control and integrate the peripheral autonomic mechanisms, such as endocrine activity, regulation of water balance, body temperature, sleep, food intake and of particular interest to this study, micturition (see Blok, 2002). Electrical stimulation of the hypothalamus excites the sacral parasympathetic pathways and causes contractions of the bladder and voiding (Gjone, 1966; Kuru, 1965). Tracing studies have shown that the hypothalamic region sends numerous and diverse projections to areas of the brain stem and the sacral spinal cord important in controlling micturition. The anterior, medial and paraventricular regions of the hypothalamus project to the raphe nuclei, from which electrical stimulation has been demonstrated to exert prominent inhibitory effects on micturition (McMahon & Spillane, 1982). The anterior hypothalamic bundle also has been shown to send direct projections to the PMC (Holstege, 1987), whereas the medial and posterior hypothalamus sends direct projections to the sacral parasympathetic nucleus and Onuf’s nucleus (Holstege, 1987). Therefore the hypothalamic function is to have a modulatory or integratory influence over micturition mainly evident from the direct projections onto pontine and spinal sites of importance, or through indirect mechanisms on other brain stem regions, e.g. the cortex (de Groat, 1993). The hypothalamic control over micturition appears to be facilitatory. Tang and Ruch (1956) used cystometry in cats, which had undergone trans hypothalamic decerebration, to
demonstrate that a tonic inhibitory drive from the cortex to the hypothalamus existed, as micturition threshold was reduced after decerebration at that level. However, after a supracollicular decerebration, eliminating the hypothalamus, the micturition threshold was significantly increased, indicating that the influence of the posterior hypothalamus is facilitatory. Axonal tracing studies have also identified spinohypothalamic pathways arising in the sacral parasympathetic nucleus (Burstein et al., 1990) and a large percentage of these neurons receive afferent input from the lower urinary tract (Birder et al., 1992). These findings support the idea that the hypothalamus in an important modulatory centre for micturition, the afferent pathways from the bladder may alter the firing of hypothalamic neurons that may in turn directly or indirectly affect micturition.

1.6 Disorders of bladder and urethral function

Disruption to the finely controlled neural pathways that innervate the bladder and urethra can result in urinary incontinence, which is a prevalent clinical problem for health services, particularly in the western world. Urinary incontinence (UI) is described as a ‘condition in which the normal storage and voiding and urine from the bladder is altered and there is an involuntary loss of urine of sufficient volume to cause problems’ (see Couture & Valiquette, 2000). As the nature of this problem is somewhat embarrassing, accurate figures of patients that suffer from UI are largely estimated as it is thought that up to 50% of all cases of UI may not be even be reported. A number of different types of UI can be defined and these will be briefly discussed.
1.6.1 Urge Urinary Incontinence

Urge UI occurs in both sexes and the probability of occurrence increases with advancing age and therefore is the most common form of UI in the very old. It is characterised by the frequent involuntary loss of moderate to large volumes of urine associated with a strong desire to void (Resnick, 1995) and is due to detrusor hyperactivity or instability. The detrusor muscle can contract unexpectedly, causing the bladder neck to open and urine to leak out. Most commonly with chronic incontinence, the origin is due to spinal and/or supraspinal neuropathy, which leads to an altered perception of bladder fullness, and the loss of the central reflex that exerts an inhibitory influence over the bladder (Couture & Valiquette, 2000). Other causes of bladder instability of a non-neurogenic origin include urinary tract infections such as interstitial cystitis, obstruction and bladder stones. An uncoupling of the bladder urethral synergy can also occur and result in vesicosphincteric dyssynergia, which when stems from a neurological etiology is termed hyperreflexia. Diseases such as dementia, Parkinson's disease and multiple sclerosis can all cause instability of the bladder (Busby-Whitehead & Johnson, 1998).

1.6.2 Stress Urinary Incontinence

Stress urinary incontinence (SUI) is the most common form of UI in women and occurs because of increased intra-abdominal pressure from physical stresses such as laughing or coughing. There are wide ranging reasons for this type of UI, but commonly childbirth can damage pelvic support so that the bladder neck and urethra are not supported and slip from their normal position above the pelvic diaphragm (Couture & Valiquette, 2000). This
results in alterations of the position and functionality of the bladder neck and urethral sphincters. Other causes of this condition are hormonal changes during menopause resulting in pelvic relaxation, which can exacerbate SUI, α-adrenoceptor antagonists, given for hypertension, and in men, urological intervention causing damage to the external urethral sphincter can result in SUI (Couture & Valiquette, 2000).

1.6.3 Overflow Urinary Incontinence

Overflow UI (OUI) can be characterised by a reduction in the force of the urinary stream, and overall a sense of incomplete voiding and micturition (Chutka et al., 1996). Outlet obstruction and bladder contractile dysfunction both lead to an increase in bladder volume. In the first instance benign prostatic hypertrophy and urogenital cancers can cause a physical blockage to urine flow. However, dysfunction in bladder contractility can also result from diabetes, spinal cord lesions, and the use of medicines with anticholinergic properties, such as neuroleptics, narcotics and muscle relaxants (Busby-Whitehead & Johnson, 1998). All of these factors interfere with normal force contractions of the bladder leading to incomplete voiding and eventually overflow incontinence.

1.6.4 Functional and Mixed Urinary Incontinence

Functional urinary incontinence occurs in otherwise continent people that are unable to reach a toilet due to various factors outside their immediate control, e.g. use of sedative, impaired immobility, dexterity problems or cognitive impairment (Busby-Whitehead &
Johnson, 1998). Mixed urinary incontinence can be a combination of both stress and urge urinary incontinence, occurring simultaneously.

1.7 Neurotransmitters in the micturition reflex pathways

The neural control of the bladder and urethra involves many different neurotransmitters. The main ones of importance are outlined briefly below with details of the receptors and the current implications in the control of micturition. However, this is not a complete list as there are a number of other neurotransmitters and regulatory molecules involved in the control of the LUT including peptides, vanilloid receptors, prostaglandins, neurokinins and opiates (see Andersson, 1993), to name a few. As this thesis is concerned with examining the role of 5-Hydroxytryptamine (5-HT; serotonin) in the control of micturition, special emphasis is placed on this monoamine, its pathways and receptors that mediate the actions of this transmitter in autonomic functions, particularly micturition.

1.7.1 Glutamate – role in micturition

Glutamic acid is an essential excitatory neurotransmitter in the central nervous system and mediates its effects via fast AMPA/kainate receptors and slow N-methyl-D-aspartic acid (NMDA) receptors. Administration of NMDA or AMPA antagonists depresses reflex evoked bladder contractions and EUS activity in urethane anaesthetized rats (Maggi et al., 1990; Yoshiyama et al., 1991). However, in awake rats an NMDA antagonist slightly facilitated micturition (Vera & Nadelhaft, 1991) and in unanaesthetized decerebrate rats,
there were no effects on micturition (Yoshiyama et al., 1994). It is proposed that the pharmacological blockade of one type of glutamate receptor is not sufficient to block micturition (Yoshiyama et al., 1995), but under urethane anaesthesia the depressant action of urethane acts to suppress all glutamatergic receptor mediated responses (Dalo & Larson, 1990; Gibbs et al., 1993) and therefore a lone antagonist at NMDA receptor does block micturition (Yoshiyama et al., 1995). Intrathecal administration of a glutamate antagonist also suppresses bladder and sphincter reflexes in anaesthetized rats (Yoshiyama et al., 1993). However, in chronic spinal rats, no significant effects were observed after systemic administration of a glutamate antagonist (Yoshiyama et al., 1993). Thus, spinal glutamatergic receptors do mediate micturition in part but are dependent on intact pathways between the brain and spinal cord (Yoshiyama et al., 1993). At a cellular level it has been shown that AMPA and NMDA receptors mediate fast and slow excitatory transmission, respectively, between spinal interneurones and preganglionic neurons in the SPN which innervate the LUT (Araki & de Groat, 1996).

Sensory afferent pathways, as well as descending pathways already mentioned, are also mediated via glutamatergic receptors. Glutamate receptors mediate nociceptive information processing in the central nervous system with glutamate antagonists reducing c-fos expression at spinal sites, after chemical irritation of the bladder (Birder & de Groat, 1992). Ascending information to the brainstem, evoked by pelvic nerve stimulation, is mediated via NMDA and AMPA receptors and they function synergistically in the processing of information from the bladder to the brain (Kakizaki et al., 1998). Supraspinal glutamate receptors also have a role in the excitatory transmission of the micturition pathway.
Glutamate analogues/agonists injected into the PMC of rats and cats facilitate micturition (Willette et al., 1988; Mallory et al., 1991).

Glutamate receptors are therefore important on each limb of the spinobulbospinal reflex loop and in the central control of micturition and could provide a potential target for the treatment of bladder dysfunction. However, while evidence shows that AMPA receptor antagonists can improve detrusor-sphincter dyssynergia in spinal cord injured rats, NMDA receptor antagonists further decrease voiding efficiency (Yoshiyama et al., 1999). NMDA receptor antagonists do, on the other hand, prevent bladder hyperactivity induced by occlusion of the middle cerebral artery (Yokoyama et al., 1999) and could therefore prevent the plasticity observed at glutamatergic synapses that control micturition during e.g. stroke if administered in time.

1.7.2 GABA – role in micturition

Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system and its effect are mediated via GABA_A, GABA_B and GABA_C receptors (see Chebib & Johnston, 1999). The evidence shows that inhibitory effects mediated via GABA receptors are complex and can be mediated at, at least, 4 distinct sites of action (Maggi et al., 1987): 1) at the supraspinal level as GABA agonists given intracerebroventriually (i.c.v.) or directly into the PMC, inhibit reflex micturition (Kontani et al., 1987; Mallory et al., 1991); 2) at the spinal level, by inhibiting neurons in the sacral parasympathetic nucleus or by reducing afferent input from the detrusor to the CNS (Maggi et al., 1987; Kontani et al., 1987); 3) at the pelvic ganglioinic level, by inhibiting excitatory
transmission in the rat and cat (Maggi et al., 1985; Mayer et al., 1983); and/ or 4) at the postganglionic level of the nerves innervating the bladder, where GABA agonists inhibit the release of ACh and therefore reduce bladder contractility of isolated tissue (Maggi et al., 1985).

GABAergic inhibitory mechanisms are tonically in the micturition reflex, as demonstrated by administration of GABA antagonist or GABA breakdown blockers, which facilitate micturition and increase bladder contraction amplitude and duration (Maggi et al., 1987).

In the peripheral tissues GABA receptors mainly mediate inhibition of mouse (Santicioli et al., 1986), guinea-pig (Maggi et al., 1985), rabbit (Santicioli et al., 1984) and rat (Kontani et al., 1988) bladder contractions. Therefore this receptor subtype may be a suitable target for treatments of overactive bladder.

Thus, GABAergic neurons are mainly implicated in the storage phase of micturition, acting to tonically inhibit bladder contractions during filling. GABAergic neurons are also known to be present in high numbers in the DGC where many local neurons projecting to Onuf’s nucleus are located (Nadelhaft & Vera, 1996). It is thought that as the PMC projects directly to the DGC, information relayed in this area controls urethral contraction during storage and relaxation during voiding (see Blok et al., 1997). Direct stimulation of the DGC causes a sharp fall in the urethral pressure (Blok et al., 1998). Therefore GABAergic mechanisms are also implicated in the control of the urethra as well as the bladder and remain a potential target for disorders of the LUT.
1.7.3 Adenosine 5’triphosphate (ATP) – role in micturition

The first studies showing the importance of adenosine-5’triphosphate (ATP) were carried out by Langley & Anderson (1895) who showed the failure of atropine to completely block the excitatory response of the urinary bladder of rabbit, cat and dog, to stimulation of sacral parasympathetic nerves, suggesting the release and action of additional neurotransmitters to acetylcholine (ACh). More recent evidence has shown that this co-transmitter released from nerves is adenosine-5’triphosphate (ATP; Burnstock et al., 1972; 1978). Using quinacrine, a fluorescent dye that binds to ATP, nerve cell bodies in intramural ganglia and ATP-positive fibres were found to innervate the smooth muscle of the bladder. ATP levels were also found to increase upon stimulation of intramural excitatory nerves, and exogenous ATP could also mimic the electrically stimulated contraction of bladder tissue. It was then proposed that ATP mediates non-cholinergic and non-adrenergic (NANC) excitatory transmission in the bladder (Burnstock et al., 1978). ATP causes contractions of bladder smooth muscle in rat (Bolego et al., 1995; Sjuve et al., 1995), human (Palea et al., 1995), dog (Creed & Tulloch, 1978), cat (Theobald, 1992, 1996), guinea pig (Burnstock et al., 1978; Harvey et al., 2002) and marmoset (McMurray et al., 1998), as well as other species. In addition, ATP receptor antagonists can block these excitatory events (Theobald, 1986; King et al., 1997).

It is widely accepted that there are two families of purinergic receptors that mediate the effects of ATP and its metabolites, P1 and P2, based on pharmacological binding profiles, cloning and tissue distribution (see Burnstock, 1978; Fredholm et al., 1997). The G-protein coupled P1 receptors are divided further into four adenosine receptor subtypes A1, A2A, A2B
and A₃ receptors (see Nyce, 1999), and are more responsive to and bind adenosine, one metabolite of ATP, rather than ATP itself. It has been shown that adenosine causes relaxation of isolated rat and guinea pig bladder through P₁ receptors (King et al., 1997) and this is likely mediated via the A₂B receptor subtype in rat (Nicholls et al., 1992) and A₂A receptor in the guinea pig (Gopalakrishnan et al., 2002). In contrast, cat bladder contraction can be mediated through A₁ adenosine receptors (Yang et al., 2000). The P₂ receptors are more responsive to ATP and have also been subdivided, into P₂X (ligand gated ion channels) and P₂Y (G-protein coupled receptors) subtypes (Burnstock & Kennedy, 1985). Within each subtype there has been further division and, to date, there are seven P₂X receptors, designated P₂X₁₋₇, and five P₂Y receptors, designated P₂Y₁, P₂Y₂, P₂Y₄, P₂Y₆, and P₂Y₁₁ that have been cloned and classified in each family (see Ralevic and Burnstock, 1998).

Using selective P₂X receptor agonists, antagonists and desensitising techniques, it has been shown that P₂X receptors can mediate contraction of the guinea pig, rat, human and dog bladder, although in dog bladder the failure of α,β-meATP to cause contraction suggests species heterogeneity with respect to expression of P₂X receptors in the tissue (Burnstock Kennedy, 1985; Palea et al., 1995; Suzuki et al., 1994; Brading & Williams, 1990). Additional work has shown that ATP also causes relaxation of rat (Suzuki et al., 1994), mouse (Boland et al., 1993) and marmoset (McMurray et al., 1998) bladder smooth muscle, which is mediated via P₂Y receptors, although lack of truly selective ligands still leaves the subtype unidentified.
The presence of differing amount of cholinergic and purinergic input to the bladder varies markedly between species. Some investigators have proposed that, as humans have a very limited supply of purinergic input to the bladder, species differences in purinergic innervation could be of an evolutionary nature. Humans are not required to territory mark and perhaps in rats and cats, where a larger proportion of their bladder innervation is non-cholinergic, ATP is an important transmitter in this respect (Brindley & Craggs, 1975; Craggs et al., 1986). It has also been proposed that purinergic input may be important in initiating bladder contractions while cholinergic transmission maintains bladder contractions and empties the bladder (see Levin & Wein, 1995; Chancellor et al., 1992). Although, Igawa et al., (1993) showed that both types of transmission are important in voiding as atropine and desensitising α, β-meATP are required to block micturition in a normal unanaesthetized rat. This was further confirmed by other findings in vitro, that block of both purinergic and cholinergic transmission, is required to effectively block electrically evoked contractions (Brading & Williams, 1990).

A further sensory role for ATP has been described, owing to findings that the P2X3 purinoceptor subtype is exclusively expressed in small diameter sensory neurones (Chen et al., 1995) and has since been localized on nerves in the bladder (Lee et al., 2000). Ferguson et al., (1997) demonstrated that ATP is released from the urothelium of rabbit bladder as a sensory mediator responding to the degree of distension of the bladder. It has also been shown that endogenous ATP is released from the rat bladder in response to bladder distension and that it is involved in the activation of mechanosensitive pelvic nerve afferents (Namasivayam et al., 1999). This has been further confirmed using P2X3 knockout mice, which show a reduction in afferent nerve activity in response to bladder
distension (Vlaskovska et al., 2000). Therefore ATP acts not only as a major excitatory neurotransmitter in the bladder but also as an important mediator in the sensory system of micturition.

1.7.4 Nitric oxide – role in micturition

Nitric oxide (NO) is a biologically active gaseous molecule that is involved in numerous physiological processes (see Moncada et al., 1991). It is derived from the amino acid L-arginine and is converted under the enzymatic catalytic action of nitric oxide synthase (NOS). NOS exists in both constitutive and inducible forms, the constitutive forms are found in endothelium, termed eNOS, and the neurones of central and peripheral nervous system where it is termed nNOS. The form induced by immunological or inflammatory events is termed iNOS (see Rand, 1992; Burnett, 1995). Nicotinamide adenine dinucleotide phosphate (NADPH)- diaphorase is used as a specific marker for nNOS (Dawson et al., 1991) and studies have shown that there is a distribution of NO positive neurones in the urethra of the pig (Persson et al., 1993), rat (Vizzard et al., 1994), rabbit (Masuda et al., 2002), cat (Keast & Kawatani, 1994) as well as other species. NO is known to be released from non-adrenergic non-cholinergic nerves (NANC), as well as other sources such as endothelium (see Burnett, 1995). NO diffuses to the target cell and utilizes guanylate cyclase as a receptor, which upon activation, generates 3’, 5’-cyclic guanosine monophosphate (cGMP). Cyclic GMP produces a wide range of effects by targeting ion channels, phosphodiesterases or protein kinases, leading to smooth muscle relaxation.
Indeed, knockout mice lacking cGMP-dependent protein kinase I (cGKI), which mediates smooth muscle relaxation, show hyperactive voiding (Persson et al., 2000).

In vitro studies indicate that NO is the main transmitter involved in mediating urethral smooth muscle relaxation in rats (Persson et al., 1992), rabbits (Andersson et al., 1992) and man (Klarskov et al., 1983), as well as other species. In addition, evidence from in vivo studies shows that NO mediates urethral smooth muscle relaxation in anaesthetized rats (Bennett et al., 1995; Wibberley et al., 2002). Barrington (1915) showed that parasympathetic nerve stimulation also caused urethral relaxations in cats and this relaxation has since been shown to be mediated via the release of NO. Therefore parasympathetic stimulation causes bladder contractions mediated via Ach and NANC transmitter ATP, but also urethral relaxations mediated via NANC nerves releasing NO. To support this idea, evidence from Persson et al., (1997) demonstrated that nerves, which produce NO in the rat lower urinary tract, are not of the sympathetic or sensory subtype. Evidence for the role of NO, as a possible mediator of bladder relaxation during urine storage, is still equivocal. Experiments have shown that systemic administration of NO pathway inhibitors in rats can decrease bladder capacity and micturition volume and increase spontaneous bladder contractions (Persson et al., 1992). However, these effects seen mimicking bladder relaxation, could be due indirectly to NO influences on the urethra. In mouse bladder, NO-donors have no inhibitory effects on smooth muscle tone, but instead, NO can functional role as an excitatory modulator (Fuijwara et al., 2000). In fetal lamb, NOS inhibitors cause increased bladder capacity; 'uninhibited' bladder contractions and incomplete bladder emptying (Mevorach et al., 1994). However, bladder distension does not increase NO release and ligands acting on or through cGMP have limited effects
of bladder activity (Haab, 2000). Therefore the exact role of NO in bladder function is not clear. However, NADPH-diaphorase positive nerves have been identified in the bladder smooth muscle of the rabbit (Masuda et al., 2002), rat (Vizzard et al., 1994), man (Smet et al., 1994) and other species. They are present in much fewer numbers in the bladder compared to the urethra and are mainly found in the bladder base.

NO has also been implicated in the control of the urethral striated sphincter, NOS is present in the human EUS (Ho et al., 1999; 2003) and has also been shown to potentiate the reflex evoked relaxations of the rat striated urethral sphincter via a mechanism involving nicotinic ACh receptors (Wibberley et al., 2002).

In addition to NO being present as a peripheral mediator, NADPH-diaphorase immunoreactivity has also identified NOS in the major pelvic ganglia (MPG), dorsal root ganglia (DRG) and sacral spinal cord (Vizzard et al., 1993; 1996; Pullen et al., 1997). Intrathecal injections of a NOS inhibitor had no significant effects on normal voiding in rats, but did reverse the effects of hyperactive bladder induced by acetic acid infusion (Kakizaki & de Groat, 1996). These findings indicate that spinal NO pathways do not play a role in normal micturition but do facilitate micturition at a spinal level when evoked by nociceptive bladder afferents. Additional evidence of a role for NO mediating sensory afferent information from the bladder comes from Vizzard et al., (1996; 1997). Findings show that NOS-immunoreactivity in DRG and bladder afferent pathways was significantly upregulated after chronic bladder irritation and spinal cord injury.
1.7.5 Acetylcholine: innervation, receptors and role in micturition

The parasympathetic nervous system provides the main excitatory input to the bladder via the release of acetylcholine (ACh) from postganglionic nerves (see section 1.3.1 of this chapter), which activates muscarinic receptors in the bladder to cause contraction. There is much evidence for a dense cholinergic input to the bladder in many species including rat, cat (Gosling & Dixon, 1975) and man (Alm, 1978). In addition, the urethra receives cholinergic innervation, which also causes contraction via muscarinic receptor activation, although this is dependent on species and location along the urethra (Mutoh et al., 1997; Alm, 1978).

To date, molecular cloning has identified gene products for five distinct muscarinic receptor subtypes and they have been designated M₁, M₂, M₃, M₄ and M₅ accordingly, although pharmacologically only M₁ to M₄ have been characterized in detail (see Eglen et al., 1996). Radioligand binding studies have revealed a dense distribution of muscarinic receptors in the bladder of the rat (Monferini et al., 1988), rabbit (Levin et al., 1988), human (Kondo et al., 1995), as well as many other species. More specifically, M₂ and M₃ receptors were subtypes that were immunoprecipitated from human, rat, guinea pig and rabbit bladder membranes, with a ratio of M₂:M₃ receptors 9:1 in rat bladder and 3:1 in other species (Wang et al., 1995). However, pharmacological studies show that the main contractile activity in the bladder is mediated by the smaller number M₃ receptors in rat (Longhurst et al., 1995), rabbit (Mutoh et al., 1997) and human (Newgreen & Naylor, 1996), rather than the predominating M₂ receptor subtype. M₃ receptors cause contraction by stimulation of phosphoinositide hydrolysis 2ⁿᵈ messenger system. The M₂ receptors can
also contract rat bladder in vitro and in vivo (Hegde et al., 1997) and are not redundant. In other smooth muscles, such as the guinea pig ileum (Thomas et al., 1993), trachea (Thomas & Ehlert, 1996) and the oesophagus of the rat (Eglen et al., 1996), M_2 receptors can mediate reversal of smooth muscle relaxation induced by adenylyl cyclase stimulatory compounds e.g. β_2-adrenoceptor agonists or forskolin. As the M_2 receptors are negatively coupled to adenylyl cyclase, one proposed mechanism by which M_2 receptor activation in the bladder causes indirect contraction of bladder smooth muscle is probably by inhibiting cAMP accumulation (Hegde et al., 1997), although this has yet to be confirmed. The presence of both receptors suggests a physiological role for a dual mechanism that may allow for more efficient and complete emptying of the bladder. Prejunctional M_2 or M_4 receptors mediate an auto-inhibitory control over ACh release on parasympathetic nerve terminals whereas the M_1 subtype is autofacilitatory (Braverman et al., 1998; D’Agostino et al., 1997; Somogyi et al., 1994). The selective recruitment of either auto-facilitatory ACh release (M_1 receptor) or auto-inhibitory (M_2 or M_4 receptor), is highly dependent on the pattern, duration and frequency of stimulation of parasympathetic nerves (Somogyi & de Groat, 1999; Somogyi et al., 1996). In fact, at physiological levels of continuous isolated rat bladder stimulation the auto-inhibitory mechanisms are turned off, suggestive of a self-amplifying mechanism to ensure full bladder contraction and efficient voiding (Somogyi & de Groat, 1999). In human detrusor the predominant muscarinic receptor subtype has been shown to be M_2 (Wang et al., 1995), but the contractile properties of the bladder are mediated by M_3 receptors (Harriss et al., 1995). Prejunctional auto-facilitatory receptors are also present and of the M_1 subtype in human (Somogyi et al., 1996) but they
are absent from the cat (Somogyi et al., 1999), although an additional auto-facilitatory mechanism is present at the parasympathetic ganglion level (de Groat & Saum, 1972).

Due to the lack of subtype selective antagonists, the role of central muscarinic receptors in the control of micturition has been sparsely researched. This is interesting as antimuscarinic agents are still the most widely used treatments for urgency and urge incontinence (see Chapple, 2000). However, evidence suggests that central muscarinic mechanisms mediate tonic excitatory influence over bladder contractions and urethral relaxations in rats (Masuda et al., 2001; Ishiura et al., 2001; Ishizuka et al., 2002).

The main side effects of antimuscarinic treatment for conditions such as overactive bladder are dry mouth, which is caused by blockage of M₃ receptors in the salivary gland, constipation, drowsiness and blurred vision (see Chapple, 2000). Overactive bladder causes urinary frequency, urgency and urge incontinence and although the etiology may involve neurogenic and myogenic dysfunction, the root cause essentially remains unclear.

It is evident that ACh-activated muscarinic receptors are involved in normal and involuntary bladder contractions (Andersson, 1993) and research has shown that patients with bladder dysfunction do have supersensitivity to carbachol (German et al., 1995) and therefore plasticity of muscarinic receptors is also a consideration in disease states.

Therefore muscarinic receptors are targeted pharmacologically. Improvements in current clinical treatments with the use of tolterodine and darifenacin, which show some selectivity for bladder tissue in animal models (Wallis et al., 1995; Nilvebrant et al., 1997), go some way to reduce unwanted side effects (Chapple, 2000).
Sacral root stimulation studies have shown that the proportion of neurogenic excitation in the bladder due to the release of ACh varies according to species, quite markedly. In man and baboons, it has been shown that bladder excitation due to stimulation of the sacral roots is almost entirely abolished by atropine (Brindley & Craggs, 1975), in comparison to cat bladder where neural evoked contraction is almost entirely resistant to atropine and rat bladder where approximately 50% of transmission is resistant (Edge, 1955; Carpenter, 1981; see Hoyle & Burnstock, 1993). This evidence illustrates the importance of the co-transmitter, now know to be adenosine 5'-triphosphate (ATP), at least in the cat and rat, which is released from parasympathetic nerves, alongside ACh, during micturition (Langley & Anderson, 1895).

1.7.6 Noradrenaline: innervation, receptors and role in micturition

The body of the bladder has relatively sparse noradrenergic innervation compared to that of the bladder neck and proximal urethra, in most species (Elbadawi, 1982; Gosling & Dixon, 1975; see Levin and Wein, 1995; see Brading, 1999; see Fletcher & Bradley 1978), suggesting a limited role for sympathetic drive in bladder function. However, studies have shown that sympathetic drive to the bladder may be important in the storage phase of micturition, but of greater consequence is the sympathetic control of the bladder neck and urethra in maintaining continence (see section 1.3.3 of this chapter).

Ahlquist (1948) described two types of adrenoceptors based on pharmacological studies with a series of agonists, these were named α- and β-adrenoceptors. Adrenoceptors are
defined as G-protein coupled receptors that respond to endogenous agonists, adrenaline and
noradrenaline, producing cellular responses. \( \alpha \)-adrenoceptors can be split into two
families, \( \alpha_1 \) and \( \alpha_2 \)-adrenoceptors and these can further divided into subtypes. The \( \alpha_1 \)
family is subdivided into \( \alpha_{1A} \), \( \alpha_{1B} \) and \( \alpha_{1D} \)-adrenoceptors. In addition, there is evidence
for another \( \alpha_1 \) adrenoceptor, which has a low affinity for prazosin, and is designated \( \alpha_{1L} \)-
adrenoceptor (Ford et al., 1997; Docherty, 1998). However, this receptor subtype has not
yet been cloned, and may not be derived from a distinct gene, instead it may represent a
conformational state of the \( \alpha_{1A} \)-adrenoceptor that is energetically favourable (Ford et al.,
1998). Activation of all \( \alpha_1 \)-adrenoceptor subtypes results, through phospholipase C, in the
formation of inositol triphosphate and diacylglycerol, resulting in release of intracellular
\( \text{Ca}^{2+} \) from stores and/or increased entry of extracellular \( \text{Ca}^{2+} \). \( \alpha_2 \)-adrenoceptor can be
divided into \( \alpha_{2A} \), \( \alpha_{2B} \) and \( \alpha_{2C} \)-adrenoceptors, with the rat \( \alpha_{2D} \)-adrenoceptor being a
species variant of the \( \alpha_{2A} \)-adrenoceptor (Harrison et al., 1991). \( \alpha_2 \)-adrenoceptors are
negatively coupled to adenylyl cyclase.

1.7.6.1 \( \alpha_1 \)-adrenoceptors – role in micturition

\( \alpha \)-adrenoceptors are expressed in low quantities in the bladder of rat, monkey and human
(Walden et al., 1997), although in most species it is possible to evoke detrusor contractions
with drugs that stimulate \( \alpha \)-adrenoceptors, preferentially \( \alpha_1 \)-adrenoceptor (Nergardh &
Boreus; 1972; Elmer, 1974; Andersson, 1993). Walden et al., (1997) found that the
\( \alpha_{1A} \)-adrenoceptor was the major subtype in the smooth muscle of the rat, monkey and
human bladder. However, more recently Malloy et al., (1998) has shown that in rat bladder
\(\alpha_{1A}, \alpha_{1B},\) and \(\alpha_{1D}\)-adrenoceptors are all equally expressed, furthermore \(\alpha_{1B/1D}\)-adrenoceptors mediate smooth muscle contraction in the rat bladder (Szell et al., 2000). In addition, it was found that there is a predominance of \(\alpha_{1D}\)-adrenoceptor over \(\alpha_{1A}\)-adrenoceptor expression in human bladder (Malloy et al., 1998) and therefore tailoring treatments for selectivity at this receptor subtype may have benefits for the patient when treating conditions such as irritative bladder or benign prostatic hyperplasia. Interestingly, Suzuki et al., (1999) demonstrated an increase contractility of rat bladder to NA in aged rats when compared to younger rats, which shows the plasticity of the sympathetic drive to bladder and how in aged patients unstable bladder may be treated by adrenoceptor ligands.

Prejunctional \(\alpha_{1A}\)-adrenoceptors have also been shown to be present on cholinergic nerve terminals in the rat bladder, mediating prejunctional facilitation of acetylcholine release (Szell et al., 2000), which could be an important mechanism to facilitate emptying of the bladder.

As already mentioned, \(\alpha\)-adrenoceptors are expressed in abundance, in the bladder neck and proximal urethral smooth muscle, compared to the sparse distribution in the bladder body, and are important in maintaining urethral tone and therefore intraurethral pressure during urine storage. The urethral smooth muscles of the rabbit (Mattiasson et al., 1990), pig (Brading et al, 1999) and human (Taki et al., 1999) have been shown to contract via \(\alpha_1\)-adrenoceptor activation. More specifically, in the male rat, reflex evoked urethral contractions appear to be mediated via \(\alpha_{1A}\) and \(\alpha_{1D}\)-adrenoceptor (Conley et al., 2001) and in the human (Ford et al., 1996) dog and rabbit (Leonardi et al., 1997) the \(\alpha_{1L}\)-adrenoceptor is mainly involved in mediating the urethral contraction in vivo. It will be important to
narrow down specific adrenoceptor subtypes for future developments in urethral-selective
drugs for the treatment of e.g. stress incontinence, with fewer or no cardiovascular side
effects (Modiri et al., 2000). As with treatments such as midodrine, increases in urethral
pressures are seen in incontinent patients with use of this sympathomimetic, but much
cautions exercised owing to cardiovascular side effects (Jonas, 1977). Additionally, there
are slight differences in expression of α-adrenoceptor subtypes between sexes with human
males having a more uniform population of α-adrenoceptors compared to females
(Nishimatsu et al., 1999; Nasu et al., 1998), but differences appear to be only marginal.

1.7.6.2 α2-adrenoceptors – role in micturition

α2-adrenoceptors have been located in the bladder and urethra of the rabbit (Latifpour et
al., 1990; Morita et al., 1987) and the urethra of the guinea pig (Trendelenburg et al.,
1997), although this receptor subtype does not appear to play any role in the control of
human bladder base and urethra (Kunisawa et al., 1985). The α2D-adrenoceptor subtype is
present in the guinea pig urethra (Trendelenburg et al., 1997) and in the rabbit the α2A-
adrenoceptor predominates (Latifpour et al., 1990), with a much higher density of receptors
in the female compared to the male. Not surprisingly therefore, the female rabbit urethra
has a larger contractile response to clonidine, an α2-adrenoceptor agonist, compared to the
male (Morita et al., 1987). Furthermore, prejunctional α2-adrenoceptors have been shown
to inhibit electrically evoked bladder contractions in the pig (Ali et al., 1998) and control
the synthesis of NO from nitrergic nerve terminals in the rabbit urethra (Seshita et al.,
2000). α-adrenoceptors have also been shown to modulate transmission in cat vesical
ganglia, with the $\alpha_2$-adrenoceptor mediating inhibitory responses to nerve stimulation and NA and the $\alpha_1$-adrenoceptor facilitating responses. The role of these two receptor subtypes in the periphery appears to be important during various stages of the voiding and storage cycle of micturition.

1.7.6.3 $\alpha$-adrenoceptors – central role in micturition

A region of the dorsolateral pontine tegmentum, the locus coeruleus (LC), innervates sympathetic and parasympathetic nuclei in the lumbosacral spinal cord, via NA containing neurons (Holstege et al., 1986). It has been demonstrated that destruction of the LC area causes urinary retention in rats (Osumi et al., 1975) and cats (Yoshimura et al., 1990). Further, intrathecal injection of $\alpha_1$-adrenoceptor antagonists modifies reflex evoked bladder contraction in anaesthetized (Jeong & Lee, 2000) and conscious rat (Yoshiyama et al., 2000) and causes an inhibition of locus coeruleus stimulated bladder contraction in cat (Yoshimura et al., 1988). $\alpha_1$-adrenoceptor antagonists have also been shown to cause a decrease in sympathetic drive to the bladder in anaesthetized cats (Ramage & Wyllie, 1995). Therefore the evidence suggests that central $\alpha_1$-noradrenergic mechanisms mainly mediate facilitatory influences on micturition, although this depends on the site of action as in the rat spinal cord $\alpha_1$-adrenoceptor inhibitory mechanisms also mediate effects (de Groat et al., 1999; Yoshiyama et al., 2000). The function of central $\alpha_2$-adrenoceptor is less clear, with studies in rats indicating both a facilitatory (Kontani et al., 1992; Ishizuka et al., 1996) and inhibitory (Maggi et al., 1985) role in the control of voiding, most likely the varying effects caused by receptors at different sites i.e. the periphery vs CNS. Urethral sphincter
function is also modulated by central noradrenergic innervation, with electrical stimulation of the LC region resulting in increased sphincter EMG in the cat (Kuru, 1965, Holstege et al., 1986) and intrathecal administration of \( \alpha \)-adrenoceptor agonist blocking relaxation of urethral sphincter in unanaesthetized rats (Durant et al., 1988). Clonidine the \( \alpha_2 \)-adrenoceptor agonist blocked reflex firing pudendal nerve efferent pathways to the urethral sphincter in the cat (Downie et al., 1991) and in chronic spinal cats clonidine i.v. or i.t. blocks bladder-sphincter dyssynergia and improves voiding efficiency (Galeano et al., 1986). In contrast, \( \alpha_1 \)-adrenoceptors appear to mediate facilitatory responses in the cat sphincter, with i.v. prazosin depressing sphincter activity and reflex firing in pudendal efferent pathways (Gajewski et al., 1984).

### 1.7.6.4 \( \beta \)-adrenoceptors –role in micturition

\( \beta \)-adrenoceptors were originally split into two subtypes, \( \beta_1 \)- and \( \beta_2 \)-adrenoceptor according to agonist potency studies (Lands et al., 1967), but since then evidence has accumulated to suggest the existence of additional \( \beta \)-adrenoceptor subtypes. \( \beta \)-adrenoceptors, resistant to the typical \( \beta \)-adrenoceptor antagonists, were shown to mediate the metabolic functions of endogenous catecholamines such as thermogeneis, lypolysis, (Arch et al., 1984) along with gastrointestinal motility and metabolic processes (Bond & Clarke et al., 1988). These findings lead to the characterization and cloning of the \( \beta_3 \)-adrenoceptor in human (Emorine et al., 1989), rat (Granneman et al., 1991) and other species.
The predominance of $\beta$-adrenoceptors over $\alpha$-adrenoceptors in the body of the bladder has been well documented and it has been proposed that these receptors mediate relaxation of the smooth muscle of the bladder during urine storage. Indeed, $\beta_1$- and $\beta_2$-adrenoceptor have been identified in rat (Maggi & Meli, 1982) and human (Levin et al., 1988) bladder, along with the presence of $\beta_3$-adrenoceptor (Fujimura et al., 1999) in these tissues. In rat bladder all the $\beta$-adrenoceptor subtypes have been shown to mediate relaxation of bladder smooth muscle (Longhurst & Levendusky, 1999) in vitro. However, studies done on other animals show species diversity with regards to the subtype that mainly mediates relaxation in the bladder smooth muscle. Yamazaki et al., (1998) found that in the rabbit $\beta_2$-adrenoceptors mainly mediate relaxation of the bladder, whereas in dogs it is the $\beta_3$-adrenoceptor, and relaxation responses in the cat (Nergardh et al., 1977) and guinea pig (Li et al., 1992) are mediated via the $\beta_1$-adrenoceptor. Even though a role for $\beta$-adrenoceptor relaxation can be identified in animals, there is still controversy over the role in humans, mainly due to the lack of effects of adrenoceptor blockade on normal detrusor function (Nordling, 1983). The functional importance of urethral $\beta$-adrenoceptor has yet to be established, but there is some evidence that $\beta$-adrenoceptor agonists can reduce intraurethral pressure, although $\beta$-adrenoceptor blockers have little effect (Thind et al., 1993). Therefore the situation requires further investigation.

1.7.7 Dopamine – role in micturition

Dopamine is another transmitter that has a role in the control of micturition, illustrated by Parkinson’s disease patients who have a degeneration of DA-containing neurons in the
This group of patients have increased rates of detrusor hyperreflexia and voiding dysfunction (Berger et al., 1987), most likely due to nigrostriatal dopamine depletion, implicating DA in the control of normal micturition.

Dopamine receptors are divided into 2 families according to their 2nd messenger systems and agonist and antagonist potencies; the D1-like receptors group, including D1 and D5 receptors and the D2-like receptor group, including the D2, D3 and D4 cloned subtypes. These groups will be referred to as D1 and D2 receptors from now on.

Peripherally DA and its metabolites have been localised in the urogenital tract of the rat (Favre et al., 1986). Likewise, autoradiographical studies have located DA receptors in the detrusor of the rat (Escaf et al., 1994). Evidence shows that DA inhibits peripheral purinergic neurotransmission (El-Mas et al., 1999), however α2-adrenoceptor antagonists attenuate these responses, so the precise role of peripheral DA is not clear. Administration of apomorphine causes bladder hyperactivity in anaesthetized rats, an effect mediated by D2 receptors and potentiated by the simultaneous stimulation of D1 receptors (Kontani et al., 1990; 1990a). Evidence has shown that D1 receptors are tonically active and act to inhibit micturition in conscious rats, as selective D1 antagonist SCH23390 caused dose dependent decreases in bladder capacity and micturition volumes, while increasing micturition pressures (Seki et al., 2001). This data concurs with work in the monkey model and also the rat model of Parkinson’s disease, where DA-containing neuron are degenerated by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP) and bladder capacity and volume voided during micturition decreases (Burns et al., 1983; Yoshimura et al.,
2003). Intravenous administration of a D1 agonist SKF38393 had the opposite effect by suppressing detrusor hyperactivity (Yoshimura et al., 1991; 1998; 2003). This is also supported by work in the cat, which showed that electrical stimulation of the substantia nigra inhibits micturition, an effect blocked by a D1 receptor antagonist (Yoshimura et al., 1992). This work in the cat and other species shows that neurons in the substantia nigra are most likely involved in the storage phase of micturition (Sakakibara et al., 2002) and indicates that the dopaminergic systems serves to tonically inhibit micturition via the D1 receptor.

In conscious rats the administration of a selective D2 receptor agonist quinpirole, facilitated micturition, but the selective D2 receptor antagonist remoxipride had no significant effects (Seki et al., 2001). In the MPTP-lesioned monkeys the administration of the selective D2 agonist bromocriptine facilitated micturition and reduced micturition volume threshold (Yoshimura et al., 1991; 1998). Interestingly, there is evidence that non-selective agonists for both D1 and D2 receptors, such as BAM-1110, also have therapeutic effects of MPTP-induced parkinsonism in Cynomolgus monkeys (Yoshimura et al., 1998). Thus, DA ligands may prove useful as treatments for LUT dysfunction.

### 1.7.8 5-Hydroxytryptamine

5-Hydroxytryptamine (or serotonin) was initially discovered and isolated in 1948 by Rapport. This substance was found in the blood and caused blood vessels to contract hence the name serotonin (a ‘serum’ factor that affected blood vessel ‘tonus’). This chemical
later identified as 5-hydroxytryptamine (5-HT). This was also the same substance that had been isolated in the gut (Erspamer & Asero, 1952), which caused increased motility and had been named enteramine. 5-HT has since been shown to be an important neurotransmitter in the central nervous system and is implicated in a wide variety of functions such as, the control of emotion, temperature regulation, circadian rhythm, sleep and central autonomic control (see Hoyer et al., 1994).
Central 5-HT pathways

Figure 1.2. Diagram illustrating central 5-HT pathways (Role & Kelly, 1991)
1.7.8.1 Central 5-HT containing pathways

Neurones expressing 5-HT are among the first to be born in the developing central nervous system and the cell bodies are located near the midline in the brainstem raphe nuclei, which are mainly contained in the basal plate of the pons and medulla in the adult nervous system (Rubenstein, 1998). There are several clusters of cell bodies and they can be classified into superior (ascending) and inferior (descending) groups depending on signals during early development. The superior group consists of four main nuclei: caudate linear nucleus, median raphe nucleus, medial lemniscus and dorsal raphe nucleus. The inferior group consist of five main nuclei: nucleus raphe obscurus, nucleus raphe pallidus, nucleus raphe magnus, lateral paragigantocellular nucleus and the intermediate reticular nucleus (see Jacobs & Azmitia, 1992). Many of the groups have a rostro-caudal orientation within the brain and Dahlstrom and Fuxe (1964) were the first to describe and trace 5-HT-containing neurones that projected directly from brain regions such as the dorsal raphe nuclei to the spinal cord. These neurones terminate in areas such as the intermediolateral cell columns of the thoracic spinal cord (regulation of sympathetic outflow) and the sacral parasympathetic nucleus in the sacral spinal cord, both areas of importance for micturition. This gave the first indication that 5-HT was likely to be an important transmitter, both spinally and supraspinally, involved in the control of micturition. Ryall & de Groat (1972) examined neurons in the spinal cords of cats. They showed that ionophoretic administration of 5-HT onto preganglionic parasympathetic bladder neurones caused inhibition in the firing rate, potentially mimicking the effects of endogenous release of this transmitter from raphe projection neurons terminating in the sacral parasympathetic nucleus. Morrison & Spillane (1982) further characterized the raphe-spinal pathway,
which mediated this inhibition of parasympathetic neurone firing, by measuring vesical nerve branches of the pelvic nerve in the cat. Electrical stimulation of the nucleus raphe magnus in spinally intact, or hemitransected cat, showed that activation of the raphe-spinal pathway on one side of the cord could inhibit pelvic nerve potentials bilaterally. In addition, work by McMahon & Spillane (1982) further confirmed these findings; they showed that electrical stimulation of the raphe nuclei could produce a strong inhibitory influence on pelvic parasympathetic nerves to the bladder. Later, Morrison & Spillane (1986) showed that the non-selective 5-HT ligand, lysergic acid (LSD), largely reversed the inhibitory effects of electrical stimulation of the raphe-spinal pathway on micturition, further confirming the importance of 5-HT containing neurones. The use of LSD, however, did nothing to clarify which receptors were involved in this inhibitory central pathway.

1.7.8.2 5-HT receptors – historical perspective

Early studies concentrated on using 5-HT on isolated peripheral tissues and Gaddum and Picarelli (1957) were the first investigators to present evidence for two pharmacologically distinct types of 5-HT receptor. In the guinea pig ileum they identified two receptors mediating two different responses, D-receptors mediated contraction of smooth muscle and M-receptors mediating depolarisation of cholinergic nerves. Subsequently, research showed a variety of smooth muscle preparations were contracted via the D-receptors. However, owing to the use of non-selective ligands, dibenzyline (phenoxybenzamine) and morphine, in this initial classification of these receptors, this particular classification scheme has been criticized. 5-HT receptors mediating smooth muscle relaxation were identified in the guinea pig ileum and cat saphenous vein (Feniuk et al., 1983). These
5-HT receptor types did not fit into the basic nomenclature scheme proposed by Gaddum and Picarelli (1957). The discovery of 5-HT as a neurotransmitter in the brain also provoked intense research and using radioactive ligands Peroutka and Snyder (1979) identified and labelled two distinct 5-HT receptor types in the cortical membranes of the rat. Based on the labelling of two sites by either nanomolar concentrations of \(^3\text{H}\)5-HT or high affinity potent 5-HT receptor antagonists such as \(^3\text{H}\)spiperone, the nomenclature for these two binding sites became 5-HT\(_1\) and 5-HT\(_2\), respectively. Functional studies on peripheral tissues, autoradiographical and behavioural studies then came together and it was found that the D-receptor did correlate well with 5-HT\(_2\) receptor sites labelled (see Fozard, 1983 and Leysen et al., 1984), and this new nomenclature was introduced, although to what the M receptor related remained to be defined. Further, the functional correlates, which could be attributed to the 5-HT\(_1\) binding site, proved more difficult to define and this was mainly due to an oversimplification of the classification system. Pedigo et al., (1981) showed that spiperone displaced \(^3\text{H}\)5-HT in rat brain, in a distinctly biphasic pattern, with at least a 3000-fold difference between its dissociation constants for the high and low affinity sites. These sites were subsequently designated 5-HT\(_{1A}\) and 5-HT\(_{1B}\), respectively. Middlemiss and Fozard (1983) demonstrated that the tetralin derivative 8-OH-DPAT displayed high affinity and selectivity for the 5-HT\(_{1A}\) binding site. While Pazos et al., (1985) subsequently designated a third subtype of 5-HT\(_1\) receptor. Binding experiments in the porcine choroid plexus showed a population of 5-HT receptors that could not be categorized in the then current nomenclature, with a high affinity for \(^3\text{H}\)mesulergine. These were named 5-HT\(_{1C}\) receptors (now the 5-HT\(_{2C}\) receptor see section 1.6.8.6.3.). Despite such intense research, there was still controversy regarding the functional
significance of the 5-HT₁ receptor group (Fozard, 1983), thus a sensible classification scheme was proposed by Bradley et al., (1986). For simplicity, the 5-HTₓ system was adopted, to allow scope for modification as the knowledge advanced. The heterogeneous group of 5-HT₁ receptors could not functionally correlate with binding sites found, and such a mixture of 5-HT₁ mediated responses found by investigators. There were also no antagonists solely selective for the 5-HT₁ receptor and so although some receptors had pharmacological similarity to the 5-HT₁ binding site originally characterized, as they did not share exact functional characteristics with the original site, it was difficult to classify them into that group. All receptors in this group were termed “5-HT₁-like” at that time.

The 5-HT₂ receptor was well characterized with selective and potent ligands and the nomenclature was appropriate and already in use. The M-receptor had no established binding site equivalent and was termed the 5-HT₃ receptor (Bradley et al., 1986). This 5-HTₓ classification scheme was to be the basis for nomenclature used today, although receptors are now grouped more specifically according to their 2ⁿᵈ messenger systems. The identification of highly potent and selective agonists and antagonists has also aided the classification of new receptors, along with improvements in molecular pharmacology and cloning techniques.

### 1.7.8.3 5-HT receptors - current status

5-HT receptors are now classified into seven families (or groups), 5-HT₁⁻⁷, comprising a total of 14 structurally and pharmacologically distinct mammalian 5-HT receptor subtypes (see Hoyer et al., 1994), to date. Molecular techniques have revealed that all but one of the 5-HT receptor families are G-protein coupled metabotropic receptors with mostly seven
transmembrane spanning domains. The 5-HT\(_3\) receptor is the only member of the 5-HT receptors that is a ligand gated ion channel. The 5-HT\(_1\) receptor group now has 5 receptors in the family which have the same 2\(^{nd}\) messenger systems. Most of the "5-HT\(_1\)-like" group of receptors originally identified, but not classified into a particular group have now been found to be best suited to the newest receptor family, the 5-HT\(_7\) receptor. The 5-HT\(_1\)C receptor has also been re-classified according to its 2\(^{nd}\) messenger system and is now better situated in the 5-HT\(_2\) receptor family as the 5-HT\(_2\)C receptor (Humphrey \textit{et al.}, 1993). The 5-HT\(_4\), 5-h\(_5\), 5-h\(_6\) and 5-HT\(_7\) classes have been cloned most recently and of these receptors the 5-HT\(_4\) and 5-HT\(_7\) receptors have been fully characterized operationally and with regards to their 2\(^{nd}\) messenger transduction systems, in isolated tissue and in vivo systems the 5-h\(_5\) and 5-h\(_6\) receptor types have been cloned yet to be fully characterized with a physiological role in intact tissues, and so are denoted with lower case and will not be discussed. The main 5-HT receptor groups of interest to this study are the 5-HT\(_7\), 5-HT\(_1\) and 5-HT\(_2\) receptors; these will be discussed in detail and a summary of all the receptor subtypes can be found in Table 1.1.
Table 1.1 Classification of G-protein coupled 5-HT receptors. Modified from Roberts *et al.*, (2002)

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<td>Inhibition adenylyl cyclase</td>
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1.7.8.4 5-HT\textsubscript{1} receptor class

5-HT\textsubscript{1} receptor family forms the largest subclass and currently comprises five receptor subtypes, 5-HT\textsubscript{1A}, 5-HT\textsubscript{1B}, 5-HT\textsubscript{1D}, 5-HT\textsubscript{1E} and 5-HT\textsubscript{1F}. These receptor subtypes preferentially couple to Gi/o, which is negatively coupled to adenylyl cyclase. This enzyme, in turn, inhibits cAMP formation. Originally these receptors were grouped together because they have a high affinity for 5-HT and 5-carboxamidotryptamine (5-CT) and were antagonized by methiothepin and methysergide (Bradley et al., 1986), although this is now not a particularly relevant classification as the 5-HT\textsubscript{1E} and 5-HT\textsubscript{1F} receptors have relatively low affinity for 5-CT, compared to 5-HT. Current classification extends to include 2\textsuperscript{nd} messenger transduction properties including preferential G-protein binding and homology in the genetic primary coding sequences of the receptors (Humphrey et al., 1993).

1.7.8.4.1 5-HT\textsubscript{1A} receptor

The 5-HT\textsubscript{1A} receptor is one of the best-characterized receptor subtypes, owing to the fact that it was the first 5-HT receptor to be fully sequenced and cloned (Fargin et al., 1988) and to the early identification of the selective agonist 8-OH-DPAT (Middlemiss & Fozard, 1983). There are several other agonists, which do have an affinity for the 5-HT\textsubscript{1A} receptor (see Table 1.2), but (R)-8-OH-DPAT is a highly selective agonist at this receptor. Interestingly its enantiomer (S)-8-OH-DPAT is only a partial agonist in functional studies.
Chapter One: Introduction

(Nelson, 1991). The agonist 5-CT is non-selective between 5-HT_1 receptor subtypes but does bind with a high affinity to the 5-HT_{1A} receptor. The production of selective 5-HT_{1A} receptor antagonists proved more problematic compared to the production of agonists as evidence shows 'apparent' antagonists, such as NAD 190, BMY73778 and WAY100135 exhibiting agonist properties in regions of the brain with a high receptor reserve (Fletcher et al., 1993). However, WAY-100635 (Fletcher et al., 1996) and NAD299 (Johansson et al., 1997) have been identified and are highly selective for the 5-HT_{1A} receptor, without any additional agonist properties identified, at present. Brain mapping using autoradiographical techniques has shown the distribution of 5-HT_{1A} receptors to be high limbic areas such as the hippocampus, septum, amygdala and cortex as well as the raphe nuclei (Radja et al., 1991). In these areas 5-HT_{1A} receptors can function as somatodendritic autoreceptors to reduce 5-HT release from terminals or as a heteroreceptor to reduce the release of other transmitters (see Barnes & Sharp, 1999). Many of these areas, especially the limbic areas, modulate of emotion and are involved in psychiatric disorders such as anxiety and depression. Central nervous system research into areas such as anxiety and depression has lead to the production of drugs such as fluoxetine (Prozac), which serves to increase the 5-HT release in the forebrain, by blocking serotonin terminal re-uptake (see Jones & Blackburn, 2002). One drawback with this and other selective serotonin reuptake inhibitors (SSRI) is their delayed onset of action. It takes 2-6 weeks for these drugs to exert their antidepressant effects, owing to the activation of somatodendritic 5-HT_{1A} autoreceptors, which decrease the firing rate of dorsal raphe neurones, and thus, the release of 5-HT in the cortex. Over time, the 5-HT_{1A} autoreceptors become desensitised thus there is large increase in 5-HT cortical regions resulting in an antidepressant and anxiolytic effect.
Romero et al., (1996) developed a 5-HT$_{1A}$ receptor antagonist, pindolol, which was produced to accelerate the effects of SSRI's. Initial trial results were promising but owing to pindolol's high affinity for $\beta$-adrenoceptors and therefore low dosage used to reduce unwanted side effects, further trials showed mixed results (see Artigas et al., 2001). Further work is being done in this area to clarify the role of 5-HT$_{1A}$ receptors in the treatment of depression (see Artigas et al., 2001).

5-HT$_{1A}$ receptors are also implicated in the control of other physiological functions and diseases e.g. central parasympathetic regulation of the heart and lungs (see Ramage, 2001), sleep, cognition, feeding, Alzheimer's disease and ischemia to name a few (see Roberts et al., 2002). Although the precise role of these receptors in feeding behaviour is equivocal as the assumption is made on agonist studies which do not support a physiological role for the receptor, merely a pharmacological one. 8-OH-DPAT, gepirone and buspirone have been shown to cause hyperphagia, which can be antagonized by pindolol (see Hoyer et al., 1994), but systemic administration of WAY-100635 does not modify feeding behaviour alone (Grignaschi et al., 1998). Conversely, infusion of 5-HT$_{1A}$ receptor agonist flesinoxan into the dorsal raphe nuclei significantly increased REM sleep, and the physiological function of 5-HT$_{1A}$ receptors is demonstrated by the selective 5-HT$_{1A}$ antagonist WAY-100635 significantly reducing REM sleep (Monti et al., 2000). 5-HT$_{1A}$ receptors have also been shown to play a fundamental role in the reflex regulation of parasympathetic outflow to the heart in rats (Bogle et al., 1990), rabbits (Futuro-Neto et al., 1993; Dando et al., 1998; Skinner et al., 2002) and cats (Wang & Ramage, 2001), to the airways in cats (Bootle et al., 1996) and guinea pigs (Bootle et al., 1998) and most importantly in terms of
this current research, 5-HT\textsubscript{1A} receptors have been shown to have a role in the control of the parasympathetic outflow to the bladder in rats (Lecci \textit{et al.}, 1992; Testa \textit{et al.}, 1999; Conley \textit{et al.}, 2001; Kakizaki \textit{et al.}, 2001; Pehrson \textit{et al.}, 2002). Lecci \textit{et al.}, (1992) showed that using a 5-HT\textsubscript{1A} receptor agonist, 8-OH-DPAT, the micturition reflex could be facilitated in the anaesthetized rat. These effects were mediated at both the spinal and supraspinal level. Selective antagonists for 5-HT\textsubscript{1A} receptors, such as WAY-100635, have also been shown to inhibit micturition in conscious rats and guinea pigs and anaesthetized rats (Testa \textit{et al.}, 1999; Conley \textit{et al.}, 2001; Leonardi \textit{et al.}, 2001; Pehrson \textit{et al.}, 2002) and also to have a ‘protective’ quality by reversing acetic acid induced hyperactivity of bladder reflexes (Pehrson \textit{et al.}, 2002). Furthermore, 5-HT\textsubscript{1A} receptor activation has been reported to inhibit bladder activity under condition of acetic acid irritation to the bladder in cats, which is the opposite to the effects seen in rats (Thor \textit{et al.}, 2002).

1.7.8.4.2 5-HT\textsubscript{1B} receptor

5-HT\textsubscript{1B} receptor was originally defined according to its pharmacological profile, with a low affinity binding site for spiperone in rat basal ganglia (Pedigo \textit{et al.}, 1981). However, in the basal ganglia of other mammals, such as the guinea pig, pig, calf, rabbit, dog, monkey and human, the pharmacological profile of the receptors present was characteristic of 5-HT\textsubscript{1D} receptor sites, with no evidence for the presence of 5-HT\textsubscript{1B} receptors (see Hoyer \textit{et al.}, 1994). Investigators speculated at an early stage that CNS distributions of 5-HT\textsubscript{1B} receptors in rodent and 5-HT\textsubscript{1D} receptors in non-rodent species were equivalent sites (Hoyer & Middlemiss, 1989), and this turned out to be the case. Cloning techniques then found two genes encoding for the 5-HT\textsubscript{1D} receptors, which were then designated 5-HT\textsubscript{1D\alpha}
and 5-HT₁D₉ (Hartig et al., 1992). Sequence homology of the cloned rodent 5-HT₁B receptor with the human 5-HT₁D₉ receptor was about 96% (Jin et al., 1992) and so a nomenclature change occurred with the non-rodent 5-HT₁D₉ receptor realigning to the 5-HT₁B receptor classification (Hartig et al., 1996). Owing to significant variation across species, prefixes are often used to denote species-specific 5-HT₁B receptors (e.g. human becomes h5-HT₁B and rat becomes r5-HT₁B; Hoyer et al., 1994). 5-HT₁D₉ has become simply 5-HT₁D and these receptors are found in only very low concentrations in rat (Bruinvels et al., 1993). Studies on rat and human transfected cell lines have shown that the 5-HT₁B receptor negatively couples to adenylyl cyclase (Adham et al., 1992) in the same way as other members of the 5-HT₁ receptor family.

Research into 5-HT₁B receptors and their physiological roles was initially hampered by the lack of selective ligands, especially those with blood brain barrier permeability. However, a large number of agonists have since been produced with a high affinity for the 5-HT₁B receptor including CP93,129, 5-CT and sumatriptan, unfortunately most are not selective. In contrast, there are now a number of 5-HT₁B receptor antagonists, such as SB-224289 and SB-236057, which are selective and potent.

5-HT₁B receptors are expressed in vascular smooth muscle and are involved in the contractile response of arteries in humans (Hamel et al., 1993), rabbits (Akin & Gurdal, 2002) and rats (Hoyer, 2001), to 5-HT. Centrally, 5-HT₁B receptors are densely expressed in the basal ganglia, substantia nigra, globus pallidus, hippocampus and cerebellum, among other brain regions, and have been shown to have a modulatory role over the control of the
suprachiasmatic nucleus (Smith et al., 2001). 5-HT\textsubscript{1B} receptors have also been shown to be involved in food intake and satiety (Lee et al., 2002) and specifically gastric accommodation in canines (De Ponti et al., 2003) as well as being implicated in drug reinforcement, stress sensitivity, mood, anxiety and aggression (see Clark & Neumaier, 2001). Indeed, knockout mutant mice lacking 5-HT\textsubscript{1B} receptors are more aggressive than wild type (Saudou et al., 1994). The 5-HT\textsubscript{1B} receptor functions not only as an autoreceptor but also as a heteroreceptor (Pazos et al., 1985) and the different locations have been shown to affect the sensitivity of the 5-HT\textsubscript{1B} receptors to agonist and antagonist binding. Measurements of tritiated neurotransmitter release from synaptosome preparations of rat brain striatum or hippocampus showed that 5-HT\textsubscript{1B} autoreceptors are more sensitive to agonist and antagonist binding, compared with 5-HT\textsubscript{1B} heteroreceptors on dopaminergic or cholinergic terminals (Sarhan & Fillion, 1999). These findings possibly indicate that 5-HT\textsubscript{1B} receptors preferentially control release of 5-HT versus other transmitters, although in a physiological setting results may vary. There is also evidence for an endogenous tetrapeptide, 5-HT-moduline, which acts as a ligand for the 5-HT\textsubscript{1B} receptor, and has the properties of an allosteric modulator (Rousselle et al., 1996). Thus far, this is a unique situation for 5-HT receptor family and the full role of this tetrapeptide is yet to be fully elucidated (Plantefol et al., 1999). The only role for the 5-HT\textsubscript{1B} receptor demonstrated in micturition has been in the mouse bladder smooth muscle, where 5-HT was shown to potentiate electrically induced contraction of mouse isolated urinary bladder strip via the 5-HT\textsubscript{1B} receptor (Holt et al., 1986).
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1.7.8.4.3 5-HT_{ID} receptor

After the re-classification of 5-HT_{1B} and 5-HT_{ID} receptors it was difficult to determine the distribution and pharmacology of 5-HT_{ID} receptors (previously called 5-HT_{1Da}), owing to the lack of selective ligands that could distinguish between the 5-HT_{1B} and 5-HT_{ID} binding sites. Since, ligands such as 5-CT and sumatriptan, bound with high affinity at the 5-HT_{ID} receptor site but were not selective, other more selective agonists were developed, such as L-775606 (Longmore et al., 2000). L-775606 was shown to be 360-times more potent in activating 5-HT_{ID} receptors compared to 5-HT_{1B} receptors in human recombinant receptor cell lines (Macleod et al., 1997). Antagonists for the 5-HT_{ID} receptor include GR127935, which binds with a high affinity to the 5-HT_{ID} and 5-HT_{1B} receptor sites, whereas BRL15572 is a selective 5-HT_{ID} antagonist, approximately 60-fold more selective for 5-HT_{ID} over 5-HT_{1B} receptor sites. Binding studies with techniques masking 5-HT_{1B} receptors, on rat brain, have shown that the 5-HT_{ID} receptor is present in the basal ganglia, hippocampus and cortex (Bruinvels et al., 1993), and in human brain receptors were present in the basal ganglia, PAG of the midbrain and the spinal cord (Castro et al., 1997). Evidence also suggests that receptors are present on terminals of both 5-HT and non-5-HT containing neurones and that 5-HT_{ID} receptor can function as a hetero- and an autoreceptor (Hamblin et al., 1992).

Functionally, 5-HT_{ID} receptors can inhibit dural plasma extravasation (Waeber et al., 1997) which is implicated in migraine relief. Originally it was thought that both 5-HT_{1B} and 5-HT_{ID} receptors both mediated cranial blood vessel constriction, but it is now evident that selective agonists for 5-HT_{ID} receptors are really devoid of any significant vasocontractile

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activity in cerebral arteries (Bouchelet et al., 2000). The 5-HT\textsubscript{1D} relief element of migraine treatments such as sumatriptan and naratriptan, mixed 5-HT\textsubscript{1B/1D} agonists, seems to be exerted through inhibition of dural plasma extravasation, as already mentioned, and an underlying mechanisms involving the inhibition of trigeminovascular afferents involved in the neurogenic inflammatory response (Goadsby & Knight, 1997). 5-HT\textsubscript{1D} receptors do seem to mediate vasoconstriction in other blood vessels, such as the human coronary artery, but with much less potency than 5-HT\textsubscript{1B} receptors, making antimigraine treatments with a more selective 5-HT\textsubscript{1D} action more preferable for reduced side effects (Longmore et al., 2000).

1.7.8.4.4 5-ht\textsubscript{1E} receptor

Little information is available on the 5ht\textsubscript{1E} receptors, they were first detected by their 5-HT\textsubscript{1}-like qualities in radio ligand binding studies, except they had a low affinity for 5-CT (Leonhardt et al., 1989). There are currently no selective ligands for 5-ht\textsubscript{1E} receptors and little is known about the physiological role of the receptor.

1.7.8.4.5 5-ht\textsubscript{1F} receptor

The 5-ht\textsubscript{1F} receptor, initially called the 5-ht\textsubscript{1E\textsubscript{B}} (Amlaiky et al., 1992), shows a similar pharmacological profile to 5ht\textsubscript{1E} receptor, although the mRNA distribution in the brain is quite different (Barnes & Sharp, 1999). Binding studies in the guinea pig, rat and human show good correlation between species, with highest levels of receptors in the cortical and hippocampal areas, claustrum and the caudate nucleus (Bruinvels et al., 1994; Waeber &
Moskowitz, 1995; Pascual et al., 1996). In a similar way to the 5-HT\(_1E\) receptor the receptor has a high affinity for 5-HT but a low affinity for 5-CT (Amlaky et al., 1992), though in contrast its high affinity for sumatriptan is what mainly discriminates 5-HT\(_1F\) from 5-HT\(_1E\) receptors. Selective ligands have also recently been identified for the 5-HT\(_1F\) receptors and have implicated the receptor as a possible target for the treatment of migraine, as the agonists inhibited dural plasma extravasation (Phebus et al., 1997; Johnson et al., 1997).

### 1.7.8.5 5-HT\(_7\) receptor class

The 5-HT\(_7\) receptor is the most recently identified and cloned 5-HT G-protein coupled receptor. The 5-HT\(_7\) receptor has been characterized by its high affinity to 5-CT, 5-HT and atypical antipsychotics such as clozapine, but there are no selective agonists, at present.

There are, however, a number of highly selective 5-HT\(_7\) receptor antagonists available such as SB269970 (Hagan et al., 2000) and SB656104 (Forbes et al., 2002) and such compounds have been useful pharmacological tools especially when labelling the central distribution of 5-HT\(_7\) receptors (Thomas et al., 2002). 5-HT\(_7\) receptors have been cloned from human (Bard et al., 1993), rat (Ruat et al., 1993) and guinea pig (To et al., 1995), and are found to positively couple to adenylyl cyclase preferentially via G\(_i\)\(\alpha\)-protein (Adham et al., 1998).

Four different splice variants have been described (5-HT\(_{7a}\), 5-HT\(_{7b}\), 5-HT\(_{7c}\) and 5-HT\(_{7d}\)), but only 5-HT\(_{7a}\) and 5-HT\(_{7b}\) are present in both rat and human tissues, (see Vanhoenacker et al., 2001). The 5-HT\(_{7c}\) isoform is found only in rat and the 5-HT\(_{7d}\) is found only in human (Heidmann et al., 1998). High levels of the mRNA for the human receptors were found in brain, coronary artery and GI tract (Bard et al., 1993), whereas in the rat and guinea pig strongest mRNA signals were found in the hypothalamus, brainstem...
and hippocampus, with low signals in the stomach and ileum (Ruat et al., 1993; Eglen et al., 1997). Work showing the distribution patterns of the specific splice variants of the human and rat 5-HT\textsubscript{7} receptors indicate that they do not match exactly (Heidmann et al., 1998) and this is likely to be a consideration for the future, but at present it is unknown whether there are differences between these receptor isoforms in their function at a physiological level. Functional responses mediated by this receptor group have been documented for a number of years. 5-HT\textsubscript{7} receptors were originally described as orphan "5-HT\textsubscript{1}-like" receptors that mediated actions such as relaxation of the isolated guinea pig ileum, cat saphenous vein (Feniuk et al., 1983), isolated neonatal porcine vena cava (Trevethick et al., 1986) and mediated other effects such as tachycardic responses to 5-HT in the cat (Saxena et al., 1985). It has now been confirmed that relaxation of various peripheral tissues is mediated via 5-HT\textsubscript{7} receptors including, the dog coronary and cerebral arteries (Terron, 1996; Terron & Falcon-Neri, 1999; Cushing et al., 1996), guinea pig ileum (Carter et al., 1995), rabbit femoral vein (Martin & Wilson, 1994), Cynomolgus monkey jugular vein (Leung et al., 1996), human colonic circular smooth muscle (Prins et al., 1999). In addition, other effects such as tachycardia in the cat (Villalon et al., 1997), hypotension in the rat (Terron, 1997), external carotid vasodilatation in vagosympathectomized dogs (Villalon et al., 1997) have also been established as being mediated via 5-HT\textsubscript{7} receptor activation. 5-HT\textsubscript{7} receptors have been implicated in the control of circadian rhythm and dense receptor expression in the suprachiasmatic nuclei of the hypothalamus (Lovenberg et al., 1993; Heidmann et al., 1998) has been demonstrated. The efficacy of antipsychotic and antidepressant compounds, such as clozapine, is also thought to be due to 5-HT\textsubscript{7} receptor binding in limbic areas (Roth et al., 1994). In addition,
in situ hybridisation techniques have revealed high levels of signals for the 5-HT\(_7\) receptor in the pontine nuclei and clear signals in the raphe nuclei in the rat (Ruat et al., 1993), important sites for the control of micturition. In fact, interference in micturition has been shown to occur by a number of non-selective 5-HT ligands, which have subsequently been shown to show an affinity for 5-HT\(_7\) receptors. 5-HT\(_7\) receptors are also important in the control of thermoregulation in the mouse and guinea pig (Hagan et al., 2002; Guscott et al., 2003), with the selective antagonist SB-269970 blocking hypothermia induced by 5-CT but the selective 5-HT\(_{1A}\) antagonist, WAY-100635, having no effects.

### 1.7.8.6 5-HT\(_2\) receptor class

Initial progress was hampered on 5-HT\(_2\) receptor research, as the ligands produced were not selective. The 5-HT\(_2\) receptor class currently comprises three receptor subtypes, 5-HT\(_{2A}\), 5-HT\(_{2B}\) and 5-HT\(_{2C}\) which are all positively coupled to phospholipase C (see Gerhardt & van Heerikhuizen, 1997). These receptor subtypes are fairly similar to each other and it was originally difficult to distinguish them, but the more recent development of selective ligands has allowed further characterisation of these receptor subtypes. Initially, using \[^3\text{H}\] ketanserin a non selective 5-HT\(_2\) receptor antagonist, 5-HT\(_2\) receptor sites were labelled in various tissues including frontal cortex from rat, rabbit, guinea pig, dog, human; striatum from rat, guinea pig, dog; platelets from cat (see Leysen et al., 1984). Isolated tissue studies have also shown the presence of 5-HT\(_2\) receptors in the periphery and they mediate contraction of the trachea in guinea pigs, and the contraction of uterus of the rat (Cohen et al., 1984). Contraction of cat (Saxena et al., 1985) and dog bladder smooth muscle are also mediated via 5-HT\(_2\) receptors, however this contrasts with rat bladder, where there is no
marked contractile responses to 5-HT (Cohen, 1990). As the 5-HT$_{2C}$ receptor is the member of this receptor family primarily of interest in this study, 5-HT$_{2A}$ and 5-HT$_{2B}$ receptor will only be discussed very briefly.

1.7.8.6.1 5-HT$_{2A}$ receptor

In terms of agonists the 5-HT$_{2A}$ receptor has a low affinity for 5-HT but a high affinity for agonists 2,5-dimethoxy-4-iodophenylpropylamine (DOI), (-)2,5-dimethoxy-4-iodoamphetamine (DOB) and a-methyl-5-HT, but these agonists are not particularly subtype selective. Where the antagonists are concerned, it is known that classical antagonists such as ketanserin and spiperone bind with 20-fold selectivity for 5-HT$_{2A}$ receptors over 5-HT$_{2B/2C}$ receptors, but both of these compounds are non selective and bind to other monoamine receptors. Newer, selective antagonists for the 5-HT$_{2A}$ receptor have been developed and include MDL100907 (see Roberts et al., 2002).

The 5-HT$_{2A}$ receptors have been shown to be widely distributed in the peripheral tissues and the effects mediated by these receptors include vascular smooth muscle contraction in rabbit aorta, rat caudal artery, dog gastroplenic vein, contraction of bronchial, uterine and bladder smooth muscle and contraction of guinea pig ileum (see Hoyer et al., 1994). Central effects mediated by 5-HT$_{2A}$ receptors include release of vasopressin and various central sympathetic effects (see Ramage, 2001), neuroexcitation of guinea pig cortical pyramidal neurones (Davies et al., 1987) and rat dorsal raphe cell bodies (Roberts & Davies, 1989) plus some neuroendocrine functions (see Hoyer et al., 1994).
1.7.8.6.2 5-HT$_{2B}$ receptor

Agonists for the 5-HT$_{2B}$ receptor include $\alpha$-methyl-5-HT and BW723C86, which do show some selectivity for this receptor subtype over the others in the group. Of the antagonists for 5-HT$_2$ receptors, it has been shown that 5-HT$_{2B}$ receptors have a lower affinity for the antagonist ritanserin and a higher affinity for yohimbine, but newer and selective antagonists have now been developed e.g. SB204741 (see Roberts et al., 2002).

5-HT$_{2B}$ receptors, were originally identified in the rat stomach fundic strip, mediating contraction, and were known as 5-HT$_{2F}$ receptors (F meaning 'fundic') until they were reclassified in the new nomenclature (Humphrey et al., 1993). Loric et al., (1992) cloned the homologue of the 5-HT$_{2B}$ receptor in the mouse and found mRNA present in the intestine, heart, and to a lesser extent in the brain and kidney. Functionally, not much is known about the role of 5-HT$_{2B}$ receptors, other than it mediates fundic smooth muscle contraction and has a role in central cardiovascular regulation (see Ramage, 2001).

1.7.8.6.3 5-HT$_{2C}$ receptor

The 5-HT$_{2C}$ receptor was initially named the 5-HT$_{1C}$ receptor before being re-classified as 5HT$_{2C}$ receptor. Upon cloning and sequencing it has become obvious that the receptor has a better fit in its new family, in terms of 2$^\text{nd}$ messenger system, structural and functional homology (Hoyer et al., 1994). Agonists $\alpha$-methyl-5-HT and mCPP have high affinity for the 5-HT$_{2C}$ receptor, although they are not selective (see Roberts et al., 2002). Mesulergine is an antagonist at the 5-HT$_{2C}$ receptors and binds with a high affinity (Closse, 1983), but is not particularly subtype selective between the 5-HT$_2$ receptor family, as are many 5-HT$_2$
antagonists. The development of selective antagonists has produced SB243213 (Bromidge et al., 2000), which is highly selective for the 5-HT\textsubscript{2C} receptor, as well as SB242084 (see Roberts et al., 2002). Initially, experimental evidence showed a high density of 5-HT\textsubscript{2C} receptor binding in the choroid plexus in a number of species. The further use of \[^{3}H\] mesulergine versus \[^{3}H\] ketanserin binding, has aided the identification of new 5-HT\textsubscript{2C} receptor sites in the central nervous system (Pazos et al., 1985). Pazos et al., (1985) postulated a role for 5HT\textsubscript{2C} receptors in the control of the composition and volume of cerebrospinal fluid. Receptors were shown to be in abundance on the epithelial cells of the choroid plexus and serotonergic terminals were known to be present on the walls of cerebral ventricles, indicating a controlling role. 5-HT\textsubscript{2C} receptors are also present in the limbic region and those areas associated with motor control (Pazos et al., 1985) and so are also implicated in a number of processes such as locomotion, feeding, migraine, anxiety and obsessive compulsive disorder (see Jones & Blackburn, 2002). Bladder function is also controlled to some extent by 5-HT\textsubscript{2C} receptors (see Chapter 4).

1.7.8.7 5-HT\textsubscript{3} receptor class

The 5-HT\textsubscript{3} receptors are not particularly pertinent to the current work and will only be discussed very briefly although there is evidence that they might play role in micturition (see below).

The 5-HT\textsubscript{3} receptor, originally termed the M-receptor (Gaddum & Picarelli, 1957) is the only member of the 5-HT receptor family that is a ligand-gated ion channel and is located in the brain and peripheral tissue (see Hoyer et al., 1994). It mediates a wide range of
affects e.g. pain sensations on peripheral afferents (Richardson et al., 1985), stimulation or inhibition of the cardiovascular system (Saxena & Villalon, 1991) and nausea and vomiting associated with chemo- and radiotherapy (Andrews et al., 1988). Saxena et al., (1985) also showed that fast tonic contraction of the cat bladder is mediated via 5-HT₃ receptors.

Further work in cats by Espey et al., (1995) has shown that intrathecal administration of 5-HT₃ receptor antagonist zatosetron decreases micturition volume in conscious spinally intact cats. After spinal transection a 5-HT₃ receptor agonist increased volume threshold in the same cats. In addition, ascending information from pelvic nerve stimulation was shown to increase during 5-HT₃ receptor blockade (Espey et al., 1998), confirming earlier findings. This work implies that 5-HT₃ receptors are tonically active and have an inhibitory role in bladder function, at the spinal level in cats. Later work by Testa et al., (2001) however, has shown that 5-HT₃ receptors do not seem to be involved in the control of micturition in rats.

1.7.8.8 5-HT₄ receptor class

5-HT₄ receptors will again be discussed very briefly. They are present in a wide variety of tissues and species, and mediate many functions by stimulation of adenylyl cyclase (see Hoyer et al., 1994). Systemic administration of 5-HT₄ receptor antagonists had no effects on the micturition reflex in anaesthetized rats (Testa et al., 2001). Central administration of a 5-HT₄ receptor agonist, RS67506, did however decrease bladder capacity and micturition volume (Ishizuka et al., 2002), although this is perhaps of little importance, as selective antagonists had no effect. 5-HT₄ receptors have also been shown to mediate relaxation of the bladder smooth muscle in the Rhesus and Cynomolgus monkeys (Waikar et al., 1994).
This is in contrast to putative 5-HT$_4$ receptors found in the urinary bladder of human, where they mediate contraction of the smooth muscle (Corsi et al., 1991; Tonini et al., 1994).
Table 1.2 Potency of various ligands at 5-HT receptors

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<th>5-HT₁A</th>
<th>5-HT₁B</th>
<th>5-HT₂D</th>
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a – Hoyer et al., 1994
b – Leysen et al., 1996
c – Napier et al., 1999
d – Roberts et al., 2001
e – Forbes et al., 2002
f – Hoyer et al., 1989
g – Wood et al., 2001
h – Beer et al., 1998
i – Macor et al., 1990
1.8 **Aims of this study**

The physiology and pharmacology of the LUT, and the central nervous system pathways that exert control over this vital area have been well studied, but are still not yet fully understood. From the introduction it is clear that previous work has outlined a great number of neurotransmitters involved in the control of the bladder and urethra, this study concentrates on just one of those neurotransmitters, 5-Hydroxytryptamine (5-HT).

As already outlined in the introduction, a number of receptors in the 5-HT receptor family have been shown to play a role in the control of parasympathetic outflow and of particular interest, outflow to the lower urinary tract. However, as the newest addition to the 5-HT receptor family, the role of the 5-HT$_7$ receptor is still being characterised. From current evidence, central 5-HT$_7$ receptors are important in the control of autonomic functions, such as temperature control (Guscott *et al.*, 2003), and may control circadian rhythm (Lovenberg *et al.*, 1993), but a role in the central control of micturition has not been demonstrated. Therefore, a portion of the studies in this thesis are concerned with determining the role of 5-HT$_7$ receptors in the control of micturition in the female anaesthetized rat, using selective 5-HT$_7$ antagonists. In addition, experiments were carried out to see if the 5-HT receptor antagonist mesulergine would have similar effects on micturition, to selective 5-HT$_7$ receptor antagonists, owing to its high affinity for 5-HT$_7$ receptors. Owing to the lack of any selective agonists for 5-HT$_7$ receptors, the ability of the non-selective 5-HT$_7$ agonist, 5-CT, to activate micturition was also investigated. This lead to further preliminary agonist studies examining the role of the 5-HT$_{1B/1D}$ receptors in the control of micturition.
The female rat offers a number of advantages over other animal models to study the role of 5-HT receptors as there is already a detailed knowledge of anatomy, physiology and pharmacology in this species, plus the function of the LUT during micturition does correlate with human voiding. In addition the female of the species is used to avoid any complications of the genital system, as the male penis has dual functionality. One portion of this study was carried out during an industrial placement at GlaxoSmithKline in Philadelphia, USA and it was possible to examine the role that 5-HT7 receptors may play in control of micturition in the cat, as it has been reported that the role of 5-HT1A receptors in control of the LUT in rat and cat may differ between the two species (de Groat, 2002), and therefore the role of 5-HT7 receptors may also differ. The simple cystometric techniques used in this study are a useful method for determining the role of various receptor subtypes in LUT function by challenging a protocol with central and systemic administration of selective drugs and comparing this to control experiments.

These investigations into the role of central 5-HT receptor subtypes in the control of micturition are an attempt to clarify and increase our understanding of the complex physiological and pharmacological control mechanisms that operate the LUT, in order to provide potential new targets for treatments of LUT dysfunction.
Chapter Two

Methods
Chapter Two: Methods

The rat experiments were carried out under the Animals (Scientific Procedures) Act, 1986. After completion of experiments, animals were killed by an overdose of pentobarbitone sodium i.v.

2.1 Rats

2.1.1 General preparation

Experiments were performed on female Sprague-Dawley rats (200 – 280 g) initially anaesthetized with isoflurane (4% in 100% oxygen), and maintained with urethane (1.2 g kg\(^{-1}\), i.v.). Depth of anaesthesia was assessed by the stability of blood pressure and heart rate, and by an absence of hind limb withdrawal in response to paw pinch. If needed, supplementary doses of urethane were given (0.1 g kg\(^{-1}\), i.v.). To maintain a patent airway the trachea was intubated. The left jugular vein was cannulated for anaesthetic and drug administration, and the right common carotid artery was cannulated with a heparinised cannula (20 units ml\(^{-1}\) heparin in 0.9% w v\(^{-1}\) saline) for the measurement of arterial blood pressure and for sampling arterial blood for gas analysis. Blood pressure was measured using a pressure transducer (Gould Statham P23Db), and heart rate (HR) derived electronically from the blood pressure signal using AcqKnowledge version 3.5.3 software (Biopac Systems Inc, U.S.A.). Body temperature was monitored with a rectal temperature probe and maintained between 36-38°C using a homeothermic blanket system (Harvard). All animals were spontaneously breathing oxygen-enriched room air (0.05–0.10 l min\(^{-1}\)).
Blood gases were maintained between 90-130 mmHg $P_{O_2}$, 40-50 mmHg $P_{CO_2}$ and pH 7.3 – 7.4 measured by a Corning pH/blood gas analyser (Model 238). The animals were infused (6 ml kg$^{-1}$ h$^{-1}$, i.v.) with a solution consisting of 10 ml plasma substitute (Gelofusine), 10 ml distilled water, 0.04 g glucose and 0.168 g sodium bicarbonate to prevent the development of non-respiratory acidosis and to maintain blood volume. All animals were placed in a stereotaxic frame. The head was tilted so the animal could lie in the supine position to prevent the weight of the animal affecting bladder and urethral pressure recordings.

2.1.2 Cannulation of the 3rd ventricle and subarachnoid space

In rats, to cannulate the lateral cerebral ventricle a stainless steel guide cannula (22 gauge) was implanted. The co-ordinates used from bregma were 3.5 mm ventral, 1.5 mm lateral and 1 mm posterior. Drug and vehicle solutions were administered through an i.c.v. injection cannula (28 gauge) attached by a length of polythene tubing to a 100 µl syringe (Hamilton). For intrathecal (i.t.) administration, in rats, a polyethylene cannula (PE-10) was inserted through a small puncture in the atlanto-occipital membrane and passed into the subarachnoid space surrounding the spinal cord (Yaksh & Rudy, 1976). The tubing was measured to the correct length externally prior to insertion and then passed caudally to the appropriate level of the spinal cord for administration. The cannula was clamped in position and drug/vehicle was delivered via a needle inserted into the guide tube, over 20 s. The location of the cannula placement for both injections sites was subsequently confirmed by the administration of 5 µl of 2% pontamine sky blue dye.
2.1.3 Measurement of bladder pressures – cystometry method

The ureters were exposed through retroperitoneal incisions, and each ureter was cannulated proximal to the kidney in order to prevent urine flow to the bladder during the experiment. The urinary bladder was exposed by a midline abdominal incision. A small incision was made in the bladder dome and a single cuffed cannula (0.86 mm internal and 1.52 external diameter) was inserted into the bladder dome. This was connected via a T-piece to a pressure transducer (Gould Statham P23Db) and a syringe pump for the infusion of saline (0.9% w/v) at a rate of 0.1 ml min⁻¹, to evoke the micturition reflex. Backflow through this cannula allowed the bladder to be emptied of residual fluid after micturition had occurred. (Figure 2.1).

2.1.4 Measurement of bladder pressures – isovolumetric method

The ureters were exposed through retroperitoneal incisions, and each ureter was cannulated proximal to the kidney in order to prevent urine flow to the bladder during the experiment. The urinary bladder was exposed and through an incision in the bladder dome, two cannulae (outer diameter: 1.2 mm, inner diameter 0.52 mm) were inserted into the lumen of the bladder. One cannula was connected to a pressure transducer (Gould Statham P23Db) to record intravesical bladder pressure (mmHg). The second cannula was connected to a syringe pump for the infusion of saline (0.9% w/v) into the bladder, to evoke the micturition reflex (0.05 ml min⁻¹). Backflow through this cannula allowed the bladder to be emptied of saline. A third cannula was inserted through the bladder dome, into the proximal urethra. This cannula comprised an outer tube (outer diameter: 1.52 mm, inner
diameter: 0.86 mm), through which saline was perfused, and an inner tube (outer diameter: 0.80 mm, inner diameter: 0.40 mm), through which urethral pressure was measured, encased in a Eppendorf pipette tip which was wedged in the neck of the bladder (see Kakizaki et al., 1997; Figure 2.1). The urethra was perfused at a constant rate of 0.075 ml min$^{-1}$, so that any alterations in urethral pressure would be reflected in changes of resistance to the flow of saline. The three cannulae were secured with purse string sutures in the bladder dome.
Figure 2.1 Diagram of methods used for A) cystometry in rat and cat experiments and B) isovolumetric methods in rat experiments.

Modified from Wibberley et al., (2002).
2.2 Cats

Experiments were approved by the Institutional Animal Care and Use Committee of GlaxoSmithKline under the Animals (Scientific Procedures) Act, 1986. After completion of experiments, animals were killed by an overdose of pentobarbitone sodium without recovery (120 mg/kg i.v.).

2.2.1 General preparation

In experiments performed on female cats (2-3.5 kg) they were initially anaesthetized with isoflurane (4% in 100% oxygen) and maintained with α-chloralose (70 mg kg\(^{-1}\) i.v.). Depth of anaesthesia was assessed by the stability of blood pressure and heart rate, and by an absence of hind limb withdrawal in response to paw pinch. The left femoral vein was cannulated for anaesthetic and drug administration, and the left femoral artery was cannulated with a heparinised cannula (20 units ml\(^{-1}\) heparin in 0.9% w v\(^{-1}\) saline) for the measurement of arterial blood pressure and for sampling arterial blood for gas analysis. Blood pressure was measured using a pressure transducer (Gould Statham P23Db), and heart rate (HR) derived electronically from the blood pressure signal using AcqKnowledge version 3.5.3 software (Biopac Systems Inc, U.S.A.). Body temperature was maintained between 36-38°C using a homeothermic blanket system (Harvard). To maintain a patent airway the trachea was intubated. All animals were artificially ventilated with room air (rate 15 min\(^{-1}\), stroke volume 15 ml kg\(^{-1}\)) by use of a positive pressure pump. Blood gases
were maintained between 90-130 mmHg P\textsubscript{O2}, 35-45 mmHg P\textsubscript{CO2} and pH 7.3 – 7.4 measured by a Corning pH/blood gas analyser (Model 238).

2.2.2 Measurement of bladder pressures – cystometry method

The urinary bladder was exposed by a midline abdominal incision. The proximal ends of each ureter, exiting from the kidney, were tied and cut. Gauze wicks were tied over the holes cut into each ureter and used to drain urine outside the peritoneal cavity. This prevented the bladder filling with urine during experiments. A small hole was punctured in the bladder dome through which tubing (size 0.86 mm internal and 1.52 external diameter)) was passed, and tied in place with purse string sutures. This was connected via a T-piece to a pressure transducer (Gould Statham P23Db) and a syringe pump for the infusion of saline (0.9% w v\textsuperscript{-1}) at a rate of 0.58 ml min\textsuperscript{-1}, to evoke the micturition reflex. Backflow through this cannula allowed the bladder to be emptied of residual fluid after micturition had occurred (see Figure 2.1). The bladder was covered in a saline soaked gauze throughout the experiment. The urethra remained patent to allow expulsion of fluids during the micturition reflex and voided fluid was collected.

2.3 Experimental protocols

2.3.1 Experiments in Chapter 3 – 5HT\textsubscript{7} receptors and micturition

All experiments were left for 30 min after completion of surgery, to stabilise. Diagrams for each of the different experimental protocols used are shown in Figures 2.2 – 2.3.
2.3.1.1 Cystometry method - Rat

Figure 2.2A (Rat protocol 1 - cystometry) represents the first experimental protocols used in Chapter 3. The bladder was infused with saline until threshold was reached and the micturition reflex was evoked. Saline was continuously infused for 15 min to 'prime' the system and cause a series of infusion-induced micturition reflexes. After 15 min the infusion was stopped and the bladder emptied, 3 min later saline was infused into the bladder until micturition was evoked, the infusion was discontinued, the bladder was emptied and the residual volume was collected and measured. This was repeated 3 times to ensure stability of the response. Then after a period of 3 min the test drug or vehicle was administered i.c.v., i.v. or i.t. After a further 3 min the infusion was continued once more to evoke the micturition reflex (a single micturition contraction) in the presence of test drug or vehicle. In some experiments the test drug caused a continuous rise in bladder pressure with no overt contraction being observed. However, in these cases, after a certain period of time, a few drops of saline leaked from the urethral opening and once this was observed the infusion was switched off. Such responses were taken to indicate that the micturition response had been abolished.

2.3.1.2 Isovolumetric method - Rat

Figure 2.2B (Rat protocol 2 - isovolumetric) represents the second experimental protocol used in Chapter 3. Saline was infused into the bladder to evoke a 'priming' reflex. After
three consecutive reflex-evoked bladder contractions of similar amplitude the infusion was discontinued. The infused saline was left in the bladder for 5 min, during which time the reflex was ongoing. The bladder was emptied and after 20 min, was infused and the infusion discontinued as above to acquire a control reflex. The bladder reflex was ongoing for 5 min before emptying the bladder of saline. After 5 min the test drug or vehicle was administered i.c.v. and after a further 5 min the infusion-evoked reflex was repeated in presence of drug or vehicle.

2.3.1.3 Cystometry method - Cat

Figure 2.2C (Cat protocol - cystometry) represents the third experimental protocol used in Chapter 3. Saline was infused into the bladder to evoke a 'priming' micturition reflex. After voiding, the bladder was emptied of residual fluid and after 10 minutes this priming reflex was repeated. A control dose of 5-CT (0.1 \( \mu \text{g kg}^{-1} \text{i.v.} \)) was given during the 10-minute rest period between voids and changes in baseline variables were measured. After a further 3 ‘control’ reflex-evoked bladder contractions, with rest periods between, the test drug or vehicle was administered i.v. The infusion was then continued to evoke the micturition reflex (a single micturition contraction) in the presence of test drug or vehicle. After 10 minutes a second reflex was evoked and once baselines were stable, subsequent to the contraction, a test dose of 5-CT (0.1 \( \mu \text{g kg}^{-1} \text{i.v.} \)) was administered.
2.3.2 Experiments in Chapter 4 – Mesulergine and micturition

Figure 2.2A (rat protocol 1 – cystometry) represents the protocol used in the first stage of experiments examining the effects of mesulergine on the micturition reflex. In additional experiments animals were pre-treated with SB-243213 (see Figure 2.3A). In these animals SB-243213 was administered i.v. after the 3 control voids. After a further 3 min saline was infused into the bladder until micturition was evoked, the infusion was discontinued and the bladder emptied and residual fluid collected, as before. SB-269970 (30 µg kg⁻¹ i.c.v.) was then administered and after a further 3 min the infusion was continued and micturition evoked in the presence of this additional drug.

2.3.3 Experiments in Chapter 5 – 5-CT and micturition

In the 5-HT receptor agonist experiments the same protocol was used as in previous experiments (Figure 2.3B). The agonist or vehicle was administered i.c.v. after 3 control voids, as previously described. After 3 min the infusion was continued to evoke the micturition reflex (a single micturition contraction) in the presence of test drug or vehicle.
Figure 2.2. Diagram showing experimental protocols. A) Rat cystometry, B) Rat isovolumetric method and C) Cat cystometry.
A) Protocol for cystometry: SB-243213 and SB269970

B) Protocol for cystometry - 5CT, CP93129, sumatriptan or vehicle

C) Protocol for cystometry: Pretreatment with GR127935 or vehicle before 5-CT

Figure 2.3 Diagram showing experimental protocols used in A) SB-243213 experiments B) agonist experiments and C) GR127935 pre-treatment experiments.
2.3.4 Data capture and analysis

Arterial blood pressure, bladder and urethral pressures were displayed on a chart recorder (Grass Instruments) and captured (1500 samples s\(^{-1}\)) by a MP 100 WSW interface (Biopac systems Inc, U.S.A.) to allow data to be acquired and analyzed off-line using AcqKnowledge version 3.5.3 software (Biopac Systems Inc, U.S.A.). Heart rates were derived electronically from the blood pressure signal using the Biopac system.

2.3.5 Analysis of reflex-evoked bladder and urethral responses

2.3.5.1 Cystometry method - Rat

Saline infusion into the bladder evoked large amplitude bladder contractions and fluid release that represent the micturition reflex (Maggi et al., 1986; see Figure 2.4). The following variables were measured; volume threshold (ml) – the volume of fluid in bladder required to evoke micturition, pressure threshold (mmHg) – the bladder pressure at the start of a micturition contraction, the evoked bladder contraction or amplitude of contraction (mmHg; maximum intraluminal pressure minus pressure threshold) and residual volume (expressed as % of the total volume infused), were measured. Baseline bladder pressure, MAP and HR were measured over a 1 min period 2 min before reflexes were evoked and again 2 min after drug/vehicle had been administered.
2.3.5.2 Isovolumetric method - Rat

Saline infusion into the bladder evoked large amplitude rhythmic bladder contractions that represent the micturition reflex. The mean amplitude (mmHg) and duration (s) of the first three bladder contractions after the discontinuation of the saline infusion were measured (see Figure 2.5). These bladder contractions were isovolumetric, i.e. they occurred when the amount of saline in the bladder, and therefore the resting pressure was constant. The mean amplitude (mmHg) and duration (s) of the three urethral relaxations that accompanied these bladder contractions were also measured. The mean amplitude (mmHg) and duration (s) of the high frequency oscillations in urethral pressure were also measured. The micturition reflex pressure thresholds were taken as the bladder pressure (mmHg) at which the first reflex bladder contraction with associated reflex urethral relaxation and high frequency oscillations, occurred. Volume threshold (ml) was calculated by taking the time to evoke this first bladder contraction, with associated urethral activity, and multiplying by the infusion rate. Baseline bladder pressure, MAP and HR were measured over a 2 min period 2 min before reflexes were evoked and again 2 min after drug/vehicle had been administered.
Figure 2.4 – Rat cystometry method, trace showing distension evoked micturition reflex and measurements taken.
Figure 2.5. Diagram explaining rat-isovolumetric method. 1,2,3 are contractions of the same height; * indicate isovolumetric bladder contractions with constant volume in the bladder.
2.3.5.3 Cystometry method - Cat

Saline infusion into the bladder evoked large amplitude bladder contractions and fluid release that represent the micturition reflex (see Figure 2.6). The following variables were measured; volume threshold (ml) – the volume of fluid in bladder required to evoke micturition, pressure threshold (mmHg) – the bladder pressure at the start of a micturition contraction, the evoked bladder contraction or amplitude of contraction (mmHg; maximum intraluminal pressure minus pressure threshold) and residual volume (expressed as a % of bladder capacity \( \text{bladder capacity} = \text{residual volume (ml)} + \text{voided volume (ml)} \)), were measured. Baseline bladder pressure was measured over a 2 min period 2 min prior to reflexes being elicited. MAP and HR baselines were measured over a 2 min period 2 min before bladder reflexes and again 5 minutes after drug or vehicle had been administered.
Figure 2.6. Trace showing typical distension-evoked bladder contraction in cat cystometry and measurements taken.
2.3.6 Statistical analysis

Changes in baselines and reflex-evoked effects were compared with vehicle controls by unpaired Students t-test. Values of $P < 0.05$ were considered statistically significant. All values are expressed as mean ± s.e.mean.

2.3.7 Drugs and solutions

Drugs and chemicals were obtained from the following sources: SB-269970 (R)-3-[(2-(2-(4-Methylpiperidin-1-yl)-ethyl)pyrrolidine-1-sulfonyl)phenol hydrochloride), SB-656104 (6-((R)-2-[[2-[4-(4-Chloro-phenoxy)-piperidin-1-yl]-ethyl]-pyrrolidine-1-sulfonyl]-1H-indole hydrochloride, SB-243213 (5-methyl-1-[[2-[[2-methyl-3-pyridyl]oxy]-5-pyridyl]carbamoyl]-6-trifluormethylindoline hydrochloride);

Sumatriptan Succinate and GR127935 were synthesized and a gift from GlaxoSmithKline, Harlow, Essex U.K.; Mesulergine was a gift from Sandoz Pharmaceuticals, Horsforth, Leeds, UK; WAY-100635, urethane, 5-
Carboxyamidotryptamine maleate (5-CT), (±)–8-Hydroxy-DPAT Hydrobromide (8-OH-DPAT), α-chloralose and dimethyl sulfoxide (DMSO) from Sigma Aldrich Chemicals., Poole, Dorset, U.K.; CP-93,129 was a gift from Pfizer, Groton, CT, USA;
Pentobarbitone sodium from Rhone Merieux Ltd, Harlow, Essex, U.K.; isoflurane from Baxter Healthcare Ltd., Thetford, Norfolk, U.K.; gelofusine, polyethylene glycol 400 (PEG) from B Braun Medical Ltd, Sheffield, U.K.; sodium chloride, glucose and sodium
bicarbonate from Merck/BDH Lab supplies, Poole, Dorset, U.K., heparin from CP Pharmaceuticals Ltd, Wrexham, U.K. SB-269970, Mesulergine, 5-CT, Sumatriptan, 8-OH-DPAT when given to rats was dissolved in 0.9% w v⁻¹ saline, SB-243213 was dissolved in 0.4% DMSO, while SB-656104 HCl was dissolved in a mixture of 50%PEG/50%DMSO which was diluted 1 in 4 in saline. CP93,129 was dissolved in 30 µl of 1% w v⁻¹ ascorbic acid and diluted in saline 0.9% w v⁻¹. GR127935 was dissolved in distilled H₂O. SB-269970 when given to cats was dissolved in sterile distilled H₂O. All i.c.v. and i.t. doses in rats were given in 5µl volume over 20 s. All i.v. doses for rats were given in 0.1 ml volume followed by a 0.1 ml flush of saline. All i.v. doses for cats were given in 1 ml distilled H₂O followed by a 1 ml flush of saline. All drugs were given as their salts.
Chapter Three

5-HT7 receptors and micturition
Chapter Three: 5-HT7 receptors and micturition

3.1 Aim - Section I

To investigate the possible role of 5-HT7 receptors in the control of micturition in anaesthetized rats.

3.2 Introduction

Little is known about the functional role of the central 5-HT7 receptor, other than its potential role in control of circadian rhythm (Lovenberg et al., 1993) and temperature regulation (Hagan et al., 2000; Hedlund et al., 2003; Guscott et al., 2003). Ligands with 5-HT7 receptor affinity have been shown to cause phase advances in the circadian neuronal activity of the hypothalamic suprachiasmatic nucleus of the rat (Lovenberg et al., 1993). Likewise, in guinea pigs, the selective 5-HT7 receptor antagonist SB-269970 (Lovell et al., 2000) was shown to reverse the hypothermic effects of 5-CT (Hagan et al., 2000). In addition, 5-HT7 receptor knock-out mice had no hypothermic responses to 5-CT, whereas wild type mice exhibit hypothermic responses, which are only blocked by selective 5-HT7 receptor antagonists, not selective 5-HT1A or 5-HT1B/1D receptor antagonists (Hedlund et al., 2003; Guscott et al., 2003). Radioligand binding studies have shown that central 5-HT7 receptors are distributed in brain areas such as the periaqueductal grey, pontine nuclei, hypothalamus, dorsal inferior colliculus, dorsal raphe, nucleus subcoeruleus and suprachiasmatic nucleus (To et al., 1995; Gustafson et al., 1996; Barnes and Sharp, 1999; see Vanhoenacker et al., 2000). These brain areas have been shown to be important in the control of autonomic function, and with particular relevance to this study, micturition (see de Groat et al., 1993). Central 5-hydroxytryptamine containing neurones project from the
brainstem directly to areas of the spinal cord, which are involved with the reflex control of micturition (Dahlstrom & Fuxe, 1964). Further, it is known that activation of other 5-HT receptor subtypes, such as central 5-HT$_{1A}$ receptors, do play an important role in the reflex control of parasympathetic outflow to the bladder in rats (Lecci et al., 1992; Testa et al., 1999, Conley et al., 2001; Kakizaki et al., 2001; Pehrson et al., 2002) as well as to the heart in rats, (Bogle et al., 1990) and rabbits (Futuro-Neto et al., 1993; Dando et al., 1998, Skinner et al., 2002) and to the airways in guinea-pigs (Bootle et al., 1998). In an examination of selective antagonists to other 5-HT receptors, the 5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_3$, 5-HT$_4$ and 5-HT$_6$ receptors, on isovolumetric bladder contractions in the rat, it was concluded that these receptors were not involved in the physiological control of parasympathetic outflow to the bladder (Testa et al., 2001).

Owing to identification of this new member of the 5-HT receptor family, the 5-HT$_7$ receptor, it has become clear that many of the original ‘classical’ 5-HT receptor ligands used to characterise the role of 5-HT receptors have since been shown to have a high affinity for the 5-HT$_7$ receptor. Experiments have shown that some of these classical 5-HT receptor antagonists interfere with micturition, such as methiothepin, which causes dose dependent disappearance of isovolumetric bladder contraction in the anaesthetized rat (Testa et al., 2001; To et al., 1995; Gustafson et al., 1996; Wood et al., 2000). In addition, methysergide, when given intrathecally in cats, decreases the volume at which micturition occurs, while when given systemically in rats, micturition is inhibited (Espey et al., 1998; Lecci et al., 1992; To et al., 1995). Mesulergine is a classical 5-HT$_{2C}$ receptor antagonist and has also been shown to inhibit bladder activity at low doses and also to interfere with the ability of selective 5-HT$_{1A}$ receptor antagonist, WAY100635, to inhibit micturition in
anaesthetized rats (Testa et al., 1999, 2001; Wood et al., 2000). There are also some classical 5-HT receptor agonists that have now been shown to have affinity at the 5-HT\textsubscript{7} receptor. They are 8-OH-DPAT, the archetypal 5-HT\textsubscript{1A} receptor agonist, which when given spinally or supraspinally facilitates micturition in rats (Wood et al., 2000; Lecci et al., 1992) and the classical 5-HT\textsubscript{2C} receptor agonist, meta-chlorophenylpiperazine (mCPP), which does the reverse, inhibiting rhythmic bladder contractions in anaesthetized rats (Steers & de Groat, 1989; Guarneri et al., 1996; see Hoyer et al., 2002). Therefore, the 5-HT\textsubscript{7} receptor affinity of the drugs and the effects on micturition suggest a potential role for 5-HT\textsubscript{7} receptors in the control of micturition.

With the development of the selective 5-HT\textsubscript{7} receptor antagonists, SB-269970 (Lovell et al., 2000; Hagan et al., 2000) and structurally distinct, but from the same chemical series, SB-656104 (Thomas et al., 2003, see Figure 3.1), it has been possible to carry out experiments to determine if 5-HT\textsubscript{7} receptors are involved in the control of the micturition reflex.
Figure 3.1. Chemical structure of SB-269970 and SB-656104, two selective 5-HT$_7$ receptor antagonists.
3.3 Results

3.3.1 Effects of SB-269970 and SB-656104 on micturition

Traces showing the effects of SB-269970 and SB-656104 on distension-evoked changes in bladder pressure are shown in Figure 3.2. SB-269970 (3 μg kg⁻¹; i.c.v.; n = 6) had no significant (P < 0.05) effects on micturition but there was a tendency towards an inhibition of the micturition reflex with volume threshold increasing by 18 ± 11% and pressure threshold by 40 ± 40% (see Figures 3.3A & D). There was no significant changes in residual volume or evoked bladder contraction (see Figures 3.3C & B). At a higher dose, SB269970 (10 μg kg⁻¹; i.c.v.; n = 5) significantly attenuated micturition. Significant increases in volume threshold of 58 ± 15% and pressure threshold of 150 ± 46% were observed, indicating that more volume and pressure from fluid within the bladder were required to initiate the micturition reflex (see Figures 3.3A & D). This attenuation of micturition was also associated with a significant reduction in the amplitude of evoked bladder contraction of -62 ± 14%, when reflex voiding was initiated (see Figure 3.3B). Residual volume remaining in the bladder after voiding was also increased by 42 ± 20% but was not significantly different from control (see Figure 3.3C). A dose dependent attenuation of micturition was observed after a higher dose of SB269970 (30 μg kg⁻¹; i.c.v.; n = 6), where volume threshold was significantly increased by 138 ± 33% (see Figure 3.3A). Pressure threshold was also significantly increased by 149 ± 60% and a significant
reduction in the amplitude of evoked bladder contraction -60 ± 11% was observed (see Figures 3.3D & B). Residual volume increased by 72 ± 39%, but this was not significantly different from control (see Figure 3.3C). At the doses of 100 and 300 µg kg⁻¹ SB-269970 (i.c.v; n = 5) totally abolished the micturition reflex and overflow incontinence was observed (this is where the pressure of the saline infusion into the bladder forces saline out through the urethral sphincter; see Figure 3.2C).

SB-656104 (30 µg kg⁻¹; i.c.v.; n=5; Figures 3.2D and 3.3) had a similar effect to SB-269970 in causing attenuation of the micturition reflex in anaesthetized rats. Volume threshold was significantly increased by 54 ± 20%. Pressure threshold was also significantly increased by 196 ± 102% and this attenuation of micturition was associated with a significant reduction in the amplitude of evoked bladder contraction of -60 ± 13%. Further, the efficiency of the bladder to void was impaired and residual volume was significantly increased by 322 ± 66%.
Figure 3.2 Urethane anaesthetized female rats: traces showing changes in bladder pressure during distension-evoked micturition reflex before and after i.c.v. injections of A) vehicle, B) 30 μg kg⁻¹ SB-269970, C) 300 μg kg⁻¹ SB-269970 and D) 30 μg kg⁻¹ SB-656104. Saline infusion begins at the start of each trace. NB effects of vehicle for SB-656104 same as Trace A).
Figure 3.3. Urethane anaesthetized female rats: histograms comparing (Δ) changes caused by i.c.v. injections of saline with that of SB-2699970 (3, 10, 30, μg kg⁻¹) and i.c.v. injections of PEG:DMSO with that of SB-656104 (30 μg kg⁻¹) on A) volume threshold, B) evoked bladder contraction, C) residual volume, and D) maximum intraluminal pressure during cystometry-evoked micturition reflex. Each bar represents the mean value and the error bars show s.e. mean. Changes caused by drugs were compared with appropriate vehicle using Student’s unpaired t-test. * P < 0.05 **P < 0.01
3.3.2 Effect of i.t. administration of SB-269970 and WAY-100635 on micturition

Intrathecal (i.t.) administration of SB-269970 (30 μg kg\(^{-1}\); 5μl; n = 4), given at the L6/S1 level of the spinal cord, had no significant effect on any of the variables measured compared to control (see Figure 3.4A & B). However, as a positive control, the selective 5-HT\(_{1A}\) antagonist WAY-100635 (10μg kg\(^{-1}\); i.t.; n = 4) administered 30 min after SB-269970 (30 μg kg\(^{-1}\)) in the same animal at the L6/S1 level of the spinal cord, significantly attenuated micturition and caused a significant increase in volume threshold of 155 ± 76 % (see Figure 3.4).

3.3.3 Administration (i.v.) of SB-269970 and the effects on micturition

Intravenous SB-269970 (10 μg – 3 mg kg; i.v.; n = 3-5) had no significant effects on any micturition variables measured, compared to a time match control. However, a larger dose of SB-269970 (10 mg kg\(^{-1}\) i.v.; 0.1ml; n = 4) caused a significant increase in volume threshold of 89 ± 22 % (see Figure 3.5).
Figure 3.4. Urethane anaesthetized female rats: traces showing changes in bladder pressure during distension-evoked bladder contractions before and after i.t. injections of A) Vehicle, B) 30 μg kg⁻¹ SB-269970 and C) 10 μg kg⁻¹ WAY100635.
Figure 3.5 Urethane anaesthetized rats: trace showing changes in bladder pressure during distension-evoked bladder contractions before and after i.v. injection of 10 mg kg\(^{-1}\) SB-269970
3.3.4 Effect of SB-266970 given i.c.v. – isovolumetric method

The selective 5-HT\textsubscript{7} antagonist SB-269970 (30 \textmu g kg\textsuperscript{-1} i.c.v., 5 \textmu l, n = 4) inhibited micturition in the same pattern as the cystometry model. After administration of SB-269970 a significant increase in volume threshold was observed of 219 ± 60%. Pressure threshold was also significantly increased by 162 ± 28% and the amplitude of the reflex evoked isovolumetric bladder contractions was significantly reduced by -17 ± 3% (see Figure 3.6). The duration of reflex bladder contractions was also reduced by -21 ± 9% but this was not significantly different from control. In addition, the frequency of the bladder contractions did not vary after addition of SB-269970.

Reflex urethral relaxations were also measured as the reflex bladder contractions were evoked, and after SB-269970 the amplitude of urethral relaxations tended to be inhibited, and were reduced by -25 ± 10%. However, this reduction was not significantly different compared to vehicle control measurements. The duration of the urethral relaxations was not significantly different from vehicle control. The rat is fairly unique as during the urethral relaxation, when voiding takes place, high frequency oscillations in urethral pressure are observed. After SB-269970 the amplitude and duration of these reflex oscillations was not significantly different from control.

3.3.5 Blood Pressure and Heart Rate

Neither SB269970 nor SB-656104 had any significant effect on baseline MAP or HR values, at the doses used.
Figure 3.6. Urethane anaesthetized female rats: traces showing changes in bladder and urethral responses during intravesical infusions of saline A) Before and B) After 30 μg kg⁻¹ SB-269970 i.c.v. The length of line under each trace represents the duration of intravesical saline infusion.
3.3.6 Baseline values - cystometric study

Infusion of saline into the bladder of 58 female rats caused distension of the bladder, and in
 turn, evoked the micturition reflex. The reflex was characterized by a rapid increase in
 bladder pressure (to a maximum of $22.4 \pm 0.4$ mmHg), the emission of fluid during the
detrusor contraction and then a return to baseline, or at least to a pressure lower than that
recorded before micturition. The contractions had a mean duration of $21.6 \pm 0.8$ s and
returned to a resting pressure of $1.9 \pm 0.3$ mmHg after micturition. The mean bladder
pressure threshold to evoke the micturition reflex was $6.3 \pm 0.2$ mmHg, which was reached
when $0.53 \pm 0.02$ ml of saline had been infused. The mean amount of residual volume left
in the bladder was $41 \pm 3\%$ of the volume infused. The mean baseline bladder pressure,
MAP and HR were $1.3 \pm 0.2$ mmHg, $135 \pm 2$ mmHg and $382 \pm 5$ beats min$^{-1}$, respectively.
The mean baseline data for individual experimental groups are shown in Table 3.1.
Table 3.1. Baseline values of control reflex-evoked changes in bladder and urethral pressures caused by intravesical infusion of saline for each experimental group in urethane anaesthetized female rats for cystometric studies. *same animals as for i.t. SB-269970

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>n</th>
<th>Bladder Thresholds</th>
<th>Micturition-evoked bladder contraction</th>
<th>Residual Volume (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pressure mmHg</td>
<td>Volume ml</td>
<td>Amplitude mmHg</td>
</tr>
<tr>
<td>Rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline i.c.v. 5 µl</td>
<td>5</td>
<td>7.1 ± 0.6</td>
<td>0.42 ± 0.03</td>
<td>20.6 ± 1.7</td>
</tr>
<tr>
<td>SB-269970 i.c.v. 3 µg kg⁻¹</td>
<td>6</td>
<td>6.9 ± 0.8</td>
<td>0.54 ± 0.05</td>
<td>13.7 ± 1.2</td>
</tr>
<tr>
<td>10 µg kg⁻¹</td>
<td>5</td>
<td>6.7 ± 0.7</td>
<td>0.65 ± 0.04</td>
<td>19.6 ± 1.0</td>
</tr>
<tr>
<td>30 µg kg⁻¹</td>
<td>6</td>
<td>7.2 ± 0.6</td>
<td>0.41 ± 0.03</td>
<td>19.4 ± 1.7</td>
</tr>
<tr>
<td>100 µg kg⁻¹</td>
<td>5</td>
<td>5.4 ± 0.6</td>
<td>0.41 ± 0.03</td>
<td>17.3 ± 1.3</td>
</tr>
<tr>
<td>300 µg kg⁻¹</td>
<td>5</td>
<td>5.6 ± 0.5</td>
<td>0.53 ± 0.06</td>
<td>16.2 ± 0.6</td>
</tr>
<tr>
<td>25% PEG/DMSO i.c.v. 5 µl</td>
<td>5</td>
<td>8.7 ± 0.6</td>
<td>0.6 ± 0.1</td>
<td>11.3 ± 0.8</td>
</tr>
<tr>
<td>SB-656104 i.c.v 30 µg kg⁻¹</td>
<td>5</td>
<td>4.8 ± 0.7</td>
<td>0.6 ± 0.1</td>
<td>12.0 ± 0.03</td>
</tr>
<tr>
<td>Saline i.t. 5 µl</td>
<td>4</td>
<td>8.7 ± 1.0</td>
<td>0.68 ± 0.10</td>
<td>11.4 ± 1.1</td>
</tr>
<tr>
<td>SB-269970 30 µg kg⁻¹ i.t.</td>
<td>4</td>
<td>4.9 ± 1.0</td>
<td>0.40 ± 0.04</td>
<td>18.0 ± 1.0</td>
</tr>
<tr>
<td>WAY-100635 10 µg kg⁻¹ i.t.</td>
<td>4*</td>
<td>4.4 ± 0.9</td>
<td>0.43 ± 0.04</td>
<td>18.1 ± 2.5</td>
</tr>
<tr>
<td>Saline i.v.</td>
<td>4</td>
<td>4.2 ± 0.7</td>
<td>0.7 ± 0.12</td>
<td>13.9 ± 0.7</td>
</tr>
<tr>
<td>SB-269970 i.v. 10 mg kg⁻¹</td>
<td>4</td>
<td>5.0 ± 0.3</td>
<td>0.47 ± 0.06</td>
<td>17.8 ± 1.1</td>
</tr>
</tbody>
</table>
3.3.7 Baseline values - isovolumetric study

Infusion of saline into the bladder in 8 female rats caused distension of the bladder, in turn evoking the micturition reflex (see Table 3.2). The reflex is characterised by the appearance of rhythmic bladder contractions of $36 \pm 2$ mmHg. The contractions had a mean duration of $45 \pm 3$ s. The mean bladder pressure threshold to evoke such contractions ($n = 8$) was $6 \pm 1$ mmHg, which was reached when $0.17 \pm 0.04$ ml of saline had been infused into the bladder. Each rhythmic bladder contraction was accompanied by a fall in urethral pressure of $9 \pm 1$ mmHg that continued for $62 \pm 3$ s before returning to baseline. High frequency oscillations occurred in urethral pressure, at the peak of each bladder contraction, that had a mean amplitude of $14 \pm 1$ mmHg and continued for $35 \pm 3$ s. Each reflex bladder contraction was accompanied by small increases in MAP and HR. The mean baseline bladder and urethral pressures, MAP and HR were $2 \pm 1$, $16 \pm 1$, $136 \pm 3$ mmHg and $360 \pm 13$ beats min$^{-1}$, respectively.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>n</th>
<th>Baseline Threshold</th>
<th>Rhythmic bladder contraction</th>
<th>Urethral contractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pressure (mmHg)</td>
<td>Volume (ml)</td>
<td>Amplitude (mmHg)</td>
</tr>
<tr>
<td>Saline 5 µl</td>
<td>4</td>
<td>4.3</td>
<td>0.2</td>
<td>36.3</td>
</tr>
<tr>
<td>30 µg kg$^{-1}$ SB-269970</td>
<td>4</td>
<td>7.2</td>
<td>0.2</td>
<td>36.4</td>
</tr>
</tbody>
</table>

Table 3.2. Baseline values of control 'initial' reflex-evoked changes in bladder and urethral pressures caused by intravesical of saline for each experimental group in urethane anaesthetized female rats. Saline and SB-269970 were given i.c.v.
3.4 Discussion – Section I

The present experiments demonstrate that both SB-269970 and SB-656104 given centrally can attenuate distension-evoked bladder contraction in anaesthetized rats. Although these ligands are from the same chemical series, they are structurally distinct. Therefore, using both these two drugs that are selective for 5-HT7 antagonists indicates that the effects observed are not compound specific but mechanism specific. In this respect, each of these compounds show high affinity and selectivity for the 5-HT7(a) receptor. SB-269970 has pKi of 8.9 on human cloned 5-HT7(a) receptors expressed in HEK 293 cells (Lovell et al., 2000) and human cerebral cortex, with an equivalent value of 8.3 being found using guinea-pig cortex (Hagan et al., 2000). SB-656104 has a pKi value of 8.7 at the human recombinant receptor (Thomas et al. 2003). The only other receptor that SB-269970 has any appreciable affinity for is the 5-HT5a receptor (Lovell et al., 2000; pKi of 7.2) while for SB-656104 a different selectivity profile is apparent, this compound having pKi’s of 7.60, 7.20, 7.04 & 7.01 for 5-HT1D, 5-HT2a, 5-HT2b and D2 receptors, respectively (Thomas et al., 2003). Thus it is can be concluded that these drugs, when given centrally, inhibit the micturition reflex by blocking central 5-HT7 receptors. It is known that there are three different splice variants of the 5-HT7 receptor in both the human and rat brain but as yet there is no evidence for a distinct pharmacology between these variants (see Vanhoenacker et al., 2000) therefore it remains to be determined which variants are involved in the micturition reflex.

These results also indicate that the 5-HT7 receptors mediating these effects on micturition are located in the brain. This is indicated by the same dose of SB-269970 administered
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i.c.v. being ineffective when administered i.v. Further, no effects of SB-269970 could be observed on the reflex when administered intrathecally into the sacral portion of the spinal cord. In addition, the relatively short half life of SB-269970 (t\(_{1/2}\) (rat) < 0.5 h when given i.v.; Forbes et al., 2002) also supports the view that it is acting within the CNS, as the compound would be rapidly removed as it redistributed from the brain into the circulation, preventing it from reaching another possible site of action. When given i.v., SB-269970 is so rapidly removed from the circulation that only at a very high dose of 10 mg kg\(^{-1}\) i.v. is a sufficient level penetrating the CNS, before being cleared, to significantly attenuate micturition in rats.

The observation that both central 5-HT\(_7\) receptors and 5-HT\(_{1A}\) receptors are involved in the central control of micturition, as both types of antagonist can completely block this reflex (present data and Conley et al., 2001), implies that central 5-HT containing neurones play a very important role in the control of micturition, at least in rats. It is intriguing that both receptors have a similar overall physiological function in the reflex control of the bladder, although having opposing actions on adenylyl cyclase, 5-HT\(_7\) receptors being positively coupled while 5-HT\(_{1A}\) receptors are negatively coupled to adenylyl cyclase (see Hoyer et al., 1994). It seems that both 5-HT\(_7\) and 5-HT\(_{1A}\) receptors are required for control of micturition at the supraspinal level (Pehrson et al., 2002; Secker et al., 2002), but 5-HT\(_{1A}\) receptor-mediated control of the bladder also occurs at the spinal level (Kakizaki et al., 2001; present experiments). This control is likely to be at the level of the sacral parasympathetic nucleus, which has been shown by autoradiographic studies to contain 5-HT\(_{1A}\) receptors (Thor et al., 1993). Also located in the sacral portion of the spinal cord is Onuf’s nucleus, which contains efferent neurones innervating the external urethral
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sphincter, and in addition contains a high density of 5-HT\textsubscript{1A} receptors (Thor \textit{et al}., 1993). However, there is no evidence that blockade of 5-HT\textsubscript{1A} receptors interferes with the reflex regulation of the urethra (Conley \textit{et al}., 2001). Further, the present experiments also indicate that central 5-HT\textsubscript{7} receptors are also not involved in the reflex control of the urethra. However, SB-656104 significantly increased residual volume, which could reflect interference with the urethra but this was not mirrored by the more selective 5-HT\textsubscript{7} receptor antagonist SB-269970. The reason why this increase in bladder residual volume occurs with SB-656104 remains to be determined but it is probably not related to 5-HT\textsubscript{7} receptor blockade.

As both the 5-HT\textsubscript{7} and 5-HT\textsubscript{1A} receptor subtypes appear to be tonically active in the control of micturition in anaesthetized rats, and blocking either this tonic stimulation or inhibition of adenyl cyclase results in the same overall effect, there must be different localisations of receptor pools on key neuronal pathways in the central micturition circuitry. The 5-HT\textsubscript{1A} receptor has been shown to be both an auto- and heteroreceptor and one theory postulates that the selective 5-HT\textsubscript{1A} antagonist WAY100635 could act to block 5-HT\textsubscript{1A} autoreceptors and therefore block the inhibition of release of 5-HT from 5-HT containing nerve terminals, leading to an increase in 5-HT release and a subsequent increase in inhibitory drive to the bladder (Testa \textit{et al}., 1999). However, an alternative theory based on disinhibition has been postulated (see Ramage, 2000). 5-HT\textsubscript{1A} receptors can also be heteroreceptors, and therefore modulate the release of other transmitters e.g. GABA, which would provide a ‘brake’ onto the activity of parasympathetic neurones. Therefore if the 5-HT\textsubscript{1A} receptors were located on GABA containing interneurones they would inhibit the release of GABA causing disinhibition (see Ramage, 2000; Wang & Ramage, 2001).
precise location of these 5-HT$_{1A}$ receptors as well as 5-HT$_7$ receptors in the micturition pathways is still to be determined. Additionally there is no evidence, to date, for 5-HT$_7$ autoreceptors modulating release in rat cortex or raphe nuclei, in contrast to 5-HT$_{1A}$ receptors (Roberts et al., 2001). However, 5-HT$_7$ receptors have been localised in other central areas involved in the control of micturition, including the hypothalamus, dorsal inferior colliculus, periaqueductal grey, pontine nucleus and nucleus subcoeruleus (see De Groat et al., 1993; To et al., 1995; Gustafson et al., 1996; see Vanhoenacker et al., 2000).

It is possible that 5-HT$_7$ receptors in these regions could be involved in the control of afferent input to the PMC regarding bladder fullness, or in the ‘switch’ circuitry that activates voiding and inhibits storage once the volume threshold of the bladder has been reached. Studies have shown that direct projections from the pre-optic area of the hypothalamus to the PMC are important in this ‘switch’ circuitry, particularly for initiating micturition (Blok & Holstege, 1998). 5-HT$_7$ receptors could be involved in controlling this ‘switch’. Further, it has been shown that 5-HT$_{1A}$ receptors can serve to augment the function of the 5-HT$_7$ receptor in the hippocampus (Thomas et al., 1999) and this may be how they are interacting in this area in the control of micturition.

In conclusion, the present results have identified a major physiological function for central 5-HT$_7$ receptors in the control of reflex induced bladder contraction in the anaesthetized rat. In addition, this study adds to the growing evidence of the importance of central 5-HT containing neurones in the control of parasympathetic outflow.
3.5 Section II - Cat study

3.5.1 Aim

The question arose as to whether 5-HT$_7$ receptors play in the control micturition in another species. The cat was chosen as data (see Introduction) suggested that 5-HT$_{1A}$ receptors play the opposite role at least in the control of micturition in this species. These experiments were carried out in the Urology Department laboratories of GlaxoSmithKline in the King of Prussia, Philadelphia, U.S.A.

3.5.2 Introduction

Micturition in the cat has been well studied and a great deal of the original work was carried out in this species (see Barrington, 1931; 1941; Blok & Holstege, 1998). Work carried out by Ryall and de Groat (1972) demonstrated that iontophoretic administration of 5-HT directly onto spinal autonomic neurones in the cat, caused inhibition of excitatory amino acid induced firing of parasympathetic preganglionic neurones in the sacral spinal cord, an area important in the co-ordination of micturition. Further, electrical stimulation of the raphe nucleus, which provides the major 5-HT input to the spinal cord, also inhibited bladder activity in anaesthetized cats (McMahon & Spillane, 1982). To further confirm the inhibitory role of 5-HT, intravenous or topical lysergic acid diethylamide (LSD), a non-selective 5-HT antagonist was used to suppress the inhibitory effect of electrical raphe stimulation (Morrison & Spillane, 1986). Espey et al. (1992) showed that intrathecal methysergide, another non-selective 5-HT$_{1/2}$ receptor antagonist with affinity for 5-HT$_7$
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receptors (To et al., 1995), and intrathecal zatoetropon, a 5-HT3 receptor antagonist (Espey & Downie, 1995), both decreased the volume threshold, at which micturition was initiated. This suggests that 5-HT1/2, possibly 5-HT7 and 5-HT3 receptors are tonically active in inhibiting bladder activity, although it is difficult to determine from this data where in the central nervous system these receptors are exerting their action, it could be spinally, supraspinally or both. Descending inhibitory control by 5-HT containing neurones has also been postulated to be crucial in the developing nervous system, with the maturing 5-HT system being involved in switching the excitatory somatovesical reflex in the cat neonate to an inhibitory reflex (Thor et al., 1990). The kitten progresses from needing perigenital stimulation from its mother, in order to micturate (excitatory somatovesical reflex), to this type of stimulation inhibiting bladder activity as supraspinal sites initiate voiding (inhibitory somatovesical reflex). Likewise, it was proposed that the hyperactive bladder and reduced volume threshold observed in spinally transected adult cats, is due in part, to removal of descending 5-HT inhibitory drive to the bladder. The hyperactive bladder was mimicked in spinally intact adult cats by suppressing the 5-HT system using low doses of 5-HT autoreceptor agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT; Thor et al., 1990). However, a number of assumptions are made when using 5-MeODMT to inhibit 5-HT descending control via autoreceptor activation. 5-MeODMT is used in other models to induce forepaw treading in rats, a behaviour characteristic of 5-HT1A heteroreceptor (postsynaptic) activation (Nisbet & Marsden, 1984). 5-HT1A heteroreceptor activation via 5-MeODMT is prominent enough in this model to be used to test the efficacy of compounds to block post-synaptic 5-HT1A receptor activity (Sanchez et al., 1996). In addition, 5-MeODMT also has affinities for 5-HT1B and 5-HT2 receptors, as demonstrated in the guinea pig epilepsy model (Nielsen, 1998). Therefore, the presumption that 5-
MeODMT is inhibiting the descending control of 5-HT neurones in the cat via 5-HT autoreceptor activation may be an oversimplification, as the agonist activates 5-HT$_{1A}$ heteroreceptors, 5-HT$_{1B}$ receptors and 5-HT$_2$ receptors. The role of 5-HT receptors in the control of micturition in cats therefore needs clarification.

The precise role of the 5-HT$_{1A}$ receptor in the control of micturition in the cat is complicated by experiments which suggest that 5-HT$_{1A}$ receptor activation may inhibit rather than stimulate bladder function. However, evidence from experiments on the role of 5-HT$_{1A}$ receptors in the reflex control of other parasympathetic outflows, to the heart (Wang & Ramage, 2001) and airways (Bootle et al., 1996) in the cat indicate that the receptors are excitatory, as they are in the rat and the guinea pig (Bogle et al., 1990; Bootle et al., 1998). Therefore the precise role for these receptors in micturition in the cat remains equivocal. It is noteworthy though, that the same 5-HT receptor subtypes can mediate the same or similar functions in different species e.g. activation of 5-HT$_7$ receptors in the mouse and guinea pig causes hypothermia (Guscott et al., 2003; Hagan et al., 2000). Therefore, experiments were carried out to determine if 5-HT$_7$ receptors played any role in the control of micturition in anaesthetized cats, using SB-269970, the selective 5-HT$_7$ receptor antagonist.
3.5.3 Results – cystometric study

3.5.3.1 Effects of SB-269970 i.v. on micturition

SB-269970 (10 mg kg⁻¹ i.v.; n = 4) in cats had no significant effects on the micturition reflex when compared with control data. (see Figure 3.7). The evoked bladder contraction amplitude, volume threshold, pressure threshold and residual volume all remained unchanged compared to control. The high intravenous dose used was, however, shown to be effective in rats and cause significant inhibition of micturition (see section 3.3.3 of this chapter).

To demonstrate that 5-HT₁ receptors were indeed blocked, at least in the periphery, for the duration of the experiment, 5-CT (0.1 µg kg⁻¹ i.v.) was administered before SB-269970 or vehicle, and at the end of the experiment. 5-CT alone caused a reduction in MAP of 16 ± 6 mmHg and a rise in HR of 11 ± 2 beats min⁻¹, while in the presence of SB-269970 caused rise in MAP of 18 ± 8 mmHg associated with no change in heart rate (0.25 ± 1 beats min⁻¹). In the vehicle control experiments, 5-CT still caused a reduction in MAP, of similar magnitude to the initial dose, -10 ± 3 mmHg, and a small rise in heart rate of 10 ± 2 beats min⁻¹ (see Figures 3.8 and 3.9).

3.5.3.2 Blood Pressure and Heart Rate

SB-269970 (10 mg kg⁻¹ i.v.; n = 4) caused a significant reduction in both MAP and HR of 33 ± 12 mmHg and 28 ± 7 beats min⁻¹, respectively compared to vehicle control.
3.5.4 Baseline values

Infusion of saline into the bladder of 8 female cats caused the distension of the bladder and, in turn, evoked the micturition reflex. The reflex was characterized by a pronounced rise in bladder pressure (to a maximum of 29.5 ± 1.4 mmHg), the release of fluid during the contraction, and then a return to baseline. The contractions had a mean duration of 105 ± 15 s and returned to a resting pressure of 4 ± 0.4 mmHg after micturition. The mean bladder pressure to evoke micturition was 9.6 ± 0.5 mmHg, which was reached when 15.8 ± 2 ml of saline had been infused (at a constant infusion rate of 0.6 ml min⁻¹, taking approximately 26 ± 3 minutes). During each micturition contraction 62 ± 6 % of bladder fluid was voided leaving a remaining 38 ± 6 % of residual volume in the bladder after each micturition contraction. The mean baseline bladder pressure, MAP and HR were 2.94 ± 1 mmHg, 118 ± 6 mmHg and 182 ± 11 beats min⁻¹, respectively. The mean baseline data for individual experimental groups are shown in Table 3.3.
Table 3.3 Baseline values of control 'initial' reflex-evoked changes in pressures caused by intravesical of saline for each experimental group in α-chloralose anaesthetized female cats.

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>n</th>
<th>Bladder Thresholds</th>
<th>Micturition-evoked bladder contraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pressure mmHg</td>
<td>Volume ml</td>
</tr>
<tr>
<td>Cats</td>
<td></td>
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</tr>
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<td>Distilled H₂O i.v.</td>
<td>4</td>
<td>9.6 ± 0.7</td>
<td>15.5 ± 3.4</td>
</tr>
<tr>
<td>SB-269970 i.v.</td>
<td>4</td>
<td>9.7 ± 0.7</td>
<td>16.0 ± 2.4</td>
</tr>
</tbody>
</table>
Figure 3.7. α-chloralose anaesthetized cat: trace showing changes in bladder pressure during saline distension-evoked micturition reflex before and after i.v. injection of 10 mg kg⁻¹ SB-269970. The infusion of saline begins at the start of each trace.
Figure 3.8. α-chloralose anaesthetized cat: histogram comparing (Δ) changes in MAP caused by i.v. 5-CT (0.1 μg kg\(^{-1}\)) before and after SB-269970 vehicle and SB-269970 (10 mg kg\(^{-1}\) i.v.). Changes caused by drugs were compared with appropriate vehicle using Student’s unpaired t-test. * P < 0.05
Figure 3.9 α-chloralose anaesthetized cat: histograms comparing (Δ) changes in HR caused by i.v. 5-CT (0.1 μg kg⁻¹) before and after SB-269970 vehicle and SB-269970 (10 mg kg⁻¹ i.v.). Changes caused by drugs were compared with appropriate vehicle using Student’s unpaired t-test. * P < 0.05
3.5.5 Discussion – Section II

SB-269970 is a selective 5-HT\textsubscript{3} receptor antagonist and has been previously shown in this chapter to attenuate micturition when given supraspinally and intravenously in the anaesthetized rat. However, the present data show that i.v. SB-269970 (10 mg kg\textsuperscript{-1}) had no significant effects on any micturition variable measured in the anaesthetized cat. This dose had previously been shown, when given i.v., to significantly increase volume threshold in the rat (see section 3.3.3 present data). SB-269970 is rapidly distributed into the brain but it is also rapidly cleared from the blood (Hagan et al., 2000). Thus, perhaps, the short half-life and rapid clearance rate of SB-269970 was too rapid for sufficient levels to penetrate the CNS and block 5-HT\textsubscript{3} receptors in the cat. However, a lower dose of SB-269970 (5 mg kg\textsuperscript{-1}) given intraperitoneally in the guinea pig did penetrate the brain and was sufficient to significantly inhibit 5-CT induced hypothermia (Hagan et al., 2000). The infusion rate used to fill the cat bladder was in line with other investigators (Thor et al., 2002), and at an appropriate and physiological level to avoid rapid stretching of the detrusor muscle, which may affect accommodation of the bladder (Klevmark, 1999). However, there was some variability in the bladder volume threshold between cats, taking 26 ± 3 mins for micturition to be evoked during constant saline infusion. It was a concern that some bladders may take some time to fill before micturition was evoked, especially considering the rapid clearance of the particular drug being used in this study. An alternative methodology would have been useful in this instance, perhaps a closed bladder system with ongoing isovolumetric bladder contractions. It may also have been more appropriate to give the 5-HT\textsubscript{3} receptor
antagonist SB-269970 i.c.v. to directly target the central 5-HT$_7$ receptors of interest, and reduce first pass metabolism. However, owing to limited time and equipment, these experiments were not carried out.

To control for the volume threshold variability and the duration of the experiment, 5-CT was given to determine if SB-269970 was still having an effect at the end of each experiment. The data demonstrates that at least peripheral 5-HT$_7$ receptors were blocked for the duration of each experiment, as shown by a reversal in the responses of MAP and HR to 5-CT. In peripheral vascular tissue there is a high expression of 5-HT$_7$ mRNA (see Vanhoenacker et al., 2000) and many vascular tissues have receptors which fit with the 5-HT$_7$ receptor pharmacology, mediating smooth muscle relaxation via increases in adenylyl cyclase and cAMP (see Terron, 1998). These include cat saphenous vein (Feniuk et al., 1983), porcine vena cava (Trevethick et al., 1986) and canine cerebral and coronary arteries (Terron & Falcon-Neri, 1999; Cushing et al., 1996). In vivo it has been demonstrated that after the removal of the sympathetic tone to the external carotid bed, and the inhibition of the vasoconstrictor responses mediated via 5-HT$_{1B/1D}$ receptors, 5-HT mediates vasodilatation in the carotid artery in dogs, via 5-HT$_7$ receptors (Villalon et al., 1997). Likewise, receptors with a pharmacological profile similar to 5-HT$_7$ receptors also mediate the long-lasting hypotensive phase in rats, after bolus i.v. 5-HT administration (Terron, 1997). Other cardiovascular effects of 5-HT include tachycardia in the cat via activation of 5-HT$_7$ receptors in the heart muscle (Villalon, et al., 1997). Tachycardia, along with a reduction in MAP did occur after giving a control dose of 5-CT. However, after SB-269970, 5-CT no longer caused a reduction in MAP or tachycardia, indicating that peripheral 5-HT$_7$ receptors were blocked at this time. Whether a sufficient number of
central 5-HT\textsubscript{7} receptors were also blocked is unknown, but the compound has been shown to have good brain permeability (Hagan et al., 2000). The reason why this dose failed to affect micturition is therefore unknown and whether 5-HT\textsubscript{7} receptors are involved in micturition in a similar way in cats as they are in rats also remains to be determined. There is also controversy over whether 5-HT\textsubscript{1A} receptors function in a similar way in cats and rats, with respect to the control of micturition. Limited evidence shows an opposite role for these receptors in control in the cat compared to the rat, particularly in mediating bladder hyperactivity induced by acetic acid infusion into the bladder (Thor et al., 2002). In rats 5-HT\textsubscript{1A} receptors facilitate micturition (Lecci et al., 1992). However, Thor et al., (2002) have shown 5-HT\textsubscript{1A} receptor activation inhibiting bladder hyperactivity in the cat. It has also been reported that blockade of 5-HT\textsubscript{1A} receptors with WAY-100635 or LY206130 has no effect on bladder or sphincter activity (Thor et al., 2002), although other combined data indicate that central 5-HT\textsubscript{1A} receptors do play a similar role in the control of parasympathetic outflow to the heart in the cat as well as in the rabbit, the rat and man (see Ramage, 2001). Therefore further investigations into this discrepancy, as well as the role of 5-HT\textsubscript{7} receptors in micturition are required in the cat.
Chapter Four

Mesulergine and micturition
4.1 Aim

To determine if the archetypical 5-HT$_{2C}$ receptor antagonist mesulergine, which has also been reported to block 5-HT$_7$ receptors, can interfere with the micturition reflex in an anaesthetized rat cystometry model.

4.2 Introduction

From the previous chapter (see Chapter 3) it has been demonstrated that supraspinal 5-HT$_7$ receptors play a major physiological role in the control of reflex evoked bladder contraction in the anaesthetised rat. In order to further elucidate the role of 5-HT$_7$ receptor physiology and pharmacology, another antagonist was used, and this was mesulergine. From the binding data available, mesulergine is one of the only available antagonists with a high affinity for 5-HT$_7$ receptors, that has a much lower affinity for the majority of other 5-HT receptor subtypes, except 5-HT$_{2C}$ receptors (Hoyer et al., 1994). Mesulergine has a pK$_i$ of 8.15 on mouse recombinant 5HT$_7$ receptors expressed in COS-7 cells (Plassat et al., 1993) and owing to a comparable affinity with the 5-HT$_7$ receptor antagonists SB-269970 and SB-656104 (pKi of 8.9 and 8.7 on human cloned receptors (Lovell et al., 2000; Thomas et al., 2003)) similar functional effects may be expected. In the literature, mesulergine has often been used to elucidate the role of 5-HT$_{2C}$ receptors (see Hoyer et al., 1994) and it seems that there are many other compounds, including clozapine, and the antagonist spiperone, that display high affinities for both 5-HT$_7$ receptors (Hoyer et al., 1994) as well as 5-HT$_2$ receptors (Saller et al., 1990). In fact, ligands that bind both receptor subtypes tend to have similar structural moieties (Kikuchi et al., 1999) and it is common for many
5-HT antagonists to have an affinity for both 5-HT\(_7\) and 5-HT\(_2\) receptor subtypes. Mesulergine also has an affinity for dopamine (D\(_2\)) receptors (pK\(_i\) of 7.82; Barwick \textit{et al.}, 1999)

Mesulergine has already been shown to interfere with micturition to some extent, by blocking the inhibition caused by selective 5-HT\(_{1A}\) receptor antagonist WAY100635, on the micturition reflex in the anaesthetized rat (Testa \textit{et al.}, 1999). It is also noteworthy that, at low doses mesulergine has been shown to inhibit isovolumetric bladder contractions in the anaesthetized rat, which could be attributed to its binding at 5-HT\(_7\) receptors, although at higher doses this effect was not observed (Testa \textit{et al.}, 2001). Therefore, the present experiments were carried to re-evaluate the effects of mesulergine in micturition in the light of the new discovery that 5-HT\(_7\) receptors play a role in micturition (Chapter 3). In addition there is also evidence for the involvement of 5-HT\(_{2C}\) receptors in micturition. The 5-HT\(_{2C}\) receptor agonist mCPP can suppress efferent nerve activity in bladder nerves and inhibit reflex evoked micturition in anaesthetized rats through activation of 5-HT\(_{2C}\) receptors (Guameri \textit{et al.}, 1996; Steers & de Groat 1989). Consequently it might be expected that 5-HT\(_{2C}\) receptors would be expected to play an inhibitory role, opposing the excitatory action of 5-HT\(_7\) receptors. This could explain the inconsistent effects on micturition reported for mesulergine. Thus the effects of a selective 5-HT\(_{2C}\) receptor antagonist were also investigated alone, and on the effect of a 5-HT\(_7\) receptor antagonist on micturition. The highly selective 5-HT\(_{2C}\) receptor antagonist SB-243213 (Bromidge \textit{et al.}, 2000) was chosen. However, this antagonist is insoluble in any vehicle that can be administered i.c.v. and therefore systemic administration of the compound was necessary. On the other hand, this antagonist does readily penetrate the brain after systemic
administration (Bromidge et al., 2000) as demonstrated by its effects in previous studies on
the firing pattern of spontaneously active midbrain neurons, where 5-HT$_{2C}$ receptors are
thought to be an important control in feedback afferent input to this neuronal type
(Blackburn et al., 2002).

Therefore the present experiments were carried out to examine whether mesulergine would
act according to its selectivity profile, as an antagonist at 5-HT$_7$ receptors and interfere with
micturition. The possibility that mesulergine’s binding affinity to other receptor subtypes,
e.g. the 5-HT$_{2C}$ receptor, may interfere with its ability to attenuate micturition, by 5-HT$_7$
receptor antagonism, was also examined.
4.3 Results

4.3.1 Effects of Mesulergine (10, 100, 300 μg kg\(^{-1}\); i.c.v.), a 5-HT\(_7\) receptor antagonist on micturition

Mesulergine 10 μg kg\(^{-1}\) (i.c.v.; \(n = 4\)) caused no significant effects to volume threshold, pressure threshold and the amplitude of the evoked micturition contraction compared to saline control i.c.v. (Figure 4.2A, B and C). At 100 μg kg\(^{-1}\) (i.c.v.; \(n = 7\)) mesulergine abolished the micturition reflex in 2 out of 7 animals tested. In those animals where the micturition reflex was not abolished 100 μg kg\(^{-1}\) mesulergine (i.c.v.; \(n = 5\)) caused no significant changes on any of the variables measured. Volume threshold, however, tended to increase (55 ± 17%) compared to the saline control (25 ± 24%). Pressure threshold also tended to increase, but was not significantly different from control (57 ± 34% compared to 30 ± 18%). There was also no change in the amplitude of evoked bladder contraction after 100 μg kg\(^{-1}\) mesulergine (i.c.v.; \(n = 5\)) compared to control.

Mesulergine (300 μg kg\(^{-1}\); i.c.v.; \(n = 7\)) abolished the reflex in 4 out of 7 animals and therefore as a group the evoked bladder contraction was significantly reduced by 73 ± 13%. In the 3 experiments where mesulergine did not abolished micturition it caused a reduction in the mean amplitude of the evoked bladder contraction of 36 ± 8% and an increase in pressure threshold of 181 ± 119%. There was also a large increase in volume threshold, 84 ± 14%. Mesulergine had no significant effects on residual volume at any of the doses tested. Traces showing examples of the effects of mesulergine on distension-evoked changes in bladder pressure at the different doses tested are shown in Figure 4.1.
4.3.2 Mesulergine - Blood Pressure and Heart Rate

Mesulergine (300 μg kg⁻¹ i.c.v.; n = 7) significantly reduced mean heart rate by 12 ± 4 beats min⁻¹ compared to saline control. Other doses of the antagonist had no significant effects on baseline variables.
Figure 4.1 Urethane anaesthetized rat: traces showing changes in bladder pressure during saline distension-evoked micturition reflex before and after i.c.v. injections of A) Mesulergine 10 μg kg\(^{-1}\) i.c.v., B) Mesulergine 100 μg kg\(^{-1}\) i.c.v. and C) Mesulergine 300 μg kg\(^{-1}\) i.c.v. Saline infusion begins at the start of each trace. For vehicle effects see Figure 3.2A Chapter 3.
Figure 4.2 Urethane anaesthetized female rats: histograms comparing (Δ) changes caused by i.c.v. injections of saline with that of Mesulergine (10, 100 and 300, μg kg⁻¹) A) volume threshold, B) pressure threshold and C) evoked bladder contraction, during cystometry-evoked micturition reflex. Each bar represents the mean value and the error bars show s.e. mean. Changes caused by drugs were compared with appropriate vehicle using Student’s unpaired t-test.
4.3.3 Effects of 5-HT<sub>2C</sub> antagonist SB-243213 on micturition

A range of doses were initially tested and SB-243213 (0.3 - 3 mg kg<sup>-1</sup> i.v.; n = 1-3) had no overt effects on any of the micturition variables measured. After the highest dose of SB-243213 (3 mg kg<sup>-1</sup> i.v.; n=3) there were no significant changes in volume threshold (20 ± 18%) and pressure threshold (4 ± 32%). The amplitude of evoked bladder contraction was also unaffected by SB-243213 at this dose and Figure 4.3A shows a trace example of a distension-evoked bladder pressure before and after SB-243213.

4.3.4 Effects of SB-269970 after pre-treatment with SB-243213 on distension-evoked micturition reflex.

SB-269970 (30 μg kg<sup>-1</sup> i.c.v.; n = 3), after the pretreatment with SB-243213 (3 mg kg<sup>-1</sup> i.v.; n = 3), still attenuated micturition, and was not significantly different from SB-269970 (30 μg kg<sup>-1</sup> i.c.v., n=5) alone. Volume threshold was increased by 91 ± 54% and pressure threshold increased by 144 ± 90%. The evoked bladder contraction was reduced by 23 ± 7%. Traces showing an example of changes in distension evoked bladder pressure before and after SB-269970 in the presence and absence of SB-243213 are shown in Figure 4.3B & C.
4.3.5 Blood Pressure and Heart Rate

SB-243213 (3 mg kg\(^{-1}\) i.v.) and SB-269970 (30 µg kg\(^{-1}\) i.c.v.) in the presence of SB-243213 had no significant effects on cardiac baseline values.

4.3.6 Cystometric study - Baseline values

Infusion of saline into the bladder of 30 female rats caused distension of the bladder, and, in turn, evoked the micturition reflex. The reflex was characterized by a rapid increase in bladder pressure (to a maximum of 20.6 ± 0.6 mmHg), the emission of fluid during the detrusor contraction and then a return to baseline, or at least to a pressure lower than that recorded before micturition. The contractions had a mean duration of 21.8 ± 1.1 s and returned to a resting pressure of 1.2 ± 0.1 mmHg after micturition. The mean bladder pressure threshold to evoke the micturition reflex was 5.2 ± 0.2 mmHg, which was reached when 0.48 ± 0.03 ml of saline had been infused. The mean amount of residual volume left in the bladder was 51 ± 3 % of the volume infused. The mean baseline bladder pressure, MAP and HR were 0.9 ± 0.2 mmHg, 132 ± 2 mmHg and 385 ± 6 beats min\(^{-1}\), respectively. The mean baseline data for individual experimental groups are shown in Table 4.1.
Figure 4.3 Urethane anaesthetized rat: trace showing changes in bladder pressure during saline distension-evoked micturition reflex, before and after A) SB-243213 (3 mg kg\(^{-1}\) i.v.), B) SB-243213 (3 mg kg\(^{-1}\) i.v.) + SB-269970 (30 µg kg\(^{-1}\) i.c.v.) and C) SB-269970 (30 µg kg\(^{-1}\) i.c.v.; control trace). Saline infusion begins at the start of each trace.
Table 4.1.

Baseline values of control reflex-evoked changes in bladder pressures caused by intravesical infusion of saline for each experimental group in urethane anaesthetized female rats for cystometric studies.

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>n</th>
<th>Bladder Thresholds</th>
<th>Micturition-evoked bladder contraction</th>
<th>Residual Volume (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pressure mmHg</td>
<td>Volume ml</td>
<td>Amplitude mmHg</td>
</tr>
<tr>
<td>Rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline i.c.v. 5 µl</td>
<td>5</td>
<td>7.1 ± 0.6</td>
<td>0.42 ± 0.03</td>
<td>20.6 ± 1.7</td>
</tr>
<tr>
<td>Mesulergine i.c.v.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 µg kg⁻¹</td>
<td>4</td>
<td>4.8 ± 0.5</td>
<td>0.72 ± 0.04</td>
<td>10.5 ± 0.9</td>
</tr>
<tr>
<td>100 µg kg⁻¹</td>
<td>7</td>
<td>4.1 ± 0.4</td>
<td>0.35 ± 0.04</td>
<td>16.3 ± 0.7</td>
</tr>
<tr>
<td>300 µg kg⁻¹</td>
<td>7</td>
<td>5.7 ± 0.4</td>
<td>0.43 ± 0.04</td>
<td>15.6 ± 1.2</td>
</tr>
<tr>
<td>Saline i.v.</td>
<td>4</td>
<td>4.2 ± 0.7</td>
<td>0.7 ± 0.12</td>
<td>13.9 ± 0.7</td>
</tr>
<tr>
<td>SB-243213 i.v. 3 mg kg⁻¹</td>
<td>3</td>
<td>5.13 ± 0.5</td>
<td>0.36 ± 0.05</td>
<td>18.6 ± 0.8</td>
</tr>
</tbody>
</table>
4.4 Discussion

The present experiments demonstrate that mesulergine given centrally can attenuate distension-evoked bladder contractions in a similar way to the selective 5-HT<sub>7</sub> receptor antagonists SB-269970 and SB-656104. The attenuation of the micturition reflex was manifested as a significant reduction in amplitude of bladder contraction as micturition was abolished in 4 out of 7 animals and inhibited in the remaining 3 animals. At the lower dose of 100 µg kg<sup>-1</sup> i.c.v. micturition still tended to be inhibited, although not significantly and was totally abolished in 2 out of 7 animals. Mesulergine has a high affinity for 5-HT<sub>7</sub> receptors (pK<sub>i</sub> of 8.15 on mouse recombinant 5HT<sub>7</sub> receptors expressed in COS-7 cells (Plassat et al., 1993;)) and this is comparable to SB-269970 and SB-656104, which have pK<sub>i</sub> of 8.9 and 8.7 at human cloned receptors, respectively. This high affinity for 5-HT<sub>7</sub> receptors explains the likely reason of why mesulergine is able to attenuate micturition (see Chapter 3). Interestingly, the present data indicate that the lowest dose of 10 µg kg<sup>-1</sup> i.c.v. mesulergine, had no significant effects on micturition. This is in contrast to reports by Testa et al., (2001), where the same dose given i.v. caused significant inhibition of micturition. However, at higher doses up to 1 mg kg<sup>-1</sup> i.v. (Testa et al., 2001) reported no significant effects on ongoing isovolumetric bladder contractions. The reason for the differences seen between the present experiments and experiments carried out by Testa et al., (2001) are unknown, but it was indicated in their work that the significant inhibition of bladder contractions caused by a low dose of mesulergine was most likely due to experimental variance, and they considered the effect of the low dose of mesulergine insignificant. These data are consistent with the hypothesis that at a low dose mesulergine
has no significant effects on micturition. It is interesting that the 5-HT$_7$ antagonist-like effects of mesulergine, on the micturition reflex, are only significant at such a high dose of 300 $\mu$g kg$^{-1}$ i.c.v., as the dose of the 5-HT$_7$ receptor antagonists SB-269970 that caused a significant inhibition of micturition was 10 $\mu$g kg$^{-1}$ i.c.v., taking into account affinity and molecular weight. However, SB-269970 is more selective than mesulergine being a 100-fold more selective for 5-HT$_7$ receptors over all other 5-HT receptors, except the 5-HT$_{5A}$ receptor where it is at least 50-fold more selective (Lovell et al., 2000). Mesulergine, in contrast, has high affinity for 5-HT$_{2C}$ receptors (pK$_i$ of 8.8 at receptors in the pig choroid plexus and 8.7 in rat cortex (Hoyer et al., 1985)), 5-HT$_{2A}$ receptors (pK$_i$ of 9.1 in rat A7r5 smooth muscle cells and pKi of 7.4 at human receptors) and a more moderate affinity for D$_2$ receptors (pK$_i$ of 7.82; Barwick et al., 1999). It was postulated that the high dose of mesulergine required, may have been due to the selectivity profile of the drug, and the high affinity it has for 5-HT$_{2C}$ receptors. It is known that 5-HT$_{2C}$ receptors may be involved in the control of micturition as the 5-HT$_{2C}$ receptor agonist mCPP has been shown to inhibit micturition (Steers & de Groat, 1989; Guarneri et al., 1996) and therefore the 5-HT$_{2C}$ antagonist qualities of mesulergine may directly oppose the inhibitory effects of the 5-HT$_7$ receptor antagonist qualities of the drug. Various effects were seen upon central administration of 100 $\mu$g kg$^{-1}$ mesulergine, with micturition abolished in 2 out of 7 animals and in the remaining animals there were no significant effects, likewise at the lowest dose of 10 $\mu$g kg$^{-1}$ there were no significant effects but a tendency towards excitation of the micturition reflex, rather than inhibition. The mixture of effects seen after mesulergine at the lower doses used in the study (10 & 100 $\mu$g kg$^{-1}$ i.c.v.), are most likely due to the lack of selectivity of the ligand. As mesulergine binds to the various receptor subtypes
mentioned, it is likely that a combination of effects at these receptors influence the overall effects measured in the micturition variables.

To further examine the effects of the 5-HT$_{2C}$ receptor antagonist qualities of mesulergine, on the micturition reflex, a selective 5-HT$_{2C}$ receptor antagonist SB-243213 was administered (pKi of 9.37 at human 5-HT$_{2C}$ receptors; (Wood et al., 2001)). Owing to solubility issues and selection of vehicles that can be put directly into the brain, the drug was given i.v. as this antagonist has been shown to have good brain penetration and high selectivity for 5-HT$_{2C}$ receptors (Bromidge et al., 2000). In expression studies there is little evidence for 5-HT$_{2C}$ receptors outside of the CNS (Hoyer et al., 1994) and SB-243213 is at least 100 fold more selective for 5-HT$_{2C}$ receptors compared to the 5-HT$_{2A}$ or 5-HT$_{2B}$ receptors (Bromidge et al., 2000), so it was assumed that central 5-HT$_{2C}$ receptors were blocked after administration of the SB-243213. Blocking 5-HT$_{2C}$ receptors had no effect on micturition. Therefore not supporting the evidence that these receptors are involved in micturition. Further in combination it was shown that SB-243213 did not influence the ability of SB-269970 to attenuate micturition. Thus indicating the effects caused by mesulergine on the micturition reflex are not related to its ability to block 5-HT$_{2C}$ receptors.

The influence of central 5-HT$_{2A}$ receptor blockade by mesulergine, on the present experiments is unknown. Testa et al., (2001) have shown that block of 5-HT$_{2A}$ receptors with ketanserin can affect micturition, but again it was reasoned this was due to experimental variance within the isovolumetric model used. The lowest dose of ketanserin (100 $\mu$g kg$^{-1}$ i.v.) caused significant inhibition of bladder contractions whereas higher doses
(1 mg kg\(^{-1}\) i.v) did not affect micturition. This is the same pattern as the results for mesulergine. It appears that the findings from Testa \textit{et al.}, (2001) need further clarification. The isovolumetric bladder contraction model can be subject to variability owing to the effects of anaesthesia and also bladder compliance over time, therefore the utmost stability is required for the model to be successfully used and for it to produce reliable data. It cannot be ruled out that in the present experiments, any effects caused by mesulergine could be due to block of 5-HT\(_{2A}\) receptors. Guarneri \textit{et al.}, (1996) showed that inhibition of bladder contractions by the 5-HT\(_2\) receptor ligand mCPP, was augmented by ketanserin pre-treatment. This indicates that the 5-HT\(_{2A}\) receptor subtype may play a role in the control of micturition. However, experiments in the present study to clarify the involvement of 5-HT\(_{2A}\) receptor block have not been carried out.

Mesulergine also has an affinity for D\(_2\) receptors and has unique properties as studies show that it can initially act as a dopamine antagonist, but that it, or its metabolites can also exerts central dopamine agonist activity in vivo (Fuxe \textit{et al.}, 1985; Barwick \textit{et al.}, 1999). This adds to the complexity of the story when using mesulergine as an antagonist. It is known that dopamine has a role in the control of micturition as administration of apomorphine, a dopamine agonist, causes bladder hyperactivity mediated by D\(_2\) receptors (Kontani \textit{et al.}, 1990). In addition, in conscious rats a D\(_2\) agonist, quinpirole, facilitates micturition, but a selective D\(_2\) antagonist remoxipride, has no significant effects (Seki \textit{et al.}, 2001). Therefore the additional properties of mesulergine acting as a dopamine receptor agonist in vivo may also have affected the 5-HT\(_7\) receptor antagonist responses that were expected at the lower doses used.
In summary the present experiments demonstrate that at a high dose mesulergine can attenuate micturition in anaesthetized rats in a similar way to selective 5-HT\textsubscript{7} receptor antagonists. However, mesulergine has a selectivity profile for other receptor subtypes that may interfere with the 'expected' 5-HT\textsubscript{7} antagonist responses seen on micturition. No interplay could be demonstrated between the 5-HT\textsubscript{2C} receptor and the 5-HT\textsubscript{7} receptor in the control of micturition and further experiments would be needed to further clarify the effects of the 5-HT\textsubscript{2A} receptor and dopaminergic properties of mesulergine and how they interfere, if at all, with the 5-HT\textsubscript{7} receptor antagonist qualities of the ligand. In addition the present data does not support a role for central 5-HT\textsubscript{2C} receptors in micturition. Mesulergine does not appear to be selective enough for 5-HT\textsubscript{7} receptors to be useful as a 5-HT\textsubscript{7} receptor antagonist in future assays and selective compounds for the 5-HT\textsubscript{7} receptor are currently being developed and are likely to prove more useful in further characterisation of physiological and disease states involving the 5-HT\textsubscript{7} receptor.
Chapter Five

5-CT and micturition
5.1 Aim

To determine whether 5-CT, a non-selective 5-HT_7 receptor agonist, can facilitate micturition.

5.2 Introduction

From the previous chapters a major role for 5-HT_7 receptors has been demonstrated in the control of micturition in the anaesthetized rat. In this respect 5-HT_7 receptors function in a similar way to that of 5-HT_{1A} receptors in this reflex, with supraspinal 5-HT_7 receptors facilitating the micturition reflex. Therefore it would be expected that an agonist for 5-HT_7 receptors will activate micturition in a similar way to that seen for the 5-HT_{1A} receptor agonist 8-OH-DPAT (Lecci et al., 1992).

Currently there are no selective agonists available for 5-HT_7 receptors, although binding studies have shown that the agonist 5-carboxyamidotryptamine (5-CT) does have a nanomolar affinity for the 5-HT_7 receptor (Bard et al., 1993; Ruat et al., 1993). In this respect, 5-CT has been shown to induce hypothermia in mice and guinea pigs, via 5-HT_7 receptor activation (Hagan et al., 2000; Guscott et al., 2003). 5-CT induced hypothermia is blocked by the selective 5-HT_7 receptor antagonist SB-269970 but not antagonists selective for 5-HT_{1A} and 5-HT_{1B/1D} receptors, in mice and guinea pigs. Thus at least in these species hypothermia does not involve other 5-HT receptors, especially the 5-HT_1 receptor subtype for which 5-CT also has high affinity. In this respect, 5-CT used in the hypothermia model in mice and guinea pigs could be described as a relatively ‘clean’ assay for 5-HT_7 receptors. Further, 5-HT_7 receptor knockout mice have no hypothermic responses to 5-CT
again confirming that this effect is purely mediated via 5-HT\textsubscript{7} receptors (Hedlund \textit{et al.}, 2003). There is a different situation in rats, where the 5-HT\textsubscript{1A} receptor agonist, 8-OH-DPAT, induces hypothermia (Testa \textit{et al.}, 1999). As 8-OH-DPAT has been found to have a moderate agonist activity at 5-HT\textsubscript{7} receptors (To \textit{et al.}, 1995; Wood \textit{et al.}, 2000), hypothermia could also be mediated by 5-HT\textsubscript{7} receptors in the rat model. Central administration of 8-OH-DPAT causes an increase in frequency of isovolumetric bladder contractions in rats, (Lecci \textit{et al.}, 1992) therefore a role for 5-HT\textsubscript{7} receptors in the excitatory micturition response to 8-OH-DPAT cannot be ruled out. In addition to 5-HT\textsubscript{7} receptors, 5-CT has a high affinity for the 5-HT\textsubscript{1} receptors (Hoyer \textit{et al.}, 1994). 5-CT will bind with a high affinity and activate the 5-HT\textsubscript{1B} and \textsubscript{1D} receptors. Therefore, from previous studies on hypothermia it may be possible that centrally administered 5-CT will facilitate micturition, however this could be either via activation of 5-HT\textsubscript{7} and/or 5-HT\textsubscript{1A} receptors.

Further agonists for 5-HT\textsubscript{1B/1D} receptors have been shown to inhibit parasympathetic outflow to the heart in rabbits (Dando \textit{et al.}, 1998) and to the airways in guinea pigs (Bootle \textit{et al.}, 1998). Thus the expected excitatory effect of 5-CT on parasympathetic outflow to the bladder, via activation of 5-HT\textsubscript{1A} and 5-HT\textsubscript{7} receptors, could be masked by an inhibitory effect caused by activation of 5-HT\textsubscript{1B/1D} receptors. Further, there is additional evidence that activation of central 5-HT\textsubscript{1B} receptors may have the opposite effect to activation of 5-HT\textsubscript{1D} receptors, in central cardiovascular control (see Ramage, 2001).

High densities of 5-HT\textsubscript{1B/1D} receptors are located in the basal ganglia, hippocampus and cortex of rats, on both the 5-HT cell bodies and terminals (Barnes & Sharp, 1999) and so
these receptors are situated in areas that could mediate or regulate the micturition reflex. Therefore the present experiments were carried out to investigate if 5-CT could activate the micturition reflex when applied i.c.v. in the presence and absence of the selective 5-HT\textsubscript{1B/1D} receptor antagonist GR127935. In addition, the effects of sumatriptan a selective agonist for 5-HT\textsubscript{1B/1D} receptor, and CP-93,129, a selective 5-HT\textsubscript{1B} receptor agonist, were also investigated.
5.3 Results

5.3.1 Effects of 5-HT$_1$ receptor agonist, 5-CT, on micturition

5-CT 3 nmol kg$^{-1}$ (i.c.v.; n = 6) caused no significant changes to any of the micturition variables, although micturition tended towards being inhibited across the group with a small reduction in amplitude of evoked bladder contraction of $25 \pm 10\%$ compared to the saline control (n = 5) where the reduction was $13 \pm 7\%$ (Figure 5.2C). A small increase in pressure threshold was also observed ($36 \pm 15\%$) compared to control ($30 \pm 18\%$; Figure 5.2B). No changes in volume threshold were observed (Figure 5.2A). Figure 5.1A shows a typical experiment, with the effects of different doses of 5-CT. In 2 out of the 6 animals, 5-CT 3 nmol kg$^{-1}$ (i.c.v) caused an increased amount of spontaneous activity in the baseline bladder pressure with a tendency towards hyperactive behaviour. These same animals also had a reduced micturition contraction time, measured of the voiding contraction (-15% and -23%). In the same way, resting pressure was also increased in these 2 animals after 5-CT, but this was difficult to measure consistently. These effects were insignificant when expressed as part of the group.

The higher dose of 5-CT had variable effects on micturition. In 3 out of 6 rats, 5-CT (10 nmol kg$^{-1}$ i.c.v.; n = 6) abolished micturition (Figure 5.1B). In the animals where the reflex was not abolished (n = 3), when these changes were compared with saline they were significant. The threshold for micturition was significantly decreased, for volume by $-33 \pm 18\%$ and for pressure by $-46 \pm 8\%$. There were no notable changes in baseline bladder...
pressure, evoked bladder contraction or resting pressure in these animals. 5-CT had no significant effects on residual volume in these experiments. Figure 5.1 shows traces from these experiments. At the 10 nmol kg⁻¹ dose of 5-CT an example is shown where micturition has been attenuated and the reflex has been abolished (n = 3) and also when it has not and the micturition reflex is in tact (n = 3). Graphical representation of the above data is shown in Figure 5.2.
Figure 5.1. Urethane anaesthetized female rats: Traces showing changes in bladder pressure during saline distension-evoked bladder contractions before and after i.c.v. injections of A) 5-CT 3 nmol kg\(^{-1}\) B) and C) 5-CT 10 nmol kg\(^{-1}\). In trace B) and C) mixed results are shown for the same dose of 5-CT – B) where micturition has been attenuated (n = 3) and C) where it has not (n = 3).
Figure 5.2 Urethane anaesthetized female rats: histograms comparing (Δ) changes caused by i.c.v. injections of saline with that of 5-CT (3 and 10 nmol kg\(^{-1}\)) on A) volume threshold, B) pressure threshold and C) evoked bladder contraction during cystometry-evoked micturition reflex. Each bar represents the mean value and the error bars show s.e. mean. Changes caused by drugs were compared with appropriate vehicle using Student’s unpaired t-test. * \(P<0.05\)
5.3.2 Effects of 5-HT\textsubscript{1B/1D} antagonist GR127935 on micturition

Traces showing the effects of GR127935 on distension-evoked changes in bladder pressure are shown in Figure 5.3A. GR127935 (100 µg kg\textsuperscript{-1} i.c.v.; n = 6) caused a significant increase in volume threshold of 94 ± 35% to evoke micturition compared to control (n = 6) where there was a much smaller increase of 29 ± 12% (See Figure 5.3A and 5.4A). This increase in volume threshold was associated with a non-significant increase in pressure threshold of 39 ± 21% and a reduction in evoked bladder contraction of 24 ± 8% when compared to control values, which were 25 ± 19% and -16 ± 7% respectively (see Figures 5.4B and C). There were no significant changes in residual volume at this dose tested.

5.3.3 Effects of 5-CT after pre-treatment with GR127935, on the micturition reflex.

When pre-treated with GR127935 (100 µg kg\textsuperscript{-1} i.c.v.), 5-CT (10 nmol kg\textsuperscript{-1} i.c.v.; n=6) caused a significant reduction in volume threshold of -60 ± 12% when compared to the time matched control, where volume threshold was reduced by only -26 ± 16% (Figure 5.5A). Figure 5.3B shows a typical example of the changes in response to 5-CT in distension-evoked bladder pressure after pre-treatment with GR127935. On the trace (Figure 5.3B), the point at which 5-CT was injected i.c.v. is included to give an example of some effects observed on baseline bladder activity. Increases in baseline bladder pressure activity were observed, even without increases in the intravesical volume. As the bladder was empty at this point, there was no release of fluid from the urethral opening in response.
to the bladder contraction. This hyperactive baseline bladder behaviour occurred in only 3 of the 6 animals and reflects the diversity of drug effects likely within a population of animals. When the infusion to the bladder is switched on, and the bladder fills with saline, more spontaneous activity of the detrusor smooth muscle compared to a control void was seen in the 3 hyperactive animals. The large decrease in volume threshold observed after 5-CT was not associated with any changes in pressure threshold or in the amplitude of the evoked micturition contraction (Figure 5.5B and C).
Figure 5.3 Urethane anaesthetized female rat: trace showing changes in bladder pressure (mmHg) during saline distension-evoked micturition reflex, before and after A) GR127935 100 µg kg\(^{-1}\) i.c.v. and B) 5-CT 10 nmol kg\(^{-1}\) i.c.v. (Pre-treated with GR127935) after pre-treatment with GR127935. Saline infusion begins at the start of each trace unless otherwise stated.
Figure 5.4 Urethane anaesthetized female rats: histograms comparing (Δ) changes caused by i.c.v. injections of saline with that of GR127935 (100 µg kg\(^{-1}\)) on A) volume threshold, B) pressure threshold and C) evoked bladder contraction, during cystometry-evoked micturition reflex. Each bar represents the mean value and the error bars show s.e. mean. Changes caused by drug were compared with appropriate vehicle using Student’s unpaired \(t\)-test. * \(P < 0.05\).
Figure 5.5. Urethane anaesthetized female rats: histograms comparing (Δ) changes caused by i.c.v. injections of 5-CT 10 nmol kg\(^{-1}\) pretreated with vehicle (control) with that of 5-CT 10 nmol kg\(^{-1}\) pretreated with GR127935 100 μg kg\(^{-1}\) on A) volume threshold, B) evoked bladder contraction and C) pressure threshold during cystometry-evoked micturition reflex. Each bar represents the mean value and the error bars show s.e. mean. Changes caused by pre-treatment were compared with vehicle using Student’s unpaired t-test. * P< 0.05.
5.3.4 Effects of sumatriptan, a 5-HT_{1B/1D} receptor agonist, on the micturition reflex

Traces showing the response of distension evoked changes in bladder pressure to sumatriptan are shown in Figure 5.6. Sumatriptan (25 nmol kg\(^{-1}\) i.c.v., \(n = 5\)) abolished the micturition reflex in 3 out of 5 animals. The mean evoked bladder contraction for the whole group was significantly reduced by 67 ± 28\% (\(n = 5\)) at this dose, compared to a time matched control, which was increased by 22 ± 10\% (\(n = 6\); see Figure 5.7A). In the remaining 2 animals, where micturition was not abolished, sumatriptan tended to cause inhibition of the micturition reflex and caused an increase in residual volume of 477 ± 450\%, compared with a reduction of 24 ± 18\% in the control experiments. Associated with this large increase in residual volume in the animals where micturition was still intact, there was again a tendency towards inhibition with a non-significant increase in volume threshold and pressure threshold of 63 ± 32\% and 30 ± 71\% respectively, compared to an increase of 14 ± 10\% for volume threshold and a reduction of 4 ± 22\% for pressure threshold control values.
Figure 5.6 Urethane anaesthetized female rat: trace showing changes in bladder pressure during saline distension-evoked micturition reflex, before and after Sumatriptan 25 nmol kg$^{-1}$ i.c.v. Saline infusion begins at the start of each trace.
Figure 5.7. Urethane anaesthetized female rats: A) histogram comparing (Δ) changes caused by i.c.v. injections of saline (control) with that of Sumatriptan 25 nmol kg\(^{-1}\) on evoked bladder contraction during cystometry-evoked micturition reflex. Each bar represents the mean value and the error bars show s.e. mean. Changes caused by drug were compared with vehicle using Student’s unpaired \(t\)-test. * \(P<0.05\).
5.3.5 Effects of 5-HT$_{1B}$ agonist, CP93,129 on micturition

Traces showing the effects of CP93,129 are shown in Figure 5.8. CP93,129 (0.5 μmol kg$^{-1}$ i.c.v.; $n = 6$) abolished the micturition contraction in 5 out of 6 animals. The mean evoked bladder contraction of the group therefore was significantly reduced by 93 ± 7% compared to the control group (i.c.v. saline $n = 6$) where there was an increase in bladder contractions of 22 ± 10% (Figure 5.10). In the one animal where the micturition reflex remained intact there was a tendency towards inhibition of micturition with a reduction in the amplitude of the evoked bladder contraction of 56%. In association with this reduction in amplitude of contraction size, an increase in volume threshold, pressure threshold and residual volume were observed of 14%, 86% and 342% respectively.

5.3.6 Bladder Pressure and Heart Rate

CP93,129 (0.5 μmol kg$^{-1}$ i.c.v.; $n = 6$) caused a significant increase in heart rate of 8 ± 1%. 5-CT, GR127935, Sumatriptan and had no significant effects on baseline values.
Figure 5.8. Urethane anaesthetized female rat: trace showing changes in bladder pressure during saline distension-evoked micturition reflex, before and after CP93,129 0.5 μmol kg⁻¹ i.c.v.. Saline infusion begins at the start of each trace.
Figure 5.9 Urethane anaesthetized female rats: histogram comparing (Δ) changes caused by i.c.v. injections of saline (control) with that of CP93,129 0.5 μmol kg⁻¹ on evoked bladder contraction during cystometry-evoked micturition reflex. Each bar represents the mean value and the error bars show s.e. mean. Changes caused by drug were compared with vehicle using Student’s unpaired *t*-test. * P< 0.05.
5.3.7 Cystometric study - Baseline values

Infusion of saline into the bladder of 41 female rats caused distension of the bladder, and, in turn, evoked the micturition reflex. The reflex was characterized by a rapid increase in bladder pressure (to a maximum of $22.4 \pm 0.4$ mmHg), the emission of fluid during the detrusor contraction and then a return to baseline, or at least to a pressure lower than that recorded before micturition. The contractions had a mean duration of $21 \pm 0.7$ s and returned to a resting pressure of $1.1 \pm 0.1$ mmHg after micturition. The mean bladder pressure threshold to evoke the micturition reflex was $6.5 \pm 0.2$ mmHg, which was reached when $0.44 \pm 0.02$ ml of saline had been infused. The mean amount of residual volume left in the bladder was $44 \pm 3\%$ of the volume infused. The mean baseline bladder pressure, MAP and HR were $0.72 \pm 0.1$ mmHg, $132 \pm 2$ mmHg and $377 \pm 5$ beats min$^{-1}$, respectively. The mean baseline data for individual experimental groups are shown in Table 5.1.
Table 5.1. Baseline values of control reflex-evoked changes in bladder pressures caused by intravesical infusion of saline for each experimental group in urethane anaesthetized female rats for cystometric studies. * and # same animals as for saline i.c.v, doses of agonist given after saline according to protocol.

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>n</th>
<th>Bladder Thresholds</th>
<th>Micturition-evoked bladder contraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rats</td>
<td>Pressure MmHg</td>
</tr>
<tr>
<td>Saline i.c.v. 5 µl</td>
<td>6*</td>
<td>5.7 ± 0.6</td>
<td>0.5 ± 0.08</td>
</tr>
<tr>
<td>Saline i.c.v. 5 µl</td>
<td>6#</td>
<td>6.5 ± 0.7</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>5-CT i.c.v. 3 nmol kg⁻¹</td>
<td>6</td>
<td>5.6 ± 0.7</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>10 nmol kg⁻¹</td>
<td>6</td>
<td>6.9 ± 0.4</td>
<td>0.61 ± 0.04</td>
</tr>
<tr>
<td>10 nmol kg⁻¹</td>
<td>6#</td>
<td>7.7 ± 1.2</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>GR127935 i.c.v.</td>
<td>100 µg kg⁻¹</td>
<td>6</td>
<td>6.1 ± 0.4</td>
</tr>
<tr>
<td>sumatriptan i.c.v.</td>
<td>25 nmol kg⁻¹</td>
<td>5</td>
<td>6.3 ± 0.7</td>
</tr>
<tr>
<td>CP 93,129 i.c.v.</td>
<td>0.5 µmol kg⁻¹</td>
<td>6</td>
<td>8.2 ± 0.8</td>
</tr>
</tbody>
</table>
5.4 Discussion

There are several findings from the present experiments. Firstly, the non-selective 5-HT\textsubscript{7} receptor agonist 5-CT cannot activate micturition when given alone. The selectivity profile of 5-CT shows that it binds to the 5-HT\textsubscript{7} receptor with nanomolar affinity (pK\textsubscript{d} of 9.5 in rat (Shen et al., 1993) but also binds with high affinity to the 5-HT\textsubscript{1} receptor family (pK\textsubscript{d} of 10.3 (human), 8.9 (rat) and 9.2 (rat) at 5-HT\textsubscript{1A}, 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} receptors, respectively). From the results it can be seen that a dose of 3 nmol kg\textsuperscript{-1} 5-CT given i.c.v. had no significant effects on micturition or any other cardiovascular baseline measured. This dose of 5-CT has been shown previously to cause significant effects on blood pressure and heart rate in rats, although under an alternative anaesthetic regime (Anderson et al., 1992). At the higher dose of 10 nmol kg\textsuperscript{-1} 5-CT i.c.v., various effects were seen on micturition. In 3 out of 6 animals the micturition reflex was abolished, in the remaining 3 animals the reflex was excited, with significant reductions in volume and pressure thresholds. It is interesting that such a range of results can be seen within this group and this is again probably due to the selectivity profile of 5-CT. The data suggest that the affinity of 5-CT for members of the 5-HT\textsubscript{1} receptor family, such as the 5-HT\textsubscript{1B} or 5-HT\textsubscript{1D} receptors, may interfere with the expected excitatory action of 5-CT on the bladder reflex, through activation of 5-HT\textsubscript{7} and/or the 5-HT\textsubscript{1A} receptors. As indicated in the introduction, activation of 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} receptors can mediate inhibition of parasympathetic outflow (Dando et al., 1998; Bootle et al., 1998), therefore it is likely 5-CT is activating these receptors to inhibit micturition and offset the excitatory influences of 5-HT\textsubscript{1A} or 5-HT\textsubscript{7} receptor activation. This is confirmed by the observation that in the presence of the selective antagonist, GR127935, which has a high affinity at the 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} receptors (pK\textsubscript{d} of 8.7 and
8.3 ; Roberts et al., (2001), 5-CT activates the micturition reflex. Interestingly GR127935 alone also significantly increased the micturition volume threshold. This is a similar effect to that observed with WAY-100635 and in this respect GR127935 does have a reasonable affinity for 5-HT\textsubscript{1A} receptors (pKi 7.2 at human receptor (Roberts et al., 1999). This data implies that 5-CT is causing excitation of the micturition reflex by activation of 5-HT\textsubscript{7} rather than by 5-HT\textsubscript{1A} receptors. However this would have to be confirmed experimentally, seeing if WAY-1000635 prevented the 5-CT effect. If the 5-HT\textsubscript{1A} receptor is downstream from the 5-HT\textsubscript{7} receptor in the pathway, this may be difficult to prove. Although the present data does suggest that it is the reverse, with the 5-HT\textsubscript{7} receptor being upstream of the 5-HT\textsubscript{1A} receptor, since in the presence of GR127935, 5-CT does activate the reflex.

Certainly from the data it is clear that 5-HT\textsubscript{7} receptor mediated activation of micturition by 5-CT is only unmasked when 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} (and 5-HT\textsubscript{1A}) receptors are blocked. This is similar to central cardiovascular regulation where a role for 5-HT\textsubscript{1B/1D} receptors is uncovered if 5-HT\textsubscript{1A} receptors are blocked (Ramage, 2001). To further characterise the role of 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} receptor subtypes in the control of micturition more selective ligands were used. The 5-HT\textsubscript{1B/1D} agonist sumatriptan has a high affinity for both 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} receptor subtypes with a pKi of 7.7 and 8.4 respectively, at human cloned receptors (Roberts et al., 2001). These data demonstrate that given i.c.v. sumatriptan can significantly attenuate micturition. In a similar way to other experiments carried out, when using a compound with a mixed selectivity profile, not all animals in the group respond in the same way, and this may reflect the number of each receptor subtypes present. There is also a complication that the site of action is undetermined in this study and it is possible that both supraspinal and spinal sites of action could affect the results. From previous work
it has been shown that central 5-HT\textsubscript{1B/D} receptor activation can attenuate reflex activation of parasympathetic outflow to the heart and airways (Bootle \textit{et al.}, 1998; Dando \textit{et al.}, 1998), these experiments confirm that receptor activation may also attenuate parasympathetic outflow to the bladder. As the 5-HT\textsubscript{1D} receptor is found in very low concentrations in the rat (Bruinvels \textit{et al.} 1993), it was postulated that the receptor most likely mediating the inhibition of micturition by sumatriptan was the 5-HT\textsubscript{1B} receptor and experiments were carried out using the selective 5-HT\textsubscript{1B} agonist CP-93,129 (Macor \textit{et al.}, 1990). CP-93,129 has a pEC\textsubscript{50} value of 7.8 in rat and is 200 times more selective for the 5-HT\textsubscript{1B} receptor over the 5-HT\textsubscript{1A} receptor and 150 times more selective for the 5-HT\textsubscript{1B} receptor over the 5-HT\textsubscript{1D} receptors, therefore is a useful pharmacological tool. Central administration of CP-93,129 significantly attenuated micturition by abolishing the reflex in 5 out of 6 animals and owing to the selectivity of the drug, this is the likely result of 5-HT\textsubscript{1B} receptor activation. It is noted that studies using agonists do not demonstrate a physiological role for a receptor subtype and therefore further work should be carried out with selective 5-HT\textsubscript{1B} receptor antagonists to identify the importance of the 5-HT\textsubscript{1B} receptor in the control of micturition. High densities of 5-HT\textsubscript{1B} receptors are found in the basal ganglia and forebrain areas (Hoyer \textit{et al.}, 1994). In addition, experiments have shown that 5-HT\textsubscript{1B} receptors, can control the release of 5-HT in the dorsal raphe nuclei, hypothalamus and hippocampus as well as the release of ACh and glutamate in other regions of the brain (Roberts \textit{et al.}, 1999; see Barnes & Sharp, 1999), and this may indicate a role for 5-HT\textsubscript{1B} receptors in the regulation of micturition pathways. There are inter-species similarities between rabbits and guinea pigs and the role of 5-HT\textsubscript{1B} in parasympathetic outflow and these data now present the possibility that the pattern continues in rats (Dando \textit{et al.}, 1998; Bootle \textit{et al.}, 1998; present experiments). However,
further work is needed and the precise role of this receptor subtype in micturition needs further clarification.

Overall these data add to the knowledge about how the 5-HT receptor family act to control and potentially modulate micturition. 5-HT\textsubscript{7} receptor activation can excite micturition and 5-HT\textsubscript{1B} receptor activation can inhibit micturition. The location, interaction and precise function of these receptor subtypes may provide new targets for treatments of lower urinary tract disorders.
Chapter Six

General Discussion
Chapter Six: General Discussion

6 General Discussion

The present experiments indicate an important role for central 5-HT\textsubscript{7} receptors in the control of parasympathetic outflow to the bladder in the anaesthetized rat. Selective antagonists were used to block central 5-HT\textsubscript{7} receptors and micturition was attenuated. This is an interesting and important finding as other members of the 5-HT receptor family are also involved in the control of micturition; in particular the well studied 5-HT\textsubscript{1A} receptor. Blocking 5-HT\textsubscript{7} receptors attenuates micturition in a similar way to 5-HT\textsubscript{1A} receptor blockade and as both these receptor subtype effects are mediated by opposing 2\textsuperscript{nd} messenger effects on adenylyl cyclase, this poses interesting questions for future studies on the location and interaction between these receptor subtypes.

The selectivity profile of the two selective 5-HT\textsubscript{7} receptor antagonists used in the initial studies, coupled with the short half life and the lack of effect when given intrathecally, indicate essentially a supraspinal role for 5-HT\textsubscript{7} receptors, rather than both spinal and supraspinal sites of action, as seen with the 5-HT\textsubscript{1A} receptors. Interestingly, 5-HT\textsubscript{7} receptor blockade had little effect on the urethral function, but additional work may be needed to entirely rule out any effects on urethral control as this may have been masked to some extent by the cannulation of the urethra from the bladder side. Systemic administration proved difficult with SB-269970, the main selective 5-HT\textsubscript{7} receptor antagonist, as it is rapidly cleared from the blood and therefore only at a very high dose were any effects observed on micturition in rats. The very rapid clearance rate of the compound was one of the reasons that at the same dose that caused significant effects on micturition in the rat, used in the cat it yielded no effects. A closed bladder system approach may have also been a useful model to use in the cat. There is however a risk
of increased damage to the bladder with this method as it involves tying a ligature around the bladder neck, but this should be compared with the likely damage of making a small yet necessary incision into the bladder dome. It is suggested that further work could be carried out on the cat model, using central administration of the drug with an altered protocol. This work would clarify further the role of 5-HT receptors in the cat, as there is currently some dispute over whether the 5-HT_{1A} receptor mediates similar effects on parasympathetic outflow in different species.

Studies were also carried out using one of the ‘classic’ 5-HT receptor antagonists, mesulergine, which has been shown to have a high affinity for the 5-HT_{7} receptor. Only at a high dose did mesulergine act in a similar way to the previous selective 5-HT_{7} receptor antagonists tested, and this was thought to be due to the selectivity profile of mesulergine. Mesulergine, although a 5-HT_{7} receptor antagonist with a similar affinity for 5-HT_{7} receptors as other selective antagonists SB-269970 and SB-656104 used in the study, also binds with high affinity to 5-HT_{2C} receptors and dopamine receptors. The affinity mesulergine has for 5-HT_{2C} receptors did not appear to interfere with the 5-HT_{7} antagonist qualities of the drug as in the presence of the 5-HT_{2C} receptor antagonist SB-243213, the selective 5-HT_{7} receptor antagonist SB-269970 could still attenuate micturition. Therefore, it is likely that the high affinity with which the drug binds to dopamine receptors somehow affects this antagonist in its ability to attenuate micturition at lower doses.

There are no selective 5-HT_{7} receptor agonists available and therefore as the non-selective agonist 5-CT has a high affinity for 5-HT_{7} receptors, it was used to try to facilitate micturition in the same way that 5-HT_{7} receptor antagonists were used to
attenuate micturition. Central administration of 5-CT alone yielded various results, again most likely owing to the selectivity profile of the agonist, which binds with high affinity to the 5-HT\textsubscript{1} receptor family. When using the selective 5-HT\textsubscript{1B/1D} receptor antagonist GR127935 to block the 5-HT\textsubscript{1} receptors, micturition could be facilitated by 5-CT, probably acting through 5-HT\textsubscript{7} receptor activation. This lead to the idea that perhaps 5-CT binding to 5-HT\textsubscript{1B/1D} receptors interfered with its ability to facilitate micturition through 5-HT\textsubscript{7} receptor activation and therefore these receptors were examined in preliminary agonist studies. Central administration of 5-HT\textsubscript{1B/1D} agonist sumatriptan and selective 5-HT\textsubscript{1B} receptor agonist CP93,129, showed that activation of these receptor subtypes, particularly the 5-HT\textsubscript{1B} receptor, inhibited parasympathetic outflow to the bladder and therefore inhibited micturition. These receptors are known to negatively couple to adenylyl cyclase in conjunction with the other members of the 5-HT\textsubscript{1} receptor family, but they have also been shown to have the opposite effect to the 5-HT\textsubscript{1A} receptor on parasympathetic outflow to the airways and heart and therefore it is likely that they would also have an inhibitory effect on outflow to the bladder. This is in contrast to the 5-HT\textsubscript{1A} receptor which, in the same way as the 5-HT\textsubscript{7} receptor seems to have a tonic facilitatory action on parasympathetic outflow to the bladder. These agonist experiments proved to be some of the most interesting and provided a new insight into the role of 5-HT\textsubscript{1B/1D} receptors in the control of micturition. Agonist studies do not demonstrate a physiological role for a receptor subtype but they do indicate a possible involvement in the control of these parasympathetic micturition pathways, and in this case had the studies continued a further examination of the role of 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} receptors would have been an interesting addition to the thesis.
Some 5-HT receptors and their effects on micturition in rats

5-HT$_7$ receptor + 5-HT$_{1A}$ receptor + 5-HT$_{1B/1D}$ receptor -

5-HT$_{1A}$ receptor + 5-HT$_{1B/1D}$ receptor -?

Figure 6.1. A summary diagram of the main findings of this thesis
The experimental design and animals models used in these studies have provided a robust method for further characterising the role of 5-HT receptors in the control of micturition. The doses of drugs used can be justified from binding studies in the literature or from test experiments used to gauge a useful working range of compounds. The criteria used to measure the micturition reflex were also appropriate for determining the effects of all the compounds tested although with hindsight, measurements of the rate of rise in bladder pressure in response to agonists or antagonists may have provided more information on the subtleties of drug effects and added to the analyses. In addition, a larger grouping of control experiments would have allowed more robust statistical analyses to occur when using the Student’s unpaired t-test, particularly when using multiple doses of the compounds compared to one control group. However, this occurred in very few experiments and was not carried out.

Future work, in continuation with these studies, would be to further clarify the role of central 5-HT$_7$ receptors in the control of micturition and use the same selective compounds in a conscious rodent cystometry model. It would then be possible to observe if the 5-HT$_7$ receptor is utilised in a similar way for micturition in the conscious animal as it is in the anaesthetized animal. It may also be useful to locate which ‘arm’ of the reflex arc these receptors mainly control, whether they are involved in processing the incoming afferent information from the bladder or located on the parasympathetic outflow to the bladder, or both. Peripheral nerve recordings and/or stimulation of specific brain regions in combination with selective antagonists may be useful for clarifying this situation. In addition, the involvement of 5-HT$_{1B}$ receptors in the control of parasympathetic outflow to the bladder was only briefly examined in this study. It would be useful to use the anaesthetized rat cystometry model with selective 5-HT$_{1B}$
and 5-HT$_{1D}$ receptor antagonists to demonstrate physiological roles, if any, for this receptor subtypes.

In terms of putting this work into a clinical context, there is a great need for new compounds to treat LUT disorders, which may have improved side effect profiles and success rates in the variety of patient populations that present with LUT disorders. Most treatments for UUI act directly on the smooth musculature of the LUT itself. A most common medication for urinary urgency and UUI is oxybutinin, owing to its spasmylytic and anticholinergic effects on detrusor smooth muscle fibres. However, this drug has a reduced success rate in older and institutionalised patients and has an associated side effect profile owing to the anticholinergic properties, which includes dry mouth, constipation, blurred vision, cognitive impairment, particularly in patients with Parkinson's disease (Donnellan, et al., 1997). Newer treatments such as the muscarinic antagonist tolterodine provide an improved control of patient symptoms with reduced adverse effects (Nilvebrant et al., 1997) but improved treatments are still sought. There is potential to exploit and manipulate 5-HT receptors to either facilitate or inhibit micturition as demonstrated by this work, with the long-term goal of improving current treatments for UI. Being that the urological literature is also now suggesting a direct link between a variety of psychiatric disorders, which can be induced by lowering monoamines such as 5-HT, and incontinence (Steers & Lee, 2001), it may be predicted that centrally acting 5-HT compounds will provide the future direction for UI research. There is certainly much scope and current growth in this field. Thus, the current studies go some way in further clarifying the role of 5-HT receptors in the control of micturition and provide an insight into new potential targets for the treatment of disorders of the lower urinary tract.
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Published work
Evidence for the involvement of central 5-HT\textsubscript{7} receptors in the micturition reflex in anaesthetized female rats

Kate E. Read, Gareth J. Sanger & Andrew G. Ramage

Introduction

Central 5-hydroxytryptamine (5-HT) containing neurones, via activation of 5-HT\textsubscript{1A} receptors, play an important role in the reflex control of parasympathetic outflow to the heart in rats (Bogle et al., 1990), rabbits (Futuro-Neto et al., 1993; Dando et al., 1998; Skinner et al., 2001) and cats (Bootle et al., 1993). Further, 5-hydroxytryptamine (5-HT) receptor antagonists SB-269970 and WAY-100635, given i.t. after SB-269970 caused a significant increase in the volume threshold. These data demonstrate that 5-HT\textsubscript{7} receptors located supraspinally in the rat are involved in the control of micturition.

Keywords: 5-HT\textsubscript{7} receptors; micturition; SB-269970; SB-656104; bladder; rats; WAY-100635; 5-HT\textsubscript{1A} receptors; blood pressure

Abbreviations: HR, heart rate; 5-HT, 5-hydroxytryptamine; i.c.v., intracerebroventricular; i.t., intrathecal; MAP, mean arterial blood pressure

1 The effects of the selective 5-HT\textsubscript{7} receptor antagonists SB-269970 (3-300 \(\mu\)g kg\(^{-1}\); \(n = 5-6\)) and SB-656104 (30 \(\mu\)g kg\(^{-1}\); \(n = 5\)) administered centrally (i.c.v.) were investigated on the 'micturition reflex' in the urethane anaesthetized female rat.

2 In cystometric recordings, SB-269970 caused significant increases in volume of 58 \pm 15 and 138 \pm 33% and pressure of 140 \pm 46 and 149 \pm 60% thresholds at 10 and 30 \(\mu\)g kg\(^{-1}\). These changes were associated with significant decreases in distension-induced bladder contraction of 62 \pm 14 and 60 \pm 11%, respectively. However, there was no change in residual volume. At the higher doses, SB-269970 blocked the micturition reflex. SB-656104 had similar effects to SB-269970 but in addition significantly increased the residual volume.

3 SB-269970 (10 \(\mu\)g kg\(^{-1}\); \(n = 5\)) given i.v. had no effect on the micturition reflex.

4 SB-269970 (30 \(\mu\)g kg\(^{-1}\); \(n = 4\)) given intrathecally (i.t.) had no effect on micturition reflex, although the selective 5-HT\textsubscript{1A} receptor antagonist WAY-100635 given i.t. after SB-269970 caused a significant increase in the volume threshold.

5 Using an isovolumetric method in which urethral changes were measured, SB-269970 (30 \(\mu\)g kg\(^{-1}\); \(n = 4\); i.c.v.) failed to have any effect on these urethral-evoked changes although they significantly reduced the amplitude of the bladder contraction.

6 These data demonstrate that 5-HT\textsubscript{7} receptors located supraspinally in the rat are involved in the control of micturition.
 METHODS

The experiments were carried out under the Animals (Scientific Procedures) Act, 1986. After completion of experiments, animals were killed by an overdose of pentobarbitone sodium intravenously (i.v.).

Experiments were performed on 67 female Sprague-Dawley rats (200–280 g), initially anaesthetized with isoflurane (4% in 100% oxygen), and maintained with urethane (1.2 g kg⁻¹, i.v.).

The depth of anaesthesia was assessed by the stability of blood pressure and heart rate (HR), and by an absence of hind limb withdrawal in response to paw pinch. If needed, supplementary doses of urethane were given (0.1 g kg⁻¹, i.v.). To maintain a patent airway, the trachea was intubated. The left jugular vein was cannulated for anaesthetic and drug administration, and the right common carotid artery was cannulated with a heparinized cannula (20U ml⁻¹ heparin in 0.9% w/v saline) and the right common carotid artery was cannulated with a heparinized cannula (20U ml⁻¹ heparin in 0.9% w/v saline) for the measurement of arterial blood pressure and for sampling arterial blood for gas analysis. Blood pressure was measured using a pressure transducer (Gould Statham P23Db) and a syringe pump for the infusion of saline to evoke the micturition reflex. Backflow through this cannula allowed the bladder to be emptied of residual fluid after micturition had occurred.

 Measurement of bladder pressures

Cystometry method The urinary bladder was exposed by a midline abdominal incision. The proximal ends of each ureter, exiting from the kidney, were exposed by retroperitoneal incisions and cannulated to prevent the bladder filling with urine during experiments. A small incision was made in the bladder dome and a single cuffed cannula (0.86 mm internal and 1.52 external diameter) was inserted into the bladder dome. This was connected via a T-piece to a pressure transducer (Gould Statham P23Db) and a syringe pump for the infusion of saline (0.9% w/v) at a rate of 0.1 ml min⁻¹, to evoke the micturition reflex. Backflow through this cannula allowed the bladder to be emptied of residual fluid after micturition had occurred.

 Measurement of bladder and urethral pressures

Isovolumetric method The urinary bladder was exposed and the proximal ends of each ureter cannulated, as above. An incision was made in the bladder dome and two cannulae (0.52 mm internal and 1.2 mm external diameter) were inserted into the bladder, one of which was connected to a syringe pump for the infusion of saline to evoke the micturition reflex and the other was connected to a pressure transducer (Gould Statham P23Db). The rate of infusion (0.046 ml min⁻¹) was chosen to simulate the maximal hourly diuresis rate (Kleemark, 1974). Urethral pressure was measured using a method developed by M. Fraser (see Kakizaki et al., 1997). For a detailed methods and diagram of this urethral pressure recording, see Conley et al. (2001).

 Experimental protocol All experiments were left for 30 min after completion of surgery, to stabilize. A diagram for the experimental protocols used is shown in Figure 1.

In Protocol 1 (cystometry), the bladder was infused with saline until threshold was reached and the micturition reflex was evoked. Saline was continuously infused for 15 min to ‘prime’ the system and cause a series of infusion-induced micturition reflexes. After 15 min, the infusion was stopped and the bladder emptied; 3 min later, the saline was infused into the bladder until micturition was evoked, the infusion was discontinued, the bladder was emptied and the residual volume was collected and measured. This was repeated three times to ensure the stability of the response. Then, after a period of 3 min, the test drug or vehicle was administered i.c.v., i.v. or i.t. After a further 3 min, the infusion was continued once more to
evoke the micturition reflex (a single micturition) in the presence of test drug or vehicle. In some experiments, the test drug caused a continuous rise in bladder pressure with no overt contraction being observed. However, in these cases, after a certain period of time, a few drops of saline leaked from the urethral opening and once this was observed the infusion was switched off. Such responses were taken to indicate that the micturition response had been abolished.

In Protocol 2 (isovolumetric method), saline was infused into the bladder to evoke a ‘priming’ reflex. After three consecutive reflex-evoked bladder contractions of similar amplitude, the infusion was discontinued. The infused saline was left in the bladder for 5 min, during which time the reflex was ongoing. The bladder was emptied and after 20 min, was infused and the infusion discontinued as above to acquire a control reflex. The bladder reflex was ongoing for 5 min before emptying the bladder of saline. After 5 min, the test drug or vehicle was administered, and after a further 5 min, the infusion-evoked reflex was repeated.

Data capture and analysis Arterial blood pressure, bladder and urethral pressures were displayed on a chart recorder (Grass Instruments) and captured (1500 samples s⁻¹) by an MP 100 WSW interface (Biopac systems Inc, U.S.A.) to allow data to be acquired and analysed offline using AcqKnowledge version 3.5.3 software (Biopac Systems Inc, U.S.A.). HRs were derived electronically from the blood pressure signal using the Biopac system.

Analysis of reflex-evoked bladder and urethral responses and baseline values Saline infusion into the bladder evoked large-amplitude bladder contractions that represent the micturition reflex. The variables mean volume threshold (ml), pressure threshold (mmHg), the evoked bladder contraction (mmHg; maximum intraluminal pressure minus pressure threshold) and residual volume (expressed as percentage of the total volume infused) were measured for the control reflexes in the cystometry method. For details of analysis of bladder and urethral pressures in the isovolumetric method, see Wibberley et al. (2002). Baseline measurements of HR and blood pressure were mean values measured 2 min before reflexes and prior to drug or vehicle administration.

Statistical analysis Changes in baselines and reflex-evoked effects were compared with vehicle controls by unpaired Students t-test and one-way ANOVA where applicable. Values of P < 0.05 were considered statistically significant. All values are expressed as mean ± s.e.m.

Drugs and solutions Drugs and chemicals were obtained from the following sources; SB-269970 ((R)-3-(2-(2-(4-methylpiperidin-1-yl)-ethyl)pyrrolidine-1-sulphonyl)phenyl hydrochloride) and SB-656104 (6-((R)-2-{2-[4-(4-chloro-phenoxo)-piperidin-1-yl]-ethyl}-pyrrolidine-1-sulphonyl)-1H-indole hydrochloride were synthesized at GlaxoSmithKline, Harlow, Essex, U.K.; WAY-100635, urethane and dimethyl sulphoxide (DMSO) from Sigma Aldrich Chemicals, Poole, Dorset, U.K.; pentobarbitone sodium from Rhone Merieux Ltd, Harlow, Essex, U.K.; isoflurane from Baxter Healthcare Ltd, Thetford, Norfolk, U.K.; gelofusine, polyethylene glycol 400 (PEG) from B Braun Medical Ltd, Sheffield, U.K.; sodium chloride, glucose and sodium bicarbonate from Merck/BDH Lab supplies, Poole, Dorset, U.K., heparin from CP Pharmaceuticals Ltd, Wrexham, U.K. SB-269970 was dissolved in 0.9% w/v saline, while SB-656104 HCl was dissolved in a mixture of 50%PEG/50%DMSO, which had been diluted 1 in 4 in saline. All i.c.v. and i.t. doses were given in 5 μl volume. All i.v. doses were given in 0.1 ml volume followed by a 0.1 ml flush of saline. All drugs were given as their salts.

Results

Cystometric study

Baseline values Infusion of saline into the bladder of 59 female rats caused distension of the bladder, and, in turn, evoked the micturition reflex. The reflex was characterized by a rapid increase in bladder pressure (to a maximum of 22.3 ± 0.4 mmHg), the emission of at least three drops of fluid...
Effects of 5-HT<sub>3</sub> receptor antagonists, SB-269970 and SB-656104

Traces showing the effects of SB-269970 and SB-656104 on distension-evoked changes in bladder pressure are shown in Figure 2. SB-269970 (3, 10, 30 µg kg<sup>-1</sup>; i.c.v.; n = 5–6) caused increases in volume threshold of 18 ± 11, 58 ± 15 and 138 ± 33%, respectively, being statistically significant compared with i.c.v. saline at the doses of 10 and 30 µg kg<sup>-1</sup>. At these doses, SB-269970 also caused significant reduction in the distension-evoked bladder contraction of −62 ± 14 and −60 ± 11% and a significant increase in pressure threshold of 150 ± 46 and 149 ± 60%, respectively. These effects were not associated with any change in residual volume (see Figure 3). At the doses of 100 and 300 µg kg<sup>-1</sup>, SB-269970 abolished the reflex (see Figure 2c).

SB-656104 (n = 5; Figures 2 and 3) i.c.v. at 30 µg kg<sup>-1</sup> had a similar effect to that of SB-269970, causing a significant increase in bladder pressure threshold (196 ± 102%) and volume threshold (54 ± 20%). Further, residual volume was significantly increased by 322 ± 66% when compared to control.

**i.t. Administration** SB-269970 (30 µg kg<sup>-1</sup>; 5 µl; n = 4) given at the L6/S1 level of the spinal cord had no significant effect on any of the variables measured, although WAY-100635 (10 µg kg<sup>-1</sup>; i.t.; n = 4) administered as a positive control 30 min after SB269970 (30 µg kg<sup>-1</sup>) caused a significant increase in volume threshold of 155 ± 76% (see Figure 4).

**i.v. Administration** SB-269970 (10 µg kg<sup>-1</sup>; n = 5) had no significant effects on the distension-evoked bladder contraction.

### Isovolumetric study

**Baseline values** Infusion of saline into the bladder in eight female rats caused distension of the bladder, in turn evoking the micturition reflex. The reflex is characterized by the appearance of rhythmic bladder contractions of 36 ± 2 mmHg. The contractions had a mean duration of 45 ± 3 s. The mean bladder pressure threshold to evoke such contractions (n = 8) was 6 ± 1 mmHg, which was reached when 0.17 ± 0.04 ml of saline had been infused into the bladder. Each rhythmic bladder contraction was accompanied by a fall in urethral

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>n</th>
<th>Pressure (mmHg)</th>
<th>Volume (ml)</th>
<th>Amplitude (mmHg)</th>
<th>Duration (s)</th>
<th>Residual volume (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline i.c.v. 5 µl</td>
<td>5</td>
<td>7.1 ± 0.6</td>
<td>0.42 ± 0.03</td>
<td>20.6 ± 1.7</td>
<td>19.8 ± 2.2</td>
<td>34 ± 5</td>
</tr>
<tr>
<td>SB-269970 i.c.v.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 µg kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>6</td>
<td>6.9 ± 0.8</td>
<td>0.54 ± 0.05</td>
<td>13.7 ± 1.2</td>
<td>19.9 ± 1.5</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>10 µg kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>5</td>
<td>6.7 ± 0.7</td>
<td>0.65 ± 0.04</td>
<td>19.6 ± 1.0</td>
<td>24.5 ± 2.0</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>30 µg kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>6</td>
<td>7.2 ± 0.6</td>
<td>0.41 ± 0.03</td>
<td>19.4 ± 1.7</td>
<td>25.6 ± 2.0</td>
<td>44 ± 4</td>
</tr>
<tr>
<td>100 µg kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>5</td>
<td>5.4 ± 0.6</td>
<td>0.41 ± 0.03</td>
<td>17.3 ± 1.3</td>
<td>17.9 ± 1.0</td>
<td>34 ± 5</td>
</tr>
<tr>
<td>300 µg kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>5</td>
<td>5.6 ± 0.5</td>
<td>0.53 ± 0.06</td>
<td>16.2 ± 0.6</td>
<td>18.4 ± 1.7</td>
<td>41 ± 7</td>
</tr>
<tr>
<td>25% PEG/DMSO i.c.v. 5 µl</td>
<td>5</td>
<td>8.7 ± 0.6</td>
<td>0.6 ± 0.1</td>
<td>11.3 ± 0.8</td>
<td>22 ± 1.2</td>
<td>33 ± 3</td>
</tr>
<tr>
<td>SB-656104 i.c.v. 30 µg kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>5</td>
<td>4.8 ± 0.7</td>
<td>0.6 ± 0.1</td>
<td>12.0 ± 0.03</td>
<td>24.3 ± 2.3</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>Saline i.t. 5 µl</td>
<td>4</td>
<td>8.7 ± 1.0</td>
<td>0.68 ± 0.10</td>
<td>11.4 ± 1.1</td>
<td>19.4 ± 1.0</td>
<td>40 ± 6</td>
</tr>
<tr>
<td>SB-269970 i.t. 30 µg kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>4</td>
<td>4.9 ± 1.0</td>
<td>0.40 ± 0.04</td>
<td>18.0 ± 1.0</td>
<td>17.1 ± 1.2</td>
<td>76 ± 22</td>
</tr>
<tr>
<td>WAY-100635 i.t. 10 µg kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>4*</td>
<td>4.4 ± 0.9</td>
<td>0.43 ± 0.04</td>
<td>18.1 ± 2.5</td>
<td>19.3 ± 2</td>
<td>63 ± 6</td>
</tr>
<tr>
<td>Saline i.v. 4.2 µl</td>
<td>4</td>
<td>4.2 ± 0.7</td>
<td>0.7 ± 0.12</td>
<td>13.9 ± 0.7</td>
<td>29.1 ± 5.7</td>
<td>50 ± 8</td>
</tr>
<tr>
<td>SB-269970 i.v. 10 µg kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>5</td>
<td>6.3 ± 1.1</td>
<td>0.76 ± 0.09</td>
<td>15.2 ± 0.6</td>
<td>22.5 ± 1.1</td>
<td>46 ± 5</td>
</tr>
</tbody>
</table>

*Same animals as for i.t. SB-269970.
pressure of $9 \pm 1$ mmHg that continued for $62 \pm 3$ s before returning to baseline. High-frequency oscillations occurred in urethral pressure, at the peak of each bladder contraction, that had a mean amplitude of $14 \pm 1$ mmHg and continued for $35 \pm 3$ s. Each reflex bladder contraction was accompanied by small increases in MAP and HR. The mean baseline bladder and urethral pressures, MAP and HR were $2 \pm 1$, $16 \pm 1$, $136 \pm 3$ mmHg and $360 \pm 13$ beats min$^{-1}$, respectively.

**Effect of SB-269970 given i.c.v.**

SB-269970 (30 µg kg$^{-1}$ i.c.v.; $n = 4$) caused a significant increase in volume threshold and pressure threshold and a significant reduction in the amplitude of bladder contractions ($219 \pm 60$, $162 \pm 28$ and $-17 \pm 3\%$, respectively; see Figure 5). However, these changes were associated with no significant effects in urethral relaxations or in urethral pressure high-frequency oscillations.

**Blood pressure and HR**

Neither antagonist had any significant effect on baseline values.

**Discussion**

The present experiments demonstrate that both SB-269970 and SB-656104 given centrally can attenuate distension-evoked bladder contraction. Although these ligands are from the same chemical series, they are sufficiently structurally distinct to minimize the possibility that the effects observed are com-

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**Figure 2** Urethane anaesthetized female rats: traces comparing the effect of i.c.v. administration of (a) saline, (b) 30 µg kg$^{-1}$ SB-269970, (c) 300 µg kg$^{-1}$ SB-269970 and (d) 30 µg kg$^{-1}$ SB-656104 on the time taken to evoke a distension-induced contraction of the bladder by infusing saline (0.1 ml min$^{-1}$) into the bladder. The saline infusion began at the beginning of the traces. It should be noted that a different vehicle control was used for SB-656104. The time base in (b, c) is the same as (a).

**Figure 3** Urethane anaesthetized female rats: histograms comparing (A) changes caused by i.c.v. injections of saline with SB-269970 (3, 10, 30 µg kg$^{-1}$) and i.c.v. injections of PEG: DMSO with SB-656104 (30 µg kg$^{-1}$) on (a) volume threshold, (b) evoked bladder contraction, (c) residual volume, and (d) pressure threshold. Each bar represents the mean value and the error bars show s.e.m. Changes caused by drugs were compared with appropriate vehicle using Student’s unpaired t-test. *$P<0.05$; **$P<0.001$. 

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variants (see Vanhoenacker et al., 2000). The reason why this increase in bladder residual volume occurs with SB-269970 remains to be determined, but it is probably not related to 5-HT1A receptor blockade.

Central areas in involved in micturition are the hypothalamus, dorsal inferior colliculus, paraquaductal grey, pontine nucleus and nucleus subcoeruleus (see De Groat et al., 1993) and 5-HT2 receptors have been identified in these regions (To et al., 1995; Gustafson et al., 1996; see Vanhoenacker et al., 2000). These regions also contain 5-HT1A receptors; in fact, 5-HT1A receptors are nearly universally throughout the brain (Verge et al., 1986; see Barnes & Sharp, 1999). Thus, precisely, how and where these two 5-HT receptor systems interact and control micturition remains to be determined. Also, whether 5-HT1 receptors are involved in micturition in a similar way in other species remains to be determined. For instance, in the cat it has been reported that blockade of 5-HT1A receptors with WAY-100635 or LY206130 has no effect on bladder or sphincter activity (Thor et al., 2002), although the other

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combined data indicate that central 5-HT\textsubscript{1A} receptors do play a similar role in the control of parasympathetic outflow to the heart in this species as well as in the rabbit, the rat and man (see Ramage, 2001). Further investigations of this discrepancy as well as the role of 5-HT\textsubscript{1A} receptors are therefore required in the cat.

In conclusion, the present results have identified a major physiological function for central 5-HT\textsubscript{1A} receptors, that of the control of reflex-induced bladder contraction, at least in the rat. In addition, this study adds to the growing evidence of the importance of central 5-HT containing neurones in the control of parasympathetic outflow.

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Effects of SB-269970, the 5-HT\textsubscript{7} receptor antagonist, on micturition.

Kate E Read\textsuperscript{1}, Gareth J Sanger\textsuperscript{2}, Andrew G Ramage\textsuperscript{1}

\textsuperscript{1}Dept Pharmacology, University College London, Royal Free Campus, Rowland Hill Street, Hampstead, London NW3 2PF United Kingdom, \textsuperscript{2}GlaxoSmithKline, Harlow, Essex United Kingdom

5-HT\textsubscript{1A} receptors play a role in the control of micturition (Conley et al., 2001, Br. J. Pharmacol., 133, 61). Experiments were performed to determine whether 5-HT\textsubscript{7} receptors might also be involved. Urethane anaesthetised (1.2 g kg\textsuperscript{-1}, i.v.) spontaneously breathing female Sprague-Dawley rats were used. Micturition was evoked by infusion of saline (0.1 ml min\textsuperscript{-1}) through a cannula inserted into the bladder dome. Drug-evoked changes were compared with saline controls by unpaired Student’s t-test. All values are means ± s.e. mean. SB-269970 (5 μl i.c.v. n = 6) at 30μg kg\textsuperscript{-1} significantly (P < 0.05) increased bladder pressure threshold (7.7 ± 1 to 17.3 ± 2.3 mmHg), volume threshold (0.51 ± 0.04 to 1.0 ± 0.1 ml) and residual bladder fluid (0.27 ± 0.03 to 0.76 ± 0.16 ml). At 100μg kg\textsuperscript{-1} ( n = 4) there was a significant reduction in peak bladder pressure during micturition (23.1 ± 1 to 15.8 ± 1.6 mmHg). Volume threshold and residual bladder fluid significantly increased (0.38 ± 0.04 to 0.78 ± 0.1 ml and 0.08 ± 0.02 to 0.70 ± 0.08 ml, respectively). Thus a role for 5-HT\textsubscript{7} receptors is indicated. KER is in receipt of CASE BBSRC studentship with GSK.