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The role of central 5-hydroxytryptamine (5-HT, serotonin) receptors in the control of micturition

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At present the most investigated 5-HT receptor that has been shown to play a role in the control of micturition is the 5-HT_{1A} receptor followed by 5-HT_7, 5-HT_2 and 5-HT_3 receptors. Most experiments focus on the control these receptors have on the parasympathetic outflow to the bladder and the somatic outflow to the external urethral sphincter (EUS) in the rat. Furthermore, 5-HT_{1A} and 5-HT_7 receptors have been identified as having an excitatory physiological role in the control of bladder function. 5-HT_{1A} receptors act, at least in the rat, at both a spinal (probably a heteroreceptor) and supraspinal (probably an autoreceptor) level, while 5-HT_7 receptors only act at a supraspinal level. Additionally, in the rat, 5-HT administered at a spinal or supraspinal site has an excitatory action, although earlier experiments have shown that activating 5-HT-containing brain areas causes inhibition of the bladder. Recent experiments have also indicated that blockade of the 5-HT_{1A} receptor pathway shows rapid tolerance. However, no data exist for the development of tolerance for the 5-HT_7 receptor pathway. Neither receptor seems to play a role in the control of the urethra. Regarding 5-HT_2 receptors, activation of this receptor subtype inhibits micturition, and this inhibitory action may occur at a spinal, supraspinal or both levels. Although no physiological role for 5-HT_{2C} receptors can yet be identified, 5-HT_{2C} receptors have been implicated in the proposed supraspinal tonically active 5-HT_{1A} autoreceptor (negative feedback) pathway. This proposition reconciles the data that central 5-HT-containing pathways are inhibitory to micturition, while 5-HT_{1A} receptors, although inhibitory to adenylyl cyclase, have an excitatory function. This is because activation of 5-HT_{1A} autoreceptors reduces the release of 5-HT thus reducing the activation of the 5-HT_{2C} receptors, which are inhibitory in the control of micturition (disinhibition). Furthermore, 5-HT_{2A} receptors in the rat and 5-HT_{2C} receptors in the guinea pig cause activation of the EUS. In this respect, 5-ht_{5A} receptors have also been identified in Onuf's nucleus, the site of somatic motoneurones controlling this sphincter. In the cat there is very little evidence to indicate that 5-HT receptors are involved in micturition except under pathological conditions in which activation of 5-HT_{1A} receptors causes inhibition of micturition. Interestingly, under such conditions 5-HT_{1A} receptors cause excitation of the EUS. Nevertheless, spinal $5HT_3$ receptors have been implicated in the physiological control of micturition in the cat, but not yet in the rat. Overall, the data support the view that 5-HT receptors are important in the control of micturition. However, many more studies are required to fully understand these roles and why there are such species differences.
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Abbreviations: 5,6-DHT, 5,6-dihydroxytryptamine; 5,7-DHT, 5,7-dihydroxtryptamine; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; EUS, external urethral sphincter; 5-HT, 5-hydroxytryptamine; i.c.v., intracerebroventricular; i.t., intrathecal; i.v., intravenous; LSD, lysergic acid diethylamide; mCPP, meta-chlorophenylpiperazine; 5-MeODMT, 5-methoxydimethyl tryptamine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; SSRI, selective serotonin reuptake inhibitor

Introduction

Along with other parts of the autonomic nervous system, for example heart, vasculature and airways (see Ramage, 2001), central 5-HT receptors have been implicated in the control of micturition (see De Groat *et al*., 1993). There are 14 different, structurally distinct 5-HT receptors, which are divided up into seven families (5-HT_{1-7}), and those receptors that have already been implicated in the control of micturition are the 5-HT_1, 5-HT_2 and 5-HT_3 receptors. These receptors seem to modulate all the pathways involved in the control of micturition, the parasympathetic, the sympathetic and the somatic (see De Groat *et al*., 1993), although there is little experimental data on the sympathetic pathway. These conclusions are based on the use of such 5-HT receptor ligands as 5-methoxydimethyl tryptamine, lysergic acid diethylamide (LSD), methysergide and 8-OH-DPAT, the archetypical 5-HT_{1A} receptor agonist. Although 8-OH-DPAT can be considered much more selective than the other compounds for a particular 5-HT receptor subtype, it is still not specific (see Ramage, 2004). More recently, the 5-HT_7 receptor (Read *et al*., 2003) and, interestingly, the least well-understood 5-HT receptor, the 5-ht_{5A} receptor, (Doly *et al*., 2004), have been added to this

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list. As with all areas of pharmacology, our understanding is always dependent on the known selectivity of the particular ligands used plus the development of novel ligands for these 5-HT receptors. In addition, it is becoming clear that different species may give a different interpretation to the function of these receptor subtypes. In this respect, a great deal of our original understanding of the mechanisms involved in the control of micturition comes from the cat. However, the rat has now taken over as the main species for the investigation of urine storage and micturition reflexes. Importantly, the guinea pig may begin to displace the rat as species of choice for micturition studies, since this species has a guarding reflex, something that is not observed in the rat. That is, in the guinea pig, before voiding, the external urethral sphincter (EUS) has an increase in activity, which shuts off during expulsion of urine. Once expulsion has finished, activity increases again before finally switching off. On the other hand, in the rat, the EUS is initially activated in bursts during voiding, and this probably aids in micturition.

In addition to the recognition that certain 5-HT receptor subtypes are involved in the control of micturition, central 5-HT-containing areas, for example, the brainstem raphé, when activated in both cat and rat, have been shown to cause inhibition of micturition (McMahon & Spillane, 1982; Chen *et al*., 1993; Sugaya *et al*., 1998). These areas also inhibit the firing of spinal dorsal horn neurones, which are activated by afferents in the pelvic nerve (Lumb, 1986a). Further, neurones in the raphé are activated by bladder afferents (McMahon & Spillane, 1982; Sugaya *et al*., 1998) as well as distension of the bladder (Oh *et al*., 1986; Lumb, 1986b). Interestingly, in the cat this inhibition of bladder reflexes can be blocked by i.v. or topical application of LSD to the sacral spinal cord (Morrison & Spillane, 1986). The problem here is knowing whether this is due to an agonist or antagonist action of LSD. Thus, from these stimulation experiments the view has evolved that 5-HT-containing pathways play an inhibitory role in the control of micturition. Nevertheless, more recent data using new pharmacological tools suggest, at least in the rat, that 5-HT pathways may be excitatory as well (see below). Thus, it is the purpose of this review to reconsider some of the older concepts and integrate the newer experimental evidence that implicates the involvement of different 5-HT receptor subtypes in micturition. This will also be carried out in the light of the newer developments in our understanding of the pharmacology of these various 5-HT receptor subtypes.

5-HT_{1A} receptors

Rats – agonist studies

Giving either 8-OH-DPAT or buspirone i.v., Lecci *et al*. (1992) showed that, in the rat, these drugs could induce rhythmic bladder contractions in bladders 8% filled (approx. 100 μl saline) but not in empty bladders. For 8-OH-DPAT this effect was not dose related and for buspirone it only occurred at the lowest dose (0.1 mg kg^{-1}). This action also occurred in spinalized rats and was inhibited by i.v. spiroxatrine. These responses did not show desensitization. Similar data were obtained with i.t. (intrathecally – lumbar level) and i.c.v. administration of 8-OH-DPAT (see Figure 1a). Depletion of 5-HT by 5,7-dihydroxtryptamine (5,7-DHT) increased the effective dose of i.c.v. 8-OH-DPAT but did not affect i.t. 8-OH-DPAT. This indicates that 5-HT_{1A} receptors in the brainstem are probably autoreceptors located on 5-HT terminals and/or on 5-HT-containing neurones (somatodendetric autoreceptors), while those at the level of the sacral spinal cord are heteroreceptors, controlling the release of another transmitter. Furthermore, distension-induced bladder contraction (isovolumetric contractions) could be blocked by i.v. buspirone (high doses), spiroxatrine and NAN-190 and a very high dose of methysergide. Further, evidence that 5-HT_{1A} receptor activation was excitatory came from anaesthetized rats (Conley *et al*., 2001) showing that 8-OH-DPAT and BMY-7378 (a partial 5-HT_{1A} receptor agonist and α_{1D}-adrenoceptor antagonist) decreased the pressure and volume threshold to evoke a micturition reflex (these values would be expected change in parallel but as volume threshold also gives an indication of any changes in compliance of the bladder i.e. if the sympathetic has been switched in to aid storage, discrepancy between these two values would indicate a change in bladder compliance); again these effects were not dose related. However, activation of 5-HT_{1A} receptors did not affect the associated urethral reflexes. Using cystometry in conscious rats (Testa *et al*., 1999; Pehrson *et al*., 2002) showed that 8-OH-DPAT i.v. decreased bladder capacity, micturition volume and pressure and this was also observed (Pehrson *et al*., 2002) for i.c.v. and i.t. administration (bladder capacity is the sum of the residual volume plus the micturition volume). Interestingly, for i.t. administration there was an associated increase in bladder pressure (this measurement indicates the level of parasympathetic drive to the bladder). Similar effects were observed for 5-HT given i.t. (Pehrson *et al*., 2002) and i.c.v. (Ishizuka *et al*., 2002; see Figure 1b). The effect of 5-HT i.c.v. lasted for 60 min and was repeatable and for higher doses 50% of the rats had urinary incontinence, presumably due to this hyperactivity of the bladder. However, BMY-7378 was with little effect when given i.v. (Testa *et al*., 1999). Nevertheless, BMY-7378 although reported not to affect cystometric variables in conscious, bladder obstructed rats, inhibited nonvoiding contractions (Velasco *et al*., 2003). These variable results with BMY-7378 may just simply reflect its lack of pharmacological selectivity. Thus, data indicate that 5-HT_{1A} receptors along with 5-HT at supraspinal and spinal sites have an excitatory effect on micturition in the rat.

Antagonist studies

In support of the view above that 5-HT_{1A} receptors are part of the mechanism by which the rat controls micturition, Lecci *et al*. (1992) showed that i.v. high doses of buspirone (partial agonist), spiroxatrine and NAN-190 could inhibit isovolumetric contractions in a dose-dependent manner. Further, the selective and archetypical 5-HT_{1A} receptor antagonist, WAY-100635, given i.v. (Testa *et al*., 1999) in conscious rats undergoing continuous cystometry, increased bladder capacity with a very steep dose–response curve and decreased micturition pressure. The latter effect was not dose related and minor. Further, in anaesthetized rats, WAY-100635 i.v. (Testa *et al*., 1999) caused a transient disappearance of regular isovolumetric bladder contractions (Figure 1c) and this action could be potentiated by pretreatment with the SSRI citalopram, which had no effect on its own. Interestingly and surprisingly this action of WAY-100635 could be blocked by pretreatment

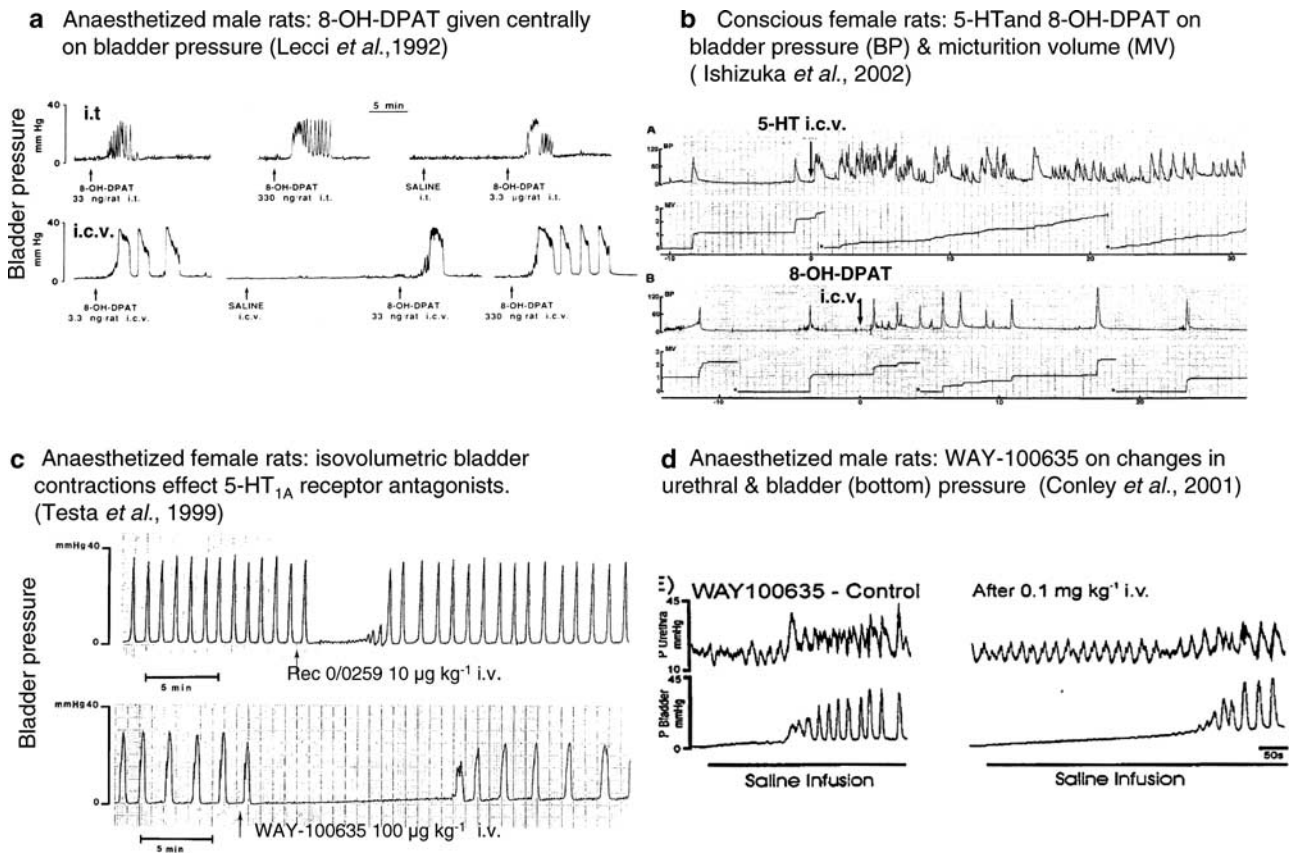

Figure 1 Rats: experimental traces showing the effects of: – (a) the 5-HT_{1A} receptor agonist 8-OH-DPAT on baseline bladder pressure given intrathecally (i.t.) and intracerebroventricular (i.c.v.), (b) 5-HT (A) and 8-OH-DPAT (B) given i.c.v. in conscious rats on bladder pressure and volume changes evoked by bladder distension induced by infusing saline into the bladder, (c) the 5-HT_{1A} receptor antagonists Rec 0/0259 and WAY-100635 given i.v. on rhythmic isovolumetric bladder contractions and (d) effect of WAY-100635 i.v. on changes evoked in bladder and urethral pressure caused by dissention induced by infusion of saline into the bladder.

i.v. with the nonselective 5-HT_{2C} receptor antagonist mesulergine (100 $\mu g\,kg^{-1}$), which also had no effect on its own (Testa *et al*., 1999; see perspectives for more details). Neither of these pretreatments affected the 'partial agonist' NAN-190. Similar results for WAY-100635 were observed in anaesthetized rats (Conley *et al*., 2001) in which it caused a dose-related increase in pressure threshold for initiation of the micturition reflex (Figure 1d) and only at high doses did it affect reflex changes evoked in the urethra and this was attributed to α_1-adrenoceptor blockade. Using a structurally similar antagonist in anaesthetized and conscious rats and guinea pigs (Leonardi *et al*., 2001) undergoing cystometry, Rec 15/3079 i.v. also caused an increase in bladder volume capacity without affecting residual volume (changes in residual volume would indicate interference in the urethra and/or reduction in the force of bladder contraction). Again, in the anaesthetized rats, citalopram potentiated the ability of neutral 5-HT_{1A} receptor antagonists to inhibit these regular isovolumetric bladder contractions, supporting the view, that 5-HT release is important in the action of these 5-HT_{1A} receptor antagonists. Further, both WAY-100635 and Rec 15/3079 could reverse the decrease in bladder volume capacity caused by irritating the bladder with acetic acid in conscious rats. Testa *et al*. (2001) also reported that another structurally related antagonist to WAY-100635, p-MPPI given i.v. had the same effect, inhibiting the regular isovolumetric bladder contractions.

Administration of WAY-100635 i.t. (Kakizaki *et al*., 2001) and i.c.v. (Yoshiyama *et al*., 2003) in anaesthetized rats also inhibited regular isovolumetric bladder contractions, and the effect was dose related (Figure 2a and b). Thus, the regulation of the bladder in micturition involves 5-HT_{1A} receptors at spinal and supraspinal sites, which overall have an excitatory action on parasympathetic supply to the bladder. In this respect, 5-HT_{1A} receptors also play an excitatory role in the reflex control of parasympathetic outflow to the heart and airways (see Ramage, 2001). Further it was suggested that supraspinal 5-HT_{1A} receptors are involved in modulating afferent input involved in the timing of the reflex while the spinal (sacral) 5-HT_{1A} receptors are involved in the activation of parasympathetic preganglionic neurones. In this respect, bladder contractions evoked by stimulation of the pontine micturition centre (PMC) were attenuated by i.t. WAY-100635 (Kakizaki *et al*., 2001). Nevertheless, the ability of WAY-100635 (Figure 2a and b) given at both central sites to inhibit the appearance of regular isovolumetric bladder contractions has been considered to intuitively favour the interruption of afferent input. However, field potentials evoked in the rostral pons (micturition centre) by stimulation of afferents running in the pelvic nerve were not affected by i.v. or i.t. WAY-100635 (Kakizaki *et al*., 2001). Interestingly, the return of these regular isovolumetric bladder contractions was reported to be only of a similar height for i.t. but not for i.c.v. WAY-100635.

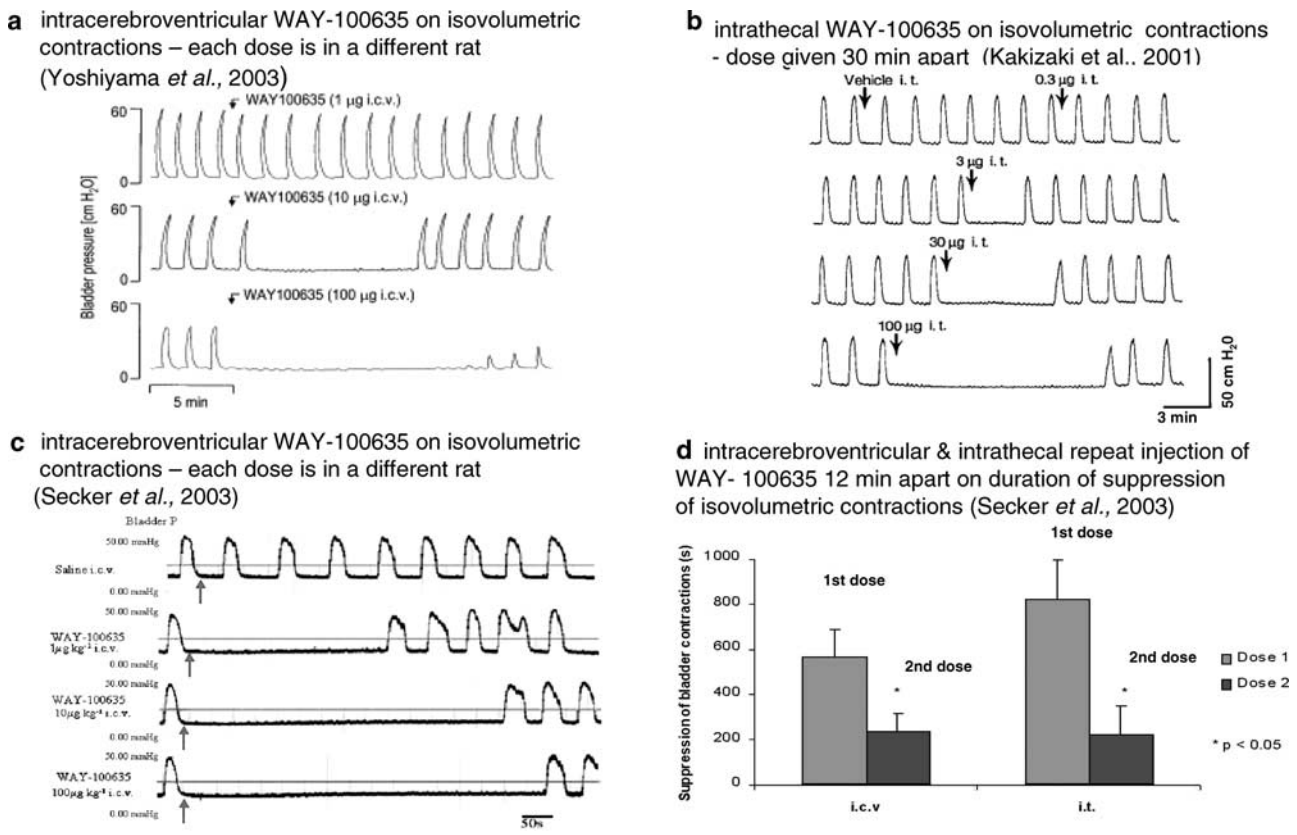

Figure 2 Anaesthetized female rats: experimental traces comparing the effects on rhythmic isovolumetric bladder of WAY-100635 given in (a) i.c.v., (b) .i.t and (c) i.c.v. and (d) histograms comparing the effects of sequential doses of WAY-100635 given i.c.v. or i.t.12 min apart on the duration (s) of suppression of rhythmic isovolumetric bladder.

This may favour the reverse interpretation, that is, the sacral spinal 5-HT_{1A} receptor interferes with afferent input while the supraspinal 5-HT_{1A} receptor interferes with efferent outflow to the bladder. However, such a phenomenon with this technique, regular isovolumetric bladder contractions, was not observed (compare Figure 2a and c, also see Sercker, 2004), and thus probably represents experimental variation with this particular technique. However, the ability of WAY-100635 to increase the pressure threshold for the micturition reflex (Conley *et al*., 2001) still intuitively favours an effect on afferent input, although this could be due to inhibition of the central 'switching' mechanism from storage to expulsion involving the raphé (dorsal?), the locus coeruleus and the PMC. Interestingly, there is evidence to suggest that there is dorsal raphé feedback on locus coeruleus to control neuronal activity within this nucleus (Kaehler *et al*., 1999). Additionally, an effect on the switching mechanism may explain the steep dose–response curve for the effects of WAY-100635. Thus, at present the precise function of these two different populations of 5-HT_{1A} receptors involved in the control of the micturition reflex remains to be determined.

More support for the central excitatory physiological role of 5-HT_{1A} receptors comes from the study of the structurally distinct 5-HT_{1A} receptor antagonist robalzotan (NAD-299) in the control of micturition in conscious rats (Pehrson *et al*., 2002). This antagonist has very little affinity for α_1 adrenoceptors compared with WAY-100635 (approx pA_2 7.5 on all subtypes). Both robalzotan and WAY-100635 i.v. increased bladder capacity and volume. Interestingly, at higher doses robalzotan was reported to decrease micturition pressure. Further, repeated doses of robalzotan at high concentrations also showed some desensitization. Both drugs given i.c.v. also increased bladder capacity while robalzotan increased micturition pressure and WAY-100635 decreased micturition pressure. Robalzotan and WAY-100635 i.t. at the doses chosen were found not have any effect although 8-OH-DPAT had the expected action. 5-HT i.t. increased micturition pressure and decreased bladder capacity and micturition volume. However, in anaesthetized rats WAY-100635 given i.c.v. or i.t (Secker *et al*., 2003) has been shown to inhibit micturition, but for both routes of administration, in this study, this action showed the development of rapid tolerance (Figure 2b). This was further extended in conscious rats to demonstrate that this effect was not compound specific, as it also occurred with robalzotan (Glew *et al*., 2004a) and was due to an upregulation of 5-HT_{1A} receptors (Glew *et al*., 2004b). More recently, another 5-HT_{1A} receptor antagonist has been developed to improve cognitive ability in Alzheimer's disease and has been reported not to cause tolerance (Schechter *et al*., 2005). It would be interesting to test this compound on micturition.

Perspective – rat 5-HT_{1A} receptors

The overall data indicate that 5-HT_{1A} receptors, although inhibitory to adenylyl cyclase, have an excitatory physiological role in the control of micturition in the rat and, it would seem, in the guinea pig, at both a supraspinal and sacral spinal level. The supraspinal receptor is believed to have an autoreceptor

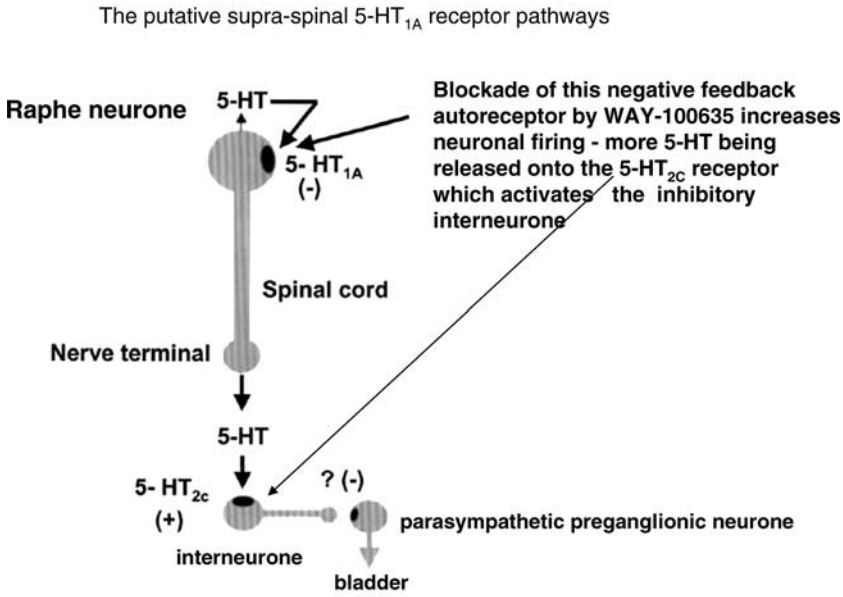

Figure 3 Diagrammatic representation of the putative supraspinal 5-HT_{1A} autoreceptor pathway involving a 5-HT_{2C} receptor probably located in the sacral region of the spinal cord which activates an inhibitory interneurone to cause inhibition of bladder contractions, adapted from De Groat (2002). 5-HT_{1A} autoreceptors are involved in negative feedback control of 5-HT release from raphé neurones. Blockade of these autoreceptors will cause an increase in firing of the raphé neurones and thus greater release of 5-HT onto the 5-HT_{2C} receptors which activate an inhibitory interneurone to 'switch off' the parasympathetic drive to the bladder. This pathway is postulated to be tonically activated. However, how the spinal 5-HT_{1A} receptor pathway integrates into this postulated pathway remains unknown.

function and thus blockade of this 5-HT_{1A} autoreceptor would cause potentiation of the supraspinal 5-HT inhibitory pathways by increasing 5-HT release, which is consistent with the citalopram data. This putative pathway would also be expected to be tonically active at rest. (It should be noted that Lecci *et al*. (1992) also favoured the presence of supraspinal 5-HT_{1A} heteroreceptor as well.) Furthermore this putative supraspinal 5-HT_{1A} pathway would have to activate another 5-HT receptor, possibly 5-HT_{2C}, to mediate its overall inhibitory action (see De Groat, 2002, Figure 3). This could explain the reported ability of mesulergine (Testa *et al*., 1999) to block the effects of WAY-100635. As this pathway is hypothesized to be a tonic inhibitory pathway mesulergine would also be expected to cause spontaneous activity in the bladder. This has not been observed. In addition, this proposed effect of mesulergine should be more overt against i.c.v. than i.v. WAY-100635. However, Yoshiyama *et al*. (2001) obtained very variable results with mesulergine i.v. against i.c.v. WAY-100635 and thus no conclusion can be drawn at present. A probable reason for the large variability may be due to the lack of selectivity of mesulergine for 5-HT_{2C} receptors. For instance mesulergine is a potent 5-HT_7 receptor antagonist (Wood *et al*., 2000) and this receptor also plays an excitatory role in the control of micturition (see later). Interestingly, in this context Testa *et al*. (2001) reported that a low dose (10 $\mu g\,kg^{-1}$, i.v.) of mesulergine did inhibit regular isovolumetric contractions, although increasing doses were ineffective. Thus, further studies are still required to identify the putative 5-HT receptor mediating this tonic inhibitory pathway and which raphé nucleus/nuclei are involved. However, the above data indicate the importance of 5-HT, *via* activation of 5-HT_{1A} receptors, in micturition control. In this respect, it would be expected that depletion of 5-HT with either *p*-chlorophenyalanine (Yoshiyama *et al*., 1994) or destruction with 5,7-DHT (Lecci *et al*., 1992) would interfere with micturition. This was not observed in these studies, although 5,6-DHT i.t., reducing spinal content by around 75%, was reported to increase micturition volume (Durant & Yaksh, 1988) favouring an excitatory action of 5-HT at least at the level of sacral spinal cord. These discrepancies may mean that the pretreatments in the first two studies may not have reduced the level of 5-HT to below that which is critically required for operation of the reflex.

Cats-5-HT_{1A} receptor agonists/antagonists and uptake inhibition

Although 5-HT_{1A} receptors play an excitatory role in the reflex control of parasympathetic outflow to heart and airways in cats (see Ramage, 2001) the limited data in cats using 8-OH-DPAT and WAY-100635 suggest the opposite, in that activation of these receptors is inhibitory to the bladder, although excitatory to the EUS. Further, these effects (Figure 4a) are only observed under conditions in which the bladder had been irritated by intravesical acetic acid (Thor *et al*., 2002). In addition, the absence of effects of WAY-100635 and LY206130 indicates that 5-HT_{1A} receptors are not physiologically activated in the cat during micturition. More recently, it was reported (Gu *et al*., 2004) that 8-OH-DPAT decreased volume threshold in cats with chronic spinal injury and this could be reversed by WAY-100635, again the effects of these ligands in intact cats were very minor. These authors concluded that 5-HT_{1A} receptors facilitate the nociceptive bladder afferent reflex pathway to the sphincter muscle and inhibit nociceptive-driven reflex micturition. Interestingly some of this group had previously shown that the 5-HT and

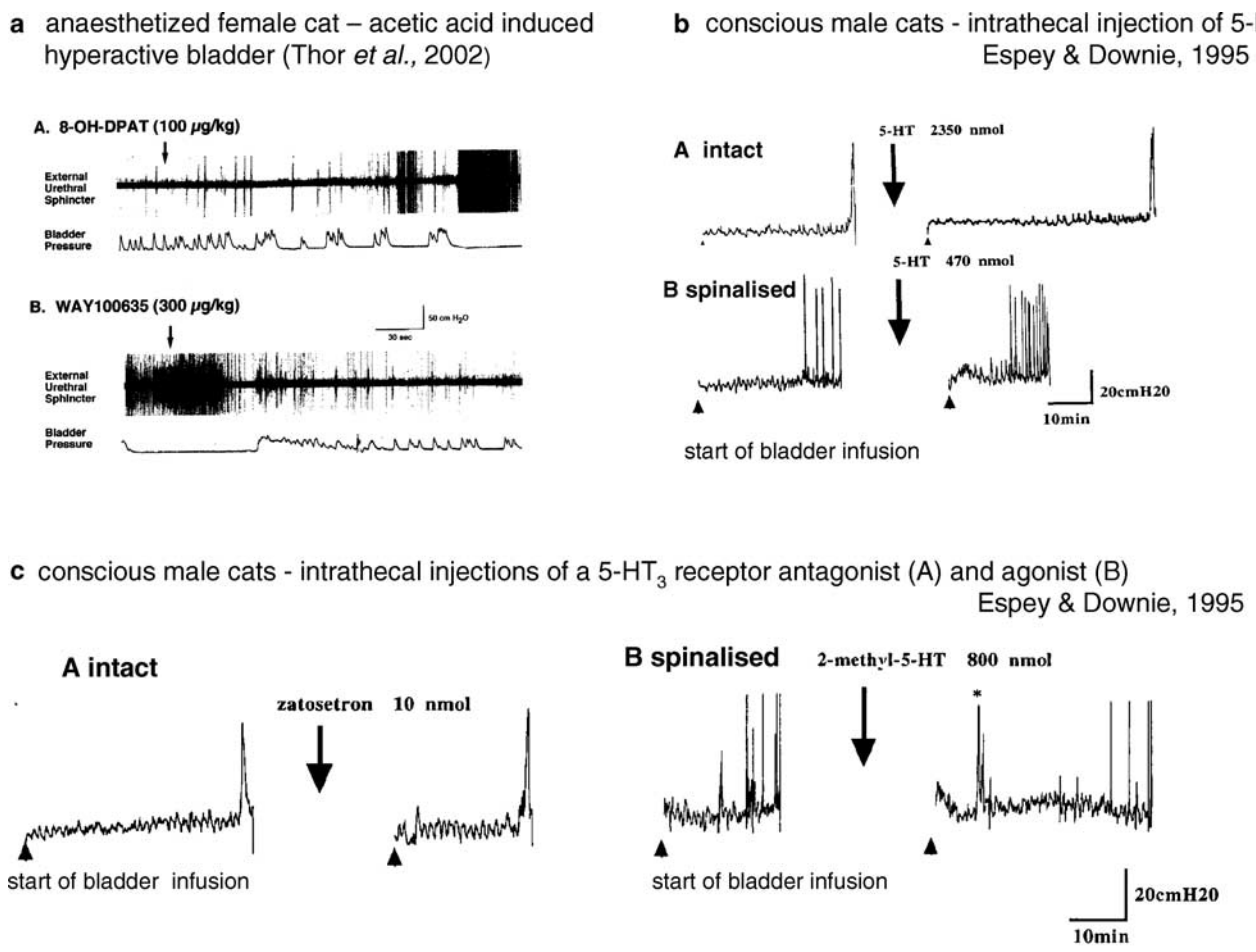

Figure 4 Cats: experimental traces showing the effects of: – (a) 8-OH-DPAT i.v. on baseline external urethral sphincter activity and bladder pressure in hyperactive bladder induced by acetic acid and the effect of WAY-1006235 i.v. on these changes, (b) 5-HT given intrathecally (i.t.) in conscious intact (A) and spinalized (B) cats and c) the effect of the 5-HT_3 receptor antagonist, zatosetron given i.t. in an intact cat and the 5-HT_3 receptor agonist 2-methyl-5-HT given i.t. in a spinalized cat.

noradrenaline uptake inhibitors duloxetine (Thor & Katofiasc, 1995) and venlafaxine (Katofiasc *et al*., 2002) had very similar effects to 8-OH-DPAT, again only in cats whose bladder had been irritated by intravesical acetic acid. These effects could be blocked by the nonselective 5-HT receptor antagonist methiothepin. Further, the selective SSRI *s*-norfluoxetine had a similar effect to duloxetine, but the selective noradrenaline uptake inhibitor thionisoxetine was ineffective (Katofiasc *et al*., 2002). These results suggest that central 5-HT may be involved in nociceptive effects on the bladder but at present there is no indication that, in the cat, the micturition reflex involves the activation of 5-HT_{1A} receptors. Interestingly, when 5-HT was given i.t. to conscious cats it caused a 200% increase in volume threshold for micturition, while in spinal cats 5-HT, as 8-OH-DPAT, reduced the volume threshold (Espey & Downie, 1995; Figure 4b). The spinal receptor/s at which 5-HT in intact cats causes inhibition of micturition remains to be determined.

5-HT_7 receptors

Another receptor subtype that needs to be considered when investigating 5-HT_{1A} receptors is 5-HT_7 receptors as the ligands used to study 5-HT_{1A} receptors also have affinity for 5-HT_7 receptors; 8-OH-DPAT has pK_i of 6.9 and WAY-100635 has a pK_i of 7 (100 × lower than at 5-HT_{1A}). Other 5-HT receptor ligands that have been used to study micturition which also have significant affinity for 5-HT_7 receptors are methysergide (Krobert & Levy, 2002), methiothepin, mesulergine (Hagan *et al*., 2000), LSD (To *et al*., 1995) and meta-chlorophenylpiperazine (mCPP; see Hoyer *et al*., 2002). However, these receptors differ from 5-HT_{1A} in that they activate adenylyl cyclase and thus would be considered excitatory. Further, it is now recognized that 8-OH-DPAT-induced hypothermia in mice, a screen sometimes used to determine if a drug has activity at 5-HT_{1A} autoreceptors (presynaptic) is mediated by 5-HT_7 receptors as for guinea pig and rat (see Hedlund *et al*., 2004).

With the development of the selective 5-HT_7 receptor antagonist SB-266970 (10–30 $\mu g\,kg^{-1}$) the role of these receptors could be investigated. SB-269970 given i.c.v. (Figure 5a) caused an increase in bladder pressure and volume threshold and at higher doses abolished the micturition reflex in anaesthetized rats but not when given i.t. although WAY-100635 was effective (Figure 5b; Read *et al*., 2003). Further, SB-656104 a 5-HT_7 receptor antagonist from the same chemical series but structurally distinct and with a different

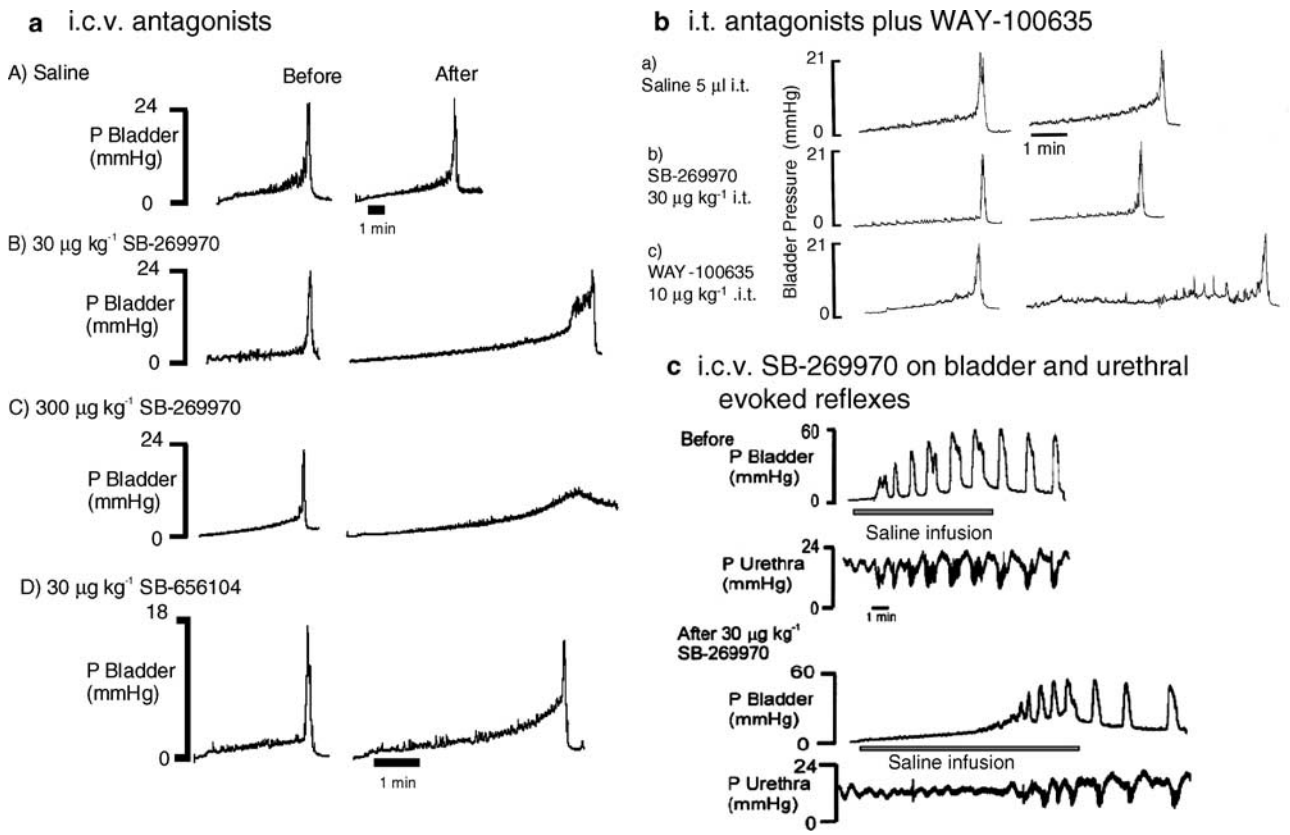

Figure 5 Anaesthetized rats showing the effects of: (a) 5-HT_7 receptor antagonists on bladder contractions evoked by saline infusion into the bladder, (b) a comparison of i.t. SB-269970 with that of WAY-100635 on the same variables and (c) SB-269970 on bladder and urethral-evoked changes caused by infusion of saline into the bladder (Read *et al*., 2003).

selectivity profile to SB-269970 had a similar effect (Figure 5a) with the additional action of causing an increase in residual volume. In this respect, it has recently been reported that 5-HT_7 receptors are located in Onuf's nucleus in the rat (Doly *et al*., 2005) however, why SB-269970 has no effect on the urethra (Figure 5c) remains to be determined but it could be related to its ability to interfere with 5-ht_{5A} receptors also found in this nucleus, see below. Thus, the effects of SB-269970 are receptor specific rather compound specific. Interestingly, central 5-HT_7 receptors also control reflex activation of parasympathetic outflow to the heart (Kellett *et al*., 2005).

5-carboxamidotryptamine (5-CT) – 5-HT_7 receptor agonist plus 5-HT_{1B} receptors

At present there are no selective agonists available to study 5-HT_7 receptors. The agonist that has been used in a large number of *in vitro* studies is 5-CT; however, this compound activates most of the 5-HT_1 receptor subtypes. As may be expected it has variable effects on micturition causing inhibition and excitation (Figure 6a) when given i.c.v. at the same dose (Read *et al*., 2004). In the presence of the 5-$HT_{1B/1D}$ receptor antagonist GR127935, which has a pK_i of 8.7 and 8.3, respectively, at these receptors (Roberts *et al*., 2001), while at rat 5-HT_{1A} it has a pK_i 6.5–6.9 (see Pauwels, 1997), 5-CT i.c.v caused a reduction in the volume threshold and evoked spontaneous bladder contractions (Figure 6b). Interestingly, in preliminary experiments GR127935 i.c.v. alone caused a large increase in the volume threshold by $94 \pm 35\%$. In this respect, the 5-HT_{1B} receptor agonist CP-93,129 (i.c.v.) abolished (Figure 6c) the micturition reflex in five out of six rats while the 5-$HT_{1B/1D}$ receptor agonist sumatriptan caused attenuation of micturition. Thus, these data support the view that 5-HT_7 receptors are excitatory but are complicated by the involvement of 5-HT_{1B} and 5-HT_{1D} receptors. Overall these data suggest that other 5-HT_1 receptor subtypes may be involved in micturition, however, further studies are required.

5-ht_{5A} receptors

These receptors, like 5-HT_{1A} receptors, are coupled negatively to adenylyl cyclase and would seem to have a similar location in the spinal cord and brain. Nevertheless, in respect to micturition it is the high density of these receptors in Onuf's nucleus (Doly *et al*., 2004) that is of interest. At present, there are no available selective ligands for these receptors. Nevertheless, they have a similar pharmacology to 5-HT_7 receptors in that the selective 5-HT_7 receptor antagonist SB-269970 has as pK_i of 7.8 at the rat 5-ht_{5A} receptor ($50 \times$ less potent than on 5-HT_7) and the rank order of agonist potency (5-CT > 5-HT > 8-OH-DPAT) is also similar (see Ramage, 2004).

5-HT_2 receptors

This receptor has three subtypes A, B and C and the subtypes that are of interest in micturition are 5-HT_{2A} and 5-HT_{2C}

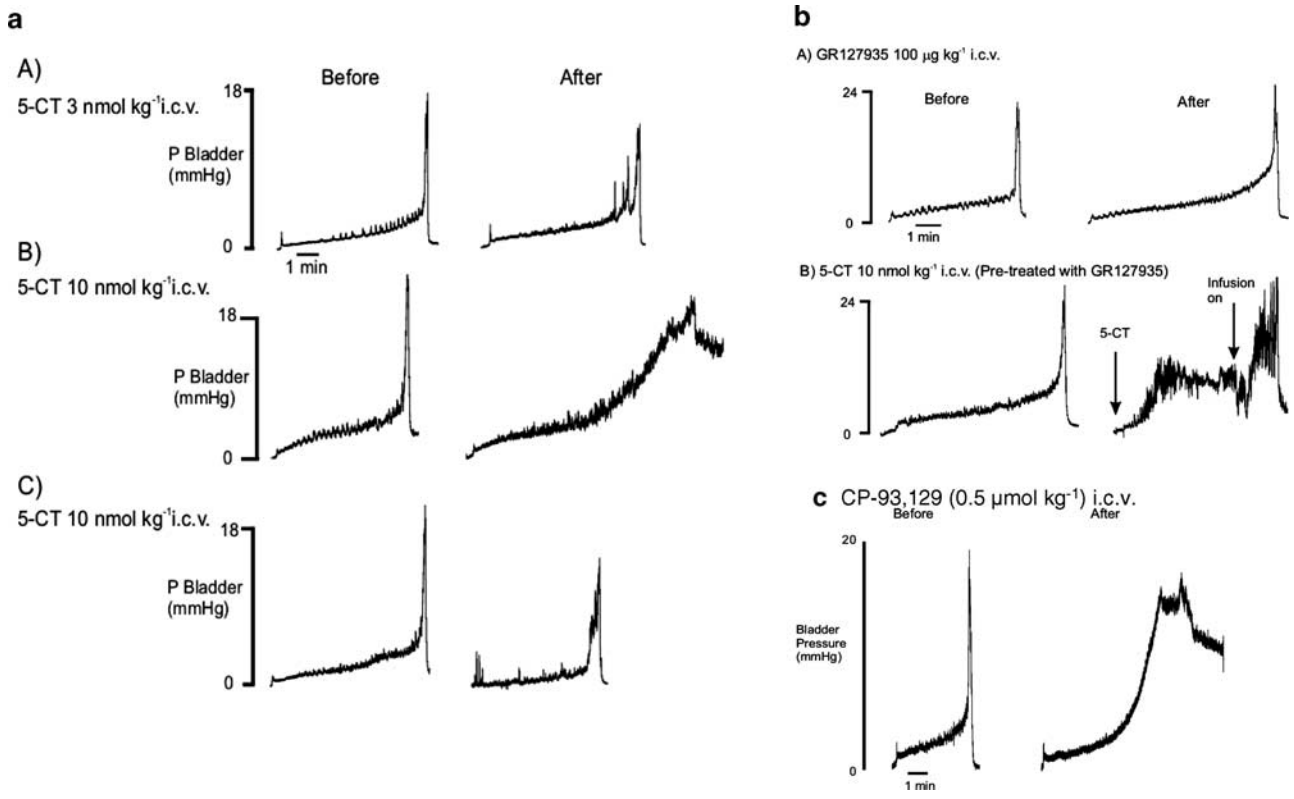

Figure 6 Anaesthetized rats comparisons of the effects of the nonselective 5-HT_7 receptor agonist 5-CT given i.c.v. (a) alone and (b) in the presence of $5HT_{1B/1D}$ receptor antagonist GR127935 on bladder contractions induced by saline infusion into the bladder; (c) shows the effect of the 5-HT_{1B} receptor agonist CP-93,129 given i.c.v. again bladder contractions induced by saline infusion into the bladder (Read *et al*., 2004).

receptors. It should be noted that activation of 5-HT_{2A} receptors causes smooth muscle contraction, thus causing contraction of the bladder, a rise in blood pressure and bronchoconstriction and centrally it is believed to cause hallucinations, while 5-HT_{2C} receptors are involved in the control of appetite as well as other functions. These receptors are considered to have an excitatory action as they couple preferentially to $G_{q/11}$ to increase the hydrolysis of inositol phosphates and elevate cytosolic [Ca^{2+}].

The indication that 5-HT_2 receptors are involved in micturition came from the study of the very unselective 5-HT_{2C} receptor agonist mCPP (Steers & De Groat, 1989). This agonist i.v. (100 $\mu g\,kg^{-1}$) caused inhibition of rhythmic isovolumetric bladder contraction caused 'by infusion 0.6 ml of saline into the bladder after the distal urethra was completely obstructed'. This was surprisingly blocked by the neuromuscular blocker pancuronium implying that this inhibition of rhythmic isovolumetric bladder contractions was related to 'somatic muscle contractions'. The ability of mCPP to block micturition was confirmed by Guarneri *et al*. (1996) and, interestingly, it was reported that these effects could by blocked by mesulergine, supposedly by blocking 5-HT_{2C} receptors, and potentiated by ketanserin a 5-HT_2 receptor antagonist, which is considered to be selective for 5-HT_{2A}. However, ketanserin has been reported to inhibit isovolumetric bladder contractions (Testa *et al*., 2001). Nevertheless, the role of 5-HT_{2C} receptors in the control of the EUS (striated muscle) has been confirmed using the selective agonist Ro 60-60175 in anaesthetized guinea pigs where the agonist activated the EUS at rest and increased bladder capacity and volume threshold although voiding occurred normally (McMurray & Miner, 2005). The selective 5-HT_{2C} receptor antagonist SB-242084 could reverse these effects. Recently, studies (Mbaki *et al*., 2006) in anesthetized rats have also confirmed that mCPP i.v. can inhibit the micturition reflex, activate the EUS and cause contraction of the urethra. However, the ability of the so-called 5-HT_{2C} receptor agonists to activate (see Figure 7a) the EUS in the rat is also mediated by 5-HT_{2A} (Mbaki *et al*., 2006), although inhibition of micturition is mediated by 5-HT_{2C} receptors. Interestingly, ketanserin was found to potentiate this inhibitory effect of 5-HT_{2C} receptor agonists on micturition (Figure 7b). Furthermore, these data are consistent with the view that the supraspinal 5-HT pathways using 5-HT_{1A} receptors mediate their actions *via* 5-HT_{2C} receptors (see above; Figure 3). However, the selective 5-HT_{2C} receptor antagonist SB 242084 alone i.v. failed to reduce bladder capacity and volume threshold. The mechanism by which ketanserin poteniates this inhibitory action of 5-HT_{2C} receptors is difficult to explain but may be due to involvement of excitatory 5-HT_{1D} receptors in micturition as ketanserin also blocks these receptors. Interestingly, a high dose (100 $\mu g\,kg^{-1}$, i.v.) of ketanserin can also inhibit micturition (Testa *et al*., 2001 and Mbaki; personal communication, Figure 7b).

The species differences between which 5-HT_2 receptor is involved in activation of the EUS could be related to a difference in the physiological role of the sphincter in these species. In the rat it is used to expel urine while in the guinea pig

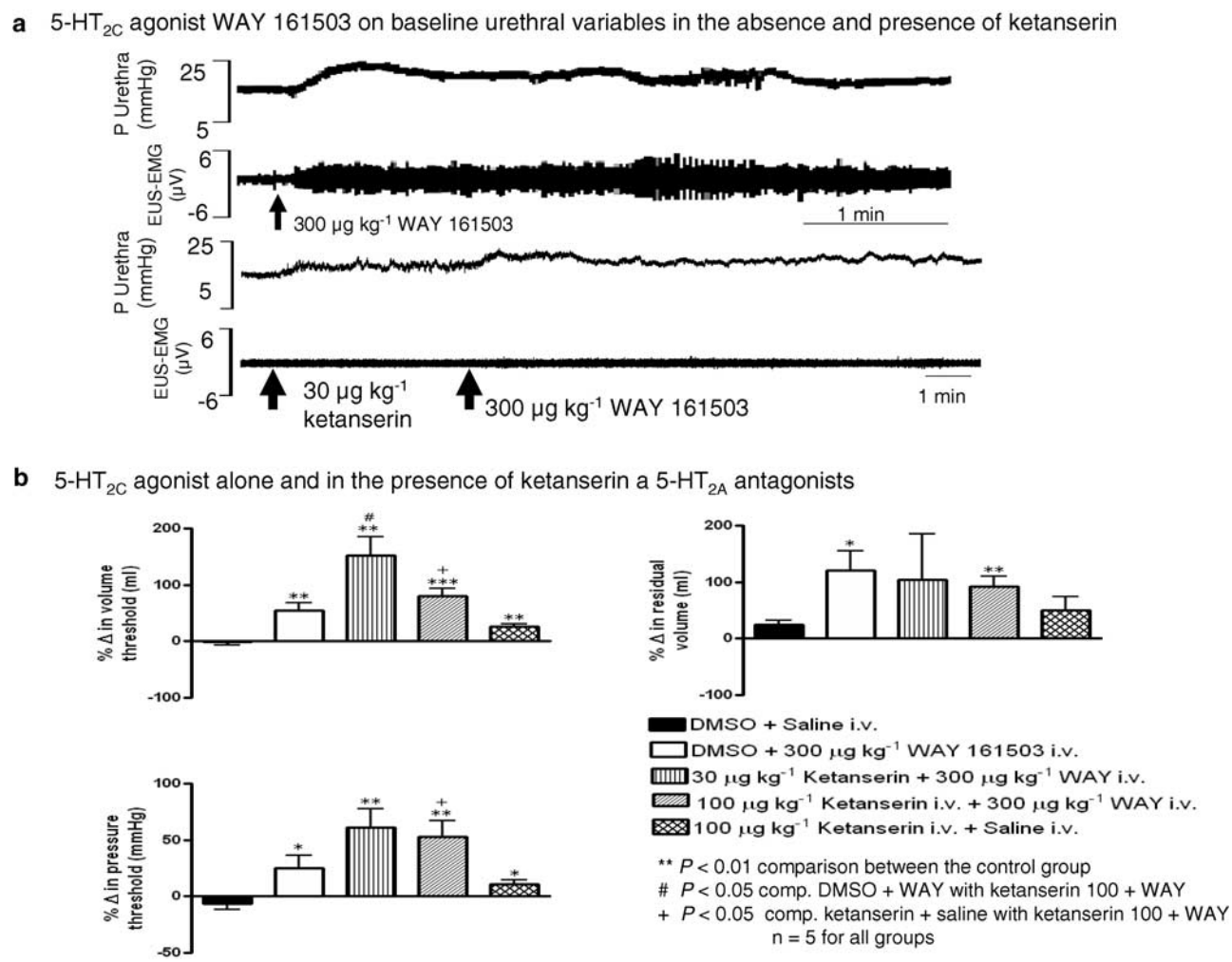

Figure 7 Anaesthetized rats: (a) experimental traces showing the effect of the 5-HT_{2C} receptor agonist WAY 161503 on external urethral sphincter EMG and urethral pressure and (b) histograms of changes caused by WAY 161503 on bladder variables during micturition evoked by a saline-induced distension of the bladder in the absence and presence of the 5-HT_{2A} receptor antagonist ketanserin.

it is used to prevent expulsion as the bladder fills, a guarding function. A possible explanation for this difference is that if 5-HT_{2C} receptors are considered inhibitory to micturition and that activation of the EUS in guinea pig is part of this inhibitory mechanism thus 5-HT_{2C} receptor activation of the EUS would be consistent with this function. However, in the rat, EUS activity is associated with the activation of micturition and thus if 5-HT_{2A} receptors are considered excitatory to micturition this activation of EUS by 5-HT_{2A} would also be consistent with that function. This would suggest that in the guinea pig 5-HT_{2A} receptor agonists might switch off EUS activity. Interestingly, in the cat, which has a guarding reflex, central 5-HT_2 receptors have also been implicated in the excitatory control of the EUS (Thor *et al*., 1990; Danuser & Thor, 1996) but this is only observed in spinalized cats. However, the role of different 5-HT_2 receptor subtypes remains to be determined, although DOI was used (Danuser & Thor, 1996) which is considered to be selective for 5-HT_{2A} over 5-HT_{2C} receptors. Interestingly, Danuser & Thor (1996) suggested that the reason for not observing excitation in the intact cat is that supraspinal 5-HT_2 receptors (5-HT_{2C}?) are inhibitory while spinally such receptors (5-HT_{2A}?) have an excitatory function.

Perspective

These data indicate that 5-HT_2 receptor stimulation activates the pudendal nerve causing an increase in EUS activity as well as causing inhibition of micturition. In the guinea pig, it is the 5-HT_{2C} receptor that has this action on the pudendal nerve while in rat it is the 5-HT_{2A} receptor, however, the involvement of both receptors in both species cannot be ruled out. Nevertheless, in both species 5-HT_{2C} receptors have an inhibitory action on the micturition reflex. However, the physiological role of these receptors remains to be determined.

5-HT_3 receptors

The 5-HT_3 receptor differs from all other 5-HT receptors in that it is a ligand-gated ion channel receptor which has been demonstrated to play an important excitatory role in the processing of cardiovascular and gut afferent input at the level of the NTS (see Jeggo *et al*., 2005). Zatosetron, a 5-HT_3 receptor antagonist given intrathecally to conscious cats decreased the volume threshold for micturition (Figure 4c).

In spinalized conscious cats the 5-HT_3 receptor agonist, 2-methyl-5-HT given intrathecally (Figure 4c) increased the volume threshold for micturition (Espey & Downie, 1995). This implies that spinal 5-HT_3 receptors are inhibitory to the reflex, at least when there is descending drive, and physiologically involved in the micturition reflex. Further along with two other 5-HT_3 receptor antagonists tropisetron (ICS 205-930) and MDL 72222, again given intrathecally, pelvic nerve-evoked neuronal activity in the lower thoracic spinal cord was found to be potentiated (Espey *et al*., 1998). This suggests that during bladder filling, 5-HT is released to activate 5-HT_3 receptors at the level of the spinal cord to inhibit afferent input. Interestingly, 5-HT_3 receptors may also have a role in the control of the EUS as the pelvic – pudendal nerve reflex was attenuated by zatosetron and potentiated by 2-methyl-5-HT also given intrathecally (Espey *et al*., 1998) implying that, at least in the cat, 5-HT_3 receptors are also involved in the control of the EUS.

In anaesthetized rats i.v. zatosetron and Y 25130 showed no overall effect on isovolumetric bladder contractions (Testa *et al*., 2001) while in conscious rats, 2-methyl-5-HT given i.c.v. also failed to have any effect on cystometric parameters (Ishizuka *et al*., 2002).

Overall, further studies are required to determine the importance of central 5-HT_3 receptors in micturition.

5-HT_4 and 5-HT_6 receptors

Drugs selective for these receptors have been tested in only two studies in rats. The 5-HT_4 receptor antagonist RS 39604 given i.v. to anaesthetized rats did not affect regular isovolumetric bladder contraction as did the 5-HT_6 receptor antagonist Ro 04-6790 (Testa *et al*., 2001). Nevertheless the 5-HT_4 receptor agonist RS 67506 given i.c.v. to conscious rats decreased bladder capacity and micturition volume (Ishizuka *et al*., 2002).

Conclusion

Traditionally, central 5-HT-pathways are considered to be inhibitory in the control of micturition. However, at least in the rat, 5-HT_{1A} and 5-HT_7 receptors have excitatory actions. Further, the use of antagonists for these two receptors indicates that both play an essential role in micturition in the rat and probably in the guinea pig. Interestingly, both receptors seem to have a similar role supraspinally, although they have opposing effects on adenylyl cyclase. At a spinal level, however, only 5-HT_{1A} receptors have a role in micturition. Whether these receptors are acting as autoreceptors, heteroreceptors or both needs to be determined. In this respect, 5-HT_{1A} receptor data suggest that supraspinal 5-HT_{1A} receptors may be predominantly acting as autoreceptors, while the spinally located receptors may be heteroreceptors. Further, the spinally located receptors seem to modulate efferent activity to the bladder parasympathetic preganglionic neurones. Nevertheless, the precise function of these receptors in the control of micturition remains to be determined. However, surprisingly, in the cat, 5-HT_{1A} receptors do not seem to play a physiological role in micturition, and may only have a pharmacological role in pathological situations. Further, in the cat, 5-HT_{1A} receptors have the opposite effect to that expected in that they are inhibitory but do cause excitation of the EUS muscle, whereas in rats and guinea pigs this is caused by activation of 5-HT_{2A} and 5-HT_{2C} receptors, respectively. There is, however, no evidence that these receptors are physiologically involved in the control of this sphincter or in bladder function except that activation of 5-HT_{2C} receptors has an inhibitory action on micturition and somewhat surprisingly, antagonists to this receptor are without effect. In this respect, only 5-HT_3 receptors have been suggested to be physiologically involved in the control of micturition in the cat, at least at a spinal level. The paucity of evidence compared with rat indicating that 5-HT plays an important role in the control of micturition may just reflect the lack of experiments carried out in this species. Overall the data indicate that 5-HT is an important transmitter involved in the control of micturition. However, further experiments are required to elucidate its precise role and the seeming difference in importance it has in this function between species.

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