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# Evidence for the presynaptic action of 5-hydroxytryptamine and the involvement of purinergic innervation in the rabbit lower urinary tract

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**1** The effects of 5-hydroxytryptamine (5-HT) were studied *in vitro* on bladder and urethral muscle strips from the rabbit. 5-HT produced dose-dependent contraction in the detrusor and urethra.

**2** The 5-HT-induced contraction could be dose-dependently inhibited by the 5-$HT_3$ antagonists MDL 72222, ICS 205-930 and BRL 43694. No effect of ketanserin, methysergide or metitepine was observed on the contractile response to 5-HT.

**3** Atropine and $\alpha,\beta$-methylene ATP both partially blocked the contractile response to 5-HT. Together they caused more inhibition than either alone.

**4** Atropine and $\alpha,\beta$-methylene ATP also inhibited the contractile response to electrical field stimulation. The 5-$HT_3$ antagonist MDL 72222 had no effect on the contraction to field stimulation.

**5** The atropine- and $\alpha,\beta$-methylene ATP-resistant components of 5-HT-induced contraction were not affected by the 5-$HT_1$ antagonists metitepine, the 5-$HT_2$ antagonists ketanserin and methysergide or the 5-$HT_3$ antagonists MDL 72222, ICS 205-930 and BRL 43694.

**6** Tetrodotoxin, hexamethonium, phentolamine and prazosin had no effect on the contractile response to 5-HT.

**7** These results suggest that in the rabbit lower urinary tract (i) there are 5-$HT_3$ receptors, (ii) the contractile response to 5-HT is mediated by presynaptic stimulation, (iii) there is non-adrenergic, non-cholinergic excitatory neurotransmission.

## Introduction

5-Hydroxytryptamine (5-HT) was discovered a little over 40 years ago by Rapport and colleagues (1948) and has been found to increase the activity of various visceral structures. In 1957, Gaddum & Picarelli showed that two distinct 5-HT effects were observed in the smooth muscle of guinea-pig ileum. One was associated with a direct contraction of smooth muscle (D-response), the other appeared to mediate depolarization of the cholinergic neurones (M-response). Scientific interest in 5-HT has increased dramatically over the past few years. There has been a recent upsurge of interest in a classification of 5-HT receptors to replace the M- and D-receptors proposed in 1957 by Gaddum & Picarelli. There is now strong evidence that 5-HT receptors can be divided into three types; 5-$HT_1$, 5-$HT_2$, and 5-$HT_3$, with further subdivisions of the 5-$HT_1$ type, and possibly of the other types as well (Bradley *et al.*, 1986).

Although it has been recognized for a long time that the mammalian urinary bladder contracts in response to 5-HT (Gyermek, 1961; 1962; Ambache & Zar, 1970; Taira, 1972; Saum & de Groat, 1973), the mechanism of action of 5-HT and the identification of the receptors in the lower urinary tract of various species is still unclear. It is known that different 5-HT receptors are present in the lower urinary tract of various species (Saxena *et al.*, 1985; Klarskov & Hørby-Petersen, 1986; Holt *et al.*, 1986).

The atropine-resistant component of the contraction evoked by parasympathetic nerve stimulation in the bladders of several species has been known for many years (Langley & Anderson, 1895; Henderson & Roepke, 1934; Ambache, 1955). This has been interpreted by most workers to be due to the presence of non-adrenergic, non-cholinergic excitatory nerves (Ambache & Zar, 1970; Burnstock *et al.*, 1972; Downie & Dean, 1977), and there is now considerable evidence that adenosine 5'-triphosphate (ATP) is an excitatory transmitter in the urinary bladder of small mammals. The ATP analogue, $\alpha,\beta$-methylene ATP, which is resistant to hydrolysis has been shown to activate and then desensitize $P_2$-purinoceptors. It not only abolishes ATP-induced contraction but also the atropine-resistant response to excitatory nerve stimulation (Kasakov & Burnstock, 1983; Fujii, 1988; Brading & Mostwin, 1989).

The purpose of the present study was (1) to investigate the mechanism and the nature of the response to 5-HT in the rabbit lower urinary tract, (2) to test whether non-adrenergic, non-cholinergic transmission is involved in the 5-HT-induced contraction, and (3) to identify the 5-HT receptor in the rabbit lower urinary tract by application of several 5-HT antagonists.

## Methods

### *Preparation of specimens*

Rabbit bladder and urethra were obtained from New Zealand White rabbits of either sex, weighing from 600 g to 2500 g. These were stunned by a blow to the neck and exsanguinated. The specimens were placed in oxygenated Krebs solution. A longitudinal cut was made from the anterior wall of the urethra up through the bladder neck to the bladder dome. The mucosa was then dissected free from the bladder and urethral muscles. Strips were cut from the anterior wall, and the posterior wall of the bladders from both sexes. The urethral strips were made in either longitudinal or transverse direction. An operating microscope was used to ensure that there was good longitudinal alignment of the muscle bundles within a strip. All strips of lower urinary tract smooth muscle measured approximately 8 mm × 1 mm × 1 mm unstretched.

### *Tension recording and stimulation*

Fine silk ligatures were tied to each end of the strip which was then mounted between platinum ring electrodes 1 cm apart in a specially constructed Perspex organ bath. The organ bath had a capacity of 0.2 ml and was continuously perfused with warmed (35–37°C) Krebs solution at a flow rate of 1 ml $min^{-1}$

(Brading & Sibley, 1983). Initially, the strips were allowed to equilibrate for at least one hour, after a resting tension of 1 g had been applied. Tension was measured isometrically with Pioden UF1 transducers and recorded on a Watanabe multichannel pen recorder after amplification.

Activation of intrinsic nerves was achieved by electrical field stimulation by pulses with the following parameters: 50 V, 0.05 ms width, 5 s trains at varying frequency. Successive trains of stimuli were given at least 5 min after the previous contraction had returned to baseline. After each drug-induced response recovery periods of 10–30 min were allowed before further drug application. Drugs and solutions were applied by dipping the ends of the feeder tubes for the perfusion system into the appropriate solutions. This allowed accurately timed exposures of the tissues to different solutions, and by following the bubbles introduced when the solution was changed the instant of tissue contact was recorded.

At the commencement of each experiment, the contractile response of the strips to a 2 min application of 126 mM KCl was obtained and subsequent responses were recorded as a percentage of the control response. This dose of KCl produced a near maximal contraction.

### *Drugs*

The following drugs were used: 5-hydroxytryptamine creatinine sulphate complex (5-HT), adenosine 5'-triphosphate (ATP), tetrodotoxin (TTX), $\alpha,\beta$-methyleneadenosine 5'-triphosphate ($\alpha,\beta$-methylene ATP), hexamethonium bromide, prazosin hydrochloride (all these drugs were obtained from Sigma); atropine sulphate (B.D.H.); methysergide bimaleate and ICS 205-930 (3$\alpha$-tropanyl-1H-indole-3-carboxylic acid ester, Sandoz); phentolamine mesylate (Ciba); BRL 43694 (Granisetron, [endo]N-(9-methyl-9-azabicyclo-[3,3,1]-non-3-yl)-1-methyl-1H-indazole-3-carboxamide hydrochloride, Beecham); ketanserin tartrate (Janssen), MDL 72222 (1$\alpha$H, 3$\alpha$,5$\alpha$H-tropan-3-yl-3,5-dichlorobenzoate, Merrell Dow); metitepine monomethanesulphonate (Roche). Drugs were, where possible dissolved in distilled water to make a concentrated stock solution, these were refrigerated until needed. MDL 72222 was made up as a stock solution of $10^{-3}$ M with 75% ethanol, prazosin was dissolved in DMA (1:9, NN-dimethylacetamide, B.D.H.) as a stock solution of $10^{-3}$ M, and diluted appropriately with Krebs solution; 5-hydroxytryptamine, ATP and $\alpha,\beta$-methylene ATP, were kept frozen. Drug concentrations presented are the final bath values. The vehicle was checked to have no effect. The Krebs solution used had the following composition (mM): NaCl 120.0, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 15.4, $NaH_2PO_4$ 1.0 and glucose 11.5. All solutions were equilibrated with 97% $O_2$, 3% $CO_2$, pH 7.4 at 35–37°C. High $K^+$ solution (126 mM) was prepared by replacing NaCl with an equimolar amount of KCl in normal Krebs solution.

### *Statistical analysis*

Student's $t$ test and analysis of variance were used to compare differences in responses between the control and experimental curves. A probability level of $P < 0.05$ was accepted as significant. When appropriate, results are presented as means ± s.e.mean. $pA_2$ values were calculated for each concentration of antagonist from a Schild plot according to: $pA_2 = -\log\{[\text{antagonist}]/(DR - 1)\}$ (Mackay, 1978).

All points of each graph are means of at least 6 muscle strips taken from 3 different rabbits.

## Results

### *Effect of 5-HT and 5-HT antagonists*

A 30 s application of 5-HT produced dose-dependent contractions in the rabbit bladder ($10^{-8}$ to $10^{-3}$ M) and in the urethra ($10^{-6}$ to $10^{-3}$ M). A larger and more rapid contraction was seen in the detrusor than in the trigone and urethra (Figures 1 and 2). Biphasic responses which consisted of a small relaxation followed by contraction were evoked by 5-HT in some rabbit urethra strips. When high concentrations ($\geq 10^{-5}$ M) or long superfusion periods (≥30 s) were used, the responses to 5-HT were easily desensitized ($n = 9$). Thus, only one dose of the higher concentrations of 5-HT was applied for 30 s in any one experiment. There was no difference in the response to 5-HT between the anterior and posterior detrusor, or between the sexes.

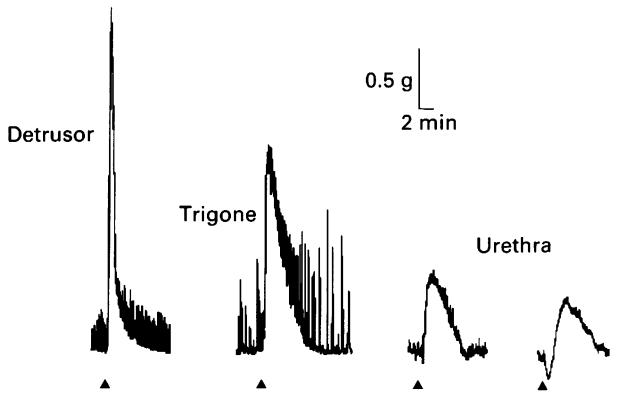


**Figure 1** Contractile response to 5-hydroxytryptamine (5-HT) $10^{-3}$ M applied for 30 s (▲) to the rabbit lower urinary tract smooth muscles. A larger and more rapid contraction was present in the detrusor than in the trigone and urethral preparations. The biphasic response of some urethral preparations is shown as well as the more common monophasic response.

Putative 5-HT antagonists were tested. Ketanserin ($10^{-7}$ to $10^{-6}$ M), methysergide ($10^{-8}$ to $10^{-7}$ M) and metitepine ($10^{-8}$ to $10^{-7}$ M) had no effect on the contractile response to 5-HT. However, BRL 43694 ($5 \times 10^{-11}$ to $2 \times 10^{-10}$ M), ICS 205–930 ($5 \times 10^{-12}$ to $1 \times 10^{-10}$ M) and MDL 72222 ($5 \times 10^{-10}$ to $5 \times 10^{-9}$ M) after 30 min exposure all competitively antagonized the 5-HT-induced contraction in the rabbit bladder strips (Figure 3). The $pA_2$ values were: for MDL 72222 9.3 ± 0.04 ($n = 9$), for BRL 43694 10.5 ± 0.01 ($n = 6$) and for ICS 205-930 12.5 ± 0.36 ($n = 8$). The Schild plot slopes were: for MDL 72222 1.52 ± 0.12, for BRL 43694 2.19 ± 0.03 and for ICS 205-930 0.68 ± 0.12. These values, although empirically useful, should be treated with some caution in view of the difference of the slope from 1.

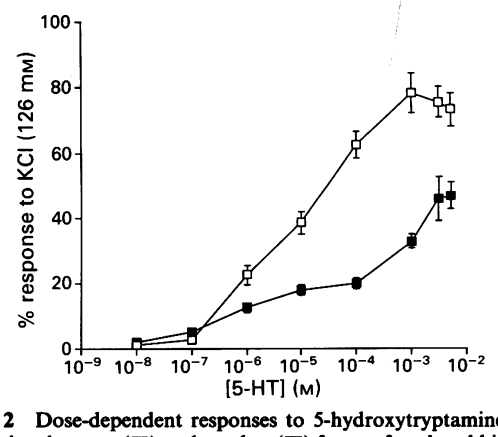


**Figure 2** Dose-dependent responses to 5-hydroxytryptamine (5-HT) in anterior detrusor (□) and urethra (■) from a female rabbit. Points represent means of 15–20 experiments of detrusor and 9–12 experiments of urethra; vertical lines show s.e.mean. Desensitization of the contractile response to 5-HT with higher concentrations ($\geq 10^{-3}$ M) can be seen for the detrusor experiments.

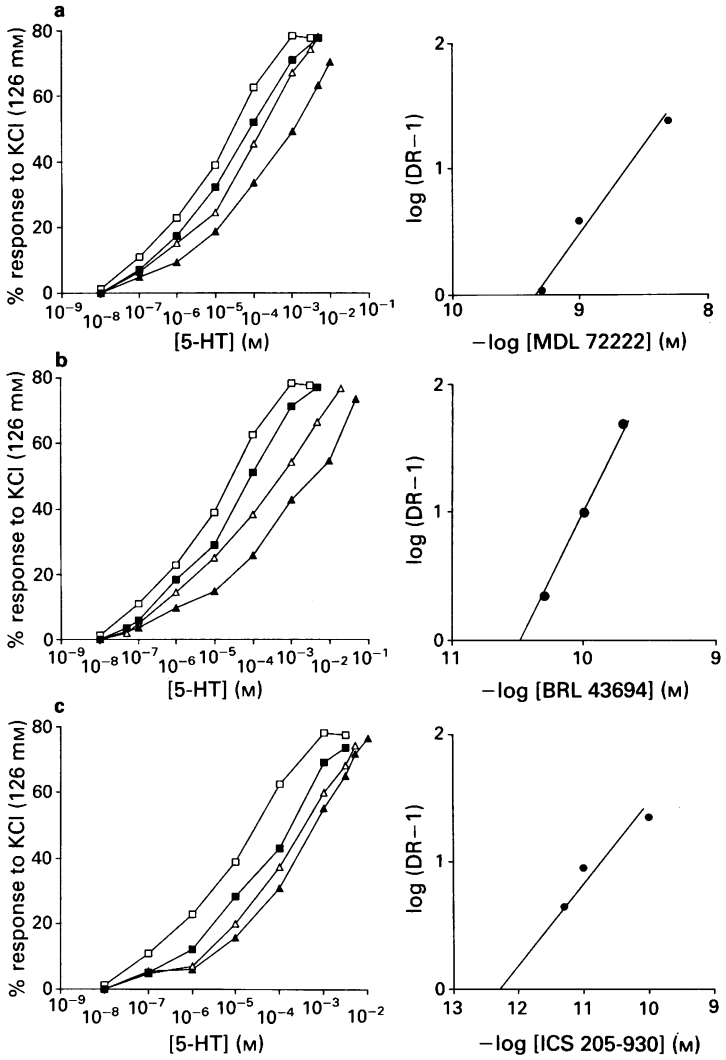


**Figure 3** Effect of the 5-hydroxytryptamine (5-HT) antagonists MDL 72222, BRL 43694 and ICS 205-930 on rabbit detrusor preparations. (a) MDL 72222 $5 \times 10^{-10}$ M (■), $10^{-9}$ M (△) and $5 \times 10^{-9}$ M (▲), control (□). The $pA_2$ value from the Schild plot was $9.3 \pm 0.04$. (b) BRL 43694 $5 \times 10^{-11}$ M (■), $10^{-10}$ M (△) and $2 \times 10^{-10}$ M (▲). The $pA_2$ value was $10.5 \pm 0.01$ ($n = 6$). (c) ICS 205-930 $5 \times 10^{-12}$ M (■), $10^{-11}$ M (△) and $10^{-10}$ M (▲), its $pA_2$ value was $12.5 \pm 0.36$ ($n = 8$). Each point represents the mean of at least 9 experiments from 6 animals.

Atropine ($10^{-7}$ M) and α,β-methylene ATP ($10^{-6}$ M) both partially inhibited the 5-HT-induced contractions. When applied together, they produced more inhibition of the contractile response to 5-HT than either alone (Figure 4). There was a small atropine and α,β-methylene ATP-resistant component of the 5-HT-induced contraction in the bladder strips, which was not abolished by either the $5\text{-HT}_1$ antagonist metitepine ($10^{-7}$ M), the $5\text{-HT}_2$ antagonists ketanserin ($10^{-7}$ M) and methysergide ($10^{-7}$ M), or the $5\text{-HT}_3$ antagonists MDL 72222 ($10^{-7}$ M), ICS 205-930 ($10^{-6}$ M) and BRL 43694 ($10^{-7}$ M).

TTX ($1.6 \times 10^{-6}$ M), hexamethonium ($10^{-6}$ M), phentolamine ($10^{-6}$ M) and prazosin ($10^{-6}$ M) did not affect the 5-HT-induced contraction of the rabbit bladder.

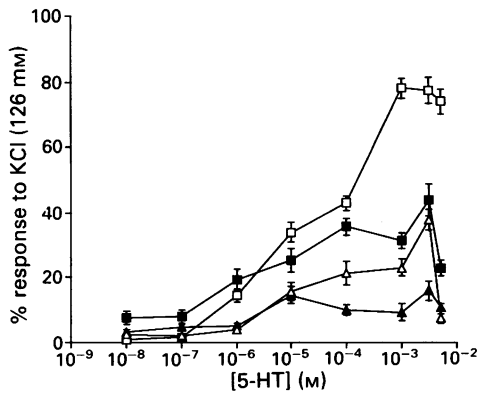


**Figure 4** Effect of atropine and α,β-methylene ATP on the contractile response to 5-hydroxytryptamine (5-HT) in anterior detrusor of male rabbits. A combination of atropine $10^{-6}$ M and α,β-methylene ATP $10^{-6}$ M (▲, $n = 12$) caused greater inhibition than either atropine (■, $10^{-6}$ M, $n = 12$) or α,β-methylene (△, $10^{-6}$ M, $n = 12$) alone. Vertical lines show s.e.mean; $P \leq 0.001$ compared to control (□) for each group at concentrations of $10^{-4}$ M, $10^{-3}$ M, $3 \times 10^{-3}$ M and $5 \times 10^{-3}$ M and for α,β-methylene ATP or combination group at $10^{-6}$ M and $10^{-5}$ M. The effect of the combination of atropine and α,β-methylene ATP was significantly different from that of atropine alone at $10^{-5}$ M to $5 \times 10^{-3}$ M ($P \leq 0.01$) and from that of α,β-methylene ATP alone at $10^{-4}$ M to $3 \times 10^{-3}$ M ($P \leq 0.01$).

### *Electrical field stimulation*

Nerve-mediated responses of the rabbit bladder were studied by use of electrical impulses at frequencies of 1, 5, 10, 20, 30, 40, and 50 Hz to stimulate the intramural nerves selectively. The contractile response increased at frequencies up to 30 Hz, and then reached a plateau. There was no difference in the response to field stimulation between male and female rabbit bladder. Abolition of the responses by TTX ($1.6 \times 10^{-6}$ M) was complete at all frequencies. A 20 min pretreatment with atropine caused dose-dependent ($10^{-8}$ to $10^{-6}$ M) inhibition of

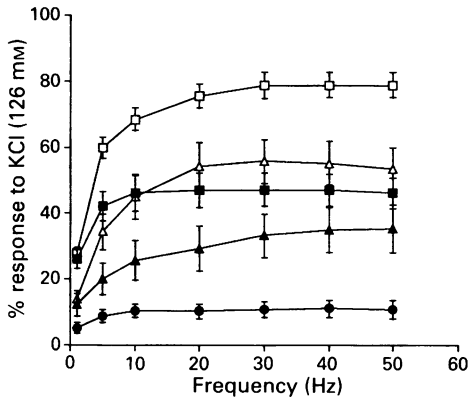


**Figure 5** Effects of atropine and $\alpha,\beta$-methylene ATP on the contractile response to electrical field stimulation in the posterior detrusor of male rabbits. Results show that a combination of $10^{-7}$ M atropine and $10^{-6}$ M $\alpha,\beta$-methylene ATP (●, $n = 9$) caused significantly ($P \leq 0.05$) greater inhibition than either atropine (■, $10^{-7}$ M, $n = 9$) or $\alpha,\beta$-methylene ATP (△, $10^{-6}$ M, $n = 12$; ▲, $10^{-5}$ M, $n = 10$) alone at 5–50 Hz. Atropine significantly decrease responses ($P \leq 0.05$) from 5–50 Hz, and both concentrations of $\alpha,\beta$-methylene ATP significantly decreased ($P < 0.05$) responses from 1–50 Hz when compared to control responses. Vertical lines show s.e.mean (□) Control responses.

the response to field stimulation in the rabbit bladder preparations, with a maximum inhibition at high frequencies of 40% (Figure 5). The residual response was termed the atropine-resistant component. $\alpha,\beta$-Methylene ATP caused a dose-dependent inhibition at all frequencies; at $10^{-6}$ M, about 35% inhibition was seen. Together, atropine ($10^{-7}$ M) and $\alpha,\beta$-methylene ATP ($10^{-6}$ M) produced a greater inhibition of the contractile response to field stimulation than either alone (Figure 5). However, the 5-$HT_3$ antagonist MDL 72222 ($10^{-7}$ M) showed no effect on the contraction to field stimulation of the strips.

## Discussion

The contractile response of the bladder and urethra to 5-HT are complicated, and may involve more than one type of receptor (Cohen, 1989). The responses could be caused either by direct effects on the smooth muscle cells, or indirectly via effects on the autonomic innervation of these organs. Both actions have been implicated in the effects of 5-HT on cat bladder (Saxena *et al.*, 1985), but the majority of the work in this field implicates indirect actions as being of major importance. The ability of 5-HT to stimulate ganglion cells in other tissues, for instance in the enteric nervous system, is well recognized (Gaddum & Picarelli, 1957; Drakontides & Gershon, 1968; Costa & Furness, 1979; Jin *et al.*, 1989), and there is evidence that 5-HT may actually be one of the neurotransmitters to ganglion cells (Richardson *et al.*, 1985; Bradley *et al.*, 1986). Since urinary tract smooth muscles may receive excitatory innervation from both parasympathetic and sympathetic pathways (for review, see Kuru, 1965), it has been of interest to determine which of these autonomic pathways was involved in the response to 5-HT. Experimental evidence strongly implicates effects via the parasympathetic pathway in all species studied (dog: Gyermek, 1962; cat: Saum & de Groat, 1973; frog: Hirai & Koketsu, 1980; rat: Aas, 1983), the indoleamine thought to be activating ganglion cells which result in the release of acetylcholine onto the smooth muscle cells. The results described in this paper also suggest the involvement of the parasympathetic pathway in the effects of 5-HT on the rabbit urinary tract smooth muscles, since atropine dose-dependently inhibited the responses. However, there was a clear atropine-resistant component of the 5-HT response in this species.

An atropine-resistant component of the parasympathetic innervation has long been recognised in most species (for review, see Brading, 1987), and ATP has been implicated as a second excitatory transmitter (Burnstock *et al.*, 1972; Fujii, 1988; Brading & Mostwin, 1989). The ATP analogue $\alpha,\beta$-methylene ATP, which has been shown to activate and desensitize $P_2$-purinoceptors, not only abolishes ATP-induced contraction but also the atropine-resistant responses to excitatory nerve stimulation (Kasakov & Burnstock, 1983; Fujii, 1988). It was therefore of interest to investigate the atropine-resistant component of the 5-HT response in the rabbit bladder and to compare it to the atropine-resistant response to excitatory nerve stimulation. The present studies revealed that after desensitization of the $P_2$-purinoceptors with $\alpha,\beta$-methylene ATP, the atropine-resistant component of the 5-HT response was inhibited, and that a combination of atropine and desensitization of the $P_2$-purinoceptors abolished all but a small component of the 5-HT response. A very similar pattern of blockade was seen with transmural stimulation of excitatory nerves. In addition, the 5-HT responses were not blocked by phentolamine, prazosin or hexamethonium. This strongly suggests that 5-HT is acting to release the excitatory transmitters from the intrinsic nerves in the rabbit and that an adrenergic mechanism is not involved.

TTX, which is known to block $Na^+$ channels in the nerve axons, and which completely blocks the excitatory effect of transmural nerve stimulation in the rabbit, had no effect on the 5-HT-induced contraction in the present study. It has also been demonstrated that TTX did not affect the 5-HT-induced responses in rabbit nodose ganglia (Higashi & Nishi, 1982), although it abolished responses to 5-HT in the mouse duodenum (Drakontides & Gershon, 1968) and in the guinea-pig intestine (Costa & Furness, 1979). A possible explanation is that 5-HT may act on receptors on the nerve terminal to induce membrane depolarization and transmitter release. Shuster *et al.* (1985) demonstrated that 5-HT can close cyclic AMP-sensitive $K^+$ channels in *Aplysia* neurones, which would lead to depolarization, and Higashi & Nishi (1982) showed that 5-HT can open $Na^+$ channels in the nodose ganglion cells of the rabbit. It has also been suggested that excitation of 5-HT receptors on nerve terminals may release acetylcholine in rat bronchi (Aas, 1983) and rabbit heart (Fozard, 1984b), and Holt *et al.* (1986) showed that 5-HT could enhance the responses to excitatory (cholinergic) innervation presynaptically in the mouse bladder.

The classes of 5-HT receptors involved in physiological responses have been extensively studied by means of radioligand binding studies and classical pharmacological organ-bath techniques. At least three classes of receptor have been demonstrated (Bradley *et al.*, 1986), one of which, the 5-$HT_3$-receptor, has clearly been identified in the peripheral nervous system (Fozard, 1984a; Richardson & Engel, 1986; Fake *et al.*, 1987; Sanger, 1987). In the present study, the 5-$HT_3$-receptor antagonists ICS 205-930, BRL 43695 and MDL 72222 were all effective at blocking the excitatory responses of the bladder, whilst the 5-$HT_1$- and 5-$HT_2$-antagonists were ineffective. Thus in the rabbit 5-$HT_3$-receptors mediate the release of ATP and acetylcholine from the nerve terminals. The relative potency of the three 5-$HT_3$-receptor antagonists obtained in the present study (ICS 205-930 > BRL 43694 > MDL 72222) is the same as seen by Fozard (1989) on the rabbit vagus, although in that preparation the $pA_2$ values were 10.2, 9.9 and 7.9, whereas for the rabbit bladder the antagonists were more potent with $pA_2$ values of 12.5, 10.5 and 9.3 respectively. The affinity of binding sites for 5-$HT_3$-receptor antagonists in rat cortical membranes showed a slightly different order, with $K_i$ values (nM) of 0.4 for ICS 205-930, 0.26 for BRL 43694 and 5.3 for MDL 72222 (Nelson & Thomas, 1989). [$^3$H]-ICS 205-930 also labels 5-$HT_3$-like binding sites in neuroblastoma cells (Hoyer & Neijt, 1987).

Although in the rabbit bladder the majority of the contractile response to 5-HT was abolished by the 5-$HT_3$-receptor antagonists, there was still a residual contractile response to 5-HT, which was not blocked by the 5-$HT_1$, 5-$HT_2$ or 5-$HT_3$

receptor antagonists. This suggests that there is another 5-HT-receptor which has a small effect either directly on the smooth muscle, or indirectly through an effect on other nerve endings. The fact that there was also a residual response to transmural nerve stimulation in the presence of atropine and desensitization of the $P_2$-purinoceptors, which was blocked by TTX, suggests that in this species a minor component of excitation involving a third transmitter may exist.

In summary, the involvement of the release of acetylcholine and a non-adrenergic, non-cholinergic neurotransmitter in the 5-HT-induced contraction is strongly suggested by the experiments. The results also identify the receptor involved in the rabbit lower urinary tract as the 5-$HT_3$ receptor.

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## References

AAS, P. (1983). Serotonin induced release of acetylcholine from neurons in the bronchial smooth muscle of the rat. *Acta Physiol. Scand.*, **117**, 477–480.

AMBACHE, N. (1955). The use and limitations of atropine for pharmacological studies on autonomic effectors. *Pharmacol. Rev.*, **7**, 467–494.

AMBACHE, N. & ZAR, M. ABOO (1970). Non-cholinergic transmission by post-ganglionic motor neurones in the mammalian bladder. *J. Physiol.*, **210**, 761–783.

BRADING, A.F. (1987). Physiology of bladder smooth muscle. In *The Physiology of the Lower Urinary Tract*. ed. Torrens, M. & Morrison, J. F. B. pp. 161–191. Berlin Heidelberg: Springer-Verlag.

BRADING, A.F. & SIBLEY, G.N.A. (1983). A superfusion apparatus to study field stimulation of smooth muscle from mammalian urinary bladder. *J. Physiol.*, **334**, 11–12P.

BRADING, A.F. & MOSTWIN, J.L. (1989). Electrical and mechanical responses of guinea-pig bladder muscle to nerve stimulation. *Br. J. Pharmacol.*, **98**, 1083–1090.

BRADLEY, P.B., ENGEL, G., FENIUK, W., FOZARD, J.R., HUMPHREY, P.P.A., MIDDLEMISS, D.N., MYLECHARANE, E.J., RICHARDSON, B.P. & SAXENA, P.R. (1986). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacology*, **25**, 563–576.

BURNSTOCK, G., DUMSDAY, B. & SMYTHE, A. (1972). Atropine-resistant excitation of the urinary bladder: the possibility of transmission via nerves releasing a purine nucleotide. *Br. J. Pharmacol.*, **44**, 451–461.

COHEN, M.L. (1989). 5-Hydroxytryptamine and non-vascular smooth muscle contraction and relaxation. In *The Peripheral Action of 5-Hydroxytryptamine*. ed. Fozard, J.R. Chapter 9, pp. 201–219. Oxford, New York, Tokyo: Oxford University Press.

COSTA, M. & FURNESS, J.B. (1979). The sites of action of 5-HT in nerve-muscle preparations from the guinea-pig small intestine and colon. *Br. J. Pharmacol.*, **65**, 237–248.

DOWNIE, J.W. & DEAN, D.M. (1977). The contribution of cholinergic postganglionic neurotransmission to contractions of rabbit detrusor. *J. Pharmacol. Exp. Ther.*, **203**, 417–425.

DRAKONTIDES, A.B. & GERSHON, M.D. (1968). 5-Hydroxytryptamine receptors in the mouse duodenum. *Br. J. Pharmacol.*, **33**, 480–492.

FAKE, C.S., KING, F.D. & SANGER, G.J. (1987). BRL 43694: a potent and novel 5-$HT_3$ receptor antagonist. *Br. J. Pharmacol.*, **91**, 335P.

FOZARD, J.R. (1984a). Neuronal 5-HT receptors in the periphery. *Neuropharmacology*, **23**, 1473–1486.

FOZARD, J.R. (1984b). Characteristics of the excitatory 5-HT receptor on the cholinergic nerves of rabbit heart. *Proceedings of IUPHAR Ninth International Congress of Pharmacology*, London, July 1984.

FOZARD, J.R. (1989). The development and early clinical evaluation of selective 5-$HT_3$ receptor antagonists. In *The Peripheral Action of 5-Hydroxytryptamine*. ed. Fozard, J. R. Chapter 15, pp. 354–376. Oxford, New York, Tokyo: Oxford University Press.

FUJII, K. (1988). Evidence for adenosine triphosphate as an excitatory transmitter in guinea-pig, rabbit and pig urinary bladder. *J. Physiol.*, **404**, 39–52.

GADDUM, J.H. & PICARELLI, Z.P. (1957). Two kinds of tryptamine receptor. *Br. J. Pharmacol.*, **12**, 323–328.

GYERMEK, L. (1961). Cholinergic stimulation and blockade on urinary bladder. *Am. J. Physiol.*, **201**, 325–328.

GYERMEK, L. (1962). Action of 5-hydroxytryptamine on the urinary bladder of the dog. *Arch. Int. Pharmacodyn.*, **137**, 137–144.

HENDERSON, V.E. & ROEPKE, M.H. (1934). The role of acetylcholine in bladder contractile mechanisms and in parasympathetic ganglia. *J. Pharmacol. Exp. Ther.*, **51**, 97–111.

HIGASHI, H. & NISHI, S. (1982). 5-Hydroxytryptamine receptors of visceral primary afferent neurones on rabbit nodose ganglia. *J. Physiol.*, **323**, 543–567.

HIRAI, K. & KOKETSU, K. (1980). Presynaptic regulation of the release of acetylcholine by 5-hydroxytryptamine. *Br. J. Pharmacol.*, **70**, 499–500.

HOLT, S.E., COOPER, M. & WYLLIE, J.H. (1986). On the nature of the receptor mediating the action of 5-hydroxytryptamine in potentiating responses of the mouse urinary bladder strip to electrical stimulation. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **334**, 333–340.

HOYER, D. & NEIJT, H.C. (1987). Identification of serotonin 5-$HT_3$ recognition by radioligand binding in NG 108-15 neuroblastom-glioma cells. *Eur. J. Pharmacol.*, **91**, 335P.

JIN, J-G., NEYA, T. & NAKAYAMA, S. (1989). Myenteric 5-HT-containing neurones activate the descending cholinergic excitatory pathway to the circular muscle of guinea-pig ileum. *Br. J. Pharmacol.*, **98**, 982–988.

KASAKOV, L. & BURNSTOCK, G. (1983). The use of the slowly degradable analog, $\alpha,\beta$-Methylene ATP, to produce desensitization of the $P_2$-purinoceptor: effect on non-adrenergic, non-cholinergic responses of the guinea-pig urinary bladder. *Eur. J. Pharmacol.*, **86**, 291–294.

KLARSKOV, P. & HØRBY-PETERSEN, J. (1986). Influence of serotonin on lower urinary tract smooth muscle in vitro. *Br. J. Urol.*, **58**, 507–513.

KURU, M. (1965). Nervous control of micturition. *Physiol. Rev.*, **45**, 425–494.

LANGLEY, J.N. & ANDERSON, H.K. (1895). The innervation of the pelvic and adjoining viscera. Part II. The Bladder. *J. Physiol.*, **19**, 71–84.

MACKAY, D. (1978). How should values of $pA_2$ and affinity constants for pharmacological competitive antagonists be estimated? *J. Pharm. Pharmacol.*, **30**, 312–313.

NELSON, D. & THOMAS, D. (1989). [$^3$H]-BRL 43694 (Granisetron), a specific ligand for 5-$HT_3$ binding sites in rat brain cortical membranes. *Br. J. Pharmacol.*, **38**, 1693–1695.

RAPPORT, M.M., GREEN, A.A. & PAGE, I.H. (1948). Serum vasoconstrictor (serotonin). IV. isolation and characterization. *J. Biol. Chem.*, **176**, 1243–1251.

RICHARDSON, B. P. & ENGEL, G. (1986). The pharmacology and function of 5-$HT_3$ receptors. *Trends Neurol. Sci.*, **9**, 424–428.

RICHARDSON, B.P., ENGEL, G., DONATSCH, P. & STADLER, P.A. (1985). Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. *Nature*, **316**, 126–131.

SANGER, G.J. (1987). Increased gut cholinergic activity and antagonism of 5-hydroxytryptamine M-receptors by BRL 24924: potential clinical importance of BRL 24924. *Br. J. Pharmacol.*, **91**, 77–87.

SAUM, W.R. & DE GROAT, W.C. (1973). The actions of 5-hydroxytryptamine on the urinary bladder and on vesical autonomic ganglia in the cat. *J. Pharmacol. Exp. Ther.*, **185**, 70–83.

SAXENA, P.R., HEILIGERS, J., MYLECHARANE, E.J. & TIO, R. (1985). Excitatory 5-hydroxytryptamine receptors in the cat urinary bladder are of the M- and 5-$HT_2$-type. *J. Auton. Pharmacol.*, **5**, 101–107.

SHUSTER, M.J., CAMARDO, J.S., SIEGELBAUM, S.A. & KANDEL, E.R. (1985). Cyclic AMP-dependent protein kinase closes the serotonin-sensitive $K^+$ channels of Aplysia sensory neurones in cell-free membrane patches. *Nature*, **313**, 392–395.

TAIRA, N. (1972). The autonomic pharmacology of the bladder. *A. Rev. Pharmacol.*, **12**, 197–208.

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