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 produces dose-dependent contraction in the detrusor and urethra.
2. The 5-HT-induced contraction could be dose-dependently inhibited by the 5-HT₃ antagonists MDL 72222, ICS 205-930 and BRL 43694. No effect of ketanserin, methysergide or metiprin was observed on the contractile response to 5-HT.
3. Atropine and α,β-methylene ATP both partially blocked the contractile response to 5-HT. Together they caused more inhibition than either alone.
4. Atropine and α,β-methylene ATP also inhibited the contractile response to electrical field stimulation. The 5-HT₃ antagonist MDL 72222 had no effect on the contract to field stimulation.
5. The atropine- and α,β-methylene ATP-resistant components of 5-HT-induced contraction were not affected by the 5-HT₃ antagonists metiprin, the 5-HT₃ antagonists ketanserin and methysergide or the 5-HT₃ 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-HT₃ 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 (1949) 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-HT₁, 5-HT₂, and 5-HT₃, with further subdivisions of the 5-HT₁ 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; Klarkov & Hafby-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 & Roepeke, 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, α,β-methylene ATP, which is resistant to hydrolysis has been shown to activate and then desensitize P₂-purinoceptors. It not only abolishes ATP-induced contraction but also the atropine-resistant response to excitatory nerve stimulation (Kasakov & Burnstock, 1983; Fujii, 1986; Braden & 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 x 1 mm x 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 Pidren UFI transducers and recorded on a Watanabe multi-channel 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), αβ-methyleneadenosine 5'-triphosphate (αβ-methylene ATP), hexamethonium bromide, prazosin hydrochloride (all these drugs were obtained from Sigma); atropine sulphate (B.D.H.); methysergide bimaleate and ICS 205-930 (3α-tropanyl-1H-indole-3-carboxamide [endo]-N49-methyl-9-azabicyclo[3,3,1]-non-3-sulphate ester, Roche). The drugs were dissolved in distilled water or 95% ethanol, tretipine 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 10⁻²⁵ M with 75% ethanol, prazosin was dissolved in DMA (1:9, NN-dimethylacetamide, B.D.H.) as a stock solution of 10⁻²⁵ M, and diluted appropriately with Krebs solution; 5-hydroxytryptamine, ATP and αβ-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, CaCl₂ 2.5, MgCl₂ 1.2, NaH₂PO₄ 15.4, NaHCO₃ 1.0 and glucose 11.5. All solutions were equilibrated with 97% O₂, 3% CO₂, 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. pA₂ values were calculated for each concentration of antagonist from a Schild plot according to: 

\[ pA₂ = -\log(\text{[antagonist]} / \text{[DR]}) - 1 \]

(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 (10⁻⁶ 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 (≥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.

Putative 5-HT antagonists were tested. Ketanserin (10⁻⁷ to 10⁻⁶ M), methysergide (10⁻⁸ to 10⁻⁷ M) and metipetamine (10⁻⁸ to 10⁻⁷ M) had no effect on the contractile response to 5-HT. However, BRL 43694 (5 × 10⁻¹¹ to 2 × 10⁻¹⁰ M), ICS 205-930 (5 × 10⁻¹² to 1 × 10⁻¹⁰ M) and MDL 72222 (5 × 10⁻¹⁰ to 5 × 10⁻⁸ M) after 30 min exposure all competitively antagonized the 5-HT-induced contraction in the rabbit bladder strips (Figure 3). The pA₂ 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 1](image1.png)  
**Figure 1** Contractile response to 5-hydroxytryptamine (5-HT) 10⁻⁶ M applied for 30 s (A) 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.

![Figure 2](image2.png)  
**Figure 2** Dose-dependent responses to 5-hydroxytryptamine (5-HT) in anterior detrusor (C) and urethra (M) 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 (≥10⁻⁵ M) can be seen for the detrusor experiments.
Contractions induced (10-6 M) was not tractile response to either single, either alone, applied methysergide (10-7 M), 72222 methysergide, and 40, bladder. Caused by atropine was complete inhibition of the contractile response. Nerve-mediated response and contractile response of the rabbit bladder. Atropine (10-7 M) and aβ-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 aβ-methylene ATP-resistant component of the 5-HT-induced contraction in the bladder strips, which was not abolished by either the 5-HT1 antagonist metipteine (10-7 M), the 5-HT2 antagonists ketanserin (10-7 M) and methysergide (10-7 M), or the 5-HT3 antagonists MDL 72222 (10-7 M), ICS 205-930 (10-6 M) and BRL 43694 (10-7 M).

TXX (1.6 × 10-6 M), hexamethonium (10-6 M), phentolamine (10-6 M) and prazosin (10-8 M) did not affect the 5-HT-induced contraction of the rabbit bladder.

**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 TXX (1.6 × 10-6 M) was complete at all frequencies. A 20 min pretreatment with atropine caused dose-dependent (10-6 to 10-4 M) inhibition of

**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 × 10-10 M ( ), 10-8 M (Δ) and 5 × 10-7 M ( ), control ( ). The pA2 value from the Schild plot was 9.3 ± 0.4. (b) BRL 43694 5 × 10-11 M ( ), 10-9 M (Δ) and 2 × 10-10 M ( ). The pA2 value was 10.5 ± 0.01 (n = 6). (c) ICS 205-930 5 × 10-12 M ( ), 10-11 M (Δ) and 10-10 M ( ), its pA2 value was 12.5 ± 0.36 (n = 8). Each point represents the mean of at least 9 experiments in 6 animals.

**Figure 4** Effect of atropine and aβ-methylene ATP on the contractile response to 5-hydroxytryptamine (5-HT) in anterior detrusor of male rabbits. A combination of atropine 10-8 M and aβ-methylene ATP 10-6 M ( , n = 12) caused greater inhibition than either atropine ( , 10-6 M, n = 12) or aβ-methylene ( , 10-6 M, n = 12) alone. Vertical lines show s.e.mean; P ≤ 0.001 compared to control ( ) for each group at concentrations of 10-8 M, 10-7 M, 3 × 10-7 M and 5 × 10-7 M and for aβ-methylene ATP or combination group at 10-6 M and 10-5 M. The effect of the combination of atropine and aβ-methylene ATP was significantly different from that of atropine alone at 10-7 M to 5 × 10-5 M (P ≤ 0.01) and from that of aβ-methylene ATP alone at 10-4 M to 3 × 10-3 M (P ≤ 0.01).

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**Graphs**

- **Figure 3**
  - Panel a: Graph showing the effect of MDL 72222 on the response to 5-HT.
  - Panel b: Graph showing the effect of BRL 43694 on the response to 5-HT.
  - Panel c: Graph showing the effect of ICS 205-930 on the response to 5-HT.

- **Figure 4**
  - Graph showing the effect of atropine and aβ-methylene ATP on the contractile response to 5-HT.
Groat, strongly involved in the experimental study of 5-HT in rabbit urogenital tissues, determined contractile responses of 5-HT on rabbit urinary bladder (Saxena et al., 1985), but the majority of results showed 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 cause cyclic AMP–sensitive K⁺ channels in Aplysia neurons, 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., 1980), one of which, the 5-HT₃-receptor, has been cloned from an enteric nervous system (Fozard, 1984a; Richardson & Engel, 1986; Fake et al., 1987; Sanger, 1987). In the present study, the 5-HT₃-receptor antagonists ICS 205-930, BRL 43695 and MDL 72222 were all effective at blocking the excitatory responses of the bladder, whilst the 5-HT₃- and 5-HT₄-antagonists were ineffective. Thus in the rabbit 5-HT₁-receptors mediate the release of ATP and acetylcholine from the nerve terminals. The relative potency of the three 5-HT₃-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 pA₂ values were 10.2, 9.9 and 7.9, whereas for the rabbit bladder the antagonists were more potent with pA₂ values of 12.5, 10.5 and 9.3 respectively. The affinity of binding sites for 5-HT₃-receptor antagonists in rat cortical membranes showed a slightly different order, with Kᵢ values (nm) of 0.4 for ICS 205-930, 0.26 for BRL 43694 and 5.3 for MDL 72222 (Nelson & Thomas, 1989). [¹³C]ICS 205-930 also labels 5-HT₁-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-HT₃-receptor antagonists, there was still a residual contractile response to 5-HT, which was not blocked by the 5-HT₁, 5-HT₂ or 5-HT₃
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 P2-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-HT3 receptor.

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References


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