Br. J. Pharmacol. (1994), **111**, 213–218

Evidence for an inhibitory 5-HT_4 receptor in urinary bladder of *Rhesus* and *Cynomolgus* monkeys

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1 The present study shows that 5-hydroxytryptamine (5-HT) inhibits electrically-evoked contractions of isolated urinary bladder strips from *Rhesus* and *Cynomolgus* monkeys via activation of 5-HT_4 receptors.

2 5-HT (0.1 nM–10 μM) produced concentration-dependent inhibition of the contractile response to electrical stimulation yielding a pEC_{50} of 7.8 (*Rhesus* monkey) and 7.6 (*Cynomolgus* monkey). This action of 5-HT was mimicked by 5-methoxytryptamine, renzapride and BIMU 8, each of which behaved as a full agonist relative to 5-HT. However, the potency estimate for BIMU 8 ($pEC_{50} = 6.5$) in *Cynomolgus* monkey was low, relative to 5-HT, indicating a possible heterogeneity of 5-HT_4 receptors.

3 The inhibitory action of 5-HT was resistant to antagonism by methysergide (1 μM) and ondansetron (5 μM), thereby eliminating a role for 5-HT_1, 5-HT_2 and 5-HT_3 receptors. The 5-HT_4 receptor antagonists, GR 113808 (10 nM), DAU 6285 (1–10 μM) and RS 23597-190 (1 μM), produced parallel, dextral displacements of the concentration-effect curves to 5-HT and other related agonists with affinity estimates in agreement with those defined previously in other 5-HT_4 receptor assay systems.

4 Experiments using direct electrical stimulation of bladder smooth muscle indicate that the 5-HT_4 receptors are located post-junctionally.

5 The inhibitory action of 5-HT in isolated urinary bladder of monkey differs from the excitatory effect of 5-HT in urinary bladder of man. Species variation and its implications for the development of therapeutic agents are discussed.

Keywords: 5-HT; 5-HT_4 receptor; urinary bladder of monkey; GR 113808; DAU 6285; BIMU 8; 5-methoxytryptamine; RS 23597-190; *Cynomolgus* monkey; *Rhesus* monkey

Introduction

Pharmacological studies on the urinary bladder have demonstrated species variation with regard to the nature of responses to 5-hydroxytryptamine (5-HT) and the subtype(s) of 5-HT receptor involved. For example, in dog, 5-HT contracts the isolated bladder via 5-HT_2 receptors (Cohen, 1990). In urinary bladder of the anaesthetized cat, biphasic excitatory concentration-effect curves to 5-HT have been reported and are mediated via 5-HT_3 and 5-HT_2 receptors (Saxena *et al.*, 1985). In isolated bladder of mouse, potentiation of electrically evoked contractions by 5-HT is mediated through 5-HT_{1B} and 5-HT_2 receptors (Cleal *et al.*, 1989). By contrast, 5-HT relaxes isolated bladder neck of pig, an effect blocked by methysergide (10 μM; an antagonist at 5-HT_1 and 5-HT_2 receptors), but not ketanserin (1 μM; a selective 5-HT_2 receptor antagonist) (Hills *et al.*, 1984). In bladder of bullfrog, 5-HT-induced inhibitions of electrically evoked contractions are insensitive to methysergide (Bowers & Kolton, 1987) as are 5-HT-induced contractions of the isolated bladder of guinea-pig (Callahan & Creed, 1981).

In view of therapeutic potential, there has been interest in the role of 5-HT in the physiology of micturition (Delaere *et al.*, 1987). However, pharmacological characterization of 5-HT receptors in urinary bladder of man has been hampered by a lack of specific ligands (Klarskov & Hørby-Petersen, 1986). Early suggestive evidence for a putative 5-HT_4 receptor can be found in the work of Hindmarsh *et al.* (1977) who reported potentiation of electrically induced contractions of human isolated bladder by low concentrations of 5-HT, an effect insensitive to blockade by methysergide and morphine. Recently, Corsi *et al.* (1991) described an 'atypical' 5-HT receptor in human bladder that mediates potentiation of contractile responses to electrical field stimulation. Elements of the agonist and antagonist profile for this receptor resemble that of the 5-HT_4 receptor, including potent agonism by 5-HT ($pEC_{50} = 8.0$) and 5-methoxytryptamine (5-MeOT), agonism by certain substituted benzamide derivatives, antagonism by micromolar concentrations of tropisetron (ICS 205-930), and resistance to inhibition by certain 5-HT_1, 5-HT_2 and 5-HT_3 receptor antagonists (see Bockaert *et al.*, 1992; Ford & Clarke, 1993). However, antagonism by tropisetron in human bladder deviates from competition and in high concentrations tropisetron behaves as an agonist (Corsi *et al.*, 1991). Furthermore, the potentiating action of 5-HT is antagonized, in part, by methysergide (1 μM).

To date, the nature of the 5-HT receptor in isolated urinary bladder of monkey has not been described. Our preliminary experiments revealed a potent inhibitory effect of 5-HT that was blocked by the 5-HT_4 receptor antagonist, DAU 6285 (Waikar *et al.*, 1992). The present study was undertaken therefore, to isolate pharmacologically and characterize further the putative 5-HT_4 receptor in the urinary bladder from *Rhesus* and *Cynomolgus* monkeys. Preliminary accounts of this work have been presented at the British Pharmacological Society Meeting, July, 1992 (Waikar *et al.*, 1992) and the 2nd International Symposium on Serotonin, September, 1992 (Ford *et al.*, 1992b).

Methods

Preparation of urinary bladder strips

Rectangular strips (2 cm × 0.5 cm) of urinary bladder from *Rhesus* and *Cynomolgus* monkeys of either sex (5–9 kg) were

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taken from the posterior medial smooth muscle layer and mounted vertically been two platinum electrodes in 10 ml tissue baths containing Tyrode solution (37°C; pH 7.4; gassed with O_2/CO_2, 95:5%). Cocaine (30 μM), corticosterone (30 μM), methysergide (1 μM), ondansetron (5 μM) and indomethacin (10 μM) were added to the Tyrode solution in order to achieve equilibrium conditions and isolate pharmacologically the putative 5-HT_4 receptor for study. Responses were recorded isometrically using a Hugo Sachs Elektronik (Biegestab K30) transducer coupled to Graphtec (linearcorder WR3310) four channel chart recorders.

Response to electrical stimulation

Electrical stimulation parameters were modified from Corsi *et al.* (1991). Tissues were placed under an initial tension of 10 mN and subjected to electrical field-stimulation (Grass S88 stimulator; Buxco Electronics Stimulus Distributor). Trains of electrical pulses (5 s duration, supramaximal voltage, 1 ms pulse width, 20 Hz) were applied at 1 train per min, yielding reproducible contractile responses. The effects of tetrodotoxin (TTX, 3 μM) and atropine (1 μM) on the responses to field stimulation were measured.

Effect of 5-HT_4 receptor agonists and antagonists on response to field stimulation

Following stabilization of electrically evoked contractions (≈30 min, washing every 10 min), cumulative concentration-inhibition (E/[A]) curves to agonist were constructed at 0.5 or 1 $\log_{10}$ unit intervals. Upon washout of agonist and subsequent recovery of the response to field stimulation (30–45 min), tissues were equilibrated with antagonist for 30–60 min and second E/[A] curves were constructed. Strips from some bladders failed to recover completely after the first E/[A] curve and were not used for antagonist studies.

Inhibition of carbachol-induced contracture

Segments of urinary bladder, not subjected to electrical stimulation, were contracted with carbachol (1 μM). Following the development of a sustained contracture (≈30 min), attempts were made to relax the tissue with 5-HT (10 nM–10 μM), isoprenaline (10 μM), forskolin (30 μM) and TTX (3 μM).

Direct stimulation of muscle

Direct electrical stimulation of the smooth muscle of bladder was conducted as described by Corsi *et al.* (1991). After a 15–30 min period of electrical stimulation (using the parameters given above), TTX (3 μM) and atropine (1 μM) were administered so as to inhibit completely the neurogenic contractile response. Subsequently, the pulse width of the electrical stimulation was increased 10 fold to 10 ms in order to evoke reproducible contractile responses of muscle similar in magnitude to those generated neurogenically. For the generation of E/[A] curves 5-HT was added cumulatively. BIMU 8 was used in a single concentration (10 μM).

Study of different anatomical regions of bladder and drug-free Tyrode solution

In order to investigate differences between results with monkey bladder (present study) and those reported in human bladder (Corsi *et al.*, 1991), strips of monkey bladder were taken from the dome region and suspended in Tyrode solution free of cocaine, corticosterone, ondansetron, methysergide and indomethacin. These conditions mimicked the experimental conditions employed by Corsi *et al.* (1991) on human bladder.

Data analysis

E/[A] curves are expressed as percentage inhibition of field stimulated contractions measured just prior to the addition of agonist. Estimates of maximal inhibition (E_{max}) and the concentration of agonist ([A]) giving half maximal inhibition (EC_{50}), were generated with a non-linear, iterative curve-fitting programme (Kaleidagraph, Synergy Software, PCS Inc., Reading, PA, U.S.A.) using the following form of the logistic function: $E/E_{max} = [A]^n/([A]^n + EC_{50}^n)$ where n is a parameter defining slope. Antagonist pA_2 estimates were made by comparing agonist EC_{50} values in the absence and presence (EC_{50}') of a single concentration ([B]) of antagonist ($CR = EC_{50}/EC_{50}'$), such that $pA_2 = -\log([B]/(CR-1))$.

Ninety five percent confidence limits (CL) and statistical significance of differences between samples (single comparisons; Student's unpaired *t* test, two-tailed) were determined using Statview IV (Brain Power Inc., Calabassas, CA, U.S.A.). Although several strips were obtained from each bladder, *n* refers to the number of bladders used.

Chemicals

The following were obtained as stipulated: BIMU 8 (endo-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-(1-methyl) ethyl-2-oxo-1H-benzimidazole-1-carboxamide hydrochloride) and DAU 6285 (endo-6-methoxy-8-methyl-8-azabicyclo [3.2.1] oct 3 yl-2,3-dihydro-2-oxo-1H-benzimidazole-1 carboxylate hydrochloride) (Dr C.A. Rizzi, Boehringer Ingelheim Italia, Institute De Angeli, Milan, Italy); 5-hydroxytryptamine creatinine sulphate, 5-methoxytryptamine hydrochloride, corticosterone, carbamylcholine chloride, indomethacin, lignocaine hydrochloride, cocaine hydrochloride, atropine sulphate, tetrodotoxin, forskolin (Sigma Chemical Co., St. Louis, MO, U.S.A.); renzapride, ondansetron, RS 23597-190 (3(piperidine-1-yl)-propyl-4-amino-5-chloro-2-methoxy benzoate HCl) and GR 113808 ([1-[2-methylsulphonyl)amino]ethyl]-4-piperidinyl]methyl-1-methyl-1H-indole-3-carboxylate) were synthesized on site (Syntex Research, Palo Alto, CA, U.S.A.); methysergide maleate (Sandoz, Basel, Switzerland); ω-conotoxin GVIA (Bachem California Inc., U.S.A.); 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT; RBI, Natick, MA, U.S.A.).

All drugs were dissolved in deionised water with the following exceptions: corticosterone and methysergide (dimethylsulphoxide; DMSO), indomethacin (0.5% Na_2CO_3 in water), ω-conotoxin GVIA (10% bovine serum albumin in Tyrode solution) and forskolin (70% DMSO in water).

Results

Response to electrical stimulation

Transmural electrical field stimulation (supramaximal voltage, 30–90 V; pulse width, 1 ms; frequency 20 Hz; pulse train, 5 s; train interval, 55 s) evoked reproducible contractile responses in strips of monkey urinary bladder (Figure 1). These responses were blocked by TTX (3 μM) and atropine (1 μM).

Effect of 5-HT_4 receptor agonists and antagonists

Figure 1 shows that cumulative addition of 5-HT (0.3 nM–1 μM) inhibited field stimulated contractile responses in a concentration-dependent manner in bladder strips from both *Rhesus* ($pEC_{50} = 7.8$; 95% CL 7.6–8.0; $n = 13$) and *Cynomolgus* monkey ($pEC_{50} = 7.6$; 95% CL 7.4–7.8; $n = 10$). Iteratively fitted mean E/[A] curves for 5-HT and other 5-HT_4 receptor agonists are shown in Figure 1b. 5-Methoxytryptamine (5-MeOT), and, possibly, renzapride ($n = 1$) acted as full agonists, mimicking the inhibitory action of 5-HT (see Table 1 for data regarding agonists potency).

BIMU 8, a selective 5-HT$_4$ receptor agonist, also behaved as a full agonist relative to 5-HT but its estimated potency (6.5, 95% CL 6.1–6.8; *Cynomolgus* monkey, $n = 4$; Figure 1b) is lower than expected for a 5-HT$_4$ receptor. The selective 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT was without effect (*Cynomolgus* monkey; data not shown).

Table 1 summarizes data with regard to antagonist affinity. 5-HT$_4$ receptor antagonists, GR 113808 (10 nM; Figure 2a) and DAU 6285 (1 μM; Figure 2b), produced parallel dextral displacements of E/[A] curves to 5-HT yielding antagonist pA$_2$ estimates of 9.5 (95% CL 9.1–9.8; $n = 5$; *Cynomolgus* monkey) and 7.0 (95% CL 6.8–7.3; $n = 4$; *Rhesus* monkey) respectively. pA$_2$ estimates ranging from 6.8–7.4 were also generated for DAU 6285 against 5-MeOT, BIMU 8 and renzapride (Table 1). Another 5-HT$_4$ receptor antagonist, RS 23597-190, yielded a pA$_2$ estimate of 7.3 (95% CL 6.8–7.9; $n = 5$) against 5-HT in strips of bladder from *Rhesus* monkey (Table 1).

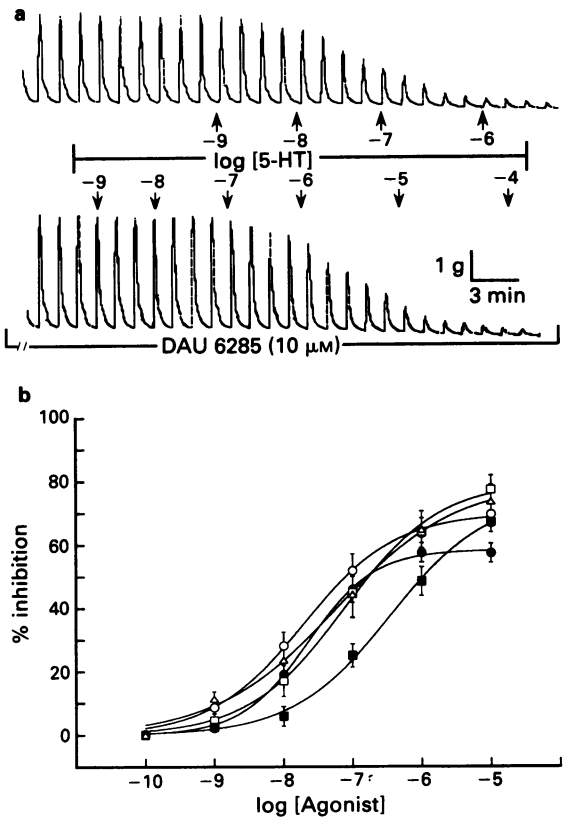

Figure 1 (a) Cumulative additions of 5-HT caused inhibition of electrically stimulated contractions in strips of *Rhesus* monkey urinary bladder (upper trace), an effect that was blocked in the presence of the selective 5-HT$_4$ receptor antagonist DAU 6285 (10 μM; lower trace). (b) Agonist concentration-inhibition curves to 5-HT (○, *Rhesus* monkey; ●, *Cynomolgus* monkey), BIMU 8 (□, *Rhesus*; ■, *Cynomolgus*), and 5-methoxytryptamine (△, *Rhesus*). Each point represents the arithmetic mean ± s.e.mean for 2–13 experiments (see Table 1 for potency estimates and *n* values).

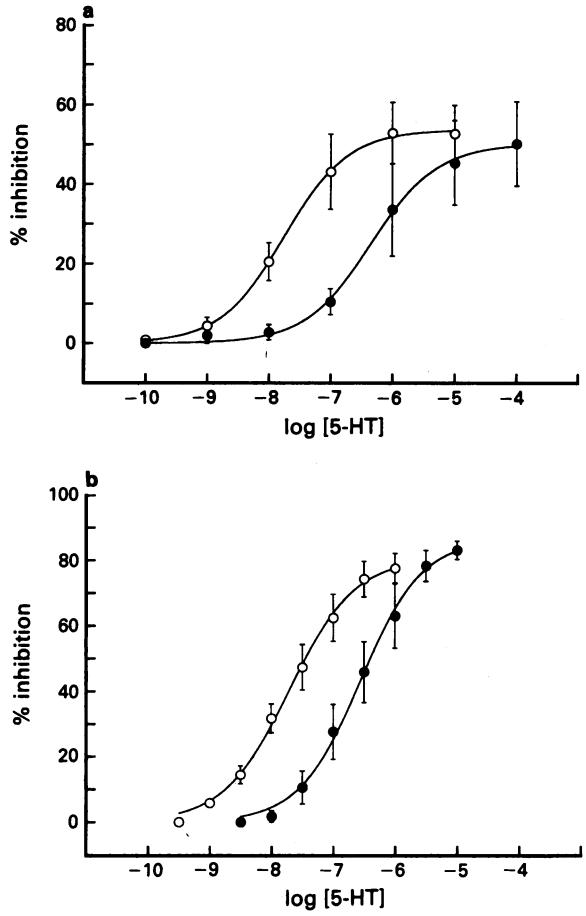

Figure 2 (a) Cumulative concentration-inhibition curves to 5-HT in the absence (○) and presence (●) of the novel, selective 5-HT$_4$ receptor antagonist, GR 113808 (10 nM) in electrically field-stimulated strips of *Cynomolgus* monkey urinary bladder. Each point represents the arithmetic mean ± s.e.mean for 5 experiments. (b) Cumulative concentration-inhibition curves to 5-HT in the absence (○) and presence (●) of DAU 6285 (1 μM) in electrically field stimulated strips of *Rhesus* monkey urinary bladder. Each point represents the arithmetic mean ± s.e.mean for 4 experiments.

Table I Agonist potencies and antagonist affinity estimates at the 5-HT$_4$ receptor in urinary bladder of *Rhesus* and *Cynomolgus* monkey

Agonist	*pEC$_{50}$ (95% CL)*	n	*Antagonist*		*[a]pA$_2$ (95% CL)*	n
Rhesus						
5-HT	7.8 (7.6–8.0)	13	DAU 6285	(1 μM)	7.0 (6.8–7.3)	4
			DAU 6285	(10 μM)	7.5 (7.5–7.6)	4
			RS 23597-190	(1 μM)	7.3 (6.8–7.9)	5
5-MeOT	7.1 (6.4–7.8)	2	DAU 6285	(1 μM)	7.0	2
Renzapride	7.2	1	DAU 6285	(3 μM)	6.9	1
BIMU 8	7.1 (6.7–7.5)	2	DAU 6285	(1 μM)	7.4	2
Cynomolgus						
5-HT	7.6 (7.4–7.8)	10	DAU 6285	(1 μM)	7.1 (7.0–7.3)	4
			GR 113808	(10 nM)	9.5 (9.1–9.8)	5
BIMU 8	6.5 (6.1–6.8)	4	DAU 6285	(1 μM)	6.8	1

[a]Single point analyses.

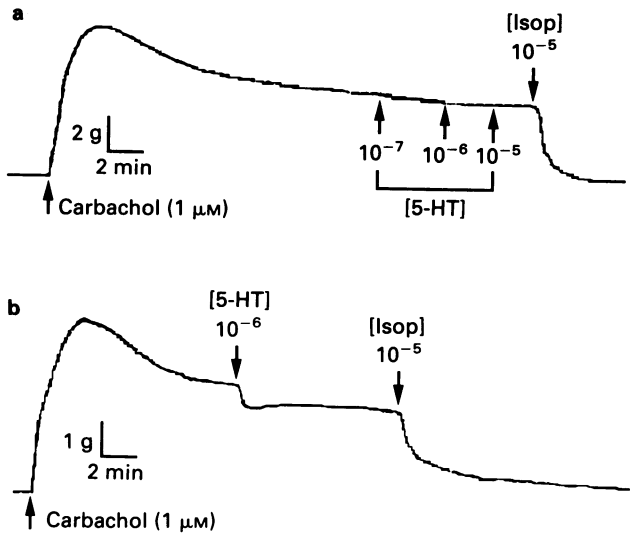

Figure 3 Two strips from the same *Rhesus* monkey urinary bladder responded differently to 5-HT following contraction with carbachol (1 μM). (a) One strip failed to relax upon addition of 5-HT whereas a corresponding strip from the same bladder (b) relaxed upon addition of 5-HT (1 μM). Further relaxation in the responsive strip (b) was achieved with isoprenaline (Isop, 10 μM). These two strips are representative of the inconsistency that hampered studies with carbachol-induced contraction of urinary bladder.

Inhibition of carbachol-induced contracture

Contractures induced by the addition of carbachol (1 μM) were strong (40–100 mN) and slow to develop (Figure 3). However, subsequent addition of 5-HT (up to 10 μM) caused relaxation in only some strips of bladder (Figure 3). Relaxation to 5-HT, when present, was unaffected by TTX (3 μM), suggesting a non-neuronally-mediated effect. Furthermore, relaxation by 5-HT versus carbachol was proportionately less than that induced by 5-HT against contractions to field stimulation (see below). Isoprenaline (10 μM) and forskolin (30 μM; data not shown), on the other hand, caused significant and consistent relaxation of the carbachol-induced contracture (Figure 3) independently of the effectiveness of 5-HT.

Localizing the 5-HT$_4$ receptor using direct muscle excitation

Figure 4 shows that electrically-evoked contractile responses were abolished by TTX (3 μM) and greatly inhibited ($\geqslant$ 90%) by atropine (1.0 μM), suggesting neuronal release of acetylcholine (ACh) as a mechanistic base for the contractile response. Lignocaine (100 μM) and ω-conotoxin GVIA (0.1 μM) also inhibited the responses (Figure 4).

In the presence of TTX (3 μM) and atropine (1 μM), electrical stimulation (using an increased pulse width of 10 ms; all other parameters unchanged) evoked direct contractions of smooth muscle that were similar in magnitude to those evoked neurogenically (Figure 4). Figure 4 shows that these atropine and TTX-insensitive contractions were also resistant to lignocaine (100 μM) and ω-conotoxin GVIA (0.1 μM), militating against the possibility of TTX and atropine-resistant neuronal transmission. However, the responses were inhibited significantly by single doses of 5-HT (1 μM; Figure 4) and BIMU 8 (10 μM; data not shown). Full E/[A] curves to 5-HT yielded a pEC_{50} estimate of 7.9 ($n = 2$; Figure 5b). Inhibition by 5-HT was reversed rapidly by addition of a selective 5-HT$_4$ receptor antagonist, GR 113808 (Figure 5a), at a concentration (3 μM) approximately $10^4 \times K_D$ (Grossman *et al.*, 1992). These findings suggest a post-junctional location

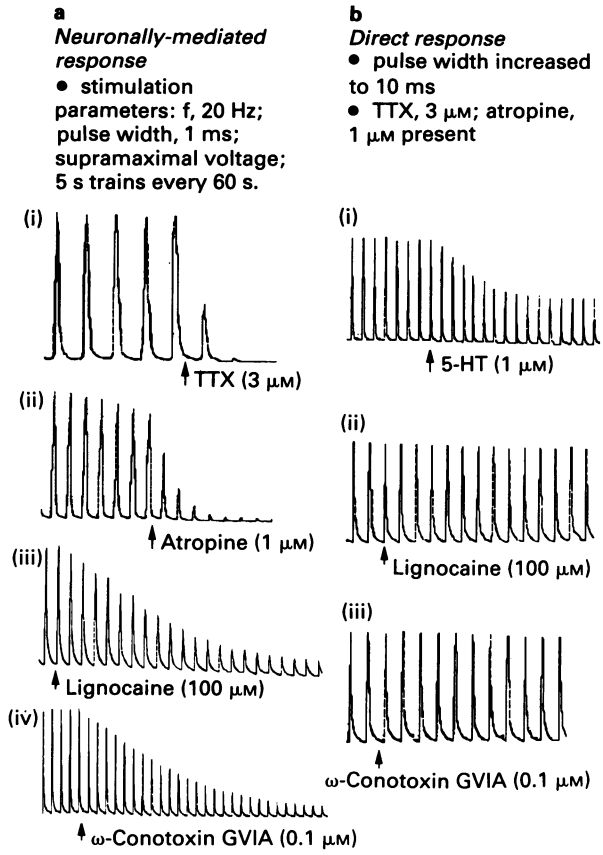

Figure 4 Location of 5-HT$_4$ receptors in strips of urinary bladder of *Rhesus* and *Cynomolgus* monkey by comparison of contractions evoked neurogenically and by direct stimulation of muscle. (a) Neurogenically-evoked contractions were abolished by tetrodotoxin TTX (3 μM; (i)) and greatly inhibited by atropine (1 μM; (ii)) indicating a neuronal release of ACh as the basis for the contractile response. Other inhibitors of neuronal function, lignocaine (100 μM; (iii)) and ω-conotoxin GVIA (0.1 μM; (iv)) also inhibited responses. (b) Contractions of similar magnitude were evoked in the presence of TTX (3 μM) and atropine (1 μM) by increasing the pulse width from 1 ms to 10 ms. These responses were resistant to inhibition by lignocaine (100 μM; b (ii)) and ω-conotoxin GVIA (0.1 μM; (b) (iii)) indicating that they were not the result of TTX-resistant neuronal transmission. Contractions elicited via direct stimulation of muscle (b(i)) were inhibited consistently by 5-HT (1 μM) or BIMU 8 (10 μM; data not shown), demonstrating the presence of post-junctional 5-HT$_4$ receptors in urinary bladder of monkey.

of the 5-HT$_4$ receptor. Due to limited tissue availability, concentration-inhibition curves to other 5-HT$_4$ receptor agonists were not constructed.

Use of different anatomical regions of the bladder and drug free Tyrode

Electrical stimulation of strips taken from other regions of the bladder (dome and bladder neck) evoked contractile responses of consistent magnitude which were inhibited by 5-HT ($n = 1$, data not shown), suggesting that the response to 5-HT (inhibitory versus excitatory) in monkey bladder is not a function of anatomical region. Furthermore, the use of Tyrode solution free from drugs to facilitate agonist equilibrium and 5-HT$_4$ receptor isolation did not affect the potency of 5-HT ($pEC_{50} = 7.7$; $n = 1$; data not shown) and BIMU 8 ($pEC_{50} = 6.4$; $n = 1$; data not shown) nor the affinity estimate for DAU 6285 ($pA_2 = 6.8$ vs. BIMU 8; $n = 1$; data not shown). Nevertheless, all other experiments conducted in the present study used Tyrode solution containing 5-HT receptor antagonists and drugs to promote equilibrium conditions.

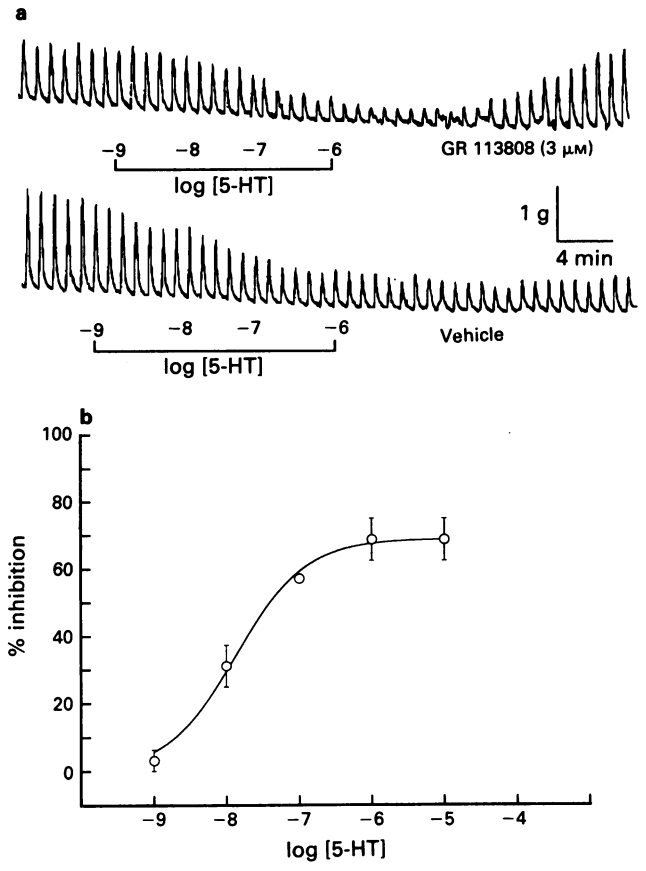

Figure 5 Determining the location of the 5-HT$_4$ receptor in monkey urinary bladder. (a) Increasing pulse width to 10 ms in the presence of tetrodotoxin (TTX, 3 μM) and atropine (1 μM) evoked contractions in strips of *Cynomolgus* monkey urinary bladder via direct stimulation of the muscle which were sensitive to inhibition by 5-HT (1 nM–1 μM). This inhibitory effect of 5-HT was readily reversed by the novel, selective 5-HT$_4$ receptor antagonist GR 113808 (3 μM; upper trace) whereas the addition of vehicle to a corresponding strip (lower trace) did not affect the inhibition caused by 5-HT. (b) Concentration-inhibition curve to 5-HT under conditions of direct stimulation of muscle yields a pEC$_{50}$ value of 7.9 in *Cynomolgus* monkey urinary bladder. Each point represents the arithmetic mean ± range for 2 experiments.

Discussion

The present study demonstrates that 5-HT inhibits electrically-evoked contractions of isolated urinary bladder from *Rhesus* and *Cynomolgus* monkeys through activation of a 5-HT$_4$ receptor. This inhibitory action of 5-HT contrasts with its excitatory action in strips of isolated bladder of man (Corsi *et al.*, 1991).

The presence of a 5-HT$_4$ receptor in the bladder of monkey is indicated strongly by the fact that 5-HT$_4$ receptor agonists from three distinct structural classes (indoleamine derivatives, 5-HT, 5-methoxytryptamine; substituted benzamides, renzapride; benzimidazolones, BIMU 8) evoked inhibition. Furthermore, three antagonists at the 5-HT$_4$ receptor caused parallel displacements of E/[A] curves to 5-HT (and other agonists) yielding single point pA$_2$ estimates similar to those generated in standard 5-HT$_4$ assay systems (GR 113808, Grossman *et al.*, 1992; DAU 6285, Bockaert *et al.*, 1992; RS 23597-190, Eglen *et al.*, 1992). Due to a limited supply of tissue, Schild regression analyses for unequivocal estimates of antagonist affinity were not possible.

One important point regarding the currently described pharmacological profile is the relatively low agonist potency of BIMU 8 (8–10 fold lower than 5-HT). This is not in accord with 5-HT$_4$ receptors defined previously where BIMU 8 is usually equipotent with 5-HT (Bockaert *et al.*, 1992). Indeed, the potency estimate for BIMU 8 in the *Cynomolgus* monkey (pEC$_{50}$ = 6.6) is lower than its estimated binding affinity at 5-HT$_4$ receptors in guinea-pig striatum (p*Ki* = 7.9; Grossman *et al.*, 1992). This result is not in agreement with receptor theory and, if reproducible, may point to the existence of heterogeneity among 5-HT$_4$ receptors as has been discussed elsewhere (Ford & Clarke, 1993).

Attempts were made to determine a pre- or postsynaptic location for the 5-HT$_4$ receptor in urinary bladder of monkey. Near-complete inhibition of electrically-stimulated contractions by TTX and atropine indicate that contractions are mediated neurogenically, largely through the release of ACh. In the presence of TTX, 5-HT inhibited carbachol-induced contracture, implying that 5-HT is not acting through neuronally based receptors. However, the interaction of 5-HT with carbachol yielded ambiguous results as the response to 5-HT was inconsistent and less effective (proportionately) than versus neuronally-mediated responses. Compared with neuronally-mediated contractile responses (evoked electrically), contractions to carbachol were sustained, greater in magnitude and slower to develop, indicating a certain lack of congruity between the two methods of evoking contractions.

In order to investigate better the location of the 5-HT$_4$ receptor, direct stimulation of the smooth muscle was employed. Under these conditions (increased pulse width, in the presence of TTX and atropine), responses were obtained which were equivalent to neuronally-mediated contractions with regard to time-course and tension development. Furthermore, ω-conotoxin GVIA (0.1 μM) and lignocaine (100 μM) failed to inhibit the responses but 5-HT and BIMU 8 caused inhibition of induced contractions. E/[A] curves to 5-HT yielded potency values similar to those generated against neurogenically-mediated contractions. Furthermore, 5-HT-induced inhibition of contractions was reversed completely by addition of the selective 5-HT$_4$ receptor antagonist GR 113808 (Grossman *et al.*, 1992), indicating that inhibition is mediated by post-junctional 5-HT$_4$ receptors. The possibility of some neuronal 5-HT$_4$ receptors, in addition to post-junctional 5-HT$_4$ receptors cannot, however, be eliminated entirely.

Previous reports have indicated that 5-HT$_4$ receptors in many tissues, including smooth muscle, couple preferentially to the stimulation of adenylyl cyclase and elevate intracellular cyclic AMP levels (see Bockaert *et al.*, 1992; Ford *et al.*, 1992a). Theoretically, if such a coupling mechanism were operational for 5-HT$_4$ receptors in the bladder of monkey, a locus of action on smooth muscle cells (where elevating cyclic AMP is characteristically relaxant) would be a more attractive explanation of the data than one on neurones (where elevating cyclic AMP characteristically enhances neurotransmitter release).

It may be postulated that the differential responses to 5-HT on the isolated urinary bladder of man (potentiation of contractions, Corsi *et al.*, 1991) versus monkey (inhibition of contractions, present study) result from the use of anatomically distinct regions (anterior dome, man; dorsal midline, monkey). Indeed, Klarshov & Hørby-Petersen (1986) found in pig that 5-HT contracts the detrusor muscle yet relaxes the trigone, bladder neck and urethral smooth muscle. Initial studies using samples from dome and neck of monkey bladder suggest that the 5-HT$_4$ receptor-mediated relaxations described above may be widespread through this hollow organ; further investigation will be required for confirmation. However, no such studies in the bladder of man were reported by Corsi *et al.* (1991). Based on our preliminary experiments, the response to 5-HT in human bladder also appears to be independent of anatomical region (Waikar, Ford & Clarke, unpublished observations). Similarly, the difference between the present study in monkey and that of Corsi *et al.* (1991) in man is not related to the use of drugs to

isolate pharmacologically the 5-HT_4 receptor (present study). Based upon previous reports (see Introduction), the most likely explanation is species variation. Such species variability in the response to 5-HT_4 receptor stimulation does not appear to be restricted to the urinary bladder. The effect of 5-HT_4 receptor activation on ileal smooth muscle, for example, is inhibitory in rat (Tuladhar *et al.*, 1991) yet excitatory in guinea-pig (contraction of ileum and potentiation of electrically-stimulated ileal contractions; Craig *et al.*, 1990). Furthermore, certain reflexes that involve both contraction and relaxation are facilitated by 5-HT_4 receptor agonism in guinea-pig (peristaltic reflex; Buchheit & Buhl, 1991; Craig & Clarke, 1991) and ferrets (emesis; Bhandari & Andrews, 1991).

Given the long-standing role of 5-HT in neuromodulation (Hen, 1993), it is conceivable that the 5-HT_4 receptor plays a key physiological role in modulating neuromuscular activity, particularly with regard to the regulation of movement and co-ordinated rhythmic activity in mammalian hollow organs (alimentary tract, urinary bladder, atria of heart). Although the source of 5-HT for such a role is not readily apparent (at least in heart and urinary bladder) it must be recalled that 5-HT has been detected in peripheral tissues, in addition to the alimentary tract, and may be released from sympathetic postganglionic neurones or chromaffin cells or both (Verbeuren, 1989; 1992). It is postulated, therefore, that the 5-HT_4 receptor is integrated into neuromuscular circuits so as to facilitate co-ordinated rhythmic activity of a functional nature (e.g. peristaltic reflex; contraction of the urinary bladder). This control may be manifested as inhibition in some species or excitation in others. Nevertheless, it provides an opportunity for the development of therapeutic agents for rhythmic disorders such as supraventricular arrhythmias, gastro-oesophageal reflux disorders, gastro-paresis, irritable bowel syndrome and urinary incontinence.

In conclusion, the present study demonstrates, for the first time, that 5-HT_4 receptors function to relax smooth muscle in the urinary bladder of two monkeys of the macaque family. A role for 5-HT_4 receptors in the physiology and pathology of urinary bladder function is postulated.

The authors wish to thank Susan Winslow, Syntex Research, for provision of the tissues used in these studies and Dr C.A. Rizzi, Institute De Angeli, Milan, Italy, for his gifts of DAU 6285 and BIMU 8.

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(Received May 4, 1993
Revised August 14, 1993
Accepted October 4, 1993)