Pharmacological analysis of 5-hydroxytryptamine effects on electrically stimulated human isolated urinary bladder

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1 5-Hydroxytryptamine (5-HT) is able to potentiate the contractions induced by electrical field stimulation of pieces of human isolated urinary bladder. On the basis of available selective 5-HT agonists and antagonists, we have further investigated the receptors involved and their site of action.

2 5-HT produced a concentration-dependent increase of the contractile response to electrical field stimulation from 0.1 nM to 1 μ M. At higher concentrations (up to 100 μ M) the effect decreased. These activities were mimicked by a variety of 5-HT agonists, for which the following rank order of potency was found: 5-HT > α -methyl 5-HT > 5-methoxytryptamine > 5-carboxamidotryptamine > 2-methyl 5-HT $\gg$ GR 43175. In addition the gastro-prokinetics agents metoclopramide, cisapride and the 5-HT₃ antagonist ICS 205-930 behaved as 5-HT agonists, their EC₅₀ values being 2.3, 0.3, and 0.5 (μ M) respectively.

3 The 5-HT potentiating effect was resistant to antagonism by ondansetron (1 μ M) and cyanopindolol (1 μ M), selective 5-HT₃ and 5-HT_{1A/1B} antagonists respectively. The 5-HT₂ antagonists ketanserin (1 μ M), spiperone (1 μ M) and methysergide (1 μ M) also showed a weak inhibitory activity. Methiothepin (0.1–1 μ M) antagonized only the inhibitory effect of 5-HT. Metoclopramide (0.1–1 μ M), cisapride (0.01–0.1 μ M) and ICS 205-930 (0.3–3 μ M) all produced a rightward displacement of the 5-HT response curve with concomitant reduction of the maximum response. The pA₂ values calculated were 7.4, 8.5 and 7.0 respectively. The antagonism of metoclopramide was receptor specific and was not apparently related to interactions with dopaminergic activity since domperidone showed no antagonism of 5-HT, and metoclopramide, itself, did not antagonize the potentiating effect of prostaglandin F_{2a}.

4 The receptor involved in the potentiating effect of 5-HT may be located prejunctionally because 5-HT did not potentiate responses to acetylcholine (ACh) or electrical field stimulation with the parameters of direct muscle excitation. Also, since the 5-HT potentiating effect was blocked by atropine, it may be attributed to a release of ACh.

5 This study suggests that in the human urinary bladder 5-HT causes two opposite effects on the contractile response to electrical field stimulation. A potentiating effect at low concentrations due to an interaction with an atypical receptor, different from the classical 5-HT₁, 5-HT₂, or 5-HT₃ subtypes and an inhibitory effect at greater concentrations probably due to an interaction with 5-HT₁-like receptors. The possibility that this atypical receptor possesses some characteristics of those found in other isolated preparations like guinea-pig ileum, rat oesophagus and mouse embryo colliculi neurones is discussed.

Keywords: Urinary-bladder; 5-HT; electrical-stimulation; ondansetron; methiothepin; ketanserin; methysergide; metoclopramide; cisapride; ICS 205-930.

Introduction

5-Hydroxytryptamine (5-HT) has a variety of actions on many cell types and knowledge of the membrane receptors which mediate its cellular responses has progressed significantly in the last decade. The current view is that there are three main groups of functional 5-HT receptors, 5-HT₁-like, 5-HT₂ and 5-HT₃ (Bradley *et al.*, 1986). The latter two equate with Gaddum & Picarelli's 'D' and 'M' receptors respectively, first identified in guinea-pig ileum (Gaddum & Picarelli, 1957).

A range of potent and specific receptor blocking drugs is now available for either 5-HT₂, e.g. ketanserin, methysergide and spiperone (Leysen *et al.*, 1981; Humphrey *et al.*, 1982) or 5-HT₃ receptors e.g. ondansetron (Brittain *et al.*, 1987), ICS 205-930 (Richardson *et al.*, 1985) and MDL 72222 (Fozard, 1984), which allows their identification and characterization in both the brain and periphery (Figure 1). No good antagonists are available for 5-HT₁-like receptors which have largely been classified on the basis of data with agonists. These receptors are clearly heterogeneous comprising several subtypes, but all can be characterized by the high agonist potency of 5-carboxamidotryptamine (5-CT) and are blocked by methiothepin (Bradley *et al.*, 1986).

5-HT has been reported to potentiate the contraction induced by electrical field stimulation of isolated urinary bladder of mouse and man. In mouse bladder, the potentiating action of 5-HT has already been analysed and the activation of facilitatory 5-HT_{1B} and 5-HT₂ receptors, located on intrinsic nerves has been demonstrated (Holt *et al.*, 1986; Clean *et al.*, 1989). In human bladder the potentiating effect of 5-HT was observed at very low concentrations and was unaffected by methysergide and morphine (Hindmarsh *et al.*, 1977). However, a detailed pharmacological analysis of this effect in human bladder is still lacking.

Exploiting currently available selective 5-HT agonists and antagonists we have now examined further the potentiating

Figure 1 Chemical structure of two 5-HT₃ antagonists, ICS 205-930 and MDL 72222.

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effect of 5-HT on electrically stimulated human bladder to characterize the receptors involved and to elucidate their site of action.

Methods

Preparation of human urinary bladder strips

Specimens from the anterior part of the dome of the urinary bladder were obtained from patients undergoing cystectomy *en bloc* because of bladder malignancy. Immediately after surgical removal, the serosal and mucosal layers were dissected away and muscular strips (approximately 10 mm long, 3 mm wide, 3 mm thick) were cut. Each bladder strip was mounted vertically between two platinum electrodes and placed in a 20 ml glass organ bath containing modified Krebs solution, aerated with 95% O₂ and 5% CO₂ and maintained at 37°C. The composition of this physiological solution was (mM): NaCl 120, KCl 4.7, MgSO₄ 7H₂O 0.6, KH₂PO₄ 1.2, NaHCO₃ 2.5, CaCl₂ 2.0 and glucose 10. Preparations were initially placed under tension of 2 g and allowed to equilibrate for at least 1 h before an experiment was started. The tension was measured with a Grass Instrument FT03 isometric force displacement transducer, connected to a Linseis model 7025 polygraph. The contractile capacity of all preparations was tested by exposure to KCl 100 mM.

Characterization of the response to electrical stimulation

Bladder strips were electrically field stimulated with a Grass S88 stimulator. Trains of electrical pulses of 5 s in duration were delivered at intervals of 1 min, at pulse width of 0.1 ms and supramaximal output voltage (30–80 V). Pulse frequency was 20 Hz producing about half maximal contraction. These trains of electrical pulses produced reproducible contractile responses for at least 3 h. An analysis of the components of the twitch response was carried out by examination of the effect of tetrodotoxin (TTX) 0.3 µM and atropine (0.001–1 µM).

Measurement of the effects of 5-hydroxytryptamine agonists

Bladder strips were electrically stimulated at the parameters listed above. When the contractile responses were stabilized (usually within 30 min) a cumulative concentration-response curve (CRC) for 5-HT or 5-HT selective agonists was run at 0.5 log₁₀ unit intervals (Van Rossum, 1963). Since the CRCs to 5-HT or 5-HT agonists were not reproducible (tachyphylaxis occurred) a single CRC was determined in each strip. In a typical experiment, from four to eight strips were obtained from a single bladder specimen. On each occasion, one of available strips was treated with 5-HT, so that the potentiating effect of 5-HT agonists could be examined in parallel with that of 5-HT. In some strips the potentiating effects of prostaglandin F_{2α} (PGF_{2α}), metoclopramide, cisapride and ICS 205-930 were also investigated.

Measurement of the effects of 5-hydroxytryptamine antagonists

Bladder strips were stimulated at the parameters listed above. When the contractile responses had stabilized, selective 5-HT antagonists were added to the bath and 2 h later a CRC to 5-HT was constructed. Comparisons of the effect of antagonists were made by comparing the CRC to 5-HT in test preparations and in matched controls. This protocol enabled the effect of antagonists on the twitch height *per se* to be examined. The effect of metoclopramide, cisapride, ICS 205-930, atropine and domperidone were also investigated following the same protocol. To minimize the binding of 5-HT antagonists to the glass, the organ baths were silanised with 5%

dimethyldichlorosilane in toluene and rinsed in distilled water before use.

Effect of 5-hydroxytryptamine on direct electrical muscle excitation

Following a 30 min period of field stimulation at the parameters listed above, TTX (0.3 µM) was administered. When the twitch response was abolished, the pulse width was increased to 6 ms and once the response to stimulation had been stabilized, 5-HT was added cumulatively.

Effect of 5-hydroxytryptamine on contractile responses to acetylcholine

The effect of 5-HT on responses to a single submaximal concentration of acetylcholine (ACh) were also investigated. Initially, concentrations of ACh that produced equivalent increases in contractile tone to electrical stimulation (20 Hz for 5 s, every 60 s, 0.1 ms pulse width, V_{max}) were determined. Concentrations of 30–100 µM ACh were most commonly used and for each preparation this selected concentration was kept constant throughout the experiment. Once reproducible responses to ACh had been obtained, each preparation was electrically stimulated for 15 min and exposed to a single concentration of 5-HT. After maximum potentiation had been obtained the stimulator was switched off and ACh added immediately. Following a 30 min wash period this cycle was repeated, and the effect of different concentrations of 5-HT analyzed. One of each pair of preparations was exposed to vehicle to monitor spontaneous changes in responses to electrical stimulation and ACh.

Data analysis

Potentiating effects were transformed into a percentage of the contractile responses to 100 mM KCl. Data are expressed as means ± s.e.mean.

Where applicable, the analysis of CRC was performed with the logistic curve fitting programme ALLFIT (De Lean *et al.*, 1978). The logistic function used was:

$$Y = E_{\max} \frac{[A]^n}{[A]^n + EC_{50}^n}$$

where Y is the observed response, [A] the concentration of agonist, E_{max} the maximal response to agonist, EC₅₀ the location and n the slope. Ratios of concentration (CR) inducing 50% of maximal response to 5-HT, in the presence or absence of antagonists are expressed as means ± s.e.mean. pA₂ values were obtained by use of the Schild equation: pA₂ = –log[Ant] + log(CR – 1) where [Ant] is the concentration of the antagonist used (Arunlakshana & Schild, 1959). The IC₅₀ estimated for atropine represents the concentration of atropine yielding 50% inhibition. This value was obtained by regression analysis. The significance of differences between mean values was determined at P < 0.05 using a Student's *t* test.

Drugs used

The following compounds were used: atropine sulphate (Sigma), acetylcholine chloride (Merck), 5-hydroxytryptamine creatinine sulphate (Sigma), prostaglandin F_{2α} (Sigma), spiperone (RBI), domperidone (Janssen), metoclopramide hydrochloride (Lepetit), cisapride (Janssen), methysergide bimalate (Sandoz).

The following compounds were gifts: ketanserin (Janssen), methiothepin maleate (Roche), cyanopindolol (Sandoz), ICS 205-930 (Sandoz) (Figure 1) and we acknowledge the generosity of these companies. GR 43175 (see Humphrey *et al.*, 1988) ondansetron, α-methyl 5-HT, 5-carboxamidotryptamine, 2-methyl 5-HT, 5-methoxytryptamine were synthesized by the Chemistry Research department at Glaxo Group Research.

All drugs were dissolved in Krebs medium, unless otherwise stated, to give a final concentration of 1–100 mM. Spiperone at a concentration of 10 mM was dissolved in dimethylsulphoxide (DMSO) and diluted in distilled water; ketanserin at a concentration of 1 mM was dissolved in distilled water containing 1% v/v of lactic acid; domperidone at a concentration of 10 mM was dissolved in dimethylformamide, 50% v/v, and diluted in distilled water. Appropriate solvent controls were found to be without effect on 5-HT-induced potentiation (results not shown).

Results

Characteristics of the contractile response of the human isolated bladder to electrical field stimulation

Electrical field stimulation of the human isolated bladder (20 Hz for 5 s every 60 s, 0.1 ms pulse width, supramaximal voltage) produced reproducible contractile responses which were abolished by TTX (0.3 μ M) and markedly inhibited by atropine (IC_{50} (with 95% C.L.) = 0.01 μ M (0.005–0.03) n = 3, maximum inhibition approximately 90–95%). The small twitch ($8.8 \pm 2.0\%$ of the original response), remaining after atropine, was insensitive to TTX.

Effects of 5-hydroxytryptamine and 5-HT agonists on contractile response of the human isolated bladder to electrical field stimulation

The cumulative addition of 5-HT produced a biphasic effect. At concentrations from 0.1 nM to 1 μ M, 5-HT induced a concentration-dependent increase of the contractile response to electrical field stimulation. At greater concentrations (from 3 to 100 μ M), 5-HT inhibited its own potentiating effect even though, at the highest concentration used, the level of contraction did not return to the control values (Figure 2).

The potentiating effect of 5-HT was mimicked by a variety of 5-HT agonists (Figure 3).

Figure 2 Experimental recording illustrating the potentiating effect of 5-hydroxytryptamine (5-HT) on the contractile response of the human bladder to neurogenic electrical field stimulation (20 Hz for 5 s, 0.1 ms pulse width, every 1 min, 60 V). Dots represent the points of 5-HT cumulative administration in 0.5 $\log_{10}$ unit intervals.

Figure 3 Potentiating effect of 5-hydroxytryptamine (5-HT) and of a variety of 5-HT agonists on the contractile response of the human isolated bladder to neurogenic electrical field stimulation. Symbols represent 5-HT (●); α -methyl-5-HT (▲); 5-methoxytryptamine (△); 2-methyl-5-HT (■); 5-carboxamidotryptamine (□) and GR 43175 (○). Values shown are the means from 12 experiments for 5-HT and 2–4 experiments for the 5-HT agonists. The s.e.mean is omitted for clarity.

The maximum responses (mean $\pm$ s.e.mean) produced by 5-CT (5-HT₁-like agonist), 5-methoxytryptamine (5-MeOTr, (5-HT₁-like agonist), α -methyl-5-HT (5-HT₂ agonist) and 2-methyl-5-HT (5-HT₃ agonist) were 55 ± 5.5 , 65 ± 10.3 , 65 ± 18 and $94 \pm 14.5\%$ respectively of the maximum response produced by 5-HT, GR 43175 (5-HT₁-like agonist) was completely ineffective. These results, together with the potency (as EC_{50}) and the equipotent molar concentration ratio (e.p.m.r.) of 5-HT and of 5-HT agonists are shown in Table 1.

The rank order of potency of these compounds was: 5-HT > α -Me5-HT > 5-MeOTr > 5-CT > 2-Me5-HT $\gg$ GR 43175.

Metoclopramide, cisapride and ICS 205-930 were all agonists and their EC_{50} ($\pm$ s.e.mean) values (μ M) were respectively: $2.3 (\pm 0.7)$; $0.3 (\pm 0.1)$; $0.5 (\pm 0.2)$. The maximal responses to metoclopramide, cisapride and ICS 205-930 were approximately 70%, 45% and 15% of the 5-HT maximum (Figure 4). These compounds were also tested as antagonists (see below).

Effect of 5-hydroxytryptamine antagonists

The potentiating action of 5-HT was substantially resistant to antagonism by ondansetron (1 μ M), a 5-HT₃ antagonist and cyanopindolol (1 μ M), which has a high affinity at 5-HT_{1A}/5-HT_{1B} recognition sites in brain radioligand binding studies (data not shown). The potentiating effect of 5-HT was also resistant to methiothepin (0.1–1 μ M), which conversely antagonized the inhibitory effect of 5-HT (Figure 5). At a low concentration (0.1 μ M) methysergide had little effect on the potentiating action of 5-HT, whilst at a higher concentration (1 μ M) it acted as an antagonist. However, this was largely

Table 1 Agonist potencies of 5-hydroxytryptamine (5-HT) and 5-HT agonists in the potentiation of responses to electrical stimulation of the human isolated urinary bladder

Agonist	n	EC_{50} (μ M)	e.p.m.r. (5-HT = 1)	% 5-HT maximum response
5-Hydroxytryptamine	12	0.01 ± 0.002	1.0	100
5-Carboxamidotryptamine	4	2.6 ± 0.8	260	55.0 ± 15.0
α -Methyl 5-HT	3	0.3 ± 0.07	30	65.0 ± 18.0
2-Methyl 5-HT	3	30 ± 1.2	3000	100.0 ± 15.0
5-Methoxytryptamine	4	0.5 ± 0.08	50	65.0 ± 10.0
GR 43175	2	NSE up to 100 μ M	—	—

Values are given $\pm$ s.e.mean. NSE = no significant effect.
 EC_{50} values were determined as described in the methods.
 e.p.m.r. = equipotent molar concentration ratio.

Figure 4 Potentiating effect of 5-hydroxytryptamine (5-HT) (○), metoclopramide (●), cisapride (■) and ICS 205-930 (△) on the contractile response of the human isolated bladder to neurogenic electrical field stimulation. Values are the mean of 12 experiments for 5-HT and 3 experiments for the other agonists, with s.e.mean shown by vertical bars.

associated with a significant depression of the maximum response to 5-HT ($P = 0.001$ versus control), there being little change in the threshold concentration of 5-HT (Figure 5). Only relatively high concentrations of 5-HT₂ antagonists, ketanserin ($1 \mu\text{M}$) and spiperone ($1 \mu\text{M}$), caused a displacement to the right of the CRC to 5-HT (approximately fourfold) yielding a pA_2 of 6.5 (Figure 6) without a significant depression of the maximum response ($P > 0.05$ versus control for both agonists). In none of these experiments did the 5-HT

Figure 5 Concentration-effect curves to 5-hydroxytryptamine (5-HT) for the potentiation of responses to electrical stimulation of the human urinary bladder in the absence (○) and in the presence of methiothepin at $0.1 \mu\text{M}$ (●) and $1 \mu\text{M}$ (■) in (a) or methysergide at $0.1 \mu\text{M}$ (■) and $1 \mu\text{M}$ (●) in (b). Values are the mean of 10 experiments for 5-HT alone and 3 experiments for 5-HT in presence of antagonists, with s.e.mean shown by vertical bars.

Figure 6 Concentration-effect curves to 5-hydroxytryptamine (5-HT) for the potentiation of responses to electrical stimulation of the human urinary bladder in the absence (○) and in the presence of $1 \mu\text{M}$ ketanserin (●) in (a) or $1 \mu\text{M}$ spiperone (●) in (b). Values are the mean of 10 experiments for 5-HT and 3 experiments for 5-HT in presence of antagonists, with s.e.mean shown by vertical bars.

receptor blocking drugs have any significant effect on the contractile response of the human isolated bladder to electrical field stimulation.

Effect of metoclopramide, cisapride and ICS 205-930

Metoclopramide (0.1 – $1 \mu\text{M}$), cisapride (0.01 – $0.1 \mu\text{M}$) and ICS 205-930 (0.3 – $3 \mu\text{M}$) all produced rightward displacement of the CRC to 5-HT. These were accompanied by a concentration-related reduction in the maximum response (Figure 7). For metoclopramide and cisapride the reduction of the maximum response was statistically significant ($P < 0.02$ versus control) for all the concentrations used. Conversely the reduction of the maximum response induced by ICS 205-930 was significant ($P < 0.05$ versus control) only at concentrations of 1 and $3 \mu\text{M}$. A calculation of the pA_2 using the ratio of concentrations inducing 50% of the effect in the absence or in the presence of antagonist was made, despite the depression of the maximum response. Thus for the concentrations of antagonists used the $\text{pA}_2 \pm \text{s.e.mean}$ was: 7.5 ± 0.03 , 8.5 ± 0.03 and 7.0 ± 0.1 for metoclopramide, cisapride and ICS 205-930 respectively. When metoclopramide was tested in the presence of methiothepin ($1 \mu\text{M}$), which inhibits the inhibitory portion of the CRC to 5-HT, the rightward displacement was still accompanied by a significant reduction ($P = 0.02$ versus control) in the maximum response (Figure 7). The antagonistic activity of metoclopramide ($1 \mu\text{M}$) was specific in nature since it did not modify the potentiating effect of $\text{PGF}_{2\alpha}$ in the same tissue ($n = 3$, data not shown). Furthermore domperidone ($1 \mu\text{M}$) which has antidopamine properties like metoclopramide, did not produce any significant effect on the potentiation produced by 5-HT ($n = 3$, data not shown).

Figure 7 Antagonism by metoclopramide (a), cisapride (b), ICS 205-930 (c) and metoclopramide combined with methiothepin (d) of 5-hydroxytryptamine (5-HT)-induced potentiation of responses to electrical stimulation of the human urinary bladder. In (a) symbols represent control responses (○) or the presence of metoclopramide at 0.1 μM (●), 0.3 μM (▲) and 1 μM (■). In (b), symbols indicate control responses (○) or the presence of cisapride at 0.01 μM (●), 0.03 μM (▲) and 0.1 μM (■). In (c) symbols represent control responses (○) or the presence of ICS 205-930 at 0.3 μM (●), 1 μM (△) and 3 μM (▲). In (d) symbols indicate control responses (○), the presence of only methiothepin 1 μM (▲), the presence of 1 μM methiothepin and 0.1 μM metoclopramide (●). Values are the mean of 10 experiments for 5-HT alone and 3 experiments for 5-HT in the presence of antagonists. The s.e.mean is shown by vertical bars.

Effect of 5-hydroxytryptamine on direct electrical muscle excitation

Marked reproducible contractions of the human urinary bladder were obtained by direct excitation of the smooth muscle. In the presence of TTX, contraction could be induced at a pulse width of 6 ms (20 Hz, for 2 s, every min, 40 V). Cumulative addition of 5-HT up to 10 μM had no effect on responses to direct muscle excitation, but at concentrations of 10–100 μM responses were very slightly potentiated with a little increase in resting tone ($n = 3$, data not shown).

Effect of 5-hydroxytryptamine on contractile responses to acetylcholine

In experiments in which both myogenic and neurogenic stimulation was used, the location of the 5-HT site of action

Figure 8 Experimental recording illustrating the effects of 0.03 μM 5-hydroxytryptamine (5-HT) on the contractile response of the human bladder to neurogenic electrical stimulation (20 Hz for 5 s, 0.1 ms pulse width, every 1 min, 50 V) or to exogenous 100 μM acetylcholine (ACh). Dots represent the points of agonist administration (5-HT or ACh) and the starting point for the electrical stimulation (EStim). W = wash.

was investigated. Myogenic contractions induced by concentrations of 30–100 μM ACh were submaximal and brought about similar contractile responses to those of nerve-mediated field stimulation (20 Hz for 5 s, every 60 s, 0.1 ms pulse width, V max). 5-HT, up to 1 μM , potentiated the electrical field stimulation, without modifying the response to ACh 100 μM (an example using 5-HT at 0.03 μM is shown in Figure 8).

Atropine (0.03 μM) attenuated by 49% the excitatory response to neuronal stimulation ($n = 3$). Furthermore it antagonized the CRC to 5-HT. This antagonism was not competitive, being largely associated with a depression of the maximum response to 5-HT (data not shown).

Discussion

In the present study, electrical stimulation of the human bladder induced reproducible contractile responses that were abolished by TTX and almost completely inhibited by atropine, thus supporting the suggestion that in man, unlike several other animal species (mouse, rat), the contractile responses to electrical stimulation of the detrusor are largely mediated by excitation of cholinergic neurones.

Neuronally induced contractions of the human bladder were potentiated in a concentration-related manner, by 5-HT from 0.1 nM to 1 μM confirming the previous findings of Hindmarsh *et al.* (1977). Our study has also revealed that at higher concentrations, 5-HT inhibited its own potentiating effect. Thus an activation of at least two receptors one excitatory, the other inhibitory would be conceivable. However there are two other possible explanations for the observed decreasing response at higher concentrations of 5-HT. Firstly, this may be a result of tachyphylaxis towards the end of the CRC. Secondly, 5-HT may be producing a direct contractile response of the preparation at the higher concentrations employed, thereby reducing the capacity for further contractions to field stimulation. However, both these hypotheses seem unlikely. In fact an antagonist (methiothepin) was found to antagonize the inhibitory effect of 5-HT without affecting the excitatory phase and on the other hand 5-HT, up to 100 μM did not modify the baseline tension (Figure 2). The inhibitory action of 5-HT was insensitive to 5-HT₂ and 5-HT₃ blockade with

ketanserin (Bradley *et al.*, 1986) and ondansetron, but was prevented by methiothepin, a potent 5-HT₁-like and 5-HT₂ antagonist. Taken together these findings suggest that only a 5-HT₁-like receptor is involved. The tryptamine analogues α -methyl 5-HT, 5-CT, 5-methoxytryptamine and 2-methyl 5-HT mimicked the potentiating effect of 5-HT, thus implicating a specific 5-HT receptor in this response. Several results clearly indicate that this receptor does not belong to the 5-HT₁-like, 5-HT₂ and 5-HT₃ families. 5-CT, a potent specific 5-HT₁ agonist, was 260 fold less potent than 5-HT, whereas 5-CT is known to have a similar or an even higher potency than 5-HT at 5-HT₁-like receptors, (Humphrey, 1984) suggesting that such a receptor subtype is not involved. This suggestion is corroborated by the absence of an effect of the 5-HT₁-like receptor agonist, GR 43175 (Humphrey *et al.*, 1988), and also the lack of antagonism by methiothepin and cyanopindolol, 5-HT_{1A} and 5-HT_{1B} antagonists. α -Methyl 5-HT, a potent 5-HT₂ antagonist, was 30 fold less potent than 5-HT, whereas, this tryptamine analogue is known to have a similar potency to 5-HT in isolated preparations containing 5-HT₂ receptors (Humphrey, 1984). The suggestion that 5-HT₂ receptors are not involved is corroborated by the weak antagonism of ketanserin and spiperone. In fact their apparent pA₂ values calculated in this study are at least 100 times less than the appropriate pA₂ values at 5-HT₂ receptors (Humphrey *et al.*, 1982). The weak agonist potency of 2-methyl-5-HT, a potent specific 5-HT₃ agonist, (3000 fold less potent than 5-HT), and the lack of antagonism by ondansetron, a selective 5-HT₃ antagonist, clearly indicate that the potentiating action of 5-HT is unlikely to be due to an involvement of 5-HT₃ receptors.

At high concentrations metoclopramide, cisapride and ICS 205-930 show agonism. The order of potency is cisapride > ICS 205-930 > metoclopramide. At lower concentrations all three compounds can antagonize the 5-HT response. Because of the inhibitory effect of 5-HT it was not easy to test whether these compounds were competitive antagonists or not. Since methiothepin antagonized only the inhibitory part of the curve, experiments were designed in the presence of methiothepin with the aim of achieving a less complicated analysis of its type of antagonism of these compounds. From these experiments it was concluded that for metoclopramide at least part of the antagonism was non-competitive since a reduction of the maximal response was still apparent. This was not due to the interaction with dopamine receptors since domperidone was completely inactive in these experiments. However, the antagonism of metoclopramide was specific in nature since it did not modify the potentiating effect of PGF_{2 α} . It is possible to make an approximate estimate of the pA₂ value for each of these antagonists and these are 8.5 for cisapride, 7.4 for metoclopramide and 7.0 for ICS 205-930. In the way in which we obtained these numbers, they represent only a relative potency of the antagonists on the 5-HT-induced potentiating effect, without showing any evidence of competitiveness for the 5-HT receptor involved and thus comparison with data, reported by other authors, where the above antagonists are competitive would be difficult. However, it is possible that the receptor identified in this study is similar to the receptors identified by other workers in guinea-pig ileum (Sanger, 1987; Kay *et al.*, 1989; Craig & Clarke, 1989), in rat oesophagus (Reeves *et al.*, 1989) or in mouse embryo colliculi neurones (Dumuis *et al.*, 1989). This

receptor as well as those present in guinea-pig ileum (Craig & Clarke, 1990), rat oesophagus (Reeves *et al.*, 1989) and mouse embryo colliculi neurones (Dumuis *et al.*, 1989) is blocked in a competitive manner by micromolar concentrations of ICS 205-930 (pK_B 6.0–6.4), is resistant to 5-HT₃ antagonists having an indole group such as BRL 43694 (ileum and neurones) and ondansetron (neurones and oesophagus). Moreover this receptor, as well as those present in guinea-pig ileum (Sanger, 1987; Craig & Clarke, 1989) and mouse embryo colliculi neurones (Dumuis *et al.*, 1989), is stimulated by high concentrations of benzamide compounds such as metoclopramide and cisapride. In both cases, cisapride was more potent than metoclopramide. The present receptor, as well as that present in rat oesophagus (Reeves *et al.*, 1989), can also be antagonized by the same benzamide compounds such as metoclopramide and cisapride but at lower concentrations. As antagonists, unlike when acting as agonists, the potencies of the two benzamides were similar in the two preparations.

5-Methoxytryptamine is reported to be equipotent with 5-HT in mouse embryo colliculi neurones (Dumuis *et al.*, 1988) and 8 times less potent than 5-HT in guinea-pig ileum (Craig & Clarke, 1990), whereas in the present study it is 50 times less potent. To explain these differences in activity, a different 'reserve' in the receptor-stimulus transduction between the tissues could be postulated or in terms of a species difference. The same variation in sensitivity should be expected also with the other 5-HT selective agonists. However 2-methyl-5-HT, although it was only a weak partial agonist in rat oesophagus (Reeves *et al.*, 1989) or ineffective in mouse embryo colliculi neurones (Dumuis *et al.*, 1989) and guinea-pig ileum (Craig & Clark, 1990), was a weak, but full, agonist in our preparation. Therefore, only a variation in the amount of the effector-couplers for the same 5-HT receptor in different tissues, could be invoked to explain these findings.

A part of this study attempted to determine the specific location of this receptor and thus the site of the potentiating action of 5-HT in human bladder. Some lines of evidence suggest that this receptor is located prejunctionally and involves the release of ACh. Firstly, the contractile responses to neuronal stimulation were potentiated in a concentration-dependent manner by 5-HT. Yet, equivalent responses to ACh in the same preparation were largely unaffected. Secondly, 5-HT (up to 10 μ M) did not alter responses to direct muscle excitation. The mechanism of action of 5-HT in potentiating responses to field stimulation may be attributed to the facilitation of the release of the excitatory transmitter ACh, since the potentiating effect of 5-HT was antagonized by atropine.

In conclusion, our study has demonstrated two opposite effects of 5-HT on the contractile responses to electrical field stimulation, a potentiation at lower doses of 5-HT and an inhibition at higher doses. Interestingly, the pharmacological characteristics of the receptor involved in the potentiating action exclude the possibility that it belongs to the known 5-HT₁, 5-HT₂ and 5-HT₃ receptor types. In several aspects the receptor resembles the atypical 5-HT receptor subtype reported in guinea-pig ileum, rat oesophagus and mouse embryo colliculi neurones. This is possibly the first finding of such an atypical 5-HT receptor in human tissue.

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