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Investigation into the 5-hydroxytryptamine receptor mediating smooth muscle relaxation in the rat oesophagus

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1 An investigation has been made into the 5-hydroxytryptamine (5-HT) receptor mediating relaxation of rat oesophagus in preparations precontracted with carbachol.

2 In tissues treated with pargyline (100 μM) and in the presence of corticosterone (30 μM) and cocaine (30 μM) the potency of 5-HT and 5-methoxytyramine (5-MeOT) was not changed but the maximum response to these agonists was reduced. Thus there was no evidence of metabolism and/or uptake through an amine depleting mechanism.

3 The relaxant concentration-effect curves to 5-HT were shifted to the left in a concentration-related manner by isobutylmethylxanthine (1 and 10 μM), suggesting the involvement of adenosine 3':5'-cyclic monophosphate in these responses.

4 5-HT produced concentration-related relaxations of rat oesophagus with an EC_{50} value of 0.24 μM. Several indole agonists were tested and the following rank order of potency of key agonists obtained: 5-HT > α-methyl-5-hydroxytryptamine = 5-carboxamidotryptamine (5-CT) > 5-MeOT. In contrast, 2-methyl-5-hydroxytryptamine, sumatriptan and 8-hydroxy-2-(di-n-propylamino) tetralin were weak or inactive.

5 The substituted benzamides, metoclopramide, cisapride, renzapride and **R,S**-zacopride acted as partial agonists, producing 60–70% of the 5-HT maximum.

6 The relaxation responses to 5-HT were neither inhibited by antagonists selective for 5-HT_1 or 5-HT_2 receptors nor by the 5-HT_3 receptor antagonists, ondansetron, granisetron or MDL 72222.

7 The relaxation responses induced by 5-HT, 5-CT, 5-MeOT and renzapride were selectively inhibited by high concentrations of ICS 205-930 with pK_B values of approximately 6.

8 The 5-HT receptor mediating relaxation in rat oesophagus cannot be designated 5-HT_1, 5-HT_2 or 5-HT_3 under the current 5-HT classification, but the observed effects are consistent with stimulation of the putative 5-HT_4 receptor.

Keywords: 5-hydroxytryptamine receptor; relaxation; rat oesophagus

Introduction

It has been observed that 5-hydroxytryptamine (5-HT) produces relaxation of rat isolated oesophageal muscularis mucosa precontracted with muscarinic agonists (Bieger & Triggle, 1985). This relaxation was not antagonized by methysergide, metergoline or ketanserin suggesting that 5-HT_1 and 5-HT_2 receptors, as classified by Bradley *et al.* (1986), were not involved in the response. Additionally, Bieger & Triggle (1985) reported that the relaxation induced by 5-HT was not affected by tetrodotoxin suggesting that it was unlikely that neuronal 5-HT_3 receptors were involved in this response. However, in contrast with this latter observation it has recently been found that the selective 5-HT_3 receptor antagonists, MDL 72222, granisetron and ICS 205-930, inhibit the effect of 5-HT in this preparation (Triggle *et al.*, 1988).

The aim of the present study was to characterize further the receptor type mediating the 5-HT-induced relaxation of rat oesophagus by use of selective agonists and antagonists. A preliminary account of this work has been presented to the British Pharmacological Society (Reeves *et al.*, 1989).

Methods

Female Wistar rats weighing between 120–180 g were killed by cervical dislocation, the abdomen opened and the most distal 2 cm of the oesophagus removed. The segments of oesophagus (one from each animal), with muscle layers and mucosa intact, were suspended in the longitudinal plane under an initial tension of approximately 0.5 g in a modified Krebs-Henseleit solution at 32°C gassed with 95% O_2/5% CO_2. The ionic composition of the Krebs-Henseleit solution (in mM) was NaCl 118.5, $NaHCO_3$ 25.0, KCl 4.7, $MgSO_4$ 0.6, KH_2PO_4 1.2, $CaCl_2$ 1.3 and glucose 11.1. This solution routinely contained indomethacin (3 μM) and except in experiments specifically aimed at investigating the effects of ketanserin, it contained ketanserin (1 μM).

Effects of agonists and antagonists

The oesophageal preparations were contracted by addition of a submaximal concentration of carbachol (1 μM) to the bathing solution, and on establishing a stable contractile response, a concentration of 5-HT was added. Once the maximum relaxation response to that concentration of 5-HT was obtained, higher concentrations were added in a cumulative manner to produce cumulative concentration-effect curves to 5-HT.

In agonist studies, a cumulative concentration-effect curve to 5-HT was constructed followed either by a second curve to 5-HT in control experiments, or by a curve to the test agonist. The relative potencies of the agonists were compared with 5-HT at the 50% response level for the 5-HT control concentration-effect curve. In antagonist studies, a control concentration-effect curve to an agonist was constructed followed by a test curve in the presence of the antagonist. Having established a stable contraction to carbachol (1 μM), antagonists were equilibrated for 30 min before construction of the test agonist concentration-effect curve.

In some experiments, following the construction of control curves to 5-HT or 5-methoxytryptamine (5-MeOT) as described above, the tissues were treated with pargyline (100 μM) for 30 min and then washed several times in fresh Krebs solution before the second application of carbachol. In these pargyline-treated preparations the effect of a mixture of

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corticosterone (30 μM) and cocaine (30 μM) on the responses mediated by 5-HT or 5-MeOT was investigated (as described above for antagonist studies). In separate experiments, the effect of isobutylmethylxanthine (IBMX, 1 and 10 μM) on the relaxant responses to 5-HT was investigated.

In all experiments, following the construction of the control concentration-effect curve, the preparations were washed with fresh Krebs solution and allowed to recover for at least 1 h, with further washes, before re-contracting with carbachol prior to the construction of the test curve.

Selectivity of antagonists

Antagonists that inhibited 5-HT-induced relaxations were also tested against isoprenaline-induced relaxations. Control cumulative concentration-effect curves to isoprenaline were constructed in carbachol-contracted preparations, followed by test curves in the presence of the antagonist, as described above for 5-HT.

Analysis of results

The relaxant responses are expressed as a percentage (arithmetic mean ± s.e.mean) of the maximum response obtained in the appropriate control concentration-effect curve. Equipotent molar ratios and EC_{50} values were calculated graphically for each preparation from the 50% response level and expressed as geometric means with 95% confidence limits in parentheses. The negative logarithm of the apparent dissociation constant for an antagonist (pK_B) was estimated by calculation of the mean (+ 95% confidence limits) of the individual results: $pK_B = \log$ (dose-ratio − 1) − log (antagonist concentration). The number of observations is indicated by n.

Drugs

Drugs obtained from commercial sources were 5-methoxytryptamine hydrochloride (5-MeOT, Sigma), 5-hydroxytryptamine hydrochloride (5-HT, Sigma), 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, Research Biochemicals Incorporated), carbachol chloride (BDH Chemicals), cisapride (Janssen), cocaine hydrochloride (May & Baker Ltd), cyanopindolol (Sandoz), (3α-tropanyl)-1H-indole-3-carboxylic acid ester (ICS 205-930, Research Biochemicals Inc.), corticosterone (Sigma), ketanserin tartrate (Janssen), 1αH,3α,5αH-tropan-3yl-3,5-dichloro-benzoate (MDL 72222, Research Biochemicals Inc), 3-isobutyl-1-methylxanthine (IBMX, Aldrich Chemical Company Ltd), isoprenaline bitartrate dihydrate (Ward Blenkinsop), mesulergine (Sandoz), metergoline (Farmitalia), methiothepin maleate (Roche), methysergide hydrogen maleate (Sandoz), metoclopramide hydrochloride (Sigma), pargyline hydrochloride (Abbott Laboratories Ltd), spiperone (Janssen) and tetrodotoxin (Sigma).

Drugs synthesized by Glaxo Group Research Ltd were α-methyl-5-hydroxytryptamine maleate (α-Me-5-HT), 2-methyl-5-hydroxytryptamine hydrochloride monohydrate (2-Me-5-HT), 5-carboxamidotryptamine maleate (5-CT), 1,2,3,9-tetrahydro-9-methyl-3-[-2-methyl-1H-imidazol-1-yl) methyl]-4H-carbazole-4-one hydrochloride (ondansetron; GR38032F), 3-[2-dimethyl-amino]ethyl-N-methyl-1H-indole-5-methane sulphonamide (sumatriptan; GR43175), endo-4-amino-5-chloro-2-methoxy-N-(1-azabi-cyclo[3.3.1] non-4-yl) benzamide hydrochloride (renzapride; BRL24924), endo-4-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)-1-methyl-1H-indazole-3-carboxamide hydrochloride (granisetron; BRL43694), and (R,S)-zacopride hydrochloride.

Results

Effects of 5-hydroxytryptamine receptor agonists

A submaximal concentration of carbachol (1 μM) produced a well maintained contraction (of between 1–2 g) for at least 60 min. 5-HT (0.01–10 μM) produced concentration-related relaxations with a mean EC_{50} value of 0.24 (0.19–0.29) μM and a mean maximum reduction of the carbachol tone of 52.3 ± 3.2% at 10 μM (n = 16); concentrations of 5-HT above 10 μM produced no further relaxation. The 5-HT responses were reproducible and two similar consecutive concentration-effect curves could be obtained in each preparation (Figure 1); thus the response to 5-HT did not change with time.

The effects of the combination of corticosterone (30 μM) and cocaine (30 μM) on responses to 5-HT or 5-MeOT in pargyline (100 μM)-treated preparations are shown in Figure 2. Treatment with pargyline, corticosterone and cocaine failed to shift the concentration-effect curves to 5-HT (ED_{50} values of 0.29(0.12–0.67) and 0.26(0.17–0.40) μM) or 5-MeOT (EC_{30} values of 7.3(4.8–11.2) and 7.2(4.3–12.5) μM) for control and test curves respectively, however, these compounds did produce marked reductions in the maximum responses to 5-HT and 5-MeOT of 27.2 ± 6.0% and 56.5 ± 2.8% (each n = 4) respectively. These reductions in carbachol tone complicated the analysis of the responses to 5-HT and 5-MeOT, and indeed this necessitated comparison of EC_{30} values for 5-MeOT since the maximum response to 5-MeOT after the drug additions did not achieve 50% of the control curve. In addition, although treatment with pargyline did not affect the size of the contraction to carbachol, the administration of corticosterone and cocaine resulted in an inhibition of 36.9 ± 2.7% (n = 8) of the carbachol tone. Preliminary experiments investigating the effects of cocaine alone indicated that the effects observed with the aforementioned mixture of compounds were predominantly due to the action of cocaine. Cocaine (10 μM) alone produced a reduction in carbachol tone of 20.5 ± 3.4% and caused a 27.2 ± 4.0% decrease in the 5-HT (10 μM) maximum (n = 4).

The effects of IBMX on the relaxant response to 5-HT are shown in Figure 3. IBMX alone (1 and 10 μM) had no direct relaxant effect but produced a concentration-related potentiation of the responses to 5-HT resulting in leftward shifts of 3.6(1.2–11.2) at 1 μM and 7.6(1.3–43.4) at 10 μM of the 5-HT concentration-effect curves.

The effects of 5-HT and other 5-HT receptor agonists are shown in Figure 4 and are summarised in Table 1. The effects of 5-HT were mimicked by 5-CT (0.1–100 μM) and α-Me-5-HT (0.1–100 μM) which exhibited equipotent molar ratios of approximately 20, and also by 5-MeOT with an equipotent

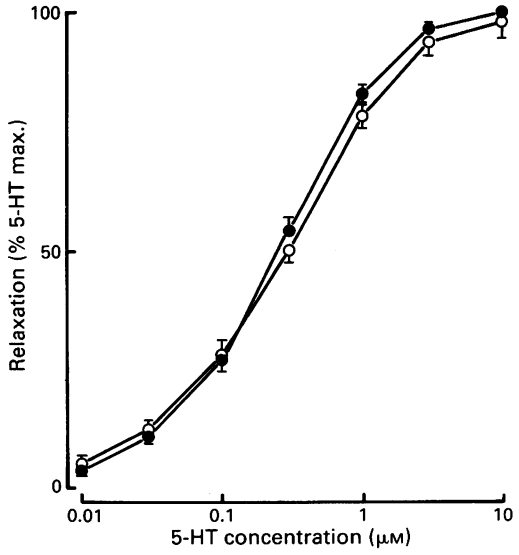

Figure 1 The relaxant effect of repeated cumulative concentration-effect curves to 5-hydroxytryptamine (5-HT) in carbachol-contracted rat oesophagus. (●) Control curve 1; (○) control curve 2. Each point is the mean of 6 observations; s.e.mean shown by vertical bars. Results expressed as % of curve 1 maximum.

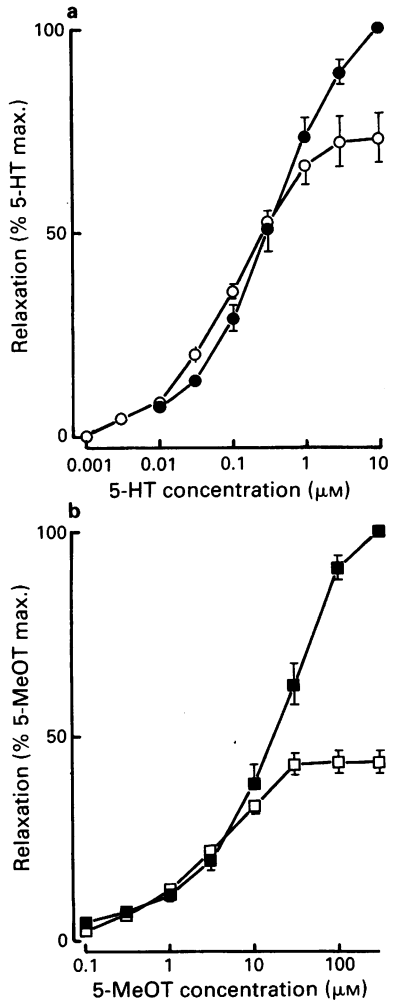

Figure 2 The effect of the combination of 100 μM pargyline (Par), 30 μM corticosterone (Cor) and 30 μM cocaine (Coc) on the relaxant responses to (a) 5-hydroxytryptamine (5-HT) and (b) 5-methoxytryptamine (5-MeOT). (●) Control concentration-effect curve to 5-HT and (○) in the presence of Par + Cor + Coc. (■) Control curve to 5-MeOT and (□) in the presence of Par + Cor + Coc. Each point is the mean of 4 observations; s.e.mean shown by vertical bars. Results are expressed as % of respective control maxima.

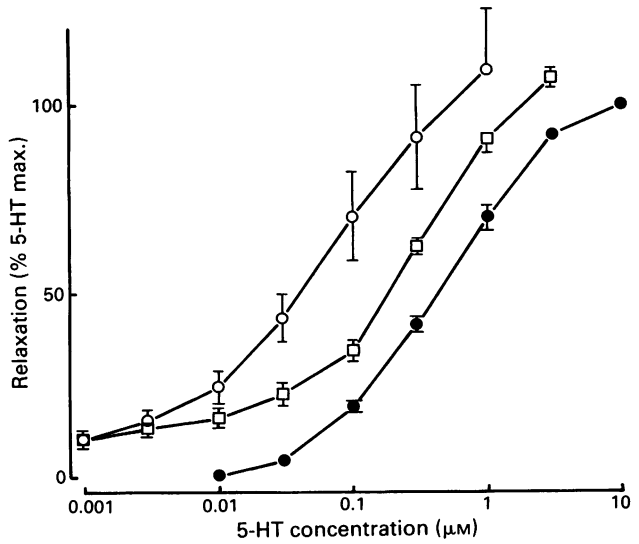

Figure 3 The effect of isobutylmethylxanthine (IBMX) on 5-hydroxytryptamine (5-HT) responses in rat oesophagus. (●) Control 5-HT responses ($n = 6$) and the effect of 5-HT in the presence of (□) 1 μM IBMX and (○) 10 μM IBMX (each $n = 3$). Each point is the mean with s.e.mean shown by vertical bars.

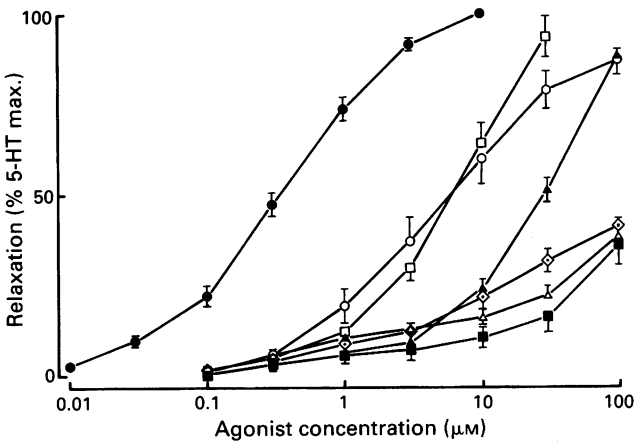

Figure 4 The effect of 5-hydroxytryptamine (5-HT) and other 5-HT receptor agonists (indole derivatives) in the rat oesophagus. (●) Control 5-HT ($n = 24$) and the agonist effect of (○) 5-carboxamidotryptamine; (□) α-methyl-5-HT; (▲) 5-methoxy-tryptamine; (■) 2-methyl-5-HT; (△) sumatriptan and (◇)8-hydroxy-2-(di-n-propylamino)tetralin. Each point is the mean of 4 observations (s.e.mean shown by vertical bars) calculated as a percentage of the control 5-HT maximum.

molar ratio of 90. The maximum responses to these agonists were close to that produced by 5-HT. The other agonists tested, 2-Me-5-HT, 8-OH-DPAT and sumatriptan were weak or inactive at concentrations up to 100 μM. The rank order of potency of the 5-HT agonists tested was: 5-HT > α-Me-5-HT = 5-CT > 5-MeOT > 2-Me-5-HT = sumatriptan = 8-OH-DPAT.

In addition to the effect of the indole analogues of 5-HT, the agonist effects of four substituted benzamides, metoclopramide, cisapride, renzapride and **R,S**-zacopride were investigated and the results obtained are shown in Figure 5 and summarised in Table 1. Each of the benzamides acted as a partial agonist producing approximately 60–70% of the 5-HT maximum, although it must be pointed out that the apparent partial agonist activity of metoclopramide and cisapride could not be examined thoroughly because high concentrations (> 100 μM) produced a non-specific relaxation of the carbachol tone that could not be inhibited by ICS205-930 (see below).

Effects of 5-hydroxytryptamine receptor antagonists

A wide range of 5-HT receptor antagonists with some degree of selectivity for the different 5-HT receptor subtypes have

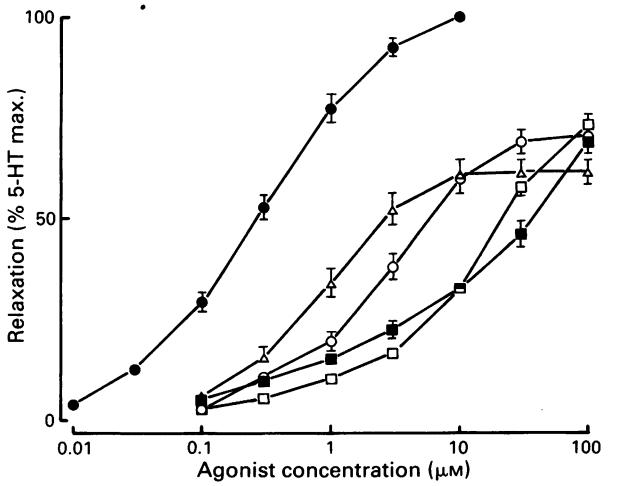

Figure 5 The agonist effect of 5-hydroxytryptamine (5-HT) and certain substituted benzamides in rat oesophagus. (●) Control 5-HT responses ($n = 16$) and the agonist responses of (△) renzapride; (○) **R,S**-zacopride; (■) cisapride and (□) metoclopramide ($n = 4$). Each point is the mean (s.e.mean shown by vertical bars) calculated as a percentage of the 5-HT maximum.

Table 1 Summary of the effects of 5-hydroxytryptamine (5-HT) receptor agonists in rat isolated oesophagus

Agonist	*5-HT receptor selectivity as agonists*	*Equipotent molar ratio where 5-HT = 1* (95% confidence limits)	*% 5-HT maximum responses**
5-HT	Non-selective	1.0	100 ± 0
5-CT	5-HT_1	20.1 (7.9–51.5)	87.1 ± 4.1
α-Me-5-HT	5-HT_2	15.6 (12.6–19.4)	93.6 ± 5.6
5-MeOT	Non-selective (but inactive at 5-HT_3)	90.4 (72.9–112.1)	88.3 ± 1.7
Renzapride	5-HT_4 ?	16.5 (6.8–40.2)	61.5 ± 3.1
R,S-zacopride	5-HT_4 ?	31.7 (17.5–56.5)	70.5 ± 3.5
Cisapride	5-HT_4 ?	90.9 (61.6–134.2)	73.3 ± 2.6
Metoclopramide	5-HT_4 ?	84.2 (16.8–175.0)	69.0 ± 2.9
Sumatriptan	5-HT_1	>300	38.6 ± 7.4
8-OH-DPAT	5-HT_{1A}	>300	41.2 ± 2.3
2-Me-5-HT	5-HT_3	>300	36.0 ± 5.4

5-CT: 5-carboxamidotryptamine; α-Me-5-HT: α-methyl-5-HT; 5-MeOT: 5-methoxytryptamine; 8-OH-DPAT: 8-hydroxy-2-(di-n-propylamino)tetralin; 2-Me-5-HT: 2-methyl-5-HT.
* at 100 μM, except for 5-HT (10 μM), α-Me-5-HT (30 μM) and 5-MeOT (300 μM).

been tested against the 5-HT-induced relaxation response in rat oesophagus. Those antagonists exhibiting no inhibitory effect are shown in Table 2. High concentrations of antagonists with affinity for 5-HT_1-like, 5-HT_2 and 5-HT_3 receptors were without effect, as was the sodium ion channel blocker, tetrodotoxin (Narahashi, 1972).

In contrast to the results obtained with the three 5-HT_3 receptor antagonists, ondansetron, MDL 72222 and granisetron (Table 2), a fourth compound, ICS 205-930, did produce antagonist effects. ICS 205-930 (1 and 10 μM) produced concentration-related, rightward shifts of the 5-HT concentration-effect curves with no depression of the maximum response to 5-HT. From these data pK_B values of approximately 6 were determined (Table 3), although it must be emphasised that the data were not consistent with a Schild slope of unity and thus ICS 205-930 did not appear to behave as a truly competitive antagonist under these conditions. ICS 205-930 (at 3 μM) was also tested against cumulative concentration-effect curves to 5-MeOT, 5-CT and renzapride. ICS 205-930 produced rightward shifts of these agonist curves with no depression of their maxima. From these data mean pK_B values of approximately 6 were again calculated (Table 3).

Isoprenaline (0.01–3 μM) elicited concentration-related and reproducible relaxations with mean EC_{50} values (μM) of 0.19 (0.13–0.29) and 0.28 (0.24–0.32) respectively for consecutive curves, and at 3 μM produced a complete reversal of the carbachol-induced contraction. ICS 205-930 (10 μM) produced no significant inhibition of the responses to isoprenaline with a mean concentration-ratio of only 1.2 (0.8–1.9) compared to the control curve, a value not significantly different from unity.

Table 2 Summary of antagonists with no inhibitory effect on 5-hydroxytryptamine (5-HT)-induced relaxation in rat isolated oesophagus ($n = 4$ throughout)

Compound	*Antagonist selectivity*	*Concentration tested* (μM)	*Concentration ratio* (95% confidence limits)
Spiperone	$5\text{-HT}_{1A}/5\text{-HT}_2$	1.0	1.0 (0.5–2.0)
Cyanopindolol	$5\text{-HT}_{1A}/5\text{-HT}_{1B}$	1.0	1.4 (0.9–2.3)
Mesulergine	$5\text{-HT}_{1C}/5\text{-HT}_2$	1.0	0.9 (0.7–1.3)
Methysergide	5-HT_1-like/5-HT_2	10	1.0 (0.4–2.9)
Methiothepin	$5\text{-HT}_1/5\text{-HT}_2$	0.3	1.0 (0.5–1.8)
Metergoline	$5\text{-HT}_1/5\text{-HT}_2$	1.0	0.5 (0.2–1.3)
Ketanserin	5-HT_2	1.0	0.4 (0.3–0.5)
Ondansetron	5-HT_3	1.0	1.1 (0.5–2.4)
Granisetron	5-HT_3	10	1.8 (0.9–3.6)
MDL 72222	5-HT_3	1.0	1.0 (0.8–1.2)
Tetrodotoxin	Na^+ channel blocker	0.3	0.8 (0.3–1.6)

Table 3 Mean pK_B values for ICS 205-930 against 5-hydroxytryptamine (5-HT) receptor agonists in rat oesophagus

Agonists used	*Concentration ICS 205-930 tested* (μM)	n	*Concentration ratio* (95% confidence limits)	pK_B value
5-HT	1.0	6	3.1 (1.7–5.5)	6.3 ± 0.1
	10	6	7.5 (2.6–21.1)	5.8 ± 0.1
5-CT	3.0	3	6.4 (1.1–38.4)	6.2 ± 0.2
5-MeOT	3.0	3	3.6 (2.6–5.1)	5.9 ± 0.1
Renzapride	3.0	4	4.2 (1.3–13.1)	6.0 ± 0.2

5-CT: 5-carboxamidotryptamine; 5-MeOT: 5-methoxytryptamine.

At the concentrations tested, none of the antagonists, with the exception of ICS 205-930, had any obvious effect on the carbachol-induced contractions. ICS 205-930 at 10 μM produced a small inhibition of 19.0 ± 1.3% of the carbachol tone, but had no effect at 1 and 3 μM.

Discussion

The present study has shown that 5-HT produces concentration-related and reproducible relaxations of rat oesophagus precontracted with carbachol, and this is in agreement with the work of Bieger & Triggle (1985). This response is apparently mediated through a direct effect on the smooth muscle, a neuronal component being unlikely since tetrodotoxin had no effect.

Since 5-HT can be a substrate for amine uptake processes and can be degraded by monoamine oxidase, the rank order of potency of 5-HT receptor agonists might have been affected by the degree of uptake and/or metabolism of each agonist. Therefore the effect of the irreversible monoamine oxidase inhibitor, pargyline, and the amine uptake inhibitors corticosterone and cocaine on the relaxant responses to both 5-HT and 5-MeOT was investigated. The addition of high concentrations of pargyline, corticosterone and cocaine produced no potentiation of the responses to either 5-HT or 5-MeOT in this study suggesting that if uptake did occur, it did not significantly modify relaxation under the conditions of these experiments. However, the mixture of these inhibitors did cause a reduction in the maximum response obtained to 5-HT, and more markedly to 5-MeOT (Figure 2), an effect that could be due in part to the large inhibition of the carbachol-induced tone produced by the addition of corticosterone and cocaine although, why the maximum response to 5-MeOT was reduced more than that to 5-HT cannot be explained. This reduction in carbachol tone complicated the analysis of the responses to 5-HT and 5-MeOT, but with this caveat in mind there was no evidence of potentiation of the relaxant responses to these agonists. Preliminary experiments suggested that these inhibitory effects were predominantly caused by cocaine, possibly through its local anaesthetic activity. These inhibitors were not used in subsequent experiments.

The relaxant responses to 5-HT were mimicked by 5-CT, α-Me-5-HT and 5-MeOT which produced responses close to the 5-HT maximum whereas 2-Me-5-HT, sumatriptan and 8-OH-DPAT were weak or inactive. As shown in Table 4 the rank order of potency of the key agonists was not typical of a 5-HT_1, 5-HT_2 or 5-HT_3-receptor. In addition, the very weak activity of sumatriptan and 8-OH-DPAT confirms that the 5-HT_1-like receptor characteristic of the dog saphenous vein (Feniuk *et al.*, 1985) and the 5-HT_{1A} receptor (Hoyer *et al.*, 1985) were not involved. Furthermore, these deductions derived from agonist data are confirmed by the lack of effect of high concentrations of 5-HT_1, 5-HT_2 and most of the 5-HT_3 receptor antagonists (Table 2).

The finding that the substituted benzamides, metoclopramide, **R,S**-zacopride, cisapride and renzapride were partial agonists in rat oesophagus was of particular interest, although high concentrations (100 μM) of metoclopramide and cisapride could not be used since non-specific relaxant responses occurred which were not inhibited by ICS 205-930, 10 μM (unpublished observations). Preliminary studies in our laboratory (Reeves *et al.*, 1989) and by others (Triggle *et al.*, 1988) have previously found that the benzamides were unsurmountable antagonists of 5-HT in rat oesophagus. Although we cannot comment on the work of Triggle *et al.* (1988), we believe that our preliminary results (Reeves *et al.*, 1989) with the benzamides were misleading. In these early experiments the benzamides were administered prior to carbachol and only tested as antagonists using an experimental protocol that would not have clearly identified any direct agonist effects. In the present studies the carbachol-induced tone was established before the addition of the benzamides, and this has allowed a quantitative study of agonist effects. The antagonist effects of the benzamides previously observed (Reeves *et al.*, 1989), were probably attributable to the affinity of the benzamides for the 5-HT receptor in rat oesophagus which then restricted the access of, and responses to, 5-HT.

The lack of involvement of 5-HT_1 and 5-HT_2 receptors in the relaxant response to 5-HT in the present study is in agreement with the results reported by Bieger & Triggle (1985). However, particularly interesting observations are derived from closer inspection of the effects of 5-HT_3 receptor antagonists. In our study, the 5-HT_3 antagonists MDL 72222

Table 4 Rank order of agonist potency of selective compounds at 5-hydroxytryptamine (5-HT) receptor subtypes

Receptor subtype	*Agonist potency*	*References*
5-HT_1-like (contraction)	5-CT > 5-HT > αMe-5-HT > 2-Me-5-HT = 0	Feniuk *et al.* (1985)
5-HT_1-like (relaxation)	5-CT ≫ 5-HT ≫ 2-Me-5-HT = α-Me-5-HT = 0	Humphrey (1984)
5-HT_2	5-HT = α-Me-5-HT > 5-CT > 2-Me-5-HT	Humphrey (1984)
5-HT_3	5-HT = 2-Me-5-HT > α-Me-5-HT ≫ 5-CT	Humphrey (1984)
Rat oesophagus	5-HT > α-Me-5-HT = 5-CT ≫ 2-Me-5-HT > 0	This study

5-CT: 5-carboxamidotryptamine; α-Me-5-HT: α-methyl-5-HT; 2-Me-5-HT: 2-methyl-5-HT.

(Fozard, 1974) and granisetron (Sanger & Nelson, 1989) did not affect the response to 5-HT. These results contrast with those of Triggle *et al.* (1988) who found that these compounds were potent antagonists of 5-HT in the rat isolated oesophageal muscularis mucosa. This discrepancy is difficult to explain; the present study used whole sections of rat oesophagus whereas Triggle *et al.* (1988) used only the muscularis mucosa, a preparation where the external striated muscle layers are removed. However, preliminary work in our laboratory would show no difference between the two isolated oesophagus preparations in this respect (unpublished observations). Indeed, 2-Me-5-HT was a weak agonist in both the present study and that of Triggle *et al.* (1988) and this result, taken with the lack of effect of ondansetron (Table 2), makes it unlikely that 5-HT_3 receptors are involved in the relaxant responses to 5-HT in rat oesophagus.

In contrast to the results obtained for ondansetron, granisetron and MDL 72222, high concentrations of the 5-HT_3 receptor antagonist ICS 205-930 (Richardson *et al.*, 1985) produced concentration-related antagonism of the 5-HT-induced relaxations. From these data pK_B values of approximately 6 were calculated, and although it did not appear to behave as a truly competitive antagonist against 5-HT, this is much lower than the reported affinity constant for ICS 205-930 at 5-HT_3 receptors in rat tissues where a pA_2 value of approximately 8.5 was obtained (Butler *et al.*, 1988). It therefore appears that ICS 205-930 interacts with a non-5-HT_3 receptor site in rat oesophagus. In addition to the effect of ICS 205-930 against 5-HT, a high concentration of ICS 205-930 also inhibited the relaxant responses to 5-CT, 5-MeOT and the benzamide, renzapride, again with pK_B values of approximately 6. The effects of ICS 205-930 were selective, since relaxant responses to isoprenaline were unaffected. The consistency of these pK_B values for ICS 205-930 suggests that both the indole and benzamide agonists were interacting with the same single population of receptors. As in the present study, high concentrations of ICS 205-930 also inhibit responses to 5-HT and the benzamides in mouse colliculi neurones (Dumuis *et al.*, 1988; 1989), guinea-pig ileum (Craig & Clarke, 1990; Eglen *et al.*, 1990) and guinea-pig hippocampus (Bockaert *et al.*, 1990) with affinity constants ranging from 6.0 to 6.5, a result which is considered to be indicative of the presence of the putative 5-HT_4 receptor (Clarke *et al.*, 1989).

The observation that the benzamides are partial agonists in rat oesophagus is also consistent with the presence of 5-HT_4 receptors in this tissue; similar results with these compounds have been reported in other tissues exhibiting 5-HT_4 receptors (Dumuis *et al.*, 1989; Bockaert *et al.*, 1990; Eglen *et al.*, 1990). With respect to the indoles, comparison of data from the present study (Table 1) with the results of Craig & Clarke (1990) in guinea-pig ileum shows that although 5-HT, 5-CT, α-Me-5-HT and 5-MeOT were agonists in both preparations (with 2-Me-5-HT being inactive), their rank orders of potency were different in these tissues. These findings could be indicative of different receptor types, although the results with ICS 205-930 (see above) do not support this contention; clearly these differences in agonist relative potency need to be resolved.

The suggestion that 5-HT-induced relaxation of rat oesophagus is mediated by 5-HT_4 receptors is also corroborated by the studies with IBMX which potentiated the responses to 5-HT. The latter result is consistent with the intracellular mediation by adenosine 3':5'-cyclic monophosphate (cyclic AMP) of smooth muscle relaxation in rat oesophagus, and indeed 5-HT_4 receptors have been shown to be positively coupled to adenylate cyclase in both mouse colliculi neurones (Dumuis *et al.*, 1988; 1989) and guinea-pig hippocampus (Bockaert *et al.*, 1990).

In conclusion, the 5-HT receptor type eliciting relaxation of rat oesophagus cannot be identified as 5-HT_1-like, 5-HT_2 or 5-HT_3, and the data presented here strongly suggest that this effect is mediated via 5-HT_4 receptors.

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