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The pharmacological characterization of 5-HT_3 receptors in three isolated preparations derived from guinea-pig tissues

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1 The pharmacological characterization of the 5-HT_3 receptors in guinea-pig isolated tissues is described. The tissues used were ileum (longitudinal muscle-myenteric plexus), colon and vagus nerve. The guinea-pig isolated colon is a novel preparation.

2 In the guinea-pig isolated ileum, 5-hydroxytryptamine (5-HT, 1×10^{-8}–3×10^{-5} M) and the selective 5-HT_3 receptor agonist 2-methyl-5-HT (3×10^{-7}–1×10^{-4} M) caused concentration-related contractions. The 5-HT concentration-response curve was biphasic whilst the 2-methyl-5-HT curve was monophasic. The EC_{50} value for the low potency portion of the 5-HT curve was 4.1×10^{-6} M. The EC_{50} for 2-methyl-5-HT was 1.23×10^{-5} M. Selective 5-HT_3 receptor antagonists caused rightward shifts of the 2-methyl-5-HT curve and the lower potency portion of the 5-HT curve. Neither ketanserin (1×10^{-6} M) nor methysergide (1×10^{-5} M) antagonized the responses to 5-HT or 2-methyl-5-HT.

3 In the guinea-pig isolated colon, 5-HT (3×10^{-7}–3×10^{-5} M; EC_{50} 2.4×10^{-6} M) caused contractions which were mimicked by 2-methyl-5-HT (1×10^{-6}–1×10^{-4} M; EC_{50} 7.2×10^{-6} M). Selective 5-HT_3 receptor antagonists caused rightward displacements of the 5-HT concentration-response curves. Neither ketanserin (1×10^{-6} M) nor methysergide (1×10^{-5} M) had any effect on responses to 5-HT or 2-methyl-5-HT.

4 In the guinea-pig isolated vagus nerve, 5-HT (1×10^{-6}–3×10^{-4} M) and 2-methyl-5-HT (1×10^{-5}–1×10^{-3} M; EC_{50} 7.6×10^{-5} M) caused depolarizations; at higher concentrations there were after-hyperpolarizations. The maximum response to 2-methyl-5-HT was less than half that to 5-HT. Selective 5-HT_3 receptor antagonists caused rightward displacements of the 5-HT concentration-response curves. Antagonists at other 5-HT receptors (ketanserin, 1×10^{-5} M and methysergide, 1×10^{-6} M) had no effect.

5 The estimated affinity values of 5-HT_3 receptor antagonists correlated well between the three models. Phenylbiguanide was inactive as an agonist or antagonist (up to 1×10^{-4} M) in each preparation.

6 Comparisons with antagonist affinity values obtained in the rat isolated vagus nerve revealed marked differences. Antagonists were generally more potent on the rat isolated vagus nerve, although the differences varied considerably between antagonists.

7 The results are discussed in terms of species-related receptor differences.

Introduction

5-Hydroxytryptamine (5-HT) receptors of the 5-HT_3 type are thought to be located exclusively on neuronal tissue. These receptors appear to be directly linked to an integral monovalent cation channel (Higashi & Nishi, 1982; Neijt *et al.*, 1986; Surprenant & Crist, 1988; Derkach *et al.*, 1989). Agonists for the receptor include 5-HT and the selective compounds, 2-methyl-5-HT (Richardson *et al.*, 1985) phenylbiguanide (Fastier *et al.*, 1959; Ireland & Tyers, 1987) and *m*-chlorophenylbiguanide (Kilpatrick *et al.*, 1990b). Antagonists include the selective compounds MDL 72222 (Fozard, 1984), ICS 205–930 (Richardson *et al.*, 1985), ondansetron (GR38032; Butler *et al.*, 1988) and granisetron (BRL 43694; Sanger & Nelson, 1989).

Drugs which act as antagonists at 5-HT_3 receptors have been shown to have differing affinities in various isolated tissue models used to examine functional 5-HT_3 receptors (Richardson *et al.*, 1985; Butler *et al.*, 1988). Such observations led Richardson & Engel (1986) to propose the presence of 5-HT_3 receptor subtypes based upon the type of neurone on which each receptor was found. However, in this latter study, tissues from different species were used so that results obtained in guinea-pig ileum were compared with those obtained in rat vagus nerve and rabbit heart. Furthermore, many of the tissues used responded to 5-HT through an indirect mechanism. For example, in guinea-pig ileum, 5-HT-induced contractions may result from the release of both acetylcholine and substance P (Buchheit *et al.*, 1985). Neurotransmitter depletion and other factors complicate interpretation of such data (Kenakin, 1984). Because of these interpretation difficulties the Richardson & Engel classification has not been widely accepted (see Bradley *et al.*, 1986).

In order to remove one of these variables, we have investigated the responses to 5-HT in three tissues obtained from the same species. The guinea-pig isolated ileum longitudinal muscle-myenteric plexus preparation is now routinely used as a bioassay for substances acting on 5-HT_3 receptors but involves an indirect response to 5-HT which can be blocked by atropine. This preparation also contains other 5-HT receptors which are not of the 5-HT_3 type (Buchheit *et al.*, 1985; Craig & Clarke, 1989). The guinea-pig vagus nerve preparation has been described previously (Burridge *et al.*, 1989; Lattimer *et al.*, 1989). Depolarizations evoked by 5-HT in the preparation are believed to be direct responses mediated through 5-HT_3 receptors.

The guinea-pig isolated descending colon preparation has been described in a communication report (Grossman *et al.*, 1989) to the British Pharmacological Society. In this tissue responses to 5-HT are believed to be largely indirect.

Methods

Guinea-pig ileum longitudinal muscle-myenteric plexus

Male, Dunkin-Hartley guinea-pigs (*Porcellus*), weighing 250–300 g, were killed by cervical dislocation. A 3 cm portion of

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ileum was excised about 1 cm from the ileo-caecal junction. The longitudinal muscle layer was removed as described previously (Butler et al., 1988). Strips approximately 1.5 cm in length were placed in 5 ml organ baths containing Krebs-Henseleit solution gassed with 95% O_2/5% CO_2 and maintained at 37°C. Tissues were placed under an initial tension of 0.5–1 g. Agonists were applied directly to the bath (volume 50 μl–150 μl) and contractions were recorded isometrically. Non-cumulative concentration-response curves were constructed for agonists with a 15 min dosing cycle to prevent desensitization. Measurements were made at the highest point of the contraction and agonists were washed out as soon as the peak responses were reached.

Antagonists were added to the reservoir containing the bathing medium and were allowed to equilibrate with the ileum preparations for 1 h before the concentration-response curves were repeated.

Guinea-pig isolated colon

Female, Dunkin-Hartley guinea-pigs (*Porcellus*), weighing 250–300 g, were killed by cervical dislocation. A segment of the descending colon was removed and cut into 2–3 cm long sections. These sections were then cut longitudinally to reveal the mucosal surface, which was removed by careful excision and discarded. The muscle strips were placed in 20 ml organ baths containing modified Krebs-Henseleit solution, gassed with 95% O_2/5% CO_2 and maintained at 37°C. Each tissue was placed under an initial tension of 1 g and allowed to equilibrate for 40 min before the experiment was started. Agonists were applied directly to the baths and contractions were recorded isometrically. Non-cumulative concentration-response curves were constructed for agonists with a 10 min dosing-cycle. Measurements were made at the highest point of the contraction and agonists were washed out as soon as the peak responses were reached.

Antagonists were added to the reservoir containing bathing medium and were allowed to equilibrate with the colon preparations for 30 min before the concentration-response curves were repeated.

Guinea-pig isolated vagus nerve

Male, Dunkin-Hartley guinea-pigs (*Porcellus*), weighing 250–350 g, were stunned by a blow to the back of the head and killed by cardiac puncture. Segments of cervical vagus nerve were excised as rapidly as possible, desheathed and mounted in two-compartment Perspex baths. Each nerve was positioned so that 50% lay in the first compartment and the other 50% projected through a greased slot into the second. A Perspex barrier was carefully inserted to separate the two compartments. The d.c. potential between the two compartments was measured with silver/silver-chloride electrodes. The preparations were maintained at 27°C since Ireland et al. (1987) and Ireland & Tyers (1987) have shown that this increases recording stability. Both compartments were perfused continuously with Krebs-Henseleit medium dripped directly onto the tissue. Drugs were added via the superfusion stream to one compartment only.

Responses were recorded on a Servogor 220 pen recorder. Non-cumulative concentration-response curves to agonists were constructed with a 3 min contact time. Nerves were allowed to repolarize completely or settle to a new resting potential before application of the next concentration of agonist. This gave a dose-cycle of 15–45 min, depending on the size of depolarization. Antagonists were applied via the superfusion stream. An initial incubation period of 30 min was allowed after which time, repeated applications of agonist at a concentration inducing approximately 50% of the maximum response were given at approximately 15 min intervals until the response was constant, indicating that equilibration of the antagonist had been achieved. The agonist concentration-response curve was then repeated.

Rat vagus nerve

The method used for the measurement of 5-HT-induced depolarizations in the rat vagus nerve (Ireland & Tyers, 1987) was similar to that used for guinea-pig vagus nerve. Male, hooded rats (Glaxo), weighing 200–250 g, were used.

[^3H]-GR67330 binding

Male, Dunkin-Hartley guinea-pigs (*Porcellus*), weighing 200–250 g were killed and brain and ileum removed. The longitudinal muscle-myenteric plexus of the guinea-pig ileum was used and 16 regions of the brain dissected (see Kilpatrick et al., 1989). Pooled tissue from 5 animals was homogenized (Ultra Turrax) in 30 vol of HEPES buffer (50 mM, pH 7.4, 4°C) and centrifuged at 4°C and 48,000 g for 10 min. The supernatant was discarded and the process repeated for the pellet. The final pellet was suspended in 30 vol of HEPES buffer.

For binding, assay tubes contained 400 μl [^{3}H]-GR67330 (synthesized by the Radiochemistry Group, Glaxo Group Research; 85 Ci mmol^{-1}) in HEPES buffer. For inhibition studies a final concentration of 0.1 nM [^{3}H]-GR67330 was used, whilst for saturation analyses 10–20 concentrations between 0.01 and 2.0 nM were used. Tubes also contained 200 μl of competing drug or its vehicle (HEPES buffer), 300 μl of HEPES buffer and 100 μl of the tissue preparation (~0.2 mg protein). Tubes were incubated at 37°C for 30 min. The incubation was terminated by rapid vacuum filtration through Whatman GF/B filters using a Brandel Cell Harvester. Filters were washed immediately with 5 × 4 ml HEPES buffer (room temperature). Filters were placed in 10 ml of Picofluor 30 scintillation fluid and left overnight before radioactivity was assessed by liquid scintillation counting. All individual assays were carried out in replicates of three. Non-specific binding was determined by the addition of metoclopramide (30 μM).

Data analysis

EC_{50} values for agonists are means ± s.e.mean of results estimated from individual concentration-response curves. Estimation was performed graphically, correction being made when curves appeared multiphasic.

For antagonists pK_B values were estimated using the equation:

$$pK_B = \log(\text{concentration-ratio} - 1) - \log(\text{concentration of antagonist}).$$

Concentration ratios were measured at the level of approximately 50% of the maximum observed response. Only one concentration of antagonist was tested on each individual tissue preparation. For most antagonists a single concentration only was tested (Table 1). In these cases, the pK_B value is the mean of single determinations made on at least four individual preparations. For some antagonists, the effects of several concentrations were tested (Table 1). For these, a mean pK_B value (± s.e.mean) was calculated providing the plot of antagonism data according to Arunlakshana & Schild (1959) had a gradient not significantly different from unity.

In the rat vagus nerve, some antagonists caused reductions in the amplitude of the maximum response to 5-HT. In quantifying the effects of such antagonists, two assumptions have been made. The first is that 5-HT has low efficacy at 5-HT_3 receptors on rat vagus nerve, the second that the antagonists, although competitive, do not dissociate appreciably from the receptor during the period of 5-HT application. To estimate the affinity of antagonists under these conditions, the method of Paton & Ward (1967) (see also Kenakin, 1984) was used, although initial quantification of experimental data was performed according to Kennedy & Roberts (1985). Estimates of pK_B are quoted as the mean ± s.e.mean of determinations in at least three separate vagus nerves.

Table 1 Concentrations of 5-HT$_3$ receptor antagonists used

	Concentration(s) tested (M)			
Antagonist	*Guinea-pig ileum*	*Guinea-pig colon*	*Guinea-pig vagus nerve*	*Rat vagus nerve*
Metoclopramide	1×10^{-5}	1×10^{-5}	1×10^{-5}–1×10^{-4}	3×10^{-6}–10^{-4}†
MDL 72222	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-8}–3×10^{-6}†
Ondansetron	1×10^{-7}–1×10^{-6}	1×10^{-6}–1×10^{-5}	3×10^{-7}–1×10^{-5}	1×10^{-8}–3×10^{-7}
R-GR38032	1×10^{-6}	NT	1×10^{-6}	1×10^{-8}–3×10^{-7}
S-GR38032	1×10^{-5}	NT	1×10^{-5}	1×10^{-8}–3×10^{-7}
GR80284	3×10^{-7}	3×10^{-7}	3×10^{-7}	1×10^{-7}
GR65630	1×10^{-6}	1×10^{-6}	1×10^{-6}	3×10^{-9}–3×10^{-7}
ICS 205-930	1×10^{-7}	1×10^{-7}	3×10^{-8}–1×10^{-6}	1×10^{-10}–3×10^{-9}†
Granisetron	1×10^{-7}	1×10^{-7}	3×10^{-8}–1×10^{-6}	1×10^{-9}
Zacopride	1×10^{-7}	1×10^{-7}	1×10^{-7}	1×10^{-8}
GR67330	1×10^{-7}	1×10^{-7}	1×10^{-7}	3×10^{-9}–1×10^{-6}

NT = Not tested.
† From Ireland & Tyers (1987).

Drugs and solutions

The composition of Krebs-Henseleit medium used was (mM): NaCl 118, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.18, $CaCl_2$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.18 and glucose 11. The bathing medium for colon experiments was the same except for the concentrations of $CaCl_2$ and $MgSO_4 \cdot 7H_2O$ which were 1.3 mM and 0.6 mM respectively.

The following drugs (sources) were used: ondansetron (RS-GR38032), (R)- and (S)-GR38032 (1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one, HCl · $2H_2O$; Glaxo), GR65630 (3-(5-methyl-1H-imidazol-4-yl)-1-(1-methyl-1H-indol-3-yl)-1-propanone maleate; Glaxo), GR67330 (1,2,3,9-tetrahydro-9-methyl-3-[(5-methyl-1H-imidazol-4-yl) methyl]-4H-carbazol-4-one maleate; Glaxo), GR80284 (endo-2,3-dihydro-N-(9-methyl-9-azabicyclo[3.3.1.]non-3-yl)-1H-indole-1-carboxamide maleate; Glaxo), BRL 24924 ([(±)-(endo)]-4-amino-N-1-azabicyclo[3.3.1]-non-4-yl-5-chloro-2-methoxybenzamide; Glaxo), granisetron (BRL 43694) (endo-1-methyl-N-(9-methyl-9-azabicyclo[3.3.1]non-3-yl-1H-indazole-3-carboxamide HCl; Glaxo), ICS 205-930 (endo-8-methyl-8-azabicyclo[3.2.1]oct-3-yl-1H-indole-3-carboxylate; Research Biochemicals Inc.), MDL 72222 (endo-8-methyl-8-azabicyclo [3.2.1]oct-3-yl-3,5-dichlorobenzoate HCl; Research Biochemicals Inc.), SDZ 206-830 (endo-9-methyl-9-azabicyclo [3.3.1]non-3-yl 5-fluoro-1-methyl-1H-indole-3-carboxylate HCl; Glaxo), 5-carboxamidotryptamine maleate (5-CT; Glaxo), haloperidol (Janssen), (±)-8-hydroxy-2-(di-N-propylamino)tetralin (8-OHDPAT; Research Biochemicals Inc.), 5-hydroxytryptamine creatinine sulphate (Sigma), ketanserin maleate (Salford Fine Chemicals), (±)-α-methyl-5-hydroxytryptamine maleate (α-methyl-5-HT; Glaxo), 2-methyl-5-hydroxytryptamine HCl · H_2O (2-methyl-5-HT; Glaxo), methysergide hydrogen maleate (Sandoz), metoclopramide HCl (Beecham), paroxetine HCl (Ferrosan), phentolamine mesylate (CIBA), 1-phenylbiguanide (Aldrich), zacopride HCl (A.H. Robins), 1,1-dimethyl-4-phenyl piperazinium iodide (DMPP, Sigma).

Drugs were dissolved in water and diluted in Krebs-Henseleit medium.

Results

Guinea-pig ileum longitudinal muscle myenteric plexus (LMMP) preparation

As described previously (Butler *et al.*, 1988), 5-HT (1×10^{-8}–3×10^{-5} M) and 2-methyl-5-HT (3×10^{-7}–1×10^{-4} M) caused concentration-related contractions of the guinea-pig isolated ileum LMMP preparation. The 5-HT concentration-response curve was biphasic whilst the 2-methyl-5-HT curve was monophasic (Figure 1a)). The EC_{50} values for 5-HT (low potency portion of the curve) and 2-methyl-5-HT were $4.1 \pm 1.0 \times 10^{-6}$ M ($n = 8$) and $1.23 \pm 0.24 \times 10^{-5}$ M ($n = 8$) respectively. α-Methyl-5-HT (1×10^{-8}–1×10^{-4} M) also induced concentration-related contractions and a biphasic concentration-response curve yielding an EC_{50} (for the whole curve) of $1.7 \pm 0.23 \times 10^{-5}$ M ($n = 7$); the maximum response was $104.5 \pm 2.6\%$ ($n = 8$) that of 5-HT (Figure 1a). 5-CT (1×10^{-6}–1×10^{-4} M) induced very small contractions (not shown), the maximum being less than 30% that of 5-HT. 8-OH DPAT (1×10^{-6}–3×10^{-5} M) had no significant effect.

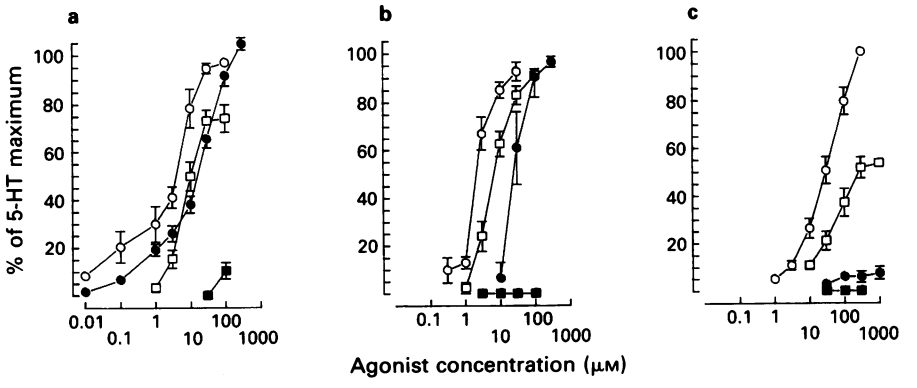

Figure 1 The effects of 5-hydroxytryptamine (5-HT, ○), 2-methyl-5-HT (□), α-methyl-5-HT (●) and phenylbiguanide (■) on the guinea-pig isolated ileum (a), isolated colon (b) and isolated vagus nerve (c). Results are expressed as the % maximum contraction to 5-HT (ileum and colon) or depolarization (% of depolarization at 3×10^{-4} M 5-HT; vagus nerve). Results are the mean of at least 4 separate observations; s.e.mean shown by vertical bars

Phenylbiguanide had no effect at concentrations up to 3×10^{-5} M but caused small contractions at 1×10^{-4} M (Figure 1a).

Because of the biphasic nature of the 5-HT concentration-response curve, 5-HT_3 receptor antagonist potencies were determined against 2-methyl-5-HT. Control concentration-response curves constructed 1 h apart on the same tissue preparation were not different from each other (not shown). Because of the low solubility and potency of 2-methyl-5-HT, the maximum shift in concentration-response curves that could be measured was small: the maximum usable concentration of 2-methyl-5-HT was 3×10^{-4} M. Selective 5-HT_3 receptor antagonists caused parallel, rightward displacements of the 2-methyl-5-HT concentration-response curve with no depression of maximum response (e.g. Figures 2a and 3a). pK_B values calculated from these shifts are shown in Table 2. Ondansetron (1×10^{-6} M) also caused a rightward shift of the low-potency portion of the α-methyl-5-HT concentration-response curve, yielding a pK_B of 7.0 which was similar to the pK_B value obtained against 2-methyl-5-HT (not shown).

Ketanserin (1×10^{-6} M), methysergide (1×10^{-5} M) (Figure 4a) and phenylbiguanide (1×10^{-4} M) (not shown) had no effect on contractions induced by 2-methyl-5-HT.

Guinea-pig isolated descending colon

5-HT (3×10^{-7}–3×10^{-5} M) and 2-methyl-5-HT (1×10^{-6}–1×10^{-4} M) induced concentration-related contractions of the guinea-pig isolated colon as exemplified by 5-HT in Figure 5. The concentration-contraction curves for both agonists appeared monophasic (Figure 1b). The EC_{50} values for 5-HT and 2-methyl-5-HT (when examined in the same tissues) were $2.4 \pm 0.4 \times 10^{-6}$ M ($n = 7$) and $7.2 \pm 1.0 \times 10^{-6}$ M ($n = 7$) respectively. The maximum response to 2-methyl-5-HT was $91.0 \pm 2.6\%$ of that to 5-HT. α-Methyl-5-HT (1×10^{-6}–1×10^{-4} M) also induced concentration-related contractions of the guinea-pig colon (Figure 1b); the EC_{50} was $2.97 \pm 0.97 \times 10^{-5}$ M ($n = 3$). The maximum response to α-methyl-5-HT was $99.2 \pm 3.8\%$ ($n = 3$) that of 5-HT. 5-CT induced small contractions ($24.3 \pm 4.4\%$ of the 5-HT maximum at 1×10^{-4} M; $n = 3$; not shown). Neither 8-OH DPAT nor phenylbiguanide caused contractions at concentrations up to 1×10^{-4} M (Figure 1b). Concentration-response curves for 5-HT constructed 30 min apart on the same tissue preparation were not significantly different from each other (not shown). 5-HT_3 receptor antagonists caused parallel, rightward shifts of the 5-HT concentration-contraction curve. The pK_B value for ondansetron was 7.1 ± 0.1 ($n = 12$). Schild analysis yielded a slope (95% confidence interval) of 1.26 (0.97–1.56) (Figure 2b). MDL 72222 (Figure 3b), also caused parallel, rightward shifts of the 5-HT concentration-contraction curve. MDL 72222 (1×10^{-6} M) had no significant effect on contractions induced by the nicotinic agonist, DMPP (1×10^{-6}–1×10^{-4} M; not shown). pK_B values calculated for 5-HT_3 receptor antagonists against 5-HT responses are given in Table 2. Ketanserin (1×10^{-6} M), methysergide (1×10^{-5} M) or phenylbiguanide (1×10^{-4} M) had no effect on contractions induced by 5-HT (Figure 4b). Atropine (1×10^{-6} M) blocked the response to 5-HT in an insurmountable manner: approximately 15% of the 5-HT maximum response remained (not shown).

Guinea-pig isolated vagus nerve

5-HT (1×10^{-6}–3×10^{-4} M) induced concentration-dependent depolarizations of the guinea-pig isolated vagus nerve. Reproducible curves to 5-HT could be obtained with up to 2 h between curves and single responses were repro-

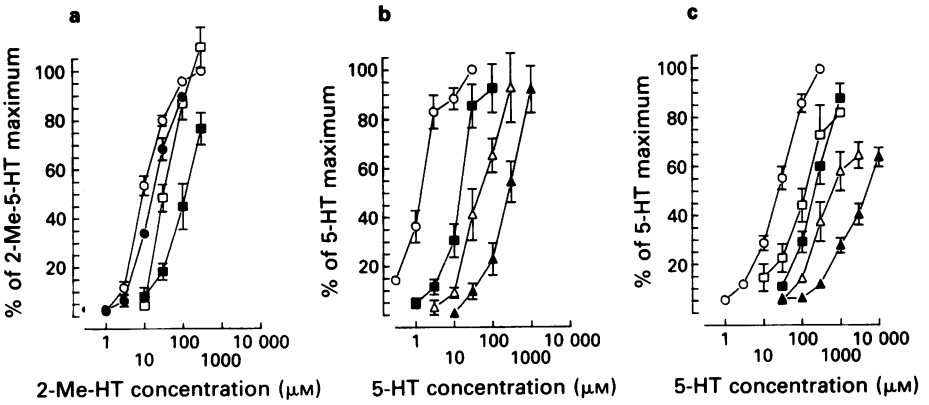

Figure 2 Antagonism by ondansetron of 2-methyl-5-HT-induced responses in guinea-pig isolated ileum (a) and 5-HT responses in guinea-pig isolated colon (b) and guinea-pig vagus nerve (c). Symbols indicate control (○) or in the presence of ondansetron 1×10^{-7} M (●), 3×10^{-7} M (□), 1×10^{-6} M (■), 3×10^{-6} M (△), and 1×10^{-5} M (▲). pK_B values derived from these experiments are presented in Table 2. Results are the mean of at least 4 separate observations; s.e. shown by vertical bars.

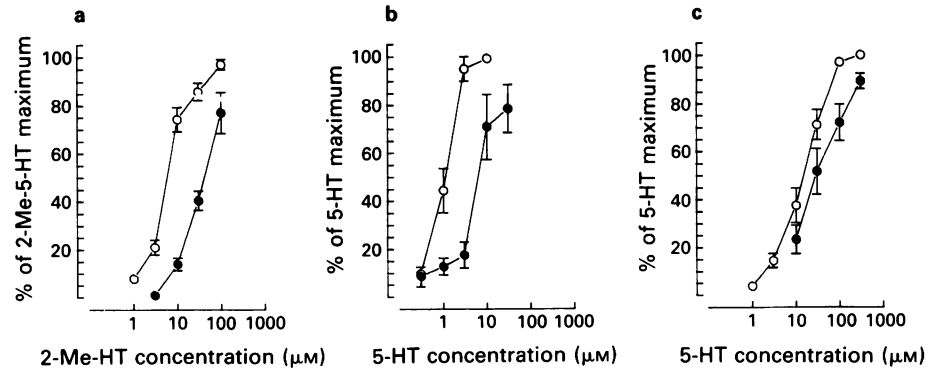

Figure 3 Antagonism by MDL 72222 of 2-methyl-5-HT responses in guinea-pig isolated ileum (a) and 5-HT responses in guinea-pig isolated colon (b) and guinea-pig isolated vagus nerve (c). Symbols indicate control (○) or in the presence of MDL 72222 at 1×10^{-6} M (●). Results are the mean of at least 3 separate observations; s.e. shown by vertical bars.

Table 2 Comparison of the affinities of 5-HT$_3$ receptor antagonists in guinea-pig isolated ileum, colon and vagus nerve and rat isolated vagus nerve

Antagonist	*Guinea-pig vs-2-Me-5-HT* pK_B	*Guinea-pig colon* pK_B	*Guinea-pig vagus nerve* pK_B	*Rat vagus nerve* pK_B
Metoclopramide	5.5 ± 0.1	5.7 ± 0.1	5.4 ± 0.1*	6.6†
MDL 72222	6.7 ± 0.1	6.7 ± 0.3	6.4 ± 0.1*	7.9†
Ondansetron	7.3 ± 0.1	7.1 ± 0.1	7.0 ± 0.1*	8.6 ± 0.1
R-GR38032	7.2 ± 0.1	NT	7.0 ± 0.1*	9.0 ± 0.1
S-GR38032	6.3 ± 0.1	NT	6.3 ± 0.1*	8.6 ± 0.1
GR80284	7.8 ± 0.1	7.6 ± 0.1	7.2 ± 0.1*	7.9 ± 0.1
GR65630	7.5 ± 0.1	7.5 ± 0.3	7.2 ± 0.1*	9.9 ± 0.1
ICS 205-930	8.0 ± 0.2↓	8.0 ± 0.1	7.8 ± 0.1*	11.0†
Granisetron	8.1 ± 0.1	8.1 ± 0.2	7.9 ± 0.1*	9.8 ± 0.1↓
Zacopride	8.1 ± 0.1	8.3 ± 0.1	8.0 ± 0.1*	9.9 ± 0.2↓
GR67330	9.0 ± 0.1	8.7 ± 0.1	8.0 ± 0.2*	10.2 ± 0.1↓

Results are the mean ± s.e. of at least four separate observations
↓ decrease in maximum observed response
NT Not tested
† From Ireland & Tyers (1987)
$P < 0.05$ (Two sample t test compared with rat vagus nerve values).
Refer to Table 1 for concentration(s) used.

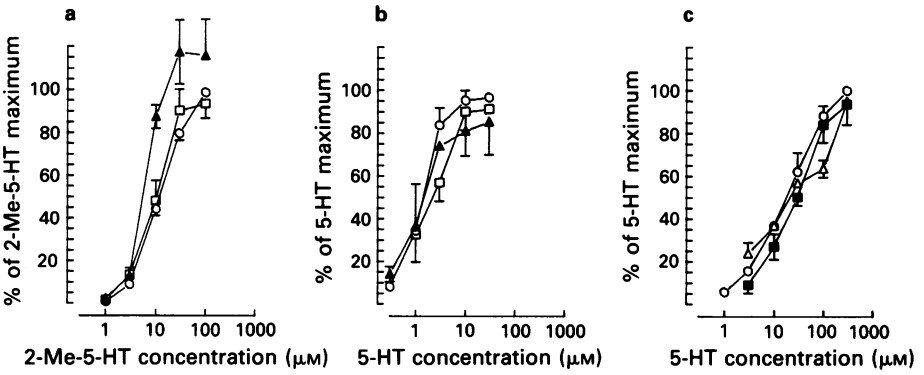

Figure 4 The effect of ketanserin and methysergide on responses to 2-methyl-5-HT in the guinea-pig isolated ileum (a) and 5-hydroxytryptamine (5-HT) in the guinea-pig isolated colon (b) and vagus nerve (c). Symbols indicate control (○) or in the presence of ketanserin at 1×10^{-6} M (□), or 1×10^{-5} M (■) and methysergide at 1×10^{-6} M (△) or 1×10^{-5} M (▲). Results are the mean of at least 3 separate observations; s.e. shown by vertical bars.

ducible with an interval between doses of 15–40 min. At higher concentrations the depolarization was followed by an after-hyperpolarization (Figure 6). Maximum depolarizations obtained were between 0.8 and 1.2 mV. True maximum responses to 5-HT were not routinely determined because the membrane potential did not recover from depolarizations evoked by concentrations of 5-HT above 3×10^{-4} M. Because of this, results are presented as a percentage of the depolarization obtained with 5-HT at 3×10^{-4} M. 2-Methyl-5-HT (1×10^{-5}–1×10^{-3} M) also induced concentration-dependent depolarizations and after-hyperpolarizations with an apparent EC_{50} for the depolarizations of $7.1 \pm 1.6 \times 10^{-5}$ M ($n = 9$). 2-Methyl-5-HT appeared to be a partial agonist, but because the true maximum response to 5-HT was not determined, a maximal response to 2-methyl-5-HT as a percentage of that to 5-HT could not be reliably calculated. Phenylbiguanide (up to 3×10^{-4} M), 5-CT and 8-OHDPAT (up to 10^{-4} M) did not induce depolarizations or hyperpolarizations of guinea-pig isolated vagus nerve. Furthermore, phenylbiguanide (1×10^{-4} M) did not antagonize 5-HT-induced responses.

The 5-HT reuptake inhibitor, paroxetine (3×10^{-7}–3×10^{-6} M) had no significant effect on the concentration-response curves for 5-HT (data not shown).

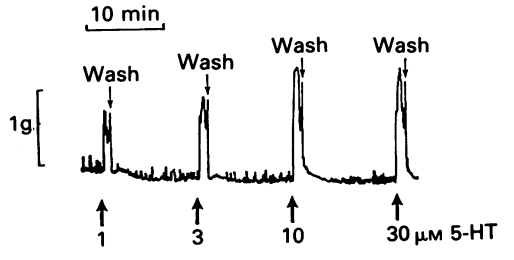

Figure 5 Typical contraction responses of the guinea-pig isolated colon to 5-hydroxytryptamine (5-HT). The traces are a continuous recording from a single preparation. Arrows indicate the application of 5-HT.

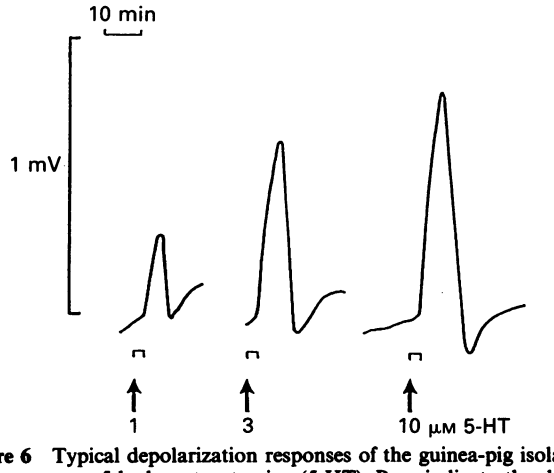

Figure 6 Typical depolarization responses of the guinea-pig isolated vagus nerve to 5-hydroxytryptamine (5-HT). Bars indicate the duration of application of 5-HT. The traces are a discontinuous recording from a single preparation.

5-HT_3 receptor antagonists caused parallel, rightward shifts of the 5-HT concentration-depolarization curves. MDL 72222 (1×10^{-6} M) caused a small shift in the concentration-response curve to 5-HT (Figure 3c). Full Schild analysis was performed on ondansetron (Figure 2c), metoclopramide, granisetron and ICS 205-930 (see Table 2 for pK_B values). For these antagonists the slopes obtained from the Schild plots did not differ significantly from unity (ondansetron, 0.89, 0.68–1.10; metoclopramide, 1.13, 0.94–1.31; granisetron 1.05, 0.85–1.25; ICS 205-930, 0.97, 0.78–1.16; slope, 95% confidence interval). For other compounds, the pK_B values were calculated from single antagonist concentrations and are shown in Table 2. The after-hyperpolarizations were also reduced by the 5-HT_3 antagonists, although this effect was not quantified. Methysergide (1×10^{-6} M) and ketanserin (1×10^{-5} M) (Figure 4c) had no effect on 5-HT-induced depolarizations in guinea-pig isolated vagus nerve.

Rat vagus nerve

As shown previously (Ireland & Tyers, 1987), 5-HT and 2-methyl-5-HT caused concentration-related depolarizations of the rat isolated vagus nerve. 5-HT_3 receptor antagonists caused rightward shifts of the concentration-depolarization response curves to 5-HT (see Table 2, Ireland & Tyers, 1987 and Butler *et al.*, 1988). For some compounds (MDL 72222, ICS 205-930, GR65630, granisetron, zacopride and GR67330) the rightward shifts were accompanied by a fall in maximum response. In these cases (except MDL 72222 and ICS 205-930, where published values are cited) a pK_B was calculated by the method of Kennedy & Roberts (1985).

[^{3}H]-GR67330 binding

Sixteen regions of guinea-pig brain were dissected (for list see Kilpatrick *et al.*, 1989). Specific binding, (defined by the inclusion of metoclopramide, 30 μM), could not be detected in any of these brain areas. Similarly, no specific binding of [^{3}H]-GR67330 could be detected with homogenates of guinea-pig ileum (longitudinal muscle-myenteric plexus).

Discussion

This study describes the pharmacological characterization of responses to 5-HT in three different tissues from the guinea-pig.

The response of the guinea-pig isolated ileum to 5-HT is well documented (Gaddum & Picarelli, 1957; Buchheit *et al.*, 1985; Richardson *et al.*, 1985; Butler *et al.*, 1988) and it is widely accepted that these responses are largely mediated via neuronally located 5-HT_3 receptors. The concentration-response curve to 5-HT is biphasic; the upper (low-potency) part of the response curve is mediated by 5-HT_3 receptors because the responses are mimicked by the selective 5-HT_3 receptor agonist, 2-methyl-5-HT and antagonized by highly selective 5-HT_3 antagonists such as ICS 205-930 and ondansetron. The lower (high potency) part of the concentration-response curve is resistant to blockade by 5-HT_3 antagonists. Craig & Clarke (1990) and Hill *et al.* (1990) have recently suggested that this response is mediated by a novel 5-HT receptor type which is also coupled positively to adenylate cyclase (Dumuis *et al.*, 1988).

The depolarizing action of 5-HT on guinea-pig isolated vagus nerve has been described previously (Burridge *et al.*, 1989; Lattimer *et al.*, 1989). Contractile effects of 5-HT in guinea-pig isolated descending colon have been described to the British Pharmacological Society (Grossman *et al.*, 1989). In contrast to responses of the guinea-pig isolated ileum, 5-HT effects in these tissues appeared to be mediated by a single 5-HT receptor type since the responses were mimicked by the selective 5-HT_3 agonist, 2-methyl-5-HT, whilst the monophasic concentration-response curves to 5-HT were shifted in a parallel fashion to the right by highly selective 5-HT_3 receptor antagonists. Furthermore, antagonists of other 5-HT receptors, such as ketanserin (5-HT_2) and methysergide (5-HT_1-like and 5-HT_2) were inactive. The depolarizing response of the guinea-pig isolated vagus nerve differs from the colon and ileum in being a directly mediated response. In the guinea-pig isolated ileum longitudinal muscle preparation and isolated descending colon preparation responses are mediated at least partially by acetylcholine because both responses are blocked insurmountably by the inclusion of atropine. Hence, estimations of agonist and antagonist potencies against the direct depolarizing response in the vagus nerve are not influenced by errors that may arise, for example, through depletion of the neurotransmitter.

In the guinea-pig isolated vagus nerve the depolarization induced by 5-HT at higher concentrations was followed by an after-hyperpolarization. This has been observed previously in other neuronal tissues in which depolarization is mediated by 5-HT_3 receptors, for example, the rabbit isolated superior cervical ganglion (Wallis & Nash, 1987) and rat isolated vagus nerve (Ireland, 1987). In the guinea-pig vagus nerve the after-hyperpolarizations, like 5-HT induced depolarizations were blocked by 5-HT_3 antagonists. This suggests that as in the rat vagus nerve, after-hyperpolarization is a consequence of depolarization and is not mediated by a different 5-HT receptor type (see Ireland, 1987).

The availability of different 5-HT_3 receptor responses in tissues from one species allows comparisons to be made between agonist potencies and antagonist affinity values. In each of the three tissues 5-HT and 2-methyl-5-HT were agonists. The EC_{50} values for 5-HT were similar in the ileum and colon ($\sim 2 \times 10^{-6}$ M) but slightly lower in the vagus nerve ($\sim 1.3 \times 10^{-5}$ M). The lower value in the vagus nerve may be due to a lower receptor reserve in this tissue which was also reflected in the lower maximum response to 2-methyl-5-HT and α-methyl-5-HT. 2-Methyl-5-HT was some three times less potent than 5-HT in each tissue which is typical of 5-HT_3 receptor-mediated responses in other species.

Phenylbiguanide has been identified as a potent 5-HT_3 receptor agonist in the rat isolated vagus nerve (Ireland & Tyers, 1987) and evokes the von Bezold-Jarisch reflex in rats (Fastier *et al.*, 1959) which has also been characterized as a 5-HT_3 receptor-mediated response. However, in each of the guinea-pig tissues described here phenylbiguanide was inactive either as an agonist or antagonist.

Conversely, α-methyl-5-HT has little effect in rat vagus nerve (Richardson *et al.*, 1985) and guinea-pig vagus nerve but we have shown it to act as a full agonist in guinea-pig ileum and guinea-pig colon. This may be explained if α-methyl-5-HT is a partial agonist and the receptor reserve in the vagus nerve is much lower than in ileum and colon.

Selective 5-HT_3 receptor antagonists inhibited the effects of 5-HT and 2-methyl-5-HT in each of the tissues described. MDL 72222 has been reported to be a weak antagonist of neuronal 5-HT contractions in the guinea-pig ileum (Fozard, 1984) and in the present study MDL 72222 was a weak antagonist in each of the three tissues. The affinity values for the 5-HT_3 antagonists were very similar in each of the guinea-pig tissues, implying that the receptors are the same. However, differences in pK_B values between the three tissues were noted for GR67330; further studies would be required before receptor heterogeneity could be suggested.

Comparison of antagonist affinities obtained in the guinea-pig tissues with those obtained against 5-HT-induced depolarization of the rat vagus nerve revealed marked differences. It is possible that the apparently low affinity of antagonists in guinea-pig tissues results from uptake and, perhaps, subsequent metabolism of agonist: the rat vagus nerve does not accumulate 5-HT actively (Ireland *et al.*, 1987). On the guinea-pig vagus nerve, the action of 5-HT was not potentiated by the 5-HT uptake inhibitor paroxetine. In addition, in no guinea-pig tissue did Arunlakshana & Schild plots of antagonism data appear non-linear or have slopes significantly less than unity. This would be expected were the apparent affinity of antagonists reduced by saturation of agonist uptake (see

Furchgott, 1972; Ireland *et al.*, 1987). In the guinea-pig, antagonist affinities were generally 10–100 fold lower. However, some compounds, such as GR80284 had similar affinities for the receptors in the guinea-pig and rat tissues whilst GR65630 had some five hundred times lower affinity for the guinea-pig receptor. These marked differences in antagonist affinities coupled with the lack of effect of phenylbiguanide in guinea-pig tissues provide convincing evidence that the 5-HT$_3$ receptors mediating responses in the guinea-pig tissues differ from those in the rat. Since all of the compounds having antagonist affinity for the guinea-pig 5-HT receptor also have affinity for the 5-HT$_3$ receptor of the rat, and are highly selective, it is reasonable to suggest that the responses in the guinea-pig tissues are also mediated by 5-HT$_3$ receptors; but these differ from the 5-HT$_3$ receptors in the rat.

This observation raises the question as to whether 5-HT$_3$ receptors also co-exist as subtypes within a species. Antagonist affinity values derived from the rat isolated vagus nerve correlate well with other affinities for 5-HT$_3$ receptor-mediated responses in other tissues in the rat such as the 5-HT$_3$-receptor binding sites in the brain (Kilpatrick *et al.*, 1987; Barnes *et al.*, 1988; Milburn & Peroutka, 1989) and vagus nerve (Kilpatrick *et al.*, 1989). There is no evidence for the 'guinea-pig-like' 5-HT$_3$ receptor in the rat. Equally, no 'rat-like' 5-HT$_3$ receptor response has been reported for guinea-pig tissues. Furthermore, using the 5-HT$_3$ receptor ligand [^{3}H]-GR67330, which labels the 5-HT$_3$ receptor in the rat (Kilpatrick *et al.*, 1990a), we could find no specific binding sites in any guinea-pig tissue tested. This could result from a lower affinity for 5-HT$_3$ receptors in the guinea-pig or from a low density of receptors.

In conclusion, the antagonist affinities of 5-HT$_3$ receptor antagonists are similar for three functional tissue responses mediated by 5-HT or 2-methyl-5-HT in the guinea-pig. The differences from affinities obtained in the rat isolated vagus nerve and the lack of effect of phenylbiguanide cannot be explained in terms of interference from other receptor types or neurotransmitter depletion. Therefore, the 5-HT$_3$ receptor in the guinea-pig tissues is almost certainly different from that in the rat. The guinea-pig and rat 5-HT$_3$ receptors can probably be termed 5-HT$_3$ receptor subtypes in the same way that 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor subtypes appear to be species variants and have similar functions in rodent (excluding the guinea-pig) and non-rodent species respectively. There are limited data on the affinities of 5-HT$_3$ receptor antagonists in other species: however, there is some indication that there may be other species variants of the 5-HT$_3$ receptor (for example in the rabbit vagus nerve, Richardson *et al.*, 1985). These observations need to be fully investigated before reaching firm conclusions, but the large differences in affinities of 5-HT$_3$ receptor antagonists between species offer a new dimension for discussion on the criteria for the classification of receptor subtypes.

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