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Pharmacological characterization of 5-hydroxytryptamine-induced depolarization of the rat isolated vagus nerve

S.J. Ireland & M.B. Tyers

Department of Neuropharmacology, Glaxo Group Research Ltd, Ware, Hertfordshire, SG12 0DJ

1 A study has been made of the pharmacology of the 5-hydroxytryptamine (5-HT)-induced depolarization responses that can be recorded extracellularly from the rat isolated cervical vagus nerve.

2 Phenylbiguanide (PBG) and 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) were found to mimic the effects of 5-HT on the vagus nerve. Their EC_{50} values were respectively 2.0 fold and 3.9 fold greater than that of 5-HT.

3 Metoclopramide behaved as a reversible competitive antagonist of depolarization induced by PBG and 2-methyl-5-HT, with pK_B values of 6.48 ± 0.04 and 6.64 ± 0.04, respectively. These agreed well with the pK_B value of 6.60 ± 0.04 obtained previously for metoclopramide against 5-HT on the rat vagus nerve. 5-HT, PBG and 2-methyl-5-HT had no demonstrable agonist effects at non-5-HT receptors on the rat vagus nerve.

4 Tropacaine and *m*-chlorophenylpiperazine were found to behave as reversible competitive antagonists of 5-HT-induced depolarization of the vagus nerve. The pK_B values were 6.29 ± 0.03 and 6.90 ± 0.03, respectively.

5 Quipazine, MDL 72222 and ICS 205-930 were also shown to be effective antagonists of 5-HT on the vagus nerve. However, although these compounds were highly potent, they all caused a marked concentration-dependent reduction in the amplitude of the maximum response to 5-HT. This behaviour was not consistent with a simple reversible competitive mechanism.

6 The results are discussed with reference to the current classification of mammalian peripheral neuronal 5-HT receptors.

Introduction

Recently, two novel, highly potent antagonists of the actions of 5-hydroxytryptamine (5-HT) on mammalian peripheral neurones have been described. These compounds, MDL 72222 (Fozard, 1984) and ICS 205-930 (Richardson *et al.*, 1985) are of special significance since they are the first 5-HT antagonists that have been demonstrated to both be effective on peripheral neurones and show clear specificity of action. Thus both MDL 72222 and ICS 205-930 are highly selective antagonists of the positive chronotropic effect of 5-HT on the rabbit isolated heart (Fozard, 1984; Richardson *et al.*, 1985), 5-HT-induced inhibition of compound action potentials in the rabbit isolated vagus nerve (Donatsch *et al.*, 1984b), and 5-HT-induced depolarization of the rabbit isolated nodose ganglion and superior cervical ganglion (SCG) (Azami *et al.*, 1985; Round & Wallis, 1986). In addition, both compounds are claimed to differentiate between sub-types of excitatory peripheral neuronal 5-HT receptors (Fozard, 1984; Richardson *et al.*, 1985). It would therefore seem desirable to use MDL 72222 and ICS 205-930 in any attempt to characterize the actions of 5-HT on other peripheral neurones.

To date, the only compound reported to behave as a reversible competitive antagonist of 5-HT-induced depolarization of the rat isolated vagus nerve is metoclopramide (Ireland *et al.*, 1982; Ireland *et al.*, 1983). In the present study, an attempt has been made to improve the pharmacological characterization of the receptors mediating this 5-HT-induced depolarization, by examining the inhibitory effects of a range of putative 5-HT antagonists – including MDL 72222 and ICS 205-930.

We also describe the effects of phenylbiguanide (PBG) and 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) on the rat vagus nerve. PBG has previously been shown to mimic selectively the effects of 5-HT on mammalian peripheral neurones (see Fastier *et al.*,

1959; Gyermek, 1964; Drakontides & Gershon, 1968). 2-Methyl-5-HT was of interest since it has been shown to mimic the effects of 5-HT on the rabbit vagus nerve and heart (Richardson *et al.*, 1985); it was about half as active as 5-HT on both these tissues. In contrast, on the smooth muscle of the rat uterus, 2-methyl-5-HT was approximately 2000 times weaker than 5-HT (Richardson *et al.*, 1985). In the present study, both PBG and 2-methyl-5-HT were found to depolarize the rat vagus nerve. The effects of metoclopramide on these depolarization responses have been quantified, and compared with the results obtained previously with this antagonist against 5-HT-induced depolarization. The effects of some non-5-HT antagonists on PBG and 2-methyl-5-HT-induced depolarization have also been examined.

A preliminary account of some of the work in this paper has been presented to the British Pharmacological Society (Fortune *et al.*, 1983; Fortune & Ireland, 1984).

Methods

Preparation of tissues

Male hooded rats weighing 200–350 g were stunned by a blow to the head and killed by cardiac puncture. Segments of cervical vagus nerve, approximately 10 to 20 mm long and minus the nodose ganglion, were excised as rapidly as possible and placed in oxygenated Krebs-Henseleit medium (greater than 25 ml per tissue) at room temperature (approximately 21°C). The connective tissue sheath around each isolated vagus nerve was then carefully removed.

Extracellular recording

Within one hour of dissection, de-sheathed vagus nerves were transferred to two-compartment Perspex baths to permit extracellular recording of agonist-induced depolarizations. Each nerve was positioned so that approximately 50% lay in the first compartment, while the remainder projected through a greased slot (Dow-Corning high vacuum grease) into the second. The d.c. potential between the two compartments was recorded via silver-silver chloride electrodes connected to the tissue preparation through agar-saline/filter paper bridges and was displayed on a potentiometric chart recorder (Servogor 220 or SE 130). Each compartment of the bath was perfused continuously at a constant rate of approximately 1–2 ml per min with Krebs-Henseleit medium dripped directly onto the tissue. Drugs were applied at known concentrations via the superfusion stream into the first compartment only.

The temperature of each preparation was maintained at 27 ± 1°C by passing solutions through heat exchangers immediately before applying them to the tissue, and by placing the recording bath and electrodes in a temperature controlled chamber. This temperature was chosen since, in preliminary experiments, recorded base-lines were more stable at 27°C than at the more physiological 37°C (results not shown).

Measurement of the effects of agonists and antagonists

In the present study, concentration-response curves for agonist-induced depolarization were constructed non-cumulatively using serially increasing concentrations. Each application of agonist was continued until apparent equilibrium was reached. In practice, this usually meant contact times of 3 min or less. Preparations were always allowed to repolarize fully between each application of agonist; typically, this took 15–30 min.

The effect of the agonists PBG and 2-methyl-5-HT were quantified in separate experiments. These were performed on a minimum of four vagus nerve preparations, each obtained from a different rat. Every preparation was exposed alternately to 5-HT and to the chosen test agonist. Half the total number of vagus nerves used in each experiment was dosed in the sequence: test agonist–5-HT–test agonist; this was reversed in the remainder.

The EC_{50} and maximum (E_{max}) for each concentration-response curve for agonist-induced depolarization were estimated by direct fit of a logistic function:

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

where y is the observed response, [A] the concentration of agonist, and n a constant. The method used for fitting logistic curves to the experimental data was based on that of Parker & Waud (1971; see also Snedecor & Cochran, 1968). The computer programme used for curve fitting in the present study was written by Miss F.J. Illingworth, Department of Computer Science, Glaxo Group Research Ltd., Greenford, Middlesex.

5-HT-induced depolarization responses of rat vagus nerve are stable and reproducible when full repolarization is allowed between each application of the agonist (see Ireland, 1984). Therefore, a standard procedure was adopted for the measurement of antagonist effects. Firstly, a control 5-HT concentration-depolarization response curve was constructed on each tissue. Then the antagonist was applied and allowed to reach apparent equilibrum. This was taken to have occured when two successive applications of an approximate EC_{50} of agonist in the presence of the antagonist gave responses equal to within 10%. Fin-

ally, a second 5-HT concentration-depolarization response curve was constructed on each tissue. Only one concentration of antagonist was applied to each vagus nerve preparation. Lateral displacements of concentration-depolarization response curves were measured at the control half-maximal response level. The negative logarithm of the apparent dissociation constant for an antagonist (pK_B) was estimated by calculation of the mean (± s.e.) of the individual results: $pK_B = \log$ (dose-ratio -1) $-\log$ (antagonist concentration).

At least four different concentrations of each antagonist were used to calculate the pK_B value. Concentrations were chosen to cover as wide a range as possible, and to give dose-ratios of between approximately 3 and 300. The effects of each concentration of antagonist were measured in at least four individual vagus nerve preparations, each obtained from a different rat.

Drugs and solutions

The composition of the Krebs-Henseleit medium used in the present study was (in mmol l^{-1}): NaCl 118, $NaHCO_3$ 25, KH_2PO_4 1.18, KCl 4.7, $MgSO_4$ 1.18, $CaCl_2$ 2.5 and glucose 11.0; it was gassed with 95% O_2 and 5% CO_2. The medium was prepared in glass-distilled water, and reagents, which were all A.R. grade, were purchased from commercial sources.

The following drugs were used: 5-HT creatinine sulphate (Sigma), 2-methyl-5-HT creatinine sulphate (Glaxo), 1-phenylbiguanide (Aldrich), 1, 1-dimethyl-4-phenylpiperazinium iodide (DMPP) (Sigma), dopamine hydrochloride (Sigma), isoprenaline sulphate (Wellcome), γ-aminobutyric acid (GABA) (Sigma), bicuculline (Sigma), phentolamine mesylate (Ciba), metoclopramide hydrochloride (Beecham), quipazine maleate (Miles), 1-(*m*-chlorophenyl) piperazine dihydrochloride (MCPP) (Aldrich), exo-8-methyl-8-azabicyclo [3.2.1]-octan-3-ol benzoate hydrochloride (tropacaine) (Aldrich), methiothepin maleate (Roche), metergoline (Farmitalia), methysergide hydrogen maleate (Sandoz), ketanserin (Salford Fine Chemicals), haloperidol (Janssen), pipamperone hydrochloride (Janssen), spiperone (Janssen), domperidone (Janssen), xylamidine (Wellcome), cinanserin hydrochloride (Squibb), MDL 72222 (1αH, 3α, 5αH-tropan-3yl-3, 5-dichlorobenzoate) gift of Dr J.R. Fozard, Merrel-Dow, Strasbourg) and ICS 205-930 ((3α-tropanyl)-1H-indole-3-carboxylic acid ester) gift of Dr G. Engel, Sandoz, Basel). All drugs were dissolved in Krebs-Henseleit medium unless otherwise stated, to give a final concentration of 1×10^{-3}–1×10^{-2} M. Solutions were prepared immediately before use. Stock solutions of (−)-noradrenaline, 1×10^{-2} M, isoprenaline 1×10^{-3} M, and dopamine, 1×10^{-2} M, contained ascorbic acid, 1×10^{-3} M, to suppress oxidation. Haloperidol, spiparone, ketanserin, metergoline, bicuculline and domperidone were all insoluble in water. The first two compounds were dissolved to give 1×10^{-2} M solutions in 0.1 M (±)-tartaric acid, metergoline was dissolved to give a 1×10^{-2} M solution in 4×10^{-2} M ascorbic acid, and bicuculline was dissolved in 0.1 M hydrochloric acid. Domperidone was dispersed in Dioxalane (Cambrian Chemicals) plus 1.5 M hydrochloric acid. All these solutions could be diluted with normal Krebs-Henseleit medium, without causing visible precipitation. Appropriate solvent controls were found to be without effect on 5-HT-induced depolarization of the rat vagus nerve (results not shown).

Results

Phenylbiguanide and 2-methyl-5-hydroxytryptamine

PBG, 1×10^{-7}–3×10^{-5} M, and 2-methyl-5-HT, 3×10^{-7}–1×10^{-4} M, induced rapid, concentration-related depolarizations of the vagus nerve that closely resembled those produced by 5-HT, 1×10^{-7}–3×10^{-5} M. The amplitude of the maximum response induced by PBG was estimated to be 79.0 ± 1.4% of the 5-HT maximum ($n = 6$); the corresponding value for 2-methyl-5-HT was 68.3 ± 3.7% ($n = 4$) (Figure 1). Both PBG and 2-methyl-5-HT were slightly less potent than 5-HT. The means of the quotients: agonist EC_{50}/5-HT EC_{50} (where both EC_{50} values were determined in the same vagus nerve preparation) were 2.0 ± 0.2 ($n = 6$) and 3.9 ± 0.3 ($n = 4$), respectively.

Metoclopramide, 1×10^{-6}–1×10^{-4} M, caused parallel rightward displacements of the concentration-depolarization response curves for PBG and 2-methyl-5-HT on the vagus nerve. Plots of the log of the dose-ratio -1 against the log of the concentration of the antagonist (Arunlakshana & Schild, 1959), were straight lines with gradients of 0.92 ± 0.05 and 0.95 ± 0.06 respectively (Figure 2). Neither was significantly different from unity ($P > 0.05$, t test). The pK_B values, calculated by constraining the gradients to unity, were 6.48 ± 0.04 ($n = 20$) against PBG, and 6.64 ± 0.04 ($n = 23$) against 2-methyl-5-HT.

Haloperidol, 3×10^{-5} M, had negligible inhibitory activity against depolarizations of the vagus nerve induced by either PBG or 2-methyl-5-HT ($n = 4$, each agonist). The pA_2 values calculated according to Schild (1947) were both less than 4.5.

Control depolarizations of the vagus nerve induced by GABA (mean EC_{50} $2.8 \pm 0.3 \times 10^{-5}$ M, $n = 3$), DMPP (mean EC_{50} $3.5 \pm 0.3 \times 10^{-5}$ M, $n = 3$) and (−)-noradrenaline (mean EC_{50} $4.0 \pm 1.0 \times 10^{-6}$ M, $n = 3$) were essentially abolished by bicuculline, 1×10^{-5} M, hexamethonium, 3×10^{-4} M, and phen-

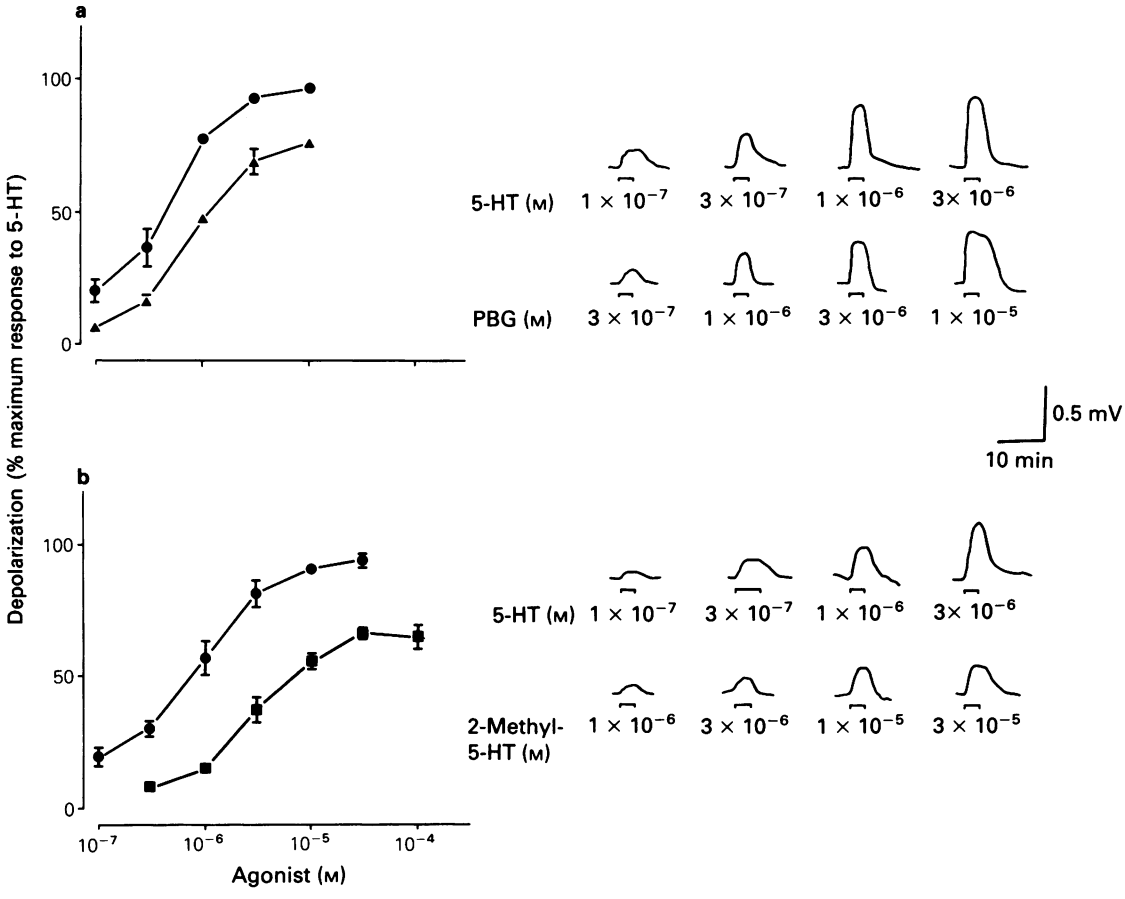


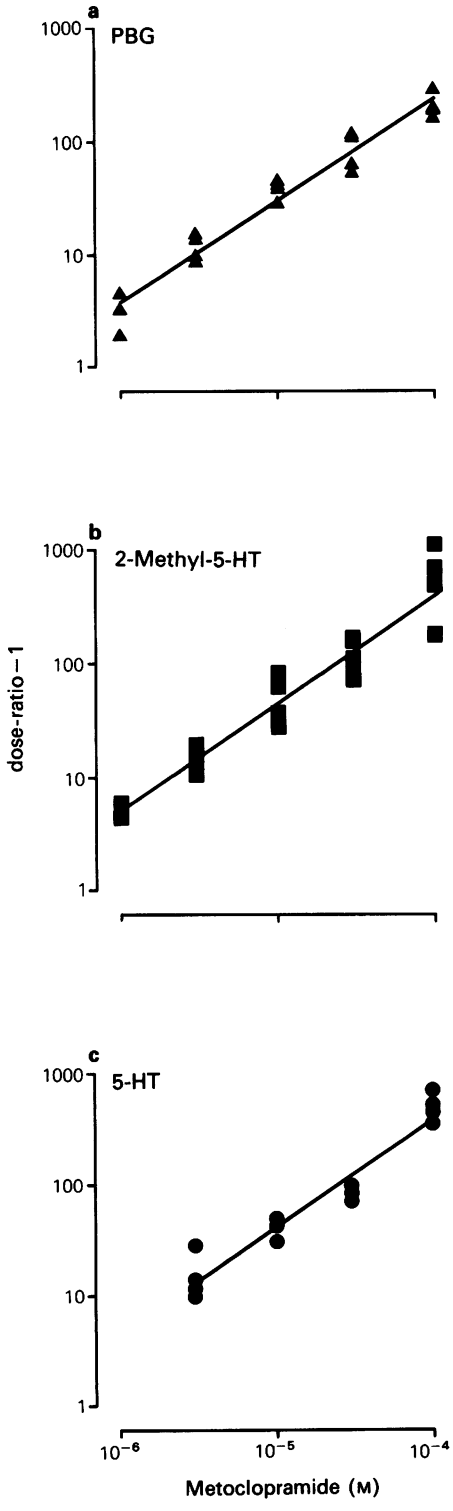
Figure 1 Comparison of the effects of 5-hydroxytryptamine (5-HT) and phenylbiguanide (PBG) (a) and 5-HT and 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) (b) on the rat isolated vagus nerve. Each point on the concentration-depolarization curves is the mean of single determinations in 6 individual tissues (a) or 4 individual tissues (b) and vertical lines show s.e.mean. Symbols indicate responses to 5-HT (●), PBG (▲) or 2-methyl-5-HT (■). Also shown are discontinuous records of the effects of the agonists on two vagus nerve preparations: one was exposed to 5-HT and PBG (a), the other to 5-HT and 2-methyl-5-HT (b). Upwards deflection indicates depolarization; the solid bar under each response shows the approximate duration of the agonist application.

tolamine, 1×10^{-6} M, respectively. In contrast, these concentrations of the antagonists did not affect concentration-response curves for the depolarization induced by 5-HT, PBG or 2-methyl-5-HT constructed either in the absence or the presence of metoclopramide, 1×10^{-4} M.

Dopamine, 1×10^{-7}–1×10^{-5} M, and isoprenaline, 1×10^{-9}–1×10^{-7} M, produced very small depolarizations of the vagus nerve. The amplitudes of the responses induced by approximately maximally-effective concentrations of dopamine (1×10^{-5} M) and isoprenaline (1×10^{-7} M) were respectively 16.4 ± 4.5% ($n = 4$) and 19.2 = 2.4% ($n = 3$) of the maximum depolarization induced by 5-HT in the same tissue preparations. Muscarine, 1×10^{-9}–1×10^{-5} M, has previously been found not to depolarize the rat vagus nerve (Ireland *et al.*, 1982).

m-*Chlorophenylpiperazine and tropacaine*

m-Chlorophenylpiperazine (MCPP), 1×10^{-6}–3×10^{-5} M, and tropacaine, 1×10^{-6}–1×10^{-4} M, produced concentration-related parallel rightward displacements of the 5-HT concentration-depolarization response curve on the vagus nerve (Figure 3). Apparent equilibrium was attained within



one hour of starting the application of either compound. Plots of the antagonism data according to Arunlakshana & Schild (1959) had gradients of 0.92 ± 0.06 and 0.99 ± 0.04, respectively (Figure 3). Neither was significantly less than unity ($P > 0.05$, t test). The estimated pK_B values were 6.90 ± 03 ($n = 17$) and 6.29 ± 0.03 ($n = 21$), respectively.

Quipazine, MDL 72222 and ICS 205-930

Quipazine, $1 \times 10^{-8} - 1 \times 10^{-6}$ M, MDL 72222, $3 \times 10^{-8} - 1 \times 10^{-6}$ M and ICS 205-930, $1 \times 10^{-10} - 3 \times 10^{-9}$ M, all produced rightward displacements of the 5-HT concentration-response curve on the vagus nerve. These were accompanied by concentration-related reductions in the maximum response. This was particularly marked with ICS 205-930 (Figure 4). Quipazine, MDL 72222 and ICS 205-930 were devoid of effects on the extracellularly-recorded membrane potential. All three compounds took 2 to 3 h to reach apparent equilibrium, as judged by stabilization of the response to an approximate EC_{50} of 5-HT in the presence of the chosen antagonist.

Effects of other putative 5-hydroxytryptamine antagonists

A range of putative 5-HT antagonists known to block the so-called 5-HT autoreceptor, or displace radioligands from 5-HT binding sites, were tested against 5-HT-induced depolarizations of the rat vagus nerve. At 3×10^{-5} M, the compounds were generally found to have only marginal activity. Typical effects observed were small (less than 10 fold) rightward displacements of the concentration response curve for 5-HT-induced depolarization, accompanied by a reduction in the maximum response. pA_2 values were calculated for the compounds according to the method of Schild (1947); these are shown in Table 1.

Figure 2 Antagonism by metoclopramide of depolarization responses of the rat isolated vagus nerve induced by (a) phenylbiguanide (PBG), (b) 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) and (c) 5-hydroxytryptamine (5-HT). Data for 5-HT are from Ireland *et al.* (1987). Each point represents the result obtained on a separate tissue. At least 4 tissue preparations, each obtained from a different rat, were used to measure the effect of each concentration of metoclopramide, on each agonist. Straight lines were fitted by linear regression.

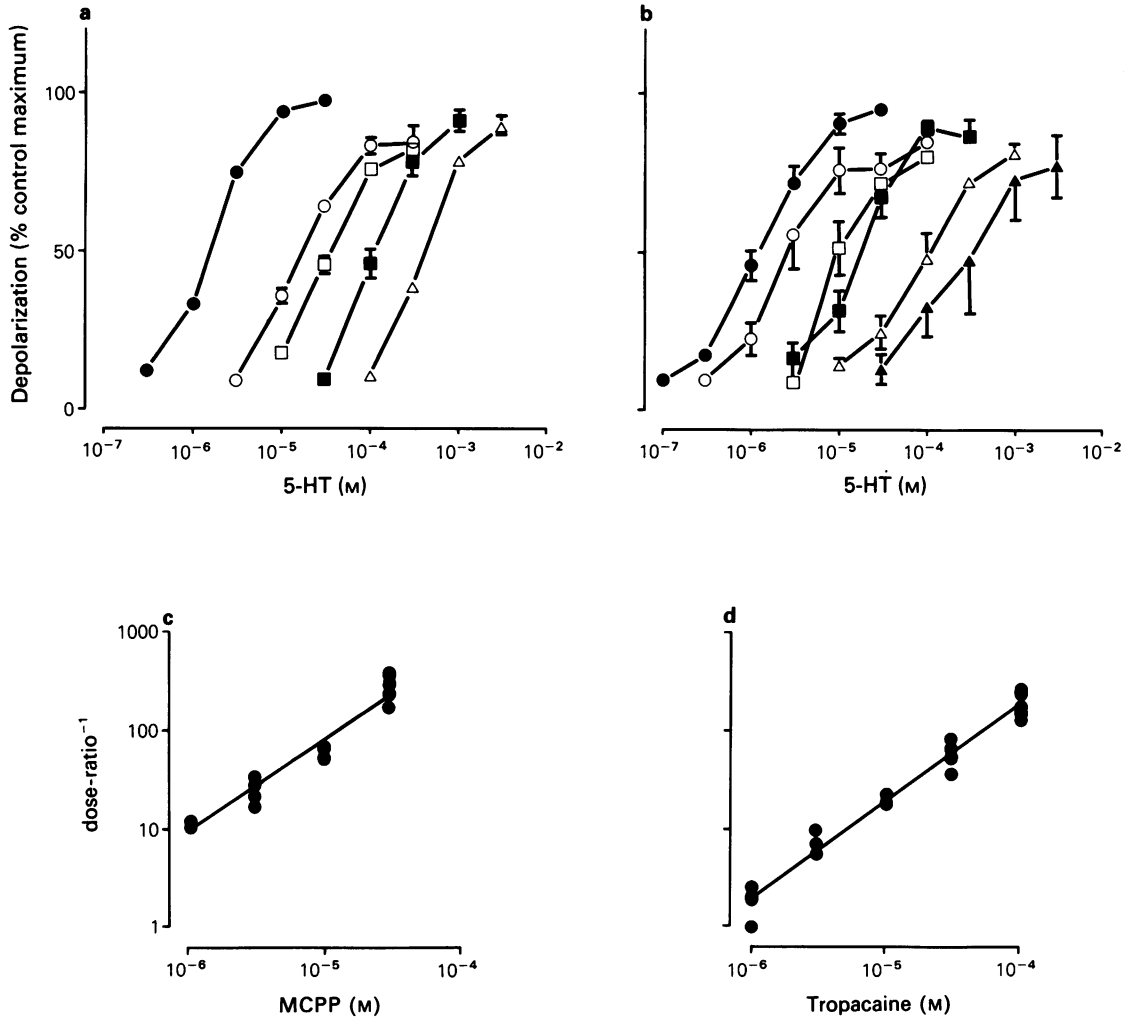


Figure 3 Antagonism by (a) *m*-chlorophenylpiperazine (MCPP) and (b) tropacaine of 5-hydroxytryptamine (5-HT)-induced depolarization of the rat isolated vagus nerve. In (a) and (b) points are means of single determinations from at least 4 separate tissues and vertical lines represent s.e.mean. Symbols indicate controls (●) or the presence of antagonist at 1×10^{-6} M (○), 3×10^{-6} M (□), 1×10^{-5} M (■), 3×10^{-5} M (△), 1×10^{-4} M (▲). (c and d) Data plotted according to Arunlakshana & Schild (1959). Each point represents the result from a separate tissue; straight lines were fitted by linear regression.

Discussion

On the rat isolated vagus nerve both PBG and 2-methyl-5-HT were found to produce depolarizations that closely resembled those induced by 5-HT. Metoclopramide was approximately equally effective as an inhibitor of the effects of all three agonists. Thus in the present study, metoclopramide behaved as a reversible competitive antagonist of depolarization induced by PBG and 2-methyl-5-HT, with pK_B values of 6.48 ± 0.04 and 6.64 ± 0.04, respectively. It has previously been shown to act similarly against depolarizations induced by 5-HT on this tissue; in this latter case, the pK_B value was 6.60 ± 0.04 (Ireland *et al.*, 1983). These results are consistent with the suggestion that 5-HT, PBG and 2-methyl-5-HT activate a common type of receptor on the rat vagus nerve.

Metoclopramide is not a specific 5-HT antagonist

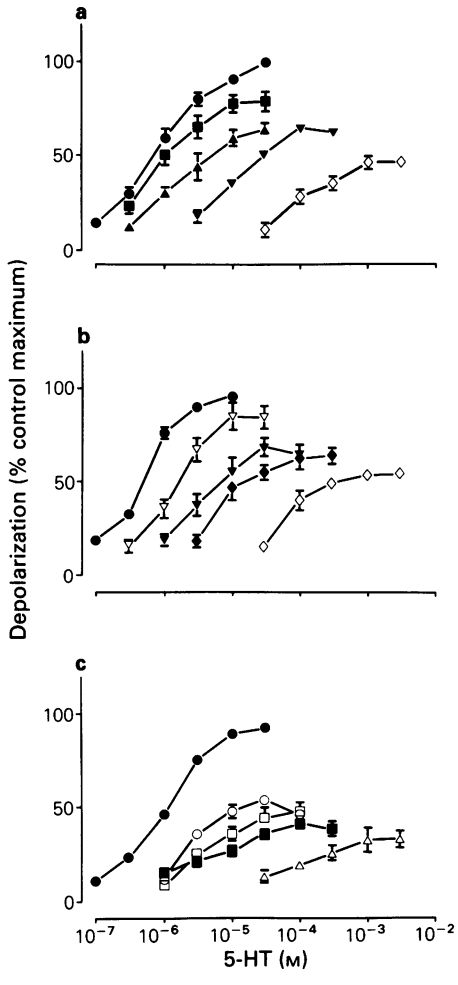


Figure 4 Antagonism by (a) quipazine, (b) MDL 72222 and (c) ICS 205-930 of 5-hydroxytryptamine (5-HT)-induced depolarization of the rat isolated vagus nerve. Points are means of single determinations from at least 4 separate tissue preparations and vertical lines represent s.e.mean. Symbols indicate controls (●), or the presence of antagonist at 1×10^{-10} M (○), 3×10^{-10} M (□), 1×10^{-9} M (■), 3×10^{-9} M (△), 1×10^{-8} M (▲), 3×10^{-8} M (▽), 1×10^{-7} M (▼), 3×10^{-7} M (◆), or 1×10^{-6} M (◇).

since it displaces [^{3}H]-spiperone and [^{3}H]-haloperidol binding in homogenates of rat corpus striatum, with pIC_{50} values of 6.95 and 5.8 respectively (Leysen *et al.*, 1978). However, it is unlikely that the antagonism by metoclopramide of depolarizations of the rat vagus nerve induced by PBG, 2-methyl-5-HT or 5-HT was due to an action at neuroleptic binding sites. Thus, haloperidol, which displaced [^{3}H]-spiperone and [^{3}H]-haloperidol binding with pIC_{50} values of 7.69 and 8.5 respectively (Leysen *et al.*, 1978), was essentially inactive against these depolarizations; the pA_2 values calculated according to Schild (1947) for antagonism of both PBG and 2-methyl-5-HT were less than 4.5, while that for 5-HT was 4.9. In addition, dopamine was a very weak agonist on the vagus nerve since the amplitude of the maximum depolarization induced by this agent was only $16.4 \pm 4.5\%$ of the maximum response to 5-HT ($n = 4$).

Evidence was also obtained to suggest that PBG, 2-methyl-5-HT and 5-HT had negligible activity at other non-5-HT receptors on the rat vagus nerve. Thus, responses by these agonists were unaffected by antagonists that blocked depolarizations induced by DMPP, GABA and (−)-noradrenaline. Further, these non-5-HT antagonists did not modify the apparent potency of metoclopramide as an antagonist of 5-HT, PBG or 2-methyl-5-HT on the vagus nerve.

PGB has previously been shown to mimic selectively some of the effects of 5-HT. For example, Fastier *et al.* (1959) have shown that both PBG and 5-HT cause reflex falls in heart rate and blood pressure in the cat, and induce pain following application to the bases of blisters in human subjects; unlike 5-HT, PBG did not contract rat blood vessels or stomach fundic strip *in vitro*. Further, Drakontides & Gershon (1968) showed cross-desensitization between PBG and 5-HT in the mouse isolated duodenum despite the fact that neither PBG nor 5-HT antagonized the effects of other spasmogens. They also found that unlike 5-HT, PBG had no direct effect on the smooth muscle of this tissue. Finally, Gyermek (1964) found that a series of biguanides, including PBG, mimicked the stimulating action of 5-HT on the cat inferior mesenteric ganglion *in situ*, while Wallis *et al.* (1982), showed that, like 5-HT, PBG depolarized the rabbit nodose ganglion *in vitro*.

2-Methyl-5-HT has previously been shown to mimic the effects of 5-HT in the rabbit isolated heart and vagus nerve, but does not appear to differentiate these two preparations from the rat vagus nerve. Thus, in the present study using the rat tissue, 2-methyl-5-HT was approximately 4 fold weaker than 5-HT in molar terms. Donatsch *et al.* (1984a) found that 2-methyl-5-HT was approximately 2 fold weaker than 5-HT on the rabbit neuronal preparations.

Tropacaine and MCPP were found to behave as reversible competitive antagonists of 5-HT-induced depolarizations of the rat vagus nerve, with pK_B values of 6.29 ± 0.03 and 6.90 ± 0.03, respectively. Like metoclopramide, tropacaine does not appear to discriminate between 5-HT-induced responses in the rat vagus nerve and rabbit heart (Table 2). Such comparative data are not available for MCPP. However, this latter compound is not a selective antagonist of the

Table 1 Effects of some putative 5-hydroxytryptamine (5-HT) antagonists on the rat vagus nerve, at 5-HT binding sites and at the 5-HT 'autoreceptor'

Compound	*Depolarization of rat vagus nerve* (pA_2)	*Effect of 5-HT inhibited Binding* (pIC_{50})		*Inhibition of 5-HT release* (pIC_{50})
		5-HT$_2$	*5-HT$_1$*	
Methysergide	4.0	8.50	7.21	6.00
Domperidone	4.5	6.58		
Ketanserin	4.5	8.88		
Pipamperone	4.5	8.58		
Cinanserin	4.8	8.18	5.88	6.00
Haloperidol	4.9	7.13		
Methiothepin	4.9		7.64	8.42
Spiperone	4.9	8.75		6.50
Metergoline	5.0	9.03	8.33	7.00
Xylamidine	5.4	8.30		
Metoclopramide	6.60*			<4.5‡
MCPP	6.90*		6.98	(Agonist)
MDL 72222	7.9	<5.0§	<5.0§	
Quipazine	8.5	6.1	6.49	6.17
ICS 205-930	11.0	<5.0†	<5.0†	

pA_2 values for blockade of 5-HT-induced depolarization of the vagus nerve were determined according to the method of Schild (1947), and are the means of single determinations on at least two individual tissue preparations. *Indicates a pK_B value. Unless stated otherwise, data for the displacement of [^{3}H]-ketanserin (5-HT$_2$) binding are from Leysen *et al.* (1981); data for the displacement of [^{3}H]-5-HT (5-HT$_1$) binding and blockade of the 5-HT 'autoreceptor' are from Martin & Sanders-Bush (1982). Symbols indicate data derived from the following sources: ‡Engel *et al.* (1983); §Fozard (1984); †Richardson *et al.* (1985). Abbreviation: MCPP = *m*-chlorophenylpiperazine.

effects of 5-HT on peripheral neurones since it has been shown to displace 5-HT$_1$-binding with a pIC_{50} of 6.98 (Martin & Sanders-Bush, 1982) and antagonize 5-HT-induced contractions of the rat isolated jugular vein with a pA_2 of 7.41 (Cohen & Fuller, 1983).

Quipazine, MDL 72222 and ICS 205-930 were all potent antagonists of 5-HT-induced depolarizations of the rat vagus nerve. However, since they caused concentration-dependent suppression of the maximum response to 5-HT, the mechanism of their antagonist actions appeared inconsistent with simple reversible competition. A full examination of the effects of quipazine against 5-HT on other mammalian neurones has not been published. MDL 72222 has been shown to cause non-parallel rightward displacements of concentration-response curves for 5-HT in the rabbit heart (Fozard, 1984), rabbit superior cervical ganglion (SCG) and rabbit nodose ganglion (Azami *et al.*, 1985; Fozard *et al.*, 1985). It is interesting to note that the latter authors observed that the nature of the antagonism produced by MDL 72222 changed from apparently competitive to insurmountable with increased time of exposure.

With ICS 205-930 there seems to be some disagreement as to the nature of the antagonism produced. In the present study on the rat vagus nerve, ICS 205-930, 1×10^{-10}–3×10^{-9} M, caused a pronounced reduction in the amplitude of the maximum depolarization induced by 5-HT. On the rabbit nodose ganglion and SCG, Round & Wallis (1986) noted similar effects with ICS 205-930, but only at concentrations of 1×10^{-9} M or greater. In contrast, Richardson *et al.* (1985) reported that ICS 205-930 behaved as a 'true competitive' antagonist when tested against the positive chronotropic effect of 5-HT in the rabbit heart, and against 5-HT-induced depression of the compound action potential in the rabbit vagus nerve. It may be of relevance that these latter effects were measured after equilibration periods of only 30 min on the heart, and 60 min on the vagus nerve, whilst the results given in the present study were obtained after at least 3 h exposure to ICS 205-930.

Given the nature of the 5-HT-antagonist effects of quipazine, MDL 72222 and ICS 205-930 on the rat vagus nerve, no attempt was made to calculate pK_B values. The approximate pA_2 values determined for these compounds according to the method of Schild (1947) (Table 2) do, however, give some guide to potency.

Quipazine, 1×10^{-6} M, has previously been shown

Table 2 Potencies of antagonists on 5-hydroxytryptamine (5-HT)-induced responses in isolated preparations of peripheral neurones

Antagonist	*Potency estimate*				
	Rat VN (pK_B)	*Rabbit Heart* (pA_2)	*Rabbit NG* (pA_2)	*Rabbit SCG* (pA_2)	*Rabbit VN* (pA_2)
Metoclopramide	6.60	7.2*, 7.1[¶]	—	—	7.3[¶]
Tropacaine	6.29	6.77†	—	—	—
MDL 72222	(7.9)	9.27‡, 8.9[¶]	7.81[§]	7.94[§]	7.9[¶]
ICS 205-930	(11.0)	10.6[¶]	9.75[§]	10.55[§]	10.2[¶]

Abbreviations: VN = vagus nerve; NG = nodose ganglion; SCG = superior cervical ganglion. MDL 72222 and ICS 205-930 did not behave as reversible competitive antagonists of 5-HT-induced depolarization of the rat VN; pA_2 values calculated according to the method of Schild (1947) are quoted. *Value from Fozard & Mobarok Ali (1978); †value from Fozard (1979); ‡value from Fozard (1984); [¶]data from Donatsch *et al.* (1984b); [§]data from Round & Wallis (1985).

to be an effective antagonist of 5-HT-induced depolarizations of the rabbit SCG and nodose ganglion, although pA_2 values were not calculated (Lansdown *et al.*, 1980; Wallis *et al.*, 1982). The potency of MDL 72222 as a 5-HT antagonist seems to depend on the tissue preparation used (Table 2). Thus, MDL 72222 appears to be approximately equipotent against 5-HT on the rat vagus nerve, rabbit vagus nerve, rabbit nodose ganglion and rabbit SCG, but is about 10 fold more potent against 5-HT in the rabbit heart. In contrast, the potency of ICS 205-930 appears to be largely independent of the tissue preparation on which it is tested (Table 2).

5-HT-induced depolarizations of the rat vagus nerve were not mimicked and were only weakly antagonized by compounds known to be potent in displacing radioligands from 5-HT_1, or 5-HT_2 binding sites, or in blocking the so-called 5-HT autoreceptor. There was no correlation between the potencies of these compounds on the vagus nerve and at the other sites in either absolute or rank-order terms (Table 1).

In conclusion, an attempt has been made to characterize 5-HT-induced depolarizations of the rat vagus nerve in terms of the effects of both agonists and antagonists. The results obtained with the two recently discovered, highly selective 5-HT antagonists MDL 72222 and ICS 205-930 were disappointing, since they did not seem to behave in a reversible competitive manner against 5-HT on this tissue preparation. This suggests caution in the use of MDL 72222 and ICS 205-930 as tools for the characterization of neuronal 5-HT receptors. Nevertheless, the observation that 5-HT-induced depolarizations of the rat vagus nerve were potently antagonized by these two compounds with approximate pA_2 values of 7.9 and 11.0 respectively, were essentially unaffected by ketanserin and methiothepin, and were mimicked by 2-methyl-5-HT with a potency similar to that of 5-HT itself, satisfy the recently proposed criteria for the involvement of a 5-HT_3-receptor (Bradley *et al.*, 1986) in their generation. However, we conclude that no good evidence is available to demonstrate convincingly any difference between the receptors that mediate 5-HT-induced depolarization of the rat vagus nerve, and those that mediate either 5-HT-induced depolarization of the rabbit nodose ganglion and SCG, or 5-HT-induced inhibition of action potential propagation in the rabbit vagus nerve.

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