An examination of the 5-HT₃ receptor mediating contraction and evoked [³H]-acetylcholine release in the guinea-pig ileum

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1 The relative contributions of two classes of 5-hydroxytryptamine (5-HT) receptor (5-HT₂ and 5-HT₃) to the contractile action of 5-HT, 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) and α -methyl-5-hydroxytryptamine (α -methyl-5-HT) were studied in the guinea-pig ileum longitudinal muscle-myenteric plexus strip (LMMP) preparation. Contractility studies were combined with an analysis of the effects of the three agonists on [³H]-acetylcholine ([³H]-ACh) release from preparations preincubated with [³H]-choline.

2 In contracting the LMMP, 5-HT was approximately one order of magnitude more active than 2-methyl-5-HT and α -methyl-5-HT, with relative activities for 5-HT: 2-methyl-5-HT: α -methyl-5-HT of 1.00: 0.13: 0.10.

3 Ketanserin (1 μ M) was without effect on the concentration-response curves for contraction to 5-HT, 2-methyl-5-HT or α -methyl-5-HT, whilst ondansetron (GR38032F; 1 μ M) produced a parallel rightward displacement of the upper part of the concentration-response curves to 5-HT and α -methyl-5-HT and of the entire curve to 2-methyl-5-HT.

4 In increasing the spontaneous release of [³H]-ACh from the LMMP, 5-HT was again approximately one order of magnitude more active than 2-methyl-5-HT and α -methyl-5-HT with relative activities for 5-HT: 2-methyl-5-HT: α -methyl-5-HT of 1.00: 0.19: 0.11.

5 Ondansetron (1 μ M) greatly attenuated the increase in spontaneous [³H]-ACh release evoked by all three agonists. pK_B estimates of 7.62 ± 0.12 and 7.64 ± 0.09 were obtained for ondansetron antagonism of 5-HT and 2-methyl-5-HT-evoked increases respectively.

6 These data suggest that the contractile action of 5-HT, 2-methyl-5-HT and α -methyl-5-HT in the guinea-pig ileum can, under these conditions, be accounted for largely in terms of 5-HT₃ receptor activation. Estimates for pK_B obtained with ondansetron are in accordance with those previously obtained from contractility studies in this preparation and these findings are discussed in terms of the postulated existence of subtypes of 5-HT₃ receptors.

Introduction

Gaddum & Picarelli (1957) showed that 5-hydroxytryptamine (5-HT) can contract the guinea-pig ileum via an interaction with two different receptor types – the neuronal 'M' receptor and the 'D' receptor located on the longitudinal smooth muscle, subsequently referred to as 5-HT₃ and 5-HT₂ receptors respectively (Bradley *et al.*, 1986). However, literature reports differ considerably with respect to the relative contribution of these pre- and post-junctional receptors to the contractile action of 5-HT in the guinea-pig ileum. For example, whilst Chahl (1983) found that most of the contractile effect of 5-HT was mediated through D (5-HT₂) receptors, others (e.g. Brownlee & Johnson, 1963; Costa & Furness, 1979) concluded that the major component was due to M (5-HT₃) receptor-mediated acetylcholine (ACh) release, although the exact contribution may vary with the region used. This lack of a significant D receptor involvement was also found by Buchheit *et al.* (1985).

However, these various conclusions were all drawn from contractility studies, using 5-HT alone, in which any neurotransmitter-mediated effects are inferred from postjunctional responses of the smooth muscle which is itself influenced by 5-HT. Also, it has been reported that the contractile concentration-response curve to 5-HT in this preparation is biphasic in form, and that substance P release mediates the neuronal actions of 5-HT (Buchheit *et al.*, 1985). Additional actions of 5-HT in the guinea-pig ileum include 5-HT₁-like-receptor-mediated relaxation (Fenuik *et al.*, 1983), a possible 5-HT₃ receptor-mediated inhibitory component

(Gunning & Humphrey, 1987), 5-HT_{1A} receptor-mediated inhibition of ACh release (Fozard & Kilbinger, 1985) and non-5-HT₃ neuronal excitation (Sanger, 1987; Craig & Clarke, 1990), which must necessarily complicate interpretation of results from any studies.

Therefore the rationale of the present experiments was to obtain direct estimates of ACh release, in parallel with contractile studies. We have re-examined the relative contribution of 5-HT₂ and 5-HT₃ receptors to the contractile action of 5-HT in the longitudinal muscle-myenteric plexus preparation using the 5-HT₃ receptor agonist 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) (Richardson *et al.*, 1985; Bradley *et al.*, 1986), and α -methyl-5-hydroxytryptamine (α -methyl-5-HT) claimed to be selective for 5-HT₂ receptors (Richardson *et al.*, 1985). Studies of the contractile effects of these three agonists were combined with studies of their ability to increase the release of [³H]-ACh (estimated as tritium overflow) from preparations prelabelled with [³H]-choline, to eliminate as far as possible other complicating actions of 5-HT mentioned above. The involvement of 5-HT₃ receptors in this ACh release was examined by the use of ondansetron (GR38032F) which behaves as a competitive reversible antagonist towards 5-HT₃-mediated contractions in the guinea-pig ileum (Butler *et al.*, 1988). Previous studies of 5-HT-evoked ACh release (Kilbinger & Pfeuffer-Friederich, 1985) used MDL 72222 which has little activity at the 5-HT₃ receptor of this system (Fozard, 1984).

The lower affinity of 5-HT₃ receptor antagonists such as ICS 205-930 and MDL 72222 in the guinea-pig ileum as compared to other preparations including the rabbit isolated heart and the rat or rabbit vagus nerve (Donatsch *et al.*, 1984; Ireland & Tyers, 1987) led to the proposal of 5-HT₃ receptor

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heterogeneity (Fozard, 1984; Richardson & Engel, 1986). More recent 5-HT₃ antagonists such as ondansetron and BRL 43694 (granisetron) also discriminate between the guinea-pig ileum and other preparations (Butler *et al.*, 1988; Sanger & Nelson, 1989). Again, however, such results in the guinea-pig ileum may be compromised by the indirect measurement of 5-HT₃ receptor activation and the complex actions of 5-HT. Here we describe a more direct measure of the affinity of ondansetron for the 5-HT₃ receptor of the guinea-pig ileum through pK_B estimates of its antagonism of 5-HT and 2-methyl-5-HT evoked increases in [³H]-ACh release.

A preliminary account of this work has been communicated to the British Pharmacological Society (Fox & Morton, 1989).

Methods

Male Dunkin-Hartley guinea-pigs (250–500 g) were killed by stunning and exsanguination. The ileum was excised approximately 10 cm from the ileo-caecal junction and strips of longitudinal muscle with adherent myenteric plexus (LMMP) were removed according to the method of Rang (1964) from distal portions of ileum.

Contractility studies

LMMP strips were mounted in 1 ml organ baths containing Krebs solution aerated with 95% O₂/5% CO₂ and maintained at 37°C. Contractile responses were recorded isometrically with Grass FT03B force-displacement transducers with a resting tension of 0.5–1.0 g. In all experiments the bathing solution contained hexamethonium (10 µM), mepyramine (1 µM), guanethidine (1 µM) and ibuprofen (1 µM).

For each experiment four tissues were obtained from each of three different animals and used as twelve preparations studied in parallel. In a balanced block design two of each set of four tissues received either ondansetron (1 µM) or ketanserin (1 µM) throughout, with the remaining two acting as concurrent controls. Concentration-response curves for 5-HT (10 nM–30 µM), 2-methyl-5-HT (0.1 µM–300 µM) and α -methyl-5-HT (0.1 µM–300 µM) were constructed, after at least 30 min equilibration with antagonist, using serial application with 30–45 s contact times and with a 15 min dose-cycle to avoid desensitization. Each preparation received 5-HT and either 2-methyl-5-HT or α -methyl-5-HT to allow direct comparison with 5-HT itself, with concentrations applied in a fully randomized order. Histamine (10 µM) was applied between each 5-HT analogue dose as an internal sensitivity control. All responses were expressed as a percentage of the maximal response to carbachol in individual preparations for the purpose of displaying averaged concentration-response curves. Data from each set of experiments using either ketanserin or ondansetron were combined and expressed in terms of mean response $\pm$ s.e.mean for each concentration with agonist activities defined in terms of pD_2 ($-\log_{10} EC_{50}$ (M)) estimates obtained from individual experiments.

³H-overflow studies as an index of acetylcholine release

Four LMMP strips were mounted in perfusion chambers on hypodermic tubing holders (o.d. 0.9 mm) and superfused at a rate of 1 ml min⁻¹ with Krebs solution aerated with 95% O₂/5% CO₂ and maintained at 37°C. The tissues were stimulated electrically via a lower platinum electrode and an upper electrode of 0.1 mm annealed stainless steel wire that also served for attachment to the transducer with 1 ms square wave pulses at 0.2 Hz and supramaximal voltage (50–70 V) delivered from Grass S44 stimulators. LMMP preparations were then incubated for 1 h in Krebs solution containing [³H]-choline (0.2 µM; 2.89 GBq mol⁻¹) in 1 ml vials inserted under each preparation within the perfusion chamber and aerated via the

muscle holder tubing. During this period electrical stimulation was maintained to enhance uptake of [³H]-choline, with concomitant contractions recorded isometrically with Grass FT03B force-displacement transducers. Superfusion was then resumed in the absence of electrical stimulation with Krebs solution containing hemicholinium-3 (10 µM) and the 5-HT uptake inhibitor citalopram (0.1 µM) and the preparations were washed for 80 min the superfusate being discarded. After this time ³H-overflow had reached a steady level and the superfusate was then collected every 1 min with 3 min agonist application periods and a 15 min dose cycle. Antagonists, when used, were present in the superfusing medium from the start of the post [³H]-choline washout period.

In experiments designed to estimate relative activities of 5-HT, 2-methyl-5-HT and α -methyl-5-HT, each preparation received one agonist at three different concentrations applied as part of a Latin square balanced block design. At the end of every experiment the selective NK₃ receptor agonist senktide, which is known to release ACh from this preparation (Wormser *et al.*, 1986), was applied to each preparation as a positive internal control. Experiments of a similar design were performed with ondansetron (1 µM) present in the superfusing medium of two preparations from the start of the post [³H]-choline washout, with the remaining two acting as concurrent controls.

To test for the possible involvement of other mechanisms or mediators, the effects of hexamethonium (10 µM), mepyramine (1 µM), guanethidine (1 µM) and ibuprofen (1 µM), (which were present throughout the contractility experiments), were assessed against responses to submaximal concentrations of 5-HT, 2-methyl-5-HT and α -methyl-5-HT. Additionally, ketanserin (1 µM) and spiperone (1 µM) were used in these experiments to test for 5-HT₂ and 5-HT_{1A} receptor-mediated influences. The possible involvement of non-5-HT₃ receptors which may mediate contraction or enhance electrically-evoked contractions in this preparation was also examined (see Sanger, 1987; Clarke *et al.*, 1989). Responses to 5-methoxytryptamine (1 µM and 10 µM), BRL 24924 (0.1 µM and 1 µM) and α -methyl-5-HT (3 µM and 30 µM), which are agonists at these receptors (Craig & Clarke, 1990; Eglen *et al.*, 1990; Hill *et al.*, 1990), were tested in the presence of ondansetron (10 µM) to ensure complete antagonism of 5-HT₃ receptors (see Hill *et al.*, 1990).

In experiments to estimate the affinity of ondansetron for the receptor mediating the responses to 5-HT and 2-methyl-5-HT, one preparation acted as a control, whilst the remaining three received ondansetron (0.1 µM, 1 µM or 10 µM) throughout. Each preparation then received three appropriately increased concentrations of agonist applied in a Latin square design to enable shifts in the agonist concentration-response line to be measured.

At the end of each experiment tissues were solubilised in NCS tissue solubiliser (Amersham); these and the superfusate samples were then assayed for radioactivity by liquid scintillation spectrometry. For each preparation the fractional release of initial ³H content was calculated as rate coefficient (min⁻¹) for each collection period (for expression of ACh release in terms of ³H overflow see, Szerb, 1976; Wikberg, 1977; Kilbinger & Pfeuffer-Friederich, 1985). Drug effects were expressed as a percentage increase in average rate coefficient during the 3 min drug treatment period over the average for the preceding 3 min control period. Results from each set of experiments were combined and expressed as mean $\pm$ s.e.mean increase in rate of loss in order to display averaged concentration-response curves. For each analogue pD_2 estimates obtained from individual experiments were combined and expressed as mean $\pm$ s.e.mean which thus reflects between-preparation variability. The equilibrium dissociation constant estimates for ondansetron expressed as the negative logarithm (pK_B) and s.e.mean, were obtained from individual dose-ratios (x) by direct calculation from the Gaddum-Schild equation ($pK_B = pA_2 = \log_{10} (x - 1) - \log_{10}[A]$) for competition, confirmed by Schild analysis, and assuming a Normal distribution of $\log (x - 1)$.

Figure 1 Log concentration-response curves for the effects of 5-hydroxytryptamine (5-HT) (●), 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) (▲) and α-methyl-5-hydroxytryptamine (α-methyl-5-HT) (■). (a) Contractile responses of longitudinal muscle myenteric plexus (LMMP) strips. Results were obtained from preparations which received 5-HT and either 2-methyl-5-HT or α-methyl-5-HT. Each point represents the mean from 24 (5-HT) or 12 (2-methyl-5-HT and α-methyl-5-HT) preparations; s.e.mean shown by vertical bars. (b) Increase in spontaneous outflow of [³H]-acetylcholine from LMMP strips preincubated with [³H]-choline. Results were obtained from experiments in which each preparation received one agonist at three different concentrations. Each point represents the mean from 8 preparations; s.e.mean shown by vertical bars.

Drugs

The following drugs were used:- [methyl-³H] choline chloride (Amersham International plc); hemicholinium-3, 5-hydroxytryptamine creatinine sulphate, 5-methoxytryptamine hydrochloride, histamine diphosphate, mepyramine maleate, hexamethonium bromide (all Sigma); guanethedine monosulphate and ibuprofen (Ciba); citalopram (Lundbeck Ltd); senktide (Suc-[Asp⁶, MePhe⁸]-substance P(6-11); gift, Dr B.N. Williams, Merck, Sharpe and Dohme Laboratories); spiperone 2-methyl-5-HT, α-methyl-5-HT, ketanserin (all RBI); BRL 24924 ([(+)-(endo)-4-amino-5-chloro-2-methoxy-N-(1-azabicyclo-[3.3.1]-non-4-yl)benzamide hydrochloride; gift, Dr G.J. Sanger, Beecham Pharmaceuticals) and ondansetron (GR38032F), (1,2,3,9-tetrahydro-3-[(methylimidazol-1-yl)methyl]-9-methyl-4H-carbazol-4-one; gift, Glaxo Group Research Ltd.).

Figure 2 Effects of ketanserin on contractile responses of LMMP strips to (a) 5-hydroxytryptamine (5-HT), (b) 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) and (c) α-methyl-5-hydroxytryptamine (α-methyl-5-HT). Symbols indicate concurrent control responses (●) or responses in the presence of ketanserin (1 μM) (○). Results were obtained from preparations which received 5-HT and either 2-methyl-5-HT or α-methyl-5-HT. In (a-c) each point represents the mean from 12 (5-HT) or 6 (2-methyl-5-HT and α-methyl-5-HT) preparations; s.e.mean shown by vertical bars.

All stock solutions of drugs were prepared in distilled water except for ibuprofen (0.01 M sodium bicarbonate) and senktide (0.01 M acetic acid).

Results

Contractility studies

5-HT, 2-methyl-5-HT and α-methyl-5-HT produced concentration-response curves of similar shape and maxima

Table 1 Comparison of agonist activities for contraction and [³H]-acetylcholine ([³H]-ACh) release in the guinea-pig ileum

Measure	<i>pD</i> ₂			Relative activities		
	5-HT	2-Me-5-HT	α-Me-5-HT	5-HT	2-Me-5-HT	α-Me-5-HT
Contraction	5.70	4.80	4.70	1.00	0.13	0.10
(s.e.mean)	(0.05)	(0.05)	(0.08)			
[³ H]-ACh release	5.73	5.00	4.76	1.00	0.19	0.11
(s.e.mean)	(0.16)	(0.14)	(0.10)			

Activities of 5-hydroxytryptamine (5-HT), 2-methyl-5-hydroxytryptamine (2-Me-5-HT) and α-methyl-5-hydroxytryptamine (α-Me-5-HT) are shown both in terms of *pD*₂ (-log₁₀ EC₅₀) estimates and relative activities calculated from these values. Each *pD*₂ estimate for contraction represents the mean ± s.e.mean as shown from 24 (5-HT) or 12 (2-methyl-5-HT and α-methyl-5-HT) determinations; *pD*₂ estimates for [³H]-ACh release are from 8 determinations for each analogue.

which allowed activity comparisons to be made in terms of the location parameter. 5-HT was approximately one order of magnitude more active than 2-methyl-5-HT and α -methyl-5-HT (Figure 1a) and relative activities of the three analogues calculated from averaged pD_2 estimates are shown in Table 1.

Responses to 5-HT, 2-methyl-5-HT and α -methyl-5-HT were unaffected by ketanserin ($1\mu M$) (Figure 2) whilst ondansetron ($1\mu M$) antagonized contractions to all three agonists. This concentration of ondansetron caused a parallel rightward shift of the upper part only of the concentration-response curves to 5-HT and α -methyl-5-HT but of the entire curve to 2-methyl-5-HT (Figure 3).

3H -overflow studies

5-HT ($0.1\mu M$ – $10\mu M$), 2-methyl-5-HT ($1\mu M$ – $100\mu M$) and α -methyl-5-HT ($1\mu M$ – $100\mu M$) all produced concentration-dependent increases in 3H -overflow, used as an index of $[^3H]$ -ACh release, which matched in time-course the concurrently recorded contractions of the tissue. Figure 4 shows a typical example of the experiments carried out in this study, including responses to the NK_3 receptor agonist senktide.

Figure 3 Effects of ondansetron on contractile responses of LMMP strips of guinea-pig ileum (a) 5-hydroxytryptamine (5-HT), (b) 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) and (c) α -methyl-5-hydroxytryptamine (α -methyl-5-HT). Symbols indicate concurrent control responses ($\bullet$) or responses in the presence of ondansetron ($\circ$). Results were obtained from preparations which received 5-HT and either 2-methyl-5-HT or α -methyl-5-HT. In (a–c) each point represents the mean from 12 (5-HT) or 6 (2-methyl-5-HT and α -methyl-5-HT) preparations; s.e.mean shown by vertical bars.

Figure 4 Effects of agonists on outflow of $[^3H]$ -acetylcholine ($[^3H]$ -ACh). LMMP strips of guinea-pig ileum were preincubated with $[^3H]$ -choline and subsequently superfused with Krebs solution containing hemicholinium-3. Perfusate samples were collected every 1 min with 3 min agonist application periods shown by horizontal bars. Individual preparations shown received (a) 5-hydroxytryptamine (5-HT), (b) 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) or (c) α -methyl-5-hydroxytryptamine (α -methyl-5-HT) at the concentrations shown with senktide (10 nM) applied lastly to all preparations. Concurrently recorded contractions are shown for 5-HT ($10\mu M$) and senktide (10 nM) (a), 2-methyl-5-HT ($100\mu M$) (b), and α -methyl-5-HT ($100\mu M$) (c).

Concentration-response lines for the three agonists are essentially parallel (Figure 1b) and relative activity estimates are very similar to those obtained from the contractility studies (Table 1).

Ondansetron ($1\mu M$) greatly attenuated the increases in 3H -overflow evoked by the three agonists, producing a decrease in response to the highest concentrations used of 5-HT ($10\mu M$), 2-methyl-5-HT ($100\mu M$) and α -methyl-5-HT ($100\mu M$) of $79.5 \pm 6.9\%$, $70.1 \pm 2.5\%$ and $87.1 \pm 12.9\%$ respectively. Hexamethonium ($10\mu M$), mepyramine ($1\mu M$), guanethidine ($1\mu M$), ibuprofen ($1\mu M$), ketanserin ($1\mu M$) and spiperone ($1\mu M$) were all without effect on responses to each of the three agonists (data not shown). Additionally, no increase in 3H -overflow was detected with either 5-methoxytryptamine, BRL 24924 or α -methyl-5-HT in the presence of ondansetron ($10\mu M$) (data not shown).

Increasing concentrations of ondansetron gave parallel rightward shifts in the concentration-response lines for 5-HT and 2-methyl-5-HT (Figure 5a) and pK_B estimates obtained from individual dose-ratios were 7.62 ± 0.12 against 5-HT

Figure 5 Ondansetron antagonism of 2-methyl-5-hydroxytryptamine-(2-methyl-5-HT) and 5-hydroxytryptamine-(5-HT) evoked increases in outflow of [^3H]-acetylcholine. (a) Parallel displacement of averaged concentration-response curves to 2-methyl-5-HT by ondansetron at three concentrations. Results were obtained from preparations receiving 2-methyl-5-HT alone (●) or in the presence of ondansetron at 0.1 μM (▲), 1 μM (■) or 10 μM (◆). Each point represents the mean from 5 preparations; s.e.mean shown by vertical bars. (b) Schild plot analysis of individual competition experiments of ondansetron against 5-HT (○) or 2-methyl-5-HT (△). The coefficient of linear regression did not differ significantly from 1.0 ($P > 0.05$) and so unity slope was imposed. The intercepts correspond to pA_2 ($= \text{pK}_B$) values of 7.62 and 7.64 respectively. Each point represents the mean from 4 (5-HT) or 5 (2-methyl-5-HT) determinations; s.e.mean shown by vertical bars.

($n = 12$) and 7.64 ± 0.09 against 2-methyl-5-HT ($n = 15$). Figure 5b shows the antagonist activity of ondansetron displayed in the conventional manner as Schild plots. Since there was no significant departure from unity slope, and the agonist and antagonist were presumed to be in an equilibrium state, the pA_2 intercept is taken to reflect the equilibrium dissociation constant K_B , expressed as a pK_B value.

Discussion

The results presented here show that in the guinea-pig ileum, under the conditions of this study, the contractile action of 5-HT, and in addition that of 2-methyl-5-HT and α -methyl-5-HT, are mediated predominantly through the activation of 5-HT $_3$ receptors. Thus, contractile responses to all three agonists were readily blocked by the selective 5-HT $_3$ receptor antagonist, ondansetron. In contrast, the selective 5-HT $_2$ receptor antagonist, ketanserin, was ineffective indicating that 5-HT $_2$ receptors have no significant involvement in responses to 5-HT and the two analogues. This conclusion is substantiated by the results from the ^3H -overflow studies, used as an estimation of ACh release, which show that the three agonists cause increases in spontaneous release in the same concentration-range as their contractile effects. Furthermore, the relative activities for 5-HT, 2-methyl-5-HT and α -methyl-5-HT in increasing ^3H -overflow are very similar to those for contraction, and this increase in release is sensitive to antago-

nism by ondansetron to a comparable extent for all three agonists. Taken together, three results are consistent with the notion that the contractile action of the three agonists can be accounted for largely in terms of 5-HT $_3$ receptor-mediated ACh release. In addition, they support the assumptions involved in estimating evoked ACh release in terms of increased ^3H -overflow.

These results also bear upon the suggested involvement of substance P (SP) in the contractile action of 5-HT in the guinea-pig ileum (Buchheit *et al.*, 1985). These authors proposed that 5-HT causes contraction through two different mechanisms. At low concentrations of 5-HT ($< 0.3 \mu\text{M}$) it was proposed that activation of a high affinity, non-5-HT $_3$ receptor releases SP which in turn liberates ACh to cause contraction; at higher concentrations ($> 0.3 \mu\text{M}$) 5-HT $_3$ receptor activation liberates SP which then causes direct contraction of the smooth muscle. It should be noted, however, that this work could not be repeated by other workers (Sanger & Nelson, 1989). Since we show that the action of 5-HT is mediated predominantly through ACh release this would seem to preclude a major direct action of SP. Furthermore, whilst from the results presented here we cannot rule out the proposal that SP, or some other neurokinin, mediates the ACh release measured following 5-HT application, our data do suggest that this is not likely under the conditions of our experiments.

In this regard, examination of the effects of ondansetron against the contractile activity of 5-HT, 2-methyl-5-HT and α -methyl-5-HT does indeed suggest the involvement of another, non-5-HT $_3$ receptor, component in the response to 5-HT. Thus, in the presence of ondansetron only the upper portions of the concentration-response curves to 5-HT and α -methyl-5-HT are shifted to the right, whereas the entire curve to 2-methyl-5-HT is shifted (see Figure 3). This effect of ondansetron on the 5-HT and 2-methyl-5-HT concentration-response curves confirms that reported by Butler *et al.* (1988), and the selective shift in the upper portion of the curve to 5-HT is seen also with other 5-HT $_3$ receptor antagonists such as BRL 43694 (Sanger & Nelson, 1989) and ICS 205-930 (Buchheit *et al.*, 1985). These results suggest that 2-methyl-5-HT contracts the ileum solely through 5-HT $_3$ receptor-mediated ACh release. It appears though, that some other receptor is involved in the response to 5-HT and also to α -methyl-5-HT, which produces the lower, ondansetron-insensitive, phase of their concentration-response curves. This receptor is likely to be the recently described excitatory neuronal non-5-HT $_3$ receptor (Clarke *et al.*, 1989; Hill *et al.*, 1990; Eglen *et al.*, 1990) which mediates ACh release and which corresponds to the high affinity receptor proposed by Buchheit *et al.* (1985; see above). However, we could not detect any increases in ^3H -overflow in response to 5-methoxytryptamine, BRL 24924 or α -methyl-5-HT (each in the presence of ondansetron), all of which are reported to be agonists at this receptor (Eglen *et al.*, 1990; Hill *et al.*, 1990). This suggests that, under the conditions of our experiments, this non-5-HT $_3$ receptor plays at the most a minor role in the contractile action of 5-HT, and it is therefore unlikely that SP mediates the ACh release measured in this study in response to 5-HT.

As mentioned in the introduction, it has been suggested that the 5-HT $_3$ receptor of the ileum differs from those found in some other preparations. However, the use of contractility studies with the guinea-pig ileum for the study of 5-HT $_3$ receptors has been questioned since their activation is measured indirectly. For this reason 5-HT $_3$ receptors have recently been studied in the guinea-pig isolated vagus nerve, a new model for 5-HT $_3$ receptors in which their activation by 5-HT is measured directly. Here too, 5-HT $_3$ receptor antagonists produce lower pA_2 estimates than, for instance, in the rat vagus nerve (Burridge *et al.*, 1989; Lattimer *et al.*, 1989).

With this in mind we carried out experiments to obtain pA_2 estimates for ondansetron against 5-HT and 2-methyl-5-HT using direct estimates of ACh release through measurements of ^3H -overflow. The increases in release evoked by 5-HT and

2-methyl-5-HT were unaffected by any other of the antagonists tested (see Results) but were markedly inhibited by ondansetron. Figure 5 shows that pA_2 estimates can be taken to reflect true pK_B values and are indistinguishable when obtained for ondansetron against 5-HT or 2-methyl-5-HT, thereby indicating that both agonists are acting on the same receptor. This similarity in pK_B values also suggests that there is no significant activation of the excitatory neuronal non-5-HT₃ receptor by 5-HT since 2-methyl-5-HT is not an agonist at this site (Eglen *et al.*, 1990; Hill *et al.*, 1990). These pK_B values, both of 7.6, are slightly higher than previously reported pK_B (7.33; Butler *et al.*, 1988) or pA_2 (7.1; Lattimer *et al.*, 1989) values for ondansetron against 5-HT in the guinea-pig ileum which may reflect the complications seen with measurements made from contractility studies. The results presented here provide the first pK_B estimate for a 5-HT₃ receptor antagonist in the guinea-pig ileum obtained through direct estimates of neurotransmitter release. These estimates support the recent suggestion that 5-HT₃ receptors show considerable

species variation, with that of the guinea-pig ileum being very similar to that of the guinea-pig vagus (Lattimer *et al.*, 1989).

Finally, it is clear from this study that α -methyl-5-HT, reported as a selective 5-HT₂ receptor agonist in preparations such as the rat uterus and rabbit aorta (see Bradley *et al.*, 1986) does not act in this way in the guinea-pig ileum. Given the fact that, under the conditions of these experiments, 5-HT₂ receptors are not involved in the response to 5-HT, α -methyl-5-HT would be expected to be inactive in this preparation. Instead, its general profile of activity is very similar to that of 5-HT and 2-methyl-5-HT, with it causing contraction and increases in [³H]-ACh release via 5-HT₃ receptor activation, thereby throwing doubt on its use as a selective 5-HT₂ receptor agonist.

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