A comparative study of functional 5-HT₄ receptors in human colon, rat oesophagus and rat ileum

Peter G. McLean,¹ Ian M. Coupar & *Peter Molenaar

School of Pharmaceutical Pharmacology, Victorian College of Pharmacy, Monash University, Parkville, Victoria 3052, Australia and *Department of Pharmacology, University of Melbourne, Parkville, Victoria 3052, Australia

1 The pharmacological properties of 5-hydroxytryptamine (5-HT), the 5-HT₄ receptor agonists, DAU 6236 and SC 53116 and the 5-HT₄ receptor antagonist, GR 113808, were studied in the rat oesophagus, rat ileum and human colon.

2 5-HT relaxed the longitudinal muscle of the rat oesophagus and rat ileum and the circular muscle of the human colon. Absolute values of relaxation were measured and showed the order of the maximum responses, rat oesophagus >> human colon > rat ileum with EC₅₀ values of 189 ± 15 nM, 157 ± 4 nM, 306 ± 72 nM, respectively. 5-HT also inhibited the spontaneous contractions of the human colon with an EC₅₀ value of 119 ± 1 nM. The effect of 5-HT on the human colon was not affected by methysergide (10 µM) or ondansetron (1 µM).

3 The use of the uptake and metabolism inhibitors, cocaine (30 µM) and pargyline (100 µM), did not increase the potency of 5-HT in the rat oesophagus or human colon. In the rat oesophagus, cocaine (30 µM) produced a reduction in carbachol-induced tone of 22.2 ± 0.6% and reduced the 5-HT maximum effect by 52.0 ± 0.4%.

4 The compounds, DAU 6236 and SC 53116, showed a different pattern of potencies and efficacies in the rat oesophagus, rat ileum and human colon compared to 5-HT. DAU 6236 relaxed the human colonic circular muscle with an EC₅₀ value of 129 ± 16 nM but its efficacy was less than that of 5-HT. DAU 6236 (1 µM) also antagonized the 5-HT-induced relaxation of the human colon with a dose-ratio of 9.9. In the rat oesophagus and rat ileum, DAU 6236 was inactive in the majority of tissues. In the minority of oesophagus tissues that did respond the EC₅₀ value was 1.2 ± 0.7 µM. DAU 6236 also antagonized the effect of 5-HT in the rat oesophagus in a non-surmountable fashion. SC 53116 relaxed the rat oesophagus with an EC₅₀ value of 91 ± 4 nM, with an efficacy less than that observed to 5-HT; however, at 200 nM it did not antagonize the 5-HT-induced relaxation of the rat oesophagus. SC 53116 showed no agonist activity in the rat ileum and human colon, but at 1 µM it did antagonize the effect of 5-HT in the human colon with a dose-ratio of 11.3 ± 0.3.

5 GR 113808 competitively antagonized the 5-HT₄ receptor-mediated relaxation of the rat oesophagus with a pA₂ value of 8.59 (8.18–9.00) against 5-HT and 9.05 (8.79–9.31) against SC 53116. GR 113808 (0.01 µM) also antagonized the 5-HT-induced relaxation of human colonic circular muscle with an apparent pA₂ value of 9.02 ± 0.12. However at 1 µM the apparent pA₂ value was significantly lower than that measured at 0.01 and 0.1 µM. GR 113808 (0.01 µM) antagonized the 5-HT₄ receptor-mediated relaxation of the rat ileum with an apparent pA₂ value of 9.30 ± 0.21.

6 In conclusion, these studies have shown that the human colon, rat oesophagus and rat ileum contain functional 5-HT₄ receptors. However, the 5-HT₄ receptor agonists displayed differences in these tissues making it necessary to be cautious when extrapolating from animal to human tissue. This emphasizes the importance of the use of human tissue in the development of therapeutic drugs.

Keywords: 5-Hydroxytryptamine; 5-HT₄ receptors; DAU 6236; SC 53116; GR 113808; rat oesophagus; rat ileum; human colon

Introduction

The neurones of the enteric nervous system contain many neurotransmitters including 5-hydroxytryptamine (5-HT). Indeed, almost all of the 5-HT in the body is found in the alimentary tract (Thompson, 1971), where it is located in the interneurons that terminate in the myenteric and submucosal plexus (Branchek & Gershon, 1987), and to a greater extent in the enterochromaffin cells of the mucosa (Erspamer, 1954). Once released, 5-HT can act as a local hormone, or a neurotransmitter (Costall & Naylor, 1990) acting on a number of different 5-HT receptors.

In the alimentary tract stimulation of 5-HT₄ receptors leads to neuronally-mediated contraction of the guinea-pig ileum (Eglen *et al.*, 1990) and colon (Elswood *et al.*, 1991), enhancement of the 'twitch' response in transmurally stimulated guinea-pig ileum (Craig & Clarke, 1990), facilitation of the peristaltic reflex in the guinea-pig ileum (Craig & Clarke, 1991; Costall *et al.*, 1993), relaxation of the smooth

muscle of the rat oesophagus (Baxter *et al.*, 1991; Reeves *et al.*, 1991) which occurs via adenylyl cyclase activation (Ford *et al.*, 1992), induction of chloride secretion by the mucosa of the rat distal colon (Bunce *et al.*, 1991) and an increase of the short circuit current response across the human small intestine mucosa (Borman & Burleigh, 1993).

5-HT has been shown to cause relaxation or contraction of the longitudinal muscle and to decrease the spontaneous activity of the circular muscle of the human colon (Fishlock & Parks, 1963; Fishlock, 1964; Misiewicz *et al.*, 1966; Wright & Sheppard, 1966). Recent studies have also shown that 5-HT mediates inhibition of the spontaneous contractions of human colonic circular smooth muscle via a 5-HT₄ receptor (Tam *et al.*, 1994; Hillier *et al.*, 1994). There is also some evidence that 5-HT mediates relaxation of the rat terminal ileum via a 5-HT₄ receptor (Tuladhar *et al.*, 1991).

The 5-HT₄ receptor is currently characterized on the basis of both agonist and antagonist activities. 5-HT₄ receptor agonists include 5-HT, and a series of indoleamine derivatives such as 5-methoxytryptamine (5-MeOT) and 5-

¹ Author for correspondence.

carboxamidotryptamine (5-CT); the substituted benzamide derivatives such as cisapride, renzapride and zacopride; the benzimidazolone derivatives, BIMU 1, BIMU 8 and DAU 6236 (Tonini *et al.*, 1991; Rizzi *et al.*, 1992) and the substituted pyrrolizidine, SC 53116 (Flynn *et al.*, 1992). 5-HT₄ receptor antagonists include GR 113808 (pA₂ value of 9.2 in the guinea-pig distal colon and 9.5 in the rat oesophagus, Grossman *et al.*, 1993a,b; Gale *et al.*, 1994), SDZ 205-557 (pA₂ = 7.4 in the guinea-pig ileum, Buchheit *et al.*, 1992), and DAU 6285 (pA₂ 6.9 and 7.0 in the rat tunica muscularis mucosae and guinea-pig ileum respectively, Waikar *et al.*, 1992). However, variations in agonist potency between CNS and peripheral tissues have led some authors to suggest the possibility of 5-HT₄ receptor subtypes (Kaumann *et al.*, 1991; Waikar *et al.*, 1994).

The present study aims to compare three previously defined preparations as assay tissues for drugs acting at the 5-HT₄ receptor, the longitudinal muscle of the rat oesophagus and rat ileum and the circular muscle of the human colon. In addition, the study aims to investigate the pharmacological properties of SC 53116 and DAU 6236, two novel compounds which are reportedly selective 5-HT₄ receptor agonists, and GR 113808 a reportedly selective 5-HT₄ receptor antagonist. These compounds are used to compare the effects of 5-HT₄ receptor activation and blockade in the rat models to the human colon.

Shortened versions of this paper have been published in the Medical Journal of Australia (McLean *et al.*, 1993a) and presented in part at the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists meeting, Brisbane, Australia, December 1993 (McLean *et al.*, 1993b) and at the XIIth International Congress of Pharmacology, Montreal, Canada (McLean *et al.*, 1994).

Methods

Rat oesophagus

The method of Reeves *et al.* (1991) was used as follows: Segments of distal oesophagus (2 cm) from female hooded Wistar rats (200–250 g) with muscle layers and mucosa intact were suspended in the longitudinal plane under a tension of 0.75 g at 37°C in Krebs-Henseleit solution containing indomethacin (3 µM) to prevent the relaxant effects of prostanooids (see Craig & Clarke, 1990) and ketanserin (1 µM) to prevent possible 5-HT₂ receptor-mediated contraction of the oesophagus (Akbarali *et al.*, 1987). Previous studies have established that pargyline does not affect the response to 5-HT in the rat oesophagus (Reeves *et al.*, 1991; Baxter *et al.*, 1991), so it was not included in the bathing solution in the present series of experiments. Reeves *et al.* (1991) reported that cocaine does not increase the potency of 5-HT in the rat oesophagus, but it was shown by Baxter *et al.* (1991) to cause a leftward shift of the 5-HT-induced relaxant concentration-effect curve in this preparation. Consequently, the effect of cocaine (30 µM) was investigated. Since cocaine did not increase the potency of 5-HT it was not included in subsequent experiments.

Effects of agonists and antagonists The oesophageal preparations were contracted by addition of a submaximal concentration of carbachol (1 µM) to the bathing solution. On establishing a stable contractile response, usually within 20 min, cumulative concentrations of 5-HT were added every 3 min and the resultant relaxations recorded.

Following the construction of the control concentration-effect curve to 5-HT, the preparations were washed with fresh Krebs-Henseleit solution containing indomethacin and ketanserin and allowed to recover for at least 1 h, with further washes, before re-contracting with carbachol prior to the construction of the concentration-effect curve to the test agonist.

In antagonist studies, a control concentration-effect curve to 5-HT was constructed. Following washout, the tissue was contracted with carbachol (1 µM), a stable contraction obtained and the tissue equilibrated with antagonist (30 min) before another agonist concentration-effect curve.

Only two concentration-effect curves were constructed to 5-HT per tissue since the third curve is not reproducible (Reeves *et al.*, 1991; this study). The first curve was constructed to 5-HT followed by washout. A second curve was then obtained to either DAU 6236, SC 53116 or alternatively to 5-HT in the presence of GR 113808. Multiple concentration-effect curves to SC 53116 were not reproducible in any of the tissues studied, therefore only one concentration effect curve was constructed per tissue. Subsequently paired tissues were used, one serving as a control and the other to compare the response in the presence of the antagonist.

Rat ileum

The method of Tuladhar *et al.* (1991, 1993, personal communication) was used as follows: 5 cm of the terminal ileum was removed from female hooded Wistar rats (200–250 g) and transferred to warm oxygenated Krebs-Henseleit solution. The mesentery was cut away, without touching the ileum itself; the lumen was flushed carefully and the tissues mounted in an organ bath and bathed with Krebs-Henseleit solution containing methysergide (1 µM) and atropine (0.1 µM) and maintained at 37°C. The tissues were equilibrated for 1 h with washes every 15 min. No tension was applied initially but was gradually applied to a maximum tension of 0.75 g over a period of 15 min. As noted by Tuladhar (1993, personal communication), any deviation from these methods results in tissues which display a high degree of spontaneous activity making interpretation of the effects of the agonists and antagonists difficult. Tuladhar *et al.* (1992) have also shown that pargyline did not effect the response to 5-HT and hence it was not included in these experiments.

Effects of agonists and antagonist Once the ileal preparations were quiescent (i.e. displayed minimal spontaneous activity) and a reasonably stable baseline was achieved, 5-HT was added cumulatively with a 2 min contact time at each concentration. In agonist studies, paired tissues were used to compare the response to 5-HT with other agonists or in the presence of an antagonist. The antagonist was incubated for a period of 30 min before the addition of the agonist. A submaximal concentration of carbachol (1 µM) was used in experiments designed to investigate the effect of pre-contracting the rat ileum.

Human colon

Specimens of ascending, transverse or sigmoid colon were obtained from male and female patients undergoing surgical resection for colonic or rectal cancer. The specimens were placed in cold Krebs-Henseleit solution as soon as possible after surgical resection. The mucosa was cut away from the muscle layers which were cut along the circular axis to give 3 to 4 strips of 3–5 cm in length and 3–5 mm in width. These were mounted in Krebs-Henseleit solution, warmed at 37°C and equilibrated under a tension of 0.75 g for 45 min. The Krebs-Henseleit solution used in this study as in that of Tam *et al.* (1994) was free of 5-HT₁, 5-HT₂, and 5-HT₃ receptor antagonists, since methysergide (10 µM) and ondansetron (1 µM) did not affect the 5-HT-induced responses. Cocaine (30 µM) and pargyline (100 µM) also did not increase the potency of 5-HT, therefore they were not included in subsequent experiments.

Effects of agonists and antagonists Within 30 min most tissues showed regular spontaneous contractions with a relatively stable baseline. A cumulative concentration-effect

curve to the agonists was determined on each strip after 45 min using a 2 min exposure time. The effect of 5-HT on the muscle strips was two fold consisting of a reduction in the basal tone in the majority of tissues and often decrease in the amplitude of the contractions. The method of analysis employed in quantifying these effects primarily involved measuring the extent of the reduction of the resting basal tone following the addition of 5-HT. The reduction of the amplitude of the spontaneous contractions was also measured in the analyses. In experiments designed to investigate the effect of pre-contracting the human colon, a submaximal concentration of carbachol (1 μ M) was used prior to the addition of 5-HT.

Four separate pieces of colon were used to study the effects of the antagonist, GR 113808. One was used to determine the control EC₅₀ value to the agonist while the other 3 tissues were incubated with 3 separate concentrations of GR 113808 for 30 min before determination of the agonist concentration-effect curve. The relative effect of the agonists in the presence or absence of antagonists were compared using the absolute responses measured.

General methods

In all experiments, tissues were mounted in 30 ml jacketed organ baths containing Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, NaHCO₃ 25, KH₂PO₄ 1.2, CaCl₂ 2.5, MgSO₄ 1.2, D-($\pm$)-glucose 11, oxygenated with 95% O₂ and 5% CO₂ and maintained at 37°C. Ugo Basile isotonic transducers connected to a Grass model 79D polygraph which amplified the responses 5–20 fold, were used to measure isotonic changes in length of the tissues.

Drugs

5-Hydroxytryptamine creatinine sulphate (Sigma, Australia), atropine sulphate (Sigma, Australia), carbachol (Sigma, Australia), ketanserin (Janssen-Cilag, Sydney, Australia), SC 53116 (1-S,8-S)-4-amino-5-chloro-N-[(hexahydro-1H-pyrrolizin-1-yl)methyl]-2-methoxy-benzamide, hydrochloride, Searle, Skokie, Illinois, U.S.A.), DAU 6236 (endo-8-methyl-8-azabicyclo [3.2.1] oct-3-yl 2,3-dihydro-3-ethyl-2-oxo-1H-benzimidazole-1-carboxylate hydrochloride, Dr Carlo Rizzi, Boehringer-Ingelheim, Milan, Italy) and GR 113808 ([1-[2-(methyl-sulphonylamino)ethyl]-4-piperidinyl)methyl 1-methyl-1H-indole-3-carboxylate, Glaxo, Melbourne, Australia) were dissolved in distilled water. Methysergide hydrogen maleate (Sandoz, Basle, Switzerland) was dissolved in ethanol 90% and diluted with (+)-tartaric acid 0.1% in distilled water. Indomethacin (Merk Sharp & Dohme, Sydney, Australia) was dissolved in 0.5% w/v sodium bicarbonate and diluted with distilled water.

Data analysis

The relaxations induced by 5-HT and 5-HT₄ receptor agonists were expressed in absolute terms, as mm cm⁻¹ of tissue to enable direct comparison of the responses between the three tissues. The resting length of tissue was measured at the end of the equilibration period. Length changes in response to the test drugs were recorded with calibrated isotonic transducers which enabled an accurate measure of the length change of the tissues. The responses were also recorded and expressed in terms of their potency as EC₅₀ values relative to individual maxima. EC₅₀ values were calculated by linear regression analysis for each preparation from the 50% response level and expressed as arithmetic means $\pm$ s.e.mean. The potency of the antagonist was expressed as the pA₂ value with 95% confidence limits (Arunlakshana & Schild, 1959) which was computed using the 3 point method of Tallarida &

Murray (1987), or as an apparent pA₂ value which was calculated using single point analysis from

$$pA_2 = \log (DR-1) - \log [B]$$

where DR is the dose-ratio of agonist used in the presence and absence of antagonist (B) and is expressed as pA₂ $\pm$ s.e.mean. The assumption of simple competition (i.e. slope of unity) between antagonist and agonist for 5-HT₄ receptors was checked with a Schild plot (Arunlakshana & Schild, 1959). The dose-ratios required for the above analysis were determined from EC₅₀ values in the presence and absence of antagonist. The number of observations is indicated by *n*.

The significance of differences between the values was determined by use of Student's unpaired two tail *t* test. The criterion for statistical significance was set at *P* < 0.05.

Results

Comparison of the responses to 5-HT in each tissue

In the rat oesophagus, a submaximal concentration of carbachol (1 μ M) produced a well-maintained contraction (of between 4–6 mm cm⁻¹ of oesophagus) for at least 60 min. 5-HT produced concentration-dependent relaxations of the precontracted oesophagus preparations (Figure 1) with a mean EC₅₀ value of 189 $\pm$ 15 nM and a mean maximum reduction of carbachol-induced tone of 61.6 $\pm$ 5.5% (*n* = 19). The mean maximum relaxation (E_{max}) caused by the maximum concentration of 5-HT (< 100 μ M) was 2.49 $\pm$ 0.43 mm cm⁻¹ of tissue (*n* = 19). These results are summarised in Table 1.

The responses to 5-HT were reproducible in the rat oesophagus and two similar consecutive concentration-effect curves could be obtained in each preparation. In experiments designed to test this, the EC₅₀ values were 227.3 $\pm$ 1.3 and 225.7 $\pm$ 1.4 nM (*n* = 4) for the first and second curves respectively. The E_{max} of the second curve was 98.5 $\pm$ 0.7% of the

Figure 1 Relaxant effect of 5-hydroxytryptamine (5-HT) on the (a) longitudinal muscle of the rat isolated oesophagus contracted with carbachol (1 μ M) in the presence of indomethacin (3 μ M) and ketanserin (1 μ M); (b) circular muscle of the human colon (spontaneous contractions returned after washing) and (c) longitudinal muscle of the rat isolated ileum in the presence of methysergide (1 μ M) and atropine (0.1 μ M).

Table 1 Summary of comparative agonist potency and maximum effect values in the rat oesophagus and human colon^a

Tissue	Agonist	EC ₅₀ (nM)	E _{max} (mm cm ⁻¹)	n ^b
Rat oesophagus	5-HT	189 ± 15	2.49 ± 0.43	19/19
	SC 53116	91 ± 4	1.27 ± 0.17	15/15
	DAU 6236	1200 ± 700	1.21 ± 0.11	4/14
Human colon	5-HT	157 ± 4	0.81 ± 0.02	19/19
	SC 53116	—	—	0/3
	DAU 6236	130 ± 2	0.23 ± 0.09	5/5

^aSee text for a summary of data obtained with the rat ileum^bIndicates the proportion of tissues responding over the total number of tissues.

first curve. The control curves were reproducible provided that at least 1 h with washes was allowed between curves. Concentrations of 5-HT up to 10 μ M had no measurable effect on the oesophagus preparation not pre-contracted with carbachol. Cocaine (30 μ M) incubated in the organ bath for 30 min did not increase the potency of 5-HT but produced a reduction in carbachol-induced tone of $22.2 \pm 0.6\%$ and reduced the 5-HT ($> 10 \mu$ M) maximum effect by $52.0 \pm 0.4\%$ ($n = 6$).

In the human colon, 5-HT usually induced three effects; direct relaxation of the tissue, inhibition of the amplitude of the spontaneous contractions, and a decrease in the frequency of the spontaneous contractions (Figure 1). Only relaxation and decrease in amplitude were used in quantifying drug effects. The relaxant effect of 5-HT was concentration-dependent with an EC₅₀ value of 157 ± 4 nM and E_{max} of 0.81 ± 0.02 mm cm⁻¹ of tissue ($n = 19$, Table 1). 5-HT also caused a marked and concentration-dependent inhibition of the amplitude of the spontaneous contractions with an EC₅₀ value of 119 ± 1 nM ($n = 6$). The potency values obtained for these two different effects of 5-HT were not significantly different ($P > 0.05$). The amplitude of the spontaneous contractions before the addition of 5-HT was 0.25 ± 0.10 mm cm⁻¹ of tissue and this was inhibited to 0.08 ± 0.01 mm cm⁻¹ of tissue ($n = 6$) by the maximum concentration of 5-HT (2 μ M). However, concentration-dependent effects of 5-HT on the amplitude of the spontaneous contractions were variable as they were either inhibited in a concentration-dependent fashion ($n = 4$) or in an all-or-none fashion ($n = 2$). In most cases spontaneous contractions were inhibited completely by high concentrations of 5-HT (approx. 4 μ M) but spontaneous contractions often returned at even higher concentrations. Consequently direct relaxation was the parameter most often used to quantify the response to 5-HT. The relaxant effect of 5-HT was not antagonized by the combination of methysergide (10 μ M) and ondansetron (1 μ M) (dose-ratio = 0.87 ± 0.30 , $P > 0.05$, $n = 3$). Likewise, cocaine (30 μ M) and pargyline (100 μ M) did not affect the sensitivities of the tissues to 5-HT (dose-ratio = 1.33 ± 0.23 , $P > 0.05$, $n = 3$). Concentrations of 5-HT up to 10 μ M had no measurable effect on the colon preparation pre-contracted with carbachol (1 μ M).

The responses of the rat ileum were inconsistent to 5-HT which relaxed only 10 of 18 tissues tested. It was also difficult to distinguish clear concentration-related relaxations in some tissues tested due to a high degree of spontaneous activity (Figure 1). The maximum absolute relaxant effect of 5-HT (6.4 μ M) was relatively small (E_{max} = 0.37 ± 0.06 mm cm⁻¹, $n = 10$). However, the tissues in which the response was quantified an EC₅₀ value of 306 ± 72 nM ($n = 10$) was calculated. The effects of 5-HT above 6.4 μ M ($< 100 \mu$ M) were variable. In most tissues no further relaxation occurred ($n = 6$); however, further relaxation occurred in a biphasic fashion in 3 tissues and the relaxation was reduced by higher concentrations of 5-HT in one tissue (as reported by Tuladhar *et al.*, 1991). The within tissue responses to 5-HT were

Figure 2 Concentration-effect curves to 5-hydroxytryptamine (5-HT) in the rat oesophagus (■), human colon (◆), and rat ileum (●). Data are means $\pm$ s.e.mean ($n = 5$). Relaxant effects are expressed as (a) mm relaxation per cm of tissue and (b) as the percentage of the maximum response elicited by 5-HT.

not reproducible in that two similar consecutive concentration-effect curves could not be obtained. Concentrations of 5-HT up to 10 μ M had no measurable effect on the ileum preparation pre-contracted with carbachol (1 μ M).

The responses to 5-HT measured in each tissue under the above conditions are compared (Figure 2) both in absolute terms to enable direct comparison of the responses between the three tissues and as a percentage of the individual maxima from which EC₅₀ values were derived (see Table 1). The order of E_{max} values in the three tissues was rat oesophagus (pre-contracted, E_{max} = 2.49 ± 0.43 mm cm⁻¹) $>>$ human colon (not pre-contracted, E_{max} = 0.81 ± 0.02 mm cm⁻¹) $>$ rat ileum (not pre-contracted, E_{max} = 0.37 ± 0.06 mm cm⁻¹). Although the efficacies of 5-HT as measured by the E_{max} values were significantly different ($P < 0.05$) in each of the three tissues, the potency of 5-HT in the rat oesophagus (EC₅₀ = 189 ± 15 nM), human colon (EC₅₀ = 157 ± 4 nM), and rat ileum (EC₅₀ = 306 ± 72 nM) were not significantly different ($P > 0.05$, Figure 2).

Effects of 5-HT₄ receptor agonists

SC 53116, a substituted pyrrolizidine which is selective for the 5-HT₄ receptor in the tunica muscularis mucosae of the rat oesophagus (Flynn *et al.*, 1992) caused concentration-dependent relaxations of the rat oesophagus with a mean EC₅₀ value of 91 ± 4 nM ($n = 15$). This potency value was around 2 fold higher than 5-HT. SC 53116 caused a maximum reduction of carbachol-induced tone of $48.0 \pm 6.1\%$ and a maximum relaxation of 1.27 ± 0.17 mm cm⁻¹ of tissue

in the rat oesophagus ($n = 15$, Table 1). This value was $51 \pm 1\%$ of the response relative to 5-HT indicating that SC 53116 might be a partial agonist. However, at a concentration of 200 nM (20 min incubation time) SC 53116 did not act as an antagonist of 5-HT ($n = 3$). In contrast to 5-HT the responses to SC 53116 were not reproducible in that a second concentration-effect curve produced a smaller response in each preparation. The comparative agonist effects of 5-HT and SC 53116 in the rat oesophagus are shown in Figure 3 and summarised in Table 1. SC 53116 ($< 10 \mu\text{M}$), in contrast to 5-HT, possessed no intrinsic activity in the human colon causing neither relaxation, nor inhibition of spontaneous contractions ($n = 3$). SC 53116 did, however, act as an antagonist in the human colon. At $1 \mu\text{M}$, it caused a rightward shift in the concentration-effect curve to 5-HT with a dose-ratio of 11.3 ± 0.3 ($n = 4$). Like 5-HT, the responses caused by SC 53116 were inconsistent in rat ileum. SC 53116 ($< 10 \mu\text{M}$) relaxed only 1 of 5 tissues studied in a non-concentration-dependent fashion.

DAU 6236, a selective 5-HT₄ receptor agonist in the guinea-pig ileum (Rizzi *et al.*, 1992), was inconsistent in its activity in the rat oesophagus. Whereas 5-HT relaxed all strips tested, DAU 6236 ($< 10 \mu\text{M}$) relaxed only 4 of 14 preparations. Of these, DAU 6236 ($E_{\text{max}} = 1.2 \pm 0.1 \text{ mm cm}^{-1}$) had a lower intrinsic activity relative to 5-HT ($E_{\text{max}} = 2.5 \pm 0.4 \text{ mm cm}^{-1}$). DAU 6236 ($EC_{50} = 1.2 \pm 0.7 \mu\text{M}$) was also less potent than 5-HT ($EC_{50} = 189 \pm 15 \text{ nM}$, Table 1). DAU 6236 did not only produce a response in well coupled tissues as there was no relationship between the potency of the individual tissues to 5-HT and the ability of DAU 6236 to evoke a response. DAU 6236 at a similar concentration to its EC_{50} in the rat oesophagus ($1 \mu\text{M}$) was shown to inhibit 5-HT-induced relaxations of the rat oesophagus where it produced a rightward shift of the 5-HT concentration-effect curve with a dose-ratio of around 7. This antagonism was not completely surmountable in that the E_{max} in the absence of DAU 6236 ($76.7 \pm 0.5\%$ reduction of carbachol-induced tone) was significantly reduced in its presence ($61.7 \pm 0.7\%$ reduction of carbachol-induced tone, $P < 0.05$, $n = 4$). DAU 6236 did not significantly reduce the response to carbachol. The dose-ratios for carbachol in the presence of DAU 6236 at 2 and $10 \mu\text{M}$ were 1.2 ± 0.8 and 2.0 ± 1.1 respectively ($P > 0.05$, $n = 4$).

The responses caused by DAU 6236 in the rat ileum were also inconsistent. DAU 6236 ($< 10 \mu\text{M}$) caused relaxation in only 1 of 5 preparations, and in this DAU 6236 possessed a similar intrinsic activity to 5-HT but a clear concentration-related effect was not observed in this tissue.

In contrast to the above tissues, DAU 6236 inhibited both the amplitude of spontaneous contractions as well as causing direct relaxation of all strips of human colonic circular mus-

cle. The relaxant effect of DAU 6236 was concentration-dependent with a mean EC_{50} value of $130 \pm 2 \text{ nM}$ ($n = 5$). This inhibition usually occurred with concentrations below $1 \mu\text{M}$. However, at higher concentrations ($1\text{--}10 \mu\text{M}$) spontaneous contractions returned or were increased. The potency of DAU 6236 in producing relaxation was similar to 5-HT itself (Figure 4). However, DAU 6236 ($E_{\text{max}} = 0.23 \pm 0.09$) was not a full agonist having an intrinsic activity less than that of 5-HT ($E_{\text{max}} = 0.81 \pm 0.02$, Figure 4, Table 1) indicating that, as with its action in the rat oesophagus, it might be a partial agonist. In this respect, DAU 6236 ($1 \mu\text{M}$) was shown to be a surmountable antagonist in the human colon, shifting the 5-HT-induced concentration-effect curve to the right with a dose-ratio of 9.86 ± 0.55 ($n = 4$).

Effects of the 5-HT₄ receptor antagonist, GR 113808

GR 113808 (0.01, 0.1, $1 \mu\text{M}$) caused parallel, dextral shifts of the concentration-effect curves to 5-HT in the rat oesophagus ($n = 9$, Figure 5a). The maximum response to 5-HT ($> 3 \mu\text{M}$) was not significantly affected ($P > 0.05$) by GR 113808. The slope of the Schild regression was 0.98 (95% CL, 0.55–1.41) which was not significantly different from 1 and yielded a pA_2 estimate of 8.59 (95% CL, 8.18–9.00, Table 2). GR 113808 (0.01, 0.1, $0.5 \mu\text{M}$) also caused parallel, dextral shifts of the concentration-effect curves to the 5-HT₄ receptor agonists, SC 53116 ($n = 9$, Figure 5b). The maximum response to SC 53116 ($> 2 \mu\text{M}$) was not significantly altered ($P > 0.05$) in the presence of GR 113808 and the slope of the Schild regression was 0.72 (95% CL, 0.39–1.04) which was not significantly different from 1 (Table 2). Affinity estimates for GR 113808 against SC 53116 gave a pA_2 value of 9.05 (95% CL, 8.79–9.31) with the slope constrained to 1 (Table 2). The pA_2 values obtained for GR 113808 against SC 53116 and 5-HT were not significantly different ($P > 0.05$).

GR 113808 caused rightward shifts of the concentration-effect curves to the relaxant effect of 5-HT in the human colon ($n = 11$, Figure 6). At $0.01 \mu\text{M}$ GR 113808 antagonized the 5-HT-induced relaxations with an apparent pA_2 value of 9.02 ± 0.12 ($n = 5$). Higher concentrations of GR 113808 (0.1, $1 \mu\text{M}$) shifted the concentration-effect curve to 5-HT to the right but the magnitude of the shift was not as great as that predicted for simple competitive antagonism (Table 3, Figure 6). The slope of the 3 point Schild regression was 0.58 (95% CL, 0.34–0.83) which is significantly different from 1 (Table 2). GR 113808 ($0.01 \mu\text{M}$) also antagonized the inhibition of spontaneous activity caused by 5-HT with an apparent pA_2 of 9.26 ± 0.14 which is not significantly different ($P > 0.05$) from the value obtained against the 5-HT-induced relaxation of the circular muscle. This antagonism of 5-HT-induced inhibition of spontaneous activity was also not com-

Figure 3 The effect of (●) 5-hydroxytryptamine ($n = 19$) and (○) the 5-HT₄ receptor agonist SC 53116 ($n = 15$) in the rat oesophagus. Each point is the mean $\pm$ s.e.mean calculated as a percentage of the carbachol-induced tone.

Figure 4 The effects of (●) 5-hydroxytryptamine ($n = 19$) and (■) the 5-HT₄ receptor agonist DAU 6236 ($n = 5$) in the human colonic circular muscle. Each point is the mean $\pm$ s.e.mean expressed as the absolute relaxant effect in mm cm^{-1} .

petitive as higher concentrations of GR 113808 did not cause the predicted increases in dose-ratios.

GR 113808 (0.01, 0.1, 1 μ M) caused rightward shifts of the concentration-effect curves to 5-HT in the rat ileum ($n = 7$). At 0.01 μ M GR 113808 antagonized the 5-HT induced relaxations with an apparent pA_2 value of 9.30 ± 0.21 ($n = 3$, Table 2). Higher concentrations of GR 113808 (0.1, 1 μ M) shifted the concentration-effect curve to 5-HT to the right but this antagonism was not of a simple competitive nature. The maximum response to 5-HT was increased in the presence of GR 113808 in 4 of 7 tissues. The slope of the Schild regression was significantly different from unity and hence a meaningful estimate of a pA_2 value could not be made (Table 2).

Figure 5 Antagonism of the concentration-effect curve to (a) 5-hydroxytryptamine (5-HT) and (b) SC 53116 by GR 113808 in the rat oesophagus pre-contracted with carbachol. Data obtained in the absence (○), or presence of GR 113808 0.01 μ M (●), 0.1 μ M (◇), and (a) 1 μ M, (b) 0.5 μ M (◆). The slope of Schild regression was (a) 0.98 (95% CL, 0.55–1.41) and (b) 0.7 (95% CL, 0.3–1.1). The pA_2 estimates were (a) 8.59 ($n = 8$) and (b) 9.1 ($n = 9$).

Discussion

This study extends the pharmacological profiles of the 5-HT₄ receptor agonists, DAU 6236 and SC 53116 and the antagonist GR 113808. This was achieved by comparing their activities in the longitudinal muscle of the rat isolated oesophagus and ileum and the circular muscle of the human colon. These tissues have been shown to contain functional 5-HT₄ receptors in earlier studies in which some 5-HT₄ receptor agonists and antagonists were screened (Reeves *et al.*, 1991; Tuladhar *et al.*, 1991; Tam *et al.*, 1994). However, the present study is the only one to date that compares the three tissues, giving an indication of their relative usefulness as assays for drugs acting at the 5-HT₄ receptor. To date there is no published data on the effects of SC 53116 and DAU 6236 on the human colon or the effects of SC 53116, DAU 6236 and GR 113808 on the rat ileum, nor is there data available on the effects of DAU 6236 and the effects of GR 113808 against SC 53116 on the rat oesophagus. An independent study (Hillier *et al.*, 1994) has shown that GR 113808 is active as an antagonist at the 5-HT₄ receptor in the human

Figure 6 Antagonism of the concentration-effect curve to 5-hydroxytryptamine (5-HT) by GR 113808 in human colonic circular muscle. Data obtained in the absence (○), or presence of GR 113808 0.01 μ M (●), 0.1 μ M (◇); 1 μ M not shown for clarity. The slope of Schild regression was 0.58 (95% CL, 0.34–0.83). The apparent pA_2 estimate at 0.01 μ M was 9.0 ($n = 5$).

Table 3 Apparent pA_2 values of GR 113808 in the human colon

[GR 113808]	Dose-ratio	pA_2	n
0.01 μ M	14 ± 0.6	9.02 ± 0.12	5
0.1 μ M	56 ± 0.8	8.72 ± 0.07	6
1 μ M	145 ± 3.7	$8.06 \pm 0.20^*$	3

*Indicates a significant difference from the apparent pA_2 values at 0.01 and 0.1 μ M ($P < 0.05$, Student's *t* test).

Table 2 pA_2 estimates for GR 113808 in the rat oesophagus, rat ileum and human colon

Agonist	Tissue	pA_2	Schild slope	n
5HT	Human colon	$9.02 \pm 0.12^*$	0.51 (0.34–0.83) ^b	5
	Rat oesophagus	8.59 (8.18–9.00)	0.98 (0.55–1.41)	9
	Rat ileum	$9.30 \pm 0.21^*$	0.59 ^c	4
SC 53116	Rat oesophagus	9.05 (8.79–9.31)	0.72 (0.39–1.04)	9

*Single point analysis at 0.01 μ M GR 113808.

^bThe slope of the three point Schild plot was different from one.

^cThe slope of the two point Schild plot was different from one.

colon. The present study aims to extend the pharmacological profile of GR 113808 in the human colon.

Tissue comparison

The three *in vitro* methods used in this study differed in the composition of the Krebs-Henseleit solution. The antagonists which were used routinely in the bathing solution in experiments on the rat oesophagus and rat ileum were present to confine the responses to those of the 5-HT₄ receptor. This study used the same concentrations of the same antagonists as used in the previous studies (Reeves *et al.*, 1991; Tuladhar *et al.*, 1991) to ensure that the results were directly comparable. Antagonists were not used routinely in experiments on the human colon because we established that the inhibitory effect of 5-HT on the spontaneous contractions of human colonic circular muscle as previously reported (Fishlock & Parks, 1963; Fishlock, 1964; Misiewicz *et al.*, 1966; Wright & Sheppard, 1966) were not altered by methysergide and ondansetron. This has also been confirmed independently in other studies (Tam *et al.*, 1994; Borman & Burleigh, 1994). Another difference in the composition of the Krebs-Henseleit solution was the inclusion of carbachol to precontract the rat oesophagus preparations (see Reeves *et al.*, 1991). The rat ileum and human colon preparations were not pre-contracted since it has been established that these tissues have sufficient intrinsic tone to reveal 5-HT₄ receptor-mediated responses (Tuladhar *et al.*, 1991; Tam *et al.*, 1994).

In an attempt to improve these established methods, however, the rat ileum and human colon were pre-contracted with carbachol. Pre-contracting these tissues did not significantly improve the responses and conversely, no response was observed to 5-HT in the rat oesophagus preparation if the tissue was not pre-contracted.

To date there is no published data with regard to absolute values for 5-HT₄ receptor-mediated relaxation in these tissues. This study provides a detailed analysis of the responses to 5-HT and 5-HT₄ receptor agonists in absolute terms which allows direct comparison of the responses displayed by each tissue and provides a convenient method for comparing the suitability of the *in vitro* methods as assay tissues for drugs acting at 5-HT₄ receptors. The order of magnitude of the 5-HT-induced relaxations in each preparation was shown to be: rat oesophagus >> human colon > rat ileum. Therefore, drug effects were relatively easy to quantify in the rat oesophagus and human colon but not in the rat ileum.

The study also compared the methods as judged by their ability to respond reproducibly. The rat oesophagus and human colon responded to 5-HT with relatively large and consistent responses and small variations between tissues. However, the rat ileum was not a reliable preparation in that not all tissues tested were relaxed by 5-HT. This is in contrast to the study by Tuladhar *et al.* (1991; 1993, personal communication) who claim that all tissues they tested were relaxed by 5-HT. The inconsistencies between the two studies can be explained by the high degree of fast and slow wave intrinsic spontaneous activity seen in this study. This highly variable basal tone masks the small effect of 5-HT₄ receptor activation making it hard or impossible to distinguish clear concentration-related responses. This problem is compounded with the drugs which are not full agonists in other preparations, such as SC 53116 and DAU 6236. In light of these findings we question the usefulness of this model as a reliable quantitative tool for drugs which act at the 5-HT₄ receptor.

Interestingly, the use of the uptake and metabolism inhibitors cocaine and pargyline did not increase the potency of 5-HT in the rat oesophagus or human colon. The results in the rat oesophagus, where cocaine was shown to reduce the efficacy of both carbachol and 5-HT, parallel the results obtained by Reeves *et al.* (1991). However, they are not in accordance with those of Baxter *et al.* (1991) who reported that cocaine increased the potency of 5-HT. A possible

explanation for this discrepancy is in the composition of the Krebs-Henseleit bathing solution. The present study, as in Reeves *et al.* (1991), used indomethacin and ketanserin routinely in the bathing solution whereas Baxter *et al.* (1991) used only methysergide when testing the effect of cocaine. Cocaine and pargyline also did not increase the potency of 5-HT in the human colon. This result is confirmed by the study of Tam *et al.* (1994).

5-HT₄ receptor agonists

Ideally animal models which are used for receptor characterization should show similarities to human tissue. However, this study showed marked differences in responses to the agonists SC 53116 and DAU 6236.

It has been suggested recently that the substituted pyrrolizidine, SC 53116, acts as an agonist at the 5-HT₄ receptor in the rat oesophagus tunica muscularis mucosae, causing relaxation (Flynn *et al.*, 1992). In the present study, SC 53116 was shown to relax the rat isolated whole oesophagus preparation via stimulation of 5-HT₄ receptors, since the pA₂ of GR 113808 against SC 53116 (pA₂ = 9.1) was characteristic of antagonism at 5-HT₄ receptors (pA₂ value of 9.2 in the guinea-pig distal colon and 9.5 in the rat oesophagus, Grossman *et al.*, 1993a). However, the intrinsic activity of SC 53116 was significantly less than that observed to 5-HT indicating that it may act as a partial agonist. Further experiments discounted such an action of SC 53116 which displayed no antagonism of 5-HT in the rat oesophagus. SC 53116 displayed tissue selectivity in that it was a relatively potent agonist in the rat oesophagus but it displayed virtually no measurable intrinsic activity in the human colon and rat ileum. Although SC 53116 was not an agonist in the human colon it did act as a weak antagonist of the 5-HT-mediated relaxation. The lack of agonist activity of SC 53116 in the human colon is possibly due to a different efficiency of the receptor coupling mechanism in that it has the properties of a silent antagonist without agonist activity. Similarly, in the rat ileum SC 53116 was variable in its action. This can be explained, as mentioned earlier, by the inherently small and variable response observed with this tissue, which would mask the effect of a weak agonist such as SC 53116.

Recently it has been suggested that DAU 6236, which is a member of a new class of compounds having a benzimidazolone structure, may act as an agonist at central 5-HT₄ receptors. This class of compound was found to stimulate adenylyl cyclase activity in mouse embryo colliculi (Dumuis *et al.*, 1991) and guinea-pig hippocampal neurones (Monferini *et al.*, 1991). DAU 6236 has also been reported to act as a 5-HT₄ receptor agonist facilitating cholinergic transmission and peristalsis in the guinea-pig ileum (Rizzi *et al.*, 1992). The present study extends the available pharmacological data on DAU 6236 to agonist activity at 5-HT₄ receptors in the human colon. This was shown by the findings that DAU 6236 inhibits spontaneous contractions and relaxes human colonic circular muscle with a similar potency to 5-HT. However, the efficacy of DAU 6236 was significantly less than that observed to 5-HT indicating that it may act as a partial agonist. Further experiments confirmed such an action of DAU 6236 in which it antagonized 5-HT-induced relaxation in the human colon.

This study revealed unexpected observations in tissue selectivity to DAU 6236. DAU 6236 was a reasonably potent agonist in the human colon but in marked contrast both the efficacy and potency of DAU 6236 were both highly variable and small compared to 5-HT in the rat oesophagus. This was a surprising result since the rat oesophagus has been used widely to assay compounds with potential activity at the 5-HT₄ receptor. A possible explanation for the variable response to DAU 6236 in the rat oesophagus (only 4 of 14 tissues responded) is that it may have only been active in tissues which were well coupled. However, this does not seem to be the case since the potency of 5-HT was no greater in

the tissues in which DAU 6236 was active. The relaxant action of DAU 6236 in the small number of oesophagus preparations may also be due to an anticholinceptor effect. This possibility was discounted since DAU 6236 (2 μ M and 10 μ M) did not antagonize the effect of carbachol. DAU 6236 was, however, a weak antagonist of 5-HT in the rat oesophagus indicating that its action in this tissue is that of a partial agonist with only weak agonist activity. DAU 6236 also displayed a lower potency and efficacy compared to 5-HT in the rat ileum which is explained by the inherently small and variable responses observed with this tissue. A previous study has established that DAU 6236 is also less potent than 5-HT in the guinea-pig ileum (Rizzi *et al.*, 1992). The relatively high agonist potency of DAU 6236 in the human colon (7–9 times higher than in the rat oesophagus and rat ileum) and its similar potency relative to 5-HT in the human colon is an important aspect of the currently described pharmacological profile.

The overall results of the present study show that SC 53116 and DAU 6236 have varying activities according to the tissue in which they are tested. This may indicate the existence of heterogeneity among 5-HT₄ receptors as has been suggested elsewhere (Kaumann *et al.*, 1990; Rizzi, 1993, personal communication; Waikar *et al.*, 1994). These differences in agonist activities may be used to explore further the possibilities that different 5-HT₄ receptors exist which can be differentiated by the order of agonist potency. However, these results may also be explained by other factors such as differences in the efficiency of the receptor coupling mechanisms between the tissues or differences in the molecular structure of the 5-HT₄ receptor. Further studies are required to examine these possibilities.

The 5-HT₄ receptor antagonist, GR 113808

GR 113808 was recently established as a novel 5-HT₄ receptor antagonist with a pA₂ of 9.2 and 9.3 in guinea-pig colon and rat oesophagus respectively (Gale *et al.*, 1994; Grossman *et al.*, 1993a). Its pA₂ values were 6.0 or less at other 5-HT receptors indicating about 1000 fold selectivity for the 5-HT₄ receptor. It has also been suggested that it antagonizes human and porcine atrial 5-HT₄ receptors (Kaumann, 1993; Medhurst & Kaumann, 1993; Gale *et al.*, 1994). The present study confirms that GR 113808 is a competitive antagonist of 5-HT in the rat oesophagus with the same pA₂ value as has been reported previously in the tunica muscularis mucosae of the rat oesophagus. Further, it was shown that GR 113808 behaved as a competitive antagonist against a 5-HT₄ receptor agonist of a different chemical class, namely SC 53116. The pA₂ value was not significantly different from that obtained against 5-HT. This indicates that GR 113808 acts as an agonist-independent antagonist and provides evidence that both 5-HT and SC 53116 act at the same receptor in the rat oesophagus.

Other workers have found that GR 113808 does not always behave as a competitive antagonist. For example Medhurst & Kaumann (1993) have shown that GR 113808 does not display competitive antagonism of 5-HT in the piglet isolated right atrium. Similarly, in the present study GR 113808 did not act competitively in the rat ileum. Although GR 113808 at the low concentration of 0.01 μ M caused a rightward displacement of 5-HT, higher concentrations (0.1 μ M, 1 μ M) produced little or no further rightward shift. This deviation from competitive antagonism may result from either the high degree of experimental error associated with the rat ileum, as discussed earlier, or a true tissue-dependent form of non-competitive antagonism. Whereas it has not been possible to establish the latter possibility in the rat ileum, the present study shows that GR 113808 apparently does act non-competitively in human colon. In this

tissue GR 113808 produced rightward shifts of the 5-HT curves, however the Schild plot slope was non linear at concentrations of GR 113808 above 0.1 μ M. This property of GR 113808 was observed previously by Hillier *et al.* (1994), who reported a pK_B value of 8.89 for GR 113808 when used at a concentration of 0.003 μ M, which is similar to the value obtained in the present study (apparent pA₂ = 9.0 at 0.01 μ M). These values are also similar to the values obtained in the rat oesophagus (pA₂ = 8.6) where GR 113808 was shown to behave competitively. However, higher concentrations of GR 113808 (0.01–0.1 μ M) in the study performed by Hillier *et al.* (1994) and in the present study (1 μ M) produced little or no further shift to the right of the 5-HT concentration-effect curve giving rise to a Schild slope which was significantly different from unity. This suggests that another receptor, in addition to the 5-HT₄ receptor, may mediate the 5-HT-induced effects in the human colon. However, the use of antagonists of 5-HT₁, 5-HT₂ and 5-HT₃ receptors, at least at the concentrations used, indicated a lack of involvement of other previously characterized 5-HT receptors in this tissue.

The results obtained in this study have highlighted no differences, with regard to the apparent affinity values of GR 113808, between the 5-HT₄ receptors mediating the relaxation response in the rat oesophagus, rat ileum and human colon. These findings are consistent with the findings in other peripheral tissues, e.g. human atrial 5-HT₄ receptors (pK_B = 8.8, Kaumann, 1993), and brain tissues from guinea-pig and rat (pK_i = 9.5 and 9.6 respectively, Grossman *et al.*, 1993a,b). Thus if differences exist between central nervous system and peripheral 5-HT₄ receptors (as was interpreted by Kaumann *et al.*, 1990; Bockaert *et al.*, 1992), the present studies in rat oesophagus and ileum and human colon suggest that they are not discriminated by GR 113808.

The apparent affinity value of GR 113808 in the human colon where direct relaxation was the parameter quantified to measure agonist potency was not significantly different from the value obtained using inhibition of spontaneous activity as the measure of agonist potency. In addition the potency of 5-HT causing direct relaxation is not significantly different from the potency causing inhibition of spontaneous activity. These results suggest that 5-HT₄ receptor activation causes both relaxation and inhibition of spontaneous activity in human colonic circular muscle. These findings extend the known pharmacology of 5-HT in the human colon as reported earlier (Fishlock & Parks, 1963; Fishlock, 1964; Misiewicz *et al.*, 1966; Wright & Sheppard, 1966) and more recently (Tam *et al.*, 1994).

In conclusion, the present study provides information on the relative usefulness of the rat oesophagus, rat ileum and human colon as assay tissues for drugs acting at 5-HT₄ receptors. The study has revealed novel data on the action of the 5-HT₄ receptor agonists, DAU 6236 and SC 53116 and the antagonist GR 113808 on these tissues. The differences between the rat tissues and the human colon has demonstrated the value of using human material in developing therapeutic drugs. Given the activity of GR 113808 and DAU 6236 in the human colon we suggest that clinical evaluation of these compounds may be worthwhile in helping to determine whether they have specific therapeutic value in treating conditions of the alimentary tract.

We thank Prof. P. Bhathal and colleagues of the Anatomical Pathology Department, Royal Melbourne Hospital, and Prof. P. Desmond and colleagues of the Gastroenterology Department, St. Vincent's Hospital, Melbourne for the specimens of human colon. We also thank Dr C. Rizzi of Boehringer-Ingelheim, Milan for DAU 6236, Glaxo Group Research for GR 113808 and Searle for SC 53116. P.M. is an NH & MRC Research Fellow.

References

- AKBARALI, H.I., BEIGER, D. & TRIGGLE, C.R. (1987). Effects of cold storage on relaxation responses in the rat oesophageal tunica muscularis mucosae. *Can. J. Physiol. Pharmacol.*, **65**, 23–29.
- ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonism. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.
- BAXTER, G.S., CRAIG, D.A. & CLARKE, D.E. (1991). 5-Hydroxytryptamine₄ receptors mediate relaxation of the rat oesophageal tunica muscularis mucosae. *Naunyn-Schmied. Arch. Pharmacol.*, **343**, 439–446.
- BOCKAERT, J., FOZARD, J.R., DUMUIS, A. & CLARKE, D.E. (1992). The 5-HT₄ receptor: a place in the sun. *Trends Pharmacol. Sci.*, **13**, 141–145.
- BORMAN, R.A. & BURLEIGH, D.E. (1993). Evidence for the involvement of a 5-HT₄ receptor in the secretory response of human small intestine to 5-HT. *Br. J. Pharmacol.*, **110**, 927–928.
- BORMAN, R.A. & BURLEIGH, D.E. (1994). Heterogeneity of 5-HT receptors in human sigmoid colon. *Br. J. Pharmacol.*, **112**, 558P.
- BRANCHECK, T.A. & GERSHON, M.D. (1987). Development of neural receptors for serotonin in the murine bowel. *J. Comp. Neurology*, **258**, 597–610.
- BUCHHEIT, K.H., GAMSE, R. & PFANNKUCHE, H.J. (1992). SDZ 205-557, a selective, surmountable antagonist for 5-HT₄ receptors in the isolated guinea-pig ileum. *Naunyn-Schmied. Arch. Pharmacol.*, **345**, 387–393.
- BUNCE, K.T., ELSWOOD, C.J. & BALL, T.T. (1991). Investigation of the 5-HT receptor mechanism mediating the short circuit current response in rat colon. *Br. J. Pharmacol.*, **102**, 811–816.
- COSTALL, B. & NAYLOR, R.J. (1990). 5-Hydroxytryptamine: new receptors and novel drugs for gastrointestinal motor disorders. *Scand. J. Gastroenterol.*, **25**, 769–787.
- COSTALL, B., NAYLOR, R.J. & TULADHAR, B.R. (1993). 5-HT₄ receptor mediated facilitation of the emptying phase of the peristaltic reflex in guinea-pig isolated ileum. *Br. J. Pharmacol.*, **110**, 1572–1578.
- CRAIG, D.A. & CLARKE, D.E. (1990). Pharmacological characterisation for a neuronal receptor for 5-hydroxytryptamine in guinea-pig ileum with properties similar to the 5-HT₄ receptor. *J. Pharmacol. Exp. Ther.*, **252**, 1378–1386.
- CRAIG, D.A. & CLARKE, D.E. (1991). Peristalsis evoked by 5-HT and renzapride: evidence for a putative 5-HT₄ receptor activation. *Br. J. Pharmacol.*, **102**, 563–564.
- CRAIG, D.A., EGLEN, R.M., WALSH, L.K.M., PERKINS, L.A., WHITING, R.L. & CLARKE, D.E. (1995). 5-Methoxytryptamine and 2-methyl-5-hydroxytryptamine-induced desensitisation as a discriminative tool for the 5-HT₃ and putative 5-HT₄ receptors in the guinea-pig ileum. *Naunyn-Schmied. Arch. Pharmacol.*, **345**, 264–269.
- DUMUIS, A., SEBBEN, M., MONFERINI, E., NICOLA, M., TURCONI, M., LADINSKY, H. & BOCKAERT, J. (1991). Azabicycloalkyl benzimidazole derivatives as a novel class of potent agonists at the 5-HT₄ receptor positively coupled to adenylate cyclase in brain. *Naunyn-Schmied. Arch. Pharmacol.*, **343**, 245–251.
- EGLEN, R.M., SWANK, S.R., WALSH, L.K.M. & WHITING, R.L. (1990). Characterisation of 5-HT₃ and 'atypical' 5-HT receptors mediating guinea-pig ileal contractions *in vitro*. *Br. J. Pharmacol.*, **101**, 513–520.
- ELSWOOD, C.J., BUNCE, K.T. & HUMPHREY, P.P.A. (1991). Identification of putative 5-HT₄ receptors in guinea-pig ascending colon. *Eur. J. Pharmacol.*, **196**, 149–155.
- ERSPAMER, V. (1954). Pharmacology of indolealkylamines. *Pharmacol. Rev.*, **6**, 425–487.
- FISHLOCK, D.J. (1964). The action of 5-HT on the circular muscle of human ileum and colon *in vitro*. *J. Physiol.*, **170**, 11P.
- FISHLOCK, D.J. & PARKS, A.G. (1963). A study of human colonic muscle *in vitro*. *Br. Med. J.*, **2**, 666.
- FLYNN, D.L., ZABRAWSKI, D.L., BECKER, D.P., NOSAL, R., VILAMIL, C.I., GULLIKSON, G.W., MOUMMI, C. & YANG, D. (1992). SC-53116: the first selective agonist at the newly identified serotonin 5-HT₄ receptor subtype. *J. Med. Chem.*, **35**, 1486–1489.
- FORD, A.P.D.W., BAXTER, G.S., EGLEN, R.M. & CLARKE, D.E. (1992). 5-Hydroxytryptamine stimulates cyclic AMP formation in the tunica muscularis mucosae of the rat oesophagus via 5-HT₄ receptors. *Eur. J. Pharmacol.*, **211**, 117–120.
- GALE, J.D., GROSSMAN, C.J., WHITEHEAD, J.W.F., OXFORD, A.W., BUNCE, K.T. & HUMPHREY, P.P.A. (1994). GR 113808: a novel, selective antagonist with high affinity at the 5-HT₄ receptor. *Br. J. Pharmacol.*, **111**, 332–338.
- GROSSMAN, C.J., GALE, J.D., BUNCE, K.T., KILPATRICK, G.J., WHITEHEAD, J.W.F., OXFORD, A.W. & HUMPHREY, P.P.A. (1993a). Development of a radioligand binding assay for the 5-HT₄ receptor: use of a novel antagonist. *Br. J. Pharmacol.*, **108**, 106P.
- GROSSMAN, C.J., KILPATRICK, G.J. & BUNCE, K.T. (1993b). Development of a radioligand binding assay for 5-HT₄ receptors in guinea-pig and rat brain. *Br. J. Pharmacol.*, **109**, 618–624.
- HILLIER, K., TAM, F.S.-F., BUNCE, K.T. & GROSSMAN, C. (1994). Inhibition of motility induced by the activation of 5-HT₁ and 5-HT₄ receptor types in isolated human colon smooth muscle. *Br. J. Pharmacol.*, **112**, 102P.
- KAUMANN, A.J. (1993). Blockade of human atrial 5-HT₄ receptors by GR 113808. *Br. J. Pharmacol.*, **110**, 1172–1174.
- KAUMANN, A.J., SANDERS, L., BROWN, A.M., MURRAY, K.J. & BROWN, M.J. (1990). A 5-hydroxytryptamine receptor in human atrium. *Br. J. Pharmacol.*, **110**, 879–885.
- KAUMANN, A.J., SANDERS, L., BROWN, A.M., MURRAY, K.J. & BROWN, M.J. (1991). A 5-HT₄-like receptor in human right atrium. *Naunyn-Schmied. Arch. Pharmacol.*, **344**, 150–159.
- MCLEAN, P.G., COUPAR, I.M. & MOLENAAR, P. (1993a). Effect of the novel 5-HT₄ receptor agonists DAU 6236 in human colon. *Med. J. Aust.*, **159**, 834.
- MCLEAN, P.G., COUPAR, I.M. & MOLENAAR, P. (1993b). The 5-HT₄ receptor in the alimentary tract. *Clin. Exp. Pharmacol. Physiol.*, **Suppl. 1**, 47.
- MCLEAN, P.G., COUPAR, I.M. & MOLENAAR, P. (1994). Characterisation of the 5-HT₄ receptor in the rat and human alimentary tract. *Can. J. Physiol. Pharmacol.*, **72** Suppl. 1, 242.
- MEDHURST, A.D. & KAUMANN, A.J. (1993). Characterization of the 5-HT₄ receptor mediating tachycardia in piglet isolated right atrium. *Br. J. Pharmacol.*, **110**, 1023–1030.
- MISIEWICZ, J.J., WALLER, S.L. & EISNER, M. (1966). Motor responses of human gastrointestinal tract to 5-hydroxytryptamine *in vivo* and *in vitro*. *Gut*, **7**, 208.
- MONFERINI, E., PARENTI, M., DONIN, L., ZOCCHETTI, A., RIZZI, C.A. & GIRALDO, E. (1991). A new class of 5-HT₄ receptor agonists positively coupled to adenylate cyclase in guinea-pig hippocampus. *Serotonin 1991 Conference*: Birmingham, U.K. July 14–17, p. 68.
- REEVES, J.J., BUNCE, K.T. & HUMPHREY, P.P.A. (1991). Investigation into the 5-hydroxytryptamine receptor mediating smooth muscle relaxation in the rat oesophagus. *Br. J. Pharmacol.*, **103**, 1067–1072.
- RIZZI, C.A., COCCINI, T., ONORI, L., MANZO, L. & TONINI, M. (1992). Benzimidazole derivatives: a new class of 5-hydroxytryptamine₄ receptor agonists with prokinetic and acetylcholine releasing properties in the guinea-pig ileum. *J. Pharmacol. Exp. Ther.*, **261**, 412–419.
- TALLARIDA, R.J. & MURRAY, R.B. (1987). *Manual of Pharmacologic Calculations with Computer Programs*. Berlin: Springer-Verlag.
- TAM, F.S.-F., HILLIER, K. & BUNCE, K.T. (1994). Characterization of the 5-hydroxytryptamine receptor type involved in inhibition of spontaneous activity of human isolated colonic circular muscle. *Br. J. Pharmacol.*, **113**, 143–150.
- THOMPSON, J.H. (1971). Serotonin and the alimentary tract. *Res. Commun. Chem. Pathol. Pharmacol.*, **2**, 687–781.
- TONINI, M., RIZZI, C.A., MANZO, L. & ONORI, L. (1991). Novel enteric 5-HT₄ receptors and gastrointestinal prokinetic action. *Pharmacol. Res.*, **24**, 5–14.
- TULADHAR, B.R., COSTALL, B. & NAYLOR, R.J. (1991). Putative 5-HT₄ receptor involvement in the relaxations induced by 5-HT in the rat ileum. *Br. J. Pharmacol.*, **104**, 151P.
- TULADHAR, B.R., COSTALL, B. & NAYLOR, R.J. (1992). Potentiation of the relaxation response to 5-methoxytryptamine in the rat ileum with mono amine oxidase inhibitors. *Br. J. Pharmacol.*, **107**, 122P.

- WAIKAR, M.V., FORD, A.P.D.W. & CLARKE, D.E. (1994). Evidence for an inhibitory 5-HT₄ receptor in urinary bladder of *Rhesus* and *Cynomolgus* monkeys. *Br. J. Pharmacol.*, **111**, 213–218.
- WAIKAR, M.V., HEDGE, S.S., FORD, A.P.D.W. & CLARKE, D.E. (1992). Pharmacological analyses of Endo-6-methoxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl-2,3-dihydro-2-oxo-1H-benzimidazolone-1-carboxylate hydrochloride (DAU 6285) at the 5-hydroxytryptamine₄ receptor in the tunica muscularis mucosae of rat oesophagus and ileum of guinea-pig: role of endogenous 5-hydroxytryptamine. *J. Pharmacol. Exp. Ther.*, **264**, 654–661.
- WRIGHT, P.G. & SHEPPARD, J.J. (1966). Some observations on the response of normal human sigmoid colon to drugs *in vitro*. *Gut*, **7**, 41.

(Received November 24, 1994

Accepted January 23, 1995)