British Journal of Pharmacology (1996) 118, 1965–1970

Neural 5-HT$_4$ receptors in the human isolated detrusor muscle: effects of indole, benzimidazolone and substituted benzamide agonists and antagonists

*S.M. Candura, Eliana Messori, **G.P. Franceschetti, §G. D'Agostino, **D. Vicini, *Monica Tagliani & [1]M. Tonini

Department of Internal Medicine and Therapeutics, Division of Pharmacology and Toxicology, University of Pavia, Piazza Botta 10, I-27100 Pavia; *Toxicology Unit, Salvatore Maugeri Foundation, Institute of Care and Scientific Research, Pavia Medical Centre; **Division of Urology, Ospedale Civile, Voghera (Pavia) and §Institute of Pharmacology, School of Pharmacy, University of Pavia, Italy

1 In strips of human isolated detrusor muscle, the 5-hydroxytryptamine (5-HT) receptor (5-HT$_4$) that mediates facilitation of neuromuscular cholinergic transmission was further characterized by using 5-HT and a series of ligands known for their 5-HT$_4$ agonist (5-methoxytryptamine: 5-MeOT, cisapride, (**R,S**)-zacopride, BIMU 8) or antagonist (RS 23597, GR 125487, DAU 6285) properties.

2 In the presence of methysergide (1 μM) and ondansetron (3 μM) to isolate pharmacologically the 5-HT$_4$ receptors, 5-HT (0.3 nM – 1 μM), 5-MeOT (10 nM – 30 μM), BIMU 8 (10 nM – 3 μM), cisapride (0.1 – 10 μM) and (**R,S**)-zacopride (0.1 – 30 μM) potentiated cholinergic contractions to electrical field stimulation in a concentration-dependent manner. RS 23597 (10 nM – 10 μM), a competitive 5-HT$_4$ receptor antagonist in other systems, also showed agonist properties. The following rank order of potency as an agonist was obtained: 5-HT (pEC$_{50}$ = 8.0) > RS 23597 (7.0) = BIMU 8 (6.9) ≥ cisapride (6.6) > 5-MeOT (6.0) ≥ (**R,S**)-zacopride (5.7). Relative to 5-HT (intrinsic activity = 1), 5-MeOT acted as a full agonist (1.03), while BIMU 8 (0.76), (**R,S**)-zacopride (0.61), RS 23597 (0.60) and cisapride (0.41) behaved as partial agonists.

3 The potentiation by 5-HT was competitively antagonized by the selective 5-HT$_4$ receptor antagonist GR 125487 (0.3 – 3 nM) with a pA$_2$ estimate of 9.75 (Schild slope of 1.09), and by DAU 6285 (1 μM; pK_B = 6.45). Additionally, GR 125487 (3 nM) antagonized the responses to 5-MeOT (pK_B = 9.72) and reversed the potentiation induced by RS 23597. As an antagonist, RS 23597 (10, 30 and 100 nM) inhibited the response to 5-HT. In addition, 30 and 100 nM RS 23597 reduced the 5-HT response maximum by 30 and 50%, respectively. The pK_B value calculated at 10 nM was 8.0.

4 Thus, in the human isolated detrusor muscle, the 5-HT$_4$ receptors mediating facilitation of cholinergic neuromuscular transmission are activated by indoleamines (5-HT, 5-MeOT), substituted benzamide (cisapride, (**R,S**)-zacopride), benzoate (RS 23597) and benzimidazolone (BIMU 8) derivatives. The activities (in terms of both potency and efficacy) of most agonists, as well as the affinity estimates of the antagonists GR 125487 and DAU 6285, are comparable to those found in other peripheral tissues. Exceptions are RS 23597, which acted either as a partial agonist or as an antagonist of the response to 5-HT, and 5-MeOT that showed an unusually low potency. The latter findings may be ascribed to differences in the efficiency of receptor coupling mechanisms and/or in the molecular structure (i.e. splice variants) of the 5-HT$_4$ receptor.

Keywords: Human urinary bladder; detrusor strips; neuromuscular cholinergic transmission; 5-hydroxytryptamine; 5-HT$_4$ receptor agonists; GR 125487; DAU 6285; RS 23597

Introduction

Since the pioneering studies of Erspamer (1952) in conscious dogs, 5-hydroxytryptamine (5-HT) has been shown to stimulate micturition in intact animals and to contract isolated bladder preparations from several mammals (see Anderson, 1993 for review), including man (Todd & Mack, 1969; Klarskov & Hørby-Petersen, 1986). The action of 5-HT may result from direct effects on the musculature and/or from indirect effects on the autonomic excitatory innervation. In resting preparations from most species, the direct contractile response to 5-HT is partly mediated by 5-HT$_{2A}$ receptors located on the effector cells (Saxena *et al.*, 1985; Klarskov & Hørby-Petersen, 1986; Cohen, 1990), while indirect effects are mediated by neural 5-HT$_3$ receptors at least in the cat (Saxena *et al.*, 1985) and the rabbit (Chen, 1990). With regard to excitatory neuromuscular transmission, 5-HT enhances the contractile activity induced by electrical field stimulation of detrusor strips from the mouse (Holt *et al.*, 1986; Clean *et al.*, 1989), guinea-pig (Messori *et al.*, 1995) and man (Hindmarsh *et al.*, 1977; Corsi *et al.*, 1991; Tonini *et al.*, 1994). In the mouse, the potentiating effect is mediated by neural 5-HT$_{1B}$ and 5-HT$_2$ receptors (Holt *et al.*, 1986; Clean *et al.*, 1989), while 5-HT-induced potentiation in the guinea-pig is mediated by three separate sites, namely 5-HT$_{2A}$, 5-HT$_3$ and 5-HT$_4$ receptors (Messori *et al.*, 1995). In the human isolated detrusor, 5-HT facilitates neuromuscular cholinergic transmission by acting on prejunctional receptors belonging to the 5-HT$_4$ subtype, as demonstrated by their sensitivity to GR 113808, a potent and selective antagonist at 5-HT$_4$ receptors (Tonini *et al.*, 1994).

In this study, we further characterized the 5-HT$_4$ receptor-mediated potentiation of cholinergic neuromuscular transmission in human isolated detrusor strips, after pharmacological isolation of 5-HT$_4$ receptors with methysergide and ondansetron. To this purpose, we utilized 5-HT and a series of

[1] Author for correspondence.

ligands known for their 5-HT_4 agonist (5-methoxytryptamine: 5-MeOT, BIMU 8, cisapride, (**R,S**)-zacopride) or antagonist (RS 23597, GR 125487, DAU 6285) properties in central and peripheral tissues (Tonini *et al.*, 1991; Schiavone *et al.*, 1992; Eglen *et al.*, 1993; Gale *et al.*, 1993).

Prior to the characterization of 5-HT_4 receptors the gastrointestinal prokinetic cisapride, which is known to promote acetylcholine release from enteric neurones (Schuurkes *et al.*, 1985), was found to improve bladder function in patients with spinal cord lesion (de Groot & de Pagter, 1988; Etienne *et al.*, 1988; Hanson & Soler, 1989). This investigation provides the mechanism by which cisapride may facilitate bladder emptying and, more generally, a rational basis for the potential use of 5-HT_4 receptor agonists as therapeutic agents in the treatment of micturition disturbances associated with detrusor hypocontractility.

Methods

Electrically stimulated detrusor strips

Specimens from the anterior part of the dome of the urinary bladder were obtained from patients undergoing total cystectomy due to bladder base malignancy. Muscular strips (20 mm long, 4 mm wide) were prepared by removing the serosal and mucosal layers and were mounted isometrically (tension: 20 mN) in 5 ml organ baths containing modified Krebs solution maintained at 37°C, and gassed with a mixture of 95% O_2 and 5% CO_2. A minimum initial equilibration period of 90 min was allowed before the experiments were started, during which time the solution was changed every 15 min. The tension was recorded by means of an isometric transducer connected to a chart recorder (Servocorder SR6221, Graphtec). Electrical field stimulation was applied via two platinum electrodes placed at the top and the bottom of the chamber, connected to a MARB ST 87 stimulator. Trains of electrical pulses at 5 Hz and 5 s in duration were delivered at 1 min intervals, at 0.1 ms pulse width and 60 V. This stimulation evoked reproducible submaximal contractions, the amplitude of which was approximately 30% of that obtained to 20 Hz stimulation (Corsi *et al.*, 1991; Tonini *et al.*, 1994).

Experimental design

Experiments were performed in the presence of 1 μM methysergide (a mixed 5-HT_1/5-HT_2 receptor antagonist) and 3 μM ondansetron (a selective 5-HT_3 receptor antagonist) to isolate pharmacologically the 5-HT_4 receptors.

Cumulative concentration-response curves to 5-HT and 5-HT-related agonists were obtained by half logarithmic or, in occasional cases, logarithmic dosing increments until the maximum effect was reached. Pilot experiments showed that the substituted benzamide ester RS 23597, a competitive 5-HT_4 receptor antagonist in other tissues (Eglen *et al.*, 1993), potentiated electrically-induced detrusor contractions. Thus, this compound was assessed for both agonist and antagonist properties. Two agonist concentration-response curves were constructed in each preparation. The second curve profile was superimposable to that of the first curve, provided that there were 60 min intervals between curves and frequent solution changes (every 10 min). In order to allow direct agonist comparisons, a series of concentration-response curves to 5-HT, 5-MeOT, BIMU 8, cisapride, (**R,S**)-zacopride and RS 23597 were constructed in single preparations.

In a separate set of experiments, the potentiating effect of 5-HT was investigated in the presence of the 5-HT_4 receptor antagonists GR 125487 and DAU 6285 and of the partial agonist/antagonist RS 23597 (incubation time: 30 min). The ability of GR 125487 to antagonize the responses to 5-MeOT and to reverse the potentiation by RS 23597 was also assessed. Only one antagonist concentration was tested in each preparation.

Data analysis

Responses to each agonist were expressed as a percentage of the maximal response to 5-HT. Values of EC_{50} (concentration of agonist producing half-maximal response) were estimated by linear regression analysis and expressed as $-\log EC_{50}$ (pEC_{50}). Any antagonist-induced inhibition of agonist response was calculated as a percentage of the maximum effect of agonist obtained under control conditions, GR 125487 pA_2 estimate was calculated following Schild regression analysis (Arunlakshana & Schild, 1959), with 5-HT concentration-ratios determined at the EC_{50} levels in control and test curves. Confidence limits (CL) at 95% probability for the slope of the regression were evaluated by a computer programme (PHARM/PCS, Version 4.1) based on a manual of pharmacological calculations (Tallarida & Murray, 1986). Apparent affinity estimates (pK_B) from single antagonist (DAU 6285 and RS 23597) concentrations were calculated by use of the Gaddum (1957) equation. All data are expressed as means $\pm$ s.e.mean of n experiments.

Solutions and drugs

The modified Krebs solution (pH 7.4) had the following composition (mM): NaCl 120, KCl 4.7, $MgSO_4.7H_2O$ 0.6, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.0 and glucose 10. Aqueous solutions of the following drugs were used: 5-hydroxytryptamine (5-HT) creatinine sulphate (from Sigma Chemical Co.); 5-methoxytryptamine (5-MeOT) hydrochloride (RBI); ondansetron ((±)-1,2,3,9-tetrahydro-3-[(methylimidazol-1-yl)methyl]-9-methyl-4H-carbazol-4-one hydrochloride) and GR 125487 ([1-[2(methylsulphonylamino)ethyl]4-piperidinyl] methyl - 5 - fluoro-2-methoxy - 1H- indole -3- carboxylate hydrochloride) (kindly donated by Glaxo); BIMU 8 (endo-N-(8-azabicyclo [3.2.1] oct-3-yl)-2,3-dihydro-3-(1-methyl)ethyl-2-oxo-1H-benzimidazole-1-carboxamide hydrochloride), RS 23597 (3-(piperidine-1-yl)-propyl-4-amino-5-chloro-2-methoxy benzoate hydrochloride), DAU 6285 (endo-6-methoxy-8-methyl-8-azabicyclo [3.2.1] oct-3-yl-2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxylate hydrochloride) and (**R,S**)-zacopride hydrochloride (kindly donated by Boehringer Ingelheim Italy). Methysergide maleate (Sandoz) and cisapride (free base) (Janssen Pharmaceutica) were dissolved in methanol and acetic acid, respectively, and then diluted in distilled water. Drugs were administered in volumes not exceeding 1% v/v of the bath volume.

Results

Effects of 5-HT and 5-HT_4 receptor agonists on electrically stimulated detrusor strips

In isolated strips of human detrusor muscle, electrical field stimulation (5 Hz for 5 s every min, 0.1 ms pulse duration, 60 V) elicited reproducible submaximal twitch contractions (Figure 1) which were 26.8 $\pm$ 5.7%, $n=8$, of those obtained by 20 Hz stimulation. We had previously shown that these contractions derive from the activation of postganglionic cholinergic nerves, since they were unaffected by hexamethonium, and abolished by tetrodotoxin and hyoscine (Tonini *et al.*, 1994).

Cumulative administration of 5-HT caused a concentration-dependent (range 0.3 nM – 1 μM) increase in the amplitude of twitch contractions. An example of the potentiating effect induced by 5-HT is shown in Figure 1. The effect of 5-HT was mimicked by other 5-HT_4 receptor agonists like 5-MeOT (an indoleamine), BIMU 8 (a benzimidazolone derivative), cisapride and (**R,S**)-zacopride (substituted benzamide derivatives). RS 23597, a substituted benzamide ester known for its 5-HT_4 antagonist properties (Eglen *et al.*, 1993), behaved as a partial agonist in our experimental model. A quantitation of the responses to 5-HT and related agonists is illustrated in Figure 2,

while Table 1 shows a comparison among their potencies and efficacies. The rank order of potency was: 5-HT > RS 23597 = BIMU 8 ≥ cisapride > 5-MeOT ≥ (R,S)-zacopride. 5-MeOT had a maximal effect superimposable to that of 5-HT. The remaining compounds acted as partial agonists: BIMU 8, RS 23597 and (R,S)-zacopride had intermediate intrinsic activity, while cisapride was the least effective agonist.

Effects of 5-HT4 antagonists

The indole derivative GR 125487 (0.3, 1 and 3 nM), a potent and selective 5-HT4 receptor antagonist, caused a parallel, concentration-dependent rightward displacement of the 5-HT curve, without depression of the maximum response (Figure 3). Schild regression analysis afforded a line with a slope of 1.09 (95% CL 0.86–1.22), which was not different from unity, and a pA_2 value of 9.75±0.06 (slope constrained to 1). GR 125487 (3 nM) also produced a parallel shift of the 5-MeOT curve to the right, without reduction in the maximum (Figure 4). The single point antagonist affinity estimate (pK_B) was 9.72±0.03. GR 125487 (30 nM) was also able to reverse by 95.2±2.3% ($n=4$) the submaximal potentiating effect caused by 1 μM RS 23597. This 'high' GR 125487 concentration was selected to obtain a rapid, clear-cut reversal of RS 23597-induced effect, since the latter slowly declined with time (see below). The benzimidazolone derivative DAU 6285 (1 μM) antagonized the response to 5-HT in a manner similar to that of GR 125487 (Figure 5), although with lower potency ($pK_B=6.45\pm0.33$). Both GR 125487 and DAU 6285 had no effect on basal twitch contractions.

As an antagonist, RS 23597 (10, 30 and 100 nM) inhibited the response to 5-HT. The antagonism caused by 30 and 100 nM, but not by 10 nM RS 23597 was associated with a depression of the 5-HT response maximum (30% and 50% depression at 30 nM and 100 nM, respectively) (Figure 6). The pK_B value calculated at 10 nM was 8.0±0.1. The enhancement of twitch contractions caused by 100 nM RS 23597 slowly decayed up to 60% of its peak effect during the 30 min contact with the tissue ($n=5$).

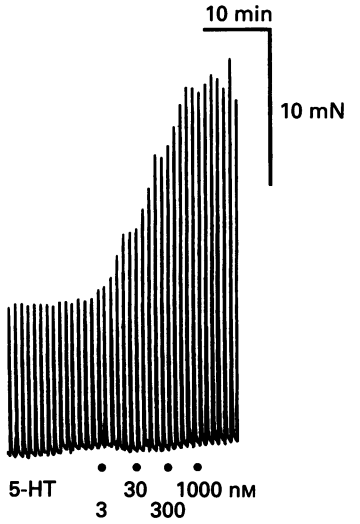

Figure 1 A tracing illustrating the potentiating effect of 5-HT (0.3 nM–1 μM) on the excitatory neuromuscular transmission elicited by repetitive trains of electrical pulses in human isolated detrusor strips.

Table 1 Potentiation by 5-HT and 5-HT4 receptor agonists of the contractile responses to electrical stimulation of human isolated detrusor strips

Agonist	n	*pEC50*	*Intrinsic activity*
5-HT	10	8.02±0.08	1.00
5-MeOT	14	6.02±0.10	1.03±0.13
BIMU 8	8	6.86±0.12	0.76±0.27
(R,S)-zacopride	5	5.67±0.05	0.61±0.08
RS 23597	4	6.96±0.06	0.60±0.17
Cisapride	4	6.61±0.14	0.41±0.09

The relative intrinsic activity was measured as a fraction of 5-HT response. Each value represents the mean±s.e.mean of n preparations.

Discussion

As in other animal species, excitatory neuromuscular transmission in the human urinary bladder is mediated by cholinergic and, to a minor extent, purinergic nerves (Ruggieri *et al.*, 1990; Anderson, 1993), the latter requiring a frequency

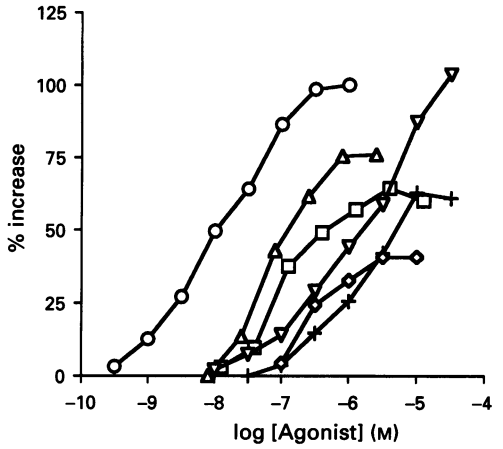

Figure 2 Concentration-response curves for 5-HT (○), 5-MeOT (□), BIMU 8 (△), RS 23597 (▽), cisapride (◇) and (R,S)-zacopride(+) in enhancing excitatory neuromuscular transmission in human isolated detrusor strips. Values are expressed as a percentage of the maximal response to 5-HT and represent the mean±s.e.mean of 4–14 determinations. For key to abbreviations used, see text.

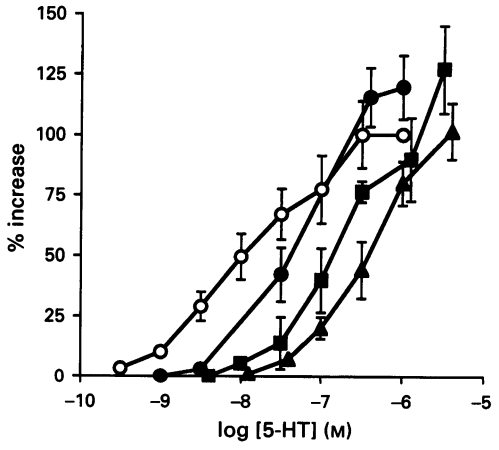

Figure 3 Concentration-response curves for the potentiating effect of 5-HT on stimulated detrusor twitch contractions under control conditions (○) or in the presence of 0.3 (●), 1 (■) and 3 nM (▲) GR 125487. Values are expressed as a percentage of the maximal response to 5-HT and represent the mean±s.e.mean (vertical lines) of 4–9 determinations.

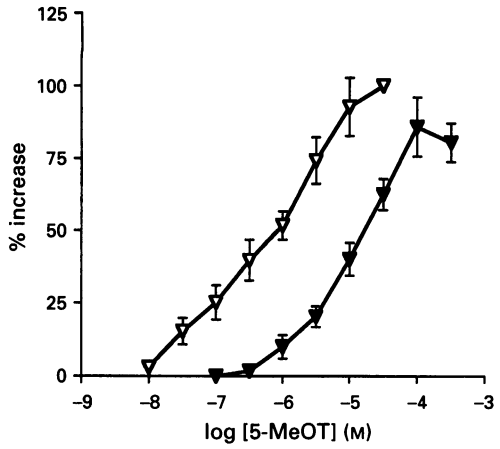

Figure 4 Concentration-response curves for the potentiating effect of 5-MeOT on stimulated detrusor twitch contractions under control conditions (▽) or in the presence of 3 nM GR 125487 (▼). Values are expressed as a percentage of the maximal response to 5-MeOT and represent the mean ± s.e.mean (vertical lines) of 4 determinations.

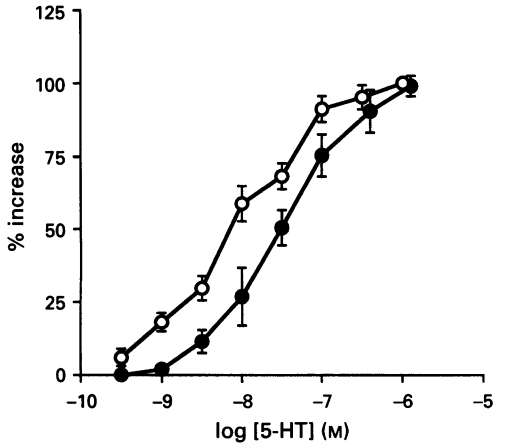

Figure 5 Concentration-response curves for the potentiating effect of 5-HT on stimulated detrusor twitch contractions under control conditions (○) or in the presence of 1 μM DAU 6285 (●). Values are expressed as a percentage of the maximal response to 5-HT and represent the mean ± s.e.mean (vertical lines) of 4 determinations.

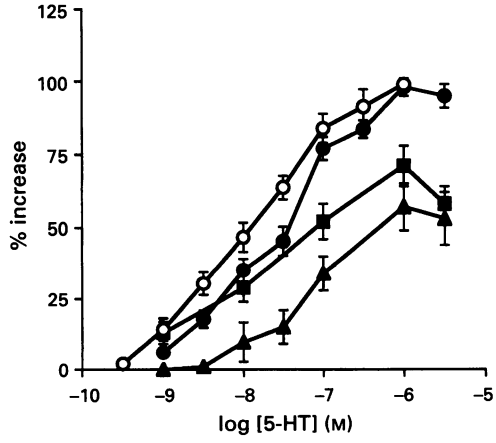

Figure 6 Concentration-response curves for the potentiating effect of 5-HT on stimulated detrusor twitch contractions under control conditions (○) or in the presence of 10 (●), 30 (■) and 100 nM (▲) RS 23597. Values are expressed as a percentage of the maximal response to 5-HT and represent the mean ± s.e.mean (vertical lines) of 4–9 determinations.

⩾10 Hz for their activation (Maggi *et al.*, 1989; Ruggieri *et al.*, 1990). Thus, under our experimental conditions, contractile responses to electrical field stimulation are typically cholinergic in nature. These contractions were enhanced by sub-micromolar concentrations of 5-HT, as previously described (Hindmarsh *et al.*, 1977; Corsi *et al.*, 1991). Recently, we have provided evidence that this potentiating effect is mediated by prejunctional 5-HT_4 receptors sensitive to the 5-HT_4 antagonist GR 113808, and that the human urinary bladder can therefore be regarded as an additional site where 5-HT_4 receptors are distributed (Tonini *et al.*, 1994). This study confirms and expands our previous findings. In fact, after pharmacological isolation of the 5-HT_4 receptors, the excitatory effect of 5-HT was mimicked, though with lower potency, by 5-HT_4 receptor agonists belonging to three separate chemical classes (5-MeOT; BIMU 8; cisapride and **(R,S)**-zacopride), and competitively inhibited by the selective 5-HT_4 antagonists GR 125487 and DAU 6285. The affinity estimates of the latter compounds (GR 125487 pA_2 value: 9.7; DAU 6285 pK_B value: 6.4) were comparable to those obtained in other 5-HT_4 receptor-containing tissues, such as the ascending colon (Gale *et al.*, 1994) and the ileum from the guinea-pig (Schiavone *et al.*, 1992; Tonini *et al.*, 1992). The apparent affinity of GR 125487 in antagonizing the responses to 5-HT and 5-MeOT was the same, in accordance with the theoretical concept that the affinity of an antagonist for a given receptor should be independent of the agonist used. Therefore, the results of the present study seem to indicate that in the human detrusor the pharmacology of the 5-HT_4 receptor is not different, with the exception of the low potency of 5-MeOT, from that in other 5-HT_4 receptor model systems, in agreement with our previous findings (Tonini *et al.*, 1994).

Further support for this hypothesis comes from the experiments with RS 23597, a substituted benzamide ester which is known to act as a high affinity, competitive antagonist at 5-HT_4 receptors in some peripheral tissues (Eglen *et al.*, 1993), but which disclosed partial agonist properties under our experimental conditions (see below). When used as an antagonist, 10 nM RS 23597 inhibited the response to 5-HT with a pK_B value of 8.0, a value similar to the pA_2 obtained by Eglen *et al.* (1993). The antagonism caused by higher (30, 100 nM) RS 23597 concentrations was associated with a depression (up to 50%) of the maximal agonist response. Partial agonists are defined as drugs that produce submaximal tissue responses and competitively block, at concentrations showing agonist properties, the effects of agonists of higher intrinsic efficacy (Kenakin, 1987). The unsurmountable nature of RS 23597 antagonism in the detrusor might be due to partial desensitization of the 5-HT_4 receptor, as observed after prolonged exposure of the tissue to this agent. In this regard, the partial agonists metaclopramide and cisapride produced unsurmountable antagonism against the response to 5-HT in the guinea-pig proximal colon, through a mechanism partially involving 5-HT_4 receptor desensitization (Elswood *et al.*, 1991).

With regard to agonist activities, 5-HT and 5-MeOT disclosed full agonist properties, while BIMU 8, cisapride and **(R,S)**-zacopride acted as partial agonists. This pharmacological profile matched that obtained in other peripheral tissues, such as the tunica muscularis mucosae of the rat oesophagus (see Bockaert *et al.*, 1992 for review), the guinea-pig ileum (Craig & Clarke, 1990; Eglen *et al.*, 1990; Tonini *et al.*, 1992) and distal colon (Wardle & Sanger, 1993), and porcine heart (Villalón *et al.*, 1991). In particular, the relatively low intrinsic activity of cisapride (as free base) in the human detrusor closely resembles that found in the guinea-pig distal colon (Wardle & Sanger, 1993). In contrast to our findings, BIMU 8 has been found to act as a full agonist in *Rhesus* and *Cynomolgus* monkey bladder (where 5-HT_4 receptors cause direct muscular relaxation) (Waikar *et al.*, 1994), and 5-MeOT has been found to possess partial agonist properties in the human isolated detrusor (Corsi *et al.*, 1991). In the latter preparation we found that BIMU 8, cisapride (as well as **(R,S)**-zacopride),

and 5-MeOT were approximately 15, 30 and 100 fold less potent than 5-HT, respectively. While the potencies of 5-HT, BIMU 8 and (**R,S**)-zacopride are comparable to those presented in the literature, the potency of 5-MeOT (and in part of cisapride) is unusually low (see also Corsi *et al.*, 1991). This might reflect degradation of 5-MeOT by monoamine oxidases (MAO) resulting in an underestimation of its potency. However, the fact that in the detrusor the potency of 5-HT, which is also a good substrate for MAO, is similar to that found in other peripheral tissues both in the presence and in the absence of pargyline (Baxter *et al.*, 1991; Reeves *et al.*, 1991) argues against this hypothesis.

The rank order of potency of agonists has long been used as a means of characterizing receptors. Indeed, the unusually low potency of 5-MeOT in the detrusor could be taken as evidence for the expression of different isoforms of the 5-HT_4 receptor, which are not distinguishable with conventional antagonists. Recently, two splice variants of the receptor, the 5-HT_{4L} and 5-HT_{4S} isoforms, differing in length and sequence of their C-terminus have been described in the brain and peripheral organs (including the bladder) of the rat (Gerald *et al.*, 1995). Since both isoforms possess identical amino acid sequencies in the transmembrane region, they have similar antagonist binding profiles (as demonstrated in displacement studies with [^{3}H]-GR 113808). By contrast, they might display a different coupling efficiency in the human detrusor, even though 5-MeOT was found to display a similar (high) potency towards central 5-HT_4 receptor variants expressed in COS-7 cells (Gerald *et al.*, 1995). Several factors such as density of receptor isoforms, their different coupling efficiencies and distinct affinity profiles for agonists, may contribute to the observed different potency and efficacy of agonists in producing functional responses. The identification of putative splice variants of the 5-HT_4 receptor in the human bladder, and their linked signal transduction mechanisms, might provide further support to our hypothesis.

In contrast to peripheral tissues with low receptor reserve in which RS 23597 disclosed clear cut antagonist properties at the 5-HT_4 receptors (Eglen *et al.*, 1993; 1995), in the detrusor this compound behaved as a partial agonist. In this context, RS 23597 has also been found to act as a partial 5-HT_4 receptor agonist in strips of guinea-pig proximal colon (A. Lucchelli: personal communication). The difference in the efficacy of RS 23597 may reflect different receptor coupling efficiency, a condition in which a ligand of G-protein coupled receptors (like the 5-HT_4 receptor) may function as a full agonist, partial agonist/antagonist, or neutral antagonist depending mainly on the tissue and the species under investigation (Kenakin, 1995). This also applies to other benzamide (cisapride, (**R,S**)-zacopride, renzapride) and benzimidazolone derivatives (BIMU 8), which act as full agonists in mouse colliculi neurones (Dumuis *et al.*, 1989; 1991), and as partial agonists in guinea-pig hippocampus (Bockaert *et al.*, 1990) and in a number of peripheral tissues (Craig & Clarke, 1990; Bockaert *et al.*, 1992). Similar observations apply to 8-(*mono*)hydroxydipropylamino tetralin (8-OHDPAT) towards 5-HT_{1A} receptors both in central neurones (Andrade & Nicoll, 1987) and in a mouse fibroblast cell line (Varrault & Bockaert, 1992).

The activation of the 5-HT_4 receptors located on cholinergic nerves explains, at least in part, the gastrointestinal prokinetic action of substituted benzamides and benzimidazolone derivatives (Tonini *et al.*, 1991; Tonini & De Ponti, 1995). The evidence that the same compounds potentiate by a similar mechanism the contractile activity of the human detrusor suggests that 5-HT_4 receptor agonists could be exploited clinically for the management of voiding disorders associated with detrusor hypocontractility, for which an enhancement of cholinergic drive is expected to be beneficial (Mundy *et al.*, 1994). In this respect, cisapride was found to increase bladder voiding efficiency in patients with bladder hypocontractility due to neurological damage of various origins (Etienne *et al.*, 1988; de Groot & de Pagter, 1988; Hanson & Soler, 1989), and to increase the frequency of micturition in patients treated for gastrointestinal motor disturbances (Boyd & Rohan, 1994; Pillans & Wood, 1994).

This work was funded in part by the Italian Ministry for University and Scientific Research (MURST, 40/60% Projects). We wish to thank the nurses and staff of the Voghera Hospital, Urology Surgical Unit, for their kind cooperation.

References

ANDERSON, K.-E. (1993). Pharmacology of lower urinary tract smooth muscles and penile erectile tissues. *Pharmacol. Rev.*, **45**, 253–308.

ANDRADE, R. & NICOLL, R.A. (1987). Novel anxiolytics discriminate between postsynaptic serotonin receptors mediating different physiological responses on single neurons of the rat hippocampus. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **336**, 5–10.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.

BAXTER, G.S., CRAIG, D.A. & CLARKE, D.E. (1991). 5-Hydroxytriptamine$_4$ receptors mediate relaxation of the rat oesophageal tunica muscularis mucosae. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **343**, 439–446.

BOCKAERT, J., FOZARD, J.R., DUMUIS, A. & CLARKE, D.E. (1992). The 5-HT_4 receptor: a place in the sun. *Trends Pharmacol. Sci.*, **13**, 141–145.

BOCKAERT, J., SEBBEN, M. & DUMUIS, A. (1990). Pharmacological characterization of 5-hydroxytryptamine$_4$ (5-HT_4) receptors positively coupled to adenylate cyclase in adult guinea pig hippocampal membranes: effects of substituted benzamide derivatives. *Mol. Pharmacol.*, **37**, 408–411.

BOYD, I.W. & ROHAN, A.P. (1994). Urinary disorders associated with cisapride. *Med. J. Aust.*, **160**, 579–580.

CHEN, H.I. (1990). Evidence for the presynaptic action of 5-hydroxytryptamine and the involvement of purinergic innervation in the rabbit lower urinary tract. *Br. J. Pharmacol.*, **101**, 212–216.

CLEAN, A., CORSI, M., FENIUK, W., GAVIRAGHI, G., TOSON, G., TREZISE, D. & TRIST, D. (1989). Potentiating action of 5-hydroxytryptamine on electrically induced contractions in the mouse urinary bladder. *Br. J. Pharmacol.*, **97**, 564P.

COHEN, M.L. (1990). Canine, but not rat bladder, contracts to serotonin via activation of 5-HT_2 receptors. *J. Urol.*, **143**, 1037–1040.

CORSI, M., PIETRA, C., TOSON, G., TRIST, D., TUCCITTO, G. & ARTIBANI, W. (1991). Pharmacological analysis of 5-hydroxytryptamine effects on electrically stimulated human isolated urinary bladder. *Br. J. Pharmacol.*, **104**, 719–725.

CRAIG, D.A. & CLARKE, D.E. (1990). Pharmacological characterization of a neural receptor for 5-hydroxytryptamine in guinea-pig ileum with properties similar to 5-hydroxytryptamine$_4$ receptor. *J. Pharmacol. Exp. Ther.*, **252**, 1378–1386.

DE GROOT, G.H. & DE PAGTER, G.F. (1988). Effects of cisapride on constipation due to a neurological lesion. *Paraplegia*, **26**, 159–161.

DUMUIS, A., SEBBEN, M. & BOCKAERT, J. (1989). The gastrointestinal prokinetic benzamide derivatives are agonists at the non-classical 5-HT receptor (5-HT_4) positively coupled to adenylate cylase in neurons. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **340**, 403–410.

DUMUIS, A., SEBBEN, M., MONFERINI, E., NICOLA, M., TURCONI, M., LADINSKY, H. & BOCKAERT, J. (1991). Azabicycloalkyl benzimidazolone derivatives as a novel class of potent agonists at the 5-HT_4 receptor positively coupled to adenylate cyclase in brain. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **343**, 245–251.

EGLEN, R.M., BLEY, K., BONHAUS, D.W., CLARK, R.D., HEDGE, S.S., JOHNSON, L.G., LEUNG, E. & WONG, E.H.F. (1993). RS 23597-190: a potent and selective 5-HT$_4$ receptor antagonist. *Br. J. Pharmacol.*, **10,** 119–126.

EGLEN, R.M., BONHAUS, D.W., JOHNSON, L.G., LEUNG, E. & CLARK, R.D. (1995). Pharmacological characterization of two novel and potent 5-HT$_4$ receptor agonists, RS 67333 and RS 67506, *in vitro* and *in vivo*. *Br. J. Pharmacol.*, **115,** 1387–1389.

EGLEN, R.M., SWANK, S.R., WALSH, L.K.M. & WHITING, R.L. (1990). Characterization of 5-HT$_3$ 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-HT$_4$ receptors in guinea-pig ascending colon. *Eur. J. Pharmacol.*, **196,** 149–155.

ERSPAMER, V. (1952). Enteramina e 5-metossitriptamina. Tossicità. Azione sulla diuresi, sulla pressione del sangue e su alcuni organi a muscolatura liscia. *Ricerca Scientifica*, **22,** 694–702.

ETIENNE, M., VERLINDEN, M. & BRASSINNE, A. (1988). Treatment with cisapride of the gastrointestinal and urological sequelae of spinal cord transection: case report. *Paraplegia*, **26,** 162–164.

GADDUM, J.H. (1957). Series of drug antagonism. *Pharmacol. Rev.*, **9,** 211–218.

GALE, J.D., GROSSMAN, C.J., DARTON, J., BUNCE, K.T., WHITEHEAD, J.W.F., KNIGHT, J., PARKHOUSE, T.J., OXFORD, A.W. & HUMPHREY, P.P.A. (1994). GR125487: a selective and high affinity 5-HT$_4$ receptor antagonist. *Br. J. Pharmacol.*, **113,** 120P.

GALE, J.D., REEVES, J.J. & BUNCE, K.T. (1993). Antagonism of the gastroprokinetic effect of metoclopramide by GR125487, a potent and selective 5-HT$_4$ receptor antagonist (Abstract). *J. Gastrointest. Motil.*, **5,** 192.

GERALD, C., ADHAM, N., KAO, H.-T., OLSEN, M.A., LAZ, T.M., SCHECHTER, L.E., BARD, J.A., VAYSSE, P.J.-J., HARTIG, P.A., BRANCHEK, T.A. & WEINSHANK, R.L. (1995). The 5-HT$_4$ receptor; molecular cloning and pharmacological characterization of two splice variants. *EMBO J.*, **14,** 2806–2815.

HANSON, P. & SOLER, J.M. (1989). Effet du cisapride sur les vessies neurologiques. *Acta Belg. Med. Phys.*, **12,** 81–88.

HINDMARSH, J.R., IDOWU, O.A., YEATES, W.K. & ZAR, M.A. (1977). Pharmacology of electrically evoked contractions of human bladder. *Br. J. Pharmacol.*, **61,** 115P.

HOLT, S.E., COOPER, M. & WYLLIE, J.H. (1986). On the nature of the receptors mediating the action of 5-hydroxytryptamine in potentiating responses of the mouse urinary bladder strip to electrical stimulation. *Naunyn-Schmiedberg's Arch. Pharmacol.*, **334,** 333–340.

KENAKIN, T. (1995). Efficacy as the molecular property of ligand. *Trends Pharmacol. Sci.*, **16,** 84–85.

KENAKIN, T.P. (1987). Drug antagonism. In *Pharmacologic Analysis of Drug-Receptor Interaction*. pp. 205–244. New York: Raven Press.

KLARSKOV, P. & HØRBY-PETERSEN, J. (1986). Influence of serotonin on lower urinary tract muscle in vitro. *Br. J. Urol.*, **58,** 507–513.

MAGGI, C.A., PATACCHINI, R., SANTICIOLI, P., TURINI, D., BARBANTI, G., BENEFORTI, P., ROVERO, P. & MELI, A. (1989). Further studies on the motor response of the human isolated urinary bladder to tachykinins, capsaicin and electrical field stimulation. *Gen. Pharmacol.*, **20,** 663–669.

MESSORI, E., RIZZI, C.A., CANDURA, S.M., LUCCHELLI, A., BALESTRA, B. & TONINI, M. (1995). 5-Hydroxytryptamine receptors that facilitate excitatory neuromuscular transmission in the guinea-pig isolated detrusor muscle. *Br. J. Pharmacol.*, **115,** 677–683.

MUNDY, A.R., STEPHENSON, T.P. & WEIN, A.J. (1994). *Urodynamics. Principles, Practice and Application*. 2nd ed. Edinburgh: Churchill Livingstone.

PILLANS, P.I. & WOOD, M.D. (1994). Cisapride increases micturition frequency. *J. Clin. Gastroenterol.*, **19,** 336–346.

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.

RUGGIERI, M.R., WHITMORE, K.E. & LEVIN, R.M. (1990). Bladder purinergic receptors. *J. Urol.*, **144,** 176–181.

SAXENA, P.R., HEILIGERS, J., MYLECHARANE, E.J. & TIO, R. (1985). Excitatory 5-hydroxytryptamine receptors in the cat urinary bladder are of the M- and 5-HT$_2$-type. *J. Auton. Pharmacol.*, **5,** 101–107.

SCHIAVONE, A., GIRALDO, E., GIUDICI, L., TURCONI, M. & SAGRADA, A. (1992). DAU 6285: a novel antagonist at the putative 5-HT$_4$ receptors. *Life Sci.*, **51,** 583–592.

SCHUURKES, J.A.J., VAN NUETEN, J.M., VAN DAELE, P.G.H., REYNTJENS, A.J. & JANSSEN, P.A.J. (1985). Motor-stimulating properties of cisapride on isolated gastrointestinal preparations of the guinea-pig. *J. Pharmacol. Exp. Ther.*, **234,** 775–783.

TALLARIDA, R.J. & MURRAY, R.B. (1986). *Manual of Pharmacologic Calculations with Computer Programs*. 2nd ed. New York: Springler-Verlag.

TODD, J.K. & MACK, A.J. (1969). A study of human bladder detrusor muscle. *Br. J. Urol.*, **41,** 448–454.

TONINI, M., CANDURA, S.M., ONORI, L., COCCINI, T., MANZO, L. & RIZZI, C.A., (1992). 5-Hydroxytryptamine$_4$ receptor agonists facilitate cholinergic transmission in the circular muscle of guinea pig ileum: antagonism by tropisetron and DAU 6285. *Life Sci.*, **50,** PL 173–PL 178.

TONINI, M. & DE PONTI, F. (1995). Serotonin modulation of gastrointestinal motility. In *Serotonin and Gastrointestinal Function*. ed. Gaginella, T.S. & Galligan, J.J. pp. 53–84. Boca Raton: CRC Press.

TONINI, M., MESSORI, E., FRANCESCHETTI, G.P., RIZZI, C.A., CASTOLDI, A.F., COCCINI, T. & CANDURA, S.M. (1994). Characterization of the 5-HT receptor potentiating neuromuscular cholinergic transmission in strips of human isolated detrusor muscle. *Br. J. Pharmacol.*, **113,** 1–2.

TONINI, M., RIZZI, C.A., MANZO, L. & ONORI, L. (1991). Novel enteric 5-HT$_4$ receptors and gastrointestinal prokinetic action. *Pharmacol. Res.*, **24,** 5–14.

VARRAULT, A. & BOCKAERT, J. (1992). Differential coupling of 5-HT$_{1A}$ receptors occupied by 5-HT or 8-OH-DPAT to adenylyl cyclase. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **346,** 367–374.

VILLALÓN, C.M., DEN BOER, M.O., HEILIGERS, J.P.C. & SAXENA, P.R. (1991). Further characterization, by use of tryptamine and benzamide derivatives, of the putative 5-HT$_4$ receptor mediating tachycardia in the pig. *Br. J. Pharmacol.*, **102,** 107–112.

WAIKAR, M.V., FORD A.P.D.W. & CLARKE, D.E. (1994). Evidence for an inhibitory 5-HT$_4$ receptor in urinary bladder of *Rhesus* and *Cynomolgus* monkeys. *Br. J. Pharmacol.*, **111,** 213–218.

WARDLE, K.A. & SANGER, G.J. (1993). The guinea-pig distal colon - a sensitive preparation for the investigation of 5-HT$_4$ receptor-mediated contractions. *Br. J. Pharmacol.*, **110,** 1593–1599.

(Received February 26, 1996
Revised April 18, 1996
Accepted April 30, 1996)