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Pharmacological characterization of two novel and potent 5-HT_4 receptor agonists, RS 67333 and RS 67506, *in vitro* and *in vivo*

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1 The pharmacology of two novel 5-HT_4 receptor agonists, RS 67333 (1-(4-amino-5-chloro-2-methoxy phenyl)-3-[1(n-butyl)-4-piperidinyl]-1-propanone HCl) and RS 67506 (1-(4-amino-5-chloro-2-methoxy-phenyl)-3-[1-(2-methyl sulphonylamino)ethyl-4-piperidinyl]-1-propanone HCl) have been assessed *in vitro* and *in vivo*.

2 RS 67333 and RS 67506 exhibited affinities ($pK_i = 8.7$ and 8.8, respectively) for the 5-HT_4 binding sites, labelled with [^{3}H]-GR 113808, in guinea-pig striatum. The Hill coefficients from these displacement curves were not significantly different from unity. The compounds exhibited lower affinities (< 6.0) at several other receptors including 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C}, dopamine D_1, D_2 and muscarinic M_1-M_3 receptors. However, RS 67333 and RS 67506 did exhibit affinities for the σ_1 ($pK_i = 8.9$ and 7.9, respectively) and σ_2 ($pK_i = 8.0$ and 7.3, respectively) binding sites.

3 At the 5-HT_4 receptor mediating relaxation of the carbachol-precontracted oesophagus, RS 67333 and RS 67506 acted as potent (pEC_{50} 8.4 and 8.6, respectively), partial agonists (intrinsic activities, with respect to 5-HT were 0.5 and 0.6, respectively) with respect to 5-HT. Relaxant responses to RS 67333 or RS 67506 were surmountably antagonized by GR 113808 (10 nM), with apparent affinities (pK_B) of 9.1 and 9.0, respectively. RS 67333 and RS 67506 induced dose-dependent increases in heart rate of the anaesthetized micropig (ED_{50} 4.9 and 5.4 $\mu g\ kg^{-1}$,i.v.), with maximal increases of 35 and 47 beats min^{-1}, respectively.

4 RS 67333 and RS 67506, therefore, acted as potent, partial 5-HT_4 receptor agonists *in vitro* and *in vivo*. These compounds, by virtue of their high potency and selectivity, may have some utility in elucidating the physiological role of 5-HT_4 receptors.

Keywords: RS 67333; RS 67506; 5-HT_4 receptors

Introduction

The 5-HT_4 receptor can be stimulated by several different classes of compounds. These include indoles, such as 5-hydroxytryptamine (5-HT) or 5-methoxytryptamine, substituted benzamides such as SC 53116, renzapride, cisapride or zacopride, substituted benzimidazolones, such as BIMU-1 or BIMU-8 and 1,8-naphthalimides, such as RS 56532 (see Ford & Clarke, 1993, for review; Eglen *et al.*, 1994a). Selective 5-HT_4 receptor agonists may enhance congnitive performance (Bockaert *et al.*, 1994), facilitate gastrointestinal motility (Gullikson *et al.*, 1992), correct micturation disturbances associated with detrusor hypomotility (Tonini *et al.*, 1994) or act as analgesics (Romanelli *et al.*, 1993).

One problem of assessing the physiological role of the 5-HT_4 receptor, and thus the therapeutic potential of selective ligands, is achieving selective stimulation of 5-HT_4 receptors *in vivo*. These difficulties primarily arise from the limited selectivity and/or the low bioavailability of the currently available compounds. For example, BIMU-8 or SC 53116, while acting as potent 5-HT_4 receptor agonists, also possess significant affinity for 5-HT_3 receptors (Dumuis *et al.*, 1992; Flynn *et al.*, 1992). 5-Methoxytryptamine, while lacking affinity at 5-HT_3 receptors, potently stimulates several other 5-HT receptor subtypes (see Eglen *et al.*, 1992, for references). Cisapride is a potent 5-HT_4 agonist used clinically for the treatment of gastro-oesophageal reflux. However, this compound also possesses affinity for dopamine, 5-HT_2, α_1-adrenoceptor and muscarinic receptors (see Wiseman & Faulds, 1994, for review).

In the present study, we describe the pharmacology of two novel 5-HT_4 receptor agonists, structurally related to the 5-HT_4 antagonist, RS 23597 (Eglen *et al.*, 1993b). The antagonist (Figure 1) possesses moderate affinity ($pK_B = 7.8$) at 5-HT_4 receptors in rat isolated oesophagus, vagus nerve and guinea-pig ileum (Eglen *et al.*, 1993b). In human isolated urinary bladder, however, RS 23597 acts as a partial agonist, albeit of low intrinsic efficacy (Rizzi *et al.*, 1994, unpublished observations). RS 23597 may, therefore, possess structural features conferring partial 5-HT_4 receptor agonism, depending upon the receptor reserve. Langlois *et al.* (1994) have described an ester analogue of RS 23597, ML 10302 (Figure 1) that acted as a potent, but partial agonist at 5-HT_4 receptors in guinea-pig ileum ($EC_{50} = 4$ nM) or rat oesophageal muscularis mucosae ($EC_{50} = 2.4$ nM).

RS 23597 is a substituted benzamide ester, and thus has limited duration of action *in vivo* as a result of plasma hydrolysis (Eglen *et al.*, 1993b). In a medicinal chemistry programme designed to augment the *in vivo* stability of RS 23597, the ester function was replaced, resulting in a series of ketone analogues (Clark *et al.*, 1994). Subsequently, two novel agonists, 4-piperidinyl congeners, RS 67333 (1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1-(2-methyl sulphonylamino)ethyl-4-piperidinyl]-1-propanone HCl) and 67506 (1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1(n-butyl)-4-piperidinyl]-1-propanone HCl), were also prepared (Figure 1). The synthetic details of these and other compounds have been described previously (Clark *et al.*, 1994).

The objective of the study was therefore to assess the pharmacology of these two agonists *in vitro* and *in vivo*. A preliminary account of these data has been communicated to the British Pharmacological Society (Eglen *et al.*, 1994b).

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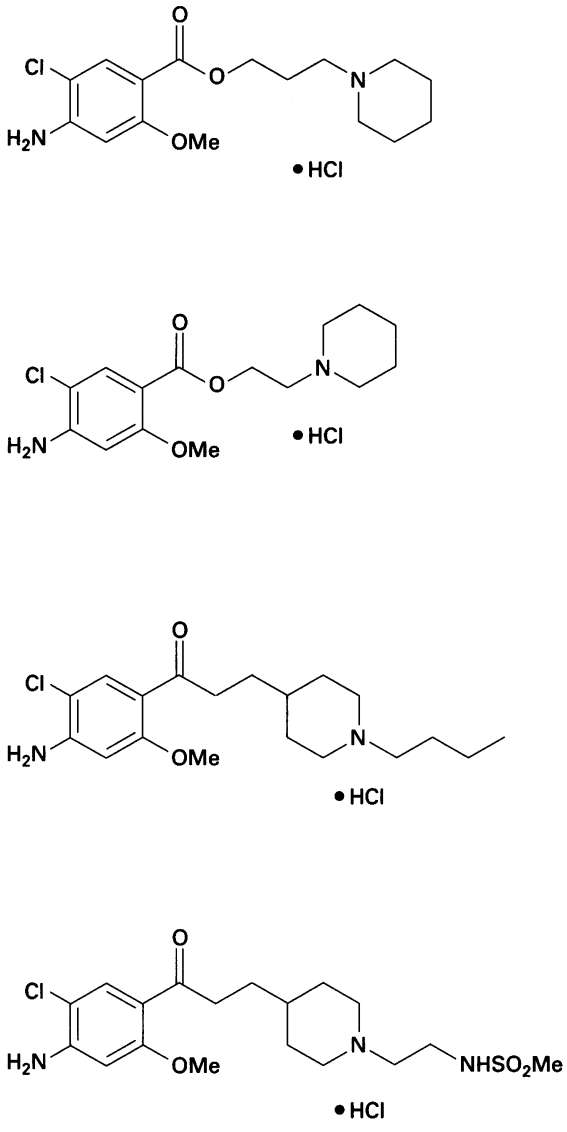

Figure 1 Structures of, reading from top to bottom, RS 23597, ML 10302, RS 67333 and RS 67506.

Methods

Competition radioligand binding studies

The affinity of the compounds at 5-HT_4 receptors was assessed using displacement of [^{3}H]-GR 113808 binding. [^{3}H]-GR 113808 binding to 5-HT_4 receptors was measured in a synaptosomal membrane preparation of guinea-pig striatum, obtained from animals previously killed by CO_2 asphyxiation. Striata were homogenized with a hand driven glass homogenizer in a Tris (10 mM), EDTA (5 mM) buffer (pH 7.4 at 4°C), containing 250 mM sucrose. The homogenate was filtered through a nylon mesh (160 μm pore) and then centrifuged at 1000 *g* for 15 min. The resulting pellet was suspended in a HEPES (50 mM) EDTA (0.5 mM) buffer (pH 7.4 at 4°C) containing choline (130 mM), glucose (25 mM) and KCl (5.4 mM). The final pellet was resuspended in a Tris (25 mM) buffer (pH 7.4 at 4°C).

Competition binding assays were conducted in a Tris buffer (25 mM) with approximately 0.1 mg striatal protein in an assay volume of 0.5 ml at room temperature. Non specific binding was determined with 1 μM unlabelled GR 113808. Previous studies have demonstrated that a 60 min incubation was sufficient for membrane binding to reach a steady state (Eglen *et al.*, 1994a). Competition binding studies were conducted with 0.1 nM [^{3}H]-GR 113808 and ten concentrations of competing ligand. Reactions were terminated by vacuum filtration, using a Brandel cell harvester, through GB/B filters pretreated for 30 min with 0.1% polyethyleneimine. Filters were then dried and the bound radioactivity determined.

The activities of the compounds, across a range of neurotransmitter receptors, were also studied. Details of the individual assays can be found in Table 1.

Rat isolated oesophageal muscularis mucosal studies

5-HT_4 agonist activity was studied in the rat isolated oesophagus (Baxter *et al.*, 1991), in which relaxation is mediated by 5-HT_4 receptor activation. Thoracic oesophagus was isolated from male Sprague-Dawley rats (Charles River, 200–250 g) and placed in Tyrode solution (composition, mM: NaCl 139.0, KCl 2.7, $MgCl_2$. $6H_2O$ 1.1, NaH_2PO_4 0.4, glucose 5.6, $NaHCO_3$ 11.8 and $CaCl_2$. $6H_2O$ 1.8). The outer striated muscle coat was cut longitudinally and gently peeled away, leaving the inner muscularis mucosae. Silk threads were

Table 1 Details of radioligand binding assays

Receptor	*Radioligand*	*Compound defining NSB*	*Tissue*
D_1	[^{3}H]-SCH 23390	Butaclamol	striatum
D_2	[^{3}H]-spiperone	Butaclamol	striatum
5-HT_{1A}	[^{3}H]-8 OH-DPAT	5-HT	brain
5-HT_{2A}	[^{3}H]-ketanserin	Methysergide	cortex
5-HT_{2C}	[^{3}H]-mesulergine	Methysergide	choroid plexus
5-HT_3	[^{3}H]-quipazine	Zacopride	NG 108–15 cells
5-HT_4	[^{3}H]-GR 113808	GR 113808	striatum
(**R**)-zacopride site	[^{3}H]-(**R**)-zacopride	Mianserin	NG 108–15 cells
M_1	[^{3}H]-pirenzepine	Atropine	cortex
M_2	[^{3}H]-NMS	Atropine	heart
β_1	[^{3}H]-CGP 12177	Isoprenaline	lung
β_2	[^{3}H]-CGP 12177	Isoprenaline	lung
α_{1A}	[^{3}H]-prazosin	Phentolamine	submaxilliary gland
α_{1B}	[^{3}H]-prazosin	Phentolamine	liver
α_{2A}	[^{3}H]-rauwolscine	Phentolamine	spleen
α_{2B}	[^{3}H]-rauwolscine	Phentolamine	kidney
σ_1	[^{3}H]-PPP	Haloperidol	hippocampus
σ_2	[^{3}H]-BIMU-1	BIMU-8	hippocampus

All tissues were from rat, except striatum and hippocampus, isolated from guinea-pig and lung and spleen isolated from rabbit. NSB-non specific binding; [^{3}H]-NMS-[^{3}H]-*N*-methyl scopolamine.

tied through the lumen on both ends of the tissue. The tissues were then mounted vertically in 10 ml organ baths containing Tyrode solution, maintained at 37°C and gassed with 95% O_2, 5% CO_2. Methysergide (1 μM), cocaine (30 μM) and corticosterone (30 μM) were present throughout the experiment. An initial resting tension of 1 g was applied to the preparation and readjusted to 0.5 g during the initial equilibration period of 60 min. After this period, pargyline (100 μM) was added to the Tyrode solution for 30 min, followed by a washout period. The tissues were then exposed to 50 mM KCl for 5 min, washed 4 times followed by an equilibration period of 30 min. Carbachol (3 μM) was added to precontract the tissues and a stable contracture allowed to develop over the succeeding 30 min. A concentration-response curve to 5-HT and subsequently to RS 67506 or RS 67333 was then established, using incremental concentrations spaced at 0.5 log intervals. The tissues were then washed and, after 45 min, a second curve established to agonists. To assess whether the relaxation was mediated by 5-HT_4 receptor activation, the tissues were washed and equilibrated in 10 μM 5-methoxytryptamine, and a third concentration-response curve established. This procedure has been shown to desensitize selectively 5-HT_4 receptor mediated relaxations (Baxter *et al.*, 1991). In seperate studies, the selective 5-HT_4 receptor antagonist, GR 113808 (10 nM) was equilibrated with the tissues for 60 min, prior to constructing a second concentration-response curve to agonists.

Tachycardia in anaesthetized micropig

The method used was that described by Villalon *et al.* (1990), modified according to Eglen *et al.* (1993a; 1994a,b). Yucatan micropigs (male and female, 17–22 kg, S & S Farms, Ranchita, CA, USA) were pretreated with ketamine (approx 30 mg kg^{-1}, i.m.), anaesthetized with pentobarbitone sodium (20 mg kg^{-1}) via the marginal ear vein, intubated and mechanically ventilated with room air by an animal respirator (Harvard, model 613). A femoral artery was cannulated for measurement of arterial blood pressure via a Gould/Statham pressure transducer (P23ID). Dual cannulae were inserted in the ipsilateral femoral vein, one cannula for continuous infusion of supplemental anaesthetic and the second cannula for compound administration. A limb lead II ECG electrode was monitored by subcutaneously placed electrodes and heart rate was determined by a cardiotachometer triggered by the R wave of the ECG. Following a ventral cervical midline incision, vagus nerves were cut bilaterally. Normal body temperature was maintained with a heated water blanket. Blood gas parameters were stabilized within a normal physiological range (pH 7.49 ± 0.05; P_{CO_2} 32 ± 5, P_{O_2} 95 ± 8 mmHg) by adjustments of ventilation rate, tidal volume and positive end expiratory pressure prior to continuing an experiment.

Each animal received 5-HT in ascending bolus i.v. doses of 1–300 μg kg^{-1} at 0.5 log intervals and with 10 min between doses. Subsequently, each animal received RS 67506 or RS 67333 at bolus i.v. doses of 0.3–300 μg kg^{-1} in a similar fashion. Mean arterial pressure and heart rate were measured immediately prior to and at peak effect following each dose of 5-HT or agonists. Additionally, arterial blood gases, pH, and the rectal temperature were periodically determined to monitor the stability of the animal preparation.

Data analysis and statistical methods

Data from competition radioligand binding studies were analyzed by fitting the data to a four parameter logistic function by an iterative curve fitting programme. The apparent affinities (pK_i) of competing ligands were calculated from IC_{50} values by the Cheng-Prusoff correction (Cheng & Prusoff, 1973). Saturation binding studies were conducted with ten concentrations of radioligand. The data from these studies were analyzed using the programme in 'LIGAND' (Munson & Rodbard, 1980), after first correcting for the free ligand concentration.

In the functional studies, agonist potencies (pEC_{50}) were determined by nonlinear regression using iterative curve fitting procedures (Leung *et al.*, 1992) and the relationship described by Parker & Waud (1971). Concentration-ratios were measured at the agonist concentration that elicited 30% of the maximal relaxation, since under some conditions the effects at higher concentrations may have reflected muscarinic receptor antagonism. The apparent affinity (pK_B) of GR 113808, a potent and selective 5-HT_4 receptor antagonist (Grossman *et al.*, 1993; Gale *et al.*, 1994), determined against responses to each agonist, estimated using a single concentration of antagonist (10 nM).

In vivo *studies*

In the micropig studies, data were expressed as absolute changes from the baseline heart rate. In studies using 5-HT, with its relatively short duration of activity, baseline heart rate refers to pre-dose heart rate; in studies using the other agonists, with more prolonged durations of action, heart rate data were expressed as cumulative changes. Dose-response data were analyzed by nonlinear iterative curve fitting procedures (Leung *et al.*, 1992). Statistically significant differences were assessed at the $P < 0.05$ level.

Compounds used

5-HT, RS 67333 and RS 67506 were dissolved in normal saline. All compounds were administered in base equivalent doses for the *in vivo* studies. GR 113808 ([1-(2-methane-sulphonamido-ethyl)-piperidin-4-yl]-methyl-indole-3-carboxylate maleate), [^{3}H]-BIMU-1 (endo-*N*-(8-methyl-8-azabicyclo{3.2.1]oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1*H*-benzimidazole-1-carboxamide hydrochloride, specific activity = 75 Ci $mmol^{-1}$), [^{3}H]-GR 113808 (specific activity = 89 Ci $mmol^{-1}$), metoclopramide, ondansetron, [^{3}H]-RS 23597 (3-(piperidine-1-yl)propyl-4-amino-5-chloro-2-methoxybenzoate hydrochloride, specific activity = 98.6 Ci $mmol^{-1}$), RS-67506 (1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1-(2-methyl sulphonylamino)ethyl-4-piperidinyl]-1-propanone HCl), RS 67333 (1-(4-amino-5-chloro-2-methoxy phenyl)-3-[1(n-butyl)-4-piperidinyl]-1-propanone HCl), [^{3}H]-(**R**)-zacopride (specific activity = 36 Ci $mmol^{-1}$), tropisetron, (**R,S**)-zacopride were synthesized at the Institute of Organic Chemistry, Syntex Discovery Research (Palo Alto, CA, U.S.A.). Methysergide was generously donated by Sandoz (Basel, Switzerland). All remaining compounds were obtained from Sigma Chemical Co., Ltd (St. Louis, MO, U.S.A.) or Research Biochemicals Inc., (Natick, MA, U.S.A.). All remaining radioligands were obtained from Dupont-NEN (Boston, MA, U.S.A.).

Results

Competition radioligand binding studies

RS 67333 and RS 67506 exhibited high affinities for the 5-HT_4 binding sites in guinea-pig striatum (Table 2). In all of these studies, the Hill coefficients were not significantly different from unity. At several other neurohormonal receptors studied, they exhibited apparent affinities (pK_i) of approximately 6.0 or less (Table 2). The notable exception to this was their apparent affinities at σ_1 and σ_2 binding sites (Table 2).

Rat isolated oesophageal muscularis mucosa

At the 5-HT_4 receptor mediating relaxation of carbachol precontracted oesophagus, all compounds studied, with the exception of 5-methoxytryptamine, acted as partial agonists, with respect to 5-HT (Table 3). RS 67333 and RS 67506 relaxed the preparation in a concentration-dependent manner (Table 3). Relaxant responses to all agonists were abolished by equilibration of the tissues with 5-methoxytryptamine (Figure

Table 2 Apparent affinities (pK_i) of several compounds at various neurotransmitter receptors and binding sites

	RS 67333	*RS 67506*
D_1	<5.0	<5.0
D_2	<5.0	<5.0
5-HT$_{1A}$	6.4	5.7
5-HT$_{2A}$	6.3	<6.0
5-HT$_{2C}$	6.1	5.7
5-HT$_3$	6.4	5.6
5-HT$_4$	8.7	8.8
(R)-zacopride site	5.9	5.9
M_1	5.2	4.4
M_2	5.3	<4.0
α_{1A} adrenoceptor	<6.7	6.4
α_{1B} adrenoceptor	5.9	<5.0
α_{2A} adrenoceptor	<5.0	<5.0
α_{2B} adrenoceptor	<5.0	<5.0
β_1 adrenoceptor	<4.0	<4.0
β_2 adrenoceptor	<4.0	<4.0
σ_1	8.9	7.9
σ_2	8.0	7.3

Values are mean, s.e.mean, n=3–4 determinations.

Table 3 Potencies of several compounds at 5-HT$_4$ receptors mediating relaxation of rat isolated oesphageal muscularis mucosae

Agonist	*pEC$_{50}$*	*% reversal of carbachol contracture*
5-HT	8.2±0.1	1.0
RS 67333	8.7±0.1	0.5
RS 67506	8.8±0.1	0.6
ML 10302[a]	8.0±0.1 (8.4)	0.5
5-MeOT[b]	8.3	1.0
SB 205149[c]	8.0	0.8
BIMU-1[b]	7.9	0.7
RS 56532-197[d]	7.9	0.8
SC 53116[b]	7.7	0.6
BIMU-8[b]	7.6	0.9
Renzapride[e]	7.6	0.8
(S)-zacopride[b]	6.7	0.9

Values are mean±s.e.mean, n=4–8 animals. [a]data from Eglen *et al.* (1994b); [b]data from Eglen *et al.* (1993a); [c]data from Baxter *et al.* (1993); [d]data from Eglen *et al.* (1994a); [e]data from Grossman *et al.* (1993). 5-MeOT-5-methoxytryptamine. The value in parentheses is that reported by Langlois *et al.* (1994).

2a and b) and surmountably antagonized by GR 113808 (10 nM; data not shown). The apparent affinities (pK_B) for this antagonist against 5-HT, RS 67333 and RS 67506 were 9.3±0.2, 9.1±0.2 and 9.0±0.3, respectively. These values, determined from 5 animals, were not significantly different from each other.

Tachycardia in anaesthetized micropig

5-HT, RS 67333 and RS 67506 (Figures 3a and b), renzapride, metoclopramide and (R,S)-zacopride elicited dose-dependent increases in heart rate in the anaesthetized micropig. Relative to 5-HT, (R,S)-zacopride acted as a full agonist, while the other compounds acted as partial agonists (Table 4). (R,S)-zacopride and metoclopramide were significantly less potent as agonists than 5-HT. In contrast, RS 67333 and RS 67506 were equipotent to 5-HT (Table 4).

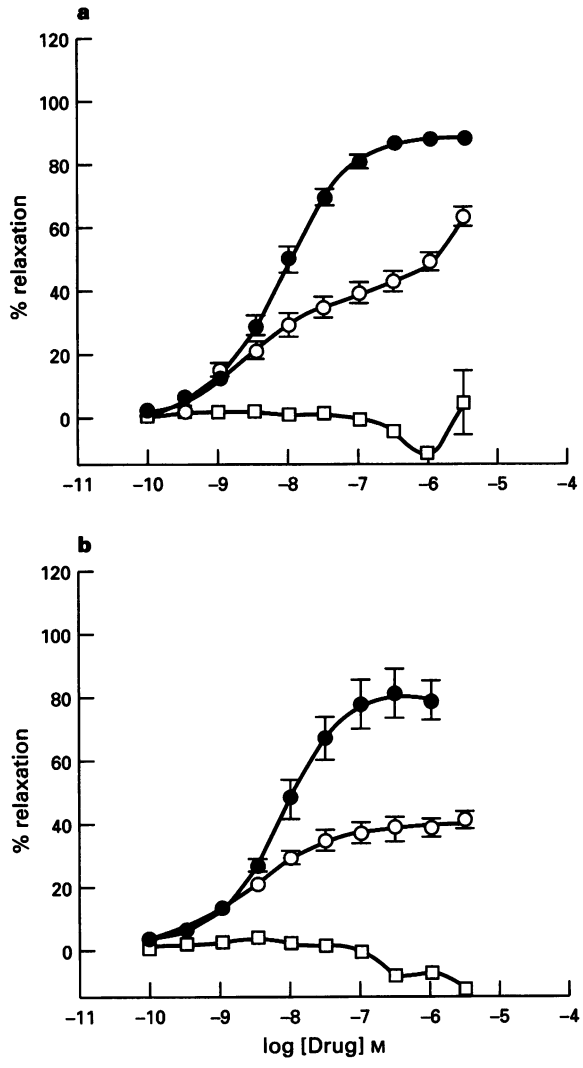

Figure 2 Concentration-response curves to 5-HT (●), RS 67333 (○; a) and RS 67506 (○; b) in rat isolated muscularis mucosae. In each figure the effect of selective desensitization of 5-HT$_4$ receptors, by 5-methoxytryptamine, is shown (□). Values are mean, with s.e.mean, n=4–8.

Discussion

The pharmacological activity of two novel 5-HT$_4$ receptor agonists are described in the present study. These compounds are analogues of the selective 5-HT$_4$ antagonist, RS 23597; a compound that lacks efficacy at 5-HT$_4$ receptors in rat oesophagus, vagus nerve, guinea-pig ileum *in vitro* or micropig myocardium *in vivo* (Eglen *et al.*, 1993b).

In radioligand binding studies, RS 67333 and RS 67506 selectively bound to the 5-HT$_4$ receptor in comparison to various other neurotransmitter receptors, with the exception of σ_1 and σ_2 binding sites. In this respect, these compounds resembled the antagonist RS 23597 (Figure 1; Bonhaus *et al.*, 1994); a compound that also discriminated σ_1 from σ_2 binding sites. The structure activity relationship for ligands interacting with σ_1 and σ_2 binding sites is not well established or, indeed, is the significance of these heterogeneous sigma binding sites (Bonhaus *et al.*, 1994). However, the potential interaction of these compounds with sigma sites *in vitro* or *in vivo* cannot be excluded. Therefore, to ascribe unambiguously a functional response of these compounds to 5-HT$_4$ receptor activation, studies should be conducted with antagonists that possess a lower affinity for sigma sites, such as GR 113808 or DAU 6285 (Bonhaus *et al.*, 1994). RS 67333 or RS 67506, therefore, act as

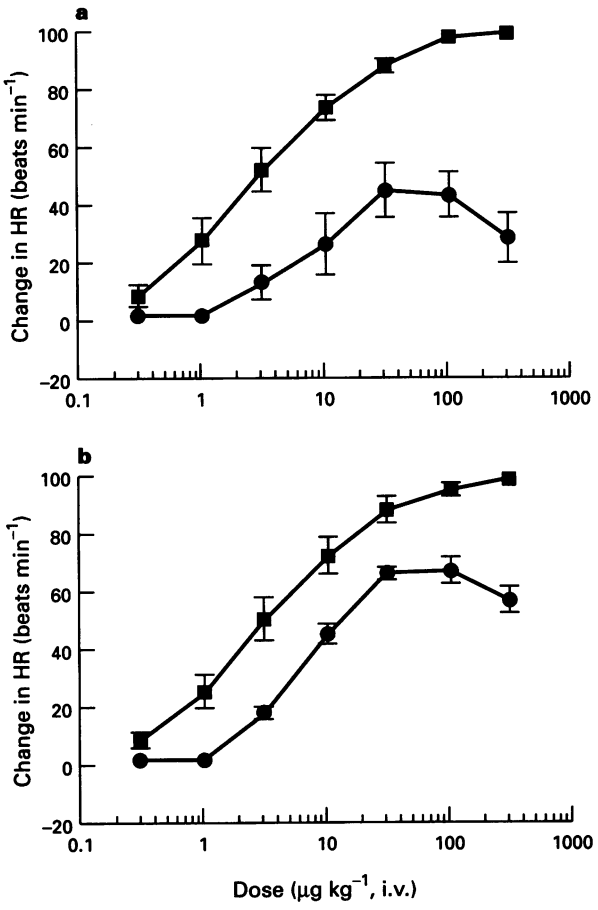

Figure 3 Dose-response curves for 5-HT, RS 67333 and RS 67506 in anaesthetized, bilaterally vagotomized, micropig. Values are mean, with s.e.mean, $n=4$–8. (a) The dose-response curve to RS 67333 (●) was established in animals following construction of the 5-HT curve (■). (b) The dose-response curve to RS 67506 (●) was established following construction of the 5-HT curve (■).

relatively high affinity ligands for the 5-HT_4 receptor, although they exhibit affinities somewhat less than for the antagonists, GR 113808 or SB 204070 ($pK_i = 10.4$ and 10.8, respectively; Grossman *et al.*, 1993; Wardle *et al.*, 1994; Bonhaus *et al.*, 1994).

RS 67333 and RS 67506 acted as partial and potent 5-HT_4 receptor agonists in rat isolated oesophageal muscularis mucosae. The potency values are shown in Table 3 together with those for several other 5-HT_4 receptor agonists previously used to define the 5-HT_4 receptor. RS 67333 and RS 67506 are thus amongst the most potent 5-HT_4 receptor agonists yet identified. They are similar in potency to ML 10302, an ester analogue of RS 23597, shown by Langlois *et al.* (1994) and the present study to act as a partial agonist. In contrast to several other 5-HT_4 receptor agonists, including SB 205149 (Baxter *et al.*, 1993), SC 53116 (Flynn *et al.*, 1993), RS 67333 and RS 67506 were selective toward the 5-HT_4 receptor (Table 2). RS 67333 and RS 67506 acted as partial 5-HT_4 receptor agonists in rat oesophagus; a property that appears to be true for most non-indole 5-HT_4 receptor agonists (Table 3). This has been ascribed to the low receptor reserve associated with 5-HT_4-mediated relaxant responses in this preparation (Ford & Clarke, 1993). Therefore, caution should be applied in interpreting the appearance (or lack thereof) of efficacy for RS 67333 and RS 67506, since their potency will be contingent upon the prevailing 5-HT_4 receptor reserve. For example, at the 5-HT_4 receptor mediating elevations of adenylyl cyclase activity in guinea-pig hippocampus, a preparation known to possess a low receptor reserve (Eglen *et al.*, 1993a), both compounds act as silent, surmountable antagonists (Alvarez & Ramsey, unpublished observations).

Table 4 Potencies of various compounds at 5-HT_4 receptors mediating tachycardia in the anaesthetized micropig

Agonist	ED_{50} (μg kg^{-1},i.v.)	*Maximum heart rate increase* (beats min^{-1})
5-HT	3.2 (2.0–5.1)	95 (84–105)
RS 67333	4.9 (2.3–10)	35 (27–43)*
5-HT	3.1 (2.3–4.2)	75 (69–80)
RS 67506	5.4 (4.0–7.2)	47 (43–52)*
5-HT	2.0 (1.6–2.5)	68 (63–73)
Metoclopramide	108 (56–210)*	28 (22–33)*
5-HT	4.2 (3.5–5.3)	95 (90–100)
Renzapride[a]	20 (17–25)*	76 (70–81)*
5-HT	4.0 (2.8–5.8)	106 (98–115)
(R,S)-zacopride[a]	28 (20–41)*	100 (98–110)

Values are mean, with 95% confidence limits in parentheses, n = 4–8 animals. *$P<0.05$ significantly different from 5-HT control. [a]data from Eglen *et al.* (1993a).

The anaesthetized micropig appears to provide a highly reproducible model in order to study 5-HT_4 receptor function *in vivo*, and the variation of the 5-HT potency (2.0–4.2 μg kg^{-1}) was small between each series of experiments. However, the maximal response to 5-HT varied between 68–106 beats min^{-1}, underlining the importance of establishing a control 5-HT dose-response curve in each animal, particularly when assessing the extent of partial agonism of novel compounds. Nonetheless, in support of the *in vitro* studies discussed above, RS 67333 and RS 67506 acted as partial agonists with respect to 5-HT. Moreover, the rank order of potency paralleled those rank orders seen *in vitro*. RS 67333 and RS 67506 are clearly structurally related (Figure 1). The incorporation of sulphonylamino moiety in RS 67506 (clog P = 2.4) suggests that it is a more polar molecule than RS 67333 (clog P = 4.5). Consequently, the penetration of the RS 67333 across the blood brain barrier would be greater than RS 67506.

It is concluded that these compounds, by virtue of their high potency and selectivity, will therefore offer some advantages over the benzamide ester, (ML 10302) described by Langlois *et al.* (1994) or non-selective compounds, such as cisapride, zacopride or SC 53116, in the *in vivo* characterization of the physiological role of 5-HT_4 receptors.

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