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The interaction of RS 25259-197, a potent and selective antagonist, with 5-HT_3 receptors, *in vitro*

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1 A series of isoquinolines have been identified as 5-HT_3 receptor antagonists. One of these, RS 25259-197 [(3aS)-2-[(S)-1-azabicyclo[2.2.2]oct-3-yl]-2,3,3a,4,5,6-hexahydro-1-oxo-1*H*-benzo[de]isoquinoline-hydrochloride], has two chiral centres. The remaining three enantiomers are denoted as RS 25259-198 (**R,R**), RS 25233-197 (**S,R**) and RS 25233-198 (**R,S**).

2 At 5-HT_3 receptors mediating contraction of guinea-pig isolated ileum, RS 25259-197 antagonized contractile responses to 5-HT in an unsurmountable fashion and the apparent affinity (pK_B), estimated at 10 nM, was 8.8 ± 0.2. In this tissue, the $-\log K_B$ values for the other three enantiomers were 6.7 ± 0.3 (**R,R**), 6.7 ± 0.1 (**S,R**) and 7.4 ± 0.1 (**R,S**), respectively. The apparent affinities of RS 25259-197 and RS 25259-198, RS 25233-197 and RS 25233-198 at 5-HT_3 receptors in membranes from NG-108-15 cells were evaluated by a [^{3}H]-quipazine binding assay. The $-\log K_i$ values were 10.5 ± 0.2, 8.4 ± 0.1, 8.6 ± 0.1 and 9.5 ± 0.1, respectively, with Hill coefficients not significantly different from unity. Thus, at these 5-HT_3 receptors, the rank order of apparent affinities was (**S,S**) > (**R,S**) > (**S,R**) = (**R,R**).

3 RS 25259-197 displaced the binding of the selective 5-HT_3 receptor ligand, [^{3}H]-RS 42358-197, in membranes from NG-108-15 cells, rat cerebral cortex, rabbit ileal myenteric plexus and guinea-pig ileal myenteric plexus, with affinity (pK_i) values of 10.1 ± 0.1, 10.2 ± 0.1, 10.1 ± 0.1 and 8.3 ± 0.2, respectively. In contrast, it exhibited low affinity ($pK_i < 6.0$) at 28 other receptors in binding assays, including adrenoceptors (α_{1A}, α_{1B}, α_{2A}, α_{2B}, β_1, β_2), muscarinic (M_1-M_4), dopamine (D_1, D_2), opioid and other 5-HT (5-HT_{1A}, 5-HT_{1D}, 5-HT_{2C}, 5-HT_4) receptors.

4 RS 25259-197 was tritium labelled (specific activity: 70 Ci mmol^{-1}) and evaluated in pharmacological studies. Saturation studies with [^{3}H]-RS 25259-197 in membranes from NG-108-15 and cloned homomeric α subunits of the 5-HT_3 receptor from N1E-115 cells expressed in human kidney 293E1 cells, revealed an equilibrium dissociation constant (K_d) of 0.05 ± 0.02 and 0.07 ± 0.01 nM, and B_{max} of 610 ± 60 and 1068 ± 88 fmol mg^{-1}, respectively. Competition studies in NG-108-15 cells indicated a pharmacological specificity entirely consistent with labelling a 5-HT_3 receptor, i.e. RS 25259-197 > granisetron > (S)-zacopride > tropisetron > (R)-zacopride > ondansetron > MDL 72222.

5 In contrast to the majority of radioligands available to label 5-HT_3 receptors, [^{3}H]-RS 25259-197 labelled a high affinity site in hippocampus from human post-mortem tissue with an equilibrium dissociation constant (K_d) of 0.15 ± 0.07 nM and density (B_{max}) of 6.8 ± 2.4 fmol mg^{-1} protein. Competition studies in this tissue indicated a pharmacological specificity consistent with labelling of a 5-HT_3 receptor.

6 Quantitative autoradiographic studies in rat brain indicated a differential distribution of 5-HT_3 receptor sites by [^{3}H]-RS 25259-197. High densities of sites were seen in nuclear tractus solitaris and area postrema, a medium density in spinal trigeminal tract, ventral dentate gyrus and basal medial amygdala, and a low density of sites in hippocampal CA1, parietal cortex, medium raphe and cerebellum.

7 In conclusion, the functional, binding and distribution studies undertaken with the radiolabelled and non-radiolabelled RS 25259-197 (S,S enantiomer) established the profile of a highly potent and selective 5-HT_3 receptor antagonist.

Keywords: 5-HT_3 receptors; radioligand binding; autoradiography; guinea-pig ileum; antagonist; allosteric interaction; autoradiographic localization

Introduction

5-Hydroxytryptamine (5-HT) receptors are classified on operational, transductional and structural criteria (see Martin & Humphrey, 1994; Boess & Martin, 1994; Hoyer *et al.*, 1994 for reviews) into 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-ht_5, 5-ht_6 and 5-ht_7 subtypes. The 5-HT_3 receptor is thought to form a ligand gated ion channel (Derkach *et al.*, 1989; Peters *et al.*, 1991; Yakel, 1992). The distribution and pharmacology of 5-HT_3 receptors have been characterized by several radiolabelled ligands including [^{3}H]-GR 65630 (Kilpatrick *et al.*, 1988), [^{3}H]-ICS 205,930 (Hoyer & Neijt, 1988), [^{3}H]-quaternary ICS 205,930 (Watling *et al.*, 1988; McKernan *et al.*, 1990), [^{3}H]-quipazine (Milburn & Peroutka, 1989; Sharif *et al.*, 1991), [^{3}H]-zacopride (Barnes *et al.*, 1989a; Pinkus *et al.*, 1989), [^{3}H]-BRL 43694 (Nelson & Thomas, 1989), [^{3}H]-LY 278,584 (Wong *et al.*, 1989), [^{125}I]-iodozacopride (Laporte *et al.*, 1992) and, most recently, [^{3}H]-RS 42358-197 (Wong *et al.*, 1993a). Consistently, these studies and those using *in situ* hybridization (see Boess & Martin, 1994, for review) suggest that central 5-HT_3 receptors are discretely localized on cortical, limbic and brain stem structures. Peripherally, 5-HT_3 receptors appear to be exclusively located on neurones, activation of which causes contractions of gastrointestinal smooth muscle (Fozard, 1984; Butler *et al.*, 1990; Eglen *et al.*, 1990) and the von Bezold-Jarisch reflex (Fozard, 1984).

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5-HT$_3$ receptors appear to exist in species variants, in that the guinea-pig 5-HT$_3$ receptor differs pharmacologically from the 5-HT$_3$ receptor in mouse, rat, ferret or man (Butler *et al.*, 1990; Newberry *et al.*, 1991; Kilpatick & Tyers, 1992; Wong *et al.*, 1993a). Recent binding studies at 5-HT$_3$ receptors in murine brain and ileum have provided some evidence for the existence of intra-species heterogeneity (Bonhaus *et al.*, 1993). Measurement of single ion conductances also support the existence of more than one receptor (see Boess & Martin, 1994, for review).

The identification of selective 5-HT$_3$ receptor antagonists has helped to establish the pharmacological relevance of these receptors. Anti-psychotic actions (Tricklebank, 1989), cognitive enhancing actions, and facilitation of withdrawal from drugs of abuse (Costall & Naylor, 1991) have all been ascribed to 5-HT$_3$ receptor antagonism (Costall *et al.*, 1990) although the neurochemical basis (Barnes *et al.*, 1989b; Johnson *et al.*, 1993), and, indeed, the behavioural actions of these antagonists *per se* remains controversial (Tricklebank, 1991; Greenshaw, 1993). In contrast, the efficacy of 5-HT$_3$ receptor antagonists as inhibitors of nausea and vomiting associated with neoplastic agents has clearly been demonstrated in dog or ferret and clinically (Oxford *et al.*, 1992; Andrews & Bhandari, 1993).

A recent investigation into the structure activity relationship of several novel 5-HT$_3$ receptor antagonists (Clark *et al.*, 1993) has identified one, RS 25259-197 [(3a**S**)-2-[(**S**)-1-azabicyclo-[2.2.2]oct-3-yl]-2,3,3a,4,5,6-hexahydro-1-oxo-1*H*-benzo [de]isoquinoline-hydrochloride] (Figure 1) as an antagonist of very high affinity. This compound is a structural analogue of RS 42358-197 (Wong *et al.*, 1993a; Eglen *et al.*, 1993), an antagonist that also exhibits high affinity and selectivity for the 5-HT$_3$ receptor (Clark *et al.*, 1993; Figure 1). In this study we have further characterized the functional, binding and autoradiographic characteristics of both radiolabelled and non-radiolabelled RS 25259-197 *in vitro*.

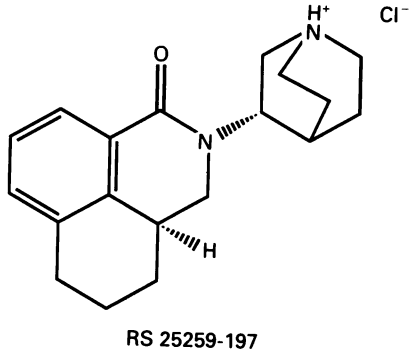
Methods

Contractility studies

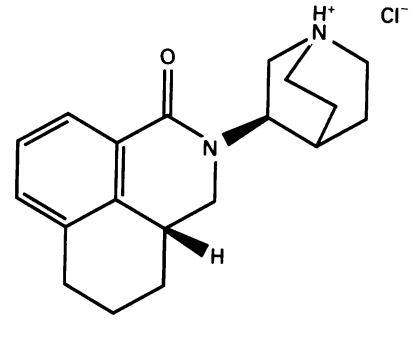
In guinea-pig isolated ileum, contractile responses to activation of 5-HT$_3$ receptors were measured according to the method of Eglen *et al.* (1990). All experiments were carried out at 37°C. Briefly, 2 cm segments of proximal ileum were suspended in Tyrode solution (composition mM: NaCl 137, KCl 2.7, $MgCl_2.H_2O$ 1.1, $NaHPO_4.2H_2O$ 0.4, glucose 5.6, $NaHCO_3$ 11.9, $CaCl_2.6H_2O$ 1.8) under 1 g resting tension. The Tyrode solution contained methysergide (1 μM) to antagonize 5-HT$_1$ and 5-HT$_2$ receptors. 5-Methoxytryptamine (10 μM) was included to desensitize 5-HT$_4$, receptors (Craig *et al.*, 1990). Concentration-response curves to 5-HT were constructed, non-cumulatively (10 nM – 10 μM) at incremental half-log concentrations, both in the absence and in the presence of antagonists, following equilibration for 60 min between consecutive concentration-response curves.

Binding studies

Radioligand binding assays at 5-HT$_3$ receptors were conducted with four 5-HT$_3$ receptor ligands, [^{3}H]-quipazine, [^{3}H]-granisetron, [^{3}H]-RS 42348-197 and [^{3}H]-RS 25259-197. Membranes were prepared according to the methods of Wong *et al.* (1993a) in a Tris-Krebs buffer (composition mM: NaCl 154, KCl 5.4, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgCl_2$ 1.0, D-glucose 11, Tris 25, pH 7.4 at 25°C) and incubations were performed in 0.5 ml total volume at 25°C for 60 min. Saturation studies were conducted with eight concentrations of radioligand, ranging from 4 pM to 4 nM. Competition studies were conducted with 0.1 to 0.4 nM of radioligand. Non-specific binding was defined with 0.1 μM (S)-zacopride. Reactions were terminated by vacuum filtration over GF/B filters pretreated with 0.3% polyethyleneimine. The filters were then



RS 25259-197



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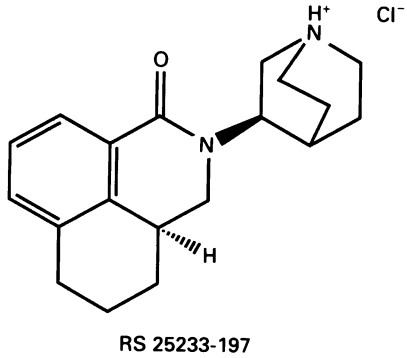

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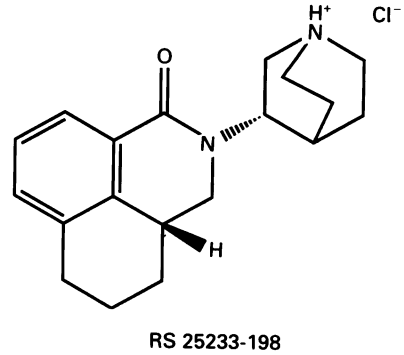

RS 25233-198

Figure 1 Structures of RS 25259-197 and enantiomers.

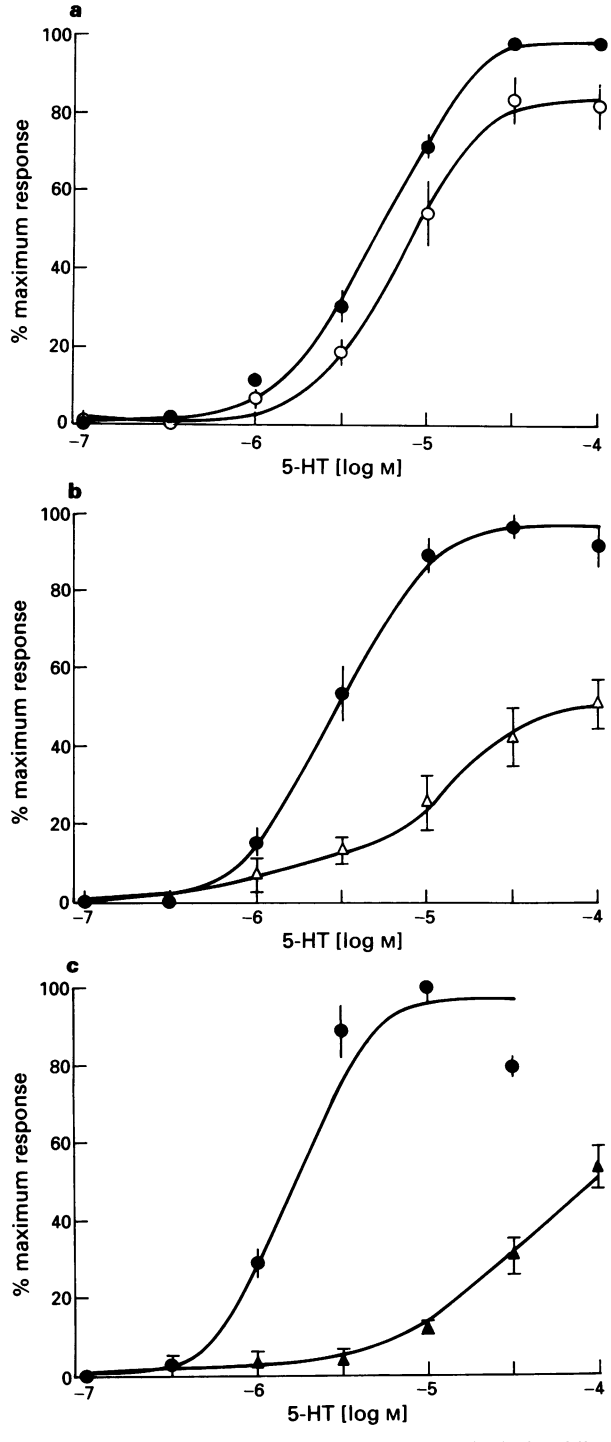

Figure 2 Antagonism of 5-HT response in guinea-pig, isolated ileum by RS 25259-197. Concentration-response curves for 5-HT were constructed in the absence (●), and presence of (a) 1 (○), (b) 10 (△) and (c) 100 (▲) nM of RS 25259-197.

washed for 10 s with ice-cold 0.1 M NaCl, dried and radioactivity retained on the filters was determined by liquid scintillation spectrometry. In all studies, the protein concentration was determined by the Biorad colormetric method with bovine gamma-globulin as the standard (Bradford, 1976). Competition curves were generated by use of 10 concentrations of non-radiolabelled compound. Association and dissociation rates for [^{3}H]-RS 25259-197 and [^{3}H]-granisetron were also determined.

Autoradiographical studies

Coronal sections of rat and mouse brains were cut at 20 μm thickness. Sections were dried and pre-incubated in Tris-HCl buffer (50 mM Tris, 120 mM NaCl, pH 7.4, 22°C) for 30 min. The sections were then covered with the same buffer containing 1.0 nM [^{3}H]-RS 42358-197 or [^{3}H]-RS 25259-197 for 60 min at 22°C. Non-specific binding was defined in the presence of 1.0 μM (S)-zacopride. The incubations were terminated by rinsing the slides for two washes of 5 min in ice cold buffer. The sections were dried and apposed, together with ^{3}H polymer standards (Amersham, Inc.) to tritium-sensitive X-ray film for 24 weeks. The autoradiograms were

Table 2 Affinity of RS 25259-197 at 5-HT$_3$ and other receptor binding assays

Assay radioligand	*p*K$_i$	
5-HT$_{1A}$	8-OH-DPAT	4.4 ± 0.1
5-HT$_{1D}$	5-HT	4.2 ± 0.3
5-HT$_{2A}$	Ketanserin	4.8 ± 0.2
5-HT$_{2C}$	Mesulergine	4.6 ± 0.3
5-HT$_3$	Quipazine	10.4 ± 0.2
5-HT upake	Paroxetine	5.3 ± 0.1
Dopamine D$_1$	SCH23390	<4.0
Dopamine D$_2$	Spiperone	<4.0
Alpha$_{1A}$	Prazosin	5.6 ± 0.3
Alpha$_{1B}$	Prazosin	5.4 ± 0.1
Alpha$_{2A}$	Rauwolscine	5.4 ± 0.2
Alpha$_{2B}$	Rauwolscine	5.4 ± 0.1
Beta$_1$	CGP-12177	<4.0
Beta$_2$	CGP-12177	<4.0
Angiotensin AT$_1$	Sar^{1}Ile^{8}Angiotensin II	<4.0
Angiotensin AT$_2$	Sar^{1}Ile^{8}Angiotensin II	<4.0
Muscarinic M$_1$	Pirenzepine	5.9 ± 0.1
Muscarinic M$_2$	NMS	4.9 ± 0.1
Muscarinic M$_3$	NMS	5.3 ± 0.1
Kappa Opioid	U69593	4.2 ± 0.4
Mu Opioid	RX783006	3.7 ± 0.2
Delta Opioid	DPDPE	4.0 ± 0.4
GABA$_A$	GABA	<4.0
GABA$_A$/BDZ	Diazepam	<4.0
GABA$_A$/Picrotoxin	TBPS	<4.0
NMDA channel	MK-801	<4.0
Ca^{2+} channel	PN200-10	<4.0
Na$^+$ channel	Saxitoxin	<4.0
NK$_1$ Substance P	<4.0	

Values are means ± s.e.mean of at least three separate determinations.

Table 1 Binding affinity and antagonist potency of RS 25259-197 and enantiomers

Compound	*p*K$_i$	n_H	*p*K$_B$
RS 25259-198 (**R,R**)	8.40 ± 0.07	0.89 ± 0.08	6.7 ± 0.1
RS 25233-197 (**S,R**)	8.63 ± 0.13	1.40 ± 0.60	6.7 ± 0.3
RS 25233-198 (**R,S**)	9.48 ± 0.05	0.91 ± 0.07	7.4 ± 0.1
RS 25259-197 (**S,S**)	10.45 ± 0.18	1.03 ± 0.10	8.8 ± 0.2
RS 25259-197 (**S,S**)[1]	10.10 ± 0.09	0.95 ± 0.05	–
RS 25259-197 (**S,S**)[2]	10.19 ± 0.06	0.91 ± 0.07	–
RS 25259-197 (**S,S**)[3]	10.14 ± 0.04	0.91 ± 0.04	–
RS 25259-197 (**S,S**)[4]	8.30 ± 0.18	0.85 ± 0.12	–

p*K*$_i$ and n$_H$ values (means ± s.e.mean; *n* = 3 animals) were generated from competition experiments using [^{3}H]-quipazine in NG-108-15 cells and [^{3}H]-RS 42358-197 in [1]NG-108-15, [2]rat cortex, [3]rabbit ileum and [4]guinea-pig ileum.
All p*K*$_B$ values were derived from guinea-pig isolated ileum using 10 nM RS 25259-197.

then analysed by digital image analysis with the MCID imaging system (Imaging Research, Inc.). Brain areas were verified on cresyl violet stained sections after autoradiography, using the areas described in the rat brain atlas of Paxinos & Watson (1985).

Data analysis

In functional studies, potencies (EC_{50}) were calculated for each tissue using the relationship of Parker & Waud (1971) by non-linear iterative curve fitting procedures (Leung *et al.*, 1992). The apparent affinity (pK_B) was derived by the method of Furchgott (1972), i.e. K_B = Antagonist concentration/Agonist concentration-ratio − 1. Statistically significant differences were assessed by Student's t test, with $P < 0.05$ being considered significant. Competition binding data were analysed by fitting the data to a four parameter logistic equation, followed by the Cheng-Prusoff correction (Cheng & Prusoff, 1973). Saturation binding data were analysed with the programme LIGAND (Munson & Rodbard, 1980).

Compounds used

[^{3}H]-quipazine (60 Ci mmol^{-1}) and [^{3}H]-ganisetron (84 Ci mmol^{-1}) were purchased from Dupont-NEN Corp. (Boston, MA, U.S.A.). [^{3}H]-RS 42358-197 (55 Ci mmol^{-1}) and [^{3}H]-RS 25259-197 (70 Ci mmol^{-1}) were synthesized in the Radiochemistry Group, Syntex Discovery Research. (**S**) and (**R**)-zacopride (4-amino-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-5-chloro-2-methoxybenzamide HCl), pancopride (*N*-(1-azabicycle-[2.2.2]-octane-3-yl)-2-cyclopropyl-methoxy-4-amino-5-chlorobenzamide), granisetron (BRL 43694, endo-1-methyl-*N*-(9-methyl-9-azabicyclo[3.3.1]-non-3-yl]-1*H*-indazole-3-carboxamide), tropisetron (ICS 205,930, endo-e-methyl-8-azabicyclo[3.2.1]oct-3-yl-1*H*-indole-3-carboxylate), ondansetron (racemic GR 38032, 1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazole-1-yl) methyl]-4*H*-carbazole-4-one HCl.2H$_2$O)) and MDL 72222 (endo-8-methyl-8-azabicyclo [3.2.1.] oct-3-yl 3,5-dichlorobenzoate HCl) were synthesized at the Institute of Organic Chemistry, Syntex Discovery Research, Palo Alto, CA, U.S.A. (1-(*meta*-chlorophenyl)-biguanide hydrochloride was purchased from Cookson Chemicals Ltd. (Southampton, U.K.). Other chemicals and reagents were purchased from Sigma Chemical Company or Research Biochemicals Incorporated (Boston, MA, U.S.A.). NG 108-15 cells were obtained from the Institute of Biochemistry and Cell Biology, Syntex Discovery Research, Palo Alto, CA, U.S.A. The human kidney 293 cell-line, permanently transfected with a homomeric alpha subunit of murine 5-HT$_3$ receptor, was a gift from Dr David Julius, Department of Pharmacology, University of California, San Francisco, U.S.A. Post-mortem human brain hippocampal tissue was obtained from Dr Gavin Reynolds, University of Sheffield, U.K.

Results

Functional studies

RS 25259-197 (**S,S**) constitutes one of four enantiomers, the other three being denoted as RS 25259-198 (**R,R**), RS 25233-197 (**S,R**) and RS 25233-198 (**R,S**) (Figure 1). These four enantiomers were tested for their apparent affinity at 5-HT$_3$ receptors in guinea-pig, isolated ileum. Non-parallel, dextral shifts in the concentration-response curves to 5-HT were seen in the presence of all four enantiomers (Table 1). The rank order of pK_B values was (**S,S**) > (**R,S**) > (**S,R**) = (**R,R**). RS 25259-197, only, unsurmountably antagonized responses to 5-HT (Figures 2a–c).

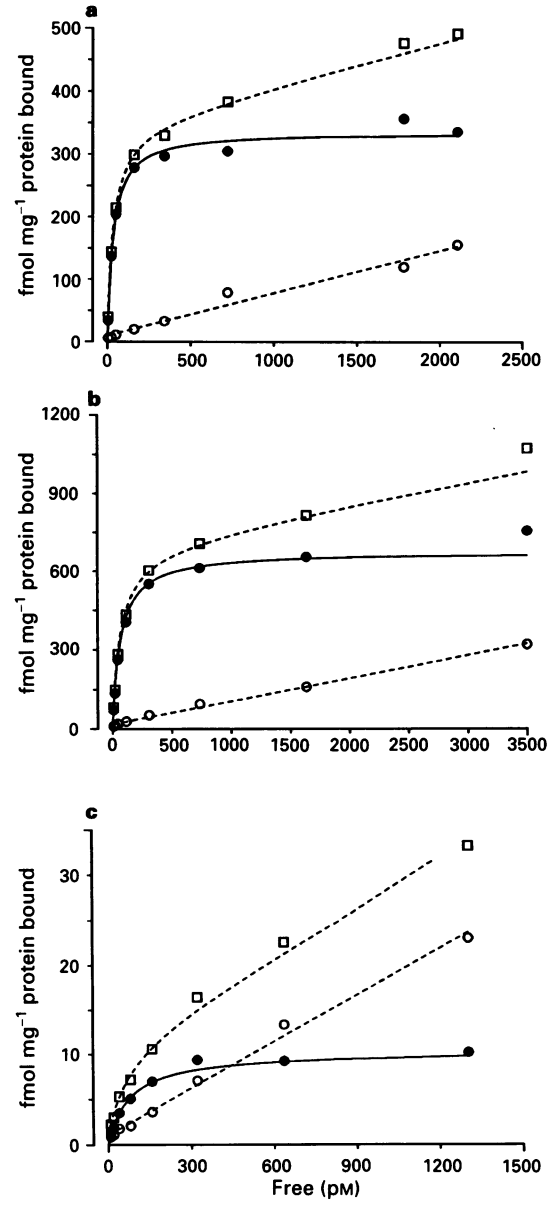

Figure 3 Saturation studies of [^{3}H]-RS 25259-197 (0.004–3.5 nM) binding in membranes from (a) NG-108-15 cells, (b) human kidney 293 cells transfected with the cloned homomeric α-subunit of 5-HT$_3$ receptor, and (c) human hippocampus. Non-specific binding was defined by 0.1 μM (S)-zacopride. (□) Total, (○) non-specific and (●) specific binding are shown.

Table 3 Saturation studies of [^{3}H]-RS 25259-197 and [^{3}H]-RS 42358-197 in membranes from NG-108-15 cells, human kidney 293E1 cells transfected with cloned 5-HT$_3$ receptor homomeric subunit and human hippocampus

Preparation	*[^{3}H]-RS 25259-197*		*[^{3}H]-RS 42358-197*	
	K_d (nM)	B_{max} (fmol mg^{-1})	K_d (nM)	B_{max} (fmol mg^{-1})
NG 108-15 cells	0.05 ± 0.02	610 ± 60	0.20 ± 0.01	660 ± 70
293E1 cells	0.07 ± 0.01	1068 ± 88	0.20 ± 0.03	888 ± 79
Human hippocampus	0.15 ± 0.07	6.9 ± 2.4	–	–

Values are means ± s.e.mean; $n = 3$ animals.

Radioligand binding studies with [^{3}H]-quipazine and [^{3}H]-RS 42358-197

In radioligand binding studies, using [^{3}H]-quipazine to label sites in membranes from NG 108-15 cells, a similar rank order of pK_i values was seen. These estimates of affinities were higher than those derived in the functional studies (Table 1). Due to the high affinity of RS 25259-197 at these 5-HT$_3$ receptors, the interaction of this enantiomer was further evaluated at other 5-HT$_3$ receptors. At 5-HT$_3$ receptors labelled by [^{3}H]-RS 42358-197, in membranes from NG 108-15 cells, rat cortex, rabbit ileum or guinea-pig ileum, RS 25259-107 exhibited apparent affinity values of 10.1 ± 0.1, 10.2 ± 0.1, 10.1 ± 0.1 and 8.3 ± 0.2, respectively. In all these studies, the Hill coefficients were not significantly different from unity (Table 1).

Pharmacological selectivity

The affinity of RS 25259-197 at other neurotransmitter receptors and ion channels was evaluated. RS 25259-197 was highly selective for the 5-HT$_3$ receptor, since low affinities (pK_i < 6.0) were seen at twenty eight neurotransmitter receptor binding assays, including other 5-HT receptors (Table 2). The lack of affinity for several ion channels was suggested by the low affinity (pK_i < 4.0) for the *N*-methyl-D-aspartate (NMDA) receptor-coupled cation channel, the voltage-sensitive Ca^{2+} channel and the Na^+ channel, as labelled by [^{3}H]-MK-801, [^{3}H]-PN200-100 and [^{3}H]-saxitoxin, respectively.

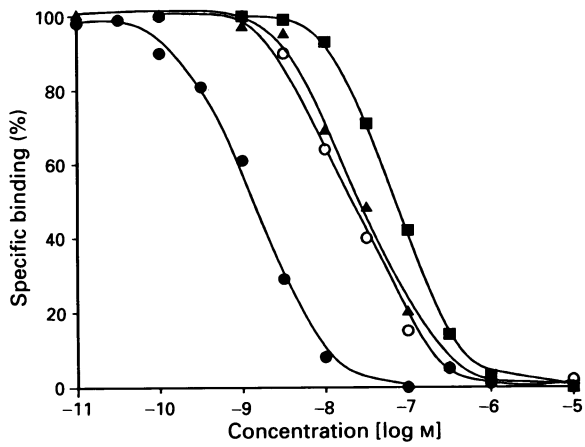

Figure 4 Displacement of [^{3}H]-RS 25259-197 binding in membranes from NG-108-15 cells by 5-HY$_3$ receptor ligands: (●) **S**-zacopride; (○) **R**-zacopride; (▲)-ondansetron; (■)*m*-chlorophenyl-biguanide.

Radioligand binding studies with [^{3}H]-RS 25259-197

The affinity of [^{3}H]-RS 25259-197 was determined in saturation binding studies, using membranes from NG 108-15 cells and 293 E1 cells expressing the alpha subunit of a murine 5-HT$_3$ receptor (Figure 3a and b; Table 3). In human hippocampal membranes, [^{3}H]-RS-25259-197 specifically labelled a site with an affinity (K_d) of 0.15 ± 0.07 nM and a density (B_{max}) of 6.8 ± 2.4 fmol.mg^{-1} protein (Figure 3c).

A stoichiometric comparison of these sites, labelled by [^{3}H]-RS 25259-197, was also made with the dehydro analogue of RS 252529-197, [^{3}H]-RS 42358-197 (Wong *et al.*, 1993a).

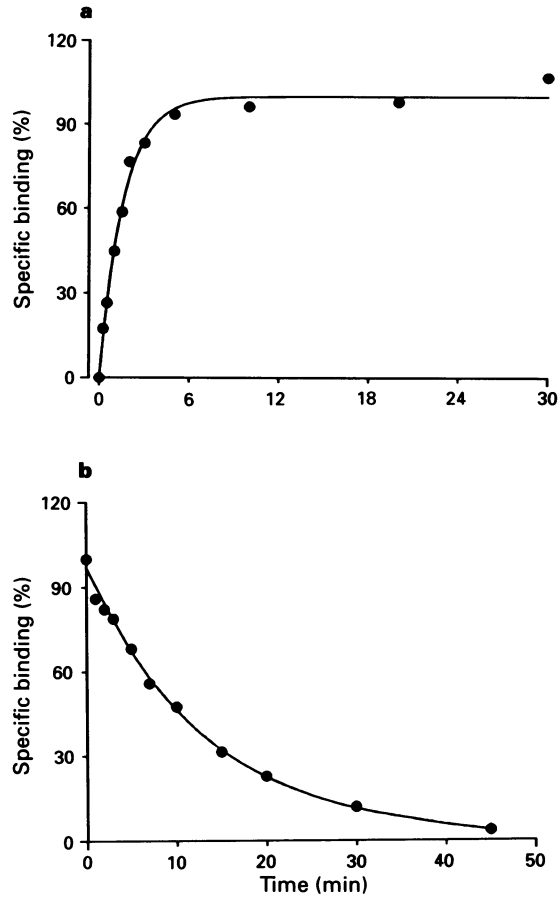

Figure 5 Kinetic analysis of [^{3}H]-RS 25259-197 (0.2 nM) binding in membranes from NG-108-15 cells: (a) association, and (b) dissociation by 1 μM RS 25259-197. Non-specific binding was defined with 0.1 μM (S)-zacopride.

Table 4 Affinities of 5-HT$_3$ compounds for [^{3}H]-RS 25259-197 at binding sites in NG 108-15 cells and human hippocampus

	[^{3}H]-RS 25259-197				*[^{3}H]-quipazine*	
	NG 108-15		*Human hippocampus*		*NG 108-15*	
	pK_i	n_H	pK_i	n_H	pK_i	n_H
RS 25259-197	10.06 ± 0.05	1.03 ± 0.02	10.01	1.69	10.45 ± 0.18	1.03 ± 0.10
(S)-zacopride	9.57 ± 0.09	1.09 ± 0.06	9.81	1.19	9.53 ± 0.08	1.12 ± 0.19
Pancopride	–	–	–	–	9.20 ± 0.30	1.10 ± 0.11
Granisetron	8.91 ± 0.03	0.87 ± 0.03	–	–	–	–
Tropisetron	8.79 ± 0.33	1.11 ± 0.12	–	–	8.68 ± 0.39	0.70 ± 0.20
(R)-zacopride	8.46 ± 0.08	0.93 ± 0.17	8.56	1.14	8.54 ± 0.08	1.26 ± 0.04
Ondansetron	8.19 ± 0.05	1.08 ± 0.10	–	–	8.39 ± 0.10	1.00 ± 0.15
mCPBG	7.75 ± 0.02	1.17 ± 0.04	–	–	–	–
MDL 72222	7.59 ± 0.02	1.12 ± 0.09	–	–	7.66 ± 0.02	1.07 ± 0.04
2-methyl-5-HT	–	–	6.76	1.69	6.30 ± 0.10	0.92 ± 0.09

Values are means ± s.e.mean; n = 3 animals in studies with NG-108-15 cell membranes.
[1]Due to a lack of tissue available, human data are obtained from a single determination.

In NG 108-15 and 293 E1 cell membranes, [^{3}H]-RS 25259-197 exhibited a 3–4 fold higher affinity than [^{3}H]-RS 42358-197, although the same density (B_{max}) of sites was labelled (Table 3).

The pharmacological specificity of [^{3}H]-RS 25259-197 was characterized in both NG 108-15 cells (Figure 4) and human hippocampus. A series of 5-HT$_3$ receptor ligands displaced [^{3}H]-RS 25259-197 binding in these tissues with a similar

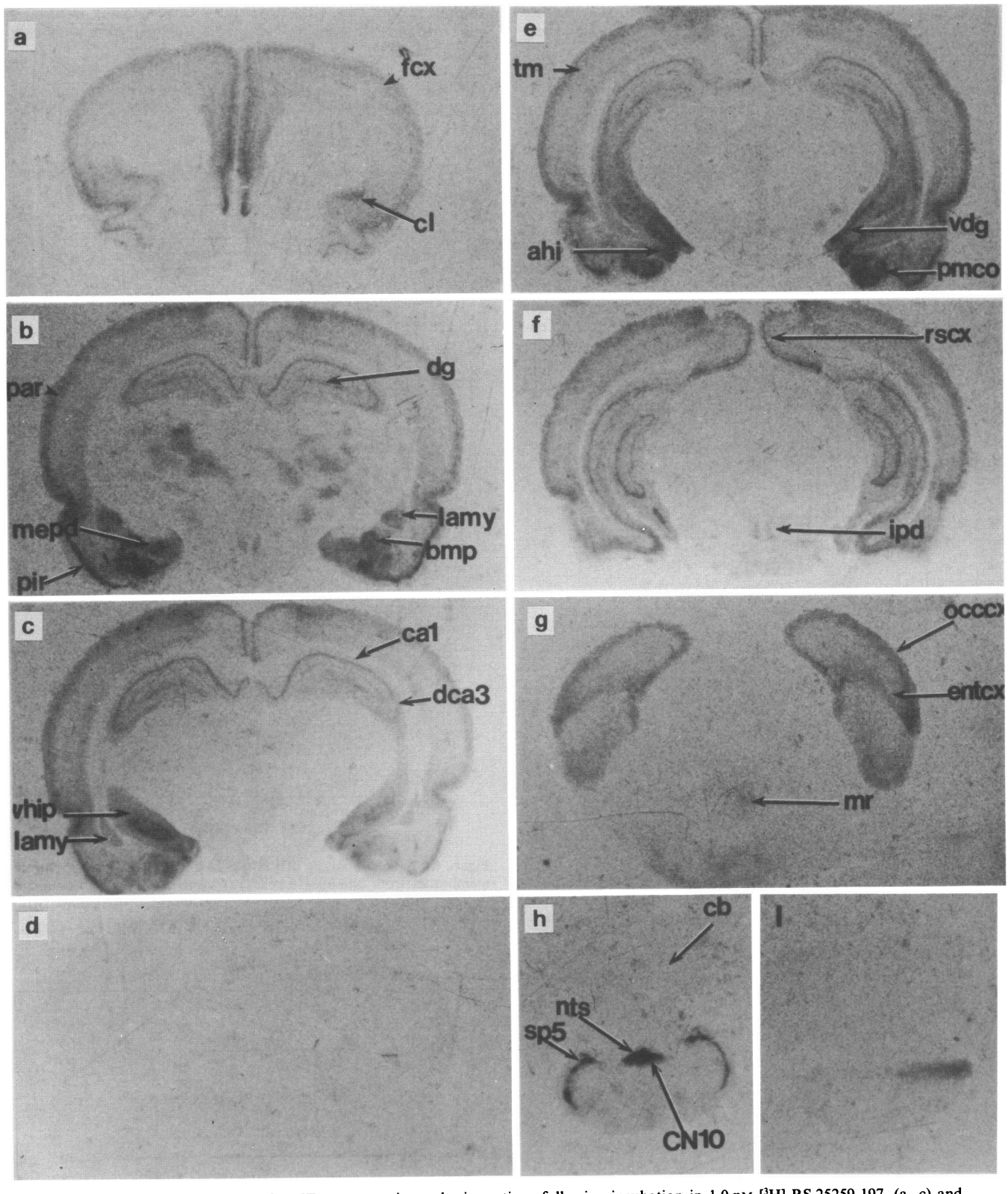

Figure 6 Autoradiography of 5-HT$_3$ receptors in rat brain sections following incubation in 1.0 nM [^{3}H]-RS 25259-197. (a–c) and (e–h) Total binding; (d) (i) non-specific binding of sections adjacent to (c) and (f), respectively, defined in the presence of 1.0 μM (S)-zacopride. Abbreviations correspond to regions in Table 5: claustrum, (cl), frontal cortex (fcx), parietal cortex (par), retrosplenial cortex (rscx), basomedial posterior amygdala (bmp), lateral amygdala (lamy), amygdalo-hippocampal region (ahi), piriform cortex (pir), ventral hippocampus (vhip), medial amygdala (mepd), posterior medial cortical amygdala (pmco), ventral dentate gyrus (vdg), temporal cortex (tm), interpeduncular nucleus (ipd), occipital cortex (occcx), entorhinal cortex (entcx), median raphe nucleus (mr), cerebellum (cb), nucleus tractus solitarius (nts), vagal motor nucleus (CN10), spinal trigeminal tract (sp5).

rank order of affinity (Table 3). A similar rank order of affinity was also observed in a binding assay using [^{3}H]-quipazine to label 5-HT$_3$ receptors in NG 108-15 cell membranes (Table 4).

Kinetics of [^{3}H]-RS 25259-197 binding

[^{3}H]-RS 25259-197 rapidly bound to a high affinity binding site in NG-108-15 cell membranes with an association constant (K_{+1}) of 2.15 nM^{-1} min^{-1} (Figure 5a). Association of the ligand/receptor complex was completed within 10 min and no further change in binding was observed for a further 80 min. Following incubation for 60 min, dissociation was induced by 1.0 μM unlabelled RS 25259-197 (Figure 5b). This followed a monophasic profile, indicating dissociation from a single population of sites, with a half-life ($t_{\frac{1}{2}}$) of 9.6 min (K_{-1} of 0.072 ± 0.004 min^{-1}). This equilibrium constant, calculated from these kinetic parameters was 0.033 nM, closely matching the K_d determined by saturation studies (Table 3). In parallel studies, the association and dissociation constants for [^{3}H]-granisetron were also measured. The corresponding K_{+1} and K_{-1} values were 0.166 nM^{-1} min^{-1} and 0.166 ± 0.009 min^{-1}, respectively, with a calculated K_d of 1.0 nM. This is comparable to the K_d value, obtained from saturation studies, of 1.8 nM (Wong *et al.*, 1993b).

Autoradiographic studies

The distribution of high affinity binding sites for [^{3}H]-RS 25259-197 in sections from rat brain (Figure 6) was characterized by quantitative ligand autoradiography *in vitro*. Parallel studies were carried out with a related 5-HT$_3$ receptor antagonist, [^{3}H]-RS 42358-197 (Wong *et al.*, 1993a). A high percentage of specific binding was measured in most of the regions studied (Table 5). Both of these ligands exhibited similar patterns of distribution, in that the regions with the highest density of binding sites were found in the hind brain vagal complex (nucleus tractus solitarius, are postrema, dorsal motor nucleus of the vagus). A moderate density of binding sites was found in the spinal trigeminal tract, amygdala, hippocampus, and cerebral cortex. Low numbers of sites were detected in median raphe, inferior colliculus and cerebellum (Table 5).

Discussion

Clark *et al.* (1993) have identified several conformationally constrained 5-HT$_3$ receptor antagonists, leading to the identification of a series of isoquinolin-1-ones. Of these, RS 25259-197 (S,S) appeared to exhibit the highest affinity (Clark *et al.*, 1993). The aim of the present study was to characterize the interaction of this enantiomer at several 5-HT$_3$ receptors.

RS 25259-197, forms one of the four enantiomers (Figure 1). It was observed from both functional and radioligand binding studies that RS 25259-197 was a 5-HT$_3$ receptor antagonist of high affinity (Figures 2 and 4, Table 3). Potent and unsurmountable antagonism was observed with RS 25259-

Table 5 Autoradiographic distribution of [^{3}H]-RS 25259-197 and [^{3}H]-RS 42358-197 binding in rats

Region	*[^{3}H]-RS 25259-197* *Specific bound* (fmol mg^{-1}) mean	(s.e.mean)	*% Specific* (specific/total) mean	(s.e.mean)	*[^{3}H]-RS 42358-197* *Specific bound* (fmol mg^{-1}) mean	(s.e.mean)
NTS (nts)	75.1	7.1	95.5	1.0	57.1	2.7
Area postrema	46.0	24.3	85.9	2.8	20.0	6.9
CN10 nucleus (cn10)	33.0	4.4	80.9	2.0	14.7	3.8
Spinal trigeminal n. (sp5)	21.0	3.3	84.2	1.6	8.9	0.9
Biriform cortex (pir)	19.9	1.4	89.0	0.9	ND	
bmp amygdala (bmp)	19.4	9.3	94.7	7.3	ND	
Ventral dentate gyrus (vdg)	18.2	1.6	84.0	6.2	14.9	1.4
Amygdala-hippocampal (ahi)	17.3	1.4	87.5	1.1	12.5	0.9
PMCO amygdala (pmco)	14.9	0.8	88.8	1.4	14.2	1.0
Perirhinal cortex	14.3	2.3	84.6	1.7	8.2	0.6
Ventral hippocampus (vhip)	11.4	0.9	84.4	0.9	11.2	1.3
Pre-subiculum	9.4	1.6	81.7	3.6	3.8	0.7
Temporal cortex (tm)	9.2	0.8	78.3	3.1	5.9	0.5
Para-subiculum	9.0	1.3	78.2	4.2	6.1	1.6
Entorhinal cortex (entcx)	8.2	0.5	81.6	1.6	7.9	1.6
Lateral amyg nucleus (lamy)	7.2	0.8	77.6	2.7	5.7	0.4
Claustrum (cl)	7.1	0.7	70.8	5.1	12.2	4.4
Occipital cortex (occcx)	7.0	0.7	77.2	3.2	4.3	0.5
CA2 hippocampus	6.0	0.5	70.8	2.2	4.8	0.5
Dorsal CA3 hippocampus (dca3)	6.0	0.4	72.1	3.5	5.3	0.6
Retrosplenial cortex (rscx)	5.7	0.3	76.6	1.0	4.0	0.4
CA1 hippocampus (ca1)	5.3	1.0	66.4	4.9	3.8	0.4
Dorsal denate gyrus (dg)	5.0	0.2	72.8	2.6	3.9	0.8
Parietal cortex L.2,3 (par)	4.6	0.4	69.0	4.4	3.6	0.3
Interpedunclar n. (ipd)	4.5	0.2	64.8	5.1	3.5	0.7
Mepd amygdala (mepd)	4.0	2.0	57.8	10.9	ND	
CN12 nucleus	2.4	0.5	37.7	5.8	1.0	0.2
Parietal cx. L.1-6	2.3	0.2	54.3	2.6	2.0	0.3
Central grey	0.8	0.2	26.7	5.8	0.6	0.7
Median raphe (mr)	0.8	0.3	19.6	7.5	1.1	0.6
Inferior colliculus	0.4	0.1	17.0	5.8	0.6	0.2
Cerebellum (cb)	0.3	0.1	12.1	5.2	0.1	0.1

Values are mean ± s.e.mean of measurements from 3–6 brains in two separate experiments. Binding density was measured following incubation in 1.0 nM [^{3}H]-RS 25259-197 or [^{3}H]-RS 42358-197. Non-specific binding was defined in the presence of 1 μM (S)-zacopride. Values are corrected for 100% receptor occupancy (K_d = 0.15 and 0.25 nM, respectively). ND not determined.
RS 25259-197 displaced [^{3}H]-RS 42358-197 binding in all brain areas to non-specific levels. Representative pK_i values were determined by autoradiography in nts (10.4 ± 0.2), bma (10.3 ± 0.2) and vhip (10.3 ± 0.2), n = 3.

197 at 5-HT$_3$ receptors in guinea-pig ileum, in that a clear depression of maximal response was observed by 10 nM of RS 25259-197. Increasing the concentration of 5-HT up to 100 μM did not overcome the blockade (Figure 2). Similar antagonism has been reported with pancopride, which also has a high affinity for 5-HT$_3$ receptors ($pK_i = 9.3$, Fernandez *et al.*, 1990). No evidence was seen for deviations from competitive antagonism in binding studies at any of the tissues, including guinea-pig ileum. The reason for such non-competitive antagonism is, therefore, likely to be related to the formation of a hemi-equilibrium state between the agonist and antagonist at the ileal 5-HT$_3$ receptor (Rang, 1966).

Several lines of pharmacological evidence support the existence of a guinea-pig variant of the 5-HT$_3$ receptor (Butler *et al.*, 1991; Newberry *et al.*, 1991; Kilpatrick & Tyers, 1992; Wong *et al.*, 1993a; Richardson *et al.*, 1985) in that most antagonists exhibited a significantly lower affinity (10 to 1000 fold) for 5-HT$_3$ receptors in this species. In support of these data, the affinities for RS 25259-197 and enantiomers at this 5-HT$_3$ receptor were less than the affinities observed at 5-HT$_3$ receptors in rat cortex, NG-108-15 cells and rabbit ileum (Table 1). RS 25259-197 did not discriminate between 5-HT$_3$ receptors in membrane preparations from the remaining species, including man (Tables 1 and 3). These competition binding studies, regardless of the species, revealed that RS 25259-197 exhibited affinities for 5-HT$_3$ receptors higher than many antagonists thus far reported (Table 4). Moreover, this high affinity was accompanied by a high degree of selectivity in that relatively low affinities at twenty eight other receptors were seen (Table 2). Indeed, up to 1 μM of RS 25259-197 did not exhibit agonist or antagonist action in the guinea-pig ileum contractile assay for 5-HT$_4$ receptor (Leung *et al.*, unpublished data).

The interaction of RS 25259-197 with 5-HT$_3$ receptors was further defined by the use of [^{3}H]-RS 25259-197. In saturation binding studies, the ligand exhibited a high affinity of 5-HT$_3$ receptors in NG-108-15 cells, human kidney 293E1 cells transfected with an α subunit of a murine 5-HT$_3$ receptor (Maricq *et al.*, 1991) and human hippocampus (Table 2). These sites appeared to be pharmacologically similar as judged by the rank orders of competing ligands, although large differences were observed in terms of receptor density. The similarity in B_{max} values between [^{3}H]-RS 42358-197 and [^{3}H]-RS 25259-197 in a given preparation (Table 3) also supports the specificity of [^{3}H]-RS 25259-197 for the 5–HT$_3$ receptor. This differed from data obtained with other radioligands, whereby potential heterogeneity was indicated when [^{3}H]-quipazine, [^{3}H]-GR 65630 and [^{3}H]-granisetron labelled a different number of sites (Wong *et al.*, 1993b). The reasons for this discrepancy remain unclear.

The high density of 5-HT$_3$ receptors in NG 108-15, NCB-20 or N1E-115 neuroblastoma cells has facilitated purification and electrophysiological studies of 5-HT$_3$ receptors (e.g. McKernan *et al.*, 1990; Peters *et al.*, 1991). In contrast, the low density of 5-HT$_3$ receptors in human brain tissue has hindered characterization and, consequently, only a limited number of studies have been reported (Barnes *et al.*, 1989a,b; Waeber *et al.*, 1989; Abi-Dargham *et al.*, 1993). The ability of [^{3}H]-RS 25259-197 to detect low but measurable levels of 5-HT$_3$ receptors, particularly in human tissue, endorses the usefulness of this radioligand in studies of this nature. Indeed, the pharmacological specificity of [^{3}H]-RS 25259-197 obtained from human hippocampus was consistent with previous studies of human brain tissues using other radioligands (Barnes *et al.*, 1989a,b; Waeber *et al.*, 1989; Abi-Dargham *et al.*, 1993). Furthermore, a comparison of this specificity to that observed in NG-108-15 cells argues against a species difference between human and murine 5-HT$_3$ receptors.

Given the fact that RS 25259-197 has the same high affinity for rat brain regions (Table 5) and in NG-108-15 cells, and the similarity in pharmacological specificity of the related radioligand [^{3}H]-RS 42358-197 at the same two preparations, the selectivity of [^{3}H]-RS 25259-197 for the 5-HT$_3$ receptor in the rat brain seems assured. Autoradiographic studies in rat brain with [^{3}H]-RS 25259-197 indicated a regional distribution of sites similar to the distribution reported with [^{3}H]-RS 42358-197 (Table 5), [^{3}H]-GR 65630 (Kilpatrick *et al.*, 1988; 1989), [^{3}H]-quipazine (Perry, 1990), [^{3}H]-tropisetron (Waeber *et al.*, 1989) or [^{3}H]-(S)-zacopride (Waeber *et al.*, 1990). The highest density of receptors was found in the hind-brain vagal complex, while medium levels were found in the spinal trigeminal tract, amygdala, hippocampus and cerebral cortex (Table 5). The higher density of binding sites in emetic centres including nucleus tractus solitarius, dorsal nucleus of the vagus or area postrema, are consistent with a central anti-emetic action of 5-HT$_3$ receptor antagonists. Distribution studies in human brain with [^{3}H]-tropisetron support this observation (Waeber *et al.*, 1989).

In conclusion, RS 25259-197 is a novel, high affinity, selective 5-HT$_3$ receptor antagonist. These properties are consistent with the potent anti-emetic action *in vivo* (Eglen *et al.*, 1995). Finally, the ability of [^{3}H]-RS 25259-197 to label selectively 5-HT$_3$ receptors in human brain or guinea-pig ileum highlight the advantage of the ligand for characterizing receptors in preparations expressing either a low density of sites or low affinities toward 5-HT$_3$ receptor ligands.

References

ABI-DARGHAM, A., LARUELLE, M., WONG, D.T., ROBERTSON, D.W., WEINBERGER, D.R. & KLEINMAN, J.E. (1993). Pharmacological and regional characterization of [^{3}H]LY278584 binding sites in human brain. *J. Neurochem.*, **60**, 730–737.

ANDREWS, P.L. & BHANDARI, P. (1993). The 5-hydroxytryptamine receptor antagonists as antiemetics: preclinical evaluation and mechanism of action. *Eur. J. Cancer*, **29A** (Suppl.), S11–S16.

BARNES, J.M., BARNES, N.M., COSTALL, B., IRONSIDE, J.W. & NAYLOR, R.J. (1989a). Identification and characterization of 5-hydroxytryptamine$_3$ recognition sites in human brain tissue. *J. Neurochem.*, **53**, 1787–1793.

BARNES, J.M., BARNES, N.M., COSTALL, B., NAYLOR, R.J. & TYERS, M.B. (1989b). 5-HT$_3$ receptors mediate inhibition of acetylcholine release in cortical tissue. *Nature*, **338**, 762–764.

BOESS, F.G. & MARTIN, I.L. (1994). Molecular biology of 5-HT receptors. *Neuropharmacol.*, **33**, 275–317.

BONHAUS, D.W., WONG, E.H.F., STEFANICH, E., KUNYSZ, E.A. & EGLEN, R.M. (1993). Pharmacological characterization of 5-hydroxytryptamine$_3$ receptor in murine brain and ileum using the novel radiologand [^{3}H]-RS-42358-197: evidence for receptor heterogeneity. *J. Neurochem.*, **61**, 1927–1932.

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254.

BUTLER, A., ELSWOOD, C.J., BURRIDGE, J., IRELAND, S.J., BUNCE, K.T., KILPATRICK, G.J. & TYERS, M.B. (1990). The pharmacological characterization of 5-HT$_3$ receptors in three isolated preparations derived from guinea-pig tissues. *Br. J. Pharmacol.*, **101**, 591–598.

CHENG, Y.C. & PRUSOFF, W.H. (1973). Relationship between inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.*, **92**, 881–894.

CLARK, R.D., MILLER, A.B., BERGER, J., REPKE, D.B., WEINHARDT, K.K., KOWALCZYK, B.A., EGLEN, R.M., BONHAUS, D.W., LEE, C.-H., MICHEL, A.D., SMITH, W.L. & WONG, E.H.F. (1993). 2-(quinuclidin-3-yl)pyrido[4,3-b]indol-1-ones and isoquinolin-1-ones. Potent conformationally restricted 5-HT$_3$ receptor antagonists. *J. Med. Chem.*, **36**, 2645–2657.

COSTALL, B. & NAYLOR, R.J. (1991). Pharmacological properties and functions of central 5-HT$_3$ receptors. *Therapie (Paris).*, **46**, 437–444.

COSTALL, B., NAYLOR, R.J. & TYERS, M.B. (1990). The psychopharmacology of 5-HT$_3$ receptors. *Pharmacol. Ther.*, **47**, 181–202.

CRAIG, D.A., EGLEN, R.M., WALSH, L.K., PERKINS, L.A., WHITING, R.L. & CLARKE, D.E. (1990). 5-Methoxytryptamine and 2-methyl-5-hydroxytryptamine-induced desensitization as a discriminative tool for the 5-HT$_3$ and putative 5-HT$_4$ receptors in guinea-pig ileum. *Naunyn-Schmied. Arch. Pharmacol.*, **342**, 9–16.

DERKACH, V., SUPRENANT, A. & NORTH, R.A. (1989). 5-HT$_3$ receptors are membrane ion channels. *Nature*, **339**, 706–709.

EGLEN, R.M., LEE, C., SMITH, W.L., JOHNSON, L.G., WHITING, R.L. & HEGDE, S.S. (1993). RS-42358-197, a novel and potent 5-HT$_3$ receptor antagonist, *in vitro* and *in vivo*. *J. Pharmacol. Exp. Ther.*, **266**, 535–543.

EGLEN, R.M., LEE, C.H., SMITH, W.L., JOHNSON, L.G., WHITING, R.L. & HEGDE, S.S. (1995). Pharmacological characterization of RS-25259-197, a novel and selective 5-HT$_3$ receptor antagonist, *in vivo*. *Br. J. Pharmacol.*, **114**, 860–866.

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.

FERNANDEZ, A.G., PUIG, J., BOU, J., FRISTWOOD, R.W. & BERGA, P. (1990). Pancopride: a novel 5-HT$_3$ antagonist with potent antiemetic action. *Eur. J. Pharmacol.*, **183**, P.we.046.

FOZARD, J.R. (1984). Neuronal 5-HT$_3$ receptors in the periphery. *Neuropharmacol.*, **23**, 1473–1486.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory In *Handbook of Experimental Pharmacology*, Vol. 33, pp. 283–335. Berlin: Springer Verlag.

GREENSHAW, A.J. (1993). Behavioral pharmacology of 5-HT$_3$ receptor antagonists: a critical update on therapeutic potential. *Trends Pharmacol. Sci.*, **14**, 265–270.

HOYER, D., CLARKE, D.E., FOZARD, J.R., HARTIG, P.R., MARTIN, G.R., MYLECHARANE, E.J., SAXENA, P.R. & HUMPHERY, P.P.A. (1994). VII. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.*, **46**, 157–203.

HOYER, D. & NEIJT, H.C. (1988). Identification of serotonin 5-HT$_3$ recognition sites in membranes of N1E-115 neuroblastoma cells by radioligand binding. *Mol. Pharmacol.*, **33**, 303–309.

JOHNSON, R.M., INOUYE, G.T., EGLEN, R.M. & WONG, E.H.F. (1993). 5-HT$_3$ receptor ligands lack modulatory influence on acetylcholine release in rat entorhinal cortex. *Naunyn-Schmied. Arch. Pharmacol.*, **347**, 241–247.

KILPATRICK, G.J., JONES, B.J. & TYERS, M.B. (1988). The distribution of specific binding of the 5-HT$_3$ receptor ligand [^{3}H] GR65630 in rat brain using quantitative autoradiography. *Neurosci. Lett.*, **94**, 156–160.

KILPATRICK, G.J., JONES, B.J. & TYERS, M.B. (1989). Binding of the 5-HT$_3$ ligand, [^{3}H] GR65630, rat area postrema, vagus nerve and the brains of several species. *Eur. J. Pharmacol.*, **159**, 157–164.

KILPATRICK, G.J. & TYERS, M.B. (1992). Inter-species variants of the 5-HT$_3$ receptor. *Biochem. Soc. Trans.*, **20**, 118–121.

LAPORTE, A.M., KOSCIELNIAK, T., PONCHANT, M., VERGE, D., HAMON, M. & GOZLAN, H. (1992). Quantitative autoradiography mapping of 5-HT$_3$ receptors in the rat CNS using [^{125}I]iodozacopride and [^{3}H]zacopride as radioligands. *Synapse*, **10**, 271–281.

LEUNG, E., MICHELSON, S., VILLARUBIA, C., PERKINS, L.A. & EGLEN, R.M. (1992). Analysis of concentration-response relationships by seemingly unrelated nonlinear regression (SUNR) technique. *J. Pharmacol. Toxicol. Methods*, **28**, 209–216.

MARICQ, A.V., PETERSON, A.S., BRAKE, A.J., MYERS, R.M. & JULIUS, D. (1991). Primary structure and functional expression of the 5-HT$_3$ receptor, a serotonin-gated ion channel. *Science*, **254**, 432–436.

MARTIN, G.R. & HUMPHREY, P.P.A. (1994). Receptors for 5-hydroxytryptamine: current perspective on classification and nomenclature. *Neuropharmacol.*, **33**, 261–273.

McKERNAN, R.M., GILLARD, N.P., QUIRK, K., KNEEN, C.O., SYEVENSON, G.I., SQAIN, C.J. & RAGAN, C.I. (1990). Purification of the 5-hydroxtryptamine 5-HT$_3$ receptors from NCB-20 cells. *J. Biol. Chem.*, **265**, 13572–13577.

MILBURN, D.M. & PEROUTKA, S.J. (1989). Characterization of [^{3}H]quipazine binding to 5-hydroxytryptamine$_3$ receptors in rat brain membranes. *J. Neurochem.*, **52**, 1787–1792.

MUNSON, P.J. & RODBARD, D. (1980). LIGAND: a versatile computerized approach for characterization of ligand systems. *Anal. Biochem.*, **107**, 220–239.

NELSON, D.R. & THOMAS, D.R. (1989). [^{3}H]-BRL43694 (Granisetron), a specific ligand for 5-HT$_3$ binding sites in rat brain cortical membranes. *Biochem. Pharmacol.*, **38**, 1693.

NEWBERRY, N.R., CHESHIRE, S.H. & GILBERT, M.J. (1991). Evidence that 5-HT$_3$ receptors of the rat, mouse and guinea-pig superior cervical ganglion may be different. *Br. J. Pharmacol.*, **102**, 615–620.

OXFORD, A.W., BELL, J.A., KILPATRICK, G.J., IRELAND, S.J. & TYERS, M.B. (1992). Ondansetron and related 5-HT$_3$ antagonists: recent advances. *Prog. Med. Chem.*, **29**, 239–270.

PARKER, P.B. & WAUD, D.R. (1971). Pharmacological estimates of drug-receptor dissociation constants. Statistical evaluation. I. Agonist. *J. Pharmacol. Exp. Ther.*, **177**, 1–12.

PAXINOS, G. & WATSON, C. (1985). *The Rat Brain in Stereotaxic Coordinates*, 2nd edition. New York: Academic Press.

PERRY, D.C. (1990). Autoradiography of [^{3}H]quipazine in rodent brain. *Eur. J. Pharmacol.*, **187**, 75–85.

PETERS, J.A., MALONE, H.M. & LAMBERT, J.J. (1991). Characterization of 5-HT$_3$ receptor mediated electrical response in nodose ganglion neurones and clonal neuroblastoma cells maintained in culture. In *Serotonin: Molecular Biology, Receptors and Functional Effects* ed. Fozard, J.R. & Saxena, P.R. pp. 84–94. Basel: Birkhauser.

PINKUS, L., SARBIN, N.S., BAREFOOT, D.S. & GORDON, J.C. (1989). Association of [^{3}H]zacopride with 5-HT$_3$ binding sites. *Eur. J. Pharmacol.*, **168**, 355–362.

RANG, H.P. (1966). The kinetics of action of acetylcholine antagonists in smooth muscle. *Proc. R. Soc. B.*, **164**, 488–510.

RICHARDSON, B.P., ENGEL, G., DONATSCH, P. & STADLER, P.A. (1985). Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. *Nature*, **316**, 126–131.

SHARIF, N.A., WONG, E.H.F., LOURY, D.N., STEFANICH, E., MICHEL, A.D., EGLEN, R.M. & WHITING, R.L. (1991). Characterization of 5-HT$_3$ binding sites in NG108-15, NCB-20 neuroblastoma cells and rat cerebral cortex using [^{3}H]-quipazine and [^{3}H]-GR 65630 binding. *Br. J. Pharmacol.*, **102**, 919–925.

TRICKLEBANK, M.D. (1989). Interactions between dopamine and 5-HT$_3$ receptors suggest new treatments for psychosis and drug addiction. *Trends Pharmacol. Sci.*, **10**, 127–129.

WAEBER, C., HOYER, D. & PALACIOS, J.M. (1989). 5-Hydroxytryptamine$_3$ receptors in the human brain: autoradiographic visualization using [^{3}H]ICS 205,930. *Neurosci.*, **31**, 393–400.

WAEBER, C., DIXON, K., HOYER, D. & PALACIOS, J.M. (1990). The (S)-isomer of [^{3}H]zacopride labels 5-HT$_3$ receptors with high affinity in rat brain. *Eur. J. Pharmacol.*, **181**, 283–297.

WATLING, K.J., ASPLEY, S., SWAIN, C.J. & SAUNDERS, J. (1988). [^{3}H] Quaternised ICS 205-930 labels 5-HT$_3$ receptor binding sites in rat brain. *Eur. J. Pharmacol.*, **149**, 397–398.

WONG, D.T., ROBERTSON, D.W. & REID, L.R. (1989). Specific [^{3}H]LY 278584 binding to 5-HT$_3$ recognition sites in rat cerebral cortex. *Eur. J. Pharmacol.*, **166**, 107–110.

WONG, E.H.F., BONHAUS, D.W., STEFANICH, E. & EGLEN, R.M. (1993a). Labelling of 5-hydroxytryptamine$_3$ receptors with a novel 5-HT$_3$ receptor ligand, [^{3}H]RS-42358-197. *J. Neurochem.*, **60**, 921–930.

WONG, E.H.F., BONHAUS, D.W., LEE, J.A., WU, I., LOURY, D.N. & EGLEN, R.M. (1993b). Different densities of 5-HT$_3$ receptors are labelled by [^{3}H]quipazine, [^{3}H]GR 65630 and [^{3}H]granisetron. *Neuropharmacol.*, **32**, 869–875.

YAKEL, J.L. (1992). 5-HT$_3$ receptors as cation channels. In *Central and Peripheral 5-HT$_3$ Receptors*, ed. Hamon, M. pp. 103–128, London, England: Academic Press.

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