(R) and (S) RS 56532: Mixed 5-HT₃ and 5-HT₄ Receptor Ligands with Opposing Enantiomeric Selectivity

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(Accepted 8 December 1993)

Summary—The pharmacological properties of the (R) and (S) enantiomers of RS 56532 have been studied in vitro and in vivo. In radioligand binding studies at 5-HT₄ receptors in guinea-pig striatum, (S) RS 56532 exhibited a higher affinity than (R) RS 56532 (−log 𝐾ᵢ = 7.6 and 6.5, respectively). (S) RS 56532 acted as a potent agonist at 5-HT₄ receptors mediating relaxation of rat oesophageal muscularis mucosae (−log EC₅₀ = 7.9) while (R) RS 56532 acted as a weaker agonist at this receptor (−log EC₅₀ < 6.0). These data suggest that at 5-HT₄ receptors, the enantiomeric selectivity of RS 56532 was (S) > (R).

In binding studies at 5-HT₃ receptors in rat cortex, (R) RS 56532, conversely, exhibited a higher affinity than (R) RS 56532 (−log 𝐾ᵢ = 9.1 and 8.0, respectively). At 5-HT₃ receptors in guinea-pig isolated ileum, (R) RS 56532 exhibited an affinity (−log 𝐾ᵢ) of 7.9, whereas (S) RS 56532 (1 nM–1 μM) was inactive. No agonism was observed at ileal 5-HT₃ receptors with either enantiomers. These data suggest that at 5-HT₃ receptors, both enantiomers acted as antagonists, with (R) > (S) RS 56532.

At the non-5-HT₃, high affinity '(R) zacopride' site, (R) RS 56532 exhibited a higher affinity than (S) RS 56532 (−log 𝐾ᵢ = 6.1 and 4.9). This site was insensitive to potent 5-HT₃ antagonists such as (R) YM 060 or ondansetron. However, it was recognized with relatively high affinity (−log 𝐾ᵢ = 7.5) by the (R), but not (S) enantiomer, of RS 42358 (−log 𝐾ᵢ = 4.7). Since (S) RS 42358 is a high affinity 5-HT₃ receptor antagonist, these data further highlight the dissimilarity between the 5-HT₃ receptor and the '(R) zacopride' site. The '(R) zacopride' site also appeared to be pharmacologically distinct from the 5-HT₄ receptor, since 5-HT₄ ligands such as renzapride, SDZ 205,557 or RS 23597-190 exhibited low affinities.

The enantiomeric selectivity of (R) and (S) RS 56532 in vivo was consistent with findings in vitro. At 5-HT₄ receptors mediating tachycardia in the pig, 5-HT induced a dose-dependent tachycardia (ED₅₀ = 3 μg kg⁻¹, i.v.; maximum response = 90–100 beats min⁻¹). (S) RS 56532 increased heart rate by 88 beats min⁻¹ with a potency of (ED₅₀) of 3 μg kg⁻¹, i.v. In contrast, a tachycardia effect (23 beats min⁻¹) of (R) RS 56532 was seen only at 1 mg kg⁻¹, i.v.

(R) RS 56532 was more potent than (S) RS 56532 (1 mg kg⁻¹, i.v.) at inhibiting the von Bezold Jarisch reflex, a response mediated by 5-HT₃ receptor activation. Similarly, (R) RS 56532, at 0.1 mg kg⁻¹ i.v., inhibited cisplatin induced emesis in the ferret, from 19.8 to 5.8 emetic episodes. In contrast, (S) RS 56532 was inactive at this oral dose. The emetic response to neoplastic agents such as cisplatin is also mediated by 5-HT₃ activation.

In summary, RS 56532 in vitro and in vivo, exhibits opposing enantiomeric selectivity at 5-HT₃ and 5-HT₄ receptors, i.e. 5-HT₃→(R) > (S); 5-HT₄→(S) > (R). The affinity of the (R) enantiomer at 5-HT₃ receptors and the potency of the (S) enantiomer at 5-HT₄ receptors render them useful pharmacological tools to further define the binding domains of these two 5-HT receptor subtypes. Furthermore, these data show that 1,8-naphthalimides, such as (S) RS 56532, represent a novel class of potent 5-HT₄ receptor agonists.

Keywords—RS 56532 enantiomers, 5-HT₃ receptor, 5-HT₄ receptor, '(R) zacopride' binding site, oesophagus (rat), guinea-pig ileum, von Bezold Jarisch reflex, porcine tachycardia, emesis (ferret), nausea (ferret).

There is presently interest in the development of compounds selective for both 5-hydroxytryptamine (5-HT₃) and 5-HT₄ receptors (Kilpatrick and Tyers, 1992; Eglen, 1993). 5-HT₃ receptor antagonists are effective in the treatment of cancer chemotherapy induced nausea and emesis (Andrews et al., 1992; Kilpatrick and Tyers, 1992; Cohen, 1992). Animal data also suggests a role in the treatment of anxiety and cognitive disorders...
(see Costall and Naylor, 1991a, b for reviews). 5-HT4 receptor agonists and antagonists may be useful for treatment of gastroesophageal reflux disease (Gullikson et al., 1992) and supra-ventricular arrhythmias, respectively (Saxena and Villalon, 1990; Bockaert et al., 1992).

It is also possible that selective 5-HT4 antagonists may be useful in the treatment of irritable bowel syndrome (Bockaert et al., 1992; Ford and Clarke, 1993; Eglen, 1993). The 5-HT4 receptor is present in foetal mouse colliculi (Dumuis et al., 1988), guinea-pig hippocampus (Shenk et al., 1987; Dumuis et al., 1989; Andrade and Chaput, 1991) and human temporal cortex (Monferini et al., 1993). Autoradiographic studies in guinea-pig using the selective ligand [3H] GR 113808 have demonstrated a high density of sites in olfactory tubercle, globus pallidus, striatum, nucleus accumbens and hippocampus (Grossman et al., 1993). In human brain, a high density of sites was observed in the striato-nigral system, with a low abundance in hippocampus, neocortex and colliculus (Waebber et al., 1993). The utility of 5-HT4 receptor ligands as potential psychotherapeutics, however, remains to be established (Ford and Clarke, 1993).

Until recently, a problem of pharmacologically defining the role of the 5-HT4 receptor has been the lack of selective ligands (see Ford and Clarke, 1993, for review). 5-HT4 receptor agonists such as the Sandox compound 9b (a quinoline derivative), BIMU-8, renzapride and metoclopramide also antagonist 5-HT receptors (Bockaert et al., 1992; Ford and Clarke, 1993).

SC-53116, in contrast, is a potent 5-HT4 agonist, with lower affinity at the 5-HT3 receptor (Flynn et al., 1992). Antagonists at the 5-HT4 receptor such as tropisetron, DAU 6285 and SDZ 205,557 have equal or higher affinities toward the 5-HT3 receptor (Dumuis et al., 1992; Eglen et al., 1993a). SC-53606 (Yang et al., 1993) is 20-fold selective, in terms of affinity, for the 5-HT4 over the 5-HT3 receptor. Antagonists with higher affinities for the 5-HT3 receptor such as RS-23597-190 (Eglen et al., 1993b), GR 113808 (Grossman et al., 1993), or SB 204070 (Wardle et al., 1993) are more selective than SC-53606 due to their low affinities at the 5-HT3 receptor. Zacopride is a mixed 5-HT4 receptor agonist/5-HT3 receptor antagonist (Smith et al., 1988; Pinkus and Gordon, 1991; Gullikson et al., 1992). The (S) isomer has a higher potency at the 5-HT3 receptor and affinity at the 5-HT3 receptor in comparison to the (R) isomer (Eglen et al., 1990; Sharif et al., 1990; Pinkus and Gordon, 1991; Baxter et al., 1991; Gullikson et al., 1992) although Coleman et al. (1991) have reported a lack of enantiomeric stereospecificity. In vivo, (S) zacopride is also more potent than the (R) isomer as an antiemetic and gastroprotective agent; effects which have been ascribed to 5-HT3 receptor antagonism and 5-HT4 agonism, respectively (Sancilio et al., 1989; Gullikson et al., 1992).

Thus, at both the 5-HT3 and 5-HT4 receptor, the enantiomeric specificity of zacopride is (S) > (R).

In animals, the behavioral effects of these enantiomers appear to be more complex. The (R) isomer is more potent as an anxiolytic in comparison to the (S) isomer (Barnes et al., 1992a; Young and Johnson, 1991). Conversely, the (S) enantiomer is more potent as an antagonist of dopamine or amphetamine induced hyperactivity (Barnes et al., 1992b). The (R) enantiomer presynaptically inhibits 5-HT release in rat cortex (Barnes et al., 1992b), although no effect was observed with the (S) isomer. Such enantiomeric selectivity is difficult to reconcile with an interaction at the 5-HT3 receptor, and it may correlate better with an interaction at an (R) zacopride site present in human brain, rat entorhinal cortex and hippocampus, NG 108-15 cells and a number of peripheral tissues including rat ileum, spleen and kidney (Kidd et al., 1992, 1993).

In the present study, the pharmacology of the N- (quinuclidin-3-yl)-1,8-naphthalimid, RS 56532 [6-amino-5-2(1-azabicyclo[2.2.2]octan-3-yl)-2,3-dihydro-1H-benz[d]isoquinone; Fig. 1], has characterized. This is a hybrid structure of zacopride and the 5-HT4 receptor antagonist RS 42358 (Wong et al., 1993; Eglen et al., 1993c). All these compounds are chiral, consisting of both (S) and (R) enantiomers (Fig. 1). Zacopride possesses a virtual ring which is formed by strong intramolecular bonding between the amide NH group and the ortho methoxy group. Naphthalimides, such RS 42348 and RS 56532 (Fig. 1), are conformationally restricted analogs of zacopride, in which the virtual ring is converted into an actual ring, thus defining spatial relationships between the aromatic rings, the carbonyl groups and the basic nitrogen containing side chains (Langois et al., 1992; Clark et al., 1993a, b).

The data obtained in the present study show that the enantiomeric selectivity of RS 56532 at the 5-HT3 receptor is the converse to that of zacopride and RS 42358 i.e. (R) > (S). However, at the 5-HT4 receptor the stereospecificity is similar to zacopride i.e. (S) > (R). At the ‘(R) zacopride’ site, the enantiomeric specificity was (R) > (S).

(A preliminary account of these data has been communicated previously to the British Pharmacological Society; Leung et al., 1993.)

METHODS

In vitro studies

Competition radioligand binding studies. The affinity of (S) and (R) RS 56532 at 5-HT4 receptors and the ‘(R) zacopride site’ was assessed using the radioligands [3H] GR 113808 and [3H] (R) zacopride, respectively. The assays were performed as follows: [3H] GR 113808 binding to 5-HT4 receptors was measured using a synaptosomal membrane preparation of guinea-pig striatum, obtained from animals previously killed by CO2 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.

buffer (pH 7.4 at 4°C), containing 250 mM sucrose.
Pharmacology of (R) and (S) RS 56532 at 5-HT\textsubscript{3} and 5-HT\textsubscript{4} receptors

The homogenate was filtered through a nylon mesh (160 µm pore) and then centrifuged at 1000 \textit{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 and 4°C).

Binding assays were conducted in a Tris buffer (25 mM) with approx. 0.1 mg striatal protein in an assay vol of 0.5 ml at room temperature. Non specific binding was determined using 1 nM unlabeled GR 113808. Preliminary studies demonstrated that a 60 min incubation was sufficient for membrane binding to reach a steady state. Competition binding studies were conducted using 0.1 nM [\textsuperscript{3}H] GR 113808 and 10 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% polyethylenimine. Filters were then dried and the bound radioactivity determined.

\[^{3}H\] (R) zacopride binding was measured using membranes prepared from NG 108-15 neuroblastoma-glioma cells. These cells were grown in Dulbecco's modified Eagles's serum, supplemented with 10% calf serum and hypoxanthine–aminopterin–thymidine. Cells were cultured at 37°C in 10% CO\textsubscript{2} in closed tissue culture flasks (Falcon, 225 cm\textsuperscript{2}) and subcultured every 4–5 days. The cells were harvested when confluent by vigorous shaking. Cells were pelleted by centrifugation at 600 \textit{g} for 6 min. The sedimented material was homogenized in 10 vol of Tris (50 mM) Na\textsubscript{2}EDTA (5 mM) buffer (pH 7.4) and centrifuged at 19,500 rpm for 15 min at 4°C. The pellet was resuspended in Tris–EDTA buffer at a protein concentration of 11 mg ml\textsuperscript{-1}. Membrane suspensions were stored at -80°C.

NG 108-15 cell membranes were incubated in a Tris (50 mM), EDTA (0.5 mM) buffer, containing 1 µM ondansetron. The ondansetron was added to prevent radiolabelled binding to 5-HT\textsubscript{3} receptors. Non-specific binding was defined using 100 µM mianserin. Competition binding studies were conducted using 0.1 nM [\textsuperscript{3}H] (R) zacopride and 10 concentrations of competing ligand. Assay tubes were incubated for 60 min at 30°C and then rapidly filtered, using a Brandel cell harvester, through GF/B filters, pretreated as described above. Filters were washed with ice cold assay buffer and dried prior to determination of bound radioactivity. The activities of (S) and (R) RS 56532 across a range of neurotransmitter receptors were also studied (Table 1). Details of the individual assays can be found in Wong \textit{et al.} (1993) and references cited therein.
Table 1. Affinities of (S) and (R) RS 56532 at various receptors in radioligand binding assays

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Radioligand</th>
<th>Compound defining NSB</th>
<th>Tissue</th>
<th>(S)</th>
<th>(R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D&lt;sub&gt;1&lt;/sub&gt;</td>
<td>[3H]SCH23390</td>
<td>butadormol</td>
<td>striatum</td>
<td>4.7</td>
<td>4.9</td>
</tr>
<tr>
<td>D&lt;sub&gt;2&lt;/sub&gt;</td>
<td>[3H]Butadormol</td>
<td>butadormol</td>
<td>striatum</td>
<td>5.2</td>
<td>5.5</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>[3H]5-HT</td>
<td>5-HT OH DPAT 5-HT</td>
<td>brain</td>
<td>4.7</td>
<td>4.6</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;2A&lt;/sub&gt;</td>
<td>[3H]ketanserin</td>
<td>methysergide</td>
<td>cortex</td>
<td>4.9</td>
<td>5.0</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;2C&lt;/sub&gt;</td>
<td>[3H]mesulergine</td>
<td>methysergide</td>
<td>CP</td>
<td>4.5</td>
<td>4.7</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;3&lt;/sub&gt;</td>
<td>[3H]quipazine</td>
<td>zacopride</td>
<td>HG108-15</td>
<td>8.0</td>
<td>9.1</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;4&lt;/sub&gt;</td>
<td>[3H]GRI 113808</td>
<td>GRI 113808</td>
<td>striatum</td>
<td>7.6</td>
<td>6.5</td>
</tr>
<tr>
<td>M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>[3H]pirenzepine</td>
<td>atropine</td>
<td>heart</td>
<td>7.0</td>
<td>6.7</td>
</tr>
<tr>
<td>M&lt;sub&gt;3&lt;/sub&gt;</td>
<td>[3H]NMS</td>
<td>atropine</td>
<td>submax.</td>
<td>6.6</td>
<td>6.5</td>
</tr>
<tr>
<td>Alpha&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>[3H]prazosin</td>
<td>phenolamine</td>
<td>submax.</td>
<td>5.9</td>
<td>6.3</td>
</tr>
<tr>
<td>Alpha&lt;sub&gt;1B&lt;/sub&gt;</td>
<td>[3H]prazosin</td>
<td>phenolamine</td>
<td>liver</td>
<td>4.8</td>
<td>4.9</td>
</tr>
<tr>
<td>Alpha&lt;sub&gt;2A&lt;/sub&gt;</td>
<td>[3H]rauwolscine</td>
<td>phenolamine</td>
<td>spleen</td>
<td>5.8</td>
<td>5.4</td>
</tr>
<tr>
<td>Alpha&lt;sub&gt;2B&lt;/sub&gt;</td>
<td>[3H]rauwolscine</td>
<td>phenolamine</td>
<td>kidney</td>
<td>5.1</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Values are means, SE mean less than 1%, n = 3-4. In all experiments the Hill coefficients were not significantly different to unity. All tissues were from rat except striatum which was from guinea-pig and spleen, which was from rabbit, CP, choroid plexus; submax, submaxillary gland.

Rat isolated oesophageal muscularis mucosa. 5-HT<sub>4</sub> agonist activity was studied using the rat isolated oesophagus (Baxter et al., 1991), in which relaxation is mediated by 5-HT<sub>4</sub> receptor-mediated enhancement adenylyl cyclase activity (Ford et al., 1992). The thoracic oesophagus was isolated from male Sprague–Dawley rats (Charles River, Wilmington, MA, 200–250 g) and placed in Tyrode solution (composition, mM: NaCl 139.0, KCl 2.7, MgCl<sub>2</sub>·6H<sub>2</sub>O 1.1, Na<sub>2</sub>HPO<sub>4</sub> 0.4, glucose 5.6, NaHCO<sub>3</sub> 11.8 and CaCl<sub>2</sub>·6H<sub>2</sub>O 1.8). The outer striated muscle coat was cut longitudinally and gently peeled away, leaving the inner muscularis mucosae. Silk threads were tied through the lumen on both ends of the tissue. Tissues were then exposed vertically in 10 ml organ baths containing Tyrode solution, maintained at 37°C and gassed with 95% oxygen, 5% carbon dioxide. 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 KC<sub>1</sub> for 5 min<sup>−1</sup>, 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 to 5-HT (0.1 nM–3 μM) 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 either (S) or (R) RS 56532. In studies using antagonists, GR 113808 was equilibrated with the tissues for 60 min, prior to constructing a curve to either 5-HT or (S) and (R) RS 56532.

Guinea-pig isolated ileum. The terminal portion of guinea-pig (male, Hartley, 300–350 g) ileum, 1 cm distal to the caecum, was gently flushed luminally with Tyrode solution. Segments (1.5 cm) were suspended under 1 g tension in 10 ml tissue baths, maintained at 37°C, pH 7.4 and continuously aerated with 95% oxygen, 5% carbon dioxide. To determine contractile responses to 5-HT, receptor activation, 5-methoxytryptamine (10 μM) was present to desensitize 5-HT<sub>4</sub> receptors (Craig et al., 1990), and methysergide (1 μM) was present to antagonize 5-HT<sub>1A</sub> and 5-HT<sub>3</sub> receptors. After an initial equilibration period, during which the bathing solution was replaced at 15 min intervals, the tissues were exposed to 50 mM KC<sub>1</sub> for 30 sec. The tissues were then washed and allowed to re-attain baseline tension. A concentration response curve to 5-HT was constructed, on a non-cumulative basis, using an exposure period of 30 sec on a 5 min dose cycle. The concentration response curve to 5-HT was repeated in the presence of either (S) or (R) RS 56532 (1 and 10 μM, respectively), after 60 min equilibration. (Preliminary experiments showed that RS 56532 did not wash out from the tissue completely after 1 hr. Consequently, only one concentration response curve was established in each tissue).

In vivo studies

Von Bezold Jarisch reflex studies in the rat. Male, Sprague–Dawley rats, (250–380 g) were anesthetized with urethane (1.5 g kg<sup>−1</sup>, i.p.). The trachea, right and left femoral veins were cannulated for facilitation of respiration, compound administration, and injection of 2-methyl-5-HT, respectively. Heart rate was derived by ECG Biotech amplifiers from a limb lead II ECG monitored via subdermal platinum electrodes. The data was recorded using a Gould recorder. Only rats with a baseline heart rate greater than 200 beats min<sup>−1</sup> were used. A stabilization period of 15–30 min was allowed after instrumentation. Each rat was challenged with 2-methyl-5-HT to establish the lowest dose that elicited the von Bezold Jarisch reflex (a rapid, transient decrease in heart rate of approx 200 beats min<sup>−1</sup>). This dose was...
usually between 10–80 μg kg⁻¹, i.v. Each rat was then challenged with this dose, every 12 min, until a consistent bradycardia was obtained.

(S) and (R) RS 56532 were administered i.v. in increasing multiple doses. Each dose of the test compound was followed, 5 min later, with a 2-methyl-5-HT challenge. Seven minutes were allowed before administration of successive doses of (S) and (R) RS 56532. This procedure was repeated until responses to 2-methyl-5-HT were completely inhibited. The change in the peak response to a fixed dose of 2-methyl-5-HT before and after administration of the vehicle or test compound was calculated. This was expressed as a percentage inhibition of the control bradycardic response.

**Tachycardia in anesthetized micropigs.** The method used was modified from that described by Villalon et al. (1990) and Eglen et al. (1993a). Yucatan micropigs of either sex (13–24 kg, Charles River Laboratories) were pretreated with ketamine (approx 30 mg kg⁻¹ i.m.), anesthetized 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 anesthetic and the second cannula for compound administration. A limb lead II ECG electrode was monitored by an animal respirator.

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Blood gas parameters were stabilized within the normal physiologic range (pH 7.52 ± 0.05; PCO₂ 36 ± 6, PO₂ 80 ± 10 mm Hg) by adjustments of ventilatory rate, tidal volume and positive end expiratory pressure prior to continuing an experiment.

All compounds were dissolved in normal saline and administered in base-equivalent doses. Each animal received 5-HT in ascending bolus i.v. doses of 1–300 μg kg⁻¹ at 0.5 log intervals and with 10–15 min between doses. Subsequently, each animal received either (S) or (R) RS 56532 at bolus i.v. doses of 1–1000 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 enantiomers of RS 56532. Additionally, arterial blood gases, pH, and rectal temperature were periodically determined to monitor the stability of the animal preparation.

**Cisplatin induced nausea and emesis in the ferret**

Adult, male, castrated ferrets (0.8–1.8 kg⁻¹, Marshall Farms, North Rose, NY, U.S.A.) were anesthetized in a sealed plexiglass anesthesia box by inhalation of methoxyflurane. The jugular vein was exposed and cannulated with intramedic tubing filled with heparinized saline (10 U ml). The cannula was exteriorized at the base of the skull, and each animal was returned to the home cage to recover. Following recovery from surgery, each animal received either vehicle (1.0 ml kg⁻¹), (S) RS 56532 (0.1 mg kg⁻¹), or (R) RS-56532 (0.1 mg kg⁻¹) orally as a solution. The animals were randomly assigned to each treatment group. Thirty minutes later, each animal received 10 mg kg⁻¹ cisplatin i.v. via the previously installed jugular cannula. Each animal was observed continuously for 5 hr following cisplatin administration for number of emetic episodes, defined as successful evacuation of stomach contents.

**Data analysis and statistical methods**

**In vitro studies.** In the radioligand binding studies, data from competition binding studies were analyzed by fitting to a four parameter logistic function using an iterative curve fitting program. The apparent affinities (−log Kᵈ) of competing ligands were calculated from IC₅₀ values by the Cheng-Prusoff equation (Cheng and Prusoff, 1973). Saturation binding studies were conducted using 10 concentrations of radioligand. Data from these studies were analyzed using the programs in ‘LIGAND’ (Munson and Rodbard, 1980) after first correcting for the free ligand concentration.

In the functional studies, agonist potencies were determined by nonlinear regression using interactive curve fitting procedures (Leung et al., 1992) and the relationship described by Parker and Waud (1971). In studies using rat isolated oesophageal muscularis mucosae, 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. Against 5-HT and (S) RS 56532, the apparent affinity was determined by the method of Arunlakshana and Schild (1957), wherein at least three concentrations of antagonists were used and the slope of the Schild plot determined by regression analysis.

**In vivo studies.** In the von Bezold Jarisch reflex studies, the dose that exhibited 50% reduction of the bradycardia response (ID₀) was obtained by using a four-parameter, modified Seemingly Unrelated Nonlinear Regression (Leung et al., 1992). The 95% confidence interval of the ID₀ was also determined. In the micropig studies, data was expressed as absolute changes from the baseline heart rate. Due to the prolonged duration of action of (S) RS 56532, heart rate data was expressed as cumulative changes from the initial baseline. Dose response data were analyzed using nonlinear iterative curve fitting procedures (Leung et al., 1992) and statistically significant differences assessed using Student's t-test. The data using (R) RS 56532 could not be analyzed by such procedures due to small size of the response. Therefore, these effects were subjected to one-way analysis of variance with pairwise comparisons to the initial predose baseline heart rate, using Fisher's LSD strategy.
Unless stated otherwise, all values were either mean ± SE mean or mean, with 95% confidence intervals in parenthesis. Statistically significant differences were determined using Student’s t-test, with P < 0.05 being considered significant.

Compounds used

BIMU-1 (endo- N- 8- methyl- 8- azabicyclo[3.1.2]oct- 3- yl)- 2,3- dihydro- 3- ethyl- 2- oxo- 1H- benzimidazole- 1- carbamoyl) hydrochloride, (S) and (R) RS 56532 (6- amino- 5- 2- (1- azabicyclo[2.2.2]octan- 3- yl)- 2,3- dihydro- 1- benz[de]isoquinoline), GR 113808 (1- (2- methanol- sulfonamido- ethyl)- piperidin- 4- yl)- methyl- indole- 3- carboxylate maleate, [3H] quipazine (sp. act. = 55 Ci mmol⁻¹), Aquasol and other radio- tracers were purchased from Du Pont New England Nuclear (Boston, MA, U.S.A.). [3H] 6- chloro- 2- methoxybenzamide hydrochloride, (S) and (R) YM 060 (5- [1- methyl- 3- indole]- carbonyl]- 4,5,6,7- tetrahydro- 9- methyl- 3- [ (2- methyl- 1H- imidazole- 1- yl)- methyl]- 2- methoxy benzamine hydrochloride), [3H] (R) zacopride (sp. act. = 36 Ci mmol⁻¹), granisetron (endo- 1- methyl- N- 9- methyl- 9- ethyl- 9- azabicyclo[3.3.2] non- 3- yl- 1H- indazolecarboxamide), renzapride (racemic endo- 4- amino- 5- chloro- 2- methoxy azabicyclo[3.3.1] non- 4- yl- benzamide), ondansetron (racemic 1,2,3,9- tetrahydro- 9- methyl- 3- [ (2- methyl- 1H- imidazole- 1- yl)- methyl]- 4H- carbazole- 4- one hydrochloride 2H₂O), SC- 53116 ((+)-exo- 4- amino- 5- chloro- N- [(5- azabicyclo[3.3.0]oct- 8- yl)- methyl]- 2- methoxy benzamide hydrochloride), SDZ 205, 557 (2- methoxy- 4- amino- 5- chloro benzoic acid 2- (diethylamino) ethyl ester) were synthesized in the Institute of Organic Chemistry, Syntex Discovery Research, Palo Alto, CA. (S) and (R) YM 060 (5- [1- methyl- (3- indole)- carbonyl]- 4,5,6,7- tetrahydro- 1H- benzimidazole hydrochloride) were generously provided by Yamanouchi Pharmaceuticals, Japan. Cisplatin, 5- hydroxytryptamine, 5- methoxytryptamine, cocaine, corticosterone and urethane were obtained from Sigma Chemical Co., Ltd. (St Louis, MO, U.S.A.) and 2- methyl- 5- HT from Research Biochemicals Inc., (Natick, MA, U.S.A.). Methysergide was generously donated by Sandoz (Basle, Switzerland). [3H] quipazine (sp. act. = 55 Ci mmol⁻¹), Aquasol and other radioligands were purchased from Du Pont New England Nuclear (Boston, MA, U.S.A.).

RESULTS

In vitro

Radioligand binding. At 5- HT₄ binding sites in guinea- pig striatum labelled by [3H] GR 113808 (Grossman et al., 1993), the specific binding was > 65% of the total binding, at 0.1 nM. The fraction of radioligand bound in the competition experiment, determined by direct measurement of the radioactivity in the assay tubes, did not exceed 20% of the amount of total ligand in the assay tube. As determined by saturation binding studies, the affinity (-log Kᵢ) of the radioligand was 10.7 and the density of sites (Bₘₐₙ) was 111.3 fmol mg⁻¹ protein (values are mean, SE mean < 10%, n = 4 determinations). The site was further characterized in competition radioligand binding studies using a range of ligands. These data are shown in Table 2, together with affinities and potencies previously estimated in functional studies in rat, isolated oesophagus (Eglen et al., 1993a, b). At 5- HT₄ binding sites the affinity of (S) RS 56532 was greater than that of the (R) enantiomer (Table 1). The displacement isotherms exhibited a Hill slope not significantly different from unity, suggesting an interaction at a single site.

At the ‘(R) zacopride site’ (Kidd et al., 1992), zacopride exhibited an enantiomeric specificity of (R) > (S).

Table 2. Comparison of affinities of ligands at the 5-HT₄ binding site in guinea-pig striatum with those estimated functionally in rat, isolated oesophagus

<table>
<thead>
<tr>
<th>Ligand</th>
<th>-log Kᵢ</th>
<th>nH</th>
<th>pA₂</th>
<th>-log EC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB 204070</td>
<td>10.9</td>
<td>1.1</td>
<td>10.4*</td>
<td></td>
</tr>
<tr>
<td>GR 113808</td>
<td>10.2</td>
<td>0.9</td>
<td>9.0*</td>
<td></td>
</tr>
<tr>
<td>SDZ 205,557</td>
<td>8.6</td>
<td>0.8</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>RS 23597-190</td>
<td>8.2</td>
<td>0.8</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>DAU 6285</td>
<td>8.0</td>
<td>0.9</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>Tropisetron</td>
<td>7.5</td>
<td>0.9</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>5-HT</td>
<td>7.5</td>
<td>0.7</td>
<td></td>
<td>8.3</td>
</tr>
<tr>
<td>5-MeOT</td>
<td>6.8</td>
<td>1.0</td>
<td></td>
<td>8.2</td>
</tr>
<tr>
<td>BIMU-1</td>
<td>7.9</td>
<td>0.8</td>
<td></td>
<td>7.8</td>
</tr>
<tr>
<td>(S) Zacopride</td>
<td>6.2</td>
<td>1.0</td>
<td></td>
<td>6.2</td>
</tr>
<tr>
<td>(R) Zacopride</td>
<td>6.2</td>
<td>1.0</td>
<td></td>
<td>6.2</td>
</tr>
<tr>
<td>SC-53116</td>
<td>7.8</td>
<td>0.9</td>
<td></td>
<td>7.7</td>
</tr>
</tbody>
</table>

Values are mean, SE mean > 1%, n = 3. pA₂ and -log EC₅₀ values are from Eglen et al. (1993a, b), except*—this study; †—value is a -log Kᵢ value determined at 1 nM; 5-MeOT, 5-methoxytryptamine.

Table 3. Affinities of various ligands at the ‘(R) zacopride sensitive’ binding site

<table>
<thead>
<tr>
<th>Compound</th>
<th>-log Kᵢ</th>
<th>nH</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R) Zacopride</td>
<td>8.2</td>
<td>1.0</td>
</tr>
<tr>
<td>(S) Zacopride</td>
<td>5.3</td>
<td>1.1</td>
</tr>
<tr>
<td>(R) YM 060</td>
<td>5.3</td>
<td>1.2</td>
</tr>
<tr>
<td>(S) YM 060</td>
<td>5.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>&gt; 5.0</td>
<td></td>
</tr>
<tr>
<td>(R) RS 42358</td>
<td>7.5</td>
<td>1.0</td>
</tr>
<tr>
<td>(S) RS 42358</td>
<td>4.7</td>
<td>1.5</td>
</tr>
<tr>
<td>(R) RS 56532</td>
<td>6.1</td>
<td>1.1</td>
</tr>
<tr>
<td>(S) RS 56532</td>
<td>4.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Renzapride</td>
<td>5.6</td>
<td>1.3</td>
</tr>
<tr>
<td>SDZ 205,557</td>
<td>5.5</td>
<td>0.9</td>
</tr>
<tr>
<td>RS 23597-190</td>
<td>6.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Prazosin</td>
<td>5.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Mianserin</td>
<td>5.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Nicotine</td>
<td>&gt; 5.0</td>
<td></td>
</tr>
<tr>
<td>Haloperidol</td>
<td>&gt; 5.0</td>
<td></td>
</tr>
<tr>
<td>Ketanserin</td>
<td>&gt; 5.0</td>
<td></td>
</tr>
<tr>
<td>Naloxone</td>
<td>&gt; 5.0</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean, SE mean less than 1%, n = 3–4 determinations. All studies were performed in the presence of ondansetron (1 μM).
the (S) isomer, although the absolute affinities of both enantiomers were lower than seen with (R) zacopride (Table 3). (R) and (S) RS 42358 exhibited a similar enantiomeric selectivity and affinities for this site, in comparison to (R) and (S) zacopride. Other ligands studied, including the (R) and (S) enantiomers of YM 060, racemic ondansetron and benzamides such as renzapride, RS 23597-190 and SDZ 205,557, exhibited negligible affinities at this site (Table 3).

Enantiomers of RS 56532 were tested for interactions at a range of other receptors using radioligand binding assays. (R) RS 56532 exhibited a higher affinity for 5-HT3 receptors, in rat cerebral cortex, than the (S) isomer (Table 1). Lower affinities for the muscarinic M1, M2, M4 receptors were also observed for (S) and (R) RS 56532. In all these studies, the inhibition curves were monophasic with Hill coefficients not significantly different to unity. At the remaining receptors studied the affinities were low (Table 1).

Functional studies. At 5-HT4 receptors mediating relaxations of precontracted rat oesophageal muscularis mucosa, (S) RS 56532 was more potent than the (R) isomer (Fig. 2). The potency (−log EC50) of the (S) isomer was 7.9 (7.7–8.4) with an intrinsic activity, relative to 5-HT, of 0.8. In contrast, the −log EC50 of the (R) RS 56532 was less than 6.0 (Fig. 2), although this could not be determined unambiguously due to additional relaxations from muscarinic receptor antagonism. To further study the interaction of (S) RS 56532 at oesophageal 5-HT4 receptors, Schild analysis was performed using the selective 5-HT4 receptor antagonist, GR 113808 (Grossman et al., 1993). The resulting pA2 value against GR 113808 was 9.6 (8.6–10.5), and the slope of the Schild regression was 0.7 (0.5–0.9) which was not significantly different to unity. When the unity constraint was imposed, the pA2 value was 9.1 (9.0–9.3). In comparison, when 5-HT was used as the agonist, the pA2 for GR 113808 was 8.7 (8.4–9.0) and the slope of the Schild regression was 1.4 (1.1–1.7). The Schild slope was not significantly different from unity and when constrained to one, the pA2 value was 9.0 (8.9–9.2).

At 5-HT3 receptors in guinea-pig ileum, neither (S) or (R) RS 56532 possessed intrinsic activity at concentrations ranging from 1 nM to 10 μM. When studied for antagonism, the (S) isomer was without effect at inhibiting responses to 5-HT at 10 μM, suggesting an affinity of less than 5.0. However, the (R) isomer, at 1 μM, dextrally shifted the concentration response curve to 5-HT and the calculated affinity (−log Kd) was 7.9 (7.7–8.2).

In vivo studies

Von Bezold Jarisch reflex. (S) and (R) RS 56532 elicited a dose-dependent inhibition of the bradycardic response induced by 2-methyl-5-HT, with IDs0 values of 78 (47–128) and 3 (2–4) μg kg−1, i.v., respectively. Compared to the vehicle group, (S) and (R) RS 56532 (1–1000 μg kg−1, i.v.) administered alone did not have a significant effect on heart rate.

Tachycardia. The respective baseline mean arterial pressure and heart rates were 114 ± 2 mm Hg and 136 ± 4 beat min−1, respectively, for the (S) RS 56532 treated animals, 90 ± 8 mm Hg and 107 ± 7 beat min−1 for the (R) RS 56532 treated animals. 5-HT and (S) RS 56532 elicited a dose-dependent tachycardia with the former being significantly more potent than the latter (Table 4). (S) RS 56532 was full agonist with respect to 5-HT. In contrast, (R) RS 56532 was much less potent (Table 4), exhibited no agonist activity until the highest dose (1000 μg kg−1) was studied at which the heart rate increased by 23 ± 6 beats min−1.

Nausea and emesis studies

Administration of cisplatin and vehicle alone caused 19.8 ± 2.8 emetic episodes. (R) and (S) RS 56532 when administered alone (0.1 mg kg−1, p.o.) did not induce emesis. When compared to the vehicle control, (S) RS 56532 (0.1 mg kg−1, p.o.) administered 30 min prior to cisplatin, did not significantly reduce the number of emetic episodes due to cisplatin (17.8 ± 1.6 emetic episodes).

<table>
<thead>
<tr>
<th>Compound</th>
<th>ED50 (μg kg−1)</th>
<th>Maximum Tachycardiac Increase (beats min−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT</td>
<td>3 (2–4)</td>
<td>88 (82–95)</td>
</tr>
<tr>
<td>(S) RS 56532</td>
<td>16 (11–21)</td>
<td>87 (81–95)</td>
</tr>
<tr>
<td>5-HT</td>
<td>3 (3–4)</td>
<td>97 (93–101)</td>
</tr>
<tr>
<td>(R) RS 56532</td>
<td>&gt;1000</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are means, with 95% confidence intervals in parenthesis, n = 5 animals. The maximum increase to 1000 μg kg−1 (R) RS 56532 was 23 ± 6 beats min−1 (mean ± SE mean from 5 animals).

![Fig. 2. Concentration response curves to 5-HT (□), (S) RS 56532 (■) and (R) RS 56532 (○) in rat, isolated oesophagus. Relaxations were determined in tissues precontracted with carbachol (3 μM). Values are mean ± SE mean, n = 4–8 animals.](image-url)
episodes). (R) RS 56532 (0.1 mg kg⁻¹, p.o.), conversely, significantly reduced the emesis caused by cisplatin (5.8 ± 2.1 emetic episodes).

**DISCUSSION**

In the present study the activity of the (S) and (R) enantiomers of RS 56532, constrained hybrid analogs of RS 42358 (a selective 5-HT₄ receptor antagonist lacking 5-HT₃ affinity; Eglen et al., 1993c) and zacopride (a mixed 5-HT₄ agonist 5-HT₃ antagonist; Pinkus and Gordon, 1991) have been investigated.

The 5-HT₄ binding site has been previously shown (Grossman et al., 1993) to be present in high density in guinea-pig striatum. The high density of specific binding sites in striatum for [³H]GR 113808 observed in the present study supports this conclusion. The radioligand appeared to act in a reversible fashion, with a high degree of specific binding. Moreover, the apparent affinities estimated in competition radioligand binding studies, were consistent with the pharmacological profile generated functionally at 5-HT₄ receptors (Table 2). The apparent affinity of SB 204070 in binding studies (−log Kᵢ = 10.9) was similar to the pA₂ value (10.8) reported by Wardle et al. (1993) in functional studies in guinea-pig colon. A higher value of GR 113808 at 5-HT₄ receptors in the binding studies was seen in comparison to those estimated functionally (this study) or reported in binding studies by Grossman et al. (1993) or Waebier et al. (1993). This was due to differences in temperature used in the binding assays, i.e. 21°C in the present study and 37°C in the studies of Grossman et al. (1993) or Waebier et al. (1993). Thus, when the assay was repeated at 37°C, the −log Kᵢ value for GR 113808 decreased to from 10.2 to 9.4 (Bonhaus and Hsu, unpublished observations). Taken together, therefore, the data obtained in the present study confirm that [³H]GR 113808 is a suitable ligand to identify 5-HT₄ binding sites, as previously concluded by Grossman et al. (1993) and Waebier et al. (1993). The apparent affinities of (R) and (S) enantiomers of RS 56532 at guinea-pig striatal 5-HT₄ binding sites demonstrated that the enantiomeric selectivity was (S) > (R). Functional studies in rat oesophagus also suggested that (S) RS 56532 was a potent 5-HT₄ receptor agonist. In contrast, (R) RS 56532 was much less potent at the 5-HT₄ receptor in this tissue. Indeed, accurate estimation of the potency of this enantiomer could not be undertaken since additional relaxations occurred due to muscarinic receptor antagonism. Comparison of the apparent affinities of 5-HT₄ agonists, estimated in the binding studies with the potencies estimated functionally (Table 2) suggest a low receptor reserve associated with 5-HT₄ receptor mediated relaxations in rat oesophagus. Consistent with this hypothesis is the finding that SC-53116, which is equipotent to 5-HT in rat oesophagus, just fails to act as a full agonist (Flynn et al., 1992; see Ford and Clarke, 1993, for further discussion).

The potencies of (S) RS 56532 (−log EC₅₀ = 7.9) in oesophagus were greater than the potencies of (S) zacopride (−log EC₅₀ = 6.7; Eglen et al., 1993a) in this preparation. However, the potency of (S) RS 56532 was similar to potencies of SC 53116, BIMU-1 or 5-methoxytryptamine (−log EC₅₀ values were 7.7, 7.6 and 8.2, respectively; Eglen et al., 1993a). (S) RS 56532 is, therefore, amongst the most potent 5-HT₄ agonists identified to date, although less selective between this receptor in comparison to the 5-HT, receptor than SC-53116 (Flynn et al., 1992). In oesophagus, a functional interaction at 5-HT₄ receptors by (S) RS 56532 was supported by antagonist studies with GR 113808. The pA₂ value for this antagonist, when 5-HT was used the agonist, was similar to that reported by Grossman et al. (1993). When (S) RS 56532 was used as the agonist, the Schid slope was not significantly from unity, indicating that (S) RS 56532 and GR 113808 interacted in a competitive manner. The displacement isotherms observed in radioligand binding studies using [³H] GR 113808 were also consistent with an interaction at a single site.

Radioligand binding and functional studies showed that both enantiomers exhibited high selectivity toward the 5-HT₃ receptor, with the (R) isomer having a greater affinity than the (S) isomer. This enantiomeric specificity is converse to that of (R) and (S) zacopride (Pinkus and Gordon, 1991) and other 5-HT₃ receptor ligands including RS 42358 (Eglen et al., 1993c; Wong et al., 1993) and ondansetron (Butler et al., 1988). However, this specificity was similar to the activity of (R) and (S) YM 060 (Miyata et al., 1991). The lower affinities for both isomers of RS 56532 at 5-HT₃ receptors in guinea-pig ileum, in comparison to those in rat tissue, supports the existence of species variants of this receptor (see Kilpatrick and Tyers, 1992 for review; Butler et al., 1990; Malone et al., 1991; Newberry et al., 1991; Peters et al., 1991; Wong et al., 1993).

Taken together, the in vitro data suggested that, at the 5-HT₃ receptor, both enantiomers of RS 56532 were antagonists, with (R) > (S). At the 5-HT₄ receptor, both enantiomers were agonists, with (S) > (R) in terms of both potency and affinity. At this receptor, (S) RS 56532 appears to possess a higher intrinsic efficacy than (R) RS 56532. These data were supported by studies in vivo. Thus, at 5-HT₃ receptors mediating the von Bezold Jarisch reflex in the rat, (R) RS 56532 was more potent than the (S) isomer at inhibiting the response to 2-methyl-5-HT. In this assay, the inhibitory potency of compounds correlates with the affinity at 5-HT₃, receptors (Fozard and Host, 1982; Fozard, 1989; Cohen et al., 1989; Sanger and Nelson, 1989; Robertson et al., 1992; Eglen et al., 1993b, c). Similar observations were seen with (S) RS 56532 in the present study. However, in terms of (R) RS 56532, a discrepancy was noted in that the high 5-HT₄ receptor affinity of this enantiomer (−log Kᵢ = 9.1) was not reflected in a high inhibitory potency in the von Bezold Jarisch reflex studies.
(ID₉₀ = 3 μg kg⁻¹ i.v.). Tropisetron, in contrast, exhibits a similar affinity at 5-HT₃ receptors (−log Kᵣ = 9.1; Wong et al., 1993) to (R) RS 56532 but exhibits an inhibitory potency in the von Bezold Jarisch reflex of less than 1 μg kg⁻¹, i.v (Richardson et al., 1985). The reasons for this lack of correlation require further investigation.

Emesis, due to administration of cisplatin, is associated with 5-HT₃ receptor activation (Andrews et al., 1992). The higher affinity of (R) RS 56532 in comparison to (S) RS 56532, at 5-HT₃ receptors was reflected in inhibitory activities of these isomers in ferret emesis studies. In should be noted that (S), but not (R) zacopride, elicited both the von Bezold Jarisch reflex in rat and emesis in ferret (King, 1991; Middlefell and Price, 1991). These effects were attributed to 5-HT₃ agonism since they were antagonized by ondansetron (Middlefell and Price, 1991). In the present study, both (R) and (S) RS 56532 failed to agonize the 5-HT₃ receptor in guineapig isolated ileum, eliciting the von Bezold Jarisch reflex in the rat or to cause emesis in the ferret. Taken together, the data suggests that RS 56532 lacks structural features required for intrinsic efficacy at the 5-HT₃ receptor both in vitro and in vivo.

Tachycardia in the pig and micropig is mediated by activation of atrial, but not ventricular, 5-HT₄ receptors (Villalon et al., 1990; Saxena and Villalon, 1990; Shoemaker et al., 1992; Eglen et al., 1993a, b). In this preparation, (S) RS 56532 was more potent than (R) RS 56532, acting as a full agonist with respect to 5-HT₄. These findings supported the in vitro data suggesting that the (S) isomer, but not the (R) isomer, was a potent 5-HT₄ receptor agonist. The enantiomeric specificity of RS 56532 i.e. (R) > (S) at 5-HT₃ receptors and (S) > (R) at 5-HT₄ receptors, was different from that of zacopride, which exhibits an enantiomeric specificity of (S) > (R) at both 5-HT₃ and 5-HT₄ receptors. The 5-HT₃ receptor pharmacophore has been previously described in detail (Hibert et al., 1990; Rizzi et al., 1990; Swain et al., 1992; Bradley et al., 1992; Robertson et al., 1992; Hyashi et al., 1993). Recently, these models have been modified to include a key lipophilic binding domain in order to accommodate structure activity relationships of novel and potent 5-HT₃ antagonists of the N-(quinuclidin-3-yl)aryl and hetero-fused pyridone series including RS 42358 (Fig. 1; Clark et al., 1993a). The stereospecificity of the interaction of RS 56532 at this receptor also accords with this refined model and this is described in more detail elsewhere (Langlois et al., 1992; Clark et al., 1993b).

The 5-HT₄ receptor pharmacophore has yet to be extensively defined due, in part, to the unavailability of many high affinity, selective 5-HT₄ receptor ligands. It is likely to be different from that of the 5-HT₃ receptor pharmacophore, as a consequence of likely differences in the tertiary structure of these receptors (i.e. a ligand gated ion channel and a guanine nucleotide binding protein coupled receptor, respectively). The opposing enantiomeric selectivity of (R) and (S) RS 56532 at these two receptors support this hypothesis.

In terms of the (R) zacopride sensitive’ binding site, the radioligand binding profile obtained in the present study, supports previous data (Kidd et al., 1992; Kidd et al., 1993). Thus, (R) zacopride exhibited a high affinity for the site, binding specifically in the presence of mianserin. The site also possessed reasonable affinity toward prazosin and mianserin (Kidd et al., 1992; this study). Pharmacologically, the site differed from the 5-HT₃ receptor, due to the insensitivity to ondansetron and the enantiomers of YM 060 (Kidd et al., 1992; this study). These pharmacological data support a recent report by Kidd et al. (1993) showing that the molecular size of the (R) zacopride’ site (approx 30 kDa) differed from that of the 5-HT₃ receptor (57 kDa). Moreover, the putative location of the site on glia, in contrast to the neuronal location of 5-HT₃ receptors, further suggests that the sites differ (Kidd et al., 1993). The (R) zacopride’ site also differs from the 5-HT₄ receptor since renzapride (5-HT₄ receptor agonist), SDZ 205,557 and RS 23597-190 (5-HT₄ receptor antagonists) exhibited negligible affinities (this study). The enantiomeric selectivity of (R) and (S) RS 42358 (Table 2) for the (R) zacopride’ site was similar to that of (R) and (S) zacopride (Kidd et al., 1992; this study). (R) RS 42358, therefore, provides a further tool to explore the pharmacology of the site. In this respect it would be of interest to study the interaction of other N-(quinuclidin-3-yl)aryl pyridones (Clark et al., 1993a, b) at this site.

To conclude, the pharmacology of (S) and (R) RS 56532 have been characterized in vitro and in vivo. The (S) isomer is a potent and selective 5-HT₃ receptor agonist and the (R) isomer is a potent and selective 5-HT₄ receptor antagonist. The compounds may facilitate studies on the physiological role of 5-HT₃ and 5-HT₄ receptors. In particular, (S) RS 56532 appears to have use to study the 5-HT₄ receptor since it displays potent and selective agonism both in vitro and in vivo. In this respect, use of the compound may be preferred over that of (S) zacopride, which also exhibits agonism at the 5-HT₄ receptor in vivo (see above). Substituted, 1,8-naphthalimides such as (S) RS 56532, therefore, represents a novel class of potent 5-HT₄ receptor agonists.

Acknowledgements—The authors wish to thank Sherry Hsu, Mohammed Khabbaz, Lorri Perkins, Marc Perry, Lorri Walsh, Sonya Wallace, Arnold Wong, Ken Zeitung for their valuable technical assistance.

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