The action of SDZ 205,557 at 5-hydroxytryptamine (5-HT₃ and 5-HT₄) receptors

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1 The interaction of the novel antagonist, SDZ 205,557 (2-methoxy-4-amino-5-chloro benzoic acid 2-(diethylamino) ethyl ester), at 5-HT₃ and 5-HT₄ receptors has been assessed *in vitro* and *in vivo*.

2 In guinea-pig hippocampus and in the presence of 0.4 µM 5-carboxamidotryptamine, 5-HT₄-mediated stimulation of adenylyl cyclase was competitively antagonized by SDZ 205,557, with a pA₂ value of 7.5, and a Schild slope of 0.81. In rat carbachol-contracted oesophagus, 5-HT₄-receptor mediated relaxations were surmountably antagonized by SDZ 205,557 with a similar pA₂ value (7.3). This value was agonist-independent with the exception of (R)-zacopride, against which a significantly lower value (6.4) was observed.

3 In functional studies of 5-HT₃ receptors, SDZ 205,557 exhibited an affinity of 6.2 in guinea-pig ileum compared with 6.9 at binding sites labelled by [³H]-quipazine in NG108-15 cells. In the anaesthetized, vagotomized micropig, SDZ 205,557 produced only a transient blockade of 5-HT₄-mediated tachycardia. This contrasted with tropisetron, which was active for over 60 min after administration. The half-lives for the inhibitory responses of SDZ 205,557 and tropisetron were 23 and 116 min, respectively.

4 In conclusion, SDZ 205,557 has similar affinity for 5-HT₃ and 5-HT₄ receptors. The apparent selectivity observed in guinea-pig is due to the atypical nature of the 5-HT₃ receptor in this species. The short duration of action of this novel antagonist may complicate its use *in vivo*. SDZ 205,557 should, therefore, be used with appropriate caution in studies defining the 5-HT₄ receptor.

Keywords: SDZ 205,557; 5-HT₃ receptors; 5-HT₄ receptors

Introduction

The 5-hydroxytryptamine (5-HT) receptor family may be classified into 5-HT₁, 5-HT₂, 5-HT₃ (Bradley *et al.*, 1986) and 5-HT₄ subgroups (Bockaert *et al.*, 1992). The 5-HT₄ receptor (Dumuis *et al.*, 1988) is stimulated by indoles (such as 5-HT, 5-methoxytryptamine, 5-carboxamidotryptamine), substituted benzamides (such as renzapride, zacopride, cisapride) and azabicycloalkyl benzimidazolones (such as BIMU-1, BIMU-8), but is insensitive to antagonists of 5-HT₁, 5-HT₂, and 5-HT₃ receptors, such as methysergide, ketanserin and ondansetron, respectively (see Craig & Clarke, 1990; Bockaert *et al.*, 1992 for reviews). The 5-HT₄ receptor is competitively antagonized by tropisetron (ICS 205,930; $-\log K_B = 6.5$) and DAU 6285 ($-\log K_B = 6.8$; Dumuis *et al.*, 1992). Receptors exhibiting this pharmacological profile have been demonstrated in embryonic mouse colliculi neuronal cultures (Dumuis *et al.*, 1992), guinea-pig ileum (Craig & Clarke, 1990; Eglen *et al.*, 1990) and colon (Elswood *et al.*, 1991), rat oesophagus (Baxter *et al.*, 1991; Waikar *et al.*, 1992), porcine myocardium (Kaumann, 1990), human myocardium (Kaumann *et al.*, 1990) and amphibian adrenal gland (Idres *et al.*, 1991). More precise definition of 5-HT₄ receptors is, however, hampered by the lack of selective, competitive antagonists (see Turconi *et al.*, 1991; Bockaert *et al.*, 1992 for reviews).

Buchheit *et al.* (1991) reported that SDZ 205,557 (2-methoxy-4-amino-5-chloro benzoic acid 2-(diethylamino) ethyl ester) selectively antagonized 5-HT₄ receptors in guinea-pig isolated ileum with an affinity ($-\log K_B$) of 7.5. This affinity was greater than that at 5-HT₃ receptors in this tissue ($-\log K_B = 6.0$). SDZ 205,557 lacked affinity at 5-HT₁ or 5-HT₂ receptors and was, therefore, suggested as a useful ligand to define the 5-HT₄ receptor (Buchheit *et al.*, 1992). Pharmacological evidence suggests that 5-HT₃ receptors are a family of species variants. The guinea-pig 5-HT₃ receptor,

notably, exhibits lower affinities for ligands than either rat or mouse 5-HT₃ receptors (see Kilpatrick & Tyers, 1992, for review). This may lead to an overestimation of the selectivity of antagonists, including SDZ 205,557, in favour of the 5-HT₄ receptor (Bockaert *et al.*, 1992). In the present study, SDZ 205,557 has been further evaluated by use of *in vitro* and *in vivo* responses in various species, on preparations sensitive to 5-HT₄ stimulation. This receptor, in contrast to the 5-HT₃ receptor, appears to exhibit affinities that are not species-dependent (Bockaert *et al.*, 1992).

A preliminary account of these studies has been previously published (Eglen *et al.*, 1992).

Methods

Guinea-pig hippocampal adenylyl cyclase

Male guinea-pigs (Hartley, 250–500 g) were killed by CO₂ asphyxiation. The hippocampi were then rapidly dissected, and homogenized (20 strokes) in a glass homogenizer in 9 ml of ice-cold buffer, of the following composition (mM): sucrose 300, Tris-HCl 20, EGTA 1, Na₂EDTA 2.5 and dithiothreitol 1 (pH 7.4, 23°C). This homogenate was then diluted, 1:8 (v/v) with buffer and centrifuged (10 min, 39,000 g, 4°C). The pellet was resuspended in 9 ml of buffer and the membrane suspension used directly in the adenylyl cyclase assay. Antagonists were added 30 min prior to addition of agonist. Measurements of adenylyl cyclase activity were performed, three times, according to the method of Alvarez & Daniels (1990) with each sample run in triplicate. The final composition of the incubation medium was (mM): [α -³²P]-adenosine 5'-triphosphate ([α -³²P]-ATP, 0.25 µCi) 0.5, MgSO₄ 5, Tris-HCl 44 (pH 7.4), 1-methyl-3-isobutylxanthine 1.0, sucrose 50, EDTA 1.0, EGTA 0.2, dithiothreitol 0.2, adenosine 3':5'-cyclic monophosphate (cyclic AMP) 2, guanosine 5'-triphosphate (GTP) 0.1, phosphoenol pyruvate 20 and pyruvate

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kinase 6 units ml⁻¹. The reaction was initiated by the sequential addition of the membrane suspension (40 µl) to the incubation medium in a total reaction volume of 200 µl. Incubations were performed for 30 min at 37°C and terminated by the addition of 20 µl of a stock solution of [³H]-cyclic AMP (0.005 µCi) in 2.2 N HCl. Labelled cyclic AMP was added to estimate and correct for recovery of the nucleotide following column chromatography. The tube contents were heated at 95°C for 4 min and then cooled in an ice-water bath. Neutral alumina (1.3 g) was dispensed into disposable polypropylene columns with a Uniflow adjustable powder measure. The columns were placed on a plexiglas rack designed to hold the columns and to fit over a box of 100 scintillation vials. An aliquot (200 µl) of the solution contained in each tube was pipetted onto the columns and allowed to flow into the dry alumina. Cyclic AMP was then eluted with 4 ml of 0.1 M ammonium acetate, pH 7. The effluent (3.2 ml) was collected into scintillation vials, mixed with 15 ml scintillation fluid and counted for ³H and ³²P in a liquid scintillation spectrometer.

Rat isolated oesophageal muscularis mucosae

The method used was that previously described by Baxter *et al.* (1991). Male rats (Sprague-Dawley, 200–250 g) from Charles River were killed by CO₂ asphyxiation, the thoracic oesophagus removed and placed in Tyrode solution (composition, mM: NaCl 139.0, KCl 2.7, MgCl₂·6H₂O 1.1, NaH₂PO₄ 0.4, glucose 5.6, NaHCO₃ 11.8 and CaCl₂·6H₂O 1.8). The outer proprial muscle coat was cut longitudinally and gently peeled away, leaving the inner muscularis mucosae. Silk threads were then tied through the lumen on both ends of the tissues, which were then mounted vertically in 10 ml tissue baths containing Tyrode solution with 1 µM methysergide, 30 µM cocaine and 30 µM corticosterone. The baths were maintained at 37°C and constantly aerated with a mixture of 95% O₂ and 5% CO₂. An initial resting tension of 1 g was applied to the tissues, then adjusted to 0.5 g tension at 15 min intervals thereafter. After 1 h of equilibration, 100 µM pargyline was added to the baths for 30 min, followed by a 15 min washout. At this point, the tissues were exposed to 50 mM KCl for 5 min, washed four times, followed by an additional 30 min equilibration period with a 15 min wash cycle.

Carbachol (3 µM) was added to the baths to contract the preparations. Once a stable contraction had been established (usually 30 min) the agonist was then added cumulatively to the bath to induce relaxation. After establishing the control concentration-response curve for the agonist, the preparations were then washed. SDZ 205,557 (1–10 µM) was applied to the baths and allowed to equilibrate with the tissues for 60 min before the second agonist concentration-response curve. Since all 5-HT₄ agonists at high concentrations have the propensity to antagonize muscarinic receptors (Baxter *et al.*, 1991), a final concentration-response curve was constructed in the presence of both 10 µM 5-methoxytryptamine and SDZ 205,557 after a further 60 min. This procedure established the agonist concentration-range attributable to 5-HT₄ agonism alone as distinct from additional relaxation due to muscarinic receptor antagonism.

Agonist potencies were determined by nonlinear iterative curve fitting procedures (Michelson *et al.*, 1992), using the relationship described by Parker & Waud (1971). Apparent antagonist affinities ($-\log K_B$) were estimated by the relationship $-\log K_B = -\log [\text{Antagonist}]/(\text{concentration ratio} - 1)$ (Furchgott, 1972). Concentration ratios were measured at the agonist concentration which elicited 30% of the maximal relaxation, since under some conditions the effects observed at higher concentrations may have reflected muscarinic receptor antagonism. Against 5-HT itself, the apparent affinity was determined by the method of Arunlakshana & Schild (1959), wherein three concentrations of SDZ

205,557 were used, and the slope of the Schild plot determined by regression analysis.

Competition radioligand binding studies

Membranes were prepared from NG108-15 cells cultured under conditions described previously (Sharif *et al.*, 1991). In competition binding studies, 5-HT₃ receptors in NG108-15 cell membranes were labelled with 0.5 nM [³H]-quipazine and non-specific binding was defined by 1 µM (S)-zacopride. The membrane homogenates were incubated in 25 mM Tris-Krebs (pH 7.4 at 25°C) with radioligand and varying concentrations of SDZ 205,557, or other standard 5-HT₃ receptor antagonists, in a final assay volume of 0.5 ml. The incubations were carried out for 45 min at 25°C and were terminated by rapid vacuum filtration over Whatman GF/B filters using a Brandell 48 cell harvester. This was immediately followed by 8 s of washing with ice-cold 0.1 M NaCl. The filters were pretreated with 0.3% polyethyleneimine in order to reduce filter binding of the radioligand and the radioactivity retained on the filters was determined by liquid scintillation spectrometry. All competition data were analyzed by iterative curve fitting procedure as described previously (Michel & Whiting, 1984). The apparent dissociation constant (K_i) of competing ligands was calculated from IC₅₀ values by the Cheng-Prusoff equation (Cheng & Prusoff, 1973).

Anaesthetized micropig studies

The method used was modified from that described by Villalon *et al.* (1990). Yucatan micropigs (male and female; 14.9–20.8 kg, Charles River Laboratories Inc., Wilmington, MA, U.S.A.) were pretreated with ketamine HCl (approx. 30 mg kg⁻¹, i.m.), anaesthetized with pentobarbitone sodium (20 mg kg⁻¹) via a marginal ear vein, intubated, and mechanically ventilated with room air by an animal respirator (Harvard, Model 613). A femoral artery was cannulated for the measurement of arterial blood pressure via a Gould/Statham pressure transducer (P231D). Dual cannulae were inserted into the ipsilateral femoral vein, one cannula for continuous infusion of supplemental anaesthetic (pentobarbitone sodium 8–15 mg kg⁻¹ h⁻¹) and the second cannula for compound administration. A limb lead II ECG was monitored via subcutaneously placed electrodes and heart rate (HR) was determined by a cardiometer triggered by the aortic pressure signal form. Following a midline cervical incision, the vagus nerves were bilaterally transected. Blood gas parameters were periodically monitored via a blood gas analyzer (Corning, Model 168) and blood gas values were stabilized within a normal (pH 7.2; Pco₂, 33 mmHg; Po₂, 96 mmHg) physiological range by adjustments of ventilatory rate, tidal volume, and positive end-expiratory pressure prior to continuing an experiment.

5-HT, tropisetron and SDZ 205,557 were administered in base equivalent doses. 5-HT, dissolved in 0.154 M NaCl, was administered at bolus i.v. doses of 1, 3, 10, 30 and 100 µg kg⁻¹ (0.05 ml kg⁻¹ doses) in each animal and a dose-response curve constructed. An ED₅₀ dose for 5-HT was determined visually from each dose-response curve. SDZ 205,557 and tropisetron were dissolved in a 3:7 propylene glycol:normal saline (v/v) mixture. Animals were assigned randomly to 3 treatment groups: vehicle (3:7 propylene glycol:normal saline), SDZ 205,557 (6 mg kg⁻¹, i.v.), or tropisetron (5 mg kg⁻¹, i.v.). Doses for SDZ 205,557 and tropisetron were determined in preliminary dose-finding experiments (data not shown). These doses represented those that maximally antagonized 5-HT-induced tachycardia and/or were the maximum feasible dose based on compound supply and micropig weight. The ED₅₀ dose of 5-HT (determined from a previous experiment) was administered 3 times at 10–15 min intervals to determine a control response (mean of 3 responses). Following administration of vehicle, SDZ 205,557, or tropisetron in a volume of 0.1 ml kg⁻¹, the ED₅₀

dose of 5-HT was administered in a volume of 0.03 ml kg⁻¹ at 3, 10, 20, 30, 45, 60, 75, 90, 105, and 120 min thereafter.

Statistical analysis

Unless stated otherwise, all values are mean $\pm$ s.e.mean. Statistically significant differences between values estimated from *in vitro* studies were determined by Student's *t* test, with $P < 0.05$ being considered significant. The data from *in vivo* studies were analysed as follows: for the SDZ 205,557 duration of action study, the SDZ 205,557 treatment group was compared to its vehicle group with respect to the changes from baseline or the ranked changes from baseline. These ranks were used whenever the data exhibited different variances from group to group, using Bartlett's test for heteroscedasticity. A repeated measures two-way analysis of variance (ANOVA) including effects for treatment, time, and treatment by time interaction was used. One-way ANOVAs were performed at each time point to determine statistical significance for individual differences between treatment groups and the data were expressed as % inhibition of control tachycardia response. Pairwise contrasts were adjusted for multiple comparisons by Dunn's procedure. The critical value for each pairwise comparison was adjusted by a Bonferroni correction, if no overall treatment or treatment-by-time effect was noted. Analysis of tachycardic dose-response curves for 5-HT, renzapride, and (R,S)-zacopride, as well as determinations of rates of return of responses to baseline in the SDZ 205,557 duration of action study, were conducted by nonlinear iterative curve fitting procedures (Michelson *et al.*, 1992) with statistically significant differences assessed from a *t* test.

Materials

NG108-15 cells were obtained from the Institute of Cancer and Developmental Biology, Syntex Research, Palo Alto, CA, U.S.A. SC-53116 was donated by Searle, Skokie, Ill., U.S.A. BIMU-1 (endo-N-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1H-benzimidazol-1-carboxamide, BIMU-8 (endo-N-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-isopropyl-2-oxo-1H-benzimidazol-1-carboxamide HCl, renzapride, MDL 72222 (1 α H, 3 α , 5 α H-tropan-3-yl-3,5-dichlorobenzoate), SDZ 205,557, ondansetron, tropisetron and zacopride (R + S; R,S) were synthesized in the Institute of Organic Chemistry, Syntex Research, Palo Alto, CA, U.S.A. 5-Hydroxytryptamine, 2-methyl-5-HT and phenylbiguanide were obtained from Research Biochemicals Incorporated, Natick, MA, U.S.A. [³H]-quipazine (specific activity 55 Ci mmol⁻¹) was purchased from DuPont NEN (Boston, MA, U.S.A.). [2,8-³H]-adenosine 3':5'-cyclic monophosphate (30–50 Ci mmol⁻¹), [α -³²P]-adenosine 5'-triphosphate (10–50 Ci mmol⁻¹) and Aquasol were purchased from New England Nuclear Corp. (NEN). Neutral alumina (ICN, Alumina N, activity grade 1) was purchased from ICN Biochemicals, Eschwege, Germany. All remaining compounds were obtained from Sigma Chemical Co, St. Louis, Mo, U.S.A.

Results

Guinea-pig hippocampal adenylyl cyclase

Adenylyl cyclase activity (basal activity = 1.8 nmol 30 min⁻¹ mg⁻¹ protein) in membranes isolated from the guinea-pig hippocampus was stimulated by 5-HT (~2 fold) with a $-\log EC_{50}$ value of 6.4 (Figure 1). The sigmoidal concentration-response curve to 5-HT appeared to be monophasic. By contrast, 5-carboxamidotryptamine (5-CT) produced a biphasic response curve. Low concentrations (0.4 μ M) of 5-CT were sufficient to stimulate maximally the initial phase (Figure 1). Previous studies have demonstrated

Figure 1 Effect of 5-hydroxytryptamine (●) and 5-carboxamidotryptamine (□) on guinea-pig hippocampal adenylyl cyclase. Values are mean $\pm$ s.e.mean (vertical bars), with the experiment repeated three times and each sample analysed in triplicate.

that the increase in adenylyl cyclase activity elicited by 5-HT in the presence of 0.4 μ M 5-CT is due to the stimulation of 5-HT₄ receptors (Shenker *et al.*, 1987). In the absence of 5-CT, maximally effective concentrations (100 μ M) of SDZ 205,557 and spiperone reduced the stimulation in response to 10 μ M 5-HT by 44% and 64%, respectively. Stimulation of adenylyl cyclase by 5-HT was abolished when the membrane preparation was incubated in the presence of both antagonists (Figure 2).

In the presence of 5-CT, responses to 5-HT were antagonized, in a concentration-dependent manner, by both tropisetron and SDZ 205,557, with $-\log IC_{50}$ values of 6.4 and 5.1 (mean, s.e.mean $< 10\%$), respectively. In contrast, responses to 5-HT were unaffected by propranolol (0.1 mM), ketanserin (1 μ M) or methysergide (1 μ M; data not shown). The apparent affinity of SDZ 205,557 at the 5-HT₄ receptor

Figure 2 Effect of spiperone, tropisetron (100 μ M) and in combination on the stimulation of hippocampal adenylyl cyclase by 5-hydroxytryptamine. Values are mean $\pm$ s.e.mean (vertical bars), with the experiment repeated three times and each sample analysed in triplicate.

Figure 3 Schild analysis of antagonism by SDZ 205,557 of 5-hydroxytryptamine-stimulated adenylyl cyclase in guinea-pig hippocampus. Values are means of three experiments, each sample analysed in triplicate.

mediating the stimulation of adenylyl cyclase was derived by Schild regression analysis. The pA_2 value was 7.5 with a Schild slope of 0.81 (Figure 3).

Rat oesophageal muscularis mucosae

In preparations pre-contracted with 10 μ M carbachol, all agonists induced a concentration-dependent relaxation. The potencies and intrinsic activities to agonists studied are shown in Table 1. The rank order was 5-HT = 5-MeOT > BIMU-1 $\geq$ SC-53116 $\geq$ BIMU-8 > (S)-zacopride > (R)-zacopride. SDZ 205,557 (1 nM–10 μ M) lacked intrinsic activity at the oesophageal 5-HT₄ receptor in that no relaxations were seen. In the presence of SDZ 205,557, concentration-response curves to all agonists were shifted to the right in a competitive fashion. Schild regression analysis performed with SDZ 205,557 (1–10 μ M) and using 5-HT as the agonist, resulted in a Schild slope not significantly different from unity (1.24, 95% confidence limits 0.98–1.50) (Figure 4). When the unity constraint was imposed, a pA_2 value of 7.3 (95% confidence limits: 7.2–7.4) was determined. The apparent affinities ($-\log K_B$) obtained with a single concentration of SDZ 205,557 against the other agonists were similar to this value (Table 1). The exception to this was the affinity

Table 1 Potencies ($-\log EC_{50}$) and intrinsic activities (α) of compounds and affinity estimates for SDZ 205,557 ($-\log K_B$) at 5-HT₄ receptors mediating oesophageal relaxation

Compound	$-\log EC_{50}$	α	$-\log K_B$
5-HT	8.3 ± 0.03	1.0	7.4 ± 0.1
5-Methoxytryptamine	8.2 ± 0.03	1.1	6.9 ± 0.2
BIMU-1	7.9 ± 0.1	0.7	7.3 ± 0.2
SC-53116	7.7 ± 0.1	0.6	7.1 ± 0.3
BIMU-8	7.6 ± 0.2	0.9	7.1 ± 0.2
(S)-zacopride	6.7 ± 0.1	0.9	6.8 ± 0.3
(R)-zacopride	6.2 ± 0.2	0.9	$6.4 \pm 0.5^*$

Values are mean $\pm$ s.e.mean, $n = 4$ –8 animals. SDZ 205,557 was employed at a concentration of 10 μ M.

*Significantly different ($P < 0.05$) from $-\log K_B$ value estimated using 5-HT.

Figure 4 Schild analysis of antagonism by SDZ 205,557 of 5-hydroxytryptamine-mediated relaxation in rat oesophagus. Values are individual concentration-ratios.

when (R)-zacopride was used as the agonist. In this case the $-\log K_B$ value was significantly lower.

Tropisetron (3 μ M) antagonized the relaxations induced by 5-HT with a concentration-ratio of 35 (95% confidence limits, 27–45) and an apparent affinity ($-\log K_B$) of 7.0 ± 0.04 . SDZ 205,557 (3 μ M) antagonized responses to 5-HT with a concentration-ratio of 98 (41–237). In the presence of both SDZ 205,557 (3 μ M) and tropisetron (3 μ M), responses to 5-HT were shifted to the right with a combined concentration-ratio of 153 (99.8–234; mean, 95% confidence limits). This was not significantly different from that predicted by the combination of two competitive antagonists interacting at the same site (i.e. 133 fold).

Interactions at 5-HT₃ receptors

Experiments in guinea-pig isolated ileum were conducted in the presence of methysergide (1 μ M) and 5-methoxytryptamine (10 μ M) to desensitize selectively 5-HT₁, 5-HT₂ and 5-HT₄ receptors. At ileal 5-HT₃ receptors SDZ 205,557 (10 μ M) antagonized responses to 5-HT, with an apparent affinity ($-\log K_B$) of 6.2 ± 0.08 . Affinities for other 5-HT₃ antagonists reported by Eglen *et al.* (1990), under similar conditions are included for comparison (Table 2). The rank order of antagonist affinity at ileal 5-HT₃ receptors was therefore: (S)-zacopride > tropisetron > ondansetron > SDZ 205,557. In binding studies, at 5-HT₃ receptors identified by [³H]-quipazine in NG108-15 cell membranes (Table 3), SDZ

Table 2 Apparent affinity in competition radioligand binding and functional studies of compounds for 5-HT₃ receptors

	NG108-15 cells		Guinea-pig ileum ^a
	$-\log pK_i$	Hill coefficient	$-\log K_B$
(S)-zacopride	9.5 ± 0.1	1.1 ± 0.2	8.1
Ondansetron	8.4 ± 0.1	1.0 ± 0.1	6.9
Tropisetron	8.3 ± 0.4	0.9 ± 0.2	7.6
MDL-72222	7.6 ± 0.1	1.1 ± 0.1	ND
SDZ 205,557	6.9 ± 0.2	1.3 ± 0.1	6.2^b
Metoclopramide	6.7 ± 0.1	1.0 ± 0.1	ND

Binding values in NG108-15 cells are mean $\pm$ s.e.mean, $n = 3$ –4 separate determinations; functional values in guinea-pig ileum are mean $\pm$ s.e.mean, $n = 6$ animals.

^aEglen *et al.* (1990).

^bUnsurmountable.

ND = not determined.

Table 3 Potencies and maximum increase in heart rate to intravenous administration of 5-HT₄ agonists in the anaesthetized, bilaterally-vagotomized Yucatan micropig

Compound	ED ₅₀ ($\mu\text{g kg}^{-1}$)	Maximum tachycardic increase (beats min ⁻¹)
5-HT	4.2 (3.3–5.3)	95 (90–100)
Renzapride	20 ^a (17–25)	76 ^a (70–81)
(R,S)-zacopride	28 ^a (20–41)	100 (98–110)

Values are mean, with 95% confidence limits in parentheses, $n = 6$ animals.

^aSignificantly different ($P < 0.05$) compared to 5-HT.

205,557 displacement isotherms yielded an apparent affinity ($-\log K_i$) of 6.9 ± 0.2 . The Hill coefficient (1.3 ± 0.1) was not significantly different from unity, suggesting an interaction of SDZ 205,557 at a homogeneous population of sites. The rank order of apparent affinities was (S)-zacopride > ondansetron > tropisetron > MDL 72222 > SDZ 205,557 > metoclopramide. The values estimated in binding sites were greater than those estimated in functional studies (Table 2), in agreement with previous reports by Butler *et al.* (1990).

Tachycardia in anaesthetized pig

5-HT, renzapride and (R,S)-zacopride elicited a dose-dependent tachycardia in the anaesthetized pig with 5-HT being significantly more potent than either benzamide (Figure 5). Preliminary studies showed that consecutive, reproducible dose-response curves to 5-HT in the pig can be constructed at intervals of 90–120 min. Heart rate responses returned to baseline within 10–15 min after each dose of 5-HT, with responses treated cumulatively after successive doses of the benzamides. 5-HT and (R,S)-zacopride acted as full agonists

Figure 5 Dose-response curves for 5-hydroxytryptamine (5-HT, ○ and □), renzapride (■) and (R,S)-zacopride (●) in the anaesthetized, vagotomized micropig. Two curves for 5-HT are shown, conducted in separate series of experiments. The curve to renzapride was established in animals following construction of the 5-HT curve shown with (○), whereas the curve to (R,S)-zacopride was established following construction of the 5-HT curve shown with (□). Values are mean $\pm$ s.e.mean (vertical bars), $n = 6$ animals.

Figure 6 Time-course of the inhibition of tachycardic responses to 5-hydroxytryptamine (5-HT) by vehicle (●), tropisetron (▲) or SDZ 205,557 (○) in the anaesthetized, vagotomized micropig. Values are mean $\pm$ s.e.mean (vertical bars), $n = 6$.

zacopride acted as full agonists and renzapride as a partial agonist (Table 3). The responses to all these agonists were antagonized by tropisetron (data not shown). To assess the inhibitory activity of tropisetron and SDZ 205,557, single doses of the antagonists were administered against the ED₅₀ dose for 5-HT ($3\text{--}10 \mu\text{g kg}^{-1}$, i.v.), previously obtained by individual titration. The variances of the control and treatment groups did not differ sufficiently to utilize ranked changes from baseline; therefore a parametric repeated measures ANOVA was used to analyze this data. In these overall ANOVAs, the effects of treatment, time and treatment $\times$ time interaction were all highly significant ($P < 0.0001$). Subsequent pairwise contrasts (one-way ANOVAs) showed that SDZ 205,557, administered at 6.0 mg kg^{-1} , i.v., significantly ($P < 0.05$) inhibited the tachycardic response to 5-HT for only 3 min following administration whereas no significant effect was seen for the remainder of the experiment. In contrast, tropisetron (5 mg kg^{-1} , i.v.) administered against similar doses of 5-HT, significantly ($P < 0.05$) inhibited responses for 120 min following administration (Figure 6). The half-lives for the inhibitory responses were 23 (17–35) and 116 (85–175) min for SDZ 205,557 and tropisetron, respectively (values are mean with 95% confidence intervals).

Discussion

Receptors can be defined on the basis of selective antagonism. Tropisetron and DAU 6285 are mixed 5-HT₃ and 5-HT₄ receptor antagonists (Dumuis *et al.*, 1992). SDZ 205,557 may be useful in 5-HT₄ characterization since it exhibits a higher affinity at guinea-pig ileal 5-HT₄ receptors (7.5) relative to 5-HT₃ receptors (6.2; Buchheit *et al.*, 1991; 1992).

In guinea-pig hippocampal membranes, two 5-HT receptors are positively coupled to adenylyl cyclase (Shenkar *et al.*, 1987). At low 5-HT concentrations (1 nM – $1 \mu\text{M}$), stimulation is mediated by an, as yet undefined 5-HT_{1-like} receptor, whereas higher 5-HT concentrations interact with a 5-HT₄ receptor (Dumuis *et al.*, 1988). The 5-HT₁ receptor component is selectively blocked by spiperone and can be maximally stimulated by 5-CT at concentrations that fail to stimulate the 5-HT₄ receptor (Shenkar *et al.*, 1987; this study). The 5-HT₄ receptor component is selectively blocked

by tropisetron but not by spiperone (Dumuis *et al.*, 1988; this study). Selective inhibition by SDZ 205,557 of the 5-HT₄ but not the 5-HT₁ component was observed in the present study. At the former, SDZ 205,557 acted in a competitive surmountable fashion, as judged by the Schild slope. The pA₂ value was similar to the value reported at 5-HT₄ receptors mediating contraction of guinea-pig ileum (Buchheit *et al.*, 1992).

SDZ 205,557 in guinea-pig hippocampus and rat oesophagus lacked intrinsic activity and Buchheit *et al.* (1992) reported similar findings in guinea-pig ileum. In rat isolated oesophageal muscularis mucosae, relaxant responses to 5-HT₄ agonists were surmountably antagonized by SDZ 205,557 and this was also observed in the hippocampus. In this respect, these findings differ from those reported by Buchheit *et al.* (1992) in guinea-pig ileum, in which unsurmountable antagonism was evident. The reason for these differences in the kinetics of antagonism by SDZ 205,557 between 5-HT₄ receptors in ileum and oesophagus is unclear. The $-\log K_B$ values for SDZ 205,557 in oesophagus were, in general, also agonist-independent suggesting an interaction by these compounds at a common site (see below). Similar observations have been made with tropisetron (Baxter *et al.*, 1991) or DAU 6285 (Waikar *et al.*, 1992) in this tissue. In guinea-pig ileum (Buchheit *et al.*, 1992), lower pA₂ values were observed for SDZ 205,557 against (R,S)-zacopride (6.8) and metoclopramide (5.4), in comparison to those against 5-HT (7.4). The reason for the agonist-independence in rat oesophagus (this study) but not in guinea-pig ileum (Buchheit *et al.*, 1992) is unclear, but the unsurmountable nature of the antagonism in the latter makes direct comparison difficult. SC-53116 is a substituted pyrrolizidine reported to be a potent and selective 5-HT₄ agonist in the rat oesophagus (Flynn *et al.*, 1992). The antagonism by SDZ 205,557 of responses to SC-53116 on the rat oesophageal muscularis mucosae (this study) is in agreement with this suggestion, since it was antagonized with a similar affinity to that observed against 5-HT.

The lower $-\log K_B$ value against (R)-zacopride observed in the present study is difficult to explain although Buchheit *et al.* (1992) reported a lower affinity for SDZ 205,557 in guinea-pig ileum when (R,S)-zacopride was used as the agonist. Combination concentration-ratio experiments (Paton & Rang, 1965) however, showed that SDZ 205,557 and tropisetron interacted at the same oesophageal site (this study) since the observed combined concentration-ratio was not significantly different from the predicted combined ratio.

The affinity of SDZ 205,557 for 5-HT₃ receptors in NG108-15, cells estimated in binding studies, was similar to the affinity obtained at 5-HT₄ receptors in guinea-pig ileum (Buchheit *et al.*, 1991; 1992), hippocampal adenylyl cyclase or

rat oesophagus (this study; Tables 1 and 3). In contrast, the affinity at the 5-HT₃ receptor in guinea-pig ileum was lower (Buchheit *et al.*, 1992; this study), a feature shared by other antagonists at this site (Table 2). Indeed, using the novel 5-HT₃ receptor ligand [³H]-RS-42358-197 to label the 5-HT₃ site in guinea-pig ileum, SDZ 205,557 gave a pK_i of 7.3 and Hill coefficient of 1.04 (Wong & Stefanich, unpublished data). These disparities suggest that the guinea-pig possesses a species variant of the 5-HT₃ receptor (see Kilpatrick & Tyers, 1992, for review; Wong *et al.*, 1992). Apart from the guinea-pig, therefore, SDZ 205,557 had equal affinity at 5-HT₃ and 5-HT₄ receptors. When using SDZ 205,557, appropriate care should therefore be taken to exclude 5-HT₃ function in bioassays in which both 5-HT₃ and 5-HT₄ receptors are involved.

In the anaesthetized, vagotomized micropig, 5-HT elicited a tachycardic response in the presence of 5-HT₁, 5-HT₂, 5-HT₃, M₂-muscarinic and β -adrenoceptor blockade. These responses were antagonized by high doses of tropisetron and mimicked by (R,S)-zacopride and renzapride. Similar observations have been reported by Villalon *et al.* (1990) in the Yorkshire pig and concur with biochemical data showing that porcine myocardial 5-HT₄ receptors stimulate adenylyl cyclase (Kaumann, 1990). The tachycardia may be due to subsequent activation of a kinase (Kaumann *et al.*, 1991) and closure of potassium channels (Bockaert *et al.*, 1992). 5-HT-induced tachycardia in the micropig, thus, provides a suitable *in vivo* assay to study 5-HT₄ compounds. The administration of SDZ 205,557 failed to antagonize, except briefly (3 min) after injection, the tachycardic responses to 5-HT. The doses of 5-HT were submaximal and adequately blocked by tropisetron. The short-lived antagonism by SDZ 205,557 *in vivo* contrasts with the sustained antagonism seen *in vitro* (at least 60 min; see Methods). The compound, at least at the dose tested, appears to be subject to rapid metabolism, probably due to hydrolysis at the ester moiety in the SDZ 205,557 molecule. It should be noted, however, that such rapid degradation may not be so marked at higher doses and additional experiments are required to study this.

In conclusion, SDZ 205,557 acted as a surmountable 5-HT₄ receptor antagonist in guinea-pig hippocampus and rat oesophagus, although it had no selectivity between mouse neuroblastoma 5-HT₃ and 5-HT₄ receptors. In guinea-pig, a 5-HT₄/5-HT₃ selectivity was evident due to the atypical nature of the guinea-pig 5-HT₃ receptor. The short duration of action *in vivo* together with this limited selectivity, suggests that caution should be exercised in its use to define the 5-HT₄ receptor.

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