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Interaction of S 21007 with 5-HT_3 receptors. In vitro and in vivo characterization

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Abstract

The interaction of S 21007 [5-(4-benzyl piperazin-1-yl)4H pyrrolo[1,2-a]thieno[3,2-e]pyrazine] with serotonin 5-HT_3 receptors was investigated using biochemical, electrophysiological and functional assays. Binding studies using membranes from N1E-115 neuroblastoma cells showed that S 21007 is a selective high affinity (IC_{50} = 2.8 nM) 5-HT_3 receptor ligand. As expected of an agonist, S 21007 stimulated the uptake of [^{14}C]guanidinium (EC_{50} ~ 10 nM) in NG 108-15 cells exposed to substance P, and this effect could be prevented by the potent 5-HT_3 receptor antagonist ondansetron. In addition, like 5-HT and other 5-HT_3 receptor agonists (phenylbiguanide and 3-chloro-phenylbiguanide), S 21007 (EC_{50} = 27 μM) produced a rapid inward current in N1E-115 cells. The 5-HT_3 receptor agonist action of S 21007 was also demonstrated in urethane-anaesthetized rats as this drug (120 μg/kg i.v.) triggered the Bezold–Jarisch reflex (rapid fall in heart rate), and this action could be prevented by pretreatment with the potent 5-HT_3 receptor antagonist zacopride. Finally, in line with its 5-HT_3 receptor agonist properties, S 21007 also triggered emesis in the ferret. Evidence for 5-HT_3 receptor antagonist-like properties of S 21007 was also obtained in some of these experiments since previous exposure to this compound prevented both the 5-HT-induced current in N1E-115 cells and the Bezold–Jarisch reflex elicited by an i.v. bolus of 5-HT (30 μg/kg) in urethane-anaesthetized rats. These data suggest that S 21007 is a selective 5-HT_3 receptor agonist which can exhibit antagonist-like properties either by triggering a long lasting receptor desensitization or by a partial agonist activity at 5-HT_3 receptors in some tissues.

Keywords: 5-HT_3 receptor; 5-HT_3 receptor agonist; N1E-115 cells; NG 108-15 cells; Bezold–Jarisch reflex; [^{14}C]guanidinium; Emesis; Inward current; (Rat); (Ferret)

1. Introduction

The 5-HT_3 receptor, first characterized as the 5-HT-M receptor (Richardson et al., 1985), differs from the other types of 5-HT receptors which all belong to the G-protein-coupled superfamily because it is the only 5-HT receptor which is a ligand-gated cation channel (Derkach et al., 1989). The 5-HT_3 receptor is exclusively localized on neurons both in the central (Waeber et al., 1989; Yakel et al., 1991) and the peripheral nervous system (Fozard, 1984a; Wallis, 1989).

Numerous selective antagonists are currently available for in vitro and in vivo studies on 5-HT_3 receptors. Among them bemesetron (Fozard, 1984b), tropisetron (Richardson et al., 1985), ondansetron (Butler et al., 1988), granisetron (Sanger and Nelson, 1989) and zacopride (Smith et al., 1988) have been the subject of extensive investigations which revealed their anti-emetic and anxiolytic-like properties in different animal models. In contrast, only few 5-HT_3 receptor agonists such as mono- and dichloro-derivatives of phenylbiguanide have been developed to date (Ireland and Tyers, 1987; Kilpatrick et al., 1990a; Kilpatrick and Tyers, 1992). Furthermore, these drugs do not generally cross the blood brain barrier, which makes their use for investigating the functional consequences of central

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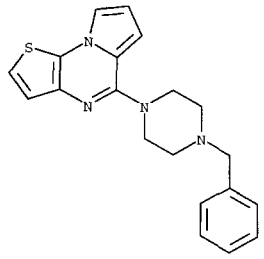

Fig. 1. Chemical structure of S 21007 [5-(4-benzyl piperazin-1-yl)4H pyrrolo[1,2-a]thieno[3,2-e]pyrazine.

5-HT_3 receptor stimulation particularly difficult. However, recently a novel agonist acting both at central and peripheral 5-HT_3 receptors, SR 57227A (4-amino-(6-chloro-2-pyridyl)-1-piperidine hydrochloride) has been shown to cross the blood brain barrier (Bachy et al., 1993). Interestingly, studies with this compound suggested that the stimulation of central 5-HT_3 receptors has potential antidepressant effects (Keane et al., 1994; Poncelet et al., 1995).

Previous studies in our laboratory have shown that S 21007 [5-(4-benzyl piperazin-1-yl)4H pyrrolo[1,2-a]thieno[3,2-e]pyrazine] (Fig. 1) is a high affinity ligand at 5-HT_3 receptors (Rault et al., 1996) that is structurally different from the above mentioned 5-HT_3 receptor agonists and antagonists but with some structural analogy with quipazine, a less selective 5-HT_3 ligand. In addition, this compound exerts potent anxiolytic-like effects in animal models of anxiety such as the light/dark box procedure (Rault et al., 1996), indicating its ability to act in the central nervous system. The structural analogy with quipazine described as a 5-HT_3 receptor antagonist in peripheral models (Ireland and Tyers, 1987; Round and Wallis, 1987) or as a 5-HT_3 receptor agonist in NG 108-15 cells (Emerit et al., 1993; Bartrup and Newberry, 1996) led us to perform appropriate in vitro and in vivo studies in order to assess the potential agonist and/or antagonist action of S 21007 at 5-HT_3 receptors.

2. Materials and methods

2.1. Chemicals

S 21007 was synthesized as described elsewhere (Rault et al., 1996). 3-Chloro-phenylbiguanide (3-Cl-PBG) was synthesized at the Institut de Recherches Internationales Servier, Courbevoie, France. [^{3}H]granisetron, [^{3}H][8-hydroxy-2-(*N*,*N*-dipropylamino)tetralin], [^{3}H]5-HT, [^{3}H]ketanserin were obtained from New England Nuclear (Du Pont de Nemours). [^{3}H]mesulergine and [^{3}H]GR113808 ([1-[2-([^{3}H]methylsulphinyl amino)ethyl]-4-piperidenyl] methyl 1-methyl-1*H*-indole-3-carboxylate) were purchased from Amersham (France). Buspirone, spiperone, pargyline, phenylbiguanide (PBG), mesulergine, propranolol, 5-HT, WB4101 (2-(2,6-dimethoxyphenoxyethyl-4-benzodioxane)) were obtained from Sigma-Aldrich (France). Quipazine was obtained from Research Biochemicals (USA), mianserin from Pharmastra (France), cisplatin from Roger Bellon Laboratories (France). Zacopride was generously given by Delalande-Synthelabo Laboratories and ondansetron by Glaxo. [^{14}C]guanidinium (59 Ci/mmol) was a gift from Commissariat à l'Energie Atomique (France).

2.2. In vitro studies

2.2.1. 5-HT_3 receptor binding assay

2.2.1.1. Cell cultures. N1E-115 neuroblastoma cells (gift of Dr. Vijverberg, University of Utrecht, The Netherlands) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% decomplemented fetal calf serum, 2 mM glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin. For binding studies, cells were grown in 75 cm^2 culture flasks.

2.2.1.2. Membrane preparation. The method was based upon that described by Hoyer and Neijt (1987). At confluency, the cells were harvested by scraping into Earle's balanced salt solution (Gibco) and centrifuged at 200 × *g* for 5 min at 4°C. The cell pellet was resuspended in Tris buffer (20 mM Tris–HCl, 154 mM NaCl, pH 7.5), homogenized with a Polytron blender (setting 9; 2 × 15 s at 4°C), and centrifuged at 900 × *g* for 8 min at 4°C. This procedure was repeated twice. The final pellet was discarded and membranes in the supernatant were kept at −70°C until used. Protein concentrations were determined according to the method of Bradford (1976).

2.2.1.3. Inhibition studies. Aliquots (~ 0.5 mg protein) of membrane suspensions were mixed with 50 mM HEPES-Na^+ buffer, pH 7.5, 1 nM [^{3}H]granisetron (80 Ci/mmol), with or without increasing concentrations of competing drugs in a total volume of 0.5 ml. Samples were incubated at room temperature for 20 min, and then rapidly filtered using a Brandel cell harvester, through Whatman GF/B filters that had been presoaked in 0.1% polyethylenimine. Filters were washed with 3 × 5 ml of ice-cold 50 mM Tris–HCl buffer, pH 7.4, dried and immersed in vials containing 4 ml of Picofluor 40 scintillation cocktail (Packard). Radioactivity was quantified by liquid scintillation counting. Nonspecific binding was determined in the presence of 10 μM ondansetron. Binding data were calculated using the computer program Lundon 2 (Lundon Software, Cleveland, OH, USA).

2.2.2. Other 5-HT receptor binding assays

5-HT_{1A} receptor binding to bovine hippocampal membranes was assayed essentially as described by Hoyer et al. (1985). Membranes were incubated at 23°C with 0.5 nM

[^{3}H]8-OH-DPAT (143 Ci/mmol). Non-specific binding was determined in the presence of 10 μM buspirone.

5-HT_{1B} receptor binding to rat frontal cortex membranes was assayed according to the procedure of Peroutka (1986). Membranes were incubated at 25°C with 2 nM [^{3}H]5-HT (25.5 Ci/mmol), 1 μM spiperone and 50 nM mesulergine. Non-specific binding was determined in the presence of 10 μM propranolol.

5-HT_{1D} receptor binding to pig frontal cortex membranes was assayed following the protocol of Waeber et al. (1990). Membranes were incubated at 25°C with 2 nM [^{3}H]5-HT (25.5 Ci/mmol), 1 μM spiperone and 50 nM mesulergine. Non-specific binding was determined in the presence of 10 μM 5-HT.

5-HT_{2A} receptor binding to bovine frontal cortex membranes was assayed according to Leysen et al. (1982). Membranes were incubated at 37°C with 0.8 nM [^{3}H]ketanserin (61.9 Ci/mmol) and 100 nM WB4101. Non-specific binding was determined in the presence of 10 μM spiperone.

5-HT_{2C} receptor binding to pig choroid plexus membranes was assayed as described by Sanders-Bush and Breeding (1988). Membranes were incubated at 25°C with 1.2 nM [^{3}H]mesulergine (80 Ci/mmol) and 1 μM spiperone. Non-specific binding was determined in the presence of 10 μM mianserin.

5-HT_4 receptor binding to pig hippocampal membranes was assayed according to Grossman et al. (1993). Membranes were incubated at 37°C with 0.1 nM [^{3}H]GR113808 (85 Ci/mmol). Non-specific binding was determined in the presence of 30 μM 5-HT.

K_i values were calculated using the computer program Lundon 2.

2.2.3. Electrophysiological recording

For electrophysiological experiments, N1E-115 cells were plated on glass coverslips in 35 mm culture dishes at a density of 50,000 cells/dish. The recording sessions were carried out 3–7 days after plating. Currents were recorded in the whole-cell mode, using standard patch-clamp techniques (Hamill et al., 1981). Patch pipettes were made from 1.5 mm (o.d.) glass electrodes in two stages using a BB CH (Mecanex) puller and were filled with a filtered (0.2 μm Millipore filter) solution containing 130 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES, and 10 mM EGTA, pH adjusted to 7.15 with KOH. N1E-115 cells were continuously superfused at a flow rate of 2 ml/min with a solution made of 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES, pH adjusted to 7.4 with NaOH. Cells were viewed with an inverted microscope with phase-contrast optics. Experiments were performed at 20–25°C. Only patches with seals of ≥ 3 MΩ were used.

Drugs were applied locally from perforated tubes positioned close to the cells. Current was monitored at -65 mV to calibrate the rate of solution exchange. The application of saline solution supplemented with 40 mM KCl led to an inward current across the cell membrane. The time from the onset of the inward current to the establishment of a new stable value was taken as the time for complete solution exchange. This time was regularly of 90 ms.

Concentration-response curves were obtained by sequentially applying increasing concentrations of drugs. Extensive washing for 15 min was managed between applications of successive concentrations of a given drug. The response was evaluated as the peak of inward current in cells voltage-clamped at -65 mV. Each concentration-response curve was fitted to the logistic equation $I = I_{max} \times [\text{agonist}]/(EC_{50} + [\text{agonist}]^{n_H})$, where n_H represents the Hill coefficient, by a nonlinear least-square curve-fitting program. In inhibition studies, S 21007 was bath-applied 2 min prior to, during and after the application of 5-HT. Voltage-clamp experiments (voltage protocol generation and data storage and analysis) were performed using an amplifier (Biologic RK300) connected by an interface (Axon Instruments) and an analog–digital converter (Scientific Solutions) to a microcomputer (IBM PC-AT) equipped with appropriate software (pClamp; Axon Instruments).

2.2.4. [^{14}C]guanidinium influx into NG 108-15 cells in the presence of substance P

Mouse neuroblastoma × rat glioma NG 108-15 hybrid cells were cultured as described (Miquel et al., 1990). Cells were grown in DMEM supplemented with 40 mM sodium bicarbonate, 1.8 mM L-glutamine, 10% decomplemented fetal calf serum (Gibco) and HAT (100 μM hypoxanthine, 1 μM aminopterine, 16 μM thymine) (Gibco) and subcultured every 2 days.

Before the experiment was started, the cell layer was washed twice with 1.5 ml of buffer A (145 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.0 mM Na_2HPO_4, 20 mM glucose and 20 mM HEPES, pH 7.4). The protocol was then as described by Emerit et al. (1993). Briefly, the incubation (10 min at 37°C) was performed in 1 ml of buffer B (135 mM NaCl, 4.5 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.0 mM Na_2HPO_4, 20 mM glucose, 20 mM HEPES, pH 7.4) supplemented with 10 mM guanidinium chloride, 200–250 nCi (7.40–9.25 kBq) [^{14}C]guanidinium, 10 μM substance P and the appropriate drugs. Assay was stopped by aspiration of the medium, and the cell layer was washed 3 times with 1.5 ml of ice-cold buffer C (same composition as buffer A except that NaCl was replaced by choline chloride). The cells were then dissolved in 0.5 ml of 0.4 M NaOH and transferred to scintillation vials. The culture dishes were rinsed with 0.5 ml 1 M HCl then 0.5 ml 0.4 M NaOH, which were mixed with the first extract for determination of radioactivity in the presence of 10 ml Aquasol (New England Nuclear). For each experiment, the protein content of a control dish was determined as above.

2.3. Studies in vivo

2.3.1. Bezold–Jarisch reflex in rats

Male Crl: CD(SD)BR rats (Charles River, Saint-Aubin-Les-Elbeuf) weighing 280–320 g were fasted for 24 h and then anaesthetized with urethane (1.25 g/kg i.p.).

In order to monitor the Bezold–Jarish reflex (an abrupt fall in heart rate following a rapid i.v. bolus injection of 5-HT, 30 μg/kg), the carotid artery was cannulated and connected to a Statham transducer, as described by Fozard (1984b).

Heart rate and blood pressure were measured using the pressure transducer and a cardiotachometer coupler, and recorded onto a Gemini polygraph (Ugo Basile, Italy).

Drugs were dissolved in 0.9% NaCl and administered intraperitoneally or intravenously (0.5 ml/kg) via a cannula placed in the jugular vein.

2.3.2. Emetic / antiemetic effects in the ferret

Male ferrets (Marshall Farms, North Rose, NY, USA) weighing 1–2 kg were housed individually and fed ad libitum prior to being used in the study. The basic procedure was that of Sancilio et al. (1991).

2.3.2.1. Emetic activity. Ferrets fasted the day before the experiments were given free access to food and water ad libitum for 30 min. Sixty minutes later, each animal received by gavage the vehicle (H_2O) followed by increasing doses of S 21007 (0.001, 0.01, 0.1, 1 mg/kg in a volume of 5 ml/kg) with intervals of at least 1 h until the emesis appeared. The dose of 10 mg/kg of S 21007 was studied 48 h later in the same animals. Two animals were treated following the same protocol with cisplatin injected intraperitoneally at 10 mg/kg in a volume of 20 ml/kg. In all cases, the last treatment was followed by two hours of observation. An emetic episode was defined as rhythmic abdominal contractions followed by the expulsion or attempt to expulse the stomach content. The number of contractions and emetic episodes were recorded.

2.3.2.2. Antiemetic activity. Ferrets were randomized into four groups of six animals treated by gavage (5 ml/kg) either with H_2O or S 21007 at the doses of 0.001, 0.01 or 0.1 mg/kg after one night of fasting, 30 min of free access to food and 30 min of fasting. Cisplastin was given intraperitoneally at 10 mg/kg in a volume of 20 ml/kg 30 min after the administration of S 21007. Animals were then observed for 5 h.

3. Results

3.1. Binding of S 21007 to various 5-HT receptors

Inhibition studies with N1E-115 cell membranes revealed an IC_{50} value of 2.8 ± 0.3 nM (mean ± S.E.M., $n = 4$ independent experiments) and a Hill number of 1.02 ± 0.20 (mean ± S.E.M., $n = 4$) for S 21007 at 5-HT_3 receptors labelled by [^{3}H]granisetron. Under the same experimental conditions, the IC_{50} value of 5-HT was equal to 140 ± 20 nM. As shown in Table 1, ondansetron, quipazine, 3-Cl-PBG and PBG also inhibited [^{3}H]granisetron binding to N1E-115 cell membranes. Interestingly, the nH value of ondansetron was also close to unity, whereas that of the other tested compounds was regularly higher, between 1.30 and 1.55 (Table 1).

Binding assays with various radioligands indicated that S 21007 had only a low affinity for other 5-HT receptors, with IC_{50} values ≥ 1.0 μM in assays for 5-HT_{1A} (IC_{50} = 4.1 ± 0.4 μM), 5-HT_{1B} (IC_{50} = 1.0 ± 0.2 μM), 5-HT_{1D} (IC_{50} = 14.1 ± 2.6 μM), 5-HT_{2A} (IC_{50} = 4.5 ± 1.0 μM), 5-HT_{2C} (IC_{50} = 1.9 ± 0.5 μM) and 5-HT_4 (IC_{50} = 2.3 ± 0.8 μM) binding sites.

3.2. Effects of S 21007 on [^{14}C]guanidinium uptake in NG 108-15 cells

The high affinity and selectivity of S 21007 for 5-HT_3 receptors led to the characterization of its agonist/antagonist activity using in vitro and in vivo assays. In the presence of 10 μM substance P, S 21007 increased the uptake of [^{14}C]guanidinium into NG 108-15 cells with an EC_{50} value of approximately 10 nM, and to the same maximal extent as that observed with a saturating concentration (1 μM) of 5-HT (see Emerit et al., 1993) (Fig. 2A). As illustrated in Fig. 2B, the stimulatory effect of 1 μM S 21007 on [^{14}C]guanidinium uptake could be prevented in a concentration-dependent manner by ondansetron. S 21007, from 0.1 nM to 1 μM, did not prevent the [^{14}C]guanidinium uptake induced by 5-HT (1 μM) in the presence of substance P (10 μM) (Fig. 2A).

3.3. Electrophysiological effects of S 21007 in N1E-115 cells

Electrophysiological studies in N1E-115 cells showed that S 21007 was able to induce an inward current with an EC_{50} value of 27 μM (Fig. 3). Under the same experimental conditions, 5-HT also induced an inward current, but

Table 1
IC_{50} and Hill coefficient values of S 21007 and different 5-HT_3 receptor ligands as inhibitors of the specific binding of [^{3}H]granisetron to N1E-115 cell membranes

Drugs	IC_{50} (nM)	Hill coefficient
S 21007	2.8 ± 0.3	1.02 ± 0.02
Ondansetron	7.1 ± 0.8	0.90 ± 0.04
Quipazine	9.1 ± 2.8	1.30 ± 0.03
3-Cl-PBG	43 ± 8	1.55 ± 0.12
5-HT	140 ± 20	1.30 ± 0.05
PBG	1900 ± 400	1.41 ± 0.23

Each value is the mean ± S.E.M. of 4 independent determinations.

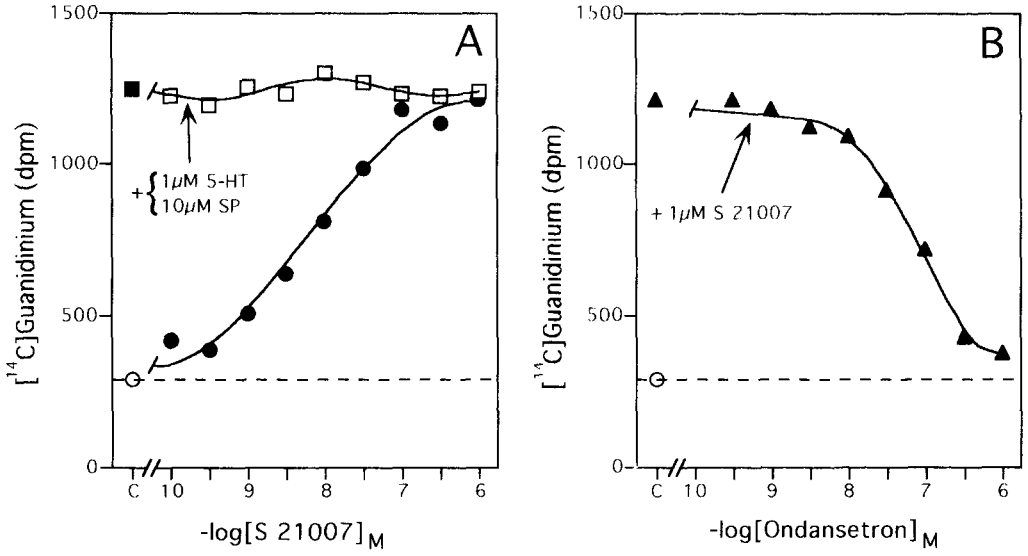

Fig. 2. (A) Concentration-dependent effect of S 21007 alone (●) or in the presence of 1 μM 5-HT (□) on the accumulation of [^{14}C]guanidinium in NG 108-15 cells exposed to 10 μM substance P. Data are expressed in dpm of [^{14}C]guanidinium taken up per culture dish (i.e. ~ 10^5 cells). Each point is the mean of triplicate determinations with less than 5% variations between them. C on abscissa: (control): assays without S 21007. (B) Concentration-dependent inhibition by ondansetron of [^{14}C]guanidinium uptake in NG 108-15 cells exposed to 1 μM S 21007 plus 10 μM substance P. The dotted line represents the basal (non-stimulated) uptake of [^{14}C]guanidinium in NG 108-15 cells incubated without S 21007, 5-HT and ondansetron.

with an EC_{50} value of 2.2 μM, whilst the two 5-HT_3 receptor agonists PBG and 3-Cl-PBG were characterized by EC_{50} values of 22 μM and 0.76 μM, respectively. To determine the full or partial agonist character of S 21007, the current amplitude induced by a saturating concentration of S 21007 (100 μM) or 5-HT (10 μM) was measured on the same cell. The inward current induced by S 21007 was 45.0 ± 8.3% (mean ± S.E.M., $n = 7$) of that induced by 5-HT showing that S 21007 behaved as a partial agonist in this preparation. To test S 21007 for possible antagonist properties, the drug was applied at concentrations where it had no effect on its own 2 min prior to 5-HT (10 μM), and the current amplitude was compared to that induced by 5-HT alone. As shown in Fig. 4, the current induced by 5-HT was completely blocked by 1 μM S 21007 whereas, at 0.1 μM, the latter drug had no inhibitory effect.

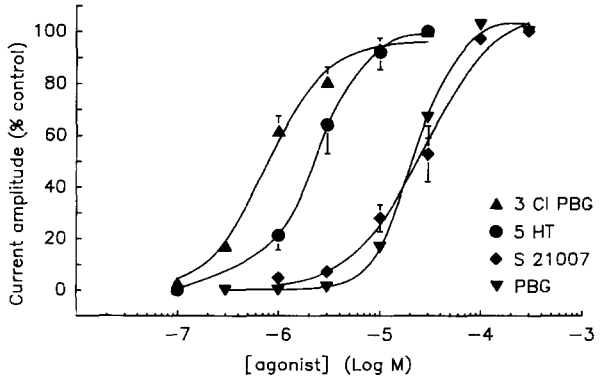

Fig. 3. Concentration–response curves of S 21007 and different 5-HT_3 receptor agonists as inducers of an inward current in N1E-115 cells. The membrane potential was continuously maintained at −65 mV. The current amplitude is expressed as a percentage of the maximal value reached for each drug. Each point is the mean ± S.E.M. of 6 independent determinations.

3.4. Bezold–Jarisch reflex

In anaesthetized rats, S 21007 induced at 120 μg/kg i.v., but not at 30 and 60 μg/kg i.v., a rapid (i.e., within 2

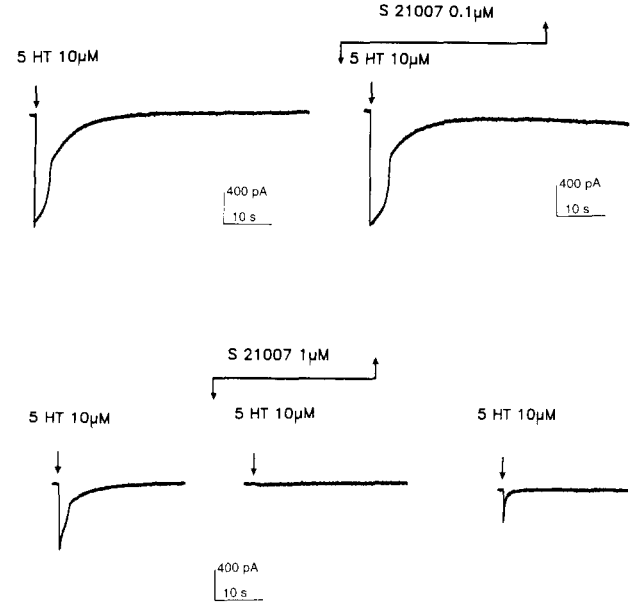

Fig. 4. Effects of S 21007 at 0.1 μM and 1 μM on the inward current induced by 10 μM 5-HT in N1E-115 cells. The membrane potential was continuously maintained at −65 mV. Upper and lower tracings are from two different cells. The time interval between the successive tracings for a given cell was 15 min.

Table 2
Effects of pretreatment with S 21007 on the bradycardia induced by 5-HT (30 μg/kg i.v.) in urethane-anaesthetized rats

S 21007 pretreatment (μg/kg i.v.)	5-HT (μg/kg i.v.) 5 min later	Reduction of heart rate (%)
0	30	−59±2
30	30	−58±3
60	30	−32±3 [a]
120	30	−9±4 [a]

The fall in heart rate due to 5-HT is expressed as a percentage of basal heart rate (320±18 beats/min, mean±S.E.M., $n=16$), prior to the administration of S 21007. Each value is the mean S.E.M. of at least 4 independent determinations.
[a] $P < 0.05$ as compared with the decrease in heart rate induced by 5-HT alone.

s) decrease in heart rate (−40±6% as compared to basal heart rate) as well as a biphasic decrease of arterial blood pressure characteristic of the Bezold–Jarisch reflex. Pretreatment with zacopride (1 μg/kg i.v.) 5 min prior to S 21007 (120 μg/kg i.v.) completely suppressed the fall in heart rate normally evoked by the latter drug (not shown). Contrary to 5-HT, whose bradycardiac and hypotensive effects could be reproduced with bolus injections at 5–10 min intervals, the efficiency of S 21007 to decrease heart rate and blood pressure disappeared rapidly, since a second or third administration 10 or 30 min after the first one was no longer able to affect cardiovascular parameters. Furthermore, S 21007 pretreatment (at 60 and 120 μg/kg i.v.), 5 min prior to 5-HT (30 μg/kg i.v.), significantly reduced the effect of the indoleamine on heart rate (Table 2) but not on blood pressure (not shown).

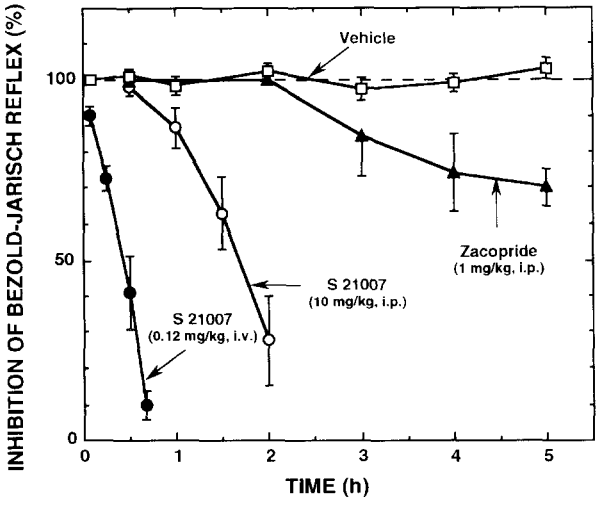

Fig. 5. Duration of the inhibition by S 21007 (i.v. or i.p.) and zacopride (i.p.) of the Bezold–Jarisch reflex evoked by 5-HT (30 μg/kg i.v.) in urethane-anaesthetized rats. S 21007, zacopride or the vehicle (0.9% NaCl) were administered at time 0 (abscissa) and 5-HT was injected at various times thereafter. The decrease in the amplitude of bradycardia due to 5-HT is expressed as a percentage (ordinate) of the maximal fall in heart rate evoked by the indoleamine prior to the administration of S 21007, zacopride or vehicle. Each point is the mean±S.E.M. of at least 4 independent determinations.

Considering the transient agonist effect of S 21007 only at high doses, its potential antagonist properties were studied in more detail. S 21007 at the dose of 120 μg/kg i.v. was able to significantly reduce the negative inotropic effect of 5-HT (30 μg/kg i.v.) during 30 min (Fig. 5). S 21007 was also active when injected i.p. at the dose of 10 mg/kg, and via this route totally antagonized 5-HT (30 μg/kg i.v.)-induced bradycardia for almost 1 h (Fig. 5). A progressive recovery of the 5-HT effect on heart rate was subsequently noted, with a response reaching ~70% of that evoked by 5-HT alone 2 h after the administration of S 21007 (Fig. 5).

Under the same experimental conditions, zacopride at 1 mg/kg i.p. still markedly reduced the effect of 5-HT on heart rate after 5 h (Fig. 5).

3.5. Emesis reflex in the ferret

In a last set of experiments, the effects of S 21007 alone or in combination with cisplatin were examined on the emesis reflex in ferrets. As shown in Table 3, per os administration of S 21007 at 1 and 10 mg/kg induced emesis within 20–25 min after the treatment. However, at 1, 10 and 100 μg/kg, the drug was inactive on its own and did not antagonize the emetic activity of cisplatin (10 mg/kg i.p.). Indeed, S 21007 at 10 and 100 μg/kg even potentiated the effect of cisplatin by increasing the number of emetic episodes as compared to cisplatin alone. However, S 21007 had no significant effect on the latency (90–100 min) before the first emesis episodes due to cisplatin (Table 3).

Table 3
Emetic effects of S 21007 and/or cisplatin in ferrets

S 21007 (mg/kg p.o.)	Cisplatin (mg/kg i.p.)	N [a]	Onset [b]	Episodes [c]
A				
0.001	0	0/8	–	–
0.01	0	0/8	–	–
1	0	4/8	18	3
10	0	8/8	26	7
B				
0	10	6/6	103	21
0.001	10	6/6	92	30
0.01	10	6/6	87	42 [d]
0.1	10	6/6	91	35 [d]

(A) Animals were observed for 2 h after the administration of various doses of S 21007 per os. (B) S 21007 was administered at various doses per os 30 min prior to the i.p. injection of 10 mg/kg cisplatin. Animals were observed for 5 h after the latter treatment
[a] Number of ferrets presenting emetic episodes/total number of ferrets.
[b] Mean interval (in min) between the injection of S 21007 (A) or cisplatin (B) and the first emesis episode. [c] Mean number of emetic episodes.
[d] $P < 0.05$ as compared with ferrets treated with cisplatin alone (Mann–Whitney's test).

4. Discussion

As expected of a selective high affinity 5-HT_3 receptor ligand, S 21007 inhibited the specific binding of [^{3}H]granisetron to membranes from N1E-115 neuroblastoma cells with an IC_{50} value of 2.8 nM, whereas micromolar concentrations of this drug were necessary to affect the specific binding of appropriate radioligands to other 5-HT receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_4). In addition, S 21007 was essentially inactive (IC_{50} > 1.0 μM) at a large series of receptors including α- and β-adrenoceptor, dopamine, muscarinic and benzodiazepine receptors (Rault et al., 1996). As compared to the 5-HT_3 receptor ligands presently available (Kilpatrick et al., 1990b; Kilpatrick and Tyers, 1992; Zifa and Fillion, 1992; Morain et al., 1994), S 21007 is therefore among the most selective and potent of these drugs.

Binding studies with [^{3}H]granisetron have previously been shown to allow the distinction between 5-HT_3 receptor agonists and antagonists as inhibition studies yielded an apparent Hill coefficient significantly higher than unity with agonists, and, in contrast, close to 1.0 with antagonists (Barnes et al., 1992a; Barnes and Barnes, 1993). Using membranes from N1E-115 cells, we confirmed this observation as the nH value for the inhibition of the specific binding of [^{3}H]granisetron by all the 5-HT_3 receptor agonists tested, 5-HT, PBG and 3-Cl-PBG, was higher than 1.0, whereas an nH value not significantly different from one was found with the potent 5-HT_3 receptor antagonist, ondansetron. Interestingly, quipazine also yielded an nH value higher that 1.0 indicating that it bound to 5-HT_3 receptors on N1E-115 cells as an agonist, in agreement with other functional studies (Emerit et al., 1993). In contrast, S 21007 inhibited [^{3}H]granisetron binding with an nH value close to 1.0, suggesting that it behaved as a 5-HT_3 receptor antagonist in this assay. Although S 21007 (see Fig. 1) and quipazine are structurally related, their interaction with 5-HT_3 receptor binding sites on N1E-115 cells was therefore fundamentally different.

Further studies on the agonist/antagonist actions of S 21007 at 5-HT_3 receptors were then performed using appropriate in vitro and in vivo tests.

In both N1E-115 (Bönisch et al., 1993) and NG 108-15 cells (Emerit et al., 1993), 5-HT_3 receptor stimulation has previously been shown to trigger a cation conductance allowing the intracellular accumulation of [^{14}C]guanidinium. Under optimal conditions for this response to occur (i.e. in the presence of 1 μM substance P, see Emerit et al., 1993), S 21007 mimicked the effect of 5-HT and other 5-HT_3 receptor agonists by markedly enhancing, in a concentration-dependent manner, the accumulation of [^{14}C]guanidinium in NG 108-15 cells. Maximal effect was noted with 1 μM of S 21007. At this concentration, the effect of S 21007 was equal to and not additive with that of a saturating concentration of 5-HT (1 μM). As expected of an effect through the stimulation of 5-HT_3 receptors, the enhanced accumulation of [^{14}C]guanidinium due to S 21007 was dose-dependently inhibited by the potent and selective 5-HT_3 receptor antagonist, ondansetron (Kilpatrick et al., 1990b). Therefore, in this test, S 21007 behaved as a full 5-HT_3 receptor agonist.

Another set of in vitro investigations consisted of recording the electrophysiological consequences of S 21007 application on N1E-115 cells using a patch clamp technique. Like 5-HT, S 21007 induced an inward current in these cells. However, at maximal stimulation, the amplitude of the current due to S 21007 (100 μM) reached only 45.0 $\pm$ 8.3% of that evoked by 5-HT (10 μM), indicating that S 21007 behaved as a partial agonist. As expected of such an action, S 21007 was able to markedly reduce the electrophysiological response of N1E-115 cells to 5-HT.

Comparison of the affinity of S 21007 for the 5-HT_3 binding sites in N1E-115 cell membranes, the 5-HT_3 receptor mediating [^{14}C]guanidinium uptake in NG 108-15 cells, and the 5-HT_3 receptor whose activation induced an inward current in N1E-115 cells revealed marked differences with IC_{50} and EC_{50} values equal to 2.8 nM, 10 nM and 27 μM, respectively. Similar differences were already noted about the interaction of another potent agonist, SR 57227A, with 5-HT_3 receptors in various preparations (Bachy et al., 1993). One possible explanation for these differences stems from the existence of variations in the pharmacological and functional properties of 5-HT_3 receptors from one cell type to another, notably between those expressed by the N1E-115 and NG 108-15 clonal cells (Boess et al., 1992). Such variations probably explain why S 21007 acted as a full agonist at 5-HT_3 receptors on NG 108-15 cells and as a partial agonist at 5-HT_3 receptors on N1E-115 cells. However, they could obviously not explain the marked difference between the IC_{50} value (2.8 nM) of S 21007 measured in binding assays, and the EC_{50} value (27 μM) of this drug determined in electrophysiological studies since both determinations were carried out using the same N1E-115 cells. In this case, the most probable explanation is the existence of different affinity states of the 5-HT_3 receptor (Sepulveda et al., 1991; Morain et al., 1994; Bartrup and Newberry, 1996). Indeed, like other receptors of the same ligand-gated cation channel superfamily (Léna and Changeux, 1993), the 5-HT_3 receptor can rapidly desensitize (Yakel et al., 1991), which leads to its structural change into a conformation with a high affinity for agonists. Thus, the high affinity of S 21007 for [^{3}H]granisetron binding sites probably reflected the fact that 5-HT_3 receptors were in their desensitized conformation in membranes prepared for binding assays, whereas those in living cells for patch clamp recordings were functional, and therefore exhibited a low affinity for the agonist (Sepulveda et al., 1991).

Bartrup and Newberry (1996) recently reported in patch clamp studies on NG 108-15 cells that a previous application of 5-HT or 3-Cl-PBG at low concentrations (0.1 and 0.01 μM) induces the desensitization of the 5-HT_3 recep-

tor, which renders the cells much less responsive to a further application of 5-HT (100 μM) than those which were not previously exposed to an agonist. The same phenomenon was observed herein since preexposure of N1E-115 cells to S 21007 markedly reduced the cell response to a second application of this drug or to a subsequent exposure to 5-HT. Indeed, under these conditions, S 21007 antagonized the effect (inward current) normally triggered by 5-HT, probably because it desensitized 5-HT_3 receptors in addition to acting as a partial agonist (see above).

Confirming the inference drawn from in vitro investigations, in vivo studies also supported that S 21007 acted as an agonist at 5-HT_3 receptors. Thus, like the i.v. injection of 5-HT, that of S 21007 triggered the Bezold–Jarisch reflex which consists of a large but transient fall in heart rate due to the stimulation of atrial 5-HT_3 receptors in anaesthetized rats (Fozard, 1984b; Cohen et al., 1989). Indeed, pretreatment with zacopride to block these receptors completely prevented the bradycardia due to S 21007. However, further studies demonstrated that S 21007 could also behave as an antagonist in this in vivo assay. Indeed, pretreatment with this drug was able to prevent the fall in heart rate normally evoked by 5-HT or S 21007 itself. Like that proposed for the in vitro studies, it is probable that this antagonism resulted from a partial agonist action of S 21007 and the ability of this drug to desensitize 5-HT_3 receptors. As compared to that due to 5-HT, the desensitization induced by S 21007 was of longer duration since an interval of only 10 min between two successive i.v. administrations of 5-HT was enough to allow the same bradycardiac response to both injections whereas no response to 5-HT could be observed for almost one hour after the systemic administration of S 21007 (10 mg/kg i.p.). This suggests that the desensitization due to the latter drug is different in nature from that due to 5-HT, in line with the differential interaction (as shown by different nH values, see Table 1) of these two agonists with the 5-HT_3 binding sites.

Finally, the emetic studies confirmed the 5-HT_3 receptor agonist properties of S 21007 since emesis appeared with a short latency (18–26 min) in all ferrets treated with 10 mg/kg of this compound, as expected of the direct stimulation of peripheral and/or central 5-HT_3 receptors (Andrews and Hawthorn, 1987; Fukui et al., 1992; Miller and Nonaka, 1992) by the drug. Indeed, emesis due to other 5-HT_3 receptor agonists such as 2-methyl-5-HT, PBG and 3-Cl-PBG is also characterized by a short latency (Leslie et al., 1990; Sancilio et al., 1991; Kamato et al., 1993). In contrast, emesis induced by cisplatin appeared much later (i.e. with a latency of $\sim$ 90 min), because it resulted from an indirect effect of the drug, ending with the stimulation of visceral 5-HT_3 receptors by 5-HT released in the gut (Schwörer et al., 1991; Fukui et al., 1993). This delayed onset could explain the absence of inhibitory effect of S 21007 on cisplatin-induced emesis because, at low doses, S 21007 was probably unable to desensitize 5-HT_3 receptors for a sufficient time to prevent subsequent receptor stimulation by the large amount of 5-HT released by cisplatin. In fact, S 21007 was found to potentiate the emetic action of cisplatin (through an increase in the number of emetic episodes) possibly by sensitizing some critical step for emesis or facilitating cisplatin-induced 5-HT release in the gut.

In conclusion, both in vitro and in vivo investigations converged to demonstrate that S 21007 is a selective and efficient 5-HT_3 receptor agonist which behaves in some models as an antagonist either by triggering a long lasting desensitization of the receptors or by acting as a partial agonist. Because of such properties, S 21007 could be a useful pharmacological tool for further exploring the functional roles of 5-HT_3 receptors (see Barnes et al., 1992b).

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