Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 511 (2005) 9–19

www.elsevier.com/locate/ejphar

Anxiolytic-like effect of a serotonergic ligand with high affinity for 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 receptors

Mercedes Delgado[a], Anne G. Caicoya[a], Virginia Greciano[b], Bellinda Benhamú[c], María Luz López-Rodríguez[c], María Soledad Fernández-Alfonso[b], Miguel A. Pozo[a], Jorge Manzanares[a,d], José A. Fuentes[a,b,*]

[a]*Unidad de Cartografía Cerebral, Instituto Pluridisciplinar, Avda. Juan XXIII, 1, 28040 Madrid, Spain*
[b]*Departamento de Farmacología, Facultad de Farmacia, Spain*
[c]*Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, 28040 Madrid, Spain*
[d]*Servicio de Psiquiatría, Hospital Universitario 12 de Octubre, 28041 Madrid, Spain*

Received 22 July 2004; received in revised form 22 December 2004; accepted 20 January 2005

Abstract

S-(-)-2-[[4-(napht-1-yl)piperazin-1-yl]methyl]-1,4-dioxoperhydropyrrolo[1,2-α]-pyrazine (CSP-2503) is a serotonin (5-HT) receptor ligand with selectivity and high affinity for 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 receptors. CSP-2503 reduced rectal temperature and 5-HT neuronal hypothalamic activity in mice, decreased electrical activity of raphe nuclei cells in rats and blocked the enhancement of adenylate cyclase activity induced by forskolin in HeLa cells transfected with the human 5-HT_{1A} receptor. This compound also blocked head-twitches induced by the 5-$HT_{2A/2C}$ receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI). Contractions of guinea pig ileum induced by the 5-HT_3 receptor agonist 2-methyl-5-HT were prevented by CSP-2503. Moreover, it reduced the bradycardia reflex induced by 2-methyl-5-HT in anaesthetized rats. In the light/dark box and social interaction tests, CSP-2503 presented anxiolytic activity, an action shared by 5-HT_1 agonists and 5-HT_3 antagonists. Taken together, these results suggest that CSP-2503 is a new 5-HT_1 receptor agonist with 5-HT_{2A} and 5-HT_3 receptor antagonist activities that might be useful in a number of conditions associated with anxiety.

Keywords: Serotonin; 5-HT_{1A} receptor agonist; 5-HT_{2A} receptor antagonist; 5-HT_3 receptor antagonist; Anxiety

1. Introduction

Serotonin (5-HT) is one of the messengers in mammals for which more targets are known. Elucidation of such a growing number of 5-HT receptor subtypes and agents that interact with them has strongly encouraged development of additional agents acting on 5-HT systems. Alterations in brain 5-HT receptors have been detected in some psychiatric disorders and drugs that work upon them and on the 5-HT transporter are useful tools for improving a variety of mental alterations. Changes in 5-HT_{1A} receptor density have been described in different brain regions of depressed, bulimic and panic disorder patients and in suicide victims (Drevets et al., 1999; Lemonde et al., 2003; Neumeister et al., 2004; Tiihonen et al., 2004). Animal models have suggested a role for the 5-HT_{1A} receptor in the development of chronic anxiety (Overstreet et al., 2003). In fact, 5-HT_{1A} receptor agonists show anxiolytic properties both in humans and animals (Hamon, 1994). On the other hand, the use of drugs that block not only dopamine D2 receptor but also 5-HT_{2A} receptors marked a new trend in the treatment of psychosis (Busatto and Kerwin, 1997). Additionally, in the handling of chemotherapy-induced emesis a breakthrough was the clinical application of 5-HT_3 receptor antagonists (Bunce et al., 1991). Later on, blockade of central 5-HT_3 receptors was also discovered to be bound to an anxiolytic action (Costall and Naylor, 1991).

* Corresponding author. Unidad de Cartografía Cerebral, Instituto Pluridisciplinar, Avda. Juan XXIII, 1, 28040 Madrid, Spain. Tel.: +34 91 394 3254; fax: +34 91 394 3264.
E-mail address: fuentes@farm.ucm.es (J.A. Fuentes).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.01.032

By using computational models for GPCR (G protein coupled receptors)–ligand-interactions (Lopez-Rodriguez et al., 2002) and QSAR (quantitative structure–activity relationships) studies (Lopez-Rodriguez et al., 1997) a new arylpiperazine derivative was designed and synthesized. CSP-2503 resulted to be a selective and high affinity 5-HT_{1A} ligand. This new compound also showed an elevated affinity for both 5-HT_{2A} and 5-HT_3 receptors. Additionally, the purpose of this work was to explore whether or not the preclinical pharmacological features of this polyvalent 5-HT receptor ligand suggest a use for it in the treatment of a variety of conditions with a common anxiety background.

2. Materials and methods

2.1. Animals

Male Swiss albino mice (24–30 g), male Sprague–Dawley rats (200–250 g) and male Hartley guinea pigs (300–400 g) were obtained from Interfauna Ibérica (Sant Feliu de Codines, Barcelona) and maintained in a temperature and light (25+1 °C, light on between 8.00 a.m. and 8.00 p.m.) controlled environment. Food and tap water were provided ad libitum. All experiments on animals were performed between 9.00 a.m. and 2.00 p.m. and were approved by the institutional ethics committee and the housing conditions were as specified by the Spanish Laws of March 14, 1988 and October 13, 1989 on the protection of laboratory animals.

2.2. Drugs

S-(-)-2-[[4-(napht-1-yl) piperazin-1-yl] methyl]-1,4-dioxoperhydropyrrolo [1,2-α] -pyrazine (CSP-2503) was designed and synthesized in the Departamento de Química Orgánica, F. C.C. Químicas, Universidad Complutense de Madrid. 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), 5-HT, 2-methyl-5-HT, forskolin, 2,5-dimethoxy-4-iodoamphetamine (DOI), N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cyclohexanecarboxamide (WAY 100,635) and ondansetron were purchased from Sigma (Madrid, Spain).

2.3. Receptor binding studies

The binding affinities of CSP-2503 for 5-HT_{1A}, 5-HT_{2A}, 5-HT_3, 5-HT_4, 5-HT_7, 5-HT transporter (5-HTT), α_1-adrenoceptor, dopamine D2 receptor and benzodiazepine receptors were evaluated by using ligand competition assays. Rats were killed by decapitation and brains were rapidly removed and dissected. Tissue was stored frozen at −80 °C. The general assay conditions are defined in Table 1. For all binding assays, incubation was terminated by rapid vacuum filtration through Whatman GF/B filters, using a Brandel Harvester. The filters were washed with 4 ml of ice-cold assay buffer and after drying, the radioactivity bound to the filters was measured by liquid scintillation spectrometry. Protein concentrations were determined in triplicate. Competition binding isotherms were analysed by using an iterative curve-fitting procedure (program InPlot, GraphPad), which provided IC50 values for test compounds. Ki values were determined by the method of Cheng and Prusoff (1973) and represent means of three to four assays.

2.4. Rectal temperature

After removal of mice from their home cages, basal rectal temperature was measured with a lubricated digital thermistor probe which was inserted into the rectum 1.5 cm for 40 s.

Table 1
Assay conditions for the [^{3}H]-ligand displacement studies

Receptor	[^{3}H]-ligand	Buffer	Non-specific ligand (μM)	T (°C)	Time (min)
5-HT_{1A}	[^{3}H]-8-OH-DPAT	A	5-HT: 10	37	15
5-HT_{2A}	[^{3}H]- ketanserin	B	Cinanserin: 1	37	15
5-HT_3	[^{3}H]-LY 278584	C	5-HT: 10	25	30
5-HT_4	[^{3}H]- GR 113808	D	5-HT: 30	37	30
5-HT_7	[^{3}H]- carboxyamide tryptamine	E	5-HT: 10	23	120
5-HTT	[^{3}H]-paroxetine	F	Fluoxetine: 10	25	60
α_1	[^{3}H]- prazosin	G	Phentolamine: 10	25	30
D_2	[^{3}H]-spiperone	H	Butaclamol: 1	37	15
Benzodiazepine	[^{3}H]-Flunitrazepam	I	Diazepam: 2	4	90

Buffers:
A: 50mM Tris-HCl, 5mM MgSO4, 0.5 mM EDTA, pH 7.4.
B: 50mM Tris-HCl, 10mM MgSO4, 0.5 mM EDTA, 0.1% ascorbic acid, 10μM pargyline, pH 7.4.
C: 50mM Tris-HCl, 10μM pargyline, 0.6 mM ascorbic acid, 5 mM CaCl2, pH=7.4.
D: 50mM HEPES, pH 7.4.
E: 50mM Tris-HCl, 4mM CaCl2, 1 mg/ml ascorbic acid, 10 μM pargyline, 1 μM (-) pindolol, pH 7.4.
F: 50mM Tris-HCl, 20 mM ClNa, 5 mM KCl, pH 7.4.
G: 50mM Tris-HCl, 2.5 mM MgCl2, pH 7.4.
H: 50mM Tris-HCl, 120 mM ClNa, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 5.7 mM ascorbic acid, pH 7.1.
I: 25 mM potassium phosphate, pH 7.4.

Rectal temperature was determined again after the appropriate treatments with CSP-2503 or vehicle. The difference between temperatures measured before and after the administration represents an index of hypothermia. A decrease of more than 1.1 °C from basal rectal temperature was considered to be an hypothermic response.

2.5. Cell culture and determination of cAMP levels after stimulation of the adenylate cyclase enzyme with forskolin

HeLa cells transfected with the human 5-HT_{1A} receptor (HA 6 cells) were kindly provided by Dr. J. del Río (Departament of Pharmacology, University of Navarra, Spain) and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum, 500 units penicillin and 500 μg streptomycin/ml and 0.3 mg/ml geneticin. Forty-eight hours before the experiment cells were plated at 75×10^3 in DMEM-P/S-foetal calf serum-geneticin medium. On the day of the experiment, cells were treated with 0.5 mM 1-methyl-3-isobutylxanthine (IBMX), 10 μM forskolin and vehicle or different concentrations of CSP-2503 and incubated at 37 °C and 5% CO_2. Ten minutes later, treatment was stopped and cells were lysed with a 65% ethanol solution for 2 h, the ethanol was then collected and evaporated at 55 °C leaving a pellet with cAMP. For the study of CSP-2503 receptor specificity, cells were preincubated for 20 min with 10^{-8} M WAY 100,635 prior to adding CSP-2503. Samples were stored at −20 °C and later analysed for their cAMP content by radioimmunoassay (RIA) using a commercial kit ([^{3}H]cAMP assay system, cod. TRK 432; Amersham). Protein was measured by the Bradford method (Bradford, 1976). Competition binding isotherms were analysed by using an iterative curve-fitting procedure (program Origin 7.0) which provided EC_{50} values for test compounds.

2.6. Neurochemical activity

Following appropriate treatments, mice were decapitated and brains were removed from the skull. The hypothalamus were dissected on ice and immediately frozen over dry ice. Tissue samples were placed in 200 μl of 0.1 M phosphate-citrate buffer (pH 2.5) containing 15% methanol and stored at −80 °C until assayed.

On the day of assay tissue samples were thawed, sonicated for 0.5 s×3 times (Vibra Cell, mod. VC-501, Sonics and Materials, Danbury, CT) and centrifuged for 60 s in a Microfuge (IEC, mod. Centra-MP4R, Needham, MA). 5-hydroxyindolacetic acid (5-HIAA), 5-HT, 3,4-dihydroxyphenylacetic acid (DOPAC) and dopamine concentrations in hypothalamic tissue extracts were measured by high performance liquid chromatography (HPLC) with electrochemical detection. Twenty microliter of the supernatant was injected onto a Nucleosil 120 5 C18 reverse-phase analytical column. The HPLC column was coupled to a single coulometric electrode conditioning cell in series with dual electrode analytical cells (Coulochem II, ESA, Bedford, MA). The conditioning electrode was set at 100 nA, and the analytical electrodes were set at +0.12 V and−0.31 V relative to internal silver reference electrodes. The HPLC mobile phase consisted of 0.1 M phosphate/citrate buffer (pH 2.8) containing 0.1 mM ethylenediaminetetracetic acid (EDTA), 0.050% sodium octylsulfate and 25% methanol. 5-HIAA, 5-HT, DOPAC and dopamine contents of each sample were quantified by comparing peak heights with those peaks of standards assayed the same day as determined by a Shimadzu integrator. The lower limit of sensitivity of this assay for these compounds was 2 to 8 pg per sample. Tissue pellets were dissolved in 1.0 N NaOH and assayed for protein (Lowry et al., 1951).

2.7. Electrophysiology

Experiments were performed on 17 male Wistar rats. Anaesthesia was induced and maintained with chloral hydrate (400 mg/kg i.p. and 100 mg/kg i.v.). The electrocardiogram was recorded and the femoral vein cannulated for the application of drugs. The animal was placed in a stereotaxic frame and the skull was opened around lambda, sparing the superior sagittal sinus. After dissection of the dura, extracellular recordings were performed with tungsten electrodes (impedance 3–5 MΩ at 1 kHz) lowered into the dorsal raphe nucleus at an angle of 22° with the caudal border of the sinus in midline, coordinates being determined from the atlas of Patxinos and Watson (1982).

Neurons with a typical firing pattern as described elsewhere were selected for treatment with a 5-HT_{1A} receptor agonist (one cell per animal). At the end of each experiment, the recording site was marked by passing a negative current through the electrode. After i.v. injection of an overdose of anaesthesia, the animals were perfused with cold saline and the brain was removed and frozen at −80 °C. For histological verification, sections of 30 μm were stained with neutral red. Only experiments with the recording mark in the dorsal raphe nucleus were included in statistics.

Drugs were dissolved in saline and applied intravenously. The 5-HT_{1A} receptor agonist 8-OH-DPAT was given in increasing doses from 0.05 to 320 μg/kg at 3 min intervals. CSP-2503 was prepared in equimolar doses (0.067 to 430 μg/kg) and applied in the same way. The 5-HT_{1A} receptor antagonist WAY 100,635 was administered as a single dose of 10 μg/kg before increasing doses of CSP-2503.

2.8. Head-twitch response

The head-twitch response in mice is analogous to wet-dog shakes in rats. The hallucinogen DOI, which exhibits high affinity for the 5-HT_{2A} receptor and acts as an agonist, induces this head-twitch response. Different doses of CSP-2503 were given 20 min prior to DOI (1 mg/kg) administration. At this dose level DOI has been shown to produce robust frequencies of head-twitch responses in mice

(Darmani and Gerdes, 1995). The number of head-twitches exhibited over the subsequent 20 min period was recorded with a multiple tally counter, by an observer blind to the drug treatment. Neither food nor water was available for the 20 min test session.

2.9. Guinea pig ileum-induced contractions

Male guinea pigs were decapitated and the ileum was isolated to prepare longitudinal muscle strips. The preparation was sustained in a 20 ml organ bath containing Tyrode solution (mM): NaCl 136.9, KCl 2.7, $MgCl_2 6H_2O$ 1.2, NaH_2PO_4 1.2, glucose 10, $NaHCO_3$ 25 and $CaCl_2 6H_2O$ 2.5 (pH 7.4) warmed at 37 °C and aerated with a mixture of 5% CO_2/95% O_2. Isometric contraction under a loading tension of 1 g was recorded. In all experiments, tissues were exposed to 50 mM KCl to obtain an estimate of the maximal size of contraction of the preparation. The tissues were washed and allowed 15 min to a regain baseline tension. Concentration–response curves were then constructed, in a non-cumulative fashion, to 2-methyl-5-HT (10^{-6}M–10^{-4} M) with an agonist exposure period of 30 s on a 10 min dose-cycle. The non-cumulative concentration response curves for 2-methyl-5-HT were obtained again in the presence of CSP-2503 or vehicle, which were applied 10 min before 2-methyl-5-HT.

2.10. 2-Methyl-5-HT-induced bradycardia (von Bezold–Jarish reflex) in urethane-anaesthetized rats

Male Sprague–Dawley rats weighing 300–350 g were used. Rats were anaesthetized with urethane (1.2 g/kg, i.p.). The femoral artery was cannulated and connected to a pressure transducer. The tube cannulating the femoral vein was connected to an infusion pump and used for 2-methyl-5-HT injection. 5-HT_3-induced bradycardia was analysed monitoring blood pressure and heart rate after bolus administration of 2-methyl-5-HT (0.1 μg/kg) at 10 min intervals. To evaluate the inhibitory effect of CSP-2503 a concentration–response curve was performed administrating this compound 5 min prior to 2-methyl-5-HT.

2.11. Light/dark box test

The light/dark test uses the rodent natural aversion to bright areas compared with darker ones (Crawley and Goodwin, 1980). In a two compartment box, rodents will prefer the dark areas, whereas anxiolytics should increase the time spent in the lit compartment. The apparatus consisted of two methacrylate boxes (20×20×14 cm) one transparent and one black and opaque separated by an opaque tunnel (5×7×10 cm). A light from a 60 W desk lamp placed 25 cm above the light box provided the room illumination. Male Swiss albino mice were housed in an isolated room for 5 days before the experiment. On the day of the experiment drugs were dissolved in distilled water and s.c. injected. After 30 min of absorption time mice were individually tested in 5 min sessions in the apparatus described above. The floor of each box was cleaned between sessions. At the beginning of the session, mice were placed in the tunnel facing the dark box. The amount of time spent by mice in the lit area was recorded over 5 min periods. A mouse whose four paws were in the new box was considered as having changed boxes.

2.12. Social interaction test

Two mice from different cages were placed together in a small plastic cage (25×25×15 cm) with a cardboard lid and fresh wood litter on the floor. In this new habitat neither had established territory, and so they engaged in social interaction. This behavioural pattern includes sniffing, following, kicking, crawling under or over the partner and touching or nearly touching their faces. The time that mice socially interacted was measured for 5 min (File, 1980).

2.13. Spontaneous motor activity

To evaluate spontaneous ambulatory activity, each animal was individually placed in a small plastic cage (26×21.5×10.5 cm) endowed with two photoelectric cells that function perpendicularly and were connected to an electromechanical counter (Actimetre Photoelectrique, J.R. Boissier, Apelab, Paris). Cages were placed in a soundproof chamber to avoid influence of disturbing noises. Spontaneous motor activity was recorded 30 min after drug administration for 5 min and was expressed as counts.

2.14. Statistical analyses

Statistical analyses were performed using analysis of variance (ANOVA) followed by the Student–Newman–Keul's test (Steel and Torrie, 1979). Differences were considered significant if the probability of error was less than 5%. For data analysis in electrophysiological experiments, the spike frequency of the spontaneously active neuron was taken as 100% and a subsequent inhibition was expressed as percentage of control (mean±S.E.M.). Statistical significance of differences between treatments was verified with paired Student *t* test ($P<0.05$).

3. Results

3.1. Radioligand binding assays

Results of the studies that evaluated the selectivity and binding affinity of CSP-2503 for serotoninergic and non-serotoninergic receptors are summarized in Table 2. The new arylpiperazine derivate displayed high affinity for 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 receptors (Ki=4.1±1.2 nM; 13.5±2.5 nM and 8.9±1.4 nM, respectively), low affinity for 5-HT_7 and D_2 receptors (Ki= 100.9±1.4 and 192.1±20.1, respectively) and very low affinity (Ki>1000 nM) for 5-HT_4

receptors, the 5-HT transporter, benzodiazepine receptors and α_1-adrenoceptors.

3.2. Regulation of body temperature

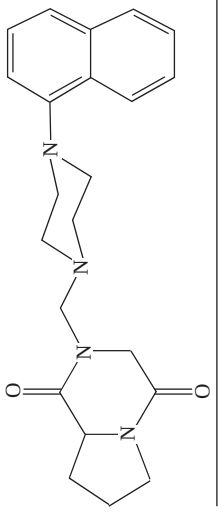
Subcutaneous administration of CSP-2503 to mice provoked a dose-related decrease in rectal temperature as shown in Fig. 1. The dose range that produced significant reductions in rectal temperature was 10–20 mg/kg. The onset of the decrease in rectal temperature was 15 min after administration of drug, and the duration of the effect at the most potent dose (20 mg/kg) was longer than 120 min.

3.3. Inhibition of forskolin-stimulated adenylate cyclase enzyme activity in cultured transfected HeLa cells

5-HT_{1A} receptor negative coupling with adenylate cyclase has been extensively used for the characterization of the pharmacological and functional properties of 5-HT_{1A} receptor ligands. Experiments carried out to determine the transduction mechanism of CSP-2503 as a potential agonist were performed by using HeLa cells expressing 5-HT_{1A} receptor. The results of these studies indicated that CSP-2503 inhibited, in a dose-dependent manner, the cAMP increase induced by 10 μM forskolin (Fig. 2). The half maximal effect (EC_{50}) observed with this compound was 0.15 μM and the maximal effect was 90.3±1.3% inhibition. As expected for an action mediated through the activation of the 5-HT_{1A} receptor, the inhibition of forskolin-stimulated adenylate cyclase by CSP-2503 could be prevented, in a concentration-dependent manner, by the 5-HT_{1A} receptor antagonist WAY 100,635 (Fig. 2).

Table 2
Binding data of CSP-2503

CSP-2503	Ki ± E.E. (nM)								
	5-HT_{1A}	5-HT_{2A}	5-HT_3	5-HT_4	5-HT_7	5-HTT	α_1	D_2	Benzodiaze pine
	4.1 ± 1.2	13.5 ± 2.5	8.9 ± 1.4	>10000	100.9 ± 1.4	976.3 ± 42.8	>1000	192.1 ± 20.1	>10000



Values are means of 2–4 experiments performed in triplicate.

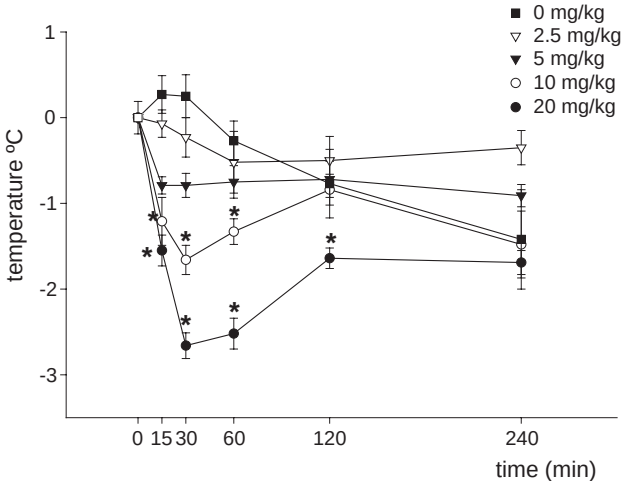


Fig. 1. Dose–response and time-course effects of CSP-2503 on rectal temperature. Mouse rectal temperature was measured at 0, 15, 30, 60, 120 and 240 min after s.c. administration of either vehicle (water 4 ml/kg), or CSP-2503. Values represent the means±S.E.M. of rectal temperature of 8–10 mice. *: values from CSP-2503-treated animals that decrease more than 1.1 °C and are significantly different ($P<0.05$) from their respective basal rectal temperature before drug administration. Vehicle alone did not alter rectal temperature.

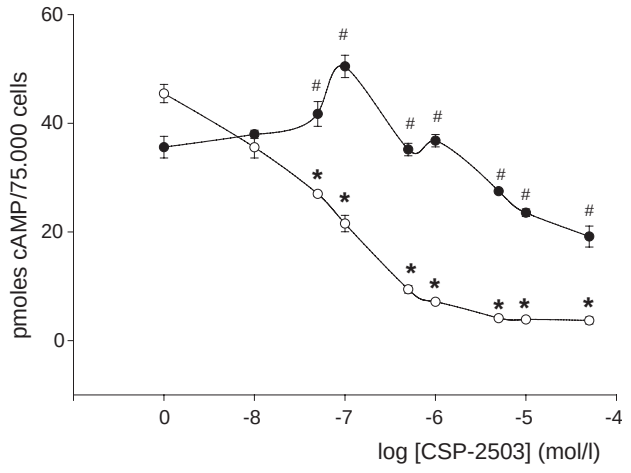


Fig. 2. Concentration–response relationships for the antagonism of forskolin-stimulated adenylate cyclase activity by CSP-2503. Adenylate-cyclase activity is expressed as picomoles cAMP/75.000 cells produced by the activity of 5-HT-sensitive forskolin-stimulated adenylate-cyclase in HeLa cells transfected with the human 5-HT_{1A} receptor in the presence of vehicle, or different concentrations of CSP-2503 (○), or CSP-2503+10^{-8} M WAY 100,635 (●). The data shown are means±S.E.M. of duplicate experiments, in each of them, 4 wells per concentration were assayed. The amount of cAMP produced by forskolin stimulation was usually around 45 pmol/well. The assay conditions are described in the Materials and methods section. *: significantly different from vehicle-treated cells; #: values of WAY 100,635+CSP-2503 treated cells that are significantly different (P<0.05) from CSP-2503 treated cells.

3.4. Neurochemical activity

As shown in Fig. 3, the administration of CSP-2503 induced a dose-related decrease in the 5-HIAA/5-HT ratio in whole hypothalamus of mice. In contrast, at the dose tested in this study no alterations were detected in the DOPAC/dopamine ratio in the hypothalamus. Values of 5-HIAA, 5-HT, DOPAC and dopamine in the vehicle-treated groups expressed in ng/mg of protein, were as follows: 5-HIAA=2.26±0.14; 5-HT=5.47±0.45; DOPAC=0.58±0.04; 0.04; dopamine = 2.04F0.28.

3.5. Electrophysiological effects on dorsal raphe nucleus neurons

Data of 14 experiments are presented that correspond to either treatment with the prototype 5-HT_{1A} receptor agonist 8-OH-DPAT (n=6) or the new compound CSP-2503 (n=8). The 5-HT_{1A} receptor antagonist WAY 100,635 was applied in 3 experiments to reverse the effect of CSP-2503 (Figs. 4 and 5).

Serotonergic dorsal raphe nucleus neurons showed regular spontaneous activity with spike frequencies between 0.7 and 5.3 Hz (mean 2.17±1.68 Hz). Experiments with 8-OH-DPAT served as control for the evaluation of the effects of CSP-2503. Application of accumulative doses of 8-OH-DPAT inhibited neuronal activity in a dose-dependent manner. The application of 8-OH-DPAT started with 0.05 μg/kg; the dose of 0.8 μg/kg showed a significant reduction (P<0.05) of the baseline activity to 58.8±12.4%. The maximal inhibitory effect was reached at 320 μg/kg. The estimated ID_{50} was 1.43 μg/kg. The new compound CSP-2503 was administrated in the same way, at equimolar doses to 8-OH-DPAT. As with 8-OH-DPAT, application of CSP-2503 caused a dose-dependent inhibition of the spontaneous ongoing activity of 5-HT neurons in the dorsal raphe nucleus. A dose of 1.04 μg/kg reduced the mean spontaneous activity to 62.0±11.5% of control in a statistically significant manner (P<0.05). The maximal effect was

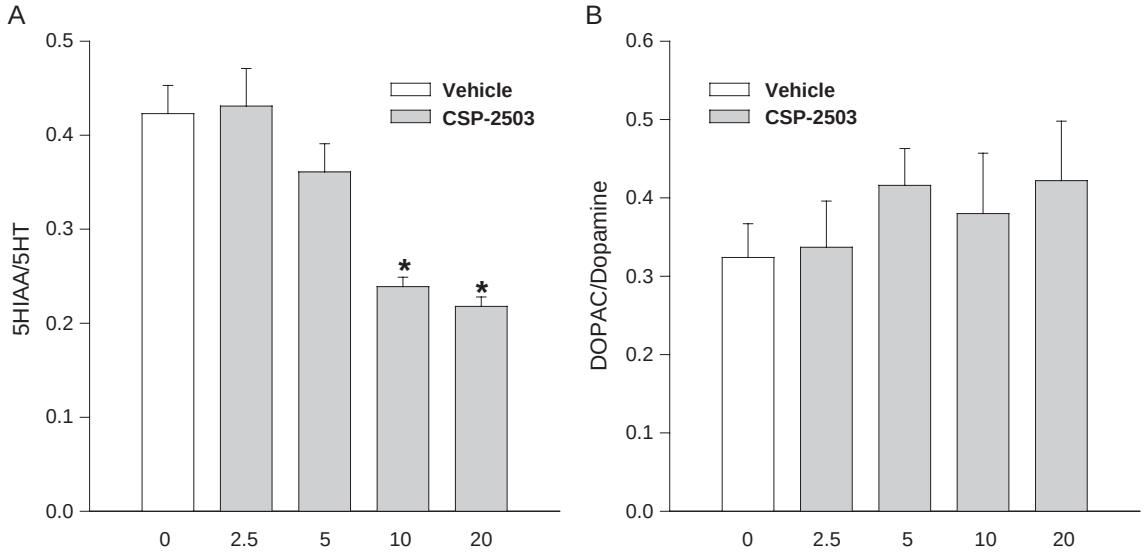


Fig. 3. Dose–response effects of CSP-2503 on 5-HIAA/5-HT and DOPAC/dopamine ratios in mouse whole hypothalamus. 5-HIAA, 5-HT, DOPAC and dopamine concentrations were measured in mouse hypothalamus 30 min after s.c. administration of the vehicle (water 4 ml/kg), or CSP-2503 (2.5, 5, 10 and 20 mg/kg). Values represent the means±S.E.M. of 5-HIAA/5-HT (Fig. 4A) and DOPAC/dopamine (Fig. 4B) ratios in vehicle or drug-treated mice. *: values from CSP-2503 treated mice, that are significantly different (P<0.05) from their respective water vehicle-treated mouse control group.

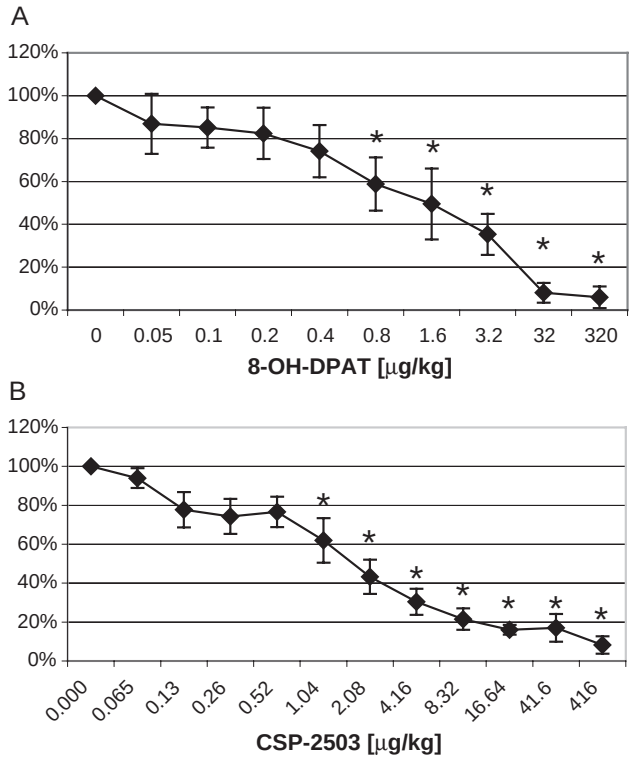


Fig. 4. Dose-dependent inhibition of neuronal activity by 8-OH-DPAT (A, n=6) and CSP-2503 (B, n=8). The control (=100%) represents the mean frequency discharge of dorsal raphe nucleus neurons before treatment. The mean inhibition is expressed as percentage of control. Error bars represent S.E.M. The inhibition of neuronal discharge is significant (P<0.05) from 0.8 μg/kg 8-OH-DPAT (A) and from 1.04 μg/kg CSP-2503 (B).

reached at 416 μg/kg, a dose of CSP-2503 that reduced baseline activity to 8±5%. The estimated ID_{50} of CSP-2503 was 1.26 μg/kg. Fig. 4B shows the dose–effect curve of CSP-2503; the slope is more pronounced than that of 8-OH-DPAT (Fig. 4A) at lower doses.

Experiments with the 5-HT_{1A} receptor-antagonist WAY 100,635 (n=3) were carried out to verify the agonist action of CSP-2503 at the 5-HT_{1A} receptor. Administration of 10 μg/kg WAY 100,635 before the injection of CSP-2503 prevented the frequency discharge of dorsal raphe nucleus neurons. No doses of CSP-2503 produced significant reductions of the basal firing rate (Fig. 5).

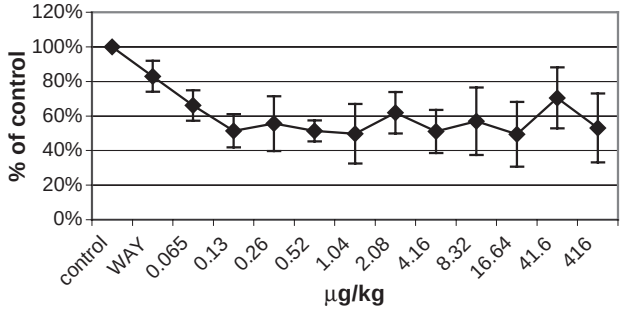


Fig. 5. Inhibition of serotonergic neuronal activity by CSP-2503 is prevented by administration of WAY 100,635. The control (=100%) represents the mean frequency discharge of dorsal raphe nucleus neurons before treatment. The mean inhibition is expressed as percentage of control. Error bars represent S.E.M. Application of WAY 100,635 (10 μg/kg) before administration of increasing doses of CSP-2503 did not produce significant reduction of the basal firing rat.

3.6. Effect on head-twitch response

Results in Fig. 6 depict the effects of the acute s.c. administration of various doses of CSP-2503 on DOI-induced head-twitch response. The compound was effective at doses as low as 0.625 mg/kg. Maximal reduction was obtained at a dose of 5 mg/kg that practically abolished blunted such a response.

3.7. Inhibition of 2-methyl-5-HT-induced contractions in guinea pig ileum

2-Methyl-5-HT produced dose-dependent contractions of the guinea pig ileum preparation with a pD_2 value of 5.2±0.1 (n=11). At concentrations of 10^{-6}–10^{-5} M preincubation of the segments with CSP-2503 elicited a non-competitive inhibition as shown in the 2-methyl-5-HT concentration–response curves (Fig. 7). When the effect of the compound under study was evaluated in the range 10^{-6}–10^{-4} M, only the 3.1×10^{-6} M concentration produced contractions in the preparation of 23.1±3.6% of the effect induced by 50 mM KCl. The rest of the concentrations assayed lacked any significant effect.

3.8. Inhibition of von Bezold–Jarisch reflex by CSP-2503

Rapid i.v. 2-methyl-5-HT injections produced a dose-dependent and transient bradycardia. In this test CSP-2503 activity was evaluated against the bradycardia induced by 2-

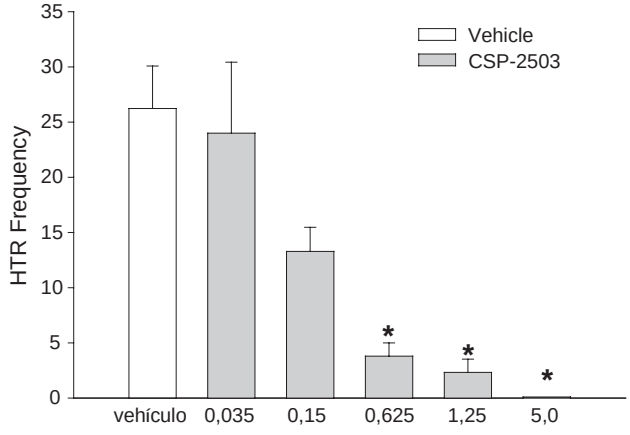


Fig. 6. Effect of CSP-2503 on DOI induced head-twitch response in mice. Animals were treated with vehicle (water 4 ml/kg s.c.) or different doses of CSP-2503 (0.035, 0.15, 0.625, 1.25 and 5 mg/kg s.c) 20 min before the experiment. Head-twitch response was analysed for 20 min after the administration of DOI (1mg/kg, i.p.). Results are means±S.E.M. of 5–9 mice. *: values from CSP-2503 treated mice, that are significantly different (P<0.05) from water vehicle-treated mouse control group.

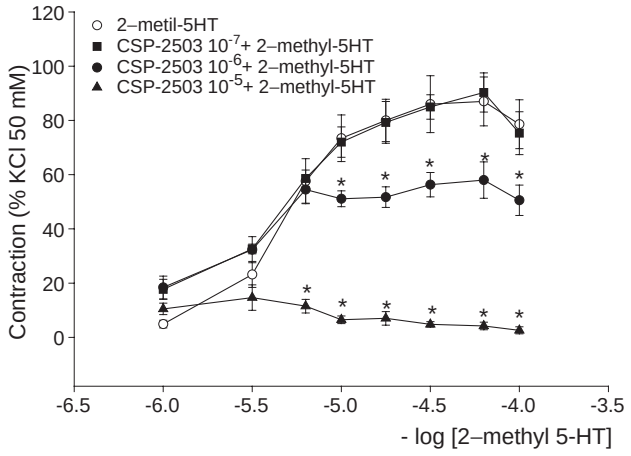


Fig. 7. Effect of CSP-2503 on non-cumulative curves of 2-methyl-5-HT-induced contractions of guinea pig isolated ileum. Vehicle or different concentrations of CSP-2503 were added 10 min before the application of non-cumulative curves of 2-methyl-5-HT. Results are expressed as percentage of maximum response to KCl 50 mM. Each point represents mean±S.E.M. of 8–9 experiments. *: values of CSP-2503 that are significantly different ($P<0.05$) from the water vehicle-treated control group.

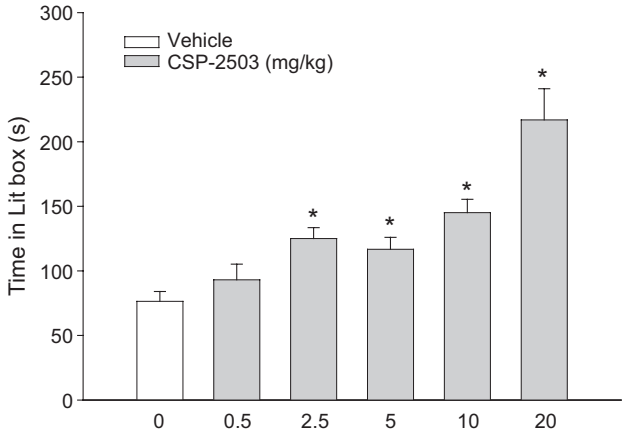


Fig. 8. Dose–response effects of s.c. administration on CSP-2503 in the mouse light/dark box test. The time spent in lit area was measured 30 min after administration of either vehicle (water 4 ml/kg s.c) or doses of CSP-2503 (0.5, 2.5, 5, 10 and 20 mg/kg). Values represent means±S.E.M. of time spent in the lit area during the 5 min test in vehicle and drug-treated mice. *: values of CSP-2503 that are significantly different ($P<0.05$) from the water vehicle-treated mouse control group.

methyl-5-HT given at an i.v. dose of 0.1 mg/kg. An i.v. 5 μg/kg dose of CSP-2503 inhibited the von Bezold–Jarisch reflex by approximately 40% (Table 3).

3.9. Light/dark box test

When mice were treated with the compound under study and exposed to this test, s.c. administration of CSP-2503 at a dose levels of 2.5 mg/kg and higher was shown to cause an increase in the time that mice spent in the light area (Fig. 8). When spontaneous motor activity was evaluated, only the 20 mg/kg dose reduced spontaneous ambulation (counts for 5 min: CSP-2503, 54±12; vehicle, 108±10; $P<0.05$). In parallel experiments, a dose of 2.5 mg/kg 8-OH-DPAT also increases the exposition time to the light of the mice treated with this 5-HT_{1A} prototype receptor agonist (time in light box during 5 min test, s: vehicle, 105.0±14.7; 8-OH-DPAT, 188.8±26; $P<0.05$). At this dose level, 8-OH-DPAT reduced spontaneous motor activity even more than CSP-2503 (counts for 5 min: 8-OH-DPAT, 21±13; vehicle, 108±10; $P<0.05$).

Table 3
Effect of CSP-2503 on the von Bezold–Jarisch reflex in anaesthetized rats

DOSE (μg/kg)	% Inhibition
0	100±0
0.1	85.7±8.2
1	74.6±16.1
5	59.6±16.3[a]
10	76.6±11.4
50	57.1±23.2
100	87.4±4.1

% Inhibition produced by CSP-2503 on 2-methyl-5-HT-induced bradycardia is shown in urethane anaesthetized rats. After the control response induced by 2-methyl-5-HT (0.1 mg/kg, i.v.) was obtained, the drug was i.v. administered 5 min before the injection of 2-methyl-5-HT with a 10 min interval. Each value represents the mean±S.E.M. of 5–8 experiments.
[a] $P<0.05$ from their respective vehicle-treated rat control group.

3.10. Social interaction test

Prior treatment (30 min) with 10 and 20 mg/kg CSP-2503 enhanced the time in which mice from separate cages (unfamiliar situation) engaged in social interaction (Fig. 9). 8-OH-DPAT (1 mg/kg, s.c.) administration also increased the interaction time in this test (time in s; vehicle: 76.4±8.6, 8-OH-DPAT: 230.6±13.4; $P<0.05$).

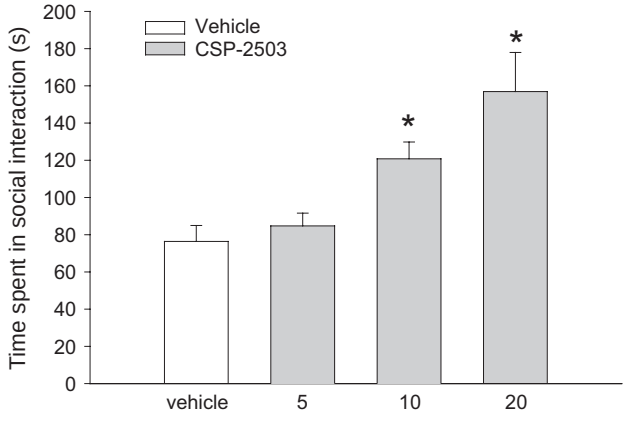


Fig. 9. Dose–response effects of s.c. administration of CSP-2503 in the social interaction test. The social interaction time was measured 30 min after administration of either vehicle (water 4 ml/kg, s.c.) or doses of CSP-2503 to pairs of mice under a faint light and unfamiliar conditions. Values represent the means±S.E.M. of social interaction time (s) in vehicle and drug–treated mice. *: values of CSP-2503 that are significantly different ($P<0.05$) from the water vehicle-treated mouse control group.

4. Discussion

5-HT_{1A} receptors operate both as somatodendritic autoreceptors and as postsynaptic receptors. Somatodendritic 5-HT_{1A} receptors are predominantly localized on dendrites and neurons of the brainstem raphe complex that project to forebrain regions in striatum, hippocampus, cortex and hypothalamus. Negative feedback inhibition of serotonergic neurons is mediated by somatodendritic 5-HT_{1A} autoreceptors (Pineyro and Blier, 1999). CSP-2503 was found to dose-dependently reduce rectal temperature in mice. In this animal species, this effect is usually considered as a consequence of the compound acting upon autoreceptors in raphe nuclei (Goodwin et al., 1985). Consistent with this notion, by measuring the 5-HIAA/5-HT ratio CSP-2503 was shown to diminish 5-HT neuronal activity in mouse whole hypothalamus, a projection area from both the medial and dorsal raphe nuclei (Hensler et al., 1994). The hypothetical action of the new compound upon 5-HT_{1A} somatodendritic receptors was proved in rats since i.v. CSP-2503 administration produced a dose-related decrease of electrical activity of dorsal raphe nucleus neurons. Interestingly, in this assay this compound showed a similar potency to the 5-HT_{1A} agonist receptor prototype 8-OH-DPAT and its effect was antagonized by selective 5-HT_{1A} receptor blockade with WAY 100,635.

Direct activation of an inwardly rectifying potassium current and adenylate cyclase inactivation are the transduction mechanisms in the brain turned on by 5-HT_{1A} receptor stimulation (Pauwels et al., 1993). Adenylate cyclase inhibition is linked to membrane hyperpolarization and is mediated by protein $G_{i/0}$. By transfecting the human 5-HT_{1A} receptor into HeLa line cells, putative agonists can be tested in a simple in vitro enzymatic assay. As previously indicated, in this system CSP-2503 showed activity in a manner sensitive to preincubation with the selective antagonist WAY 100,635. Collectively, these data suggest that the new compound behaves as a specific agonist of 5-HT_{1A} receptors with a demonstrated action upon their somatodendritic sites.

A large body of evidences indicates that 5-HT_{1A} receptor deficiency or alteration may be involved in mood and anxiety disorders (Griebel, 1995). In fact, mice with 5-HT_{1A} receptor gene inactivation display a phenotype associated with an increase of anxiety-related behaviour and stress reactivity (Lesch et al., 2003; Toth, 2003). In accordance, the prototypic 5-HT_{1A} receptor selective agonist, 8-OH-DPAT, and the partial agonists buspirone, ipsapirone and gepirone presented anxiolytic activity in rodents (De Vry, 1995). Similarly, CSP-2503 increased the time that the mice individually spent in the light–dark box test or interacted with each other in the social interaction test. As in the case of the 5-HT_{1A} agonist receptor prototype 8-OH-DPAT, a reduction of spontaneous motor activity that was shown for the highest dose of CSP-2503 (20 mg/kg) did not interfere with the anxiolytic-like action of this arylpiperazine derivative in these tests. As with other 5-HT_{1A} agonists, the dose-dependent anxiolytic effect of CSP-2503 correlates with the inhibition of serotonergic neuronal firing, the decrease of serotonin turnover and the reduction in 5-HT signalling at postsynaptic target receptors.

In humans, stimulation of 5-HT_{1A} somatodendritic receptors appears to be associated with anxiolytic effects, whereas postsynaptic 5-HT_{1A} receptor activation may relate to antidepressant action (Jolas et al., 1995; Matsuda et al., 1995). Evidence obtained in recent association studies further indicates a role for the 5-HT_{1A} receptor in anxiety and depression. In this regard, it has been shown that transcription of the human 5-HT_{1A} gene is modulated by a common C-1016G single nucleotide polymorphism. Reported data indicate that transcriptions factors that repress this gene are inhibited by this promoter polymorphism (Lemonde et al., 2003). The frequency of both the G(−1019) and C(−1019) polymorphisms was studied in separate cohorts of depressed patients and completed suicide cases versus controls. Data obtained were consistent with the hypothesis that the G(−1019) allele depresses 5-HT_{1A} autoreceptor gene expression thus reducing serotonergic transmission and probably increasing vulnerability to depression and suicide (Lemonde et al., 2003). Moreover, in another report when healthy individuals were genotyped for these 5-HT_{1A} receptor gene variants the G allele was found to be associated with depression- and anxiety-related personality traits (Strobel et al., 2003).

5-HT_{2A} receptors activate phospholipase C and in contrast to 5-HT_{1A} they are only localized postsynaptically. On the basis of studies with selective antagonists (Darmani et al., 1996), even though DOI has a similar affinity for both 5-HT_{2A} and 5-HT_{2C} receptors (Zifa and Fillion, 1992), the head-twitch response induced by this hallucinogenic compound is considered as a 5-HT_{2A} receptor-mediated action (Darmani and Gerdes, 1995). Consistent with its affinity for the 5-HT_{2A} receptor, CSP-2503 dose-relatedly antagonized the head-twitch response induced by DOI in mice. As opposed to classical antipsychotics that only block D_2-dopamine receptors, antagonism of 5-HT_{2A} receptors is now shared by a good number of antipsychotic agents considered as atypical because they are associated with substantially lower risks of adverse extrapyramidal effects. This feature may be a consequence of the fact that 5-HT_{2A} receptor antagonists produce a depolarization block of dopamine neurons of the ventral tegmental area but not of those of the substantia nigra (Sorensen et al., 1993). With regard to the possible clinical prospects of this new compound, due to the multiple cohorts of symptoms of most of the psychiatric conditions, blockade of 5-HT_{2A} receptor together with a major 5-HT_{1A} agonistic and thus anxiolytic action would be desirable for a drug to be used in certain types of psychotic patients.

5-HT_3 is the unique ligand-gated ion channel 5-HT receptor. Intracellular microelectrode recordings performed

on the central and peripheral autonomic, sensory and enteric neurons have shown that 5-HT_3 receptors mediate a rapid depolarising response (Sugita et al., 1992). 2-Methyl-5-HT that has relatively selective affinity for this serotonergic receptor is known to contract guinea pig ileum at low concentrations. The antagonism afforded by CSP-2503 in the guinea pig ileum was not entirely overcome by the 5-HT3 receptor agonist 5-methyl-5-HT thus indicating a non-competitive mode of receptor blockade. With few exceptions (for instance ondansetron), other 5-HT_3 receptor antagonists have been reported to attenuate the effect of agonists through competitive but also non-competitive mechanisms (Costall and Naylor, 1997).

Transient bradycardia induced by i.v. administration of 5-HT (von Bezold–Jarisch reflex) is the consequence of a reflex stimulation of the vagus nerve following activation of sensory afferent fibers in the right ventricle. Such a response is sensitive to 5-HT_3 antagonists implying that this receptor is involved in the von Bezold–Jarisch reflex (Fozard and Host, 1982; Nagakura et al., 1993). Our data obtained in the case of the von Bezold–Jarisch reflex test also support a role for CSP-2503 as a 5-HT_3 antagonist since this compound was able to partially prevent 2-methyl-5-HT induced bradycardia in urethane-anaesthetized rats.

Location of 5-HT_3 binding sites throughout cortical and limbic brain regions suggested the clinical application of their antagonists to treat anxiety (Gehlert et al., 1991). As already indicated by using the light/dark box test, the compound under study was found to behave as an anxiolytic agent in mice. On the basis of our present data implication of both the 5-HT_{1A} and 5-HT_3 receptors in the anxiolytic-like action of CSP-2503 may be considered. Potency of 5-HT_3 receptor antagonists as anxyolitic-like agents has been shown in varied species and tests. In fact, these results encouraged clinical trials of the 5-HT_3 receptor antagonists ondansetron, tropisetron and zacopride in patients with anxiety related disorders. Their beneficial effects in psychiatric conditions were limited by the dependency on dose and stage of disease (Costall and Naylor, 2004). In another regard nowadays there is increasing acceptance that the central nervous system plays an important role in symptomtic production of bowel conditions. The existence of central but also gastrointestinal tract 5-HT_3 receptors justifies the current studies of ondansetron and other antagonists in these intestinal alterations. 5-HT_3 receptor antagonists that not only reduce gut secretions and motility but also are endowed with an anxiolytic effect might be of indication in conditions such as the irritable bowel syndrome (Ye et al., 2001).

In summary, binding assays carried out on compound CSP-2503 showed its selectivity and high binding affinity for 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 receptors. Further pharmacological tests for each of these receptors rendered data consistent with predictions made on CSP-2503 receptor affinity as this new arylpiperazine derivative behaved as a 5-HT_{1A} receptor agonist with 5-HT_{2A} and 5-HT_3 receptor antagonistic features. Data obtained suggest that this compound might be therapeutically useful for conditions with symptoms of anxiety in which 5-HT_{2A} and 5-HT_3 serotonergic receptors are involved.

Acknowledgements

This work was supported by CEPA Schwartz Pharma (CSP) as part of a university–industry agreement and by grants BQU2001-1459 and SAF2000-04487-C02-02 (Ministerio de Ciencia y Tecnología) and 08.5/0079/2000 (Comunidad de Madrid). V.G. is a fellow supported by Universidad Complutense de Madrid. M.D. and A.G.C. are postdoctoral fellows supported by CSP and Comunidad de Madrid, respectively. The cooperation in some of the experiments of the undergraduate student Belén Marzal and the technician Laura Peláez, both supported by Comunidad de Madrid, are also acknowledged.

References

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.

Bunce, K., Tyers, M., Beranek, P., 1991. Clinical evaluation of 5-HT3 receptor antagonists as anti-emetics. Trends Pharmacol. Sci. 12, 46–48.

Busatto, G.F., Kerwin, R.W., 1997. Perspectives on the role of serotonergic mechanisms in the pharmacology of schizophrenia. J. Psychopharmacol. 11, 3–12.

Cheng, Y., Prusoff, W.H., 1973. Relationship between the inhibition constant (k_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108.

Costall, B., Naylor, R.J., 1991. Anxiolytic effects of 5-HT3 antagonists in animals. In: Rodgers, R.J., Cooper, S.J. (Eds.), 5-HT1A Agonists, 5-HT3 Antagonists and Benzodiazepines, Their Comparative Behavioural Pharmacology. CRC, Boca Raton, pp. 133–157.

Costall, B., Naylor, R.J., 1997. Neuropharmacology of 5-HT3 receptor ligands. In: Baumgarten, H.G., Göthert, M. (Eds.), Serotoninergic neurons and 5-HT Receptors in the CNS. Springer, Berlin, pp. 409–438.

Costall, B., Naylor, R.J., 2004. 5-HT3 receptors. Curr. Drug Targets. CNS Neurol. Disord. 3, 27–37.

Crawley, J.N., Goodwin, F.K., 1980. Preliminary report of a simple animal behavior model for the anxyolitic effects of benzodiazepines. Pharmacol. Biochem. Behav. 13, 167–170.

Darmani, N.A., Gerdes, C.F., 1995. Temporal differential adaptation of head-twitch and ear-scratch responses following administration of challenge doses of DOI. Pharmacol. Biochem. Behav. 50, 545–550.

Darmani, N.A., Shaddy, J., Gerdes, C.F., 1996. Differential ontogenesis of three DOI-induced behaviors in mice. Physiol. Behav. 60, 1495–1500.

De Vry, J., 1995. 5-HT1A receptor agonists: recent developments and controversial issues. Psychopharmacology (Berl.) 121, 1–26.

Drevets, W.C., Frank, E., Price, J.C., Kupfer, D.J., Holt, D., Greer, P.J., Huang, Y., Gautier, C., Mathis, C., 1999. PET imaging of serotonin 1A receptor binding in depression. Biol. Psychiatry 46, 1375–1387.

File, S.E., 1980. The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. J. Neurosci. Methods 2, 219–238.

Fozard, J., Host, M., 1982. Selective inhibition of the von Bezold–Jarisch effect of 5-HT in the rat by antagonists of neuronal 5-HT receptors. Br. J. Pharmacol. 77 (520 pp.).

Gehlert, D.R., Gackenheimer, S.L., Wong, D.T., Robertson, D.W., 1991. Localization of 5-HT3 receptors in the rat brain using [3H]LY278584. Brain Res. 553, 149–154.

Goodwin, G.M., De Souza, R.J., Green, A.R., 1985. The pharmacology of the hypothermic response in mice to 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT). A model of presynaptic 5-HT1 function. Neuropharmacology 24, 1187–1194.

Griebel, G., 1995. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol. Ther. 65, 319–395.

Hamon, M., 1994. Neuropharmacology of anxiety: perspectives and prospects. Trends Pharmacol. Sci. 15, 36–39.

Hensler, J.G., Ferry, R.C., Labow, D.M., Kovachich, G.B., Frazer, A., 1994. Quantitative autoradiography of the serotonin transporter to assess the distribution of serotonergic projections from the dorsal raphe nucleus. Synapse 17, 1–15.

Jolas, T., Schreiber, R., Laporte, A.M., Chastanet, M., De Vry, J., Glaser, T., Adrien, J., Hamon, M., 1995. Are postsynaptic 5-HT1A receptors involved in the anxiolytic effects of 5-HT1A receptor agonists and in their inhibitory effects on the firing of serotonergic neurons in the rat? J. Pharmacol. Exp. Ther. 272, 920–929.

Lemonde, S., Turecki, G., Bakish, D., Du, L., Hrdina, P.D., Bown, C.D., Sequeira, A., Kushwaha, N., Morris, S.J., Basak, A., Ou, X.M., Albert, P.R., 2003. Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. J. Neurosci. 23, 8788–8799.

Lesch, K.P., Zeng, Y., Reif, A., Gutknecht, L., 2003. Anxiety-related traits in mice with modified genes of the serotonergic pathway. Eur. J. Pharmacol. 480, 185–204.

Lopez-Rodriguez, M.L., Rosado, M.L., Benhamu, B., Morcillo, M.J., Fernandez, E., Schaper, K.J., 1997. Synthesis and structure–activity relationships of a new model of arylpiperazines: 2. Three-dimensional quantitative structure–activity relationships of hydantoin-phenylpiperazine derivatives with affinity for 5-HT1A and alpha 1 receptors. A comparison of CoMFA models. J. Med. Chem. 40, 1648–1656.

Lopez-Rodriguez, M.L., Vicente, B., Deupi, X., Barrondo, S., Olivella, M., Morcillo, M.J., Behamu, B., Ballesteros, J.A., Salles, J., Pardo, L., 2002. Design, synthesis and pharmacological evaluation of 5-hydroxytryptamine(1a) receptor ligands to explore the three-dimensional structure of the receptor. Mol. Pharmacol. 62, 15–21.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Matsuda, T., Somboonthum, P., Suzuki, M., Asano, S., Baba, A., 1995. Antidepressant-like effect by postsynaptic 5-HT1A receptor activation in mice. Eur. J. Pharmacol. 280, 235–238.

Nagakura, Y., Kadowaki, M., Tokoro, K., Tomoi, M., Mori, J., Kohsaka, M., 1993. Pharmacological characterization of FK1052, a dihydropyridoindole derivative, as a new serotonin 3 and 4 dual receptor antagonist. J. Pharmacol. Exp. Ther. 265, 752–758.

Neumeister, A., Bain, E., Nugent, A.C., Carson, R.E., Bonne, O., Luckenbaugh, D.A., Eckelman, W., Herscovitch, P., Charney, D.S., Drevets, W.C., 2004. Reduced serotonin type 1A receptor binding in panic disorder. J. Neurosci. 24, 589–591.

Overstreet, D.H., Commissaris, R.C., De La, G.R., File, S.E., Knapp, D.J., Seiden, L.S., 2003. Involvement of 5-HT1A receptors in animal tests of anxiety and depression: evidence from genetic models. Stress 6, 101–110.

Patxinos, G., Watson, C., 1982. The Rat Brain in Stereotaxic Coordinates. Academic Press, London.

Pauwels, P.J., Van Gompel, P., Leysen, J.E., 1993. Activity of serotonin (5-HT) receptor agonists, partial agonists and antagonists at cloned human 5-HT1A receptors that are negatively coupled to adenylate cyclase in permanently transfected HeLa cells. Biochem. Pharmacol. 45, 375–383.

Pineyro, G., Blier, P., 1999. Autoregulation of serotonin neurons: role in antidepressant drug action. Pharmacol. Rev. 51, 533–591.

Sorensen, S.M., Kehne, J.H., Fadayel, G.M., Humphreys, T.M., Ketteler, H.J., Sullivan, C.K., Taylor, V.L., Schmidt, C.J., 1993. Characterization of the 5-HT2 receptor antagonist MDL 100907 as a putative atypical antipsychotic: behavioral, electrophysiological and neurochemical studies. J. Pharmacol. Exp. Ther. 266, 684–691.

Steel, R.G.D., Torrie, J.H., 1979. Principles and Procedures of Statistics. McGraw Hill, New York.

Strobel, A., Gutknecht, L., Rothe, C., Reif, A., Mossner, R., Zeng, Y., Brocke, B., Lesch, K.P., 2003. Allelic variation in 5-HT1A receptor expression is associated with anxiety- and depression-related personality traits. J. Neural Transm. 110, 1445–1453.

Sugita, S., Shen, K.Z., North, R.A., 1992. 5-hydroxytryptamine is a fast excitatory transmitter at 5-HT3 receptors in rat amygdala. Neuron 8, 199–203.

Tiihonen, J., Keski-Rahkonen, A., Lopponen, M., Muhonen, M., Kajander, J., Allonen, T., Nagren, K., Hietala, J., Rissanen, A., 2004. Brain serotonin 1A receptor binding in bulimia nervosa. Biol. Psychiatry 55, 871–873.

Toth, M., 2003. 5-HT1A receptor knockout mouse as a genetic model of anxiety. Eur. J. Pharmacol. 463, 177–184.

Ye, J.H., Ponnudurai, R., Schaefer, R., 2001. Ondansetron: a selective 5-HT(3) receptor antagonist and its applications in CNS-related disorders. CNS Drug Rev. 7, 199–213.

Zifa, E., Fillion, G., 1992. 5-Hydroxytryptamine receptors. Pharmacol. Rev. 44, 401–458.