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Preclinical pharmacology of B-20991, a 5-HT_{1A} receptor agonist with anxiolytic activity

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Abstract

The purpose of this study was to characterize the pharmacological effects of 2-[[4-(*o*-methoxyphenyl)piperazin-1-yl]methyl]-1,3-dioxoperhydroimidazo[1,5-a]pyridine (B-20991) by using several biochemical and behavioral assays. Results of binding studies showed that B-20991 binds with high affinity to the 5-HT_{1A} receptor ($K_i = 31.7 \pm 1.7$ nM), moderate affinity to 5-HT_3 receptor ($K_i = 269.4 \pm 23.2$ nM) and low affinity ($K_i > 1000$) to 5-HT_{2A} receptor, dopamine D_2 receptor, benzodiazepine receptors and α_1-adrenoceptor. The administration of B-20991 produced a dose and time related decrease in mouse rectal temperature, increased both lower lip retraction and flat body posture behavioral scores in rat, decreased 5-hydroxytryptamine (5-HT, serotonin) neuronal activity in mouse hypothalamus, and did not alter dopamine neuronal activity nor locomotor activity. The anxiolytic activity of B-20991 was assessed by using both the social interaction and light/dark box tests. The results of these tests indicated that B-20991 caused a dose-related increase in the social interaction and light/dark box behavioral scores. Taken together, these results suggest that B-20991 is a 5-HT_{1A} receptor agonist that exhibits anxiolytic activity. © 1998 Elsevier Science B.V.

Keywords: 5-HT_{1A} receptor agonist; Anxiolytic; Hypothermia; Behavior, 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin)

1. Introduction

5-Hydroxytryptamine (5-HT, serotonin) has been implicated in the regulation of nociception, locomotor behavior, endocrine secretion, cardiovascular function, appetite and mood disorders (Simonovic et al., 1984; Osborne and Hamon, 1988; Glennon, 1990; Davids and Lesch, 1996; Graeff et al., 1996). It is well accepted that the variety of its pharmacological actions occur through an activation of multiple 5-HT receptors that exist in the central nervous system. To date, following pharmacological, functional and structural criteria, seven different 5-HT receptors families have been identified in the central nervous system. They comprise the 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7 receptors still to be operationally and transductionally completely characterized (Peroutka, 1995; Lacau-Mengido et al., 1996). The 5-HT_1 receptor family group consists of the 1A, 1B, 1D, 1E and 1F receptor subtypes (Leonhardt et al., 1989; Peroutka et al., 1990; Adham et al., 1993).

The 5-HT_{1A} subtype is specially relevant due to its implication in the regulatory processes of anxiety and depression (Traber and Glaser, 1987; Gleeson et al., 1989; Heller et al., 1990; Taylor and Moon, 1991; Remy et al., 1996). 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) was the first compound identified as a selective 5-HT_{1A} receptor agonist (Ahlenius et al., 1981; Hjorth et al., 1982; Dourish et al., 1987). However, this compound produces additional pharmacological actions by acting upon dopamine D_2 receptor and α_2-adrenoceptor (Simonovic et al., 1984; Crist and Surprenant, 1987). Subsequently, a number of compounds with a variety of chemical structures have been identified as 5-HT_{1A} receptor agonists. They can be divided into four different groups: (1) 8-substituted-2-(di-*n*-propylamino)tetralins, derivatives of 8-OH-DPAT (Schaus et al., 1990; Yu et al., 1993, Barf et al., 1996), (2) 6-substituted-4-(di-*n*-propylamino)-1,3,4,5-

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tetrahydrobenz[c,d]indoles structurally very similar to 5-HT and represented by LY293284 ((−)-4*R*-6-acetyl-4-(di-*n*-propylamino)-1,3,4,5-tetrahydrobenz[*c*,*d*]indole) (Foreman et al., 1994), (3) 6-substituted-'ABC' partial ergolines (Mason et al., 1987; Flaugh et al., 1988) and (4) arylpiperazine derivatives headed by buspirone, ipsapirone, gepirone (Traber and Glaser, 1987) and tandospirone (Ishizumi et al., 1991), the benzodioxopiperazine (+)-flesinoxan (Hartog and Wouters, 1988), the halogenated phenylpiperazine LY165,163 (1-(2-(4-aminophenyl)ethyl-4-(3-trifluoromethylphenyl)-piperazine) (Ransom et al., 1986), and the naphtylpiperazine S14671 (1-[2-(2-thenoylamino)ethyl]-4[1-(7-methoxynaphtyl)]piperazine) (Millan et al., 1992), among others. Most of these compounds act as partial agonists and/or as agonists or antagonists at other serotonergic or non-serotonergic receptors. Thus, receptor selectivity limits the use of some of these 5-HT_{1A} receptor agonists.

Buspirone, an arylpiperazine derivative that acts as a partial agonist on 5-HT_{1A} receptor, was the first agent of this kind to be approved for clinical use (Taylor and Moon, 1991; Levy and Van de Kar, 1992). However, it is not optimal in terms of selectivity and pharmacokinetical properties, and shows a slow onset of action. In order to increase the selectivity for the 5-HT_{1A} binding site, several structural changes have been made on the imide and arylpiperazine moieties of buspirone. Nevertheless, most of these compounds still exhibit a high level of undesired affinity for the α_1-adrenoceptor (e.g. NAN-190 (1-(2-methoxyphenyl)-4-[4-(2-phtalimido)butyl]piperazine): K_i (5-HT_{1A}) = 0.6 nM, $K_i(\alpha_1)$ = 0.8 nM), and attempts have been made to identify and then eliminate the structural features of these compounds that account for their affinity for α_1-adrenergic binding sites (Raghupathi et al., 1991; El-Bermawy et al., 1992). Several reports indicate that a certain lipophilic character of the imide portion is required for the molecule to bind to 5-HT_{1A} receptors with high affinity (Abou-Gharbia et al., 1988; Raghupathi et al., 1991; Van Steen et al., 1993). It has been postulated that steric factors play an important role in ligand receptor interactions (Van Steen et al., 1994). In order to evaluate the influence of non-steric factors on the affinity and selectivity for 5-HT_{1A} binding sites, our research group has analyzed a new set of novel arylpiperazines (López-Rodríguez et al., 1996) in which the imide moiety has been replaced by a bicyclohydantoin (V_w = 77.04 cm^3/mol), preserving the steric requirements of the phtalimido (V_w = 69.47) and the azaspirodione (V_w = 89.72) moieties.

B-20991 (2-[[4-(*o*-methoxyphenyl)piperazin-1-yl]methyl]-1,3-dioxo-perhydroimidazo[1,5-a]pyridine) has proved to be the most selective member of this series. This manuscript presents the experiments that have been carried out to determine the in vitro and in vivo pharmacological activity of B-20991. Taken together the results of the study indicates that B-20991 is a 5-HT_{1A} receptor agonist endowed with anxiolytic properties.

2. Materials and methods

2.1. Animals

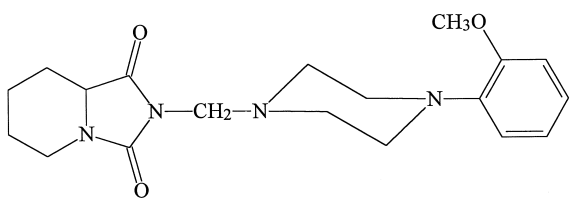
Male Swiss albino mice (20–25 g) and male Sprague–Dawley rats (200–250 g) were obtained from Interfauna Ibérica (Sant Feliú de Codines, Barcelona) and maintained in a temperature and light (25 ± 1°C, light on between 8.00 a.m. and 8.00 p.m.) controlled environment. Food and tap water were provided ad libitum. All experiments were performed between 10.00 a.m. and 2.00 p.m.

2.2. Drugs

2-[[4-(*o*-methoxyphenyl)piperazin-1-yl]methyl]-1,3-dioxoperhydroimidazo[1,5-a]pyridine (B-20991) (Fig. 1) was designed and synthesized in the Department of Organic Chemistry, School of Chemistry, Complutense University, Madrid, SPAIN. 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin was purchased from Sigma (Madrid).

2.3. Receptor binding studies

The binding affinities of B-20991 for the following receptors: 5-HT_{1A}, 5-HT_{2A}, 5-HT_3, α_1-adrenoceptor, dopamine D_2 receptor and benzodiazepine receptor 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 assays 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. Proteins were determined by the method of Lowry et al. (1951), with bovine serum albumin as the standard. All determinations were performed in triplicate. Competition binding isotherms were analyzed by using an iterative curve-fitting procedure (program InPlot, GraphPad), which provided IC_{50} values for test compounds. K_i values were determined by the method of Cheng and Prusoff (1973) and are means of two to four assays.



B-20991

Fig. 1. Structure of B-20991. 2-[[4-(*o*-methoxyphenyl)piperazin-1-yl]methyl]-1,3-dioxoperhydroimidazo[1,5-a]pyridine.

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

Receptor	^{3}H-ligand (nM)	Buffer	Nonspecific ligand (μM)	min/°C	Ref.
$5HT_{1A}$ receptor	8-OH-DPAT; 0.6	A	5-HT; 10	15/37	Clark et al. (1990)
$5HT_{2A}$ receptor	Ketanserin; 0.4	B	Cinanserin; 1	15/37	Titeler et al. (1987)
$5HT_3$ receptor	LY 278584; 0.7	C	5-HT; 10	30/25	Wong et al. (1989)
α_1-adrenoceptor	Prazosin; 0.2	D	Phentolamine; 10	30/25	Ambrosio et al. (1984)
Dopamine D_2 receptor	Raclopride; 0.8	E	(+)-butaclamol; 1	60/25	Hall et al. (1988)
Benzodiazepine	Flunitrazepam; 0.25	F	Diazepam; 2	90/0–4	Orensanz et al. (1990)

Buffers: A = 50 mM Tris–HCl, 5 mM $MgSO_4$, 0.5 mM EDTA, pH 7.4 at 37°C; B = 50 mM Tris–HCl, 0.5 mM Na_2EDTA, 10 mM $MgSO_4$, 0.1% ascorbic acid, 10 μM pargyline, pH 7.4 at 37°C; C = 50 mM Tris–HCl, 10 μM pargyline, 0.6 mM ascorbic acid, 5 mM $CaCl_2$, pH 7.4 at 25°C; D = 50 mM Tris–HCl, 2.5 mM $MgCl_2$, pH 7.4 at 25°C; E = 50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid, pH 7.4 at 25°C; F = 25 mM potassium phosphate (KPi), pH 7.4.

2.4. Rectal temperature

After removal of mice from their home cages, basal rectal temperature was measured with a lubricated digital thermistoprobe which was inserted into the rectum 1.5 cm for 40 s. Rectal temperature was determined again after the appropriate treatments with 8-OH-DPAT, B-20991 or vehicle. The difference between the temperature 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 a hypothermic response.

2.5. Evaluation of 5-HT_{1A} receptor-mediated behavior in the rat

Male Sprague-Dawley rats were acclimatized to the housing environment and handled everyday for one week before testing. Rats were housed in groups of 3–4 per cage. Lower lip retraction and flat body posture were used to evaluate the 5-HT_{1A} receptor-mediated behavior. Both responses were measured 5, 10, 20 and 30 min after drug administration by using a 0–3 scale as previously described (Smith and Peroutka, 1986).

2.6. Neurochemical activity. 5-HIAA / 5-HT and DOPAC / dopamine ratios

Following appropriate treatments, mice were decapitated and brains were removed from the skull. Hypothalami 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 the assay tissue samples were thawed, sonicated for 3 s (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-hydroxyindoleacetic acid (5-HIAA), 5-hydroxytryptamine (5-HT), 3,4-dihydroxyphenylacetic acid (DOPAC) and dopamine concentrations in hypothalamus tissue extracts were measured by high performance liquid chromatography (HPLC) with electrochemical detection. Twenty μl of the supernatant were injected onto a C18 reverse-phase analytical column (5 μm spheres; 250 × 4.6 mm; Nucleosil; Scharlau, Barcelona) which was protected by a pre-column cartridge filter (5 μm spheres; 30 × 4.6 mm). 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 potential was set at 100 nA, and the analytical electrodes were set at +0.12 V and −0.31 V relative to internal Ag 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 octylsulphate and 25% methanol. 5-HIAA, 5-HT, DOPAC and dopamine contents of each sample were quantitated by comparing peak heights with those peaks of standards assayed the same day as determined by Shimadzu integrator (Shimadzu, mod. C-R4AX-CHROMATOPAC, Kyoto). 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. Locomotor activity

Open field locomotor activity was measured in male Swiss albino mice, after the appropriate treatments with B-20991 and 8-OH-DPAT. The apparatus consisted of a square box (30 × 30 cm) with 10 cm high walls. The floor was a white plastic sheet divided into 25 (5 × 5) equally sized squares painted in fine black. Mice were maintained in the animal house at least 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. Each mouse was tested by placing it in one corner of the square field. Its behavior was recorded on a videotape and locomotor activity analysis was subsequently performed from the recording. The number of total squares crossed were counted during 5-min test. A mouse whose four paws were in a new square was considered as having crossed a square.

Table 2
Affinity of B-20991 for a variety of receptor binding sites

Receptor	K_i (nM)	
	B-20991	8-OH-DPAT
5-HT_{1A} receptor	31.7 ± 1.7	0.5 ± 0.1
5-HT_{2A} receptor	> 1000	> 1000
5-HT_3 receptor	269.4 ± 23.2	NT
Dopamine D_2 receptor	> 10 000	88 ± 7
α_1-adrenoceptor	> 1000	> 2,427
Benzodiazepine receptor	> 10 000	NT

NT: not tested.

2.8. Social interaction

When two mice from separated cages are placed together in a small chamber in which neither has established territory, they engage in a social interaction which includes a variety of behavioral patterns: sniffing, following, grooming, kicking, crawling under or over the partner, and touching or nearly touching their faces (File and Hyde, 1978; File, 1980). Male Swiss Albino mice were housed (12 per cage) 5 days before the experiment. On the day of the experiment drugs were dissolved in distilled water and s.c. injected into pairs of mice belonging to different home-cages. During the first 30 min of absorption time mice remained housed in their 5-day-home-cage. After this absorption time, the pairs of mice from different home cages were placed together in a small plastic cage (21 × 13 cm) with a cardboard lid and fresh wood litter on the floor (no change in the light level). The time that mice socially interacted was visually measured for 5 min.

2.9. 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 5 days before the experiment. On the day of the experiment drugs were dissolved in distilled water and s.c. injected. After 30

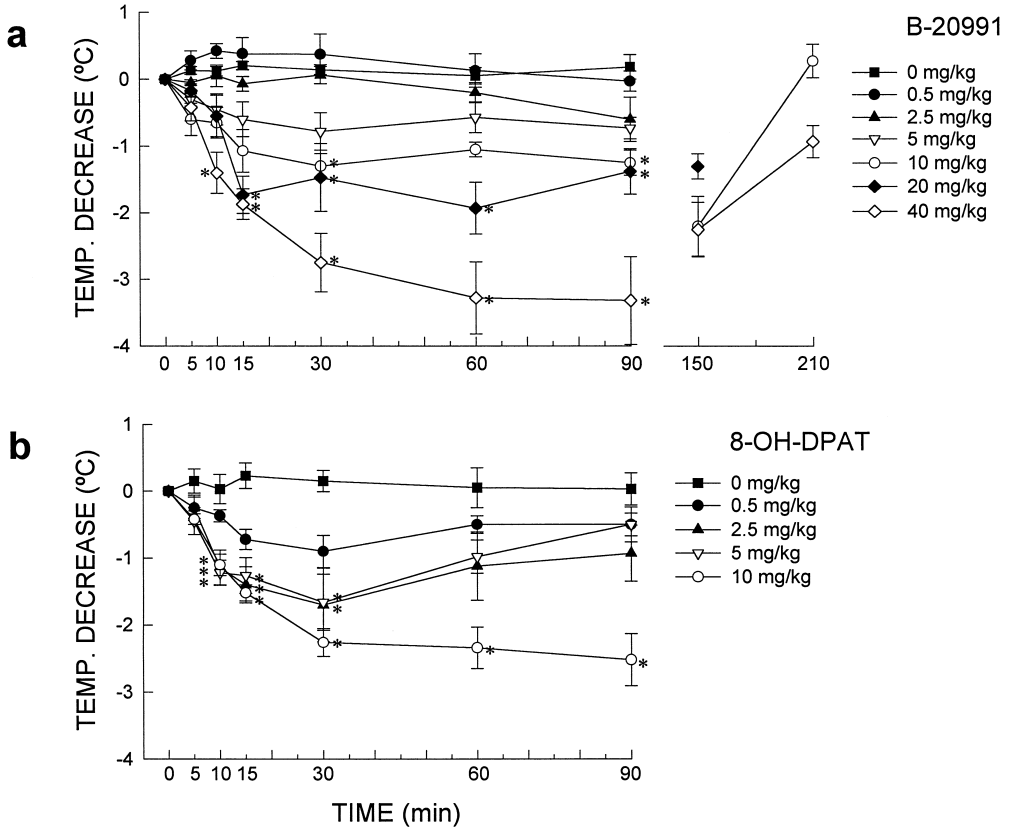


Fig. 2. Dose–response and time-course effects of B-20991 (panel a) or 8-OH-DPAT (panel b) on rectal temperature. Mouse rectal temperature was measured at 0, 5, 10, 15, 30, 60, 90, 150, and 210 min after s.c. administration of either vehicle (water 4 ml/kg), or doses of B-20991 (0.5, 2.5, 5, 10, 20 and 40 mg/kg) or 8-OH-DPAT (0.5, 2.5, 5 and 10 mg/kg). Values represent the means ± S.E.M. of rectal temperature in 6–8 mice. * Values from B-20991 or 8-OH-DPAT 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.

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.

2.10. Statistical analyses

Statistical analyses were performed using analysis of variance 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%.

3. Results

3.1. Radioligand binding assays

The results of the studies that evaluated the binding affinity of B-20991 as compared with the 5-HT_{1A} receptor agonist prototype for serotonergic and non-serotonergic receptors are summarized in Table 2. The new arylpiperazine derivative displayed high affinity for 5-HT_{1A} receptors ($K_i = 31.7 \pm 1.7$ nM), low affinity for 5-HT_3 receptors ($K_i = 269.4 \pm 23.2$ nM) and very low affinity ($K_i >$ 1000 nM) for 5-HT_{2A}, dopamine D_2, benzodiazepine receptors or α_1-adrenoceptors.

3.2. Hypothermic effect in mice

The effects of s.c. administration of either B-20991 or 8-OH-DPAT on rectal temperature in mice are summarized in Fig. 2. The dose ranges that produced significant reductions in rectal temperature were 0.5–10 and 5–40 mg/kg for 8-OH-DPAT and B-20991, respectively. The onset in the decrease of rectal temperature occurred 5–10 min after the administration of both compounds tested, and the duration of the effects at their respective most potent dose 40 mg/kg (B-20991) and 10 mg/kg (8-OH-DPAT) was longer than 90 min.

3.3. Evaluation of 5-HT_{1A} receptor-mediated behavior in the rat

B-20991 and 8-OH-DPAT induced increases in the flat body posture and in the lower lip retraction scores in rats (Fig. 3). B-20991 produced a dose-related effect on flat body posture score. The dose of 0.5 mg/kg was without effect whereas both the 2 and 8 mg/kg doses increased the score. In contrast, all the doses of 8-OH-DPAT used in this study increased in a similar manner the flat body posture score. All the doses of B-20991 and 8-OH-DPAT tested produced significant increases in the lower lip retraction score. However, this increase was not dose-related since the highest dose of 8-OH-DPAT produced the lowest lower lip retraction score. In addition, there were no

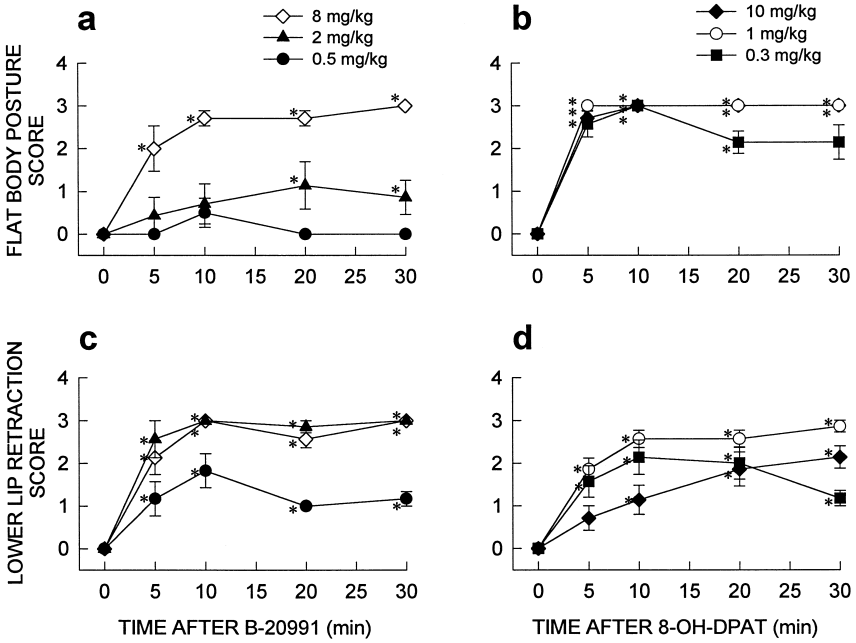


Fig. 3. Dose–response and time-course effects of B-20991 or 8-OH-DPAT on flat body posture (panels a, b) and lower lip retraction (panels c, d). Flat body posture and lower lip retraction were evaluated 5, 10, 20 and 30 min after s.c. administration of either vehicle (water 1 ml/kg), or doses of B-20991 (0.5, 2 and 8 mg/kg) or 8-OH-DPAT (0.3, 1 and 10 mg/kg) according to an arbitrary scale going from 0 (no effect) to 3 (maximum effect). Values represent the means ± S.E.M. of flat body posture and lower lip retraction score in 7 rats. * Values from B-20991 or 8-OH-DPAT that are significantly different ($P < 0.05$) from vehicle-treated rats at each time point. Mean scores of vehicle-treated rats was 0.

significant differences in the lower lip retraction score between rats treated with the 2 and 8 mg/kg doses of B-20991.

3.4. Neurochemical activity. 5-HIAA / 5-HT and DOPAC / dopamine ratios

As shown in Fig. 4, the administration of B-20991 and 8-OH-DPAT induced a dose-related decrease of the 5-HIAA/5-HT ratio in whole hypothalamus of mice. In contrast, neither compound at the doses tested in this study altered 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: controls for the B-20991 treated group (5-HIAA = 2.99 ± 0.39; 5-HT = 4.78 ± 0.72; DOPAC = 0.74 ± 0.06; dopamine = 2.11 ± 0.39) and controls for the 8-OH-DPAT treated group (5-HIAA = 3.60 ± 0.36; 5-HT = 5.30 ± 0.62; DOPAC = 0.68 ± 0.09; dopamine = 2.02 ± 0.34).

3.5. Locomotor activity. Open field

Results from the open-field observations are depicted in Fig. 5. The number of squares counted in the open field include both central and peripheral squares. The administration of 8-OH-DPAT produced a dose-related decrease in the locomotor activity in mice. The lowest dose that decreases the locomotor activity was 2.5 mg/kg. In contrast, no changes were found after the administration of B-20991 at any of the dose levels used.

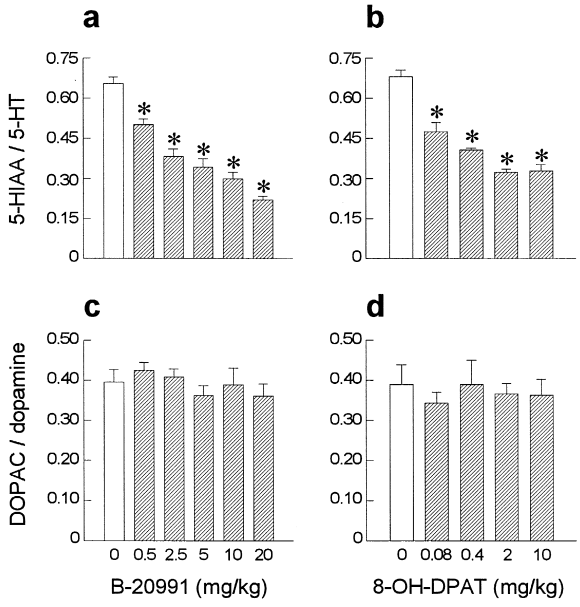


Fig. 4. Dose–response effects of B-20991 or 8-OH-DPAT on 5-HIAA/5-HT (panels a, b) and DOPAC/dopamine (panels c, d) ratios in mouse whole hypothalamus. 5-HIAA, 5-HT, DOPAC and dopamine concentrations were measured 30 min after administration of the vehicle (water, 4 ml/kg; s.c.) or doses of B-20991 (0.5, 2.5, 5, 10 and 20 mg/kg) or 8-OH-DPAT (0.08, 0.4, 2 and 10 mg/kg) in mouse hypothalamus. Values represent the means + S.E.M. of 5-HIAA/5-HT (panels a, b) and DOPAC/dopamine (panels c, d) ratios in vehicle or drug-treated mice. * Values from B-20991- or 8-OH-DPAT-treated mice, that are significantly different ($P < 0.05$) from their respective water vehicle-treated mouse control group.

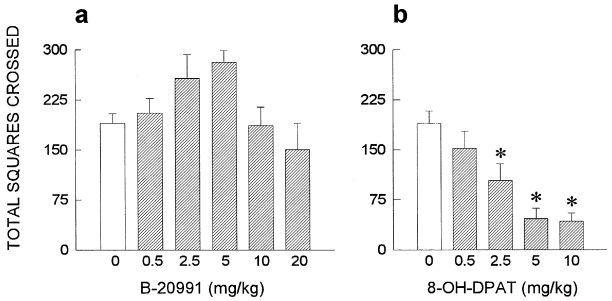


Fig. 5. Dose–response effects of B-20991 (panel a) or 8-OH-DPAT (panel b) on locomotor activity in mice. The total squares crossed by a mouse were counted, 30 min after administration of either vehicle (water, 4 ml/kg; s.c.), or doses of B-20991 (0.5, 2.5, 5, 10 and 20 mg/kg) or 8-OH-DPAT (0.5, 2.5, 5 and 10 mg/kg). Values represent the means + S.E.M. of total squares crossed during 5-min test in vehicle and drug-treated mice. * Values from 8-OH-DPAT-treated mice, that are significantly different ($P < 0.05$) from their respective water vehicle-treated mouse control group.

3.6. Social interaction

B-20991 and 8-OH-DPAT caused a dose-related increase in the time that mice were socially interacting (Fig. 6). The effect of 8-OH-DPAT was found to be more potent than that of B-20991. While a dose of 0.5 mg/kg of 8-OH-DPAT produced significant effects in the social interaction test, a dose of 2.5 mg/kg of B-20991 was

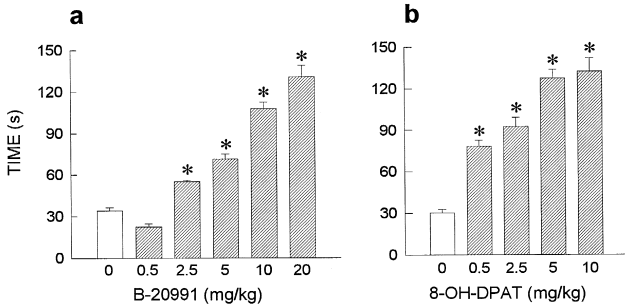


Fig. 6. Dose–response effects of s.c. administration of B-20991 (panel a) or 8-OH-DPAT (panel b) in the mice social interaction test. The social interaction time was measured 30 min after administration of either vehicle (water 4 ml/kg; s.c.), or doses of B-20991 (0.5, 2.5, 5, 10 and 20 mg/kg) or 8-OH-DPAT (0.5, 2.5, 5 and 10 mg/kg) to pairs of mice under bright light and unfamiliar conditions. Values represent the means + S.E.M. of social interaction time (s) in vehicle and drug-treated mice. * Values from B-20991- or 8-OH-DPAT-treated mice, that are significantly different ($P < 0.05$) from their respective water vehicle-treated mouse control group during 5-min test.

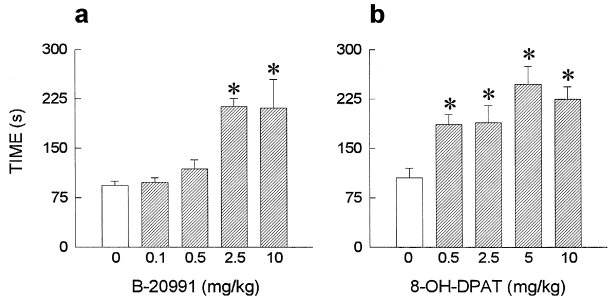


Fig. 7. Dose–response effects of s.c. administration of B-20991 (panel a) or 8-OH-DPAT (panel b) in the mouse light/dark box test. The time spent in the lit area was measured 30 min after administration of either vehicle (water 4 ml/kg; s.c.), or doses of B-20991 (0.1, 0.5, 2.5, and 10 mg/kg), or 8-OH-DPAT (0.5, 2.5, 5 and 10 mg/kg). Values represent the means + S.E.M. of time (s) spent in the lit area during 5-min test in vehicle and drug-treated mice. * Values from B-20991- or 8-OH-DPAT-treated mice, that are significantly different ($P < 0.05$) from their respective water vehicle-treated mouse control group.

needed to obtain similar effects. However, the dose of 10 mg/kg of both compounds rendered comparable results.

3.7. Light / dark box test

B-20991, like 8-OH-DPAT increased dose-dependently the time spent in the light area of the test chamber (Fig. 7). In a similar way to the results observed in the social interaction test, 2.5 mg/kg of B-20991 was the minimum dose that produced significant effects in this test whereas a dose 0.5 mg/kg of 8-OH-DPAT was found to be sufficient to increase the time that mice spent in the light area. However, analogous results were obtained with both compounds at higher doses (5 and 10 of both mg/kg).

4. Discussion

The results of the present study demonstrate that B-20991 is a 5-HT_{1A} receptor agonist endowed with anxiolytic activity. In vitro ligand displacement studies showed that B-20991 binds with high affinity for the 5-HT_{1A} receptor ($K_i = 31.7$ nM) and 9 times less affinity ($K_i =$ 269.4 nM) for the 5-HT_3 receptor. B-20991 binds for the 5-HT_{1A} with 30 times more affinity than to the 5-HT_{2A} receptor and α_1-adrenoceptor and with 300 times more affinity to either one of the dopamine D_2 and benzodiazepine receptors.

Two types of anxiolytic drugs are mainly used at present, the benzodiazepines and the 5-HT_{1A} receptor partial agonist buspirone. As adverse effects, benzodiazepines produce ataxia, sedation, amnesia, ethanol and barbiturate potentiation, tolerance, dependence, and the potential for drug abuse plagues their clinical use (Costa and Guidotti, 1996). Buspirone, used for the treatment of chronic anxiety, does not cause sedation, and has no known potential for abuse. However, some authors question its efficacy (Murasaki and Miura, 1992). Therefore, there is a need for the discovery of new anxiolytics deprived of so many adverse effects and with a short latency period.

The in vivo pharmacological studies conducted with B-20991 were designed to explore its effects on both inhibitory somatodendritic 5-HT_{1A} autoreceptor and postsynaptic 5-HT_{1A} receptors and to evaluate its preclinical efficacy as a potential anxiolytic.

The hypothermic response to 5-HT_{1A} receptor stimulation has been proposed by different authors to be an index of either inhibitory somatodendritic 5-HT_{1A} autoreceptor or postsynaptic receptor activation (Hjorth, 1985; Green and Goodwin, 1987; Hillegaart, 1991). Recent studies in rats (O'Connell et al., 1992) strongly suggest that postsynaptic receptors mediate 5-HT_{1A} receptor agonist-induced hypothermia. In contrast, it has been suggested that hypothermia induced by 5-HT_{1A} receptor agonist in mice is mediated by somatodendritic 5-HT_{1A} autoreceptors (Goodwin et al., 1985). In this study, B-20991 decreased rectal temperature in mice with 3 times less potency than 8-OH-DPAT. These results suggest that in this test B-20991 is less active at the somatodendritic site than 8-OH-DPAT.

Systemic administration of 5-HT_{1A} receptor agonists such as 8-OH-DPAT produces a behavioral syndrome consisting of flat body posture, hind limb abduction, head weaving and forepaw treading (Tricklebank et al., 1984). This '5-HT syndrome' seems to be mediated through stimulation of postsynaptic 5-HT_{1A} receptors. In addition, a number of 5-HT_{1A} receptor agonists, including 8-OH-DPAT, induce a contraction of the musculature of the lower lip, and this behavioral response is considered to be mediated by inhibitory somatodendritic 5-HT_{1A} autoreceptor (Berendsen et al., 1989). Flat body posture and lower lip retraction were chosen in this study since they represent sensitive behavioral measures of 5-HT_{1A} receptor activation in the rat. In the present study, the administration of either 8-OH-DPAT or B-20991 at various doses was found to induce a high score in the lower lip retraction. In contrast, in the flat body posture test, only the highest dose of B-20991 rendered similar results to those obtained with much lower doses of 8-OH-DPAT.

The precise assessment of 5-HT_{1A} somatodendritic functional activity requires to measure the ability of the compounds under study to decrease the activity of 5-HT neurons. In our experiments, the whole hypothalamus was chosen to determine 5-HT neuronal activity, since it is a brain area that receives projections from both the medial and the dorsal raphe nuclei (Azmitia and Segal, 1978; Hensler et al., 1994) and thus it is quite abundant in 5-HT terminals. Systemic administration of B-20991 or 8-OH-DPAT caused a dose-related decrease in the 5-HIAA/5-HT ratio in the hypothalamus, but had no effect on the DOPAC/dopamine ratio. These results further indicate that B-20991 is a 5-HT_{1A} receptor agonist at the somatodendritic site. Interestingly, similar doses of B-20991 and 8-OH-DPAT produce comparable results. On the other

hand, these data suggest that B-20991 had no effect on dopaminergic neuronal activity.

In agreement with previous results (Hillegaart et al., 1989), the administration of 8-OH-DPAT decreased locomotor activity in the open field test. In contrast, B-20991 did not significantly alter locomotor activity, suggesting that it has less sedative properties than 8-OH-DPAT.

Preclinical evaluation of anxiolytic activity of B-20991 was carried out by using the social interaction and the light/dark box tests. In both tests, B-20991 or 8-OH-DPAT increased the time that mice interacted with each other (social interaction test) and the time that mice spent in the light area (light/dark box test). These data suggest that both compounds displayed anxiolytic activity. Although at low doses B-20991 presented less anxiolytic potency than 8-OH-DPAT, when doses were sufficiently increased comparable results were obtained.

The structural changes that have been carried out to synthesize this new arylpiperazine derivative succeeded in the replacement of the imide moiety of buspirone by a bicyclohydantoin, preserving the steric requirements of the phtalimido and the azaspirodione moieties. A number of 5-HT_{1A} receptor agonists display high activity at this 5-HT receptor but are also active at α_1-adrenoceptor and dopamine D_2 receptor. In conclusion, the results of the present study suggest that B-20991 is a new 5-HT_{1A} receptor agonist that presents anxiolytic activity, and therefore may be potentially useful in the treatment of anxiety related-disorders.

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