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Short communication

Effects of 5-HT_4 receptor antagonists on rat behaviour in the elevated plus-maze test

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Abstract

The anxiolytic-like effects of a variety of 5-HT receptor agonists and antagonists have been intensively studied in animal models. However, no direct effects of agents modulating 5-HT_4 receptors have been reported, in spite of their suggestive location in the brain. The objective of the present study was the determination of the effects of two selective 5-HT_4 receptor antagonists, SB 204070 [1-butyl-4-piperidinylmethyl)-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate] and GR 113808 [[1-[2-methylsulphonyl)amino]ethyl]-4-piperidinyl]methyl 1-methyl-1*H*-indole-3-carboxylate], in the elevated plus-maze test in rats. Results have shown that both 5-HT_4 receptor antagonists exhibit an anxiolytic-like profile, although only at the dose of 1.0 mg/kg (s.c.). At this dose, both compounds significantly increased the percentage of time spent in open arms exploration, while other variables evaluated remained unaffected at the dose range tested. Results suggest that 5-HT_4 receptor antagonists could have some anxiolytic-like properties, although their effects seem more limited and less consistent than those presented by classic anxiolytics, such as diazepam. However, they are similar to those exhibited by granisetron [endo-1-methyl-*N*-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)-1*H*-indazole-3-carboxamide], a 5-HT_3 receptor antagonist.

Keywords: Anxiety; 5-HT (5-hydroxytryptamine, serotonin); 5-HT_4 receptor antagonist; Elevated plus-maze

1. Introduction

It has traditionally been accepted that a reduction in serotonin (5-HT) neurotransmission tends to reduce anxiety, whereas stimulation of the 5-HT system usually results in an anxiogenic effect (Chopin and Briley, 1987). This hypothesis is supported by the effects of 5-HT agents in a varied range of animal anxiety models. However, available clinical data are not so conclusive, and contradictory evidence has been found in animal models, particularly in acute administration of these substances. The variation in responses to 5-HT modulators is particularly notable in the elevated plus-maze. Acute administration of 5-HT_{1A} receptor agonists or 5-HT_2 receptor antagonists were found to be anxiolytic, anxiogenic or without effects in the elevated plus-maze test (revised in Handley and McBlane, 1993). Similarly, acute administration of 5-HT_3 receptor antagonists, such as ondansetron, was also reported to induce anxiolysis within a narrow dose range (Vasar et al., 1993), or to have no effects whatsoever (Wright et al., 1992) in the elevated plus-maze test.

Recently, 5-HT_4 receptors have been identified in the human (Doménech et al., 1994; Monferini et al., 1993) and animal (Doménech et al., 1994; Grossman et al., 1993) brains. The zones of highest density include several limbic areas, such as nucleus accumbens, caudate, striatum, frontal cortex, colliculus and hippocampus. It is known that 5-HT_4 receptor activation induces stimulation of cAMP production and inhibition of K^+ channels (Fagni et al., 1992). Moreover, studies in rats have demonstrated that 5-HT_4 receptor activation facilitates the release of acetylcholine in the frontal cortex (Consolo et al., 1994). Therefore, anatomical and neurochemical data suggest that 5-HT_4 receptors could be involved in a variety of central phenomena, such as learning and memory, as well as anxiety-related behaviours.

However, there is a lack of information concerning the effects of 5-HT_4 agents on anxiety. Indeed, the direct effects of 5-HT_4 receptor modulators on anxiety have not been demonstrated, although a role for central 5-HT_4 receptors in anxiety has been claimed (Costall and Naylor, 1993; Naylor et al., 1993). In fact, it has been demonstrated that the 5-HT_4/5-HT_3 receptor antagonist, SDZ 205-557, was able to block the anxiolytic-like effects of

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both diazepam in the dark-light test in mice (Naylor et al., 1993), and ritanserin in either dark-light or social interaction tests in rodents, always after the co-administration of 5-hydroxytryptophan (5-HTP) to increase baseline anxiety levels (Costall and Naylor, 1993). Indeed, SDZ 205-557 per se had no effect (Costall and Naylor, 1993; Naylor et al., 1993), which would suggest the absence of an endogenous 5-HT_4 receptor tone. However, SDZ 205-557 is a non-specific 5-HT_4 agent with a very short plasma half life (3-4 min). Both features add difficulties in the interpretation of the above-mentioned results.

The objective of this study was the determination of the role of 5-HT_4 receptor in anxiety using two selective 5-HT_4 receptor antagonists, SB 204070 and GR 113808, in the elevated plus-maze model in rats. Both GR 113808 and SB 204070 have been described as very potent, selective and competitive 5-HT_4 receptor antagonists with pK_B values against 5-HT of 9.7 and 10.4, respectively (see Hoyer et al., 1994). Moreover, the results obtained with 5-HT_4 receptor antagonists have been contrasted with diazepam used as standard control agent, and granisetron, a 5-HT_3 receptor antagonist, in order to include a serotonergic agent as a reference.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (232 ± 2.3 g, Janvier, France) were housed in groups of 5–6 in wire mesh cages at 21–22°C with a 12-h light-dark cycle (on 06:30, off 18:30). The animals had access to food and water ad libitum during a 5-day acclimatisation period before the study.

2.2. Drugs

SB 204070 [1-butyl-4-piperidinylmethyl)-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate], GR 113808 [[1-[2-methylsulphonyl)amino]ethyl]-4-piperidinyl]methyl 1-methyl-1*H*-indole-3-carboxylate] (both synthesized in the Almirall Medicinal Chemistry Department), granisetron [endo-1-methyl-*N*-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)-1*H*-indazole-3-carboxamide] (Research Biochemicals International, USA) and diazepam (Sigma, USA) were suspended in a vehicle composed of 0.5% methylcellulose and 0.1% Tween-80 in distilled water.

2.3. Apparatus and procedure

The elevated plus-maze apparatus was made of clear Plexiglas (adapted from Pellow et al., 1985) and consisted of two open arms, 50×10 cm, and two enclosed arms, $50 \times 10 \times 40$ cm, with an open roof, arranged in such a way that the two open arms were opposite each other. The maze was elevated to a height of 50 cm and a single white incandescent lamp (60 W) was positioned 120 cm above the centre of the apparatus. The apparatus was placed in an isolated and sound proofed room contiguous to the observer room. The subjects' behaviour was recorded with a video camera mounted vertically above the apparatus, and simultaneously analysed by means of an image analyser programme (SMART, Letica, Spain). Subjects were placed individually in the centre of the maze facing an open arm and the following parameters were automatically scored for 5 min: percentage (%) of time spent in open arms, percentage (%) of entries into open arms respect to total arms entries, and total activity (total distance travelled). The apparatus was cleaned with water after each trial to remove any trace of odour.

2.4. Drug administration studies

SB 204070 (0.3, 1.0 and 3.0 mg/kg) and GR 113808 (0.3, 1.0 and 3.0 mg/kg) were administered 30 or 10 min prior to testing, while granisetron (0.01, 0.1 and 1.0 mg/kg) and diazepam (0.3, 1.0 and 3.0 mg/kg) were administered 30 min before testing only. All experiments, each compound and each trial of 10 and 30 min before testing, were independently carried out by duplicate in independent groups ($n = 5–7$) along different days using daily prepared drug suspensions. All compounds, as well as the vehicle, were administered subcutaneously (s.c.) at a volume of 1 ml/kg.

2.5. Statistical analysis

An analysis of variance (MANOVA) with planned between-group contrasts was used for between-group comparison. Since some compounds can affect not only the time spent and entries into open arms but also the total activity, and these behaviours could co-vary, an analysis of co-variance (MANCOVA) was applied, with the total activity as the co-varied variable. The standard conditions of normality of the data and homogeneity of variance were assessed by means of the Kolmogorov-Smirnov and Cochran tests, respectively.

3. Results

As Table 1 shows, diazepam (3.0 mg/kg) increases the percentage of time spent in open arms [$F(1,40) = 14.91$, $P < 0.001$], as well as the percentage of entries into open arms respect to total arms entries [$F(1,40) = 7.95$, $P < 0.01$]. There was a significant increase in total activity [$F(1,40) = 4.42$, $P < 0.05$]. However, the MANCOVA also demonstrated significant increase of both the percentage of time spent in open arms and the percentage of entries into open arms [$F(1,39) = 12.61$, $P < 0.01$; $F(1,39) = 5.25$, $P < 0.05$, respectively]. Furthermore, di-

Table 1
Effects of diazepam, granisetron (administered 30 min prior to testing), SB 204070 and GR 113808 (both administered 10 min before testing) in the elevated plus-maze test in rats

Treatment	(mg/kg)	n	% TOA	% EOA	Activity
Vehicle		11	18.7±5.8	40.5±4.4	2422±199
Diazepam	0.3	11	20.5±5.6	46.9±4.1	2649±200
	1.0	11	27.0±3.0	50.9±2.8 [c]	2659±318
	3.0	11	45.8±4.8 [d]	55.4±3.3 [e]	3123±201 [b]
Vehicle		12	12.8±3.5	35.8±4.3	2567±129
Granisetron	0.01	11	18.3±2.7	39.5±3.8	2293±134
	0.1	11	28.5±4.1 [a]	41.2±2.1	2700±81
	1.0	12	15.4±4.0	35.9±2.6	2293±164
Vehicle		13	15.0±3.6	41.1±2.7	2696±108
SB 204070	0.3	12	17.0±2.8	44.5±2.6	2725±182
	1.0	12	24.7±2.8 [b]	47.2±3.2	2911±139
	3.0	12	14.9±4.1	38.4±5.6	2556±166
Vehicle		12	11.8±1.6	41.9±3.8	2889±132
GR 113808	0.3	12	13.0±2.4	32.2±4.0	2639±110
	1.0	12	21.2±2.6 [a]	41.7±4.2	2746±121
	3.0	12	9.6±2.0	34.3±3.6	2670±137

The following parameters are shown: % TOA (percentage of time spent in open arms); % EOA (percentage of entries into open arms respect to total arms entries); and Activity (total distance travelled expressed in cm). Results are expressed as means±S.E.M. Significant differences calculated from MANOVA are expressed by [a] $P < 0.01$, [b] $P < 0.05$ and [c] $P < 0.1$. Since diazepam affected not only the time spent and entries into open arms but also the total activity, a MANCOVA was used, with the activity as the co-varied variable. Significant differences are expressed by [d] $P < 0.001$ and [e] $P < 0.01$.

azepam at the dose of 1.0 mg/kg presented a clear statistical trend to increase the percentage of entries into open arms respect to total arms entries [$F(1,40) = 3.88$, $P = 0.056$], although the other parameters were unaffected at this dose.

Only at the dose of 0.1 mg/kg did granisetron increase the percentage of time spent in open arms [$F(1,42) = 9.23$, $P < 0.01$] while neither the percentage of entries into open arms nor the total activity were statistically affected within the dose range tested.

SB 204070 and GR 113808 had no effect at any of the doses tested when administered 30 min prior to testing (data not shown). However, when administered 10 min before testing both SB 204070 and GR 113808 only at the dose of 1.0 mg/kg increased the percentage of time spent in open arms [$F(1,44) = 8.55$, $P < 0.01$ and $F(1,45) = 4.15$, $P < 0.05$, respectively], suggesting a bell-shaped dose-response curve. However, the percentage of entries into open arms respect to total arms entries and the total activity were not affected by either SB 204070 or GR 113808.

4. Discussion

The present study demonstrates that two selective 5-HT_4 receptor antagonists, SB 204070 and GR 113808, have an anxiolytic-like profile in the elevated plus-maze test in rats. However, these effects appear to be limited and less consistent than those produced by the classic anxiolytic diazepam.

Diazepam exhibited a typical anxiolytic agent profile in elevated plus-maze, where it increased the percentage of both time spent and entries into open arms, as well as augmenting total activity in the elevated plus-maze. The 5-HT_4 receptor antagonists SB 204070 and GR 133808 as well as the 5-HT_3 receptor antagonist granisetron only increased the percentage of time spent in open arms exploration, leaving the other parameters unaffected, so indicating a less consistent effect. Thus, while diazepam affected all anxiolytic-related parameters, the 5-HT_4 receptor antagonists affected only the percentage of time spent in open arms, which was increased by 65 and 80% for SB 204070 and GR 113808, respectively. Moreover, this effect was weaker than those presented by diazepam or granisetron, which increased this parameter by 145 and 123%, respectively.

Furthermore, these effects were observed only when 5-HT_4 receptor antagonists were administered 10 min before testing, while no effects were observed when 5-HT_4 receptor antagonists were administered 30 min beforehand. This would be explained if both compounds had a short plasma half life and limited in vivo activity due to hydrolysis. Hence, limited data suggest that these compounds have a limited duration of action and poor bioavailability being rapidly degraded in vivo (Eglen et al., 1995; Hedge et al., 1994). Furthermore, no direct evidences of brain bioavailability in vivo have been reported for either SB 204070 or GR 113808 after systemic administration. To explain their pharmacological actions, further kinetic and distribution are required.

In addition, the effects of these compounds were only observed at the dose of 1.0 mg/kg, while administration of higher or lower doses failed to present any effects, suggesting a bell-shaped dose-response curve. Nevertheless, it has been reported that other non-classic anxiolytic compounds, such as 5-HT_3 receptor antagonists, also show limited elevated plus-maze profiles affecting only one variable linked with an anxiolytic effect, and also present a bell-shaped dose-response curve (Vasar et al., 1993), so supporting the data herein concerning granisetron.

The present results contrast with those which found that the 5-HT_4/5-HT_3 receptor antagonist SDZ 205-557 blocked the anxiolytic-like effects of diazepam (Naylor et al., 1993) and of ritanserin (Costall and Naylor, 1993), although in these studies SDZ 205-557 per se failed to have any effect (Costall and Naylor, 1993; Naylor et al., 1993). However, the lack of selectivity and low 5-HT_4 receptor affinity of SDZ 205-557 compared to both SB 204070 and GR 113808, in addition to the very short half life of SDZ 205-557, the time lapse between administration and testing as well as the different animal models used, could explain the discrepancies.

To conclude, the results of this study indicate that

5-HT_4 receptor blockade can induce, although at a low level, anxiolytic-like effects in an animal model, such as the elevated plus-maze test in rats. Thus, results agree with the traditionally accepted hypothesis that a functional reduction in 5-HT neurotransmission tends to reduce anxiety (Chopin and Briley, 1987), all the more so considering the location in the limbic areas of the 5-HT_4 receptors. However, although these results indicate an involvement of the 5-HT_4 receptors in anxiety, the small number of parameters affected and weak efficacy presented by 5-HT_4 receptor antagonists compared to diazepam, and even when compared to granisetron, would suggest that this action is indirect and limited.

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