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Anxiolytic-like activity of SB-205384 in the elevated plus maze test in mice

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Recent studies point to a major role for α2-containing GABA-A receptors in modulating anxiety. However, the possible implication of GABA-A receptors containing the α3 subunit on anxiety is less known. The aim of this study was to examine the effects of SB-205384 (0.5-4 mg/kg, ip), an α3 subunit positive modulator of GABA-A receptor, on anxiety tested in the elevated plus-maze in male mice, using classical and ethological parameters. Mice treated with SB-205384 showed an increase in the frequency of entries and the time spent in open arms, as well as a reduction in the time spent in closed arms, as compared with the control group. A notable increase of «head-dipping» unprotected and a reduction of «stretched-attend posture» protected was also evident. These findings indicate that SB-205384 exhibits an anxiolytic-like profile in the elevated plus-maze test, suggesting that GABA-A receptors which contain the α3 subunit might be involved in regulation anxiety.

Actividad ansiolítica del SB-205384 en el laberinto elevado en cruz en ratones. Recientes investigaciones indican que los receptores GABA-A que contienen subunidades α2 desempeñan un importante papel en la modulación de la ansiedad. Sin embargo, la posible implicación de los receptores GABA-A que contienen la subunidad α3 es menos conocida. El objetivo de este trabajo fue examinar los efectos del SB-205384 (0.5-4 mg/kg, ip), un modulador positivo de los receptores GABA-A que contienen la subunidad α3, sobre la ansiedad evaluada en el laberinto elevado en cruz en ratones machos, utilizando parámetros clásicos y etiológicos. Los animales tratados con SB-205384 mostraron un incremento en la frecuencia de entradas y el tiempo pasado en brazos abiertos, así como una reducción en el tiempo pasado en los brazos cerrados del laberinto, en comparación con el grupo control. Igualmente, se observó un notable aumento en la frecuencia de «head-dipping» desprotegidos y una disminución del número de «stretched-attend posture» protegidos. Estos resultados indican que el SB-205384 exhibe un perfil ansiolítico en el test de laberinto elevado en cruz, sugiriendo que los receptores GABA-A que contienen la subunidad α3 podrían estar involucrados en la regulación de la ansiedad.

GABA-A receptors belong to a superfamily of ligand-gated ion channels that mediate fast inhibitory neurotransmission in the mammalian brain. These receptors are hetero-pentamers with individual subunits sharing a common structure. To date, numerous subunits have been cloned and divided into subunit classes based upon the deduced aminoacid sequence homology: α (1-6), ß (1-4), γ (1-4), δ, ε, π and θ (Kneussel, 2002; Fritschy and Brünig, 2003; Steiger and Russek, 2004).

Potentiation of GABA-A receptor mediated inhibition underlies the therapeutic efficacy of benzodiazepine site ligands as anxiolytics, sedatives, muscle relaxants and anticonvulsants (Möhler, Crestani and Rudolph, 2001). Recent molecular genetic approaches have begun to define which of the specific pharmacological features of benzodiazepines are associated with particular (i.e., α1, α2, α3 or α5-containing) subtypes of GABA-A receptor (Rudolph and Möhler, 2004). Whereas the anxiolytic and myorelaxant actions of benzodiazepines appear to be mediated by α2 subunit-containing receptors (Crestani et al, 2001; Low et al, 2000), α1-containing receptors appear responsible for the sedative action and, at least in part, for the anticonvulsant effect of these drugs (Rudolph et al, 1999; McKerman et al, 2000). Nonbenzodiazepine hypnotic compounds, including the imidazopyridine zolpidem and the pyrazolopyrimidine zaleplon have also shown to interact selectively with GABA-A receptors containing the α1 subunit (Martín-López and Navarro, 2002; Sanna et al, 2002). Overall, these findings suggest the α2 GABA-A receptors are specific targets for the development of future selective anxiolytic drugs.

The GABA-A receptor α3 subunit is expressed throughout the forebrain, being a component of approximately 17% of GABA-A receptors. Among other structures, α3-GABA-A receptors are highly expressed in numerous brain regions involved in the modulation of anxiety, such as amygdala or medial septum (Fritschy and Brüning, 2003). However, to date few studies have specifically analyzed the implication of GABA-A receptors containing the α3 subunit in anxiety using animal models (Low et al, 2000; Collins et al, 2002; Griebel et al, 2003). Atack et al (2005) have comunicated recently that inverse agonists selective

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for GABA-A receptors containing an α3 subunit are anxiogenic, suggesting that agonists selective for this subtype should be anxiolytic. SB-205384 is an α3 subunit positive modulator of GABA-A receptors This compound belongs to the group of benzothiophene compounds that act as allosteric modulators of GABA-A receptor function, but which do not bind to the benzodiazepine site (Meadows et al, 1998). The aim of this study was to examine the role of α3-containing GABA-A receptors in anxiety. For this purpose, we designed an experiment to explore the effects of a wide dose range of SB-205384 on anxiety tested in the elevated plus-maze in male mice. This laboratory test is routinely used to study anxiety-related behaviors in rodents (Lister, 1987; Manzaneque, Brain and Navarro, 2002; Navarro and Maldonado, 2002; Navarro, Burón and Martín-López, 2002; Navarro, Luna, García and Pedraza, 2003; Navarro, Luna and García, 2005).

Methods

Animals

88 albino adult male mice of the OF.1 strain weighing 25-30 g were used. Animals were housed in groups of five in plastic cages (24 × 13.5 × 13 cm) under reversed 12h light/dark cycle (white lights on 20:00-8:00), a constant temperature (20 ºC), and food and tap water available ad libitum, except during behavioral tests. Cage maintenance was undertaken twice weekly, but never on the day of testing. Mice were housed 7 days before the experiment.

This experiment was carried out in accordance with the Guiding Principles for Care and Use of Laboratory Animals approved by the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Drug administration

Five groups of mice were used. Animals were randomly allocated to four experimental groups (n= 17-19 each) receiving SB 205384 injections. SB-205384 (Tocris Laboratories) was diluted in physiological saline (90%) plus dimethylsulphoxide (DMSO; 10%) to provide appropriate doses for injections and administered acutely in four doses: 0.5, 1, 2 and 4 mg/kg. Animals of control group received physiological saline (90%) plus dimethylsulphoxide (DMSO; 10%) (n= 17). Drug or vehicle was injected intraperitoneally in a volume of 10 ml/kg. Tests were performed 30 min after injections. Doses were chosen on the basis of a pilot study carried out in our laboratory. At these doses, SB 205384 did not produce sedation or reduction of motility.

Apparatus and behavioral test

Plus-maze test consisted of two open arms (30 × 5 cm, surrounded by a 0.25-cm-high border) and two closed arms (30 × 5 cm, surrounded by 15-cm-high walls) with the two pairs of identical platform, which emerged from a central platform (5 × 5 cm), positioned opposite each other. The apparatus was elevated 40 cm above the floor.

Mice were tested on the maze in randomized order. The test was initiated by placing the mouse on the central platform of the maze, facing one of the open arms, and letting it move freely. Each session lasted 5 min, being recorded by a videocamera. All tests were carried out under dim red lighting between the second and seventh hour of the dark phase. After each test, the maze was thoroughly cleaned. Behavioral analysis was performed by a trained experimenter who was blind to treatment condition.

A number of classical parameters were collected during the session: (a) Open arm duration: the total amount of time the mouse spent in the open arms; (b) Closed arm duration: the total amount of time the mouse spent in the closed arms; (c) Central platform duration: the total amount of time the mouse spent in the central platform; (d) Open arm frequency: the frequency of mouse entry with all four paws into the open, unprotected arms; (e) Closed arm frequency: the frequency of mouse entry with all four paws into the closed, protected arms, and (f) Total number of entries in the arms.

Likewise, different ethological measures were also quantified: (a) Rearings: a body stance in which the animal sets his forepaws onto the wall of a closed arm while keeping his rear legs on the floor; (b) Stretched attend posture (SAP): a body posture in which the mouse stretches forward and then retracts to its original position without moving the feet, and (c) Head-dipping (HD): movement of the head over the side of the maze and down towards the floor. In the case of the last two behavioral categories, a distinction was made between «protected» (if the behavior was displayed from the central plarform or closed arm) and «unprotected» (if it occurred while being in an open arm).

Data analyses

Nonparametric Kruskal-Wallis tests were initially used to assess the variance of the behavioural measures over different treatment groups. Subsequently, if necessary, appropriate paired comparisons were performed using Mann-Whitney U tests to contrast the parameters in the different treatment groups. The analysis was carried out using nonparametric statistics since the criteria for parametric statistics were not met by the data.

Results

Table 1 illustrates medians (with ranges) of duration and number of entries in the different areas of the maze, and Table 2 shows the medians (with ranges) of the ethological behavioral measures examined. Kruskal-Wallis analysis showed that there were significant differences in the number of entries ($p<0.02$) and the time spent in open arms ($p<0.003$) and in the number of entries ($p<0.04$) and the time spent in closed arms ($p<0.002$), as well as in the frequency of SAP protected ($p<0.01$) and HD unprotected ($p<0.02$).

Paired comparisons using Mann-Whitney U tests revealed that SB-205384 (all doses) produced a significant increase in the time spent in open arms ($p<0.002$-$p<0.05$) as well in the number of entries in this area (1 and 4 mg/kg, $p<0.05$), as compared with the control group. Likewise, a reduction in the time spent in the closed arms was observed after treatment with SB-205384 (with a tendency toward significance, $p<.1$: 0.5-2 mg/kg; 4 mg/kg: $p<0.05$), whereas the number of entries in this arm was decreased with the highest dose used, in comparison with the control group (4 mg/kg, $p<0.05$). Furthermore, HD unprotected significantly increased with SB-205384 (all doses) and SAP protected reduced (2 and 4 mg/kg), as compared with the control group ($p<0.005$-$p<0.05$).

Table 1
Median values with ranges of classical behavioral measures in the elevated plus-maze after SB-205384 treatment

Treatment (mg/kg)	Doses	Duration (s)			Frequency		
		Open[a]	Closed[c]	Center	Open[b]	Closed[d]	Total entries in the arms
Vehicle	0.0	47.2 (0-102)	171.1 (128-264)	65.41 (35-106)	4 (0-9)	16 (7-31)	41 (24-81)
SB-205384	0.5	86.81** (0-151)	157.6# (92-244)	55.44 (17-103)	6 (0-17)	14 (9-19)	42 (20-62)
	1.0	71.18** (5-149)	155.3# (81-273)	60.85 (18-158)	7 * (1-16)	17 (4-29)	50 (14-73)
	2.0	76.16** (13-155)	152.3# (103-219)	61.55 (22-127)	5 (2-11)	14 (7-25)	40 (22-62)
	4.0	99.04** (11-153)	140.8* (70-218)	61.60 (22-120)	6.5 * (1-12)	13* (6-22)	8.5 (25-64)

[a] p<0.003, [b] p<0.02, [c] p<0.002 , [d] p<0.04, Kruskal-Wallis test
** p<0.002-p<0.05, Mann-Whitney U test (as compared with the vehicle group)
* p<0.05, Mann-Whitney U test (as compared with the vehicle group)
# p<0.1, Mann-Whitney U test (as compared with the vehicle group)

Table 2
Median values with ranges of ethological behavioral measures in the elevated plus-maze after SB-205384 treatment

Treatment (mg/kg)	Doses	Frequency				
		Rears	HD unprotected[a]	HD protected	SAP unprotected	SAP protected[b]
Vehicle	0.0	14 (6-31)	3 (0-17)	7 (1-13)	2 (0-10)	11 (4-23)
SB-205384	0.5	15 (3-33)	7 * (0-33)	7 (0-13)	5 (0-11)	9 (2-20)
	1.0	15.5 (2-34)	5.5 * (0-16)	5.5 (2-14)	4 (0-11)	9 (2-22)
	2.0	13 (2-28)	6 * (0-12)	7 (2-10)	3 (0-11)	6 * (0-18)
	4.0	16.5 (5-26)	7 * (0-19)	5 (1-11)	3 (0-10)	5.5 * (1-17)

HD: head-dipping; SAP: stretched attend posture
[a] p<0.02, [b] p<0.01, Kruskal-Wallis test
* p<0.005-p<0.05, Mann-Whitney U test (as compared with the vehicle group)

Discussion

Exploratory behavior in novel environments is the basis for many psychopharmacological tests of anxiety, including the elevated plus maze. In this test, mice typically avoid the open arms of the maze and spend most of their time in the two enclosed arms. It is based upon the behavioral repertoire of rodents faced with threatening situations (i.e. it has ethological validity) and it is sensitive to both anxiogenic and anxiolytic drugs (Belzung and Griebel, 2001). Furthermore, the inclusion of ethological measures results in a more sensitive methodology to characterize drug effects than if only are included spatial (classical) measures (Ohl, 2003).

Analysis of the behavior of mice in the elevated plus-maze showed that acute treatment with SB-205384 increased the time spent and the number of entries in the open arms, also reducing the time spent in the closed arms, as compared with the control group (Table 1). No significant differences were found in the total number of entries in the arms of the maze between the experimental and control groups. Overall, this behavioral profile suggests that SB-205384 could exhibit an anxiolytic-like activity in the elevated plus-maze test. A number of postural elements considered indicative of «risk assessment», such as head-dipping (HD) and stretched-attend posture (SAP), have been shown to be sensitive to drug effects in the elevated-plus maze (Rodgers and

Johnson, 1995). In our study, the probable anxiolytic-like properties of this compound are also reinforced by the notable increase of HD unprotected and the reduction of SAP protected (Table 2). On the other hand, the effects on anxiety were no dose-dependent, probably due to the narrow dose range used.

The regulation of anxiety is associated with function of the GABA-A receptor system. Available evidence points to a major role for α2-containing GABA-A receptors in modulating anxiety (Löw et al, 2000; Griebel et al, 2003), although a recent study also suggests a possible implication for α5 subunit (Navarro et al., 2002). The results of the present study suggest that α3-subunit-containing GABA-A receptors could be involved in anxiety. Localization studies in rodents have revealed that the α3 subunit is expressed in different structures modulating anxiety, such as amygdala and medial septum (Fritschy and Brünig, 2003). Likewise, α3-GABA-A receptors are the main GABA-A receptor subtype expressed by monoaminergic and basal forebrain cholinergic neurons (Gao, Fritschy, Benke and Möhler, 1993), which innervate most of the brain by dense and widespread axonal arbors. Furthermore, our findings are in line with a recent study of Atack et al. (2005) who have demonstrated that α3IA, an inverse agonist selective for GABA-A receptors containing an α3, is anxiogenic in the elevated plus maze test, suggesting that agonists selective for this subtype should be anxiolytic.

SB-205384 has a novel mechanism of action. Thus, in addition to potentiating the GABA-activated current it prolongs the half-life for decay of current after GABA removal. This effect seems to be selective for the α3ß2α2 subunit combination of GABA-A receptors (Meadows et al, 1997, 1998). Our findings are in concordance with those described with tracazolate, a compound that exhibits very similar modulatory properties to SB-205384 (Thompson et al, 2002) and also displays anxiolytic activity (Patel and Malick, 1982; Young et al, 1987).

In conclusion, in this study we have shown that SB-20538, an α3 subunit positive modulator of GABA-A receptors, could exhibit an anxiolytic-like profile in the elevated plus-maze in male mice. Overall, these results suggest that GABA-A receptors which contain α3 subunits might be involved in regulation anxiety. Further studies with more selective compounds for α3-subunit-containing GABA-A receptors are needed to confirm these results.

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