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Effects of SB-205384, a positive modulator of α3-subunit-containing GABA-A receptors, on isolation-induced aggression in male mice

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GABA-A receptors are involved in the control of aggressive behaviour. Various studies suggest a role for α1-containing GABA-A receptors in modulating aggression. However, the possible involvement of α3 subunit of GABA-A receptors has not been examined. In this study, we analysed the effect of SB-205384 (0.5-4 mg/kg, i.p), a positive modulator of GABA-A receptors containing α3 subunit, on agonistic behaviour elicited by isolation in male mice. Half of the mice were housed during 30 days and employed as experimental or control animals; the remainder were used as «opponents» and were temporally rendered anosmic by zinc sulphate. Individually housed mice were exposed to anosmic opponents in a neutral area 30 minutes after the drug administration and encounters were videotaped and evaluated using an ethopharmacologically-based analysis. The results indicated that SB-205384 did not produce any significant behavioural changes, suggesting that GABA-A receptors which contain the α3 subunit may not be involved in the modulation of aggression.

Efectos del SB-205384, un modulador positivo de los receptores GABA-A que contienen la subunidad α3, sobre la agresión inducida por aislamiento en ratones machos. Los receptores GABA-A están involucrados en el control de la conducta agresiva. Diversos estudios sugieren un papel de los receptores GABA-A que contienen la subunidad α1 en la modulación de la agresión. Sin embargo, la posible implicación de los receptores que contienen la subunidad α3 no ha sido examinada. En este trabajo analizamos el efecto de la administración de SB-205384 (0.5-4 mg/kg, i.p), un modulador positivo de los receptores GABA-A que contienen la subunidad α3, sobre la conducta agonística en ratones machos, utilizando un modelo de agresión inducida por aislamiento. La mitad de los ratones fueron aislados durante 30 días y empleados como animales experimentales o controles; la otra mitad fueron utilizados como «oponentes», siendos anosmiados temporalmente mediante sulfato de zinc. Treinta minutos después de la administración del fármaco se llevaron a cabo interacciones agonísticas de diez minutos de duración entre un animal aislado y un oponente anósmico en un área neutral, grabadas en vídeo para su posterior análisis etológico/conductual mediante ordenador. Los resultados indicaron que el SB-205384 no produjo cambios conductuales significativos, sugiriendo que los receptores GABA-A que contienen la subunidad α3 podrían no estar implicados en la modulación de la agresión.

Gamma-aminobutyric acid (GABA) is the primary mediator of inhibitory transmission in the mammalian central nervous system. GABA-A receptor belongs to the super-family of ligand-gated ion channels. It is assembled as a pentameric complex (i.e. composed of five subunits), arranged symmetrically to enclose the transmembrane chloride ion channel. Each subunit of GABA-A receptors consists of four trans-membrane domain (TM1-4) modulating the receptor activity. To date, eight subclasses of GABA-A receptor subunits have been cloned in the CNS (α1-6, β1-3, γ1-3, δ, ε, θ, π and ρ1-3) (Steiger & Russek, 2004; Korpi & Sinkkonen, 2006; Rudolph & Möhler, 2006).

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GABAergic system is clearly involved in the modulation of aggression. Thus, many substances that act on the GABA-A receptors provoke marked effects on aggressive behaviour (Beltrán & Navarro, 2002; Miczek et al., 2002; De Almeida et al., 2005). Drugs with a high affinity for GABA-A receptors which contain the α1 subunit, such as zolpidem, exhibit antiaggressive effects in isolated male mice, suggesting a role for these receptors in the modulation of aggression (Martín-López & Navarro, 2002). Likewise, selective ligands for α5-GABA-A receptors also reduce significantly aggression, although this action seems to be unselective (Navarro et al., 2004). To date, there have been few functional studies involving the α3-GABA-A receptors and, to our knowledge, no studies have focused on the possible role of these receptors in aggression.

SB-205384 is an α3 subunit positive modulator of GABA-A receptors. This substance belongs to the group of benzothiophene compounds that act as allosteric modulators of GABA-A receptor function (Meadows et al., 1998). The aim of this study was to examine the role of α3-containing GABA-A receptors in

aggression. For this purpose, we designed an experiment to analyze the effects of SB-205384 (0.5-4 mg/kg) on agonistic encounters in isolated male mice using an ethopharmacological approach. The term «agonistic behaviour» comprises all elements of behaviour present in situations of conflict, including attack, defence and flight. Although this model mainly represents offensive aspects of agonistic behaviours, defensive aspects are also present, which renders the model useful to measure more subtle activity of drugs as well (Krsiak, 1979).

Materials and methods

Animals

A total of 206 albino male mice of the OF.1 strain (provided by CRIFFA, Barcelona, Spain) weighing 25-30 g were used. Animals were housed under standardised lighting conditions (white lights on: 20:00-8:00) at a constant temperature (21 ºC) with food and tap water available *ad libitum* except during behavioural trials. Upon arrival in the laboratory, mice were allocated to two different categories. Half were housed individually in transparent plastic cages (24 x 13.5 x 13 cm) as experimental animals and the remainder housed in groups of five to be used as «standard opponents» and were rendered temporally anosmic by intranasal lavage with 4% zinc sulphate solution (Sigma Laboratories) on both days 1 and 3 before testing. We used this type of opponents because it elicits attack but never initiates such behaviour (Brain et al., 1981).

All experimental animals were kept in isolation for 30 days prior to behavioural testing (isolation-induced aggression model) since social isolation is an effective form of increasing the level of aggressiveness in different species of animals, particularly in laboratory mice (Valzelli, 1969; Navarro, 1997).

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

Six groups of mice were used. Animals were randomly allocated to two control group (n= 16-17 each) receiving only physiological saline or physiological saline (90%) plus DMSO (10%), and four experimental groups (N= 16-19 each) receiving SB-205384 injections. SB-205384 (Tocris Laboratories) was diluted in physiological saline (90%) plus DMSO (10%) to provide appropriate doses for injections and administered in four doses: 0.5, 1, 2 and 4 mg/kg. Drug or vehicle was injected intraperitoneally in a volume of 10 ml/kg. Tests were performed 30 min after injections. The doses were chosen on the basis on a previous study carried out in our laboratory with SB-205384 (Navarro et al., 2006).

Agonistic encounters and behavioural analysis

30 minutes after injection, an isolated animal and a «standard opponent» were allowed to confront each other in a neutral area for 10 min. This neutral cage consisted of an all glass area, measuring 50 x 26 x 30 cm with a fresh sawdust substrate. Before the encounter, the animals were allowed 1 min of adaptation to the neutral cage, remaining separated by means of a plastic barrier throughout this time. The social encounters were videotaped using a Sony-V8 camera. All tests were conducted under red light between the second and seventh hours of the dark phase of the artificial cycle of the animals. After each encounter, the neutral cage was washed out and the sawdust bedding was replaced. The tapes were analyzed using a microprocessor and a custom-developed programme (Brain et al., 1989), which facilitated estimation of times allocated to ten broad behavioural categories. The names of the categories and their constituent elements are as follows: (i) body care (which includes groom, self-groom, wash, shake, scratch); (ii) digging (dig, kick dig, push dig); (iii) non-social exploration (explore, rear, supported rear, scan); (iv) exploration from a distance (approach, attend, circle, head orient, streched attention); (v) social investigation (crawl over, crawl under, follow, groom, head groom, investigate, nose sniff, sniff, push past, walk around); (vi) threat (aggressive groom, sideways offensive, upright offensive, tail rattle); (vii) attack (charge, lunge, attack, chase); (viii) avoidance/flee (evade, flinch, retreat, ricochet, wheel, startle, jump, leave, wall, clutch); (ix) defense/submission (upright defensive, upright submissive, sideways defensive), and (x) immobility (squat, cringe). This ethoexperimental procedure allows a complete quantification of the behavioural elements shown by the subject during the agonistic encounters. Only the behaviour of the isolated animal was assessed. The analysis was carried out by a trained experimenter who was unaware of the treatment administered to the groups.

Data analyses

The medians for times allocated to each behavioural category were determined. Non-parametric Kruskal-Wallis tests were used to assess the variance of the behavioural measures over different treatment groups. Subsequently, if necessary, appropriate paired comparisons would be carried out using Mann-Whitney U-tests to contrast the behaviour in the different treatment groups. The analysis was performed using non-parametric statistics since the criteria for the parametric statistics were not met by the data.

Results

The effects of acute administration of SB-205384 on agonistic interactions between male mice are shown in table 1 (medians with ranges). Kruskal-Wallis analysis showed that there were no significant differences between control and experimental groups in any of the behavioural parameters examined.

Discussion

This study represents an attempt to explore the effects of SB-205384, a positive modulator of GABA-A receptors which contain the ·3 subunit, on agonistic interactions between male mice. The results obtained in the present study indicate that SB-205384 did not exhibit an antiaggressive / proaggressive activity in isolated male mice. To our knowledge, this is the first report in which the behavioural profile of drugs acting on these receptors has been examined in agonistic encounters between mice.

Clinical studies show that the benzodiazepines usually reduce aggressive behaviour. These compounds enhance the GABAergic

activity via their positive modulation of the GABA-A receptor subtype. In animal studies, numerous ligands for GABA-A receptors appear to display antiaggressive activity. Benzodiazepines such as clobazam (Martín-López & Navarro, 1996), diazepam (Martín-López & Navarro, 1997), bentazepam (Martín-López & Navarro, 1998) and midazolam (Martín-López & Navarro, 1999), as well cyclopyrrolones and imidazopyridines such as zopiclone and zolpidem, respectively (Martín-López et al., 1994; Martín-López & Navarro, 2002) have demonstrated to possess antiaggressive properties in isolation-induced aggression models. Likewise, L-655,708, a selective ligand for the benzodiazepine site of GABA-A receptors which contain the α5 subunit, also reduced aggressive behaviour, although this effect seemed to be unselective (Navarro et al., 2004). On the other hand, GABA-A receptors has also been implicated in escalated aggression. Thus, positive modulators of GABA-A receptors with specific subunit configuration may be relevant for heightening aggression. These effects on aggressive behaviour might be related to the modulation of GABA-A receptors by serotonin in corticolimbic projection areas. In fact, there is wide neurochemical and behavioural evidence that 5-HT and GABA interact in diverse brain regions (De Almeida et al., 2005).

The expression pattern of the various GABA-A receptor subunits varies extensively between different brain regions. The subunit combination α1β2γ2 represents ~ 50% of the total GABA-A population. In contrast, the α3 subunit is expressed at lower levels. The α3 subunit most frequently coassembles with β2/3 and γ2 subunits, and this minor receptor subtype accounts for ~ 15% of the total complement of receptors. SB-205384 is a positive modulator of α3-subunit containing GABA-A receptors with a novel mechanism of action. Thus, in addiction to potentiating the GABA-A activated current, it prolongs the half-life for decay of current after GABA removal. This effect seems to be selective for the α3β2γ2 subunit combination of GABA-A receptors (Meadows et al., 1997, 1998).

Pharmacological studies indicate that anxiolytic effects of benzodiazepines are mainly mediated by GABA-A receptors containing the α3 subunit (Atack et al., 2005; Dias et al., 2005), which are highly expressed, among other structures, in several brain regions involved in the modulation of anxiety (such as amygdala or medial septum) (Fritschy & Brüning, 2003). On the other hand, in a recent study with SB-205384 (an α3 subunit positive modulator of GABA-A receptor) was found a clear anxiolytic-like profile in mice tested in the elevated plus maze (Navarro et al., 2006). Although the distribution of these receptors could also suggest a possible role in the regulation of aggressive behaviour, our results show that these receptors might not be implicated in the modulation of aggression. Further studies with other more selective compounds for α3 subunit-containing GABA-A receptors and a greater dose range are needed to confirm these findings.

Table 1
Medians values (with ranges) for times (in seconds) allocated to broad behavioural categories in animals receiving acute treatment with SB 205384 (0.5-4 mg/kg, ip)

Behavioural categories			**Doses SB205384 (mg/kg)**			
	Saline	**Vehicle (DMSO 10%)**	**0.5**	**1**	**2**	**4**
Body care	7.4 (1-20)	5.2 (0-37)	8.6 (0-20)	7.3 (0.5-48)	5.1 (1.3-34)	4.3 (0.3-21)
Digging	10.6 (0.8-43)	6.2 (0.2-26)	7.1 (0.2-42)	3 (0-25)	4.7 (0-48)	3.8 (0-33)
Non-social Exploration	344 (257-434)	352 (283-407)	342 (243-385)	357 (179-489)	370 (266-418)	356 (260-421)
Exploration From a distance	27.6 (4.4-56)	25.7 (8.1-51)	26.4 (16-63)	22.3 (6-58)	22 (6-58)	28.8 (17-108)
Social Investigation	79.6 (17.6-159)	85.5 (5-160)	79.5 (15-169)	85.5 (18-263)	82.1 (25-123)	66.6 (10-173)
Threat	56 (5.3-118)	64 (0-110)	74 (1.5-112)	38.4 (0-228)	35.5 (0-85)	51 (0-189)
Attack	14.1 (0-71)	7.4 (0-52)	5.5 (0-62)	3.75 (0-29)	4.6 (0-45)	9.8 (0-54)
Avoidance/flee	0 (0-3.9)	0 (0-1.3)	0 (0-2)	0 (0-3)	0 (0-1.6)	0 (0-6.5)
Defence/Submission	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1.8)	0 (0-5.5)
Immobility	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)

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