Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 586 (2008) 217–220
www.elsevier.com/locate/ejphar

Short communication

JNJ16259685, a selective $mGlu_1$ antagonist, suppresses isolation-induced aggression in male mice

José Francisco Navarro *, Vanessa De Castro, Mercedes Martín-López

Department of Psychobiology, Faculty of Psychology, University of Málaga, Spain

Received 19 July 2007; received in revised form 7 February 2008; accepted 20 February 2008
Available online 29 February 2008

Abstract

$mGlu_1$ receptors are present in brain regions involved in aggression modulation. This study examines the effects of 3-4-Dihydro-2*H*-pyrano [2,3-b]quinolin-7-yl-(cis-4-methoxycyclohexyl)-methanone (JNJ16259685; 0.125, 0.25, 0.5, 1, 2, 4 and 8 mg/kg, i.p), a selective antagonist of the $mGlu_1$ receptors, on agonistic interactions between male mice. Individually housed mice were exposed to anosmic "standard opponents" 30 min after drug administration. Ten minutes of diadic interactions was staged between a singly housed and an anosmic mouse in a neutral area. The encounters were videotaped and the accumulated time allocated by subjects to ten broad behavioural categories was estimated using an ethologically based analysis. JNJ16259685 (all doses) produced a significant reduction of offensive behaviours (threat and attack), without affecting immobility. These findings suggest for the first time a role for $mGlu_1$ receptors in aggression regulation.

Keywords: Aggression; Metabotropic glutamate receptor; $mGlu_1$ receptor; (Mouse)

1. Introduction

Glutamate plays a critical role as excitatory neurotransmitter in the central nervous system. It acts at several types of receptors, including ionotropic (cation-specific ion channels divided into three groups: NMDA, AMPA and kainate receptors) and metabotropic receptors (mGlu), which are members of the wider G-protein-coupled receptor family. To date, eight glutamate mGlu receptor subtypes, together with splice variants, have been cloned and characterized in functional studies. These receptors are grouped into three classes based on structural homology, pharmacology, and signal transduction mechanisms: group I (mGluR 1 and 5), group II (mGluR 2 and 3) and group III (mGluR 4, 6, 7 and 8) (Kew and Kemp, 2005; Ferraguti and Shigemoto, 2006).

Glutamate is involved in the modulation of aggression in laboratory animals. Agents which reduce activity of ionotropic *N*-methyl-D-aspartate (NMDA) receptors inhibit isolation-induced aggression in mice (Belozertsseva and Bespalov, 1999; Lumley et al., 2004). Likewise, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) subtype of glutamate receptors has been recently implicated in aggression. Thus, Vekovischeva et al. (2004) found a reduced aggression in AMPA-type glutamate receptor GluR-A subunit-deficient mice, and Shimshek et al. (2006) also communicated that male mice with a lack of GluR-B containing AMPA receptors exhibit aggressive behaviours. Moreover, the glutamate receptor subunit AMPA3 gene appears to be involved in aggression of mice (Brodkin et al., 2002).

By contrast to ionotropic receptors, the implication of mGlu receptors in aggression is scarcely known. In a recent study, Navarro et al. (2006) found that the acute administration of 2-methyl-6-(phenylethylnyl)pyridine (MPEP), a selective antagonist of $mGlu_5$ receptors, reduces offensive behaviours (threat and attack) in male mice, suggesting a role for these receptors in aggression regulation. $mGlu_1$ receptors are present in brain structures involved in aggression, like the central periaquaductal gray, amygdala or lateral septum (Lavreysen et al., 2004a). So far, no studies have examined

* Corresponding author. Department of Psychobiology, Faculty of Psychology, Campus de Teatinos s/n, University of Málaga, 29071 Málaga, Spain. Tel.: +34 952 132501; fax: +34 952 132621.
E-mail address: navahuma@uma.es (J.F. Navarro).

0014-2999/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2008.02.070

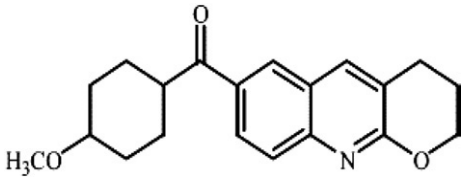

Fig. 1. Chemical structure of 3-4-Dihydro-2*H*-pyrano[2,3-b]quinolin-7-yl-(cis-4-methoxycyclohexyl)-methanone (JNJ16259685).

the functional role of $mGlu_1$ antagonists in animal models of aggression. Therefore, this work was designed to analyze the effects of 3-4-Dihydro-2*H*-pyrano[2,3-b]quinolin-7-yl-(cis-4-methoxycyclohexyl)-methanone (JNJ16259685), a selective antagonist at the $mGlu_1$ receptors, on agonistic behaviour elicited by isolation in male mice (see Fig. 1). Although this model mainly represents offensive aspects of agonistic behaviours, defensive and social aspects are also present, which renders the model useful to measure more subtle activity of drugs as well.

2. Materials and methods

A total of 264 albino male mice of the OF.1 strain weighing 25–30 g were used. Animals were housed under standardized lighting conditions (white lights on: 20:00–08:00) at a constant temperature (21 °C) with food and tap water available *ad libitum* except during behavioural trials. Upon arrival in the laboratory, mice were allocated to two categories. Half were housed individually in transparent plastic cages (24×13.5×13 cm) to be used as experimental animals. All experimental subjects were isolated for 30 days prior to behavioural testing since this housing is an effective means of increasing the level of aggressiveness, particularly in laboratory mice (Brain et al., 1989). The remainder were housed in groups of five to be used as "standard opponents". These animals were rendered temporally anosmic by intranasal lavage with 4% zinc sulfate solution (Sigma Laboratories) on both days 1 and 3 before testing. This type of opponent elicits attack but never initiates such behaviour (Brain et al., 1981). Consequently, fighting is always unidirectional, and easily quantified.

Nine groups of mice were used. Animals were randomly allocated to two control groups ($n=15$ each) receiving only saline or saline (90%) plus DMSO (10%), and seven experimental groups ($N=14$–16 each) receiving JNJ16259685 injections. JNJ16259685 (Tocris Laboratories) was diluted in saline (90%) plus DMSO (10%) to provide appropriate doses for injections and administered in seven doses: 0.125, 0.25, 0.5, 1, 2, 4 and 8 mg/kg. The doses were chosen on the basis of recent behavioural studies using this compound (Steckler et al., 2005a, b; Dravolina et al., 2007). Drug or vehicle was injected intraperitoneally in a volume of 10 ml/kg.

Thirty 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 arena, measuring 50×26×30 cm with a fresh sawdust substrate. Before the encounter, the animals were allowed 1 min of adaptation to the neutral cage, whilst separated by means of a plastic barrier. 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 lighting condition. After each encounter, the neutral cage was washed and the sawdust bedding was replaced. The tapes were analyzed using a microprocessor and a custom-developed program (Brain et al., 1989), which facilitated estimating time and frequency allocated to ten broad behavioural categories. Only the behaviour of the isolated animal was assessed and the analysis was carried out by a trained experimenter 'blind' to the treatment administered to the experimental subjects. The categories and their constituent elements were as follows: (i) body care (including 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, stretched 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) defence/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.

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 in the different treatment groups. Subsequently, appropriate paired comparisons were carried out using Mann–Whitney U-tests to contrast behaviours in different treatment groups. The analysis was performed using non-parametric statistics, since criteria for parametric statistics were not met by the data.

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).

3. Results

The effects of acute administration of JNJ16259685 on agonistic interactions between male mice are shown in Table 1 (medians with ranges). Kruskall–Wallis analysis showed that there was significant variance in the categories of body care, digging, non-social exploration, threat and attack ($P<0.0001$). Paired comparisons using Mann–Whitney U-tests revealed that JNJ16259685 significantly reduced the time spent in digging behaviours (0.25–8 mg/kg; $P<0.0001$–$P<0.05$), threat (all doses; $P<0.0001$–$P<0.01$) and attack (all doses; $P<0.0001$–$P<0.05$), in comparison with vehicle group. Body care behaviours were also decreased after treatment with the drug (2, 4 and 8 mg/kg; $P<0.0001$–$P<0.002$), whereas non-social exploration behaviours (0.25–8 mg/kg) were increased, as compared with vehicle group ($P<0.0001$–$P<0.001$). The median values for the categories of defence/submission and immobility were zero for all groups. No

Table 1
Medians values (with ranges) for times (in seconds) allocated to broad behavioural categories in animals receiving acute treatment with JNJ16259685 (0.125–8 mg/kg, i.p)

Behavioural categories	Saline	Vehicle	Doses of JNJ16259685 (mg/kg)						
			0.125	0.25	0.5	1	2	4	8
Body care [a]	10.07 (4–18)	7.94 (0–38)	8.9 (0.5–22)	6.2 (0–31)	9.8 (0–26)	7.32 (0.4–31)	3.2 [e] (0–11)	0.7 [e] (0–22)	1.2 [e] (0–12)
Digging [a]	12.04 (0–28)	4.2 (0–42)	4.6 (0–24)	0.32 [b] (0–6)	0.71 [b] (0–9)	0.33 [b] (0–14)	0 [b] (0–8)	0 [b] (0–22)	0 [b] (0–9)
Non-social exploration [a]	339 (199–462)	368 (246–459)	403 (250–519)	447 [c] (338–506)	492 [c] (289–561)	444 [c] (320–521)	489 [c] (290–525)	485 [c] (434–527)	480 [c] (352–547)
Exploration from a distance	21.93 (0.6–82)	24.17 (3.4–101)	7.4 (0.7–36)	10.3 (0.7–27)	21.8 (2.7–92)	15.9 (2.4–64)	17.6 (5.5–74)	29.2 (2.3–55)	21.1 (6–55)
Social investigation	54.85 (2.4–232)	73.35 (11.7–196)	87.3 (21.–243)	113.6 (30–256)	73.3 (9–128)	89.6 (0–170)	69.6 (37–284)	62.6 (27–123)	76.3 (32–191)
Threat [a]	132.1 (0–208)	88.54 (0–210)	28.3 [d] (0–131)	0 [d] (0–118)	0 [d] (0–100)	0 [d] (0–173)	0 [d] (0–115)	0 [d] (0–0.3)	0 [d] (0–97)
Attack [a]	32.17 (0–92.4)	33.87 (0–98)	6.1 [b] (0–88)	0 [b] (0–25)	0 [b] (0–97)	0 [b] (0–66)	0 [b] (0–35)	0 [b] (0–0)	0 [b] (0–76)
Avoidance/flee	0 (0–4)	0 (0–2.6)	0 (0–3.5)	0 (0–4.8)	0.5 (0–5.2)	0 (0–2.1)	0.9 (0–7.2)	0.25 (0–7.1)	0 (0–5.7)
Defence/submission	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Immobility	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–3)	0 (0–46)	0 (0–62)	0 (0–81)

Kruskal–Wallis test showed significant variance: [a] $P<0.0001$.
Differs from vehicle on Mann–Whitney U-tests: [b] $P<0.0001$–$P<0.05$; [c] $P<0.0001$–$P<0.01$; [d] $P<0.001$–$P<0.01$; [e] $P<0.0001$–$P<0.002$.

significant differences were observed between both control groups (saline and vehicle) in any of the behavioural categories analyzed.

4. Discussion

mGlu receptors exert an important role in mediating glutamate neurotransmission in the brain. $mGlu_1$ receptors have been implicated in the modulation of memory and learning (Steckler et al., 2005a), epilepsy (Shannon et al., 2005), drug abuse (Dravolina et al., 2007), anxiety (Steckler et al., 2005b) or pain (Sevostianova and Danysz, 2006). However, in spite of the distribution of $mGlu_1$ receptors in brain structures related to aggressive behaviour (such as the central periaquaductal gray, amygdala or lateral septum), to date no studies have explored the role of these receptors in aggression regulation.

JNJ16259685 is a selective $mGlu_1$ receptor antagonist with excellent potencies in inhibiting $mGlu_1$ receptor function and binding and in occupying the $mGlu_1$ receptor after systemic administration (Lavreysen et al., 2004b). As Table 1 shows, the results of this study indicate that acute administration with JNJ16259685 in a dose wide range (0.125–8 mg/kg) produced a strong reduction of offensive behaviours (threat and attack), without affecting immobility, as compared with vehicle group. To our knowledge, the present results represent the first data describing the effects of a selective ligand for $mGlu_1$ receptors on aggression in experimental animals. The doses employed in this study are very similar to those used by other authors (0.63–10 mg/kg) in mice (Steckler et al., 2005a), without affecting motility.

The reduction of aggressive behaviours after treatment with JNJ16259685 was accompanied by an increase in non-social exploration behaviours as well as a decrease in digging behaviours with all doses of the drug used. Digging is involved in aggressive behaviour together with the threats and attacking behaviour. In fact, there is usually a correlation between these behavioural domains (Kudryavtseva et al., 2000), and they may be induced by electrical stimulation in the lateral hypothalamus (Lammers et al., 1987).

JNJ16259685 produced robust antiaggressive effects in male mice. With all doses used (from 0.125 to 8 mg/kg) the reduction of offensive behaviours (threat and attack) is statistically significant. These findings indicates that a dose of 0.125 mg/kg would be sufficient to produce a reduction of aggression (being specially marked since 0.25 mg/kg), perhaps provoking a "ceiling" effect. Thus, the administration of greater doses of JNJ16259685 would produce a similar decrease of aggression. The antiaggressive action induced by the blockade of $mGlu_1$ receptors is similar to that described with MPEP, a selective antagonist for $mGlu_5$ receptors. In this sense, Navarro et al. (2006) recently communicated a notable reduction of offensive behaviours using the same animal model of isolation-induced aggression in male mice.

The antiaggressive effects of JNJ16259685 are mediated through blockade of $mGlu_1$ receptors since this drug is a potent and highly selective antagonist at this receptor subtype. In conclusion, this is the first demonstration that an antagonist of $mGlu_1$ receptors decreases aggressive behaviours (threat and attack) in mice. These findings suggest a role for $mGlu_1$ receptors in the regulation of aggression. Further studies using other selective ligands for $mGlu_1$ are necessary to confirm these results.

References

Belozertsseva, I.V., Bespalov, A.Y., 1999. Effects of NMDA receptor channel blockade on aggression in isolated male mice. Aggress. Behav. 25, 381–396.

Brain, P.F., Benton, D., Childs, G., Parmigiani, S., 1981. The effects of the type of opponent in test of murine aggression. Behav. Processes 6, 319–327.

Brain, P.F., McAllister, K.H., Walmsey, S., 1989. Drug effects on social behaviour: methods in ethopharmacology. In: Boulton, A.A., Baker, G.B., Greenshaws, A.J. (Eds.), Neuromethods. Humana Press, New Jersey, pp. 687–739.

Brodkin, E.S., Goforth, S.A., Keene, A.H., Fossella, J.A., Silver, M., 2002. Identification of quantitative trait loci that affect aggressive behavior in mice. J. Neurosci. 22, 1165–1170.

Dravolina, O.A., Zakharova, E.S., Shekunova, E.V., Zvartau, E.E., Danysz, W., Bespalov, A.Y., 2007. $mGlu_1$ receptor blockade attenuates cue- and nicotine-induced reinstatement of extinguished nicotine self-administration behavior in rats. Neuropharmacology 52, 263–269.

Ferraguti, F., Shigemoto, R., 2006. Metabotropic glutamate receptors. Cell Tissue Res. 326, 484–504.

Kew, J.N.C., Kemp, J.A., 2005. Ionotropic and metabotropic glutamate receptor structure and pharmacology. Psychopharmacology 179, 4–29.

Kudryavtseva, N.N., Bondar, N.P., Alekseyenko, O.V., 2000. Behavioral correlates of learned aggression in male mice. Aggress. Behav. 26, 386–400.

Lammers, J.H., Meelis, W., Kruk, M.R., van der Poel, A.M., 1987. Hypothalamic substrates for brain stimulation-induced grooming, digging and circling in the rat. Brain Res. 418, 1–19.

Lavreysen, H., Nóbrega, S., Leysen, J.E., Langlois, X., Lesage, A.S.J., 2004a. Metabotropic glutamate 1 receptor distribution and occupancy in the rat brain: a quantitative autoradiographic study using [3H]R214127. Neuropharmacology 46, 609–619.

Lavreysen, H., Wouters, R., Bischoff, F., Nóbrega, S., Langlois, X., Blockland, S., et al., 2004b. JNJ16259685, a highly potent, selective and systemically active $mGlu_1$ receptor antagonist. Neuropharmacology 47, 961–972.

Lumley, L.A., Robinson, C.L., Slusher, B.S., Wozniak, K., Dawood, M., Meyerhoff, J.L., 2004. Reduced isolation-induced aggressiveness in mice following NAALADase inhibition. Psychopharmacology 171, 375–381.

Navarro, J.F., Postigo, D., Martín-López, M., Burón, E., 2006. Antiaggressive effects of MPEP, a selective antagonist of $mGlu_5$ receptor, in agonistic interactions between male mice. Eur. J. Pharmacol. 551, 67–70.

Sevostianova, N., Danysz, W., 2006. Analgesic effects of $mGlu_1$ and $mGlu_5$ receptor antagonists in the rat formalin test. Neuropharmacology 51, 623–630.

Shannon, H.E., Peters, S.C., Kingston, A.E., 2005. Anticonvulsant effects of LY456236, a selective $mGlu_1$ receptor antagonist. Neuropharmacology 49, 188–195.

Shimshek, D.R., Bus, T., Grinevich, V., Single, F.N., Mack, V., Sprengel, R., et al., 2006. Impaired reproductive behaviour by lack of GluR-B containing AMPA receptors but not of NMDA receptors in hypothalamic and septal neurons. Mol. Endocrinol. 20, 219–231.

Steckler, T., Oliveira, A.M., Van Dyck, Ch., van Craenendonck, H., Mateus, A.M., Langlois, X., et al., 2005a. Metabotropic glutamate receptor 1 blockade impairs acquisition and retention in a spatial water maze task. Behav. Brain Res. 164, 52–60.

Steckler, T., Lavreysen, H., Oliveira, A.M., Aerts, N., van Craenendonck, H., Prickaerts, J., et al., 2005b. Effects of mGlu1 receptor blockade on anxiety-related behaviour in the rat lick suppression test. Psychopharmacology 179, 198–206.

Vekovischeva, O.Y., Aitta-Aho, T., Echenko, O., Kankaanpaa, A., Seppala, T., Honkanen, A., et al., 2004. Reduced aggression in AMPA-type glutamate receptor GluR-A subunit-deficient mice. Genes Brain Behav 3, 253–265.