Rev Psiquiatr Salud Ment (Barc.) 2009;**2(3)**:133-137

Revista de Psiquiatría y Salud Mental

www.elsevier.es/saludmental

SHORT ORIGINAL

Effects of the administration of (RS)-3,4-DCPG, a mixed AMPA receptor antagonist/mGluR8 receptor agonist, on aggressive behaviour in mice

José Francisco Navarro,* Vanessa de Castro and Mercedes Martín-López

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

Received 20 January 2009; accepted 6 May 2009

KEYWORDS
Aggression;
Glutamate;
mGlu8 receptor;
AMPA receptor;
Agonistic behavior

Abstract
Introduction: Ionotropic and metabotropic (mGlu) receptors of glutamate have been suggested to be involved in the modulation of aggression. Thus, recent studies found reduced aggression in AMPA-type glutamate receptor GluR-A subunit-defi cient mice. Likewise, mGlu1 and 5 receptors have also been implicated in aggression regulation. (RS)-3,4-DCPG is a mixed antagonist of AMPA receptors and an agonist of mGluR8. The AMPA antagonist activity of this compound is determined by its R isomer while the S isomer is responsible for its mGluR8 agonistic properties.
Methods: We analyzed the effects of (RS)-3,4-DCPG (5, 10 and 20 mg/kg, ip) on agonistic encounters between male mice. Individually housed mice were exposed to anosmic opponents 30 min after drug administration. Ten min of dyadic interactions were 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 10 broad behavioral categories was estimated using an ethologically based analysis.
Results and conclusions: The results indicated that (RS)-3,4-DCPG produced no signifi cant behavioral changes, suggesting that antagonism of AMPA receptors by the R isomer and stimulation of mGluR8 by the S isomer do not act synergistically on aggression in the racemic form of 3,4-DCPG.

*Corresponding author
E-mail: navahuma@uma.es (J.F. Navarro).

1888-9891/$ - see front matter

PALABRAS CLAVE
Agresión;
Glutamato;
Receptores mGlu8;
Receptores AMPA;
Conducta agonista

Efectos de la administración de (RS)-3,4-DCPG, un antagonista mixto de los receptores AMPA y agonista de los receptores mGlu8, en la conducta agresiva de los ratones

Resumen
Introducción: Los receptores ionotrópicos y metabotrópicos (mGlu) de glutamato han sido implicados en la modulación de la agresión. Así, estudios recientes han encontrado una reducción de la agresión en ratones que carecen de la subunidad GluR-A de los receptores AMPA. Asimismo, los receptores mGlu1 y mGlu5 también se han involucrado en la regulación de la agresión. (RS)-3,4-DCPG es un antagonista mixto de receptores AMPA y agonista de receptores mGlu8. Su actividad antagonista AMPA está determinada por su isómero R, mientras que el isómero S es causal de sus propiedades agonistas mGlu8.
Métodos: En este estudio, se analiza el efecto de la administración de (RS)-3,4-DCPG (5, 10 y 20 mg/kg, intraperitoneal) en los encuentros agonistas entre ratones machos; 30 min después de la administración del fármaco se llevaron a cabo interacciones agonistas de 10 min de duración entre un animal aislado y un oponente anósmico en un área neutral. Dichos encuentros se grabaron en vídeo para su análisis etológico mediante un programa de ordenador y se estimó el tiempo pasado por los ratones en cada una de diez categorías conductuales.
Resultados y conclusiones: Los resultados indicaron que el (RS)-3,4-DCPG no produjo cambios conductuales significativos, esto apunta a que el antagonismo de los receptores AMPA por el isómero R y la estimulación de los receptores mGlu8 por el isómero S no parecen actuar sinérgicamente sobre la agresión en la forma racémica del 3,4-DCPG.

Introduction

Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS). Once it is released from the presynaptic vesicles, it bonds with postsynaptic ionotropic receptors (NMDA, AMPA and kainate). Furthermore, it activates metabotropic receptors (mGluR), which modulate their release, the postsynaptic response and the activity of other synapses. To date, eight subtypes of metabotropic glutamate receptors (mGluR1-8) have been described. These are divided into three different groups based on their amino acid sequence homology, the mechanisms of signal transduction, the secondary messenger system to which they are coupled and their pharmacology. Thus, we have metabotropic receptors in group I, which includes mGluR1 and mGluR5, those of group 11, comprised by the mGluR2 and mGluR3 receptors and, finally, those of group III, which includes the mGluR4, 6, 7 and 8 receptors.[1-3]

Numerous studies have shown that glutamate is involved in regulating aggression. Thus, initial studies examining the administration of NMDA glutamate receptor antagonists, such as phencyclidine (PCP) or dizocilpine (MK-801), observed changes in the aggressive behaviour of animals, although with contradictory results, and a pro-aggressive and anti-aggressive effect was observed depending on the ligands used, the doses administered and the animal species selected as experimental subjects.[4-6] More recently, Belozertseva et al.,[7] via an aggression model induced by isolation, analysed the effect of agents that acted on the NMDA receptor with different pharmacodynamic characteristics: non-competitive antagonists (PCP, MK-801), antagonists with little affinity to the NMDA receptor (memantine and MRZ 2/579) and competitive antagonists (D-CPPene). The results showed that, at low doses, although there were no significant differences, there was a pro-aggressive trend following the administration of non-competitive NMDA receptor antagonists. All agents that showed an anti-aggressive effect did so at doses which also caused deterioration of motor activity and the majority of them did not increase non-aggressive social behaviour, with the exception of D-CPPene, the only competitive antagonist assessed.

In addition, the involvement of the AMPA receptor in aggressive behaviour was also observed. Thus, in a first study, Vekovischeva et al.[8] found a reduction of agonistic behaviour in genetically modified male mice lacking the GluR-A subunit of this receptor. In a later study, the effects of different AMPA receptor antagonists were compared (two competitive and less selective [CNQX and NBQX] and one non-competitive and very selective [GYKI 52466] in terms of the aggressive behaviour of mice with differing degrees of innate aggression. The results obtained showed that

the administration of competitive antagonists reduced the "bite" component of attack behaviour in the two groups of mice, while the administration of the non-competitive antagonist reduced all components of the attack behaviour in both groups. Although all of them showed a reduction in some category of aggressive behaviour, levels of anxiety also increased during the agonistic encounter.[9] Furthermore, other drugs that modulate (at least partially) the activity of the AMPA receptors, such as topiramate, also showed anti-aggressive effects in animal models.[10]

A recent line of research is focusing on the use of N-acetylated-#a-linked-acidic dipeptidase (NAALADase), which is involved in the formation of glutamate in the CNS. This enzyme is responsible for the hydrolysis of the neurotransmitter N-acetylaspartylglutamate (NAAG) into glutamate and N-acetylaspartate and therefore its inhibition would prevent the transformation of NAAG into glutamate, thus reducing the amount of cerebral glutamate. Thus, Lumley et al.[11] observed that the administration of GPI-2232, a pharmacological agent inhibiting the NAALADase enzyme, produced an anti-aggressive effect in an aggression model induced by isolation. The inhibition of this enzyme reduces cerebral glutamate through different means. On the one hand, the fact that NAAG is not transformed into glutamate causes a drop in glutamate levels in the CNS and, on the other, this inhibition causes an accumulation of NAAG in the brain, which acts as a total agonist for the presynaptic mGluR3 glutamate receptors, which is also a partial agonist for NMDA receptors.

In contrast with the ionotropic glutamate receptors, there has been little research into the possible involvement of the mGlu receptors in the modulation of aggressive behaviour.[12-16] Navarro et al.[12] have found that the administration of a non-competitive mGluR5 receptor antagonist (MPEP) causes a significant reduction in offensive behaviour (threat and attack) in male mice. More recently, it has been observed that blocking of the mGluR1 receptors (with the selective antagonist JNJ16259685) is associated with a significant reduction in aggression.[14] Furthermore, although the results are less clear, the mGluR2/3 and mGluR7 receptors (but not mGluR8) may also be involved in regulating aggressive behaviour in rodents.[15,16]

To analyse in more detail the role of AMPA and mGluR8 glutamate receptors in the modulation of aggressive behaviour, we conducted an experiment to analyse the effect of the administration of (RS)-3,4-DCPG (5, 10 and 20 mg/kg, intraperitoneal [i.p.]), a mixed AMPA receptor antagonist/mGluR8 receptor agonist, on agonistic interactions between male mice, via an aggression model induced by isolation. Its AMPA antagonist activity is determined by its R-isomer, while the S-isomer explains its mGluR8 agonist properties.[17]

Methodology

Animals

A total of 94 adult albino mice of the OF1 strain were used. On arrival to the laboratory, all animals were housed in groups of five for one week to allow them to adapt to our installations and the cycle of light/darkness imposed (light from 20.00 to 08.00), before beginning the isolation period. At the end of the adaptation period, half of the animals (n=47) were divided into experimental and control groups and were housed individually (during a 30 days period) in transparent plastic cages (Tecniplast-Letica, Madrid) measuring 24 x 13.5 x 13cm. At the end of the isolation period, these animals were randomly distributed to the different experimental conditions (control and doses of 5, 10 and 20mg/kg of (RS)-3,4-DCPG). The other mice were used as anosmic opponents and were housed in groups of five in cages of the same characteristics as the others up to the moment when the behaviour test was performed. For all animals, food and drink were administered *ad libitum*. The atmospheric conditions in the laboratory where the animals were located were carefully controlled, maintaining constant temperature (20 °C) and humidity.

This experiment was performed pursuant to the principles of the guide for the care and use of laboratory animals approved by European Directive 86/609/EEC.

Aggression model induced by isolation and description of anosmia

For this research, an aggression model induced by isolation was used; this is one of the models of choice for provoking offensive behaviour between male mice. The procedure consisted of housing male animals in individual cages for a period of 30 days, during which they were provided with food and drink *ad libitum*. At the end of this period, the aggression behaviour encounters were held, lasting 10 min. The dominant animal displays threatening and attack behaviour aimed at the opponent, while the subordinate does not attack and adopts defensive postures.

There is much evidence of the involvement of the olfactory system in triggering aggressive behaviour in rodents. Thus, it has been shown that once the olfactory bulb has been removed, animals no longer display offensive behaviour, not even when bitten by members of the same species.[18] The most common procedure for producing peripheral anosmia (and transitory) is the administration of zinc sulphate (at 4%) through the nasal orifices, which causes reversible necrosis of the nasal epithelium. The reason why the male mice do not fight against their opponents is most likely due to the fact that they are unable to smell the pheromones in the urine of the mice, which is a trigger for offensive behaviour in mice with normal olfactory sense.[19] In our case, anosmia was produced by instilling a 0.025 ml volume of zinc sulphate at 4% in each nasal orifice of the rodents. This procedure was performed on day 1 and 3 prior to each behaviour test.

Drug administration

(RS)-3,4-DCPG was acquired from Tocris laboratories (United Kingdom), and was dissolved in saline serum to prepare the corresponding doses. The doses selected for treatment were 5, 10 and 20mg/kg (n = 11 per group), while the animals in the control group received saline serum (n = 14 per group). In both cases, administration was i.p. and in proportion to the animal's weight (constant volume of 10 ml/kg). Both the drug and the vehicle were administered 30min before the behaviour test.

Behavioural assessment

The agonistic encounters between the isolated animals (experimental and control) and their anosmic opponents took place in a neutral area. The neutral zone consisted of a transparent glass vessel measuring 50 × 26 × 30cm. Following each encounter, the sawdust in the vessel was refreshed to avoid any possible interference of smells.

Before beginning the behaviour test, the two animals were kept at opposite sides of the neutral area for an adaptation period of 1min, after which the separator was removed and the behaviour test, which lasted 10min, began. All agonistic encounters were videoed for subsequent ethological analysis using a computer program designed for this purpose.[20] All behaviour tests were performed 30min after the administration of the drug and the aggressive encounters began during the second hour of darkness (and therefore activity) for the mice.

The ten behaviour categories analysed were as follows: *a)* body care; *b)* scratching; *c)* non-social exploration; *d)* exploration at a distance; *e)* social research; *f)* threat; *g)* attack; *h)* avoidance/flee; *i)* defence/submission, and *j)* immobility. Each category represents a sum of different elements and postures. A more detailed description of these categories, as well as their constituent elements can be found in Brain et al.[20] The behavioural assessment was performed only on the behaviour displayed by the experimental animal. The assessment was blind; the researcher initially did not know the experimental condition of each animal assessed.

Statistical analysis

In order to establish whether there were statistical differences between the two experimental groups (control and treatment) in each of the behaviour categories assessed, non-parametric variance analysis was used (Kruskal-Wallis test). To draw comparisons between pairs of groups, a non-parametric test was used for independent samples (Mann-Whitney U test).

Results and discussion

Table 1 shows the average values (with their intervals) of the time accumulated (in seconds) in each one of the behaviour categories for each treatment group. The Kruskal-Wallis test indicated that there were no statistically significant differences between the different experimental groups. Similarly, the Mann-Whitney test did not show statistically significant differences between pairs of groups.

The aggression model induced by isolation (offensive aggression model) appeared to have an apparent significant validity and, in particular, excellent predictive validity in terms of human aggression.[21] In this respect, the offensive behaviour assessed in this model has been compared with the impulsive/reactive/hostile violence defined in humans.[22] However, many of the drugs that have been useful for controlling aggression in humans have shown a clear anti-aggressive profile on their assessment in this aggression model.[23]

Pharmacological studies assessing the functional role of mGluR8 receptors have been limited due to the lack of selective ligands for said receptors. Recently, however, various drugs that act as agonists of said receptors and are active when administered systemically have been characterised.[24] These receptors are mainly located at the presynaptic level and it is thought that they act as autoreceptors; in recent years, their involvement in several functions has been described. Thus, it has been suggested that these receptors may have a potential role in regulating anxiety,[24,25] the self-administration of alcohol[26] and the modulation of pain in mice.[27]

As shown in table 1, none of the doses of (RS)-3,4-DCPG used in this study (5, 10 and 20mg/kg, i.p.) caused any statistically significant change in offensive behaviour (threat and attack), in comparison with the control group. Similarly, nor were any significant differences observed in the other behaviour categories assessed. These results are in line with those found following the administration of enantiomer (S)-3,4-DCPG16, which acts selectively on mGluR8 receptors, without showing affinity to the AMPA receptors.

Table 1 Averages (intervals) for the time(s) assigned to the 10 behaviour categories assessed in mice treated with (RS)-3,4-DCPG (5-20mg/kg).

Behaviour categories	Doses of (RS)-3,4-DCPG (mg/kg)			
Parametres	Control (n = 14)	5 (n = 11)	10 (n = 11)	20 (n = 11)
Body care	8,9 (0-22)	10 (1,5-28)	10 (2-44)	12,4 (3-32)
Scratching	17 (1-56)	8,7 (0,5-52)	4,2 (0-48)	9,2 (0-57
Non-social exploration	376 (241-484)	328 (255-446)	361 (203-392)	416 (231-503)
Exploration at a distance	14,3 (2,3-52)	16,9 (5,4-52)	14,2 (5,7-35)	20,4 (6-47,5)
Social research	76,6 (2-125)	75,7 (8-268)	60 (19-301)	59,6 (18-173)
Threat	89,8 (0-289)	84,4 (0-177)	116,7 (45-200)	49,1 (0-118)
Attack	6,6 (0-34)	11,3 (0-69)	23,7 (6-53)	6,7 (0-35)
Avoidance/flee	0 (0-21)	0 (0-12)	0 (0-5)	0 (0-19)
Defence/submission	0	0	0	0
Inmobility	0	0	0	0

To conclude, the results obtained in the present experiment indicate that the antagonism of the AMPA receptors by the R isomer and the stimulation of the mGluR8 receptors by the S isomer do not seem to act together on aggression in the racemic form of 3,4-DCPG. A limitation of this work is that it only assessed the behavioural profile of the racemic form of 3,4-DCPG. As such, additional experiments are required to compare both DCPG isomers.

References

1. Luján-Miras R. Receptores metabotrópicos del glutamato: nuevas dianas moleculares en la terapia de enfermedades neurológicas y psiquiátricas. Rev Neurol. 2005;40:43-53.
2. Kew JNC, Kemp JA. Ionotropic and metabotropic glutamate receptor: structure and pharmacology. Psychopharmacology. 2003;179:4-29.
3. Ferraguti F, Shigemoto R. Metabotropic glutamate receptors. Cell Tissue Res. 2006;326:483-504.
4. Mustly RE, Consroe PF. Phencyclidine produces aggressive behaviour in rapid eye movement sleep-deprived rats. Life Sci. 1982;30:1733-8.
5. McAllister K. Ethological analysis of the effects of MK-801 upon aggressive male mice: similarity to chlordiazepoxide. Pharmacol Biochem Behav. 1990;37:101-6.
6. Miczek KA, Hanney M. Psychomotor stimulant effects of Damphetamine, MDMA and PCP-aggressive and schedule-controlled behaviour in mice. Psychopharmacology. 1994;115: 358-65.
7. Belozertseva IV, Bespalov AY. Effects of NMDA receptor channel blockade on aggression in isolated male mice. Aggressive Behav. 1999;25:381-96.
8. Vekoviskeva OY, Aitta-aho T, Echenko O, Kankaanpaa A, Sépala T, Honkanen A, et al. Reduced aggression in AMPA-type glutamate receptor GluR-A subunit-defi cient mice. Genes Brain Behav. 2004;3:253-65.
9. Vekoviskeva OY, Aitta-aho T, Verbitskaya E, Sandnabba K, Korpi ER. AMPA-type glutamate receptor antagonists on intermale social behaviour in two mouse lines bidirectionally selected for offensive aggression. Pharmacol Biochem Behav. 2007;87:241-7.
10. Navarro JF, Burón E, Martín-López M. Antiaggressive effects of topiramate in agonistic encounters between male mice. Methods Find Exp Clin Pharmacol. 2007;29:195-8.
11. Lumley LA, Robinson LC, Slusher BS, Wozniak K, Dawood M, Meyerhoff JL. Reduced isolation-induced aggressiveness in mice following NAALADase inhibition. Psychopharmacology. 2004;171:375-81.
12. Navarro JF, Postigo D, Martín M, Burón E. Antiaggressive effects of MPEP, a selective antagonist of mGlu5 receptors, in agonistic interactions between mice. Eur J Pharmacol. 2006;551:67-70.
13. Huertas P, Martín-López M, Burón E, Navarro JF. Efectos de la administración de (RS)-2-cloro-5-hidroxifenilglicina sobre la conducta agonística en ratones. Psiq Biol. 2007;14:199-203.
14. Navarro JF, De Castro V, Martín-López M. JNJ16259685, a selective mGlu1 receptor antagonist, suppresses isolation-induced aggression in male mice. Eur J Pharmacol. 2008;586:217-20.
15. Navarro JF, Luque MJ, Martín-López M. Effects of LY379268, a selective agonist of mGlu2/3 receptors, on isolation-induced aggression in male mice. The Open Pharmacol J. 2009;3:17-20.
16. Navarro JF, De Castro V, Martín-López M. Behavioural profi le of selective ligands for mGlu7 and mGlu8 glutamate receptors in agonistic encounters between male mice. Psicothema. 2009;21: 475-9.
17. Owssowska K, Pietraszek M, Wards J, Wolfarth S. Potencial antipsychotic and extrapyramidal effects of (RS)-3,4-dicarboxyphenylglycine ((RS)-3,4-DCPG), a mixed AMPA antagonist/mGluR8 agonist. Pol J Pharmacol. 2004;56:295-304.
18. Bergvall AH, Matuszczyk JV, Dahöf LG, Hansen S. Peripheral anosmia attenuates female-enhanced aggression in male mice. Physiol Behav. 1991;10:79-81.
19. Mugford RA, Novell NM. Pheromones and their effect on aggression in mice. Nature. 1970;226:967-8.
20. Brain PF, McAllister KH, Walmsey S. Drug effects on social behaviour. En: Boulton AA, Baker GB, Greenshaw AJ, editores. Neuromethods. New Jersey: Humana Press; 1989. p. 687-739.
21. Oliver B, Young LJ. Animal models of aggression. En: Davis KL, Charney D, Coyle JT, Nemeroff CH, editores. Neuropsychopharmacology: The fi fth generation of progress. American College of Neuropsychopharmacology; 2002. p. 1699-708.
22. McEllistrem JE. Affective and predatory violence: A bimodal classifi cation system of human aggression and violence. Aggr Viol Behav. 2004;10:1-30.
23. Siegel A, Bhatt S, Bhatt R, Zalcman SS. The neurobiological bases for development of pharmacological treatment of aggressive disorders. Curr Neuropharmacol. 2007;5:135-47.
24. Linden AM, Bergeron M, Baez M, Schoepp DD. Systemic administration of the potent mGlu8 receptor agonist (S)-3,4-DCPG induces c-Fos in stress-related brain regions in wild-type, but not mGlu8 receptor knockout mice. Neuropharmacology. 2003;45:473-83.
25. Duvoisin RM, Zhang C, Pfankuch TF, O'Connor H, Gayet-Primo J, Quraishi S, et al. Increased measures of anxiety and weight gain in mice lacking the group III metabotropic glutamate receptor mGluR8. Eur J Neurosci. 2005;22:425-36.
26. Bäckström P, Hyytiä P. Suppression of alcohol self-administration and cue-induced reinstatement of alcohol seeking by the mGlu2/3 receptor agonist LY379268 and the mGlu8 receptor agonist (S)-3,4-DCPG. Eur J Pharmacol. 2005;528:110-8.
27. Marabese I, Rossi F, Palazzo E, De Novellis V, Starowicz K, Cristino L, et al. Periaqueductal gray metabotropic glutamate receptor subtype 7 and 8 mediate opposite effects on amino acid release, rostral ventromedial medulla cell activities, and thermal nociception. J Neurophysiol. 2007;98:43-53.