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Short communication

The anxiolytic-like effect of metabotropic glutamate receptor antagonists after intrahippocampal injection in rats

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Abstract

Using the conflict drinking Vogel test in rats as a model we examined the anxiolytic-like activity, after their intrahippocampal administration of (*S*)-4-carboxy-3-hydroxyphenyl-glycine (S-4C3H-PG), (*RS*)-α-methylserine-*O*-phosphate-monophenyl ester (MSOPPE) and (*RS*)-α-methylserine-*O*-phosphate (MSOP), the antagonists of the I, the II and the III group of metabotropic glutamate receptors (mGlu receptors), respectively. The results indicate that S-4C3H-PG and MSOP produced a dose-dependent anticonflict effect in rats, which was unrelated to a reduced perception of the stimulus or to an increased thirst drive. The hippocampus may be one of the neuroanatomical sites of the anxiolytic-like effects of both mGlu receptor antagonists.

Keywords: Metabotropic glutamate receptor; S-4C3H-PG ((*S*)-4-carbonyl-3-hydroxyphenylglycine); MSOP ((*RS*)-α-methylserine-*O*-phosphate); Drinking conflict test; Intrahippocampal injection

1. Introduction

Glutamate (Glu) is one of the major neurotransmitters in the brain involved in several physiological and pathological conditions (Wróblewski and Danysz, 1989). It acts via several receptor subtypes: ionotropic glutamate receptors with the NMDA receptors attracting major attention, and metabotropic glutamate receptors (mGlu receptors) (Pin and Duvoisin, 1995; Wróblewski and Danysz, 1989). Anticonflict effects of NMDA receptor antagonists have been reported by several authors (Bennett et al., 1989; Jessa et al., 1996; Stephens et al., 1986). As the potential, wide clinical use of NMDA receptor antagonists is hampered by their undesirable side-effects, (Wróblewski and Danysz, 1989), the major research effort has been directed towards the development of substances which modulate the function of NMDA receptors acting within the NMDA receptor complex. Indeed the anticonflict effect of 1-aminocyclopropanecarboxylic acid (ACPC), which acts on glycine receptors associated with the NMDA receptor complex, has been described (Trullas et al., 1991). MGlu receptors play an important role in effects mediated by glutamate, including modification of NMDA receptor function (see Glaum and Miller, 1994). Hence we decided to investigate if antagonists of mGlu receptors also evoke an anticonflict effect in rats. Using the conflict drinking Vogel test as a model (Vogel et al., 1971), we now studied the anxiolytic-like activity of (*S*)-4-carboxy-3-hydroxyphenylglycine (S-4C3H-PG), (*RS*)-α-methylserine-*O*-phosphate (MSOP) and (*RS*)-α-methylserine-*O*-phosphate-monophenyl ester (MSOPPE), the antagonists of the I, II and III group of mGlu receptors, respectively (Thomas et al., 1996; Watkins and Collingridge, 1994), after administration into the hippocampus, a structure which is involved in anxiety (Gray, 1982).

2. Materials and methods

2.1. Animals and housing

Male Wistar rats, weighing 250 ± 20 g, were used in the study. The animals were caged individually ($40 \times 27 \times 15$ cm), on a natural day-night cycle and at a room temperature of 19–21°C, with free access to food and tap water before the experiment. All experimental procedures

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were approved by the IF PAN Animal Care and Use Committee.

2.2. Intrahippocampal injections

The rats were operated under equithesin anesthesia. A socket with two stainless steel guide cannulae (0.4 mm o.d., 0.3 mm i.d., 8.0 mm long) was implanted stereotaxically 2 mm above the CA1 region of the dorsal hippocampus (A 5.2 mm, L 2.0 mm, H 7.3 mm from the interaural line), and was fixed to the skull with stainless steel screws and dental acrylic cement. Seven days later the rats were subjected to behavioral testing. Intrahippocampal injections of drugs were made using Hamilton microsyringes connected, via polyethylene tubing, with two stainless steel needles (0.3 mm o.d.). The injection needles were lowered 2 mm below the tip of the guide cannula, i.e., at a level of the CA1 region of the dorsal hippocampus. Solutions were administered bilaterally over 60 s. The injection needle remained in place for an additional 30–60 s before it was removed and replaced with a stylet. S-4C3H-PG, MSOP and MSOPPE were dissolved in sterile saline with the addition of a minimal amount of 0.1 M NaOH and injected into hippocampus in a volume of 1 μl/site 10 min before the test. Control rats received vehicle.

2.3. Conflict drinking test (Vogel test)

A modification of the method of Vogel et al. (1971) was used. On the first day of the experiment, the rats were adapted to the test chamber for 10 min. After the adaptation period, the animals were deprived of water for 24 h and were then placed in the test chamber for 10 min with free access to the drinking bottle. Afterwards, they were allowed a 30-min free-drinking session in their home cage. After another 24-h water deprivation the rats were again placed in the test chamber and were allowed to drink for 30 s. Immediately afterwards, drinking attempts were punished with an electric shock (0.5 mA). The impulses were released every 2 s (timed from the moment when a preceding shock was delivered) in 1-s periods, between the grid floor and the spout of the drinking bottle. The number of shocks accepted throughout a 5-min experimental session was recorded.

2.4. Shock threshold and free-drinking tests

To check for the possibility of drug-induced changes in the perception of the stimulus or in the thirst drive, which might have contributed to the activity in the conflict drinking test, a stimulus threshold measurement and a free-drinking experiment were also carried out. In both cases the rats were treated in a manner similar to that described in the conflict drinking test. In the shock threshold test, the rats were placed individually in the box, and electric shocks were delivered through the grid floor. The shock threshold was determined stepwise by increasing manually the current (0.1, 0.2, 0.3, 0.4, 0.5 mA) delivered through the grid floor until a rat showed an avoidance reaction (jump, jerk, or alike – recorded by an observer blind to the treatment) to an electric stimulus. There was a 15-s shock-free interval between steps.

In the free-drinking test each animal was allowed to drink from the water spout. Licking was not punished. The total amount of water (ml), consumed in 5 min, was recorded for each rat. In these tests and in the Vogel test the animals were used only once.

2.5. Histological analysis

After intrahippocampal injections, the animals were killed and their brains were stored in a 10% formalin solution. The frozen tissue was dissected, and the injection sites were verified visually. Only the data from rats in which the cannulae were located bilaterally in the intended structure were included in the results for calculation.

2.6. Analysis of the data

All the data are expressed as the means ± S.E.M. The results obtained from the vehicle-treated rats (0 nmol) and two doses of a single antagonist were obtained on the same day and were analysed with a one-way analysis of variance (ANOVA). Specific comparisons were carried out with the Dunnett test.

2.7. Drugs

MSOP, MSOPPE and S-4C3H-PG were purchased from Tocris-Cookson, UK.

3. Results

Our results show that S-4C3H-PG after intrahippocampal injection induced a dose-dependent, more than 4-fold (after the higher dose of 30.1 nmol) increase in the number of shocks accepted during the experimental session in the conflict drinking test. The results were statistically signifi-

Table 1
Effects of metabotropic glutamate receptor antagonists and the shock threshold and the amount of water consumed by water-deprived rats

Compound	Dose (nmol)	*n*	Shock threshold (mA)	Water consumption (ml)
Vehicle	–	6	0.37 ± 0.02	8.6 ± 1.0
S-4C3H-PG	30.125	6	0.48 ± 0.02	7.7 ± 1.0
MSOP	12.5	6	0.40 ± 0.04	7.4 ± 0.8
MSOP	30.125	6	0.43 ± 0.03	6.9 ± 0.7

S-4C3H-PG and MSOP were administered intrahippocampally 10 min before the test. *n* = number of rats per group. The values are means ± S.E.M.

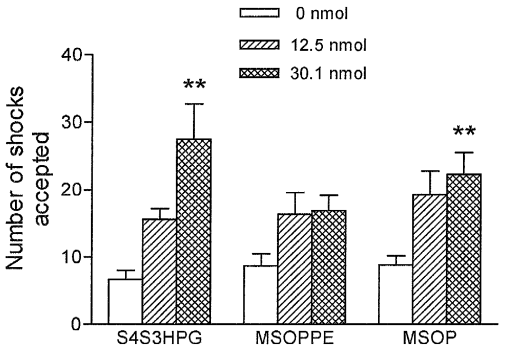

Fig. 1. Effects of S-4C3H-PG, MSOP and MSOPPE in the conflict drinking Vogel test in rats. Drugs were administered intrahippocampally 10 min before the test at doses of 12.5 and 30.125 nmol. Values represent the means ± S.E.M. of the number of shocks accepted during a 5-min experimental session. The number of rats in each group was 7–10. Data were analyzed using separate ANOVAs for two doses of each compound versus vehicle (0 nmol) treatment. When the ANOVA indicated significance ($P < 0.05$), post-hoc comparisons were performed using the Dunnett multiple comparison test. * $P < 0.05$; ** $P < 0.01$ versus vehicle treatment.

cant: $F(2,18) = 10.442$, $P < 0.01$. MSOP also produced a dose-dependent, statistically significant, almost 3-fold increase in the number of shocks accepted during the experimental session in the conflict drinking test: $F(2,23) = 5.835$, $P < 0.01$ (Fig. 1). The effect of MSOPPE did not reach the level of statistical significance; however, a 2-fold increase in the number of shocks accepted during the experimental session in the conflict drinking test was observed. The possibility that the efficacy of effective doses of S-4C3H-PG or MSOP was related to a reduced perception of the stimulus or to an increased thirst drive was excluded, as both drugs, tested at doses effective in the Vogel test, did not increase significantly either the threshold current or the water intake (Table 1). In our experiments seven cannula placements fell outside the CA1 hippocampus target area and the scores for these animals were discarded. The results from discarded rats were at the control level.

4. Discussion

In spite of the fact that mGlu receptors are ubiquitous in the brain and are present at a variety of central nervous system synapses, the data concerning behavioral consequences of mGlu receptor activation and/or inhibition are scarce, largely due to lack of selective antagonists. However, several antagonists for different groups of mGlu receptors have recently been developed (e.g., Thomas et al., 1996; Watkins and Collingridge, 1994), therefore we decided to investigate their effects in the Vogel test after intrahippocampal administration. Our results indicate that blockade of the I and the III group of mGlu receptors exerts anxiolytic-like activity in rats. The most efficacious was S-4C3H-PG, an antagonist of the I group of mGlu receptors. This may be related to high expression of mRNA for the I group of mGlu receptors in the CA1 region of hippocampus (see Testa et al., 1994). MSOP, an antagonist of the III group of mGlu receptors with some activity at mGlu II receptors, was less effective, which may reflect the low levels of mRNA expression in the hippocampus for the III group of mGlu receptors (Testa et al., 1994). The II type of mGlu receptors is absent in the CA1 region (Testa et al., 1994), which might explain the lack of significant effects of MSOPPE. However, one must be aware that ligands specific for different types of mGlu receptors have been not developed and the detailed characteristic of mGlu receptor antagonists, especially for the native cells, is not known. Both MSOP and S-4C3H-PG exert an anxiolytic-like effect with an efficacy similar to that of NMDA receptor antagonists and ACPC (e.g., Przegalinski et al., 1996). ACPC is a partial agonist at strychnine-insensitive glycine receptors, and acts as functional antagonist at glycine receptors in vivo (Marvizon et al., 1989), leading to a decrease in NMDA-mediated neurotransmission. Since glutamatergic transmission via mGlu receptors can potentiate the ionotropic glutamate response in various preparations (see Glaum and Miller, 1994) and potentiation of NMDA currents by mGlu receptor agonists, including agonists of the I group of mGlu receptors has been observed in the CA1 neurons (see Fitzjohn et al., 1996), the blockade of mGlu receptors may also lead to a decrease in NMDA receptor-mediated neurotransmission. Our results may indicate that the hippocampus is one of the neuroanatomical sites of anticonflict activity of S-4C3H-PG and MSOP, which is consistent with the notion that the hippocampus is involved in anti-anxiety effects of different anxiolytic drugs (Gray, 1982). The anxiolytic-like effect of mGlu receptor antagonists may represent an important new feature of that group of drugs. Further work with other mGlu receptor antagonists and agonists (as some agonist potency of S-4C3H-PG at mGlu receptors has been observed (Watkins and Collingridge, 1994)) as well as use of other tests to detect anxiolytic activity will be necessary to more fully elucidate the possibility of involvement of mGlu receptors in the anti-anxiety effect.

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