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Stimulation of group II metabotropic glutamate receptors or inhibition of group I ones exerts anxiolytic-like effects in rats

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Summary. Using the conflict drinking Vogel test in rats as a model we examined the anxiolytic-like activity of (S)-4-carboxyphenylglycine (S-4CPG), an antagonist of group I metabotropic glutamate receptors (mGlu receptors), of (RS)-α-methylserine-O-phosphate-monophenyl ester (MSOPPE), an antagonist of group II mGlu receptors, and of (2S,1′S,2′S)-2-(carboxycyclopropyl)glycine (L-CCG-I), an agonist of group II mGlu receptors. The obtained results indicate that intrahippocampal administration of S-4CPG and L-CCG-I, but not MSOPPE to rats produces a dose-dependent anticonflict effect, which is unrelated to the 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 either agent.

Keywords: Amino acids – Metabotropic glutamate receptors – (S)-4-carboxyphenylglycine (S-4CPG) – (2S,1′S,2′S)-2-(carboxycyclopropyl) glycine (L-CCG-I) – Drinking conflict test – Intrahippocampal injection

Glutamate (Glu) is one of the major neurotransmitters in the brain: it is estimated that ca. 50% of neurons may utilize glutamate as a neurotransmitter (McGeer et al., 1987). Glutamate stimulates two major groups of receptors: ionotropic glutamate receptors and metabotropic glutamate ones (mGlu receptors) (Conn and Pin, 1997; Wroblewski and Danysz, 1989). Eight different subtypes of mGlu receptors have been cloned so far (mGlu 1–8). mGlu receptors have been subdivided into three groups: group I mGlu receptors (mGlu1 and mGlu5), positively coupled to phospholipase C; group II mGlu receptors (mGlu2 and mGlu3), and group III mGlu receptors (mGlu4, mGlu6, mGlu7 and mGlu8), negatively coupled to adenylate cyclase (Conn and Pin, 1997). Several data indicate crucial involvement of glutamate receptors in the mechanism of action of anxiolytics (for review see Wiley

and Balster, 1993). Some recent findings point to a role of mGlu receptors in anxiety. Anticonflict effects of (S)-4-carboxy-3-hydroxyphenyl-glycine (S-4C3H-PG), which is an antagonist of group I mGlu receptors and an agonist of group II mGlu receptors (Sekiyama et al., 1996), have been reported by Chojnacka-Wójcik et al. (1997). Helton et al. (1998) have also found that LY 354740, an agonist of group II mGlu receptors, produced anxiolytic responses in a fear-potentiated startle and in an elevated plus-maze model of anxiety in rats, while Klodzinska et al. (1999) have shown that peripheral administration of LY 354740 produced an anxiolytic-like effects in a conflict drinking test in rats and a four-plate test in mice. In order to resolve the problem of involvement of group I mGlu receptor antagonists, we decided to investigate the anxiolytic-like activity of agonists and antagonists of different subtypes of mGlu receptors including (S)-4CPG, an antagonist of group I mGlu receptors, L-CCG-I, an agonist of group II mGlu receptors, and MSOPPE, an antagonist of the II group of mGlu receptors, using the conflict drinking Vogel test as a model (Vogel et al., 1971). All those substances were administered to the hippocampus.

Methods

Animals and housing

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

Intrahippocampal injections

The rats were operated under equithesin anaesthesia. 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 a behavioral testing procedure. Intrahippocampal injections of the 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)-4CPG , L-CCG-I and MSOPPE were dissolved in sterile saline with an addition of minimal amount of 0.1 M NaOH, and were injected into hippocampus in a volume of 1 μl/ site, 10 min before the test. Control rats received the vehicle.

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

period, 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.

Histological analysis

After the final testing day, the animals were killed and their brains were removed and 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 target structure were accepted for calculation of the results.

Analysis of the data

All the data are expressed as means ± SEM and evaluated by a one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test, where $P < 0.05$ was considered significant.

Drugs

(S)-4CPG, L-CCG-I and MSOPPE were purchased from Tocris-Cookson, England.

Results

Conflict drinking test in rats

(S)-4CPG, which is a competitive group I mGlu receptors antagonist, with selectivity for mGlu 1a over mGlu 5a (Brabet et al., 1995), used in a dose of 20 μg, induced a dose-dependent, almost four-fold increase in the number of shocks accepted during the experimental session in the conflict drinking test after its intrahippocampal injection; the results were statistically significant: $P < 0.01$. Effects of the group II mGlu receptors agonist L-CCG-I (Wright and Schoepp, 1996) also were tested. L-CCG-I in a dose of 10 μg significantly increased (by 215%) the number of shocks accepted during the experimental session in the Vogel test ($P < 0.01$). The effect of MSOPPE, an antagonist of group II mGlu receptors (Thomas et al., 1996), did not alter the number of shocks accepted during the experimental session in the conflict drinking test.

Table 1. Effects of metabotropic glutamate receptor ligands in the Vogel test in rats

Compound	Dose (μg)	Number of shocks Accepted (mA)	N
Vehicle	–	9.0 ± 1.1	7
S-4CPG	20	33.6 ± 5.6**	8
L-CCG-I	10	28.4 ± 4.6**	8
MSOPPE	15	16.9 ± 2.3	8

S-4CPG, L-CCG-I and MSOPPE were administered intrahippocampally 10 min before the test. *N* the number of rats per group. The values are means ± S.E.M. $P < 0.01$**.

The possibility that the efficacy of effective doses of (S)-4-CPG or L-CCG-I was related to a reduced perception of the stimulus or to an increased thirst drive, was excluded since both agents, tested at the effective doses in the conflict drinking test, did not change the threshold current, nor the water intake compared to vehicle treatment (results not shown).

Conclusions

The rat Vogel test (Vogel et al., 1971) is a procedure widely employed as a screening method for anxiolytics. Our results indicate that blockade of group I as well as stimulation of group II mGlu receptors exerts an anxiolytic-like activity in rats after intrahippocampal administration of drugs. The present results corroborate and extend the data of Helton et al. (1998) and Klodzinska et al. (1999), who reported that LY 354740, an agonist of group II mGlu receptors, produced anxiolytic responses in different models of anxiety in rats and mice. Also Chojnacka-Wójcik et al. (1997), reported that S-4C3H-PG, an antagonist of group I mGlu receptors with an agonist activity to wards group II mGlu receptors, exerted an anxiolytic-like effect in the Vogel test.

The anxiolytic-like efficacy of S-4CPG, an antagonist of group I mGlu receptors, may be related to a high expression of mRNA for that group of mGlu receptors in the CA1 region of the hippocampus (see Testa et al., 1994), as well as the high immunoreactivity of group I mGlu receptors in that structure (Shigemoto et al., 1997; Blumcke et al., 1996), manifested by an intense immunolabeling by mGluR5 antibody. The effectiveness of the group II mGlu receptor agonists L-CCG-I may also be due to an intense group II mGlu receptor immunolabeling in the CA1 area (Shigemoto et al., 1997), possibly related to the presence of this receptor subtype in that structure. The lack of significant effects of MSOPPE, which is a group II mGlu receptor antagonist, indicates that stimulation of group II mGlu receptors, rather then their blockade, which is responsible for the antianxiety effect. Our results may indicate that the hippocampus is one of neuroanatomical sites of the anticonflict activity of L-CCG-I and S-4CPG, which is consistent with the notion that this structure is involved in anti-anxiety effects of different anxiolytic drugs (Gray, 1982). The anxiolytic-like effect of mGlu receptor antagonists may be a new important feature of drugs of this group.

The mechanism of anxiolytic-like effects of the substances acting on mGlu receptors may be related to a decreased glutamatergic neurotransmission in the brain: a variety of effects may lead to such an action. Glutamatergic neurotransmission can be attenuated by stimulation of group II mGlu receptors. Group II mGlu receptors are present on presynaptic glutamatergic nerve terminals (Shigemoto et al., 1997), and stimulation of these receptors leads to a decrease in glutamate release (Attwell et al., 1995). Group I mGlu receptors are predominantly postsynaptically localized (Lujan et al., 1996). It has been shown that activation of group I mGlu receptors in the CA1 region of the hippocampus is responsible for the depolarization of pyramidal neurons

(Davies et al., 1995) – probably as a result of inhibition of potassium channels (Saugstad et al., 1996). Therefore blockade of group I mGlu receptors may also lead to a decrease in glutamatergic neurotransmission. The decreased glutamatergic transmission, which leads to overall inhibitory effects in the central nervous system, may have consequences similar to the effect of increased GABA-ergic transmission (which is connected with the mechanism of action of benzodiazepines – the most popular anxiolytic drugs) and may thus be an important outcome of stimulation of group II mGlu receptors and/or inhibition of group II mGlu receptors in the brain. Further studies with other mGlu receptor antagonists and agonists as well as the use of other tests for the detection of an anxiolytic activity will be necessary to satisfactorily elucidate the possibile involvement of mGlu receptors in the anti-anxiety effect.

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