ELSEVIER

European Journal of Pharmacology 294 (1995) 361–365

ejp

Short communication

Effects of the selective metabotropic glutamate receptor agonist, L-CCG-I, on acquisition of a Morris task by rats

F. Josef Van der Staay [a,*], Horst Antonicek [b], Barbara Helpap [b], Wolf-Dietrich Freund [a]

[a] *Institute for Neurobiology, Troponwerke GmbH & Co. KG, D-51063 Cologne, Germany*
[b] *Central Research, Bayer AG, D-51368 Leverkusen, Germany*

Received 17 October 1995; accepted 31 October 1995

Abstract

L-Glutamate, a major excitatory neurotransmitter in the central nervous system, plays an important role in a variety of neuronal events associated with learning and memory, neuronal plasticity, neurotoxicity, and neurodegeneration. We assessed the effects of L-CCG-I ((2*S*,3*S*,4*S*)-α-(carboxycyclopropyl)glycine), a conformationally restricted glutamate analogue, in a standard Morris water escape task with young adult rats. L-CCG-I is considered to be a selective agonist of the metabotropic glutamate receptor. Vehicle, 5, 50, or 500 nmol L-CCG-I was injected intra-cerebroventricularly (i.c.v.) into the right lateral ventricle 30 min before the start of each of five daily acquisition sessions. The data indicate that L-CCG-I had a centrally mediated mode of action; rats treated with 500 nmol L-CCG-I were clearly impaired in acquiring the standard Morris water escape task. The no-effect dose was 5 nmol.

Keywords: Metabotropic glutamate receptor; Metabotropic receptor agonist; L-CCG-I ((2*S*,3*S*,4*S*)-α-(carboxycyclopropyl)glycine); Morris maze; (Rat)

1. Introduction

L-Glutamate, a major excitatory neurotransmitter in the central nervous system (CNS), plays an important role in a variety of neuronal events, such as learning and memory formation, neuronal plasticity, neurotoxicity and neurodegenerative diseases. There are two major classes of glutamate receptors in the mammalian CNS. The ionotropic receptors are gated ion channels which are subdivided, according to their selective agonists, into NMDA (*N*-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)/kainic acid subtypes. Activation of these receptors leads to ion flux through the respective channels, which have different conductances for Na^+, K^+ and Ca^{2+}. In contrast, the metabotropic glutamate (mGlu) receptors mediate their effects via GTP-binding proteins and are coupled to intracellular signal transduction. Depending on the receptor subtype stimulated, signal transduction involves increased phosphoinositide hydrolysis with subsequent formation of inositoltrisphosphate and diacylglycerol and mobilization of intracellular Ca^{2+}, or decreased levels of cyclic AMP (Pin and Duvoisin, 1995). mGlu receptors are supposed to be involved in the acute modulation of synaptic transmission and in the induction of long-lasting changes in synaptic function, including long-term potentiation in the hippocampal CA1 region (Schoepp and Conn, 1993) and the dentate gyrus (Riedel and Reymann, 1993).

Metabotropic glutamate receptor antagonists such as MCPG ((*R*,*S*)-α-methyl-4-carboxyphenylglycine; Richter-Levin et al., 1994), or L-AP4 (L-2-amino-4-phosphonobutonic acid; Hölscher, 1994) have amnestic effects in a Morris water escape task with rats and in a one-trial passive avoidance task with chicks, respectively. It has been hypothesized that these effects might be due to the inhibitory action of mGlu receptor agonists on long-term potentiation (Riedel et al., 1995a), an electrophysiological effect that has been described by Richter-Levin et al. (1994).

* Corresponding author. Institute of Neurobiology, Troponwerke GmbH & Co. KG, Berliner Straße 156, D-51063 Cologne, Germany. Tel.: (+49) 221-6472629, fax: (+49) 221-6472255.

SSDI 0014-2999(95)00743-1

ACPD (1*S*,3*R*-1-amino-cylopentyl-1,3-dicarboxylic acid), a specific agonist of mGlu receptors, is able to prevent the amnestic effects of MCPG (Hölscher, 1994). It has been suggested that mGlu receptors play a role in the induction and maintenance of long-term potentiation (Riedel and Reymann, 1993). Therefore, mGlu receptor agonists might facilitate learning through their effects on the induction and maintenance of long-term potentiation, although there is no experimental evidence to support this hypothesis. In normal Wistar rats, ACPD at a dose of approximately 58 nmol per animal per day attenuated performance in the Morris water escape task, whereas a lower dose, 5.8 nmol per animal per day, was without effect (Pettit et al., 1994). The mGlu receptor agonist, *trans*-azetidine-2,4-dicarboxylic acid (tADA), was found to impair the 24-h retention performance of rats in a shock-motivated spatial alternation task (Riedel et al., 1995b). It did not affect the acquisition of the alternation behavior.

In the present study we investigated whether daily pre-session application of 5, 50, or 500 nmol of the metabotropic glutamate receptor agonist, L-CCG-I ((2*S*,3*S*,4*S*)-α-(carboxycyclopropyl)glycine), would improve spatial learning in young adult rats. L-CCG-I is a conformationally restricted glutamate analogue and is considered to be a selective agonist of the metabotropic glutamate receptor (Hayashi et al., 1992). This compound is a member of the α-(carboxycyclopropyl) glycine family of eight isomers (Kawai et al., 1992).

The dose range of L-CCG-I used was comparable to that of ACPD in the study of Pettit et al. (1994). L-CCG-I is more potent than ACPD at the $mGlu_2$ receptor subtype (Cavanni et al., 1994), which is highly expressed in the hippocampus (Schoepp and Conn, 1993), a structure known to be relevant for spatial learning. To assess the effects of i.c.v. infusions of L-CCG-I on spatial orientation learning in rats, we used the standard Morris water escape task. This task, in which a rat is required to localize a submerged platform in a circular water tank, measures predominantly spatial reference memory, which holds trial-independent information about, for example, the position of the escape platform in the water tank.

2. Material and methods

All experiments and animal care were performed according to international guidelines.

2.1. Test substance

L-CCG-I ($C_6H_9NO_4$) was freshly dissolved in deionized water shortly before the first set of experiments. The pH was adjusted to 7.5–8.5 by careful addition of very small amounts of solid $NaHCO_3$. During the 5 days of training, the prepared stock solution was stored at 4°C. Selected concentrations (1, 10, and 100 mmol/l) were administered i.c.v. in a volume of 5.0 μl per animal. The amount injected was 5, 50, 500 nmol per animal.

2.2. Animals

Thirty-two Wistar rats (WISW:BOR, new name HsdCpb:WU) weighing 200–300 g were obtained from Harlan-Winkelmann (Borchen, Germany). They were randomly assigned to one of four conditions ($n = 8$ per group): vehicle, L5 (receiving 5.0 nmol L-CCG-I), L50 (receiving 50 nmol L-CCG-I), or L500 (receiving 500 nmol L-CCG-I). One animal from the L500 group died during the testing period. The cause of death was unknown.

2.3. Apparatus

The Morris water tank consisted of a circular black tub with a slightly sloping wall (material: polyethylene; inner dimensions: diameter at top 153 cm, diameter at bottom 143 cm, depth 63 cm), filled with 43.5 cm of clear tap water at a temperature of approximately 22°C. The escape platform was a black polyethylene cylinder (diameter 10.8 cm), submerged 1.5 cm below the surface of the water. Note that the water was *not* made opaque, because the black escape platform was invisible in the black water tank, even when clear tap water was used.

The water tank was situated in a room illuminated by white fluorescent strip lights. Abundant extra-maze cues were provided by the furniture in the room, which included desks, computer equipment, a second water tank, the presence of the experimenter, and by a radio on a shelf. The radio was playing softly. All testing was done between 10:00 and 15:00 h.

A video camera, mounted in the centre above the circular pool, provided a picture of the pool on a TV monitor. Lines on the monitor defined quadrant boundaries and the position of the escape platform. Each quadrant was further subdivided by a pattern of lines (a 4×4 matrix of squares; corresponding to a distance of 16.9 cm between grid lines in the pool). Crossing a line was scored when a rat moved across it with its whole body. The movements of the rat were recorded manually and stored in an MS-DOS compatible microcomputer.

2.4. Procedure

2.4.1. Surgery

Two weeks before the start of behavioral testing, the rats were equipped with a permanent guide cannula in the right ventral ventricle. The rats were anaesthetized

with Hypnorm (0.7 ml/kg). The cannula was made of polyethylene (outer diameter: 0.75 mm). Except during injection, the cannula was firmly closed with a removable polyethylene husk.

Because of the preparatory surgery and the route of application of L-CCG-I, only a restricted number of rats could be handled at one time during behavioral testing. Therefore, the experiment was performed with two identical series, separated by 1 week. During each series, half the animals (i.e. four rats per treatment condition) were tested; their order was randomized.

Vehicle or L-CCG-I was administered i.c.v. 30 min before each first daily acquisition trial.

2.4.2. Behavioral testing

The animals received four acquisition trials during each of five daily sessions. A trial was started by placing a rat in the pool, facing the wall of the tank. Each of four starting positions (north, east, south, and west) was used once in a series of four trials in a randomized order. The escape platform was always in the west quadrant. A trial was terminated as soon as the rat had climbed onto the escape platform or when 90 s had elapsed, whichever event occurred first. A rat was allowed to stay on the platform for 30 s before it was removed and the next trial was started. Rats that did not find the platform within 90 s were put on the platform by the experimenter and were allowed to stay there for 30 s.

After the fourth trial of the fifth daily acquisition session, an additional trial was given as a probe trial. The platform was removed, and the time a rat spent in the four quadrants was measured for 30 s. In the probe trial, all rats started from the same start position, opposite to the quadrant where the escape platform had been positioned during acquisition.

2.5. Statistical analysis

As the results for the two series were virtually identical, the pooled data were evaluated statistically.

2.5.1. Acquisition

We analysed *escape latency*, that is, the time taken to find, and escape onto, the submerged platform, the *total number of line crossings* as a measure of the distance swum to reach the escape platform, and *swimming speed* (number of line crossings divided by escape latency). The scores within each session were averaged per rat. Treatment effects on the acquisition of the water escape task were assessed with an analysis of variance (ANOVA) with repeated measures over sessions. Where appropriate, the results of ANOVAs on differences between treatment groups for particular sessions are included. Least significant difference (LSD) post-hoc tests were performed to evaluate the effects of different doses of L-CCG-I in more detail. Differences with an associated probability < 0.05 were considered as statistically reliable.

2.5.2. Probe trial

Treatment effects on the swimming time per quadrant were assessed with a repeated measures ANOVA *over* quadrants (time in the quadrant north, east, south, and west are considered as levels of the repeated measures factor).

3. Results

3.1. Acquisition of the Morris water escape task

The results are summarized in Fig. 1.

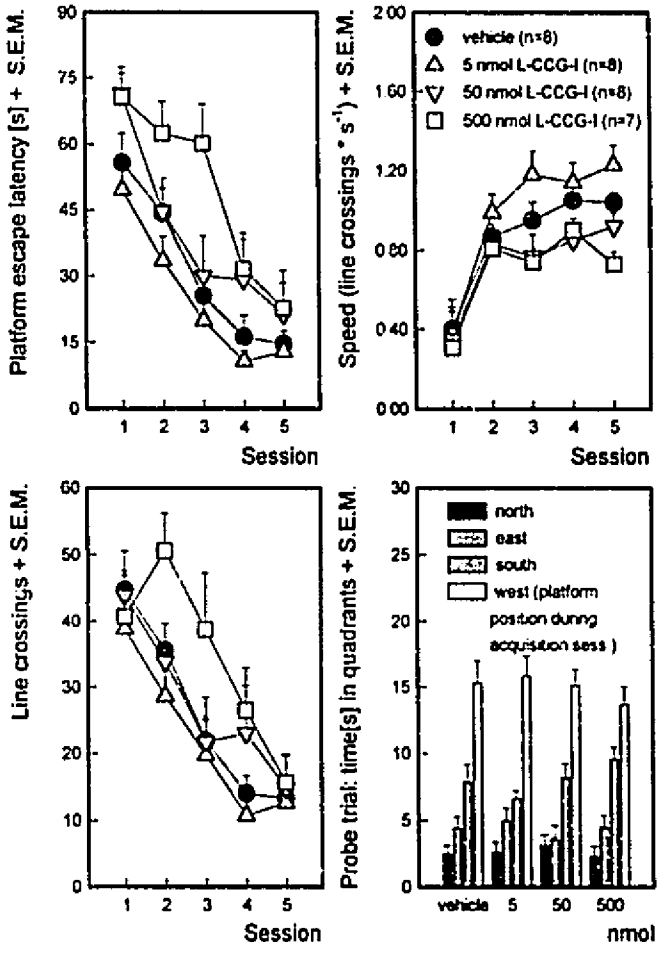

Fig. 1. Acquisition of a water escape task in a circular pool by 3-month-old male Wistar rats treated i.c.v. daily with 5, 50, or 500 nmol L-CCG-I, or with vehicle only ($n = 7$–8 per group). Session means and standard errors of the means (S.E.M.s) are depicted for latencies [s] to escape onto a submerged platform (upper left panel), swimming speed (i.e. line crossings per second; upper right panel), number of line crossings (lower left panel), and time spent in quadrants [s] during the probe trial (lower right panel).

3.1.1. Escape latency

Averaged over the five acquisition sessions, L-CCG-I affected the latency to escape onto the platform (General mean: $F(3,27) = 4.96$, $P < 0.01$). Post-hoc analysis of the general means revealed that the L500 group had, on average, a longer escape latency than the vehicle and the L5 group. The average escape latency of the L5 group was shorter than that of the L50 group, whereas the average escape latencies of the L5 and L50 groups were statistically indistinguishable from that of the vehicle group. Over sessions, the rats improved their escape performance (Sessions: $F(4,108) = 45.63$, $P < 0.01$); the four groups had similar learning curves for the escape latencies (Sessions by Treatment interaction: $F(12,108) = 1.37$, n.s.).

3.1.2. Total number of line crossings

Averaged over sessions, L-CCG-I affected the number of line crossings (General mean: $F(3,27) = 3.45$, $P < 0.05$). LSD post-hoc comparisons revealed that the L500 rats swam a longer distance to reach the escape platform than the vehicle and the L5 group. No other pairwise comparisons showed statistically reliable differences. Over sessions, the rats reduced the number of line crossings (Sessions: $F(4,108) = 27.01$, $P < 0.01$); the rate of improvement was similar for the four groups (Sessions by Treatment interaction: $F(12,108) = 1.14$, n.s.).

3.1.3. Swimming speed

The effects of L-CCG-I on swimming speed were different from those on the other two measures. Averaged over sessions, L-CCG-I affected swimming speed (General mean: $F(3,27) = 7.64$, $P < 0.01$). The animals in the L5 groups swam, on average, faster than the rats in the L50 and L500 group. In addition, the vehicle group swam faster than the L500 group. Swimming speed changed over sessions (Sessions: $F(4,108) = 29.45$, $P < 0.01$), but the change was similar for all groups (Session by Treatment interaction: $F(12,108) < 1.00$, n.s.).

3.2. Probe trial

In the probe trial, all groups showed a preference for the quadrant (Quadrants: $F(3,81) = 76.17$, $P < 0.01$) in which the escape platform had been located previously. The preference was similar for the vehicle- and the L-CCG-I-treated rats (Quadrants by Treatment interaction: $F(9,81) < 1.00$, n.s.).

4. Discussion

This study demonstrates that unilateral i.c.v. infusions of the mGlu receptor agonist, L-CCG-I, at a dose of 500 nmol retarded the acquisition of the standard Morris water escape task by Wistar rats. This was evident from both the platform escape latency and the distance swum to reach the submerged platform. The two lower doses (50 and 5 nmol) had no effect on spatial orientation. Our data confirm the finding of Pettit et al. (1994) that i.c.v. pre-session infusion of the selective mGlu receptor agonist, $ACPD_{(1S,3R)}$, attenuated learning in the Morris water escape task. Our data are also in line with the report by Riedel et al. (1995b) that i.c.v. administration of the mGlu receptor agonist, tADA, disrupted memory performance in a shock-motivated spatial alternation task. Although the modulation of mGlu receptors might be a process that is crucially involved in the acquisition of spatial tasks, the data available so far led Riedel et al. (1995c) to hypothesize that both saturated stimulation and blocking of mGlu receptors interfere with learning or memory.

The shapes of the learning curves for all treatment groups were similar. Over the acquisition sessions, all groups reduced the time needed and the distance swum to reach, and escape onto, the submerged platform. After five acquisition sessions, all groups had reached the same level of performance.

During the i.c.v. administration of the highest dose of L-CCG-I, the animals sometimes showed short-lasting, convulsion-like reactions. Many other mGlu receptor agonists also induce convulsions (Klitgaard and Laudrup, 1993). The convulsions were no longer seen when the rats were tested in the Morris maze. In non-systematical observations *after* the administration of the compound, a number of side-effects were noticed in the animals treated with 500 nmol L-CCG-I. These rats appeared to be sedated; they showed ptosis, gnawing, and piloerection.

The above mentioned side-effects were never observed during behavioral testing in the Morris task. Therefore we assume that the observed adverse effects which occurred prior to testing were not the cause of the impaired performance in the acquisition sessions. However, during a trial, the animals treated with 500 nmol L-CCG-I sometimes floated in the water without moving. This behavioral abnormality most likely affected the escape latencies directly. On the other hand, the floating does not explain the increased distance swum to reach the platform. This measure indicates that the rats treated with the highest dose of the mGlu receptor agonist transiently adopted a less efficient searching behavior than the animals in the other groups.

Because the two higher dosages (50 and 500 nmol) of L-CCG-I considerably reduced the swimming speed, the escape latency must be considered as a biased measure that cannot be used to assess the effects of L-CCG-I on learning and memory. However, as the highest dosage also increased the distance swum to

reach and escape onto the submerged platform, it can be concluded that daily administration of 500 nmol L-CCG-I retarded the acquisition of the standard Morris water escape task by young male Wistar rats. This indicates that L-CCG-I, which is considered to be a selective agonist of the metabotropic glutamate receptor (Hayashi et al., 1992), indeed has a centrally mediated mechanism of action.

Whether mGlu receptor agonists are neurotoxic or neuroprotective is a matter of debate (e.g. Lipartiti et al., 1993; Bruno et al., 1994). On the basis of our behavioral observations, there are no indications that repeated administration of L-CCG-I in doses up to 500 nmol had persistent neurotoxic effects. If brain damage were induced by L-CCG-I, one would expect that the learning curves would diverge, i.e. the acquisition of the Morris water escape task by the L-CCG-I groups should have been disrupted compared with that of the control group. This disruptive effect should have become more pronounced with repeated administration of the compound. However, such an effect was not seen. In contrast, the L-CCG-I-treated rats eventually reached the same level of performance as did the controls. Likewise, during the probe trial all groups showed a similar bias towards the quadrant where the escape platform had been positioned previously.

However, we cannot completely rule out the possibility that neurotoxic changes were induced. If brain tissue was damaged by administration of the high dose of L-CCG-I, then this appears to have been efficiently compensated for at the behavioral level. As no histological or biochemical examination of the brains was performed, however, further studies are needed to clarify this point.

In summary, our results confirm earlier negative findings reported for the mGlu receptor agonists, ACPD (Pettit et al., 1994) and tADA (Riedel et al., 1995b). L-CCG-I, a potent agonist at the $mGlu_2$ receptor subtype, which might be of particular relevance for learning processes, did not facilitate learning in the Morris water escape task. Further experiments are needed to substantiate the potential of mGlu receptor agonists as putative cognition-enhancing compounds.

Acknowledgements

The authors are grateful to Mrs. Doris Winkler for her skilful technical assistance.

References

Bruno, V., A. Copani, G. Battaglia, R. Raffaele, H. Shinozaki and F. Nicoletti, 1994, Protective effect of the metabotropic glutamate receptor agonist, DCG-IV, against excitotoxic neuronal death, Eur. J. Pharmacol. 256, 109.

Cavanni, P., V. Pinnola, M. Mugnaini, D. Trist, F.Th.M. Van Amsterdam and F. Ferraguti, 1994, Pharmacological analysis of carboxyphenylglycines at metabotropic glutamate receptors, Eur. J. Pharmacol. Mol. Pharmacol. 269, 9.

Hayashi, Y., Y. Tanabe, I. Aramori, M. Masu, K. Shimamoto, Y. Ohfune and S. Nakanishi, 1992, Agonist analysis of 2-(carboxycyclopropyl)glycine isomers for cloned metabotropic glutamate receptor subtypes expressed in Chinese hamster ovary cells, Br. J. Pharmacol. 107, 539.

Hölscher, C., 1994, Inhibitors of metabotropic glutamate receptors produce amnestic effects in chicks, NeuroReport 5, 1037.

Kawai, M., Y. Horikawa, T. Ishihara, K. Shimamoto and Y. Ohfune, 1992, 2-(Carboxycyclopropyl) glycines: binding, neurotoxicity and induction of intracellular free Ca^{2+} increase, Eur. J. Pharmacol. 211, 195.

Klitgaard, H. and P. Laudrup, 1993, Metabotropic excitatory amino acid receptor agonists selectively potentiate behavioral effects induced by ionotropic excitatory amino acid receptor agonists in mice, Eur. J. Pharmacol. 250, 9.

Lipartiti, M., E. Fadda, G. Savoini, R. Siliprandi, J. Sautter, R. Arban and H. Manev, 1993, In rats, the metabotropic glutamate receptor-triggered hippocampal neuronal damage is strain-dependent, Life Sci. 52, PL 85.

Pettit, H.O., D. Lutz, C. Gutierrez and D. Eveleth, 1994, I.c.v. infusions of $ACPD_{(1S,3R)}$ attenuate learning in a Morris water maze paradigm, Neurosci. Lett. 178, 43.

Pin, J.-P. and R. Duvoisin, 1995, The metabotropic glutamate receptors: structure and functions, Neuropharmacology 34, 1.

Richter-Levin, G., M.L. Errington, H. Maegawa and T.V.P. Bliss, 1994, Activation of metabotropic glutamate receptors is necessary for long-term potentiation in the dentate gyrus and for spatial learning, Neuropharmacology 33, 853.

Riedel, G. and K. Reymann, 1993, An antagonist of the metabotropic glutamate receptor prevents LTP in the dentate gyrus of freely moving rats, Neuropharmacology 32, 929.

Riedel, G., G. Casabona and K.G. Reymann, 1995a, Inhibition of long-term potentiation in the dentate gyrus of freely moving rats by the metabotropic glutamate receptor antagonist MCPG, J. Neurosci. 15, 87.

Riedel, G., W. Wetzel, A.P. Kozikowski and K.G. Reymann, 1995b, Block of spatial learning by mGluR agonist tADA in rats, Neuropharmacology 34, 559.

Riedel, G., W. Wetzel and K.G. Reymann, 1995c, Metabotropic glutamate receptors in neuronal plasticity and learning and memory in rats, Eur. J. Neurosci. Suppl. 8, 108.

Schoepp, D.D. and P.J. Conn, 1993, Metabotropic glutamate receptors in brain function and pathology, Trends Pharmacol. Sci. 14, 13.