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On the role of inhibitory glutamate receptors in N-methyl-D-aspartate- and dopamine-receptor mediated motor behavior of rats*

U. O. Kronthaler[1] and **W. J. Schmidt**[2]

[1] Aventis Behring GmbH, Marburg, and
[2] Department of Neuropharmacology, University Tübingen,
Federal Republic of Germany

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Summary. The physiological function of inhibitory group II metabotropic glutamate-receptors, a family of second messenger coupled glutamate-receptors, for motor behavior is almost unknown. The aim of this study is to address this topic by quantifying motor effects of the preferential group II agonist (2S,3S,4S)-alpha-(carboxycyclopropyl)-glycine, administered i.c.v. (62.5, 125.0, 187.5, 250.0, 500.0 nmol/4 μl), in an open-field equipped with a hole-board. (2S,3S,4S)-alpha-(carboxycyclopropyl)-glycine decreased spontaneous locomotor and exploratory behavior, which was blocked be the group II antagonist (2S)-alpha-ethylglutamic acid (250.0 nmol/4 μl). Locomotion induced by the N-methyl-D-aspartate-receptor antagonist dizocilpine (0.08, 0.16, 0.32 mg/kg) was counteracted by the group II agonist (2S,3S,4S)-alpha-(carboxycyclopropyl)-glycine, but an antagonism towards dizocilpine did not occur in all aspects of motor behavior evaluated. In contrast to the antagonism of dizocilpine induced locomotion, D,L-amphetamine (1.0, 2.0, 3.0 mg/kg) induced locomotion was not antagonised by (2S,3S,4S)-alpha-(carboxycyclopropyl)-glycine.The results suggest that group II agonists may be devoid of psychotomimetic effects in humans and even may antagonise this side effect of N-methyl-D-aspartate receptor antagonists. Since group II activation and N-methyl-D-aspartate-receptor blockade very efficiently protects against excitotoxic neurodegeneration, selective group II agonists may allow novel pharmacotherapeutical approaches in pathophysiological conditions characterised by a glutamatergic hyperactivity, like epilepsy, ischemia and trauma.

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Until recently, the transmitter glutamate was classified in general as an excitatory amino acid transmitter. This partially reflects the predominance of studies performed on ionotropic glutamate-receptors mediated effects. By the identification of the metabotropic glutamate receptors (mGluRs) this simplistic view of glutamate as a solely excitatory transmitter was replaced by the more complex view as a transmitter with a dual, excitatory and inhibitory, function: Activation of most mGluRs subtypes hyperpolarises neurons. mGluRs are a class of heterogenous receptors and mGluRs subtypes are assigned to three groups, I, II and III (Schoepp and Conn, 1993). Group I mGluRs have excitatory and inhibitory effects and stimulate phosphatidylinositol 4,5-bisphosphate hydrolysis (producing inositol 1,4,5-trisphosphate and diacylglycerol) (Schoepp and Conn, 1993; Fiorillo and Williams, 1998). Group II mGluRs have inhibitory effects, as they are negatively linked to adenylyl cyclase (Tanabe et al., 1993). Group III mGluRs have the same effects on cyclic AMP formation as group II mGluRs, i.e. activation of group III has inhibitory effects as well (Tanabe et al., 1993). Group III and group II receptors however, differ in sequence homology and pharmacology.

mGluRs in the basal ganglia, structures crucial in controlling motor behavior, clearly contribute to this function (Schoepp et al., 1992). Nevertheless studies on the function of glutamate, mediated by ionotropic receptors still predominate. Recently it has been reported, that: 1. coactivation of group I and group II mGluRs decreases motor behavior and even antagonises motor stimulation induced by blockade of N-methyl-D-aspartate (NMDA) receptors but less pronouncedly when induced by activation of dopamine-receptors (Kronthaler and Schmidt, 1996); 2. activation of group I mGluRs in the basal ganglia input structures, which are crucial for the control of motor behavior, increases motor behavior (Kearney et al., 1997). Taken together, these results lead to the hypothesis that group II mGluRs activation counteracts motor stimulation, at least when induced by NMDA-receptor blockade. Since such an antagonism would predict anti-psychotic effects in humans, mGluRs group II agonists may represent a possible new approach to treat psychotic symptoms.

To test this hypothesis we studied the motor functions of group II mGluRs in an open-field with hole-board. Since head dips are a valuable measure for exploratory behavior this test allows a detailed analysis of locomotor and exploratory behavior. First we tested the motor function of group II mGluRs by quantifying the motor effects exerted by the preferential group II agonist (2S,3S,4S)-alpha-carboxycyclopropyl-glycine (L-CCG I). Second we tested whether motor stimulation induced by NMDA-receptors blockade or indirect dopamine-receptor activation, namely by dizocilpine (MK-801) or D,L-amphetamine, would be counteracted by activation of group II mGluRs.

Material and methods

Subjects

Subjects were 98 male CD Sprague-Dawley rats (Charles-River, F. R. G.) weighting 240–290 g from the surgery until the end of the experiment, housed in groups of seven to ten in standard macrolon cages. Animals were fed with 12 g/day/animal standard rat chow following the behavioral testing, water was available ad libitum. Housing conditions were a constant 12-h-light-dark cycle, light on at 6 a.m. and temperature of 22° ± 3°C.

Cannula implantation

Stainless steel guide cannulas (outer diameter 0.8 mm, length 15.0 mm) were aimed at the lateral ventricle under chloral hydrate anaesthesia (400.0 mg/kg i.p.). Coordinates for the implantation relative to bregma were: AP = −0.8 mm, L = 1.4 mm, DV = −2.8 mm according to a stereotaxic atlas (Paxinos and Watson, 1986). Guide cannulas were firmly closed with stainless steel stylets of the same length. Following the surgery animals were allowed to recover for 3–5 days before behavioral testing.

Drugs

I.c.v. infusion was executed by infusion needles extending 0.8 mm over the tip of the guide cannulas. Infusion volume of 4 μl was administered over a period of 2 min, infusion needles were left in place for 1 min to allow diffusion. L-CCG I and (2S)-alpha-ethylglutamic acid (EGLU) (Tocris, Köln, F. R. G.) were dissolved in sterile 0.2 M phosphate-buffer, adjusted to pH = 7.4 with 0.1 M NaOH. Chloral hydrate (E. Merck, Darmstadt, F. R. G.), MK-801, (RBI, Biotrend, Köln, F. R. G.) and D,L-amphetamine sulphate (Geyer, Stuttgart, F. R. G.), were dissolved in sterile saline and injected i.p. Injection volume of chloral hydrate was 10.0 ml/kg, the injection volume of MK-801 and D,L-amphetamine was 1.0 ml/kg. Control animals received the respective vehicle. Animals were randomly assigned to groups receiving 250.0 nmol EGLU, 62.5, 125.0, 187.5, 250.0, 500.0 nmol L-CCG I, 0.08, 0.16, 0.32 mg/kg MK-801, 1.0, 2.0, 3.0 mg/kg D,L-amphetamine and respective combinations with 250.0 nmol L-CCG I n = 8–12/group.

Quantification of motor behavior

10 min following i.c.v. administration of 250.0 nmol EGLU, 62.5–500 nmol L-CCG I or vehicle, animals were gently placed in an open-field for 8 min. In the case of combined administration MK-801, D,L-amphetamine or vehicle were injected i.p. 30 min before testing and L-CCG I or vehicle were administered i.c.v. 10 min before gently placing the animals for 8 min in the open-field. Prior to the first test animals were habituated to the open-field for 5 min in order to avoid aversive reactions.

The open-field (69 × 69 cm) was equipped with a hole-board, divided into 16 equal-sized squares with a hole (diameter 4 cm), located in the centre of each field. The open-field was placed inside a wooden box and illuminated with four red bulbs (20 W), providing non-aversive conditions. Background noise was masked by a fan. Behavior was recorded on video tape for subsequent analysis; the number of line-crossings as a measure of locomotor activity, the number and duration of head-dips and rearings as a measure of exploratory activity and the duration of sitting without visible motor activity were manually analysed using a PC. As it has been reported that drugs can influence mean duration of head dips, we additionally calculated this parameter and furthermore we calculated mean duration of rearings.

Histological verification

At the end of the experiments location of the guide cannulas was verified histologically. 50 μm slices were stained with a standard cresyl violett staining technique.

Statistical analysis

One-way or two-way factorial ANOVA was used to detect significant ($P < 0.05$) group differences, followed by Fisher's Least Significant Difference (Protected t) Test.

Results

Histological analysis

A small fraction of the animals exhibited incorrect cannula placements, diverging more than 1.0 mm from the position, the cannulas were aimed to, thus drug or vehicle solutions may not been infused into the lateral ventricle. Those animals were excluded from statistical analysis, as were animals exhibiting tissue damage due to infections. There was no apparent tissue damage suggesting toxic effects of the L-CCG I infusions.

The preferential group II agonist L-CCG I induces akinesia, which is antagonised by the group II antagonist EGLU

187.5, 250.0 and 500.0 nmol L-CCG I reduced the number of spontaneous line-crossings (Fig. 1a) ($F_{(9,88)} = 3.9$; $p < 0.001$) and rearings (Fig. 1b) ($F_{(9,88)} = 3.0$; $p < 0.01$), while the two lower doses, 62.5 and 125.0 nmol L-CCG I, were inefficient in these respects. 125.0 nmol and the two highest doses, 250.0 and 500.0 nmol L-CCG I also reduced the number of spontaneous head dips (Fig. 1c) ($F_{(9,88)} = 7.1$; $p < 0.001$). The doses that decreased locomotor activity, increased the total duration of inactivity ($F_{(9,88)} = 17.8$; $p < 0.001$), but did not affect the mean duration of head dips or rearings (Table 1).

To investigate whether the preferential group II agonist L-CCG I depressed motor behavior by activating group II receptors, L-CCG I was coadministered with the selective group II antagonist EGLU. When 250 nmol L-CCG I was coadministered with 250.0 nmol EGLU there was no significant difference to the vehicle control group, in respect to the number of line-crossings, rearings, head dips (Fig. 1a–c) and the total duration of inactivity.

Table 1. Effects of L-CCG I on spontaneous motor activity (vehicle controls N = 8–10, L-CCG I group N = 11–12; data are MEAN ± S.E.M.)

	Dose L-CCG I (nmol)	62.5	125	187.5	250	500
Mean duration/ Head dip (sec)	L-CCG I groups	1.2 ± 0.2	0.9 ± 0.1	1.2 ± 0.1	0.6 ± 0.3	1.1 ± 0.2
	Vehicle controls	1.9 ± 0.6	0.9 ± 0.1	1.4 ± 0.2	0.9 ± 0.1	0.9 ± 0.1
Mean duration/ Rearing (sec)	L-CCG I groups	2.0 ± 0.2	1.7 ± 0.3	2.4 ± 0.3	1.4 ± 0.5	3.2 ± 0.7
	Vehicle controls	2.8 ± 0.9	1.8 ± 0.1	2.6 ± 0.2	1.7 ± 0.2	2.1 ± 0.2
Duration inactivity (sec)	L-CCG I groups	15.6 ± 15.6	60.3 ± 31.5	42.3 ± 29.2*	406.6 ± 40.4**	226.4 ± 63.1**
	Vehicle controls	15.6 ± 15.6	7.8 ± 5.6	9.4 ± 9.1	1.1 ± 0.6	22.5 ± 17.5

*indicates a difference of $P < 0.05$ and ** of $P < 0.01$ as compared to respective vehicle controls.

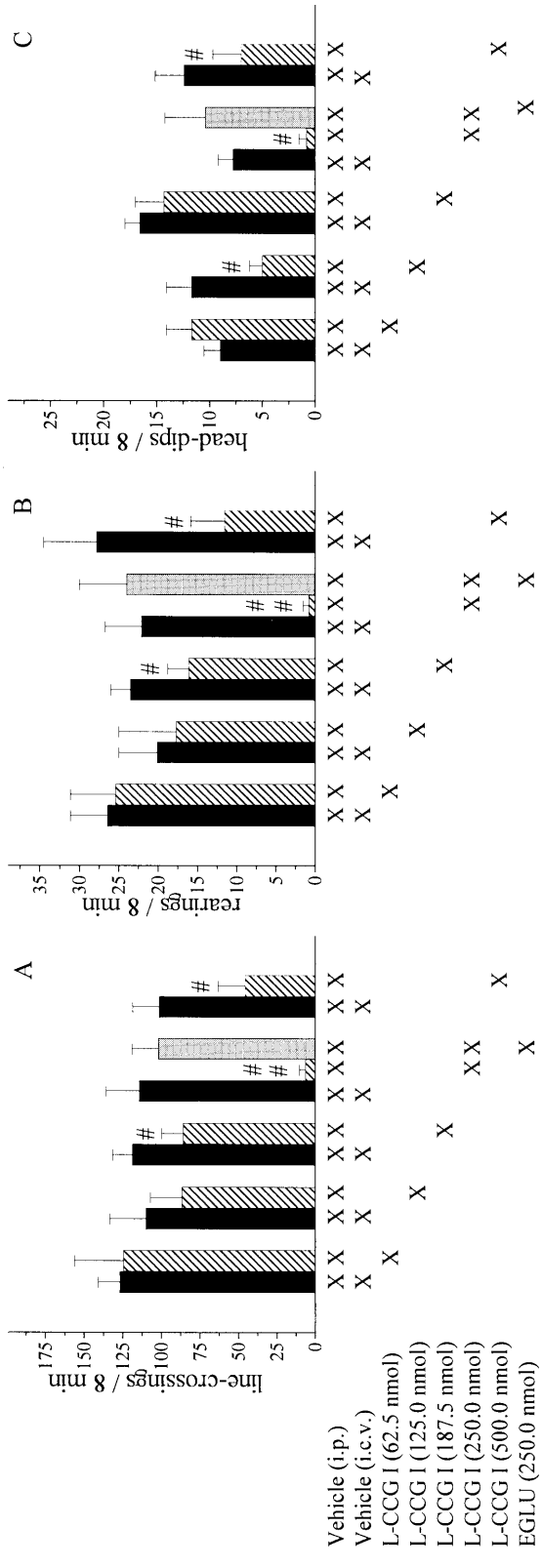

Fig. 1A–C. Effects of L-CCG I on spontaneous behaviour, antagonised by EGLU (vehicle controls N = 8 – 10, L-CCG I group N = 11–12; L-CCG I plus EGLU group N = 10; data are MEAN ± S.E.M.); # indicates a difference of $P < 0.05$ and ## of $P < 0.01$ as compared to respective vehicle controls

L-CCG I antagonises MK-801-induced locomotor stimulation

Figure 2a shows the dose-dependent induction of locomotor activity by 0.08–0.32 mg/kg MK-801. I.c.v. administration of 250 nmol L-CCG I antagonised MK-801-induced locomotor stimulation [$F_{L\text{-}CCG(1,76)} = 17.0$, $p < 0.001$, $F_{MK\text{-}801(3,76)} = 12.9$, $p < 0.001$, $F_{L\text{-}CCG\times MK\text{-}801(3,76)} = 5.4$, $p < 0.05$]. 250.0 nmol L-CCG I also reduced the number of rearings, but MK-801 and also coadministrations with L-CCG I had no effects in this respect (Fig. 2b). 250 nmol L-CCG I reduced the number of head-dips compared to vehicle controls as did 0.32 mg/kg MK-801, but 0.08 and 0.16 mg/kg MK-801 were inefficient in this respect. Combined administration of these inefficient doses of MK-801 with 250.0 nmol L-CCG I reduced the number of head dips (Fig. 2c) [$F_{L\text{-}CCG(1,76)} = 12.0$, $p < 0.001$, $F_{MK\text{-}801(3,76)} = 5.3$, $p < 0.01$, $F_{L\text{-}CCG\times MK\text{-}801(3,76)} = 4.1$, $p < 0.05$]. There was no effect of MK-801 or L-CCG I, nor any coadministration on the calculated mean duration of a head-dip (Table 2). MK-801 however, dose-dependently reduced the mean duration of a rearing, as did L-CCG I in this experiment, but this effect was not additive (Table 2) [$F_{L\text{-}CCG(1,63)} = 5.5$, $p < 0.05$, $F_{MK\text{-}801(3,63)} = 8.6$, $p < 0.001$, $F_{L\text{-}CCG\times MK\text{-}801(3,63)} = 1.7$, $p > 0.05$]. As for the number of line-crossings MK-801 antagonised the L-CCG I-increased duration of inactivity (Table 2) [$F_{L\text{-}CCG(1,83)} = 44.1$, $p < 0.001$, $F_{MK\text{-}801(3,83)} = 5.7$, $p < 0.05$, $F_{L\text{-}CCG\times MK\text{-}801(3,83)} = 5.1$, $p < 0.05$].

Table 2. Effects of MK-801 on 250.0 nmol L-CCG I mediated akinesia (vehicle controls N = 10, L-CCG I N = 8, 0.08 mg/kg MK-801 N = 12, 0.16 mg/kg MK-801 N = 10, 0.32 mg/kg MK-801 N = 11, 0.08 mg/kg MK-801 plus L-CCG I N = 12, 0.16 mg/kg MK-801 plus L-CCG I N = 10, 0.32 mg/kg MK-801 plus L-CCG I N = 11, data are MEAN ± S.E.M.)

	Dose MK-801 (mg/kg)	0.00	0.08	0.16	0.32
Mean duration/ Head dip (sec)	L-CCG I group (250 nmol)	1.4 ± 0.4	1.3 ± 0.2	1.1 ± 0.2	0.6 ± 0.3
	Vehicle controls (0 nmol L-CCG I)	1.4 ± 0.2	1.2 ± 0.1	1.1 ± 0.1	0.9 ± 0.2
Mean duration/ rearing (sec)	L-CCG I group (250 nmol)	1.4 ± 0.2**	2.2 ± 0.3	1.2 ± 0.3	0.9 ± 0.1
	Vehicle controls (0 nmol L-CCG I)	2.6 ± 0.2	2.3 ± 0.3	1.6 ± 0.3**	1.1 ± 0.1**
Mean duration/ inactivity (sec)	L-CCG I group (250 nmol)	270.5 ± 68.0**	157.4 ± 49.9$$	216.6 ± 59.8$$	8.9 ± 8.0
	Vehicle controls (0 nmol L-CCG I)	9.4 ± 9.1	0.0 ± 0.0**	0.0 ± 0.0**	0.0 ± 0.0**

** indicate a difference of $P < 0.01$ as compared to controls exclusively treated with vehicles, $$ indicate a difference of $P < 0.01$ as compared to groups treated with the same dose of MK-801 but 0 nmol L-CCG I.

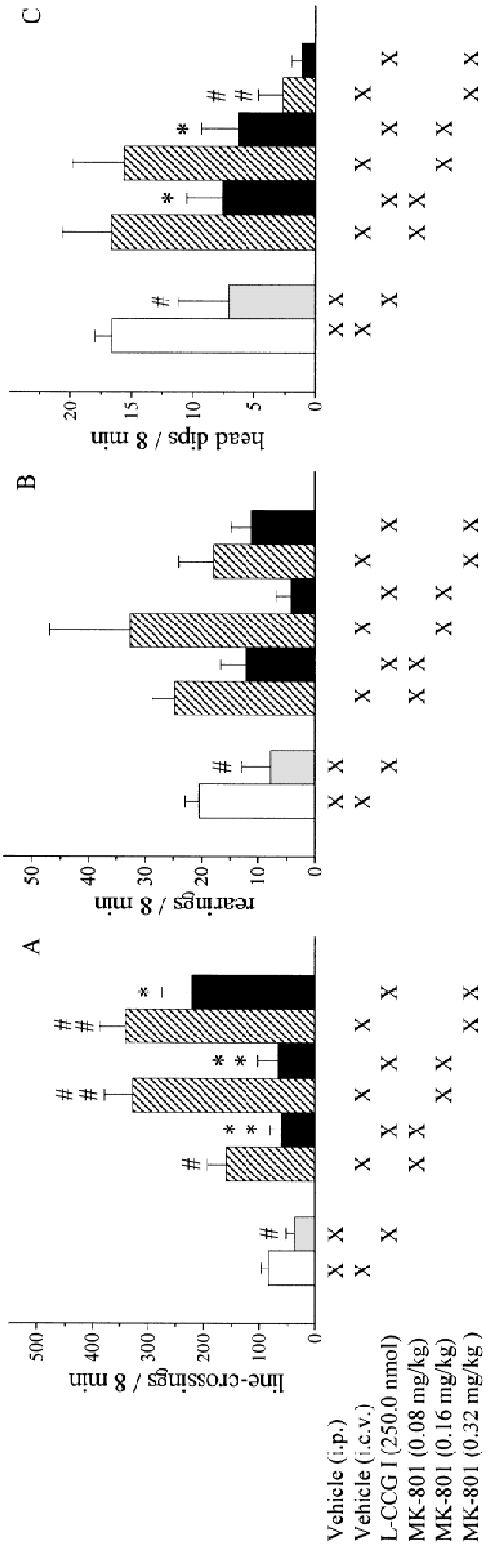

Fig. 2A–C. Effects of 250.0 nmol L-CCG I on motor behaviour modulated by MK-801 (vehicle control group N = 10; group treated with 250.0 nmol L-CCG I i.c.v. and vehicle i.p. N = 8; groups treated with MK-801 and i.c.v. vehicle N = 10–12; groups treated with MK-801 and 250.0 nmol L-CCG I N = 10–12; data are MEAN ± S.E.M.); # indicates a difference of $P < 0.05$ and ## a difference of $P < 0.01$ as compared to vehicle controls, * indicates a difference of $P < 0.05$ and ** of $P < 0.01$ as compared to the respective MK-801 group

L-CCG I does not antagonise D,L-amphetamine-induced locomotor stimulation

Figure 3a shows the induction of locomotor activity by 2.0 and 3.0 mg/kg D,L-amphetamine. In contrast to MK-801-induced locomotion, 250 nmol L-CCG I did not antagonise D,L-amphetamine-induced locomotor stimulation [$F_{L\text{-}CCG(1,84)} = 6.4$, $p < 0.05$, $F_{D,L\text{-}amphetamine(3,84)} = 18.0$, $p < 0.001$, $F_{L\text{-}CCG\times D,L\text{-}amphetamine(3,84)} = 2.6$, $p > 0.05$]. 2.0 and 3.0 mg/kg D,L-amphetamine increased and L-CCG I decreased the number of rearings, but there was no interaction in this respect, as it was the case for MK-801 (Fig. 3b) [$F_{L\text{-}CCG(1,84)} = 5.1$, $p < 0.05$, $F_{D,L\text{-}amphetamine(3,84)} = 10.9$, $p < 0.001$, $F_{L\text{-}CCG\times D,L\text{-}amphetamine(3,84)} = 0.4$, $p > 0.05$]. L-CCG I reduced the number of spontaneous head dips, but opposite to the coadministration with MK-801, there was no interaction with coadministered D,L-amphetamine (Fig. 3C) [$F_{L\text{-}CCG(1,84)} = 6.8$, $p < 0.05$, $F_{D,L\text{-}amphetamine(3,84)} = 1.4$, $p > 0.05$, $F_{L\text{-}CCG\times D,L\text{-}amphetamine(3,84)} = 0.2$, $p > 0.05$]. Mean duration of head dips and rearings were affected neither by L-CCG I nor D,L-amphetamine (Table 3). Surprisingly D,L-amphetamine and L-CCG I had antagonistic effects on the duration of inactivity, opposite to their effects on locomotion (Table 3) [$F_{L\text{-}CCG(1,84)} = 44.7$, $p < 0.001$, $F_{D,L\text{-}amphetamine(3,84)} = 18.3$, $p < 0.001$, $F_{L\text{-}CCG\times D,L\text{-}amphetamine(3,84)} = 19.6$, $p < 0.001$]. This antagonism, however, was only observed at the lowest dose of D,L-amphetamine tested, which failed to stimulate locomotion.

Table 3. Effects of D,L-amphetamine on 250.0 nmol L-CCG I mediated akinesia (vehicle controls N = 10, L-CCG I N = 9, 1.0 mg/kg D,L-amphetamine N = 10, 2.0 mg/kg D,L-amphetamine N = 10, 3.0 mg/kg D,L-amphetamine N = 13, 1.0 mg/kg D,L-amphetamine plus L-CCG I N = 10, 2.0 mg/kg D,L-amphetamine plus L-CCG I N = 10, 3.0 mg/kg D,L-amphetamine plus L-CCG I N = 13, data are MEAN ± S.E.M.)

	Dose D,L-amphetamine (mg/kg)	0.00	1.0	2.0	3.0
Mean duration/ Head dip (sec)	L-CCG I group (250 nmol)	1.6 ± 0.8	0.8 ± 0.3	0.5 ± 0.1	0.7 ± 0.1
	Vehicle controls (0 nmol L-CCG I)	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.2
Mean duration/ Rearing (sec)	L-CCG I group (250 nmol)	1.5 ± 0.3	1.7 ± 0.1	1.7 ± 0.2	1.4 ± 0.1
	Vehicle controls (0 nmol L-CCG I)	1.7 ± 0.2	1.7 ± 0.2	0.8 ± 0.2	1.8 ± 0.3
Mean duration/ inactivity (sec)	L-CCG I group (250 nmol)	327.7 ± 56.9**	171.9 ± 56.0$$	0.0 ± 0.0	0.0 ± 0.0
	Vehicle controls (0 nmol L-CCG I)	1.1 ± 0.6	2.0 ± 0.8	0.0 ± 0.0	12.8 ± 8.9

** indicate a difference of $P < 0.01$ as compared to the control group exclusively treated with vehicles, $$ indicate a difference of $P < 0.01$ as compared to the group treated with the same dose of D,L-amphetamine but 0 nmol L-CCG I.

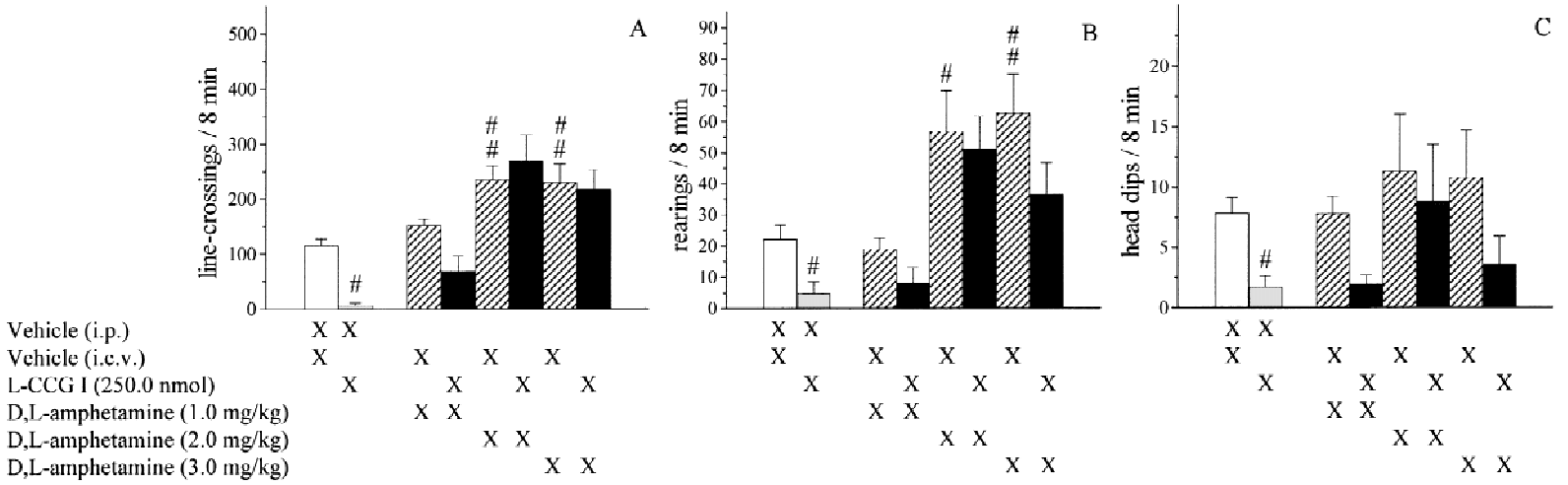

Fig. 3A–C. Effects of 250.0 nmol L-CCG I on motor behaviour modulated by D,L-amphetamine (vehicle control group N = 10; group treated with 250.0 nmol L-CCG I i.c.v. and vehicle i.p. N = 9; groups treated with D,L-amphetamine and i.c.v. vehicle N = 10 − 13; groups treated with D,L-amphetamine and 250.0 nmol L-CCG I N = 10 − 13; data are MEAN ± S.E.M.); # indicates a difference of $P < 0.05$ and ## a difference of $P < 0.01$ as compared to vehicle controls

Discussion

These results show, that i.c.v. infusions of the preferential group II agonist L-CCG I reduces locomotor and exploratory behavior in an open-field, as shown by the reduction of line crossings, rearings and head-dips. The motor effects of L-CCG I were antagonised by the selective group II antagonist EGLU. MK-801 but not D,L-amphetamine induced locomotor stimulation was antagonised by L-CCG I.

L-CCG I mediates its motor effects by activating group II mGluRs

L-CCG I activates not only group II subtypes but also mGluR1 and mGluR4. The EC_{50} for those subtypes, however, is 50- to 150-fold higher than for group II mGluRs, their expression in the basal ganglia is low while it is high for group II mGluRs and mGluR4 knockout mice are not impaired in novelty induced exploration (Testa et al., 1994; Pin and Duviosin, 1995; Pekhetski et al., 1996). The motor coordination of mGluR1 deficient mice on the other hand is impaired (Conquet et al., 1994). Activation of group I mGluRs however increases motor behaviour, an effect which might contribute to the rebound of the motor response observed with the highest dose L-CCG I (Kearney, et al., 1997). Indeed motor activity of a group treated with a lower dose of L-CCG I (250.0 nmol) and the selective group II antagonist EGLU (250.0 nmol) did not differ from a vehicle control group (Jane et al., 1996; Manahan-Vaughan, 1997).

Motor effects of a decreased glutamate release in the basal ganglia input structures, mediated by activation of group II mGluRs

Three independent lines of evidence, immunohistochemical, electrophysiological and microdialysis studies suggest, that group II mGluRs in the input structures of the basal ganglia function at least partially as auto-receptors of glutamatergic cortico-striatal afferents, which activation subsequently reduces glutamate release release (Cozzi et al., 1997; Manzoni et al., 1997). This reduction might at least partially mediate the motor effects of L-CCG I and would affect two major classes of postsynaptic glutamate receptors: ionotropic, i.e. NMDA and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and mGluRs. Striatal and accumbal infusions of NMDA reduce locomotor and exploratory behavior, while NMDA-receptor antagonists produce a strong locomotor stimulation (for review see Schmidt and Kretschmer, 1997). Against this background it is very unlikely that reduced NMDA-receptor activation, due to L-CCG I mediated reduction of glutamate release, contributes to the motor depression observed (but see also Konieczny et al., 1998). While results from striatal and accumbal AMPA infusions are partially controversial, systemic injections of high doses of AMPA-receptor antagonists reduce locomotion, but do not elicit catalepsy, when administered alone (for review see Schmidt and Kretschmer, 1997).

Thus, taken together with the fact, that L-CCG I can elicit catalepsy, it is also unlikely that reduced AMPA-receptor activity mediates the akinesia induced by L-CCG I (Kronthaler and Schmidt, 1998). Much less is known about the role of postsynaptic mGluRs than about the role of ionotropic glutamate-receptors for basal ganglia mediated motor behavior behavior (Smith and Beninger, 1996; Kearney et al., 1997; Zalewska and Wisniewski, 1997; Attarian and Amalric, 1997; Camón et al., 1998; Kim and Vezina, 1998). Given the as yet poor knowledge about the *in vivo* function of mGluRs and the partially unclear pharmacological effects of mGluRs ligands, when used in vivo, several contradictions exist. In addition it might be reasonable to take neurotoxic effects into account in order to explain some behavioral effects reported so far (Wang et al., 1997). Taken together, the motor effects of L-CCG I can not be explained by the reduced activation of solely one group of glutamate-receptors, but rather by a reduced activation of multiple glutamate-receptor groups, but the specific contribution of each group is yet not clear.

Interaction of activated group II mGluRs with NMDA- but not dopamine-receptor mediated behavior

MK-801 induced locomotor stimulation was antagonised by L-CCG I. MK-801 binds within the ion-channel of the NMDA-receptor complex, thus it can antagonise NMDA-receptors only in the activated status (for review see Schmidt and Kretschmer, 1997). Since L-CCG I decreases glutamate release thus decreasing the activation of NMDA-receptors, this might explain the antagonism of MK-801 and L-CCG I. If this use-dependency of MK-801 would be responsible for this effect, the antagonism should not be restricted to locomotion, but it was not observed for the number and mean duration of rearings. Thus, such a mechanistic explanation of the antagonism appears to be unlikely. Recently it was directly shown by Moghaddam et al. (1997), that blockade of NMDA-receptors increases glutamate release, which in consequence would cause the motor hyperactivity observed. Indeed, activation of group II mGluRs counteracts increased glutamate release in the nucleus accumbens which correlates with the counteraction of motor stimulation, mediated by this structure structure (Moghaddam and Adams, 1998). One might be cautious concluding a causal relationship from this. It is very well known that not only psychotomimetic NMDA-receptor antagonists but also one of the most frequently used antipsychotics, haloperidol, increases glutamate release (Daly and Moghaddam, 1993; Moghaddam and Bunney, 1993; Yamamoto and Cooperman, 1994). Although D,L-amphetamine increases glutamate release as well, L-CCG I had no prominent effects on stimulated locomotion and all other D,L-amphetamine induced effects, in contrast to MK-801 stimulated locomotion (Reid et al., 1997). Reducing dopamine release by lesioning dopaminergic terminals on the other hand, decreases D,L-amphetamine but not MK-801 stimulated motor behavior (Ouagazzal et al., 1994; U. O. Kronthaler, M. Bubser, and W. J. Schmidt,

unpublished observation). Taken together this argues in favour of a different neurochemical basis of MK-801 and D,L-amphetamine mediated motor stimulation and suggests that an increased glutamate release is not crucial for D,L-amphetamine mediated effects. In addition it is controversial, whether microdialysis quantifies at all that portion of glutamate, which is vesicularly released by neurons (for Review see Timmermann and Westerink, 1997). A quantitative correlation exists between the increased dopaminergic activity and the motor stimulation mediated by NMDA-receptor antagonists, but this relationship is not causal (Ouagazzal et al., 1994; Bubser et al., 1997; Adams and Moghaddam, 1998). For this, even if a correlation exists between the possibly increased glutamate release and motor stimulation mediated by NMDA-receptor antagonists, this relationship is not necessarily causal. Finally infusions of the NMDA-receptor antagonists into and excitotoxic lesions of the basal ganglia input structures induce hyperactivity and disrupt the prepulse inhibition of the startle reflex (Kodsi and Swerdlow, 1994; Reijmers et al., 1995; for further Ref. see Schmidt and Kretschmer, 1997). Taken together the assumption, that a glutamate hyperactivity is underlying the motor stimulation induced by a NMDA-receptor antagonist is not convincing.

Group II receptors are not exclusively expressed presynaptically. For this it is tempting to speculate, whether these postsynaptic group II receptors, namely mGluR3, at least partially contribute to the differential effects of L-CCG I and indeed in the striatum and nucleus accumbens show a marked postsynaptic expression of mGluR3 (Testa et al., 1994; Petralia et al., 1996). Since activation of group I mGluR positively modulates NMDA-receptors one might speculate that the opposite might occur by activation of mGluR3, taken the opposite effects on their second messenger systems into account (Pisani et al., 1997). Indeed, in the nucleus accumbens NMDA-receptor mediated synaptic transmission can be postsynaptically inhibited by group II receptors (Martin et al., 1997). It would have to be elucidated how this negative modulation would induce differential effects on locomotion and exploration, but locomotion is mainly controlled by the nucleus accumbens, while exploration possibly represents a striatal function (Ouagazzal et al., 1994; U. O. Kronthaler, M. Bubser, and W. J. Schmidt, unpublished observation). Unfortunately the possible differential interactions of mGluRs with NMDA- and dopamine-receptors have not been addressed so far, neither on the biochemical nor on the behavioral level. This is surprising given the numerous potential therapeutic applications of mGluRs ligands (Knöpfel et al., 1995).

Therapeutical considerations

The present results may have important implications for pathophysiological situations characterised by an acute glutamatergic hyperactivity, such as epilepsy, ischemia or trauma. Most clinical trials with NMDA-receptor antagonists, favoured as pharmacotherapeutical approach, have been stopped

due to unwanted effects such as psychotomimesis and amnesia (Rockstroh et al., 1996). The antagonism of NMDA-receptor blockade induced motor stimulation by group II activation suggests however, that group II activation agonists may counteract psychosis, at least when induced by NMDA-receptor antagonists. For this group II agonists may be the more suitable approach than the blockade NMDA-receptor antagonists in this respect. However, it is possible that a therapy basing on group II agonists might be hampered by unwanted extrapyramidal and amnestic effects at least given a chronic treatment (Van Der Staay et al., 1995; Kronthaler and Schmidt, 1998; but see also Helton et al., 1998).

Conclusions

To our knowledge this is the first study directly examining the interactions of inhibitory, second messenger coupled glutamate-receptors with ionotropic glutamate- and dopamine-receptors in the control of locomotor and exploratory behavior. It reveals, that activation of group II mGluRs may decrease motor behavior and that group II receptors might be more closely linked to NMDA- than to dopamine-receptor mediated motor behavior. Since both NMDA-receptor antagonists and group II agonist are neuroprotective to a sub-maximal degree, when administered alone, one may consider to coadminister such ligands. By this one possibly might at least add their neuroprotective effects effects (Bruno et al., 1994) and at the same time, achieve a reduction of NMDA-receptor antagonists induced psychotomimetic side effects.

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Authors' address: Dr. Ulrich O. Kronthaler, Aventis Behring GmbH, Pharmacology/ Toxicology, P.O. Box 1230, D-35002 Marburg, Federal Republic of Germany, Fax +49(0)6421 39-5310, e-mail: Ulrich.Kronthaler@aventis.com

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