ELSEVIER

European Journal of Pharmacology 366 (1999) 151–158

ejp

Induction of long-lasting depression by (+)-α-methyl-4-carboxyphenylglycine and other group II mGlu receptor ligands in the dentate gyrus of the hippocampus in vitro

Lingqian Huang [a], Michael J. Rowan [b], Roger Anwyl [a,*]

[a] *Department of Physiology, Trinity College, Dublin, 2, Ireland*
[b] *Department of Pharmacology and Therapeutics, Trinity College, Dublin, 2, Ireland*

Received 2 July 1998; revised 1 December 1998; accepted 4 December 1998

Abstract

Application of several well characterized group II mGlu receptor ligands was found to induce a long-lasting depression of synaptic transmission in the medial perforant path of the dentate gyrus. These ligands were *N*-acetylaspartylglutamate (NAAG), which is a dipeptide located in the brain and possibly functioning as a neurotransmitter, two agents widely used previously as mGluR antagonists, (+)-α-methyl-4-carboxyphenylglycine (MCPG), and (*S*)-α-ethylglutamate (EGLU), and the well characterized group II mGluR agonist (2*S*,1*R*,2*R*,3*R*)-2-(2*S*,1′*R*,2′*R*,3′*R*)-2(2′3′-dicarboxycyclopropyl)glycine (DCG-IV). It is postulated that all these ligands induced the long-lasting depression by an agonist/partial agonist action at group II mGlu receptor. The long-lasting depression induced by the ligands showed mutual occlusion with low frequency stimulation-induced long-term depression, demonstrating common induction or maintenance mechanisms. The induction of the long-lasting depression by the mGlu receptor ligands are suggested to occur postsynaptically as the induction was not associated with a change in paired pulse depression of excitatory postsynaptic potentials (EPSPs).

Keywords: mGluR receptor, group II; MCPG ((+)-α-methyl-4-carboxyphenylglycine); EGLU ((*S*)-α-ethylglutamate); DCG = IV ((2*S*,1*R*,2*R*,3*R*)-2-(2*S*,1′*R*,2′*R*,3′*R*)-2 (2′3′-dicarboxycyclopropyl)glycine); NAAG (*N*-acetylaspartylglutamate); Hippocampus; Long-term depression

1. Introduction

Long-term depression is a long-lasting reduction in excitatory transmission which can be induced in vitro by a period of low frequency stimulation in CA1 (Dudek and Bear, 1992; Mulkey and Malenka, 1992) and dentate gyrus (O'Mara et al., 1995; Wang et al., 1997). There is evidence for an essential role of metabotropic glutamate receptors (mGlu receptors) in the induction of long-term depression in the hippocampus, as the induction of homosynaptic long-term depression in hippocampus was blocked by the mGlu receptor antagonist (+)-α-methyl-4-carboxyphenylglycine (MCPG) (Bashir and Collingridge, 1994; Bolshakov and Siegelbaum, 1994; Yang et al., 1994; O'Mara et al., 1995). Moreover, the mGluR agonist 1*S*,3*R*-ACPD induced long-term depression in the hippocampus (Bolshakov and Siegelbaum, 1994; O'Mara et al., 1995).

Recent studies have provided evidence that activation of group II mGlu receptor is necessary for long-term depression induction. In a study from the present authors, the group II mGlu receptor antagonist 2*S*,1*S*′,2*S*′-2-methyl-2-(2′-carboxycyclopropyl)glycine (MCCG) (Hayashi et al., 1994; Jane et al., 1994, 1995; Knopfel et al., 1995) was found to inhibit the induction of long-term depression in the medial perforant path of the dentate gyrus in vitro (Huang et al., 1997a). In addition, the mGlu receptor group II antagonists (*RS*)-α-methylserine-*O*-phosphate monophenyl ester (MSOPPE) and (2*S*)-α-ethylglutamic acid (EGLU) were found to block the induction of late-phase long-term depression in CA1 in vivo (Manahan-Vaughan, 1997). Furthermore, mutant mice lacking $mGlu_2$ receptor had strongly impaired long-term depression induction at the mossy fibre-CA3 pathway in which long-term depres-

* Corresponding author. Tel.: +353-1-6081-624; Fax: +353-1-6793-545; E-mail: ranwyl@mail.tcd.ie

0014-2999/99/$ - see front matter
PII: S0014-2999(98)00918-2

sion is presynaptically induced (Kobayashi et al., 1996; Yokoi et al., 1996).

In the present studies, we have investigated the effects of four group II mGlu receptor ligands on the induction of a long-lasting depression in the medial perforant path of the hippocampus in vitro.

2. Materials and methods

All experiments were carried out on transverse slices of the rat hippocampus (age 3–4 weeks, weight 40–80 g). The brains were rapidly removed after decapitation and placed in cold oxygenated (95% O_2/5% CO_2) media. Slices were cut at a thickness of 350 μm using a Campden vibroslice, and placed in a storage container containing oxygenated media at room temperature (20–22°C). The slices were then transferred as required to a recording chamber for submerged slices and continuously superfused at a rate of 5–6 ml/min at 30–32°C.

The control media contained: (mM) NaCl, 120; KCl 2.5, NaH_2P0_4, 1.25; $NaHC0_3$ 26; $MgS0_4$, 2.0; $CaCl_2$, 2.0; D-glucose 10. All solutions contained 100 μM picrotoxin (Sigma) to block $GABA_A$ receptor-mediated activity.

Presynaptic stimulation was applied to the medial perforant pathway of the dentate gyrus. Field excitatory postsynaptic potentials (EPSPs) were recorded at a control test frequency of 0.033 Hz from the medial perforant pathway. In each experiment, an input–output curve (afferent stimulus intensity versus EPSP amplitude) was plotted at the test frequency, and the amplitude of the test EPSP was adjusted to one-third of maximum, usually about 1–1.2 mV. Paired pulse stimulation was given at 0.017 Hz with 40 ms interval. Paired pulse ratio was calculated as (E1–E2)/E1 (E1, the amplitude of the first EPSP; E2, the amplitude of the second EPSP). Long-term depression was evoked by low frequency stimulation consisting of 900 stimuli at 1 Hz for 15 min, with the test stimulation voltage remaining at the same amplitude during the low frequency stimulation. In two sets of experiments, multiple low frequency stimulation (consisting of one low frequency stimulation for 900 stimuli at 1 Hz followed by a further 3 sets of 300 stimuli at 1 Hz) was given in order to induce the maximal long-term depression. The magnitude of the ligand induced long-term depression was measured at 40–60 min following washout of the ligand, while the low frequency stimulation-induced long-term depression was measured 30–40 min after low frequency stimulation.

Drugs used were MCPG, EGLU, (2*S*,1*R*,2*R*,3*R*)-2-(2*S*,1′*R*,2′*R*, 3′*R*)-2 (2′ 3′-dicarboxycyclopropyl)glycine (DCG-IV) (all Tocris Cookson) and *N*-acetylaspartylglutamate (NAAG) (Sigma). Recordings were analysed using p-clamp. Values are the mean ± SEM for *n* slices and two-tailed Student's *t*-test was used for statistical comparison.

3. Results

NAAG is an endogenously located neuropeptide which was demonstrated to selectively activate group II mGlu receptor (Wroblewska et al., 1993, 1997). Perfusion of NAAG (200 μM, 20 min) caused, in the presence of the ligand, a small significant enhancement of the EPSP of 9 ± 4% ($P < 0.05$, $n = 5$) which was not associated with a change in paired pulse depression (0.21 ± 0.06 and 0.24 ± 0.07) prior to and during the perfusion of NAAG, respectively, ($P > 0.05$) (Fig. 1A,B). Following washout of NAAG, a long-lasting depression measuring 29 ± 3% ($P < 0.01$, $n = 5$) was induced which was also not associated with a change in paired pulse depression (0.24 ± 0.07, $P > 0.05$). NAAG induced a long-lasting depression in all experiments, although the time course and amplitude of such long-term depression was quite variable, as illustrated by three individual examples shown in Fig. 1B.

The NAAG-induced long-lasting depression showed mutual occlusion with low frequency stimulation-induced long-term depression. Thus the induction of long-term depression by low frequency stimulation was strongly inhibited by prior NAAG-induced long-lasting depression, the low frequency stimulation-induced long-term depression measuring 8 ± 4% ($P > 0.05$, $n = 5$) (Fig. 1A). Furthermore, following low frequency stimulation-induced long-term depression measuring 44 ± 7%, $P < 0.01$, $n = 5$), NAAG-induced long-lasting depression measured 10 ± 5%, significantly inhibited ($P < 0.01$, $n = 5$) compared to control (Fig. 1C).

MCPG is a well known antagonist of mGlu receptors, including mGlu receptor group I (Eaton et al., 1993) and group II (Hayashi et al., 1994; Thomsen et al., 1994). Perfusion of MCPG (500 μM) for 20 min caused, in the presence of the ligand, either a small decrease or a small increase in the EPSP in separate experiments, although on the average, no significant change occurred (96% ±2%, $P > 0.05$, $n = 6$). No significant change in paired pulse depression occurred in the presence of MCPG (paired pulse depression measured 0.23 ± 0.04 and 0.24 ± 0.05 prior to and during the perfusion of MCPG, $P > 0.05$). Following washout of MCPG, a consistent long-lasting depression was induced in all of six experiments, the long-lasting depression measuring 19 ± 4% ($P < 0.01$, $n = 6$) (Fig. 2A, B). No change in paired pulse depression occurred during the induction of the long-lasting depression (paired pulse depression measured 0.22 ± 0.03 and 0.21 ± 0.03, $P > 0.05$) prior to and following perfusion of MCPG. MCPG induced long-term depression in all experiments, although the time course and amplitude of such long-term depression was quite variable, as illustrated by three individual examples shown in Fig. 2B.

The MCPG-induced long-lasting depression showed mutual occlusion with low frequency stimulation-induced long-term depression. Following MCPG-induced long-lasting depression, subsequent low frequency stimulation-in-

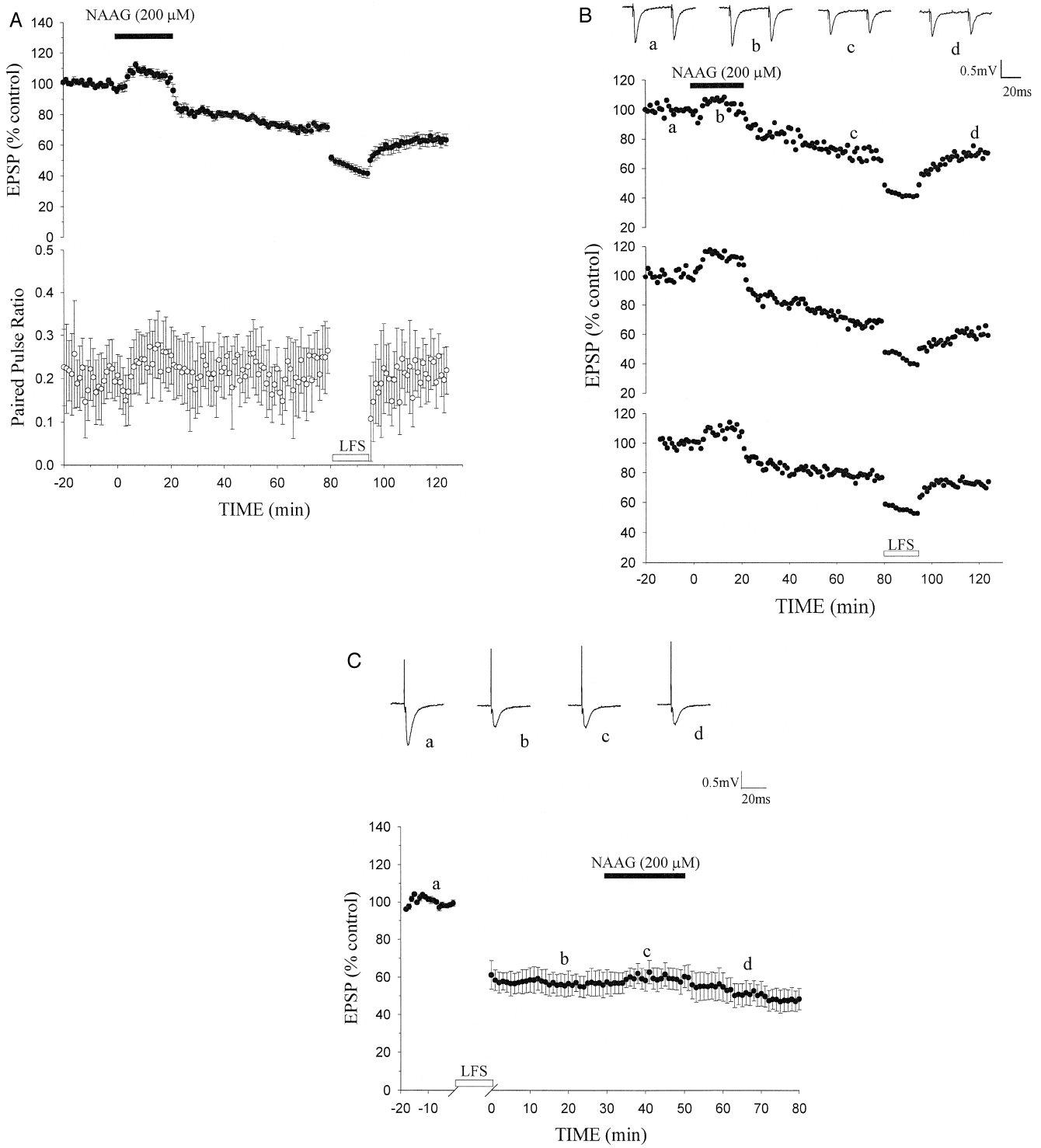

Fig. 1. Induction of a long-lasting depression by the group II mGluR ligand NAAG. (A) The upper graph is a measure of the EPSP (closed circles) and the lower graph a measure of the paired pulse depression (open circles). Perfusion of NAAG (200 μM) for 20 min induced a small enhancement which was not associated with a change in paired pulse depression, followed, after washout of NAAG, by expression of a long-lasting depression which was also not associated with a change in paired pulse depression. Subsequent low frequency stimulation-induced long-term depression was strongly occluded, $n = 5$. (B) Three examples of individual experiments in which perfusion of NAAG induced a small enhancement of the EPSP during perfusion, and in which a long-lasting depression was expressed following washout of NAAG, and in which subsequent low frequency stimulation-induced long-term depression was occluded. The traces shows the amplitude of the EPSPs and paired pulse depression of EPSPs prior to (a), during (b) and 60 min following (c) perfusion of NAAG, and following low frequency stimulation (d). (C) The induction of long-term depression by low frequency stimulation resulted in an inhibition of subsequent NAAG-induced long-lasting depression, $n = 5$. The traces shows the amplitude of the EPSPs prior to low frequency stimulation (a), 20 min following low frequency stimulation (b), during perfusion of NAAG (c) and 20 min following perfusion of NAAG (d).

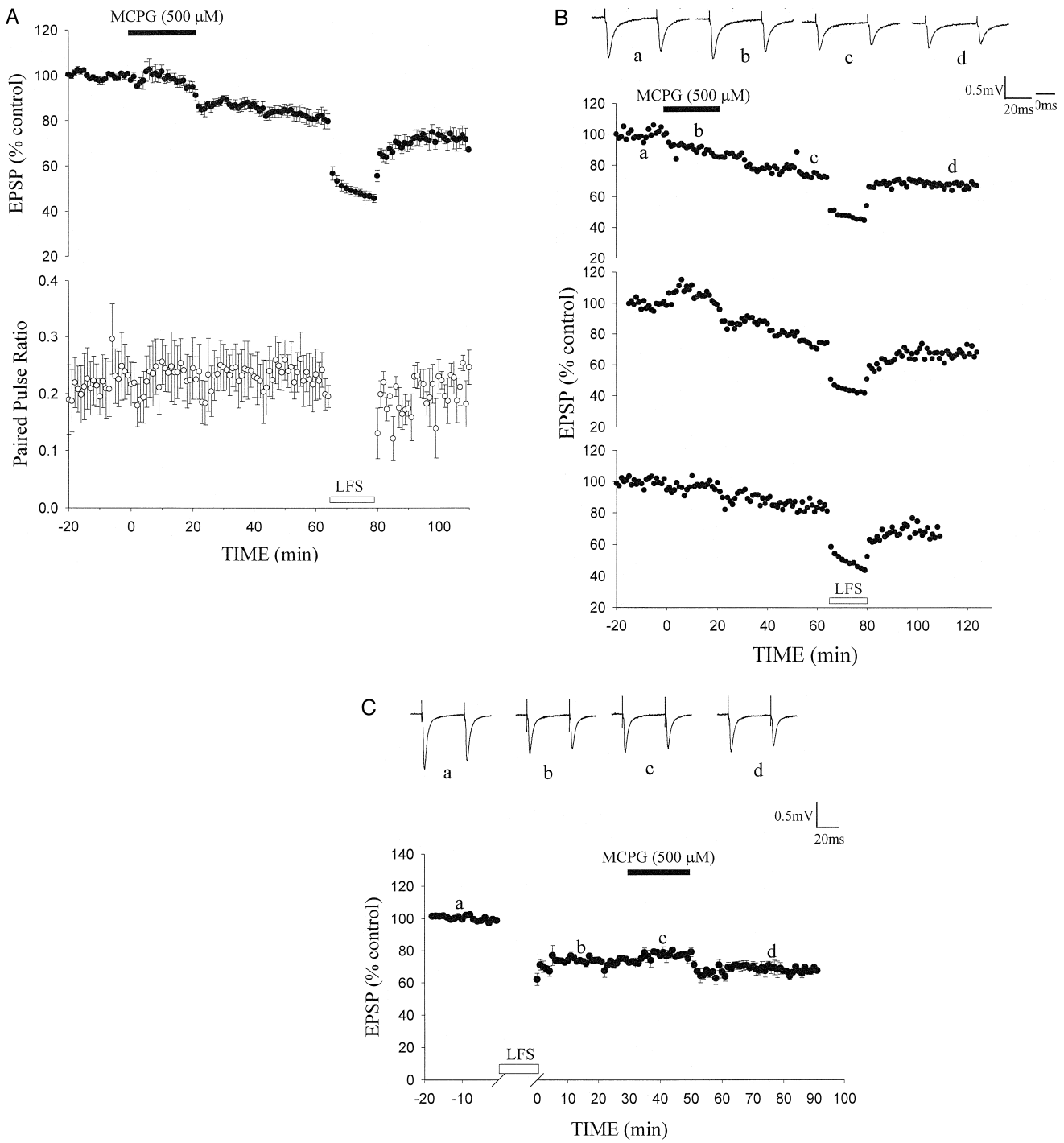

Fig. 2. Induction of a long-lasting depression by the group II mGluR ligand MCPG. (A) The upper graph is a measure of the EPSP (closed circles) and the lower graph a measure of the paired pulse depression (open circles). Perfusion of MCPG (500 μM) for 20 min induced a small depression which was not associated with a decrease in paired pulse depression, followed, after washout of MCPG, by expression of a long-lasting depression which was also not associated with a change in paired pulse depression. Subsequent low frequency stimulation-induced long-term depression was strongly occluded, $n = 5$. (B) Three examples of individual experiments. The traces shows the amplitude of the EPSPs and paired pulse depression of EPSPs prior to (a), during (b) 45 min following perfusion of MCPG (c), and following low frequency stimulation (d). (C) The induction of large amplitude long-term depression by low frequency stimulation resulted in an occlusion of the MCPG-induced long-term depression, $n = 4$. The traces shows the amplitude of the EPSPs prior to low frequency stimulation (a), following low frequency stimulation-induced long-term depression (b), during perfusion of MCPG (c) and 30 min following perfusion of MCPG (d).

duced long-term depression was strongly occluded, measuring $10 \pm 2\%$, a significant reduction compared to control ($P < 0.01$) (Fig. 2A, B). Moreover, following induction of low frequency stimulation-induced long-term de-

pression measuring $28 \pm 2\%$, ($P < 0.01$, $n = 4$, with no associated change occurring in the paired pulse depression), perfusion and subsequent washout of MCPG (500 μM) did not induce significant long-lasting depression ($3 \pm 3\%$, $n = 4$, $P > 0.05$) (Fig. 2C). Such experiments demonstrated that low frequency stimulation-induced long-term depression had occluded the MCPG-induced long-lasting depression and also that MCPG did not block the maintenance of long-term depression. It should be noted that a small nonsignificant enhancement of the EPSP occurred during the application of MCPG when the ligand was applied following the induction of low frequency stimulation-induced long-term depression ($7 \pm 3\%$, $P > 0.05$, $n = 4$).

EGLU has been shown to be an antagonist of the action of the presynaptic action of (1*S*,3*S*)-ACPD in the rat spinal cord, with a K_d of 66 μM (Jane et al., 1996). In the present studies, perfusion of EGLU (200 μM, 30 min) was found to cause a small depression measuring $13 \pm 4\%$ ($P < 0.05$, $n = 5$) (in the presence of the ligand) which was at least partially mediated presynaptically as it was asociated with a small but significant change in paired pulse depression (0.28 ± 0.04 and 0.22 ± 0.05 prior to and during the perfusion of EGLU, $P < 0.05$). Following washout of the EGLU, a large long-lasting depression measuring $32 \pm 5\%$ ($P < 0.01$, $n = 5$) was induced. Subsequent low frequency stimulation-induced long-term depression was strongly occluded, measuring $5 \pm 2\%$ ($P > 0.05$, $n = 5$) (Fig. 3A). EGLU induced a long-lasting depression in all experiments, although the time course and amplitude of such long-lasting depression was quite variable, as illustrated by three individual examples shown in Fig. 3B.

DCG-IV is a potent mGlu receptor group II agonist with an EC_{50} for inhibition of forskolin elevated cAMP of 0.3 and 0.2 μM at mGluR2 and 3, respectively (Hayashi et al., 1994). Perfusion of DCG-IV (200 nM) for 20 min induced a rapid and reversible depression of the EPSP measuring $41 \pm 6\%$ ($P < 0.01$, $n = 6$), which was maintained in the presence of DCG-IV. This reversible depression was associated with a change in paired pulse depression from the control value of 0.17 ± 0.05 to a paired pulse facilitation of 0.06 ± 0.10 ($P < 0.01$, $n = 6$). Following washout of DCG-IV, a long-lasting depression measuring $22 \pm 3\%$ ($P < 0.01$, $n = 6$) was induced (Fig. 4). No change in paired pulse depression was associated

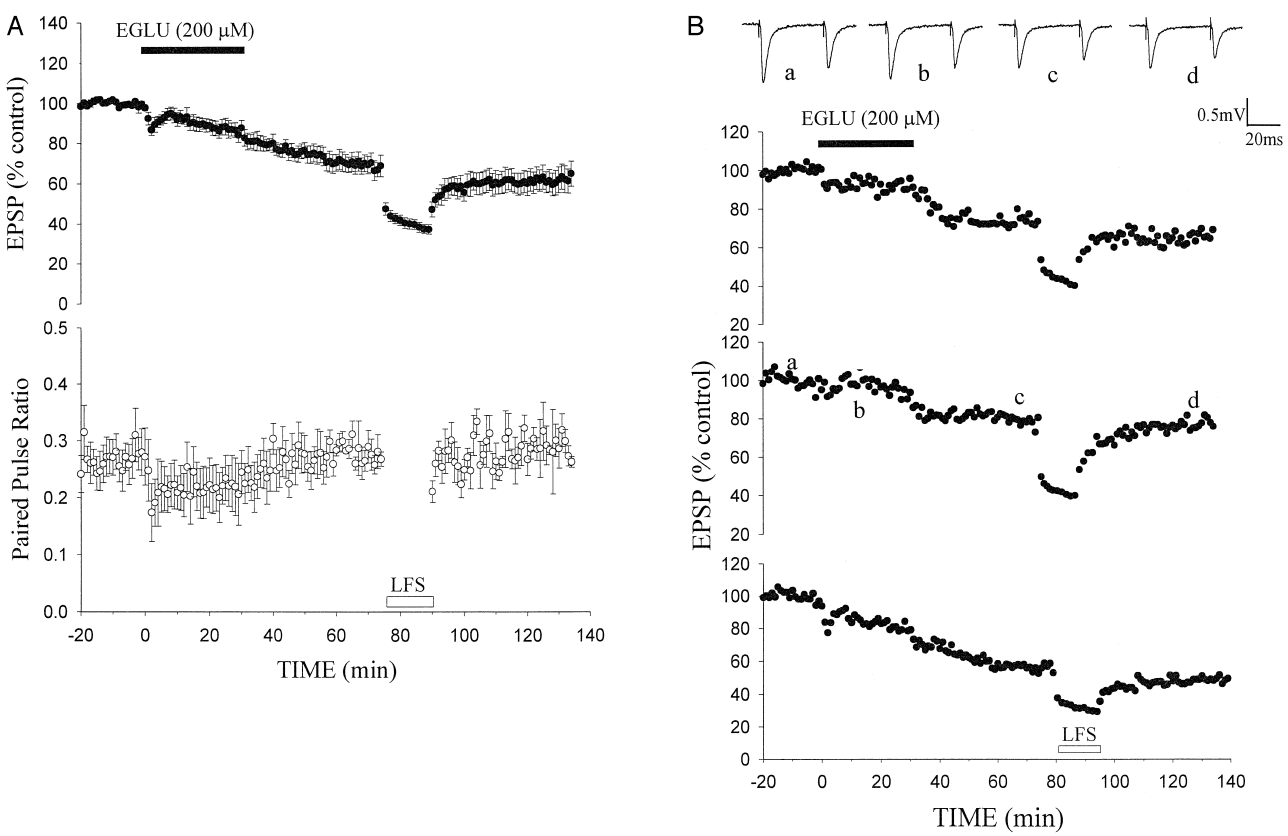

Fig. 3. Induction of a long-lasting depression by the group II mGluR ligand EGLU. (A) The upper graph is a measure of the EPSP (closed circles) and the lower graph a measure of the paired pulse depression (open circles). Perfusion of EGLU (200 μM) for 20 min induced a small depression which was associated with a change in paired pulse depression. Following washout of EGLU, a long-lasting depression was induced which was not associated with a change in paired pulse depression. Subsequent low frequency stimulation-induced long-term depression was significantly inhibited, $n = 5$. (B) Three examples of individual experiments. The traces shows the amplitude of the EPSPs and paired pulse depression of EPSPs prior to (a), during (b) 45 min following perfusion of EGLU (c), and following low frequency stimulation (d).

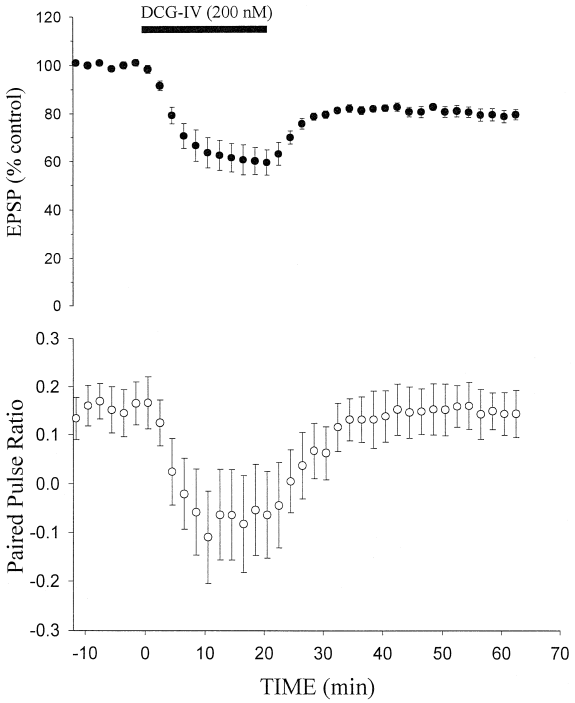

Fig. 4. DCG-IV induces a reversible depression and a long-lasting depression. (A) Upper graph shows that application of DCG-IV (200 nM) for 20 min resulted in the induction of a reversible depression of the EPSP (closed circles) which was followed, upon washout of the DCG-IV, by a long-lasting depression. The lower graph shows the reduction of the paired pulse depression (open circles) during the reversible depression induced by DCG-IV, and also that no change in paired pulse depression occurred during the expression of long-term depression, $n = 6$.

with the induction of the long-lasting depression (0.14 ± 0.03, $P > 0.05$, $n = 6$).

4. Discussion

The present experiments show that the mGlu receptor ligands NAAG, MCPG, EGLU and DCG-IV all induced a long-lasting depression in the medial perforant path of the dentate gyrus. The long-lasting depression induced by these ligands showed a common maintenance mechanism with low frequency stimulation-induced long-term depression, as mutual occlusion occurred between the ligand-induced long-lasting depression and the low frequency stimulation-induced long-term depression. Such occlusion demonstrates a common induction and/or maintenance pathway between the ligand-induced long-lasting depression and the low frequency stimulation-induced long-term depression. It is postulated that the mGluR ligands NAAG, MCPG, EGLU and DCG-IV all induce long-lasting depression by an agonist/partial agonist action at group II mGluR. This theory is strong for the action of DCG-IV, for previous electrophysiological studies have shown that DCG-IV is a potent agonist at mGluRII, evoking a presynaptic reversible inhibition of monosynaptic excitatory transmission in several regions of the central nervous system including the medial and lateral perforant paths of the dentate gyrus (Bushell et al., 1996; Macek et al., 1996). A partial agonist action of MCPG in the present studies is somewhat surprising, as MCPG has previously been shown to be an mGlu receptor antagonist (Eaton et al., 1993). However, certain previous studies have shown evidence for an agonist action of MCPG. Thus in the amygdala, MCPG reduced glutamate-evoked EPSPs by an agonist action at mGluR (Keele et al., 1995), and MCPG induced PKC translocation in rat cortical synaptosomes through phospholipase D activation (Pastorini et al., 1998). An alternative theory for the action of MCPG, EGLU and NAAG, is that these ligands are evoking the long-lasting depression by acting as antagonists. In this theory, the long-lasting depression would be evoked as a result of a compensatory change in the receptor that occurs during the antagonist exposure. We consider this unlikely, as compensatory changes in the receptor are unlikely to occur in such a short time course (20 min).

Immunocytochemical studies have shown a high density of presynaptic $mGlu_2$ receptor and a low density of postsynaptic $mGlu_2$ receptor and $mGlu_3$ receptor in the middle molecular layer of the dentate gyrus (Petralia et al., 1996; Shigemoto et al., 1997). It is most likely that the presynaptic reversible inhibition evoked by EGLU and DCG-IV is mediated via an action at presynaptic $mGlu_2$ receptor, as DCG-IV is known to be a potent agonist at this receptor (Hayashi et al., 1994). Although EGLU has previously been demonstrated to be an antagonist at group II mGlu receptor (Jane et al., 1996), it is clear from the present studies that it also has a weak agonist action at presynaptic $mGlu_2$ receptor. The lack of induction of a reversible presynaptic depression by MCPG and NAAG demonstrates that these ligands do not activate presynaptic $mGluR_2$. The action of NAAG, MCPG, EGLU and DCG-IV associated with the induction of long-term depression is likely to be mediated via a postsynaptic activation of group II mGlu receptor, as it was not associated with a change in paired pulse inhibition. It is therefore most likely that the induction of the long-lasting depression by NAAG, MCPG, EGLU and DCG-IV is mediated via an agonist action at postsynapatic $mGlu_2$ receptor or $mGlu_3$ receptor.

The agonist/partial agonist action of MCPG and NAAG is interesting in relationship to LTP induction. Evidence for an involvement of mGlu receptor in the induction of hippocampal long-term potentiation (Bashir et al., 1993; Riedel et al., 1993; Richter-Levin et al., 1994; Breakwell et al., 1998) and long-term depression (Bashir and Collingridge, 1994; Bolshakov and Siegelbaum, 1994; O'Mara et al., 1995; Oliet et al., 1997) has been based on the assumption that MCPG only acts as an mGluR antagonist. However, the discovery in the present studies that MCPG can actually induce a long-lasting depression via an agonist action at group II mGluR raises the possibility that the inhibition of long-term potentiation by MCPG observed in previous studies may have been a result of

activation of group II mGluR via an agonist action at group II mGlu receptor. This theory is supported by further evidence from studies in the present laboratory. Firstly, a low concentration of DCG-IV which evoked little reversible depression was found to inhibit long-term potentiation induction in the dentate gyrus (Huang et al., 1997a). Secondly, PCCG-IV inhibited long-term potentiation induction via an action at group II mGlu receptors in the dentate gyrus (Huang et al., 1997b). Thirdly, MCPG and 1*S*,3*S*-ACPD have been found to inhibit long-term potentiation by an agonist action at group II mGlu receptor in CA1 (Breakwell et al., 1998). A mixed agonist-antagonist action of MCPG may also explain the difficulty encountered in certain studies in observing a block of long-term potentiation by MCPG (Selig et al., 1995).

NAAG is a dipeptide found in the central nervous system that has received much attention as many studies have shown that it meets the criteria for a neurotransmitter (Wroblewska et al., 1993, 1997; Moffett and Namboodiri, 1995). Improved immunochemical studies have shown the location of NAAG in the dentate gyrus (Moffett and Namboodiri, 1995). In recent studies, NAAG has been shown to bind to $mGlu_3$ receptor with an EC_{50} of 65 μM. It is therefore possible that NAAG acts as a cotransmitter in the hippocampus, being released during low frequency stimulation and binding selectively to $mGlu_3$ receptor, thereby resulting in the mediation of the induction of long-term depression. NAAG does activate *N*-methyl-D-aspartate receptors (NMDAR) with low affinity (EC_{50} = 666 μM) (Trombley and Westbrook, 1990). However, the induction of the long-lasting depression by NAAG in the present study is not a result of activation of NMDAR by NAAG. Several previous studies, including one from the present laboratory, have shown that application of NMDA induces a large reversible depression of the EPSP in the presence of NMDA, which is followed, upon washout, by a recovery to baseline and hence a short-term potentiation (Collingridge et al., 1983; Kauer et al., 1988; McGuiness et al., 1991). In contrast, the application of NAAG resulted in a small increase in the EPSP in the presence of NAAG, while washout of NAAG led to induction of a long-lasting depression.

Acknowledgements

We would like to thank the Health Research Board, Ireland, the Wellcome Trust and the European Union (Grants BMH4-CT96-0228 and BIO4 CT960049) for financial support.

References

Bashir, Z.I., Collingridge, G.L., 1994. An investigation of depotentiation of long-term potentiation in the CA1 region of the hippocampus. Exp. Brain Res. 100, 437.

Bashir, Z.I., Bortolotto, Z.A., Davies, C.H., Berrtta, N., Irving, A.J., Seal, A.J., Henley, J.M., Jane, D.E., Watkins, J.C., Collingridge, G.L., 1993. Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. Nature 363, 347.

Bolshakov, V.Y., Siegelbaum, S.A., 1994. Postsynaptic induction and presynaptic expression of hippocampal long-term depression. Science 264, 1148.

Breakwell, N.A., Rowan, M.J., Anwyl, R., 1998. (+)-MCPG blocks induction of LTP in CA1 of rat hippocampus via agonist action at an mGluR group II receptor. J. Neurophysiol. 79, 1270.

Bushell, T.J., Jane, D.E., Tse, H.-W., Watkins, J.C., Garthwaite, J., Collingridge, G.L., 1996. Pharmacological antagonism of the actions of group II and group III mGluR agonists in the lateral perforant path of rat hippocampal slices. Br. J. Pharmacol. 117, 1457–1462.

Collingridge, G.L., Kehl, S.J., McLennan, H., 1983. Excitatory amino acids in synaptic transmission in the Schaffer-collateral commissural pathway of the rat hippocampus. J. Physiol. 334, 33–46.

Dudek, S.M., Bear, M.F., 1992. Homosynaptic long-term depression in area CA1 of the hippocampus and effects of *N*-methyl-D-aspartate receptor blockade. Neuron 9, 967–974.

Eaton, S.A., Jane, D.E., Jones, P.L., Porter, R.H.P., Pok, P.C.-K., Sunter, D.C., Udvarhelyi, P.M., Roberts, P.J., Salt, T.E., Watkins, J.C., 1993. Competitive antagonism at metabotropic glutamate receptors by (*S*)-4-carboxyphenylglycine and (*RS*)-α-methyl-4-carboxyphenylglycine. Eur. J. Pharmacol. 244, 195.

Hayashi, Y., Sekiyama, N., Nakanishi, S., Jane, D., Sunter, D.C., Birse, E.F., Udvarhelyi, P.M., Watkins, J.C., 1994. Analysis of agonist and antagonist activities of phenylglycine derivatives for different cloned metabotropic glutamate receptor subtypes. J. Neurosci. 14, 3370–3381.

Huang, L.Q., Rowan, M.J., Anwyl, R., 1997a. Group II mGluR agonist inhibition of LTP induction, and group II mGluR antagonist inhibition of long-term depression induction, in the dentate gyrus in vitro. NeuroReport 8, 687–691.

Huang, L.Q., Breakwell, N.A., Rowan, M.J., Anwyl, R., 1997b. PCCG-IV inhibits the induction of long-term potentiation in the dentate gyrus in vitro. Eur. J. Pharmacol. 332, 161–165.

Jane, D.E., Jones, P.L., Pook, P.C.-K., Tse, H.-W., Watkins, J.C., 1994. Actions of two new antagonists showing selectivity for different sub-types of metabotropic glutamate receptor in the neonatal rat spinal cord. Br. J. Pharmacol. 112, 809–815.

Jane, D.E., Pittaway, K., Sunter, D.C., Thomas, N.K., Watkins, J.C., 1995. New presynaptic glutamate receptors in neonatal rat spinal cord. Neuropharmacology 34, 851–856.

Jane, D.E., Thomas, N.K., Tse, H.W., Watkins, J.C., 1996. Potent antagonists at the L-AP4 and (1*S*,3*S*)-ACPD-sensitive presynaptic metabotropic glutamate receptors in the neonatal rat spinal cord. Neuropharmacology 35, 1029–1037.

Kauer, J.A., Malenka, R.C., Nicoll, R.A., 1988. NMDA application potentiates synaptic transmission in the hippocampus. Nature 334, 250–254.

Keele, N.B., Arvanov, V., Holmes, K., Shinnick-Gallagher, P., 1995. Agonist action of (*RS*)-α-methyl-4-carboxyphenylglycine (MCPG) in the amygdala. NeuroReport 6, 1058–1062.

Knopfel, T., Lukic, S., Leonardt, T., Flor, P.J., Huhn, R., Gasparini, F., 1995. Pharmacological characterization of MCCG and MAP4 at the mGluR1b, mGluR2 and mGluR4a human metabotropic glutamate receptor subtypes. Neuropharmacology 34, 1099.

Kobayashi, K., Manabe, T., Takahashi, T., 1996. Presynaptic long-term depression at the hippocampal mossy fibre-CA3 synapse. Science 273, 648–652.

Macek, T.A., Winder, D.G., Gereau, R.W., Ladd, C.O., Conn, J.P., 1996. Differential involvement of group II and group II mGluRs as autoreceptors at lateral and medial perforant path synapses. J. Neurophysiol. 76, 3798–3806.

Manahan-Vaughan, D., 1997. Group 1 and 2 metabotropic glutamate receptors play differential roles in hippocampal long-term depression

and long-term potentiation in freely moving rats. J. Neurosci. 17, 3303–3313.

McGuiness, N., Rowan, M.J., Anwyl, R., 1991. The effects of external calcium on the *N*-Methyl-D-Aspartate induced short-term potentiation in the rat hippocampal slice. Neurosci. Lett. 131, 13–17.

Moffett, J.R., Namboodiri, M.A.A., 1995. Differential distribution of *N*-acetylaspartylglutamate and *N*-acetylaspartate immunoreactivities in rat forebrain. J. Neurocytol. 24, 409–416.

Mulkey, R.M., Malenka, R.C., 1992. Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9, 967–974.

Oliet, S.H.R., Malenka, R.C., Nicoll, R.A., 1997. Two distinct forms of long-term depression coexist in CA1 hippocampal pyramidal cells. Neuron 18, 969–978.

O'Mara, S.M., Rowan, M.J., Anwyl, R., 1995. Metabotropic glutamate receptor-induced long-term depression and depotentiation in the dentate gyrus of the rat hippocampus in vitro. Neuropharmacology 34, 983–991.

Pastorini, L., Di Luca, M., Sciuto, A., Gardoni, F., Cattabeni, F., 1998. MCPG induces PKCε translocation in rat cortical synaptosomes through PLD activation. Forum Eur, Neurosci., 125.

Petralia, R.S., Wang, Y.-X., Niedzielski, A.S., Wenthold, R.J., 1996. The metabotropic glutamate receptors mGluR2 and mGluR3 show unique postsynaptic, presynaptic and glial localizations. Neuroscience 71, 949–957.

Richter-Levin, G., Errington, M.L., Maegawa, H., Bliss, T.V.P., 1994. Activation of metabotropic glutamate receptors is necessary for long-term potentiation in the dentate gyrus and for spatial learning. Neuropharmacology 33, 853–861.

Riedel, G., Wetzel, W., Reymann, K.G., 1993. (*R*,*S*)-a-methyl-4-carboxyphenylglycine (MCPG) blocks spatial learning in rats and long-term potentiation in the dentate gyrus in vivo. Neurosci. Lett. 167, 141–146.

Selig, D.K., Lee, H.-K., Bear, M.F., Malenka, R.C., 1995. Reexamination of the effects of MCPG on hippocampal LTP, long-term depression and depotentiation. J. Neurophysiol. 74, 1075–1084.

Shigemoto, R., Kinoshita, A., Wada, E., Nomura, S., Ohishi, H., Takada, M., Flor, P.J., Neki, A., Abe, T., Nakanishi, S., Mizuno, N., 1997. Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. J. Neurosci. 17, 7503–7514.

Thomsen, C., Boel, E., Suzdak, P.D., 1994. Action of phenylglycine analogs at subtypes of the metabotropic glutamate receptor family. Eur. J. Pharm. Mol. Pharm. Sec. 267, 77–81.

Trombley, P.Q., Westbrook, G.L., 1990. Excittory synaptic transmission in cultures of rat olfactory bulb. J. Neurophysiol. 64, 598.

Wang, Y., Rowan, M.J., Anwyl, R., 1997. Induction of long-term depression is NMDAR-independent, but dependent on Ca influx via low voltage activated Ca channels and release of Ca from intracellular stores in the dentate gyrus in vitro. J. Neurophysiol. 77, 812–821.

Wroblewska, B., Wroblewska, J.T., Saab, O.H., Neale, J.H., 1993. *N*-acetylaspartylglutamate inhibits forskolin-stimulated cyclic AMP levels via a metabotropic glutamate receptor in cultured granule cells. J. Neurochem. 61, 943–949.

Wroblewska, B., Wroblewska, J.T., Pshenichkin, S., Surin, A., Sullivan, S.E., Neale, J.H., 1997. *N*-acetylaspartylglutamate selectively activates mGluR3 receptors in transfected cells. J. Neurochem. 69, 174–178.

Yang, X.D., Connor, J.A., Faber, D.S., 1994. Weak excitation and simultaneous inhibition induce long-term depression in hippocampal CA1 neurons. J. Neurophysiol. 71, 1586–1594.

Yokoi, M., Kobayashi, K., Manabe, T., Takahashi, T., Sakaguchi, I., Katssura, G., Shigemoto, R., Ohishi, H., Nomura, S., Nakamura, K., Nakao, K., Katsuki, M., Nakanishi, S., 1996. Impairment of hippocampal mossy fibre long-term depression in mice lacking mGluR2. Science 273, 645–648.