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PCCG-IV inhibits the induction of long-term potentiation in the dentate gyrus in vitro

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Abstract

The effects of two ligands with previously established high and selective potency for metabotropic glutamate receptors (mGlu receptors) group II have been investigated on the high frequency stimulation (HFS) induced long-term potentiation of the field excitatory postsynaptic potential (EPSP) in the dentate gyrus of the rat hippocampus in vitro. The ligands investigated were (2*S*,1′*S*,2′*S*,3′*R*)-2-(2″-carboxy-3′-phenylcyclopropyl)glycine (PCCG-IV) and (*R*,*S*)-α-methyl-4-tetrazolylphenylglycine (MTPG). PCCG-IV (10 μM) strongly inhibited the induction of long-term potentiation of the field EPSP by high frequency stimulation. MTPG (50 μM) did not inhibit the induction of long-term potentiation, but prevented the inhibition of long-term potentiation induction by PCCG-IV. The inhibition of long-term potentiation induction by PCCG-IV is suggested to be due to an agonistic action on mGlu receptor group II, probably $mGlu_3$ receptor, as the inhibition of long-term potentiation can be reversed by the application of MTPG, a well-known selective and potent antagonist of mGlu receptor group II. © 1997 Elsevier Science B.V.

Keywords: mGlu receptor; Long-term potentiation; Hippocampus; PCCG-IV ((2*S*,1′*S*,2′*S*,3′*R*)-2-(2″-carboxy-3′-phenylcyclopropyl)glycine); MTPG ((*R*,*S*)-α-methyl-4-tetrazolylphenylglycine)

1. Introduction

Substantial evidence has been presented in a number of studies that metabotropic glutamate receptors (mGlu receptors) are involved in, and most likely essential for, the induction of long-term potentiation in the hippocampus. Thus the mGlu receptor agonist (1*S*,3*R*)-aminocyclopentane-1,3-dicarboxylate ((1*S*,3*R*)-ACPD) potentiated the induction of long-term potentiation (McGuinness et al., 1991) and directly induced long-term potentiation (Bortolotto and Collingridge, 1992; O'Connor et al., 1995; Breakwell et al., 1996). In addition, the mGlu receptor antagonist L-AP3 inhibited the induction of the late phase of long-term potentiation (Behnisch et al., 1991) while the mGlu receptor antagonist (*R*,*S*)-α-methyl-4-carboxyphenylglycine (MCPG) completely blocked the induction of long-term potentiation in vitro (Bashir et al., 1993; Wang et al., 1995; Little et al., 1995) and in vivo (Riedel et al., 1994; Richter-Levin et al., 1994). However, the involvement of mGlu receptors in the induction of long-term potentiation is controversial, as several studies have failed to demonstrate the ability of MCPG to inhibit the induction of long-term potentiation (Chinestra et al., 1994; Manzoni et al., 1994; Selig et al., 1995).

mGlu receptors are divided into three major groups, I, II and III. Stimulation of group II mGlu receptors is associated with an inhibition of forskolin-stimulated cAMP formation (Casabona et al., 1992; Schoepp et al., 1992) and a potentiation of cAMP responses to agonists of other receptors that are directly coupled to adenylyl cyclase (Schoepp and Johnson, 1993). Moreover, an increase of cAMP formation was found to be mediated by a synergistic interaction between mGlu group I and group II receptors (Schoepp et al., 1996). Group II agonists have been found to produce presynaptic depression at many synapses (Pook et al., 1992; Ishida et al., 1993; Jane et al., 1994; Brown and Reymann, 1995; Lovinger and McCool, 1995; Manzoni and Bockaert, 1995; Ugolini and Bordi, 1995; Bushell et al., 1996).

(2*S*,1′*S*,2′*S*,3′*R*)-2-(2″-carboxy-3′-phenylcyclopropyl) glycine (PCCG-IV) has been shown to be a potent and

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selective antagonist of group II metabotropic glutamate receptors, with a K_d of 8 μM in studies involving forskolin-induced cAMP formation at expressed $mGlu_2$ receptor in kidney cells (Thomsen et al., 1996). However, in the same study it was also shown that PCCG-IV had neuroprotective effects similar to other agonists of group II and III. (*R,S*)-α-methyl-4-tetrazolylphenylglycine (MTPG) has been shown to be a potent mGlu receptor group II antagonist, with an apparent K_d of 28 μM for the antagonism of (1*S*,3*S*)-ACPD evoked presynaptic inhibition in the rat lateral perforant pathway of the hippocampus (Bushell et al., 1996) and 77 μM for the antagonism of (1*S*,3*S*)-ACPD presynaptic inhibition of synaptic transmission in the neonatal rat spinal cord (Jane et al., 1995).

In the hippocampus, mGlu receptor group II have been found to be particularly concentrated in the terminals of perforant path fibres, especially in the middle third of the molecular layer (Petralia et al., 1996). In the present studies, the effects of two mGlu receptor group II ligands, PCCG-IV and MTPG, have been investigated on the induction of long-term potentiation in the medial perforant pathway of the dentate gyrus.

2. Materials and methods

This study was carried out using male Wistar rats weighing 30–60 g (BioResources Unit, Trinity College, Dublin, Ireland). Rat hippocampal slices were cut at a thickness of 350 μm using a Camden vibroslice. The slices were submerged in a media containing: (mM) NaCl, 120; KCl 2.5, NaH_2PO_4, 1.25; $NaHCO_3$ 26; $MgSO_4$, 2.0; $CaCl_2$, 2.0; D-glucose 10. The solution was bubbled with 95% O_2/5% CO_2 at a temperature of 32°C and perfused at a rate of 5 ml per min.

Presynaptic stimulation and recordings of field EPSPs were carried out from the medial perforant pathway of the dentate gyrus. In all experiments, test EPSPs were evoked at a frequency of 0.033 Hz, and an input–output curve (stimulus intensity versus EPSP amplitude) plotted for each experiment at this test frequency. For the test EPSPs, the stimulation voltage intensity was adjusted to give an EPSP amplitude around 1 mV, an amplitude which was about one-third of maximum I/O amplitude in control media. Long-term potentiation was induced using high-frequency stimulation consisting of 8 trains of 8 stimuli, inter-stimulus interval 5 ms (200 Hz) and inter-train interval 2 s. The stimulation intensity was increased to give an EPSP of two-thirds of maximum amplitude during high-frequency stimulation. Long-term potentiation was measured at 30 min post high-frequency stimulation.

mGlu receptor group II ligands were perfused for at least 40 min prior to application of high-frequency stimulation in order to ensure full equilibration.

PCCG-IV was kindly provided by Dr. Pellicciari (University of Perugia, Perugia, Italy) and MTPG was obtained from Tocris Cookson.

Two-tailed Student's *t*-test was used for statistical significance. Values are the mean ± S.E.M. for *n* slices in each experiment.

3. Results

Test EPSPs remained at a constant amplitude when evoked at the control frequency of 0.033 Hz in the medial

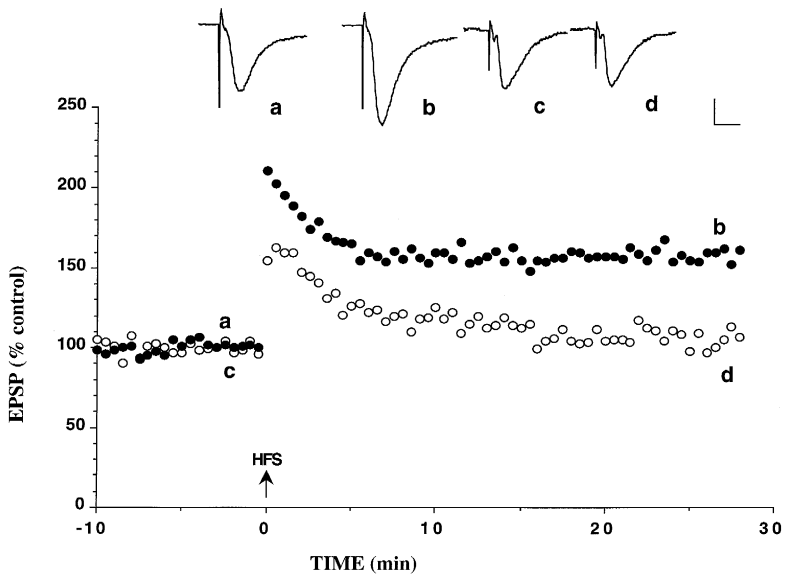

Fig. 1. PCCG-IV (10 μM) inhibits the induction of long-term potentiation in the medial perforant pathway of the dentate gyrus of the hippocampus. The graph shows typical experiments in which high-frequency stimulation consisting of a series of high frequency trains (arrow) induced long-term potentiation measuring 161% at 30 min post high-frequency stimulation in control (closed circles) and 107% in the presence of PCCG-IV (10 μM) (open circles). The traces of the field EPSPs are control (a) and PCCG-IV (c) prior to, and following (b and d, respectively) application of high-frequency stimulation. Each trace is the average of 3 consequent individual EPSPs. Calibration bar is 0.5 mV, 5 ms.

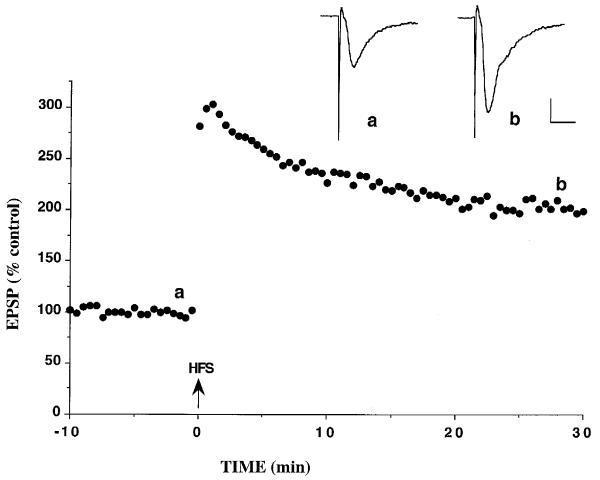

Fig. 2. MTPG (50 μM) does not inhibit the induction of long-term potentiation. The graph shows a typical experiment in which long-term potentiation measured 199% at 30 min post high-frequency stimulation in the presence of MTPG. The traces of the field EPSPs are before (a) and after (b) high-frequency stimulation. Calibration bar is 0.5 mV, 5 ms.

perforant pathway of the dentate gyrus. In control slices, high-frequency stimulation induced a stable long-term potentiation measuring $158 \pm 7\%$ ($P < 0.01$, $n = 10$) at 30 min post high-frequency stimulation. In the presence of a low concentration of PCCG-IV (10 μM), the induction of long-term potentiation following high-frequency stimulation was strongly inhibited, long-term potentiation measuring $107 \pm 2\%$ ($n = 8$) which was significantly different from control ($P < 0.01$) (Fig. 1 and Fig. 4).

A selective and potent antagonist of mGlu receptor group II, MTPG, was then tested on the induction of long-term potentiation. In the presence of MTPG (50 μM), high-frequency stimulation induced long-term potentiation

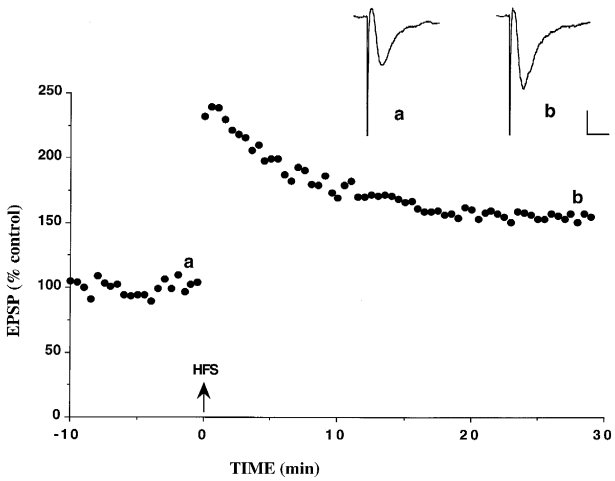

Fig. 3. MTPG (50 μM) does prevent the inhibition of the induction of long-term potentiation by PCCG-IV (10 μM). In a typical experiment, long-term potentiation measured 155% at 30 min post high-frequency stimulation in the presence of both MTPG (50 μM) and PCCG-IV (10 μM). The traces of the field EPSPs before (a) and after (b) high-frequency stimulation are shown. Calibration bar is 0.5 mV, 5 ms.

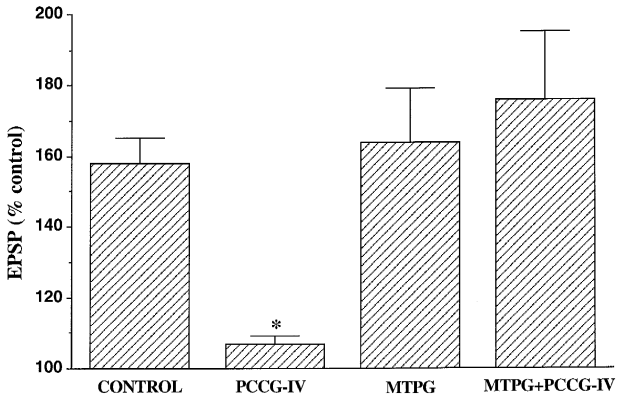

Fig. 4. Summary of the effect of mGlu receptor group II ligands on the induction of long-term potentiation. Each bar represents the amplitude of field EPSPs (mean ± S.E.M.) at 30 min post high-frequency stimulation. The star represents statistic significance.

measuring $164 \pm 15\%$ ($n = 5$, $P < 0.01$), a value not significantly different from control (Fig. 2). However, application of MTPG reversed the block of induction of long-term potentiation by PCCG-IV. In all 5 experiments, following perfusion of both MTPG (50 μM) and PCCG-IV (10 μM), robust long-term potentiation was induced by high-frequency stimulation measuring $176 \pm 19\%$ ($P < 0.01$), which was not significantly different from the control long-term potentiation (Fig. 3).

A summary of the effect of mGlu receptor group II ligands on the induction of long-term potentiation is given in Fig. 4.

4. Discussion

The major findings of the present studies are that low concentrations of the mGlu receptor group II ligand PCCG-IV strongly inhibited the induction of long-term potentiation in the medial perforant pathway of the dentate gyrus. Furthermore, the well established mGlu receptor group II antagonist MTPG did not inhibit the induction of long-term potentiation but did prevent the inhibition of induction of long-term potentiation by PCCG-IV.

The inhibition of long-term potentiation induction by PCCG-IV was considered to be produced by an action at mGlu receptor group II because at the concentration (10 μM) used in these studies, PCCG-IV has been previously shown to act as an antagonist only on mGlu receptor group II, with a K_d of 8.2 μM for $mGlu_2$ receptor (Thomsen et al., 1996). PCCG-IV was only found to act on mGlu group I and group III receptors at much higher (20–40 fold) concentrations (Thomsen et al., 1996). Is this inhibition of long-term potentiation induction by PCCG-IV due to an agonistic or antagonistic action? To address this question, we tested the effect of MTPG on the induction of long-term potentiation. In previous studies, MTPG was found to be a potent mGlu receptor group II antagonist with a K_d of 28

μM in the lateral perforant pathway of the dentate gyrus in electrophysiological studies (Bushell et al., 1996). In the present studies, application of MTPG (50 μM) alone had no effect on long-term potentiation induction. However, MTPG (50 μM) was found to prevent the inhibition of long-term potentiation induction by PCCG-IV. These experiments demonstrate that the inhibition of long-term potentiation induction by PCCG-IV is due to an agonistic action at an mGlu group II-like receptor. Therefore we propose that PCCG-IV inhibits long-term potentiation induction by acting as a partial agonist at an mGlu group II-like receptor, with MTPG antagonizing such action. It is most likely that PCCG-IV and MTPG are acting at $mGlu_3$ receptor, as it has been previously reported that MTPG is inactive at $mGlu_2$ receptor (Thomsen et al., 1996). An agonist action of PCCG-IV in cultured cortical neurons was also previously suggested in the studies of Thomsen et al. (1996) in which PCCG-IV was found to elicit a neuroprotective action in a similar way to other agonists of group II.

An inhibition of long-term potentiation by agonist action at an mGlu group II-like receptor has been shown in other studies from this laboratory. Thus DCG-IV, a very potent and selective presynaptic mGlu receptor group II agonist (Ishida et al., 1993), inhibited long-term potentiation induction in the medial perforant pathway of the dentate gyrus in vitro, an effect reversed by application of the mGlu receptor group II antagonist MCCG (Huang et al., 1997). In addition, the mGlu receptor group II agonist (1*S*,3*S*)-ACPD (Jane et al., 1994, 1995) inhibited the induction of long-term potentiation in CA1 in vivo, with the mGlu receptor group II antagonists MCCG and MTPG reversing the inhibition (Hölscher et al., 1997).

Acknowledgements

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