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Short communication

Antagonists of the metabotropic glutamate receptor do not prevent induction of long-term potentiation in the dentate gyrus of rats

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Abstract

The effects of two competitive metabotropic glutamate (mGlu) receptor antagonists, (*RS*)-α-methyl-4-carboxyphenylglycine (MCPG) and (*S*)-4-carboxylphenylglycine (4CPG), were studied on long-term potentiation in the dentate gyrus of rats under urethane anaesthesia. Intracerebroventricular (i.c.v.) injection of MCPG or 4CPG 30 min prior to tetanic stimulation of the perforant path in rats did not affect the induction of long-term potentiation measured by extracellular recording. As a control, i.c.v. injections of the NMDA receptor antagonist, *dl*(−)-2-amino-5-phosphonopentanoic (dl-AP5), effectively blocked long-term potentiation. These results suggest that the mGlu receptor subtype blocked by MCPG and 4CPG is not involved in long-term potentiation in the dentate gyrus.

Keywords: Long-term potentiation; Hippocampus; Dentate gyrus; Neural plasticity; Metabotropic glutamate receptor; NMDA (*N*-methyl-D-aspartate)

1. Introduction

The long-term potentiation of synaptic efficacy produced by high frequency afferent stimulation is a well established synaptic model of memory and neural plasticity (Bliss and Lømo, 1973; Bliss and Collingridge, 1993). Evidence indicates that activation of NMDA receptors is frequently implicated in long-term potentiation. In the hippocampus, NMDA receptor antagonists prevent long-term potentiation when given before tetanization both in slice preparations (Collingridge et al., 1983) and in vivo (Morris et al., 1986; Abraham and Mason, 1988). Recently, the metabotropic glutamate (mGlu) receptors have been implicated in the induction of long-term potentiation in the hippocampus. Facilitating effects of the mGlu receptor agonist, *trans*-(±)-1-amino-1,3-cyclopentadicarboxylic acid (ACPD), in generating long-term potentiation have been shown in hippocampal CA1 slices, using different experimental procedures (Otani and Ben Ari, 1991; Bortolotto and Collingridge, 1993). However, the role of the mGlu receptor antagonists in blocking long-term potentiation induction is still controversial and currently under scrutiny. Bashir et al. (1993) reported that (*RS*)-α-methyl-4-carboxyphenylglycine (MCPG), a weak but selective mGlu receptor antagonist, blocked long-term potentiation in CA1 rat slices. Similar results were found in vivo with the dentate gyrus of rats (Riedel and Reymann, 1993). Other investigators failed to block long-term potentiation with MCPG slices, however (Chinestra et al., 1993). These contrasting results may be due to the lack of availability of selective and potent antagonists for mGlu receptors. Recently, the potency and specificity of a number of putative mGlu receptor antagonists, the phenylglycine derivatives, were reported (Hayashi et al., 1994). In the present study we investigated the effects of MCPG and, for the first time, of a more potent mGlu receptor antagonist, (*S*)-4-carboxylphenylglycine (4CPG), on long-term potentiation induced in the dentate gyrus by stimulation of the perforant path in vivo. The NMDA receptor antagonist, *dl*-2-amino-5-phosphonopentanoate (dl-AP5), was also studied for comparison.

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2. Materials and methods

Male Sprague-Dawley rats weighing 250–350 g were anesthetized with urethane (1.5 g/kg body weight) and placed in a Kopf stereotaxic frame adjusted so that the surface of the skull was levelled between lambda and bregma (Bordi and LeDoux, 1994). Body temperature was regulated at 37 ± 1°C by means of a heating pad. A bipolar stimulating electrode was placed in the left perforant path (AP −8.0; ML 4.1) and evoked potentials were recorded extracellularly with a stainless steel electrode (1–2 MΩ impedance) from the hilus of the ipsilateral dentate gyrus (AP −4.0; ML 2.3). Both electrodes were driven ventrally using hydraulic micropositioners to search for the best location and optimize the amplitude of the population spike obtained in the test pulses. An additional cannula was lowered into the ipsilateral ventricle for drug administration (AP −0.9; ML 1.3; DV −3.5). Neural potentials were amplified (×200) by an a.c. amplifier, band-pass filtered (2–2000 Hz) and recorded digitally on a personal computer (Axobasic system, Axon Instruments). Test pulses (0.1 ms duration, 0.033 Hz, 150–300 μA) were applied for at least 30 min prior to drug administration at a level that evoked a population spike amplitude of about 1/3 of the maximum. Tetanic stimulation (three trains, 10 s apart, 400 Hz, 33 impulses in each train) was applied at the same intensity of test pulse. Recordings of the evoked potential continued for 2–3 h after tetanization. The population spike amplitude was calculated as the vertical distance from the peak of the negative spike to a tangent drawn between the two positive e.p.s.p. peaks. The e.p.s.p. slope was determined with a least-squares fit of a linear portion of the rising phase of the evoked synaptic response. Both the e.p.s.p. slope and the population spike were calculated on-line for each evoked potential with custom-made software and are expressed as percent change from baseline. Vehicle (0.9% NaCl) or drug solutions were injected intraventricularly (i.c.v.) 30 min before tetanization at a volume of 5 μl with a microsyringe at a rate of 1 μl/min. MCPG and 4CPG (Tocris Neuramin) were dissolved in equimolar NaOH (1 M, Sigma) and further diluted with saline (0.9% NaCl). dl-AP5 (Tocris Neuramin) was dissolved in saline.

3. Results

In control animals ($n = 5$) injection of NaCl (0.9%, 5 μl) had no effect on baseline and tetanic stimulation in all cases resulted in a clear potentiation of both e.p.s.p. slope and population spike amplitude recorded for at least 2 h post-tetanus (Fig. 1A). Population spike amplitude, but not e.p.s.p. slope, was slightly depressed by the NMDA receptor antagonist, dl-AP5 ($n = 7$; 40 mM), compared to pre-injection baseline. This effect is often found in in vivo studies (Errington et al., 1987; Abraham and Mason, 1988), and is explained as the influence of some extrinsic afferent pathway (Abraham and Mason, 1988). The induction of long-term potentiation was effectively blocked by dl-AP5 (Fig. 1B).

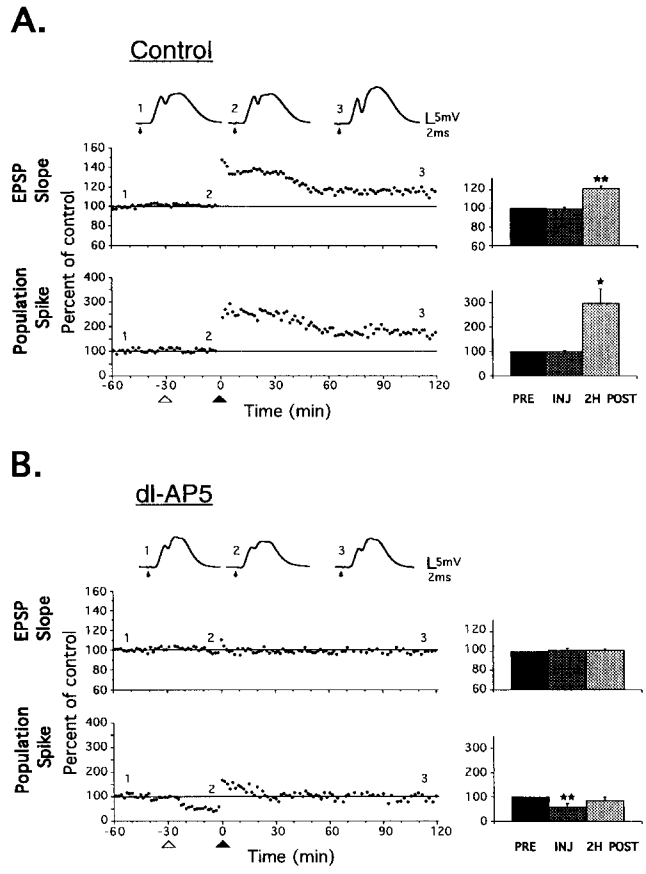

Fig. 1. Effects of vehicle ($n = 5$; NaCl 0.9%/5 μl) or dl-AP5 ($n = 7$; 40 mM/5 μl) i.c.v. administration on perforant path-dentate gyrus long-term potentiation. Consistent long-term potentiation was induced in control animals without affecting baseline neural potentials (A). dl-AP5 effectively blocked the induction of long-term potentiation of both the e.p.s.p. slope and the population spike amplitude (B). Top traces are representative e.p.s.p.s taken from one experiment during the pre-injection period (1), during the application of the drug or the vehicle (2), and 2 h post-tetanus (3). White triangles show the start of the i.c.v. injection. Black triangles show when the tetanus was induced (three trains 10 s apart, 400 Hz, 33 impulses each). Each point is the average of three evoked potentials. Histograms show the mean of e.p.s.p. slope and population spike recorded pre-injection (PRE), post-injection (INJ), and 2 h post-tetanus (2H POST). Small arrows denote statistical difference between baseline PRE and the other two groups ($^{*}P < 0.05$; $^{**}P < 0.01$; paired t-test).

The mGlu receptor antagonists, MCPG and 4CPG, however, did not prevent the induction of long-term potentiation in this pathway (Fig. 2). MCPG ($n = 7$; 200 mM, 5 μl) did not affect baseline potentials, while 4CPG ($n = 9$; 25–150 mM, 5 μl) depressed population spike amplitude and e.p.s.p. slope. The three doses

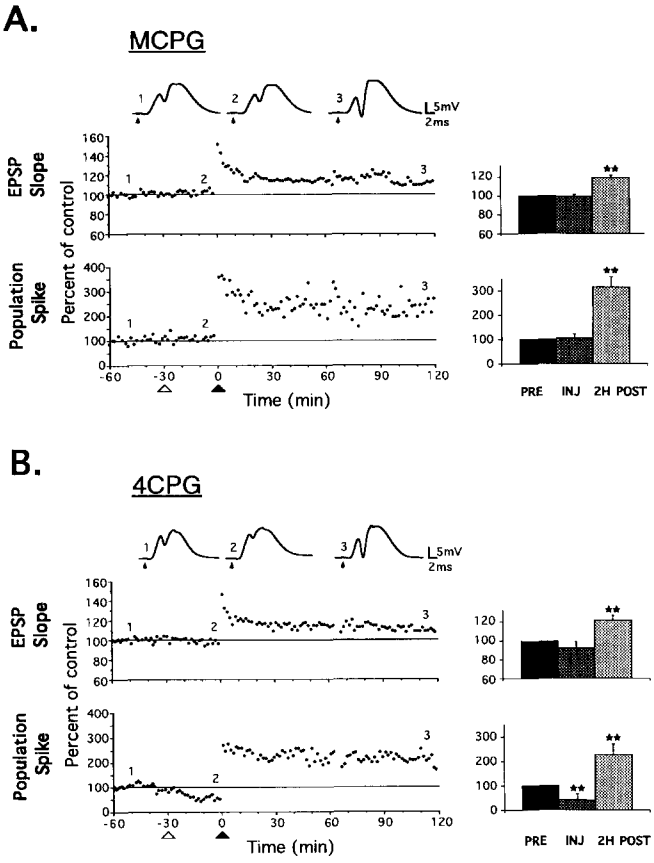

Fig. 2. Long-term potentiation induction was not blocked by i.c.v. injection of either MCPG ($n = 7$; 200 mM/5 μl) or 4CPG ($n = 8$; 50–150 mM/5 μl). MCPG did not affect synaptic transmission measured before tetanization (A). 4CPG, however, depressed neural potentials without blocking long-term potentiation (B). Top traces are taken from one representative experiment and show e.p.s.p.s during the pre-injection period (1), during the application of the mGlu receptor antagonist (2), and 2 h post-tetanus (3). Each point is the average of three evoked potentials. Histograms represent the mean of the results for each group.

used for 4CPG (25, 50 and 150 mM, 5 μl) had dose-dependent effects on population spike amplitude and e.p.s.p. slope, with the latter being affected especially by the higher doses. Since the results for the different doses of 4CPG on long-term potentiation were quite similar, for the purpose of this study we combined the three groups.

4. Discussion

Our results show that the mGlu receptor antagonists, MCPG and 4CPG, did not prevent the induction of long-term potentiation in the dentate gyrus in vivo. This is in contrast with results of a recent study by Riedel and Reymann (1993) conducted in freely moving animals, where a block of long-term potentiation after MCPG injection was seen from the population spike amplitude, although only after 2 h post-tetanus. In the present study we measured e.p.s.p. slope as well as population spike amplitude, but did not observe any decline in either population spike amplitude or slope even at 2 h after tetanization. A possible difference between that study and ours regards recording and/or stimulating sites. Different results obtained with the same drug have been found in the dentate gyrus depending on the place of stimulation in the perforant path (Bramham et al., 1988). Riedel and Reymann (1993) recorded from the granule cell layer, while we placed the electrode in the hilus and recorded from the molecular layer of the dentate gyrus. We also tested for the first time the mGlu receptor antagonist, 4CPG, which was found to be at least 7 times more potent in vitro than MCPG as antagonist of the mGlu receptor (Hayashi et al., 1994). The lack of effects of 4CPG to block long-term potentiation confirms the results obtained in our study with MCPG.

The mGlu receptor consists of at least seven different subtypes coupled to intracellular transduction via G proteins (Schoepp and Conn, 1993). MCPG and 4CPG effectively antagonize the $mGlu_1$ receptor subtype (Hayashi et al., 1994). The failure to block long-term potentiation by MCPG or 4CPG in our study suggests the involvement of mGlu receptor subtypes different from $mGlu_1$ receptor. Alternatively, our results can be explained by the lack of $mGlu_1$ receptor subtype in dentate gyrus neurons. A heterogeneous distribution of the $mGlu_1$ receptor subtype within the hippocampus could also be responsible for these contrasting findings (Chinestra et al., 1993). The availability of more antagonists of different subtypes will enable us to study further the role of mGlu receptor in long-term potentiation and neural plasticity.

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References

Abraham, W.C. and S.E. Mason, 1988, Effects of the NMDA receptor/channel antagonists CPP and MK801 on hippocampal field potentials and long-term potentiation in anesthetized rats, Brain Res. 462, 40.

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

Bliss, T.V.P. and G.L. Collingridge, 1993, A synaptic model of memory: long-term potentiation in the hippocampus, Nature 361, 31.

Bliss, T.V.P. and T. Lømo, 1973, Long-lasting potentiation of synap-

tic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path, J. Physiol. 232, 331.

Bordi, F. and J.E. LeDoux, 1994, Response properties of single units in areas of rat auditory thalamus that project to the amygdala. II. Cells receiving convergent auditory and somatosensory inputs and cells antidromically activated by amygdala stimulation, Exp. Brain Res.98, 275.

Bortolotto, Z.A. and G.L. Collingridge, 1993, Characterisation of LTP induced by the activation of glutamate metabotropic receptors in area CA1 of the hippocampus, Neuropharmacology 32, 1.

Bramham, C.R., M.L. Errington and T.V.P. Bliss, 1988, Naloxone blocks induction of long-term potentiation in the lateral but not in the medial perforant pathway in the anesthetized rat, Brain Res. 449, 352.

Chinestra, P., L. Aniksztein, D. Diabira and Y. Ben-Ari, 1993, (*RS*)-α-Methyl-4-carboxyphenylglycine neither prevents induction of LTP nor antagonizes metabotropic glutamate receptors in CA1 hippocampal neurons, J. Neurophysiol. 70, 2684.

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

Errington, M.L., M.A. Lynch and T.V.P. Bliss, 1987, Long-term potentiation in the dentate gyrus: induction and increased glutamate release are blocked by D(−) aminophosphonovalerate, Neuroscience 20, 279.

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

Morris, R.G.M., E. Anderson, G.S. Lynch and M. Baudry, 1986, Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist, AP5, Nature 319, 774.

Otani, S. and Y. Ben-Ari, 1991, Metabotropic receptor-mediated long-term potentiation in rat hippocampal slices, Eur. J. Pharmacol. 205, 325.

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

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