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Metabotropic glutamate group II receptors are responsible for the depression of synaptic transmission induced by ACPD in the dentate gyrus

Annarosa Ugolini, Fabio Bordi *

Pharmacology Department, Glaxo Research Laboratories, Via Fleming 4, 37100 Verona, Italy

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Abstract

The functional role of metabotropic glutamate (mGlu) receptors in the rat dentate gyrus was investigated. By using extracellular recording techniques in slices, it was found that the depression induced by the mGlu receptor agonist (1*S*,3*R*)-1-amino-cyclopentane-1,3-dicarboxylate (ACPD) was mediated through the mGlu group II receptors. The mGlu receptor antagonist α-methyl-4-carboxyphenylglycine (MCPG) (500 μM), active at group I and group II subtype receptors, was effective in antagonizing the ACPD (30 μM)-induced depression of the excitatory field potentials. An antagonist selective for group I, (*S*)-4-carboxyphenylglycine (4CPG), did not block the effects induced by ACPD, but by itself produced a dose-dependent depression of the field potentials. This ACPD-like effect shown at high concentrations of 4CPG (300 μM) is explained by its group II receptor agonistic properties and was blocked by bath application of MCPG (500 μM). A selective agonist of group I, (*S*)-3-hydroxyphenylglycine (3-HPG), did not cause any depression of synaptic transmission. However, the selective mGlu group II receptor agonist, (2*S*,3*S*,4*S*)-α-(carboxycyclopropyl)glycine (L-CCG-I), induced a marked dose-dependent depression and its action was blocked by MCPG (500 μM). Furthermore, the selective mGlu group III receptor antagonist, α-methyl-L-2-amino-4-phosphonobutyrate (MAP4) (500 μM), was not able to antagonize the depression induced by ACPD (30 μM), but was effective in blocking the action induced by the selective mGlu group III agonist, L-2-amino-4-phosphonobutyrate (L-AP4) (100 μM). These results indicate that mGlu group II receptors, but not groups I or III, are involved in the depression of synaptic transmission in the dentate area of the hippocampus induced by ACPD.

Keywords: Hippocampus; Dentate gyrus; Glutamate metabotropic receptor; Synaptic transmission; Electrophysiology, in vitro; Extracellular recording

1. Introduction

The metabotropic glutamate (mGlu) receptors are a class of excitatory amino acid receptors linked to G protein and second messenger systems. Molecular biological studies have revealed the existence of at least seven subtypes of these receptors ($mGlu_1$–$mGlu_7$) which differ in their sequence and second messengers cascades (Nakanishi and Masu, 1994). The mGlu receptor subtypes can be arranged into three groups based on agonist pharmacology, specific signal transduction pathways and sequence similarity (Nakanishi, 1994; Hollman and Heinemann, 1994).

The mGlu receptors are involved in a variety of important physiological functions in the central nervous system (CNS) and they may play a major role in some pathological processes (Schoepp and Conn, 1993). The selective mGlu receptor agonist, (1*S*,3*R*)-1-amino-cyclopentane-1,3-dicarboxylate (ACPD), has allowed some characterization of the physiological roles of mGlu receptor activation (Baskys and Malenka, 1991; Schoepp et al., 1991). The lack of specific antagonists for the mGlu receptor subtypes, however, has limited the precise characterization of the role of the individual receptor subtypes.

Recently, the potency and the specificity of a num-

* Corresponding author. Tel.: 39/45/921-8845; fax: 39/45/921-8153; e-mail: fb23261@ggr.co.uk.

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ber of putative mGlu receptor antagonists, the phenylglycine derivatives, were reported (Hayashi et al., 1994) and the effects of these compounds were studied in different areas of the brain by using electrophysiological techniques (Eaton et al., 1993; Birse et al., 1993; see Watkins and Collingridge, 1994, for a review). For example, (*RS*)-α-methyl-4-carboxyphenylglycine (MCPG), active at mGlu groups I and II (Hayashi et al., 1994), was reported to antagonize the ACPD-induced depression in hippocampal slices (Bashir et al., 1993; Manzoni et al., 1994), thalamic neurons (Eaton et al., 1993), and motoneurons (Kemp et al., 1994). Another mGlu receptor antagonist, (*S*)-4-carboxyphenylglycine (4CPG), active at group I, blocked the excitatory but not the depressant effects of ACPD in thalamic neurons (Eaton et al., 1993).

In this paper we investigated, using an extracellular recording technique, the role of the mGlu receptors in synaptic transmission in the dentate area in vitro. We demonstrate that the depression induced by ACPD can be accounted for by activation of mGlu group II receptor subtypes.

2. Materials and methods

Experiments were performed on hippocampal slices obtained from male Wistar rats (100–150 g). Standard extracellular recording techniques were used. Rats were anesthetized with ether and decapitated. Transverse slices (450 μm) were cut in 4°C Ringer's solution using a Vibroslice (Campden Instruments) and placed in a holding chamber. After being maintained for at least 1 h at room temperature in the Ringer's medium, each slice was submerged in a constant flow (2–3 ml/min) recording chamber maintained at 32°C during the experiment. The superfusing medium contained (in mM): 126 NaCl, 3.5 KCl, 1.3 $MgCl_2$, 2.5 $CaCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4, and 10 glucose, equilibrated with 95% O_2/5% CO_2. Electrodes were initially placed visually

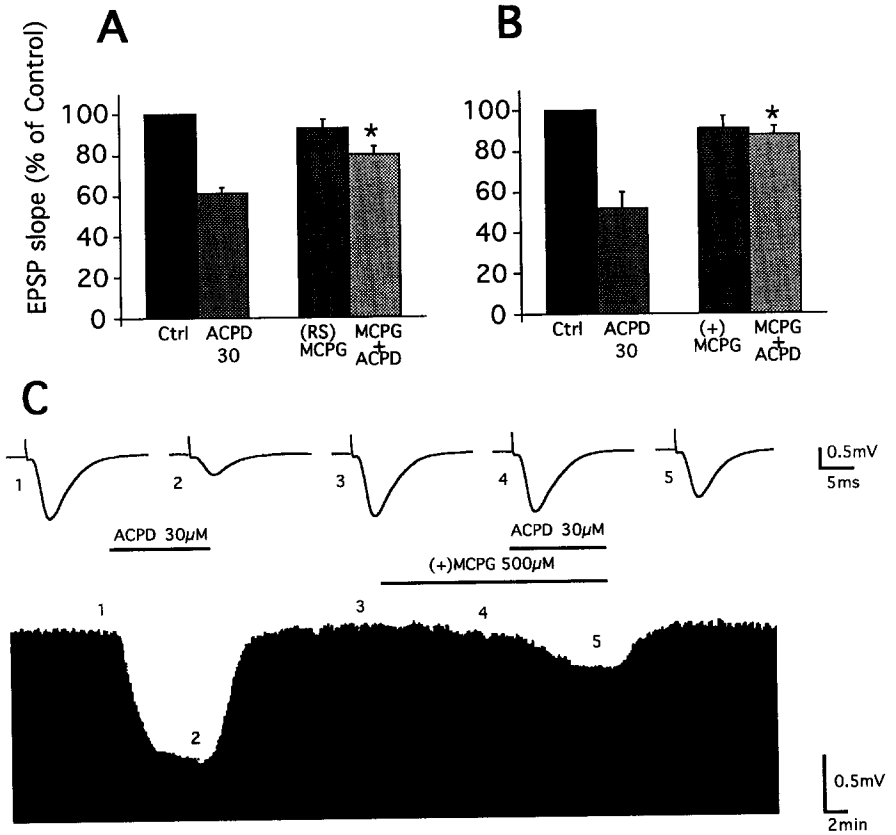

Fig. 1. Blockade of ACPD-induced depression of synaptic transmission by MCPG. Histograms represent the mean ± S.E.M. of the change in slope of the initial phase of the synaptic response (see Materials and methods). A: 30 μM ACPD induced a 40% depression of EPSP slope. Pre-treatment with 500 μM (*RS*)-MCPG (applied 10 min before ACPD) successfully blocked the effects of ACPD. B: Pre-treatment with the active isomer (+)-MCPG (500 μM, 20 min) fully antagonized the ACPD (30 μM, 8–10 min)-induced depression ($n = 5$). C: Result from an experiment in which (+)-MCPG (500 μM, 20 min) blocked the action of ACPD (30 μM, 10 min). Top traces are representative EPSPs recorded at the time point indicated by the number: control (1), during the application of ACPD (2), after wash (3), during the (+)-MCPG (4), and during the application of MCPG and ACPD together (5). Lower chart recording shows the peak negative amplitude of each response, evoked at 0.1 Hz, during the entire experiment. Individual deflections cannot be distinguished due to the slow chart speed. * $P < 0.01$, *t*-test for repeated measures (ACPD vs. MCPG + ACPD).

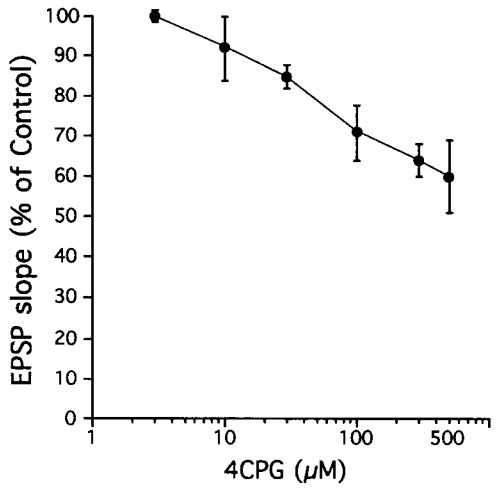

Fig. 2. Depression of synaptic transmission by 4CPG. Log dose-response relationship showing the relation between percent change in EPSP slope (ordinate scale) and applied agonist concentration (abscissa scale). Each point represents mean ± S.E.M. values pooled from 23 different slices.

and then adjusted to give the best response. In the dentate gyrus there are two electrophysiologically and spatially different responses to perforant path stimulation: the first recorded in the outer molecular layer is evoked by lateral path stimulation, the second in the middle layer by medial perforant path stimulation. Evoked potentials were screened in order to obtain responses resulting from the activation of the medial rather than the lateral perforant path fibers. The criterion for identifying a medial perforant path response was the shape of the dendritic waves (Collingridge et al., 1983; Dahl and Sarve, 1989). Extracellular field excitatory postsynaptic potentials (EPSPs) were evoked by repetitive stimulation of the medial perforant pathway (0.1 Hz, 0.1 ms duration, 7–12 V) with a bipolar stimulating electrode and recorded in the middle third of the molecular layer of the dentate gyrus with a glass micropipette containing 154 mM NaCl (resistance 3–5 MΩ). EPSPs were amplified (Axoprobe-1A, Axon Instruments), digitized at 10 kHz using a laboratory interface (TL-1 DMA Interface, Axon Instruments), and displayed and stored on a computer system, which was also used for subsequent off-line analysis of the EPSP slope and peak. The initial negative slope of the EPSP was used as an index of synaptic excitation and was calculated as the least-squares fit of an approximately linear portion of the initial phase of the evoked synap-

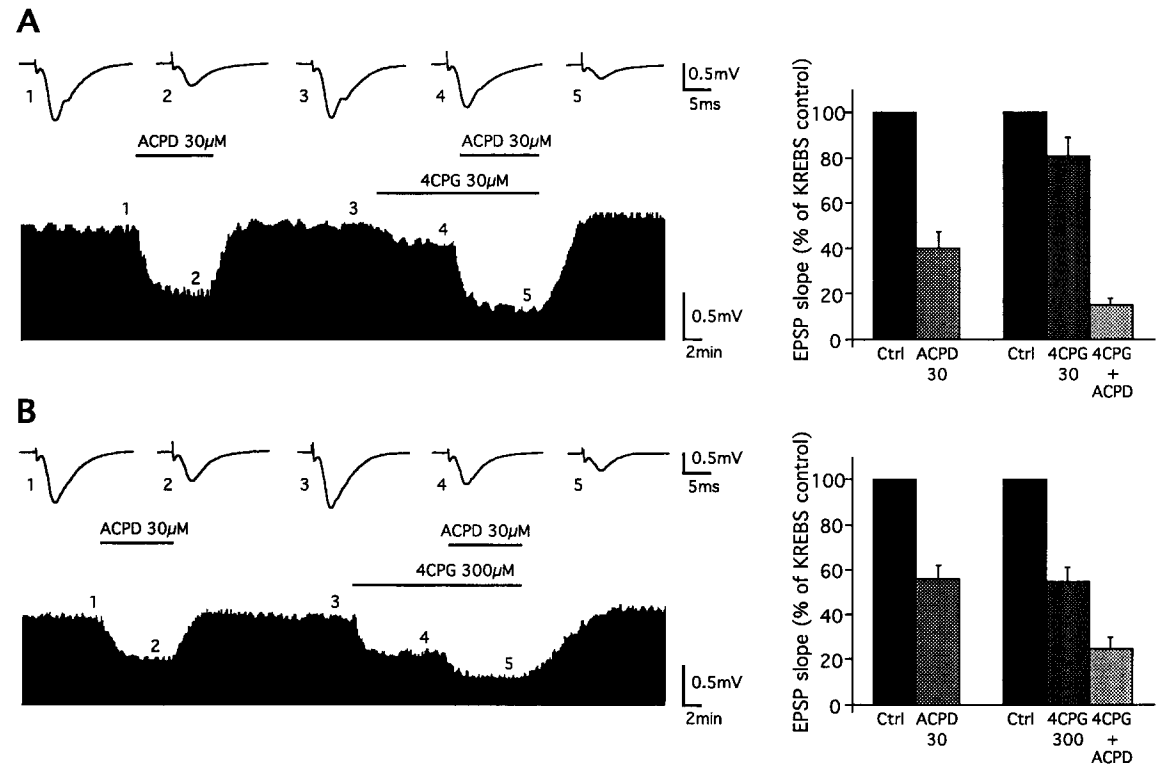

Fig. 3. Effect of 4CPG on the depression of EPSP induced by ACPD. Details of the traces chart recording and histograms are the same as in Fig. 1. A: Individual experiment showing the failure of 30 μM 4CPG to block the depression caused by 30 μM ACPD. Histogram summarizes the results of 4 separate experiments. The action of ACPD (30 μM, 10 min) induced about 60% of depression of the pre-treatment EPSP slope. Bath application of 4CPG alone (30 μM, 20 min) depressed the field potentials by 20%; together with ACPD (30 μM, 10 min) induced a further depression to less than 20% of control. B: Higher doses of 4CPG (300 μM vs. 30 μM) were also ineffective in blocking the effect of ACPD. The traces and chart recording show the results of a single experiment; combined results from 3 separate experiments are summarized in the histogram.

tic response (between 20 and 80% of the peak amplitude). The peak amplitude was calculated as the vertical distance from the baseline level before the stimulus artefact and the peak of the negative EPSP. EPSP slope and peak were calculated for each evoked potential. Drugs were applied by bath perfusion. Each experiment was carried out on a slice from a separate rat. Mean differences were evaluated by the paired t-test and were considered significant if $P < 0.01$. The following drugs were employed (all from Tocris Neuramin): (1*S*,3*R*)-ACPD; (*RS*) and (+)-α-methyl-4-carboxyphenylglycine; (*S*)-4-carboxyphenylglycine; (2*S*,3*S*,4*S*)-α-(carboxycyclopropyl)glycine; (*S*)-3-hydroxyphenylglycine; L-2-amino-4-phoshonobutyrate; α-methyl-L-AP4.

3. Results

In all slices analyzed ($n = 42$) the mGlu receptor agonist ACPD (30–50 μM) caused an immediate and reversible depression of the field EPSP, which recovered to its pre-treatment level within a few minutes after a 5–6 min period of drug application (cf. left side of Fig. 1C). These results in the dentate gyrus confirm the results of previous studies of other brain areas. We noted, however, that successive treatments with the same dose of ACPD caused larger reductions of the potentials than the first ACPD application. No further reduction of the field potentials was usually found after the second or third treatment. We therefore applied ACPD 2 or 3 times, each application separated by a 10- to 15-min wash period, to acquire a stable action of the drug before challenging it with an mGlu receptor antagonist.

We first examined the ability of the mGlu receptor antagonist MCPG to antagonize the depression induced by ACPD in our extracellular slice preparation. With 50 μM ACPD, which reliably produced a depression of about 50–60% of the field EPSP, 500 μM (*RS*)-MCPG did not antagonize the effect of ACPD ($n = 6$, not shown). With only 30 μM ACPD, however, 500 μM (*RS*)-MCPG did block the depression (Fig. 1A, $n = 6$). An even clearer antagonism was found with the active isomer of MCPG, (+)-MCPG ($n = 5$), as shown in Fig. 1B and C. Neither (*RS*)-MCPG nor (+)-MCPG produced any effect by itself to the field EPSP when perfused in the bath. These results show that in the dentate gyrus MCPG is an effective mGlu receptor antagonist, although it is only able to block the action of relatively low concentrations of the mGlu receptor agonist ACPD.

A more potent mGlu receptor antagonist, 4CPG, was recently reported to antagonize the effects of ACPD in motoneurons and thalamic neurons (Eaton et al., 1993). To use 4CPG in the dentate gyrus, we first measured its effect alone, as we found it decreased the field potentials. Fig. 2 shows the dose-dependent depression induced by 4CPG. Since 4CPG was described in vitro to act as potent mGlu group I antagonist, but as an mGlu group II agonist at higher concentrations (Hayashi et al., 1994), we selected both a low and a high concentration to block the effects of ACPD in the dentate gyrus. However, neither 30 μM ($n = 4$) nor 300 μM ($n = 3$) of 4CPG was able to reverse the effects of ACPD (Fig. 3). The action was, instead, additive to

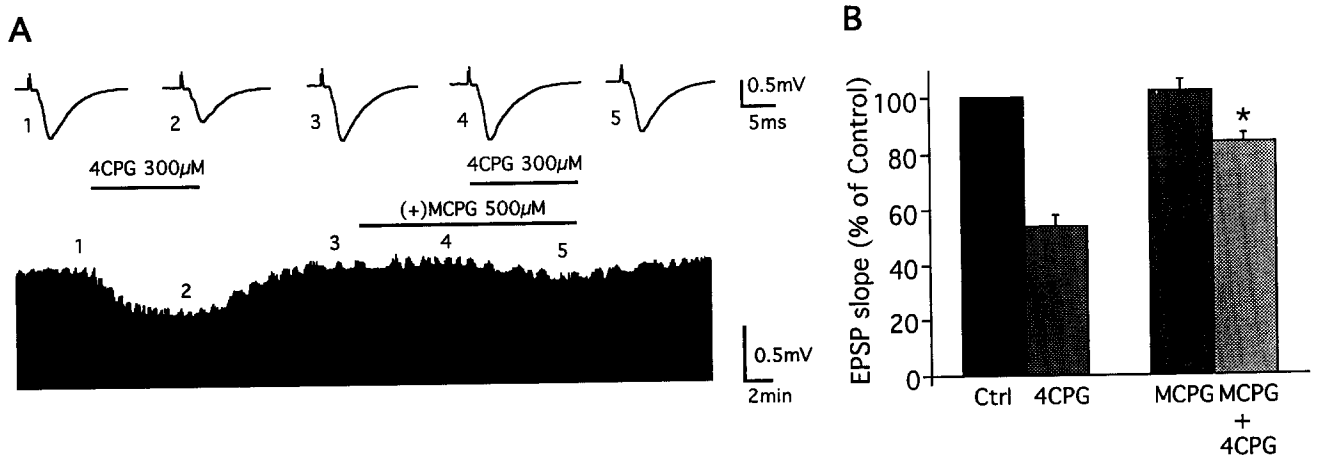

Fig. 4. Effect of (+)-MCPG on the depression of EPSP induced by high concentrations of 4CPG. A: Individual experiment shows that pre-treatment for 10 min with 500 μM (+)-MCPG blocked the depression produced by 300 μM 4CPG. Individual traces and chart recording as in Fig. 1D. B: Summary of results from 5 separate experiments; details of the histograms as in Fig. 1. Response levels of 4CPG (300 μM, 10 min) were compared to 10 min stable pre-4CPG baseline (CTRL). (+)-MCPG (500 μM, 10 min) had no significant effect on the dentate gyrus EPSP by itself (MCPG), but markedly blocked the depression caused by 4CPG (MCPG + 4CPG) when perfused 10 min before 4CPG application. * $P < 0.01$, t-test (4CPG vs. MCPG + 4CPG).

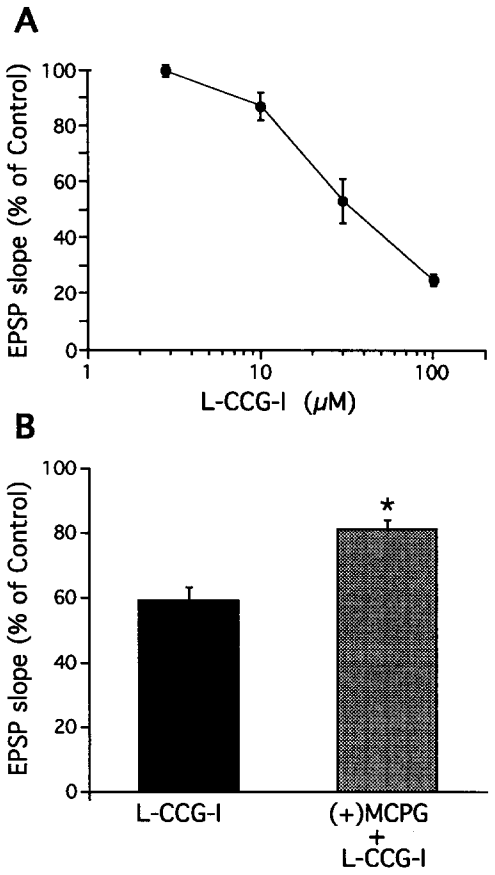

Fig. 5. The mGlu group II receptor agonist L-CCG-I depressed synaptic transmission in the dentate gyrus, and its action was effectively antagonized by (+)-MCPG. A: Dose-response curve for the effect of L-CCG-I on EPSP slope ($n = 3$). Details as in Fig. 2. B: Summary of results from 4 separate experiments; details of the histograms as in Fig. 1. The action of L-CCG-I (30 μM, 10 min) caused a 40% reduction of EPSP slope. (+)-MCPG (500 μM, 20 min) largely blocked this depression produced by the agonist. * $P <$ 0.01, t-test (L-CCG-I vs. MCPG + L-CCG-I).

that of ACPD. When applied together with ACPD, the lower dose of 4CPG induced an even greater depression than the higher one, although the final level of depression (right hand columns in Fig. 3A and B) was approximately the same. Similar results were found by Pook et al. (1993) in the spinal cord preparation. We also tried to antagonize the ACPD-induced depression by using 1 μM or 10 μM 4CPG concentrations that should avoid mGlu group II agonism. Both doses failed to block ACPD effects (data not shown). The effects of 4CPG on synaptic transmission in the dentate gyrus confirm earlier reports of its mGlu receptor agonistic properties in vitro (Hayashi et al., 1994), although at much lower concentrations. Our data do not confirm the results of studies conducted in the thalamus and in motoneurons that showed an antagonist action of 4CPG (Eaton et al., 1993).

The mGlu receptor antagonist MCPG is effective as an antagonist at both receptor groups I and II, with about the same potency (Hayashi et al., 1994). To test whether the depression induced by 4CPG on synaptic transmission in the dentate gyrus was due to its group II receptor agonist properties, we examined whether MCPG could antagonize these effects. Fig. 4 shows that (+)-MCPG blocked nearly all the depression induced by 300 μM 4CPG ($n = 5$). These results, taken together with the inability of 4CPG to antagonize the effects of ACPD, suggest that ACPD depresses the EPSP by acting on group II of the mGlu receptor subtypes.

To test the idea that the depressive effects of ACPD were indeed mediated through group II receptors, we examined the actions of selective agonists of group I and group II mGlu receptors. 3HPG, a selective group I agonist, had no effect on the EPSPs at concentrations between 10 and 500 μM (data not shown). In contrast,

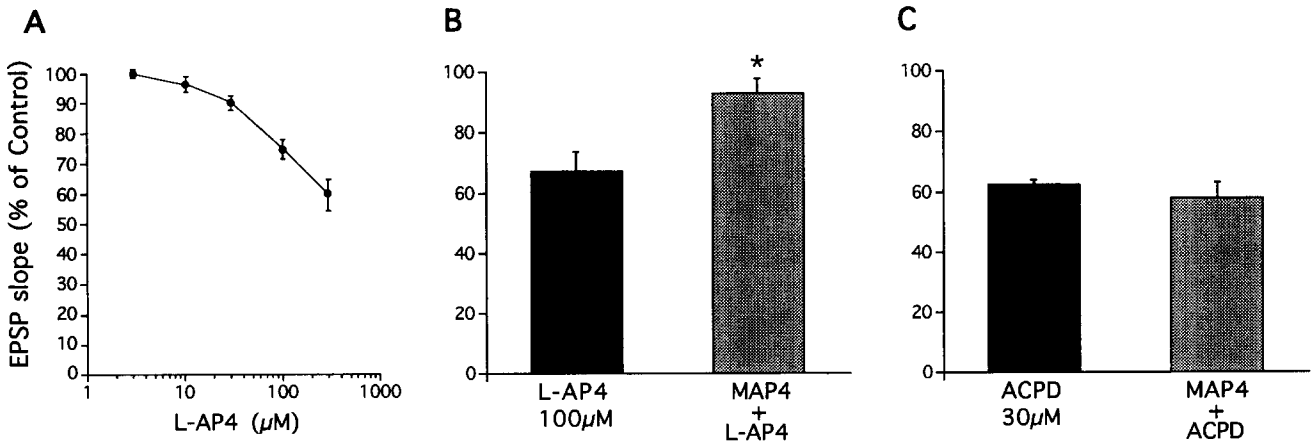

Fig. 6. The mGlu group III receptor agonist L-AP4 depressed synaptic transmission. The group III receptor antagonist MAP4 antagonized this action, but failed to block the ACPD-induced depression. A: Dose-response curve for the effect of L-AP4 on EPSP slope ($n = 10$). Details as in Fig. 2. B: The action of L-AP4 (100 μM, 6 min) caused a reduction of about 30% of the EPSP slope ($n = 4$). MAP4 (500 μM, 20 min) fully blocked this depression induced by the agonist. C: MAP4 (500 μM, 20 min, $n = 5$) failed to block the depression induced by ACPD (30 μM, 6 min). * $P < 0.01$, t-test (L-AP4 vs. MAP4 + L-AP4).

L-CCG-I, a selective group II agonist, blocked the EPSP at low concentrations (50% at 30 μM) and in a dose-dependent fashion (Fig. 5A, $n = 3$). Furthermore, MCPG effectively antagonized these effects (Fig. 5B, $n = 4$), just as it did those of ACPD.

There is some evidence that group III mGlu receptor subtypes are present at the medial perforant path synapses in the dentate (Kroona et al., 1991; Kahle and Cotman, 1993). The specific group III receptor agonist L-2-amino-4-phosphonobutyrate (L-AP4) was reported to reduce synaptic transmission in the spinal cord (Jane et al., 1994) and in the dentate gyrus excited by lateral perforant path (Bushell et al., 1995). To address the contributions of group III receptors in the synaptic transmission of the medial perforant path stimulation, we examined the effects of L-AP4 in this synapse. Fig. 6A shows the dose-response-dependent depression induced by L-AP4. A concentration of 100 μM of the agonist produced a reduction of only 25% of the EPSPs, compared to the 50% reduction induced by 10 μM L-AP4 in the lateral perforant path synapse (Bushell et al., 1995). The group III receptor antagonist α-methyl-L-AP4 (MAP4) (500 μM) fully antagonized the 100 μM L-AP4-induced depression (Fig. 6B, $n = 4$). MAP4, however, was not able to block any depression induced by the agonist ACPD (30 μM) (Fig. 6C, $n = 5$). To futher exclude any involvement of group III mGlu receptor subtypes in the ACPD-induced synaptic depression, we repeated the experiment in which (*RS*)-MCPG (500 μM) was not able to block the effects produced by 50 μM ACPD. This time we added the group III receptor antagonist MAP4 (500 μM) to the bath, during the perfusion with (*RS*)-MCPG. MAP4, together with (*RS*)-MCPG, was unable to reverse the ACPD-induced depression ($n = 3$, data not shown), indicating that the lack of effect of (*RS*)-MCPG (500 μM) alone is due to its relatively low potency as group II receptor antagonist, and not to activation of a group III mGlu receptor subtype with higher concentrations of ACPD.

4. Discussion

The metabotropic glutamate (mGlu) receptor agonist ACPD is known to depress synaptic transmission in the hippocampal CA1 area (Baskys and Malenka, 1991), neostriatal area (Lovinger, 1991; Lovinger et al., 1993), and spinal cord (Pook et al., 1992; Ishida et al., 1993). In this paper we confirm similar results extending them to the dentate gyrus of the rat.

In our study the mGlu receptor antagonist MCPG blocked the ACPD-induced effect only at a low dose of ACPD (30 vs. 50 μM), and a complete block was seen (at 30 μM ACPD) only with the active isomer of MCPG (at 500 μM). MCPG has been found to antagonize ACPD-induced depression both in pyramidal cells in CA1 (Manzoni et al., 1994; Bashir et al., 1993) and in motoneurons in the spinal cord (Kemp et al., 1994). Chinestra et al. (1993), however, did not find this antagonism, perhaps because of this compound's low potency (Hayashi et al., 1994). Although the distribution of the mGlu receptor subtypes within the hippocampal areas and in particular in the CA1 and dentate gyrus is not completely the same (Fotuhi et al., 1994), our study seems to confirm a relatively low potency of MCPG in antagonizing ACPD-induced depression. Therefore, our findings show that MCPG is a metabotropic glutamate receptor antagonist, extending these findings to the dentate gyrus, although relatively high concentrations are required.

The second mGlu receptor antagonist used in the present study, 4CPG, failed to block the depression induced by ACPD. By itself this compound caused a depression of the field potentials in a dose-dependent fashion and potentiated the depression produced by ACPD. A similar potentiation of ACPD-induced synaptic depression was reported in neonatal rat spinal cord (Pook et al., 1993). 4CPG is found in transfected cells to be an antagonist for the group I receptor subtype, but also an agonist for group II (Hayashi et al., 1994). Our results in brain slices confirm these studies, although at unexpectedly low concentrations. Similar results were obtained in vivo in the dentate gyrus by stimulating the perforant path (Bordi and Ugolini, 1995). However, 4CPG did not induce any depression of the field EPSP when recordings were made from slices of CA1 and CA3 areas of the hippocampus (Ugolini and Bordi, unpublished results). Our findings can be explained by the high concentration of mGlu subtype 2 receptors in the dentate gyrus suggested by in situ hybridization studies (Ohishi et al., 1993), although these studies only indicate that $mGlu_2$ receptor mRNA is expressed in dentate neurons and only immonocytochemical studies using subtype-specific antibodies will precisely determine the location of the receptors. 4CPG was reported to inhibit the synaptic excitation of thalamic neurons evoked by noxious stimuli in vivo, but not to block the depressant effects of ACPD (Eaton et al., 1993). A hypothesis consistent with all these findings is that mGlu group I ($mGlu_1$ and $mGlu_5$) receptors are involved in synaptic excitation but not in synaptic depression, while activation of group II receptors ($mGlu_2$ and $mGlu_3$) does produce depression. This conclusion is in line with the interpretation of results obtained with neonatal rat spinal cord (Watkins and Collingridge, 1994).

MCPG is an antagonist of group II receptors (Hayashi et al., 1994) and effectively antagonized the depression induced by 4CPG in the present study. To confirm that this action of MCPG is mediated through

mGlu group II receptors, we used the selective mGlu group II agonist L-CCG-I (Hayashi et al., 1994). The effect of L-CCG-I on the field potentials of the dentate area was very similar to that exerted by ACPD, as reported for spinal motoneurons (Ishida et al., 1993), and MCPG successfully blocked it, as it did the effects of ACPD. The selective mGlu group I receptor agonist 3HPG (Hayashi et al., 1994), on the other hand, did not have any effect on the field potentials in the dentate gyrus.

Group III mGlu receptor subtypes are also involved in the depression of synaptic transmission (Fagg and Lanthorn, 1985; Kroona et al., 1991; Jane et al., 1994; Bushell et al., 1995). In the dentate gyrus, the group III receptor agonist L-AP4 has a strong depressant action when the lateral perforant path is excited, but shows a much weaker effect when the middle perforant path is excited (Fagg and Lanthorn, 1985; Kroona et al., 1991; Bushell et al., 1995). Our findings show that indeed in the middle perforant path synapse L-AP4 produced a weak effect. Recently, Bushell et al. (1995) reported that 10 μM L-AP4 decreased the EPSPs in the lateral perforant path synapse by about 50%, while our findings show a 40% depression with 300 μM L-AP4.

Our results show that the group III receptor antagonist MAP4 successfully antagonized the depressant action produced by L-AP4, as reported by others in the spinal cord (Jane et al., 1994) and in dentate gyrus stimulated by the lateral perforant path (Bushell et al., 1995). We also found that MAP4 did not reverse the effect induced by ACPD, in agreement with studies performed in the neonatal rat spinal cord (Jane et al., 1994). The lack of effect of MAP4 demonstrates that the depressant action induced by ACPD is mediated by group II mGlu receptors in the dentate gyrus. However, not all the depression of synaptic transmission mediated via mGlu receptors in the medial perforant path can be ascribed to group II receptor subtypes. The action produced by L-AP4 suggests the participation of a mGlu receptor that belongs to the group III in the modulation of such depressant effects. The nature of the receptor subtype mediating this depression, however, is currently unknown (see Jane et al., 1994, for a discussion). The relatively small effect of L-AP4 in our study suggests that the group III receptors mediating synaptic depression might play a minor role in the middle perforant path synapse.

In conclusion, our study demonstrates for the first time that the depressant action induced by the mGlu receptor agonist ACPD in the hippocampus is due to activation of mGlu group II receptors. Group I and group III mGlu receptors, on the other hand, can be excluded from any involvement in this action. These findings help elucidate one aspect associated with an individual mGlu receptor subtype. The availability of more potent and specific antagonists for different mGlu receptor subtypes will enable a better understanding of the role of mGlu receptors in CNS functions.

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