Pergamon

0028-3908(95)00060-7

Neuropharmacology Vol. 34, No. 8, pp. 965–971, 1995

0028-3908/95 $9.50 + 0.00

Pharmacology of Metabotropic Glutamate Receptors at the Mossy Fiber Synapses of the Guinea Pig Hippocampus

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(Accepted 11 April 1995)

Summary—We have tested the ability of several specific agonists of glutamate metabotropic receptors (mGluRs) to depress synaptic transmission at mossy fiber synapses in the CA3 region of the guinea pig hippocampus. 1*S*,3*R*-1-amino-cyclopentyl-1,3-dicarboxylate (ACPD) reversibly inhibited monosynaptic mossy fiber field potentials, presumably by a presynaptic mechanism, with an EC_{50} of $2.0 \pm 0.4\ \mu M$ ($n = 3$), suggesting the presence of mGluRs on mossy fiber synaptic terminals of the group 1 or 2 category. L-2-amino-4-phosphono butanoate (L-AP4) also inhibited responses with an EC_{50} of $1.1 \pm 0.2\ \mu M$ suggesting that mGluRs of the group 3 (mGluR4, 6, 7 and 8) category of receptors are also present on mossy fiber terminals. Both (2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine (L-CCG1) and (S)-4-carboxy-3-hydroxy phenylglycine (4C3HPG) were also efficacious at blocking mossy fiber transmission, with an EC_{50} of $1.1 \pm 0.1\ \mu M$ ($n = 4$) and $4.8 \pm 0.6\ \mu M$ ($n = 3$) respectively. The latter finding indicates the involvement of mGluRs belonging to the group 2 (mGluR2, 3) category of receptors. The effects of L-AP4 and L-CCG1 were both antagonized by (+)-α-methyl-4-carboxyphenylglycine [(+)MCPG]. MAP4, an antagonist of group 3 mGluRs in other systems, blocked the effect of L-AP4, but not the effect of L-CCG1, while MCCG, an antagonist of group 2 mGluRs in other systems, blocked the effect of L-CCG1, but not the effect of L-AP4. These pharmacological findings provide strong evidence for the coexistence of group 2 and 3 mGluRs on the terminals of mossy fibers in the guinea pig.

Keywords—Hippocampus, mossy fiber, CA3, metabotropic glutamate receptors, phenylglycines, L-AP4, ACPD.

Synaptic transmission between mossy fibers and CA3 pyramidal neurons of the hippocampus of the guinea pig, but not the rat, is suppressed by the phosphonic derivative of glutamate (Glu), L-2-amino-4-phosphono butanoate (L-AP4) (Lanthorn *et al.*, 1984; Yamamoto *et al.*, 1983). Other studies have shown that the L-AP4-sensitive receptors are likely to be presynaptic "auto-receptors" (Cotman *et al.*, 1986; Forsythe and Clements, 1990; Harris and Cotman, 1983). Since then, considerable progress have been made in the molecular characterization of Glu receptors and in addition to the ionotropic receptors (i.e. ligand-gated ion channels), a family of at least 8 distinct receptors coupled to various intracellular effectors via GTP-binding proteins has been described (Nakanishi, 1992; Pin and Duvosin, 1995; Schoepp and Conn, 1993). These receptors, termed metabotropic glutamate receptors (mGluRs) are currently divided into 3 subgroups: group 1 (mGluRs 1 and 5), group 2 (mGluRs 2 and 3), and group 3 (mGluRs 4, 6, 7, and 8). The group 3 mGluRs have been shown to be specifically activated by L-AP4 (Aramori and Nakanishi, 1992; Kristensen *et al.*, 1993; Nakajima *et al.*, 1993; Okamoto *et al.*, 1994; Tanabe *et al.*, 1992, 1993). The cloning of mGluRs also enabled the characterization of new pharmacological tools to study these receptors (Hayashi *et al.*, 1992, 1994; Thomsen *et al.*, 1994; Watkins and Collingridge, 1994). For instance, these latter studies proposed that (2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine (L-CCG1) is a selective agonist for the group 2 mGluRs and that (*S*)-4-carboxy-3-hydroxy phenylglycine (4C3HPG) acts both as a antagonist of group 1 mGluRs and a specific agonist at group 2 mGluRs. Finally, a number of antagonists have been developed. MCPG is a relatively broad spectrum mGluR antagonist, while MAP4 selectively blocks the activation

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of group 3 receptors (Bushell *et al.*, 1995; Jane *et al.*, 1994) and MCCG blocks responses evoked by group 2, but not group 3 mGluRs (Jane *et al.*, 1994). In the present report, we took advantage of this newly described pharmacology in order to investigate the subtypes of receptors responsible for the depression of the mossy fiber-CA3 synaptic transmission in hippocampal slices of the guinea pig.

METHODS

Hippocampal slices

Standard procedures for preparing and maintaining guinea pig hippocampal slices were used (Nicoll and Alger, 1981). Transverse hippocampal slices 400–500 μm thick were cut in ice cold Ringer's solution using a vibratome, placed in a holding chamber for at least 1 hr, and then transferred to a superfusing chamber for recordings. The superfusing medium contained (in mM): 119 NaCl, 2.5 KCl, 1.3 $MgCl_2$, 2.5 $CaCl_2$, 26 $NaHCO_3$, 1 NaH_2PO_4, and 10 Glucose, and was equilibrated with 95% O_2/5% CO_2. All experiments were done at room temperature.

Electrophysiological recordings and data analysis

Field potential recordings were made with electrodes filled with 3 M NaCl. The restricted anatomy of the mossy fiber input as well as the reversal of the waveform as the recording electrode is moved from *S. lucidum* to *S. radiatum* served to define mossy fiber inputs. At the end of all experiments 20 μM CNQX was added to the bath to assess the fiber volley component of the response. Baseline transmission was monitored at 0.033–0.05 Hz. An Axopatch-1D (Axon Instruments) was used to record the data, which was filtered at 2 kHz, digitized at 2–5 kHz on a TL-1 DMA interface (Axon Instruments) and collected on a 486 IBM compatible computer. A modified version of P-clamp was used for analysis. All values are given as mean $\pm$ SEM. The fitting curves were drawn according to $y = \{y_{max} - y_{min}/1 + (x/EC_{50})^n\} + y_{min}$, using Kaleidagraph software.

Drugs

Drugs used were CNQX (Research Biochemicals Inc.); L-AP4 (Sigma); 1*S*,3*R*-ACPD, L-CCG1, 4C3HPG, (+)MCPG, MAP4, and MCCG (Tocris Neuramin). 1*S*,3*R*-ACPD, L-CCG1, 4C3HPG and (+)-MCPG were made up as a 100 mM stock in 100 mM NaOH.

RESULTS

Mossy fiber synaptic transmission is depressed by metabotropic glutamate receptors agonists

We examined the ability of various mGluR agonists to depress synaptic transmission at mossy fibers synapses.

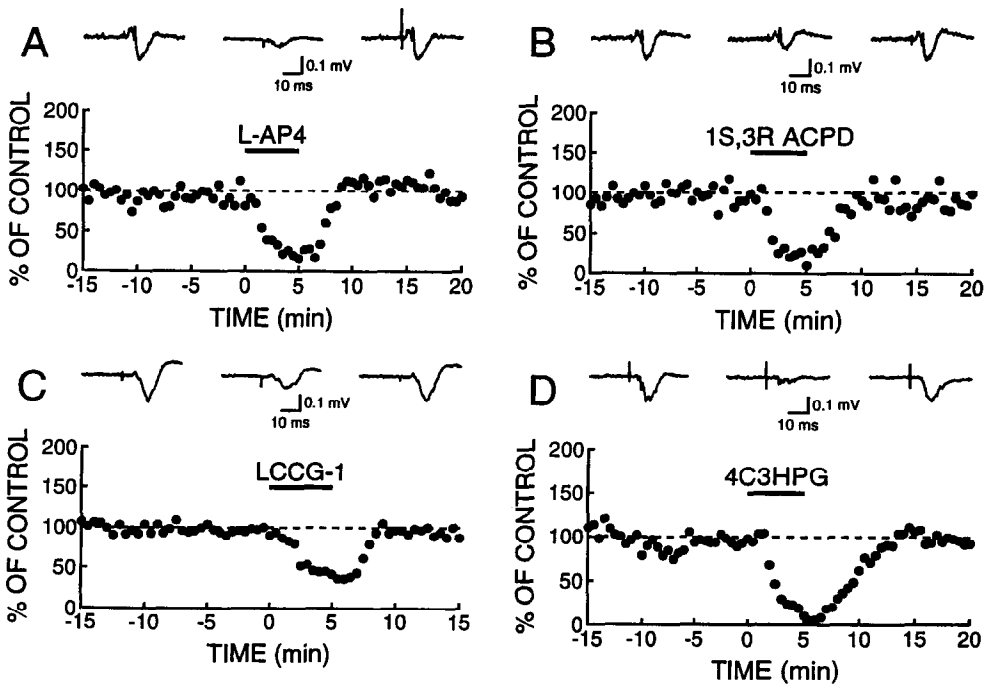

Fig. 1. Inhibitory effect of mGluR agonists on mossy fiber synaptic transmission. (A) Under normal conditions, the metabotropic glutamate agonist L-AP4 (1.25 μM) strongly diminished the EPSP field. The effect of L-AP4 was completely reversed within 5–10 min of washing (similar results were observed in 8 other slices). (B) 1*S*,3*R*-ACPD (2.5 μM) strongly diminished the EPSP field. This effect was completely reversed within 5–10 min of washing (similar results were observed in 4 other slices). (C) L-CCG1 (1 μM) clearly diminished the EPSP field. Reversal was complete within 5–10 min of washing (similar results were observed in 10 other slices). (D) 4C3HPG (10 μM) reduced the field EPSP. Reversal was complete with 5–10 min of washing (similar results were observed in 4 other slices).

Presynaptic inhibition of excitatory synaptic transmission is a classical effect of metabotropic receptor activation (Baskys and Malenka, 1991). As shown in Fig. 1(A) (top left) the specific group 3 agonist L-AP4 (1.25 μM) (Aramori and Nakanishi, 1992; Hayashi *et al.*, 1992; Kristensen *et al.*, 1993; Nakajima *et al.*, 1993; Okamoto *et al.*, 1994; Tanabe *et al.*, 1992, 1993) caused a large and reversible reduction in the size of the field excitatory post-synaptic potential (EPSP) ($n = 9$). Figure 1(B) shows a typical experiment in which the specific metabotropic receptor agonist 1*S*,3*R*-ACPD (2.5 μM), which activates group 1 and 2 mGluRs, also caused a reproducible depression in the field EPSP ($n = 4$). We then tested the ability of two newly described molecules, L-CCG1 and 4C3HPG to inhibit synaptic transmission. In Fig. 1(C), L-CCG1 at a concentration of 1 μM, which specifically activates group 2 mGluRs (Hayashi *et al.*, 1992), reversibly reduces the field EPSP ($n = 11$). Finally, the mGluR1 antagonist/mGluR2 agonist 4C3HPG (Hayashi *et al.*, 1994; Thomsen *et al.*, 1994) also inhibited synaptic transmission [Fig. 1(D)] ($n = 5$).

Dose dependent inhibition by L-AP4, 1S,3R ACPD, L-CCG1 and 4C3HPG

We next perform complete dose response curves of L-AP4, 1*S*,3*R* ACPD, L-CCG1 and 4C3HPG on mossy fiber synaptic transmission. Figure 2 shows the summary of all these experiments. The EC_{50} was determined in each individual experiment by fitting the curves according to $y = \{y_{max} - y_{min}/1 + (x/EC_{50})^n\} + y_{min}$. Then, the EC_{50} values were averaged and we found that the rank order of agonist potencies was: L-CCG1 $\geqslant$ L-AP4 > 1*S*,3*R* ACPD > 4C3HPG with EC_{50} values of $1.1 \pm 0.2\,\mu$M ($n = 4$), $1.1 \pm 0.1\,\mu$M ($n = 3$), $2.0 \pm 0.4\,\mu$M ($n = 3$) and $4.8 \pm 0.6\,\mu$M ($n = 4$), respectively.

Effects of mGluR antagonists on L-AP4 and L-CCG1-induced depression

In a final set of experiments we tested the ability of various mGluR antagonists, including, (+)-MCPG (Jane *et al.*, 1993), MAP4 (Bushell *et al.*, 1995; Jane *et al.*, 1994), and MCCG (Jane *et al.*, 1994) to antagonize

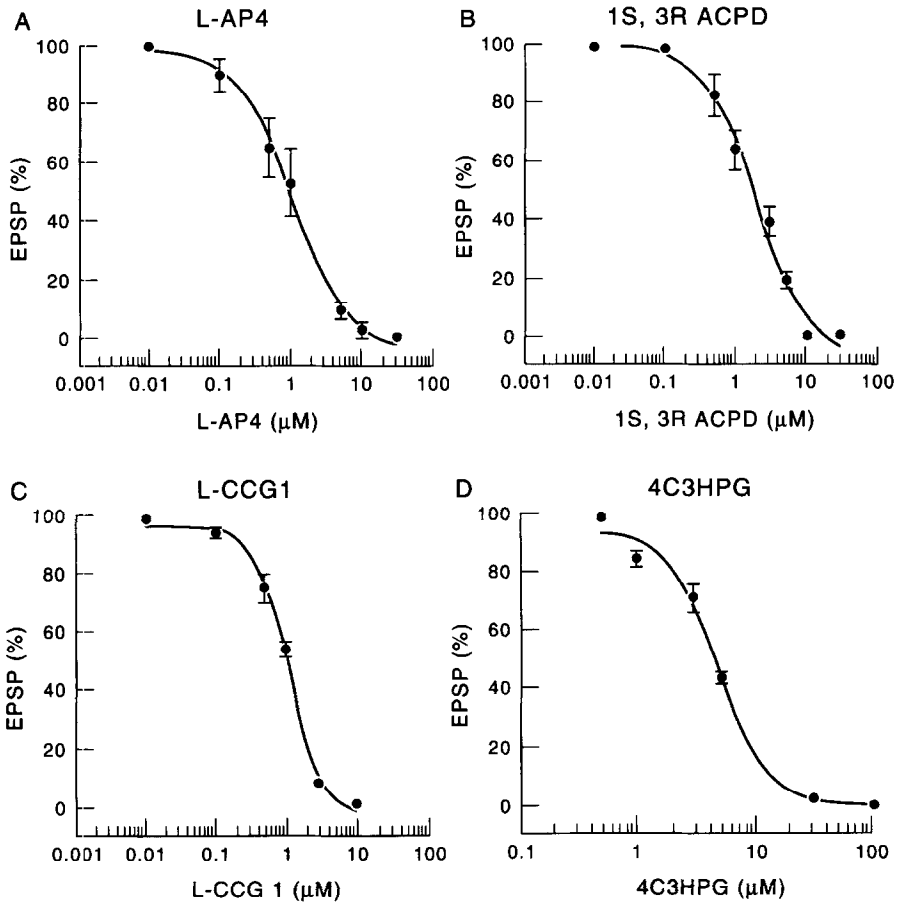

Fig. 2. Dose–response relationship of L-AP4 (A), 1S,3R-ACPD (B), L-CCG1 (C) and 4C3HPG (D) on mossy fiber synaptic transmission. Each point represents the mean ± SEM of 3–4 measurements and is expressed as the percentage of control responses at the maximum of the depression. The rank order of agonist potencies was: L-CCG1 $\geqslant$ L-AP4 > 1*S*,3*R*-ACPD > 4C3HPG with EC_{50} values of $1.1 \pm 0.2\,\mu$M ($n = 4$), $1.1 \pm 0.1\,\mu$M ($n = 3$), $2.0 \pm 0.4\,\mu$M ($n = 3$) and $4.8 \pm 0.6\,\mu$M ($n = 4$), respectively.

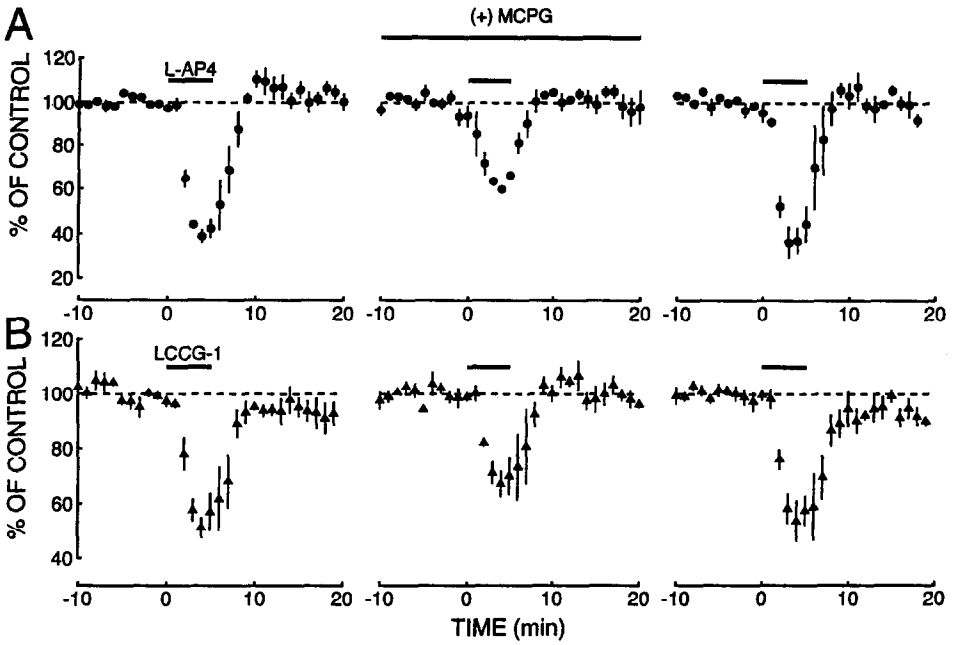

Fig. 3. Antagonism of the L-AP4- and L-CCG1-induced depression of the EPSP by (+)MCPG. (A) Pooled data of 5 experiments showing the effect of 1 μM L-AP4 alone (36 $\pm$ 4.2% of basal), in the presence of (+)MCPG (200 μM) (61 $\pm$ 2% of basal) and after washout (37 $\pm$ 5% of basal). (B) Pooled data of 5 experiments showing the effect of 1 μM L-CCG1 alone (51 $\pm$ 3% of basal), in the presence of (+)MCPG (200 μM) (68 $\pm$ 4% of basal) and after washout (54 $\pm$ 3% of basal).

the depressant action of mGluR agonists on mossy fiber responses. We have previously shown that (±)MCPG inhibits the 1*S*,3*R* ACPD-induced depression of the mossy fiber responses (Manzoni *et al.*, 1994). Since L-AP4 and L-CCG1 both caused an inhibition of mossy fiber responses at concentrations which selectively activate group 3 and group 2 mGluRs, respectively (Aramori and Nakanishi, 1992; Hayashi *et al.*, 1992; Kristensen *et al.*, 1993; Nakajima *et al.*, 1993; Okamoto *et al.*, 1994; Tanabe *et al.*, 1992, 1993), we compared the relative effectiveness of (+)MCPG on the responses to the two agonists. Figure 3 summarizes the results obtained from experiments on 5 slices which were carried out in the following manner. After recording the response to L-AP4 (1 μM, 36 $\pm$ 4.2% of basal transmission at the peak of depression) and L-CCG1 (1 μM,

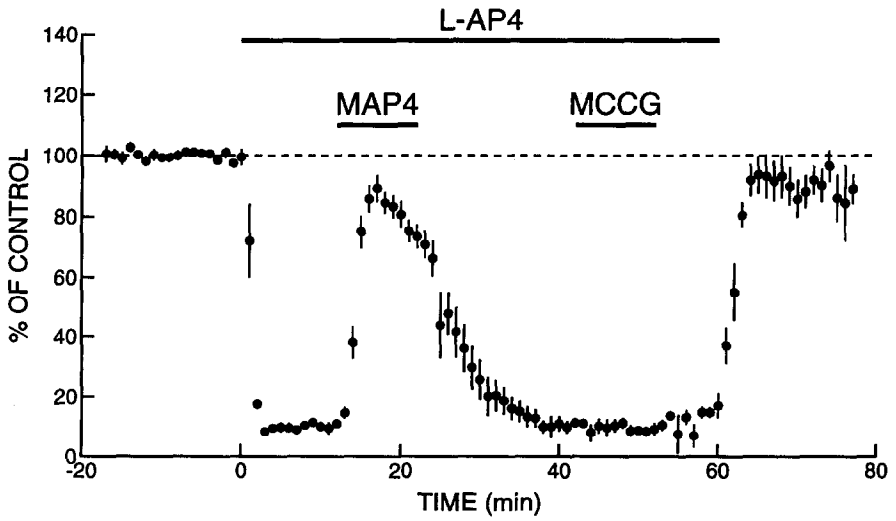

Fig. 4. MAP4, but not MCCG, antagonizes the depressant actions of L-AP4. The graph summarizes three experiments (mean $\pm$ SEM) where the actions of 500 μM MAP4 and 500 μM MCCG were tested during blockade of mossy fiber synaptic transmission by 10 μM L-AP4.

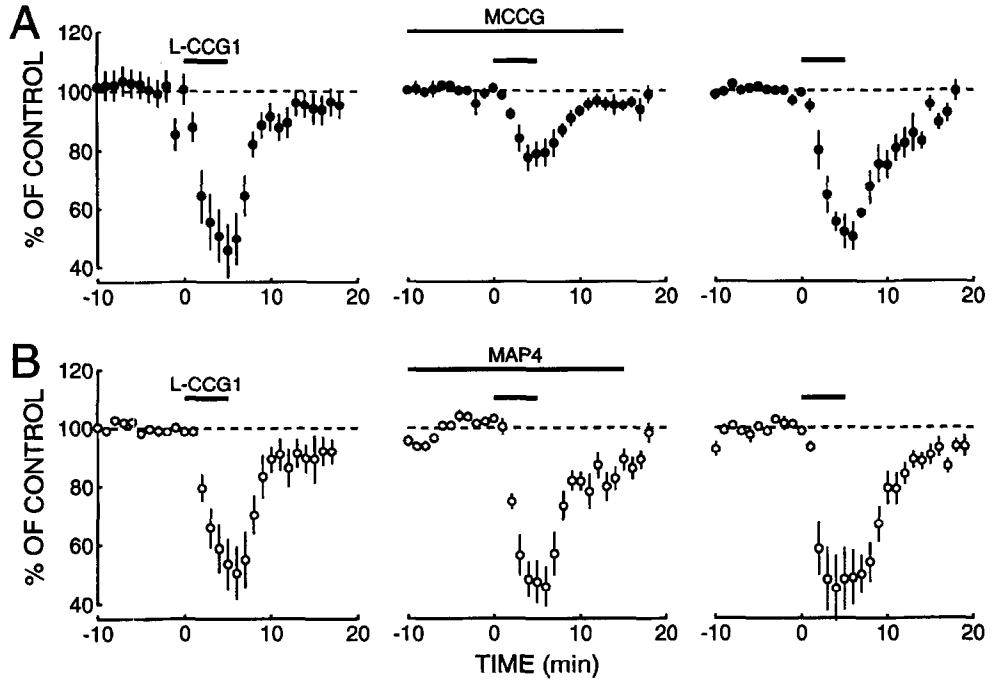

Fig. 5. Selective antagonist effects of MCCG- on L-CCG1-induced depression of mossy fiber responses. Each graph plots the field potential amplitude (mean ± SEM) for four experiments. After establishing a stable baseline, the effects of 1 μM L-CCG1 were examined. (A) The blockade of synaptic transmission by L-CCG1 was compared before (47 ± 7% of basal), during 500 μM MCCG (79 ± 4% of basal), and after 30 min washout (49 ± 4% of basal). (B) Same protocol as in (A) using 500 μM MAP4 instead, did not antagonize the L-CCG1–induced blockade.

51 ± 3% of basal), (+)MCPG (200 μM) was added to the superfusing medium for 20 min. (+)MCPG, by itself, had no effect on baseline transmission in any of these experiments. L-AP4 and L-CCG1 were then reapplied and it is clear that the responses were reduced by the (+)MCPG, The L-AP4 response before (+)MCPG was 36% of basal and in the presence of (+)MCPG it was reduced to 61 ± 2% of basal responses, while the L-CCG1 response was reduced to 68 ± 4% of basal in the presence of (+)MCPG. These values were significantly different from the controls as tested with the Student's t-test, ($P < 0.001$, $n = 5$). After 30 min washout of (+)MCPG, the mGluR-induced depression completely recovered (37 ± 5 and 54 ± 3% of basal, for L-AP4 and L-CCG1 respectively, $n = 5$). We next compared the ability of MAP4 and MCCG to antagonize the response to L-AP4 and L-CCG1. In the L-AP4 experiments the agonist was applied and after the depression had stabilized the ability of the 2 antagonists to reverse the depression was tested. As can be seen in Fig. 4 MAP4 (500 μM) almost completely reversed the depression evoked by 10 μM L-AP4 whereas MCCG had no effect on the L-AP4 induced depression ($n = 3$). Neither one of these antagonists had any effect on baseline responses in the absence of agonist ($n = 3$). Since responses recovered incompletely following prolonged applications of L-CCG1, we performed the antagonist experiments as shown in Fig. 3. MCCG (500 μM), but not MAP4 (500 μM), antagonized the depression evoked by 1 μM L-CCG1 (Fig. 5).

DISCUSSION

In the present paper, we tested the ability of specific mGluR agonists to inhibit mossy fiber synaptic transmission in the CA3 region of the guinea pig hippocampus, in an attempt to determine the subtypes of mGluR involved. In agreement with previous reports we found that L-AP4 (Cotman *et al.*, 1986; Lanthorn *et al.*, 1984; Yamamoto *et al.*, 1983) and 1*S*,3*R*-ACPD (Manzoni *et al.*, 1994) are both able to reversibly depress mossy fiber synaptic transmission in the guinea pig. 1*S*,3*R*-ACPD activates group 1 (subtypes 1 and 5) and 2 (subtypes 2 and 3) mGluRs (Manzoni *et al.*, 1990; Palmer *et al.*, 1989). The molecular cloning of several mGluR subtypes revealed that L-AP4 is a specific agonist at a family of mGluRs (4, 6, 7, 8) that are negatively coupled to cyclic AMP signal transduction (Nakanishi, 1992; Pin and Duvosin, 1995). Together these mGluRs are referred to as group 3 mGluRs. Thus the inhibitory action of L-AP4 suggests than an mGluR of the group 3 category is present on mossy fiber terminals of the guinea pig. Furthermore, *in situ* mRNA hybridization studies in the rat brain have demonstrated that mGluR6 mRNA is solely expressed in the retina (Nakajima *et al.*, 1993). While, the mGluR7 subtype is strongly expressed in pyramidal cells throughout CA1–CA4 regions as well as in granule cells of the dentate gyrus of the rat (Okamoto *et al.*, 1994), the EC_{50} for the mGluR7 in expression systems for L-AP4 is about 100 μM compared to 1 μM in the present study. The high sensitivity

of mossy fibers to L-AP4 is more consistent with an mGluR4 subtype.

Expression of single receptor clones in animal cells has enabled the determination of the selectivity and the potency of new agonists and antagonists at mGluRs (Hayashi *et al.*, 1992, 1994; Thomsen *et al.*, 1994). In particular these studies have shown that L-CCG1 is a selective agonist at receptors of the group 2 category (at micromolar concentrations) and that 4C3HPG is both an agonist at group 2 receptors and an antagonist at receptors belonging to group 1. In our hands, both compounds proved to be effective, with EC_{50}s in the low micromolar range. This data with selective agonists suggests that, in addition to the presence of a group 3 mGluR, a group 2 mGluR (Tanabe *et al.*, 1992) is also present on mossy fiber synaptic terminals.

We extended the agonist studies by examining the effects of various mGluR antagonists. Recently, the action of MCPG, an antagonist of mGluRs, has been studied in the hippocampal slice (Bashir *et al.*, 1993; Manzoni *et al.*, 1994), and pharmacological studies on cloned receptors suggested that MCPG was inactive ($IC_{50} > 1$ mM) at mGluR4 receptors (Hayashi *et al.*, 1994; Thomsen *et al.*, 1994) but is active at most other mGluRs. We therefore tested the effects (+)MCPG, the active form of MCPG (Jane *et al.*, 1993) on both L-AP4- and L-CCG1-induced depression and found (+)MCPG to be effective in blocking both L-AP4- and L-CCG1-induced effects. This finding suggests that, although the group 3 mGluR on mossy fiber terminals has a high affinity for L-AP4, the depression is unlikely to be mediated by an mGluR4. MAP4 selectively blocked the actions of L-AP4, while MCCG selectively antagonized the action of L-CCG1. These antagonist studies extend the agonist studies and clearly indicate that guinea pig mossy fiber terminals express mGluRs of group 2 and 3. Our results compliment studies on the spinal cord of the newborn rat in which similar pharmacological evidence suggests the presence of group 2 and 3 mGluRs on the terminals of primary afferent fibers (Jane *et al.*, 1994).

In conclusion, we have provided pharmacological evidence for the involvement of two subtypes of mGluRs in the depression of mossy fiber synaptic transmission. While our results indicate that both group 2 and 3 mGluRs participate in this depression, we were unable to identify definitively which specific mGluR within each group was responsible for the inhibition. Further development of subtype-specific agonists and antagonists will be needed to unequivocally address these issues.

Acknowledgements—This research was supported by the NIMH. R.A.N. is a member of the Keck Center for Integrative Neuroscience and the Silvio Conte Center for Neuroscience Research.

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