Pergamon

0028-3908(95)00115-8

Neuropharmacology, Vol. 34, No. 11, pp. 1567–1572, 1995

0028-3908/95 $29.00 + 0.00

Evidence Against a Role for Metabotropic Glutamate Receptors in Mossy Fiber LTP: the Use of Mutant Mice and Pharmacological Antagonists

A. Y. HSIA[2], P. A. SALIN[1], P. E. CASTILLO[1], A. AIBA[3*], A. ABELIOVICH[3], S. TONEGAWA[3] and R. A. NICOLL[1†]

[1]Departments of Cellular and Molecular Pharmacology and Physiology, and [2]Neuroscience Graduate Program, University of California, San Francisco, San Francisco, CA 94143-0450, U.S.A. and [3]Howard Hughes Medical Institute, Center for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA 02139, U.S.A.

(Accepted 12 July 1995)

Summary—We have used a number of approaches to address a possible role of metabotropic glutamate receptors (mGluRs) in mossy fiber long-term potentiation (LTP) in the hippocampus. We have used two types of mutant mice—one lacking the mGluR1 subtype of receptor and one lacking the gamma isoform of protein kinase C. In neither type of mouse did we find any alteration in the magnitude of mossy fiber LTP. We next examined whether mGluRs might modulate the magnitude and/or threshold for the induction of mossy fiber LTP. In these experiments we used tetani that were either just subthreshold or just suprathreshold for generating LTP. The mGluR antagonist (+)-α-methyl-4-carboxyphenylglycine [(+)MCPG] did not convert a subthreshold tetanus into a suprathreshold tetanus, nor did (+)MCPG have any effect on the small amount of LTP that was generated by a just suprathreshold tetanus. Based on our studies, we have been unable to identify a role for mGluRs in mossy fiber LTP.

Keywords—Long-term potentiation (LTP), metabotropic glutamate receptors (mGluRs), mossy fibers, hippocampus, protein kinase C.

Although the discovery of glutamate receptors that couple to GTP binding proteins (mGluRs) is relatively recent, molecular cloning and pharmacological studies have provided considerable insight into this class of glutamate receptor (Pin and Duvosin, 1995; Watkins and Collingridge, 1994). These receptors can occur presynaptically and when activated by exogenous agonists inhibit the release of transmitter. They can also occur postsynaptically and when activated produce a slow blockade of potassium channels. However, the physiological roles for this class of receptor remain poorly understood.

Based on experiments utilizing mGluR antagonists, it has been proposed that these receptors play an essential role in mossy fiber LTP in the hippocampus. Specifically, it has been reported that the mGluR antagonist AP3 partially antagonized mossy fiber LTP (Ito and Sugiyama, 1991) and that (+)MCPG completely blocked mossy fiber LTP (Bashir *et al.*, 1993). Although results from our laboratory confirmed that (+)MCPG was an effective mGluR antagonist, we found that it had no effect on mossy fiber LTP (Manzoni *et al.*, 1994). More recently, it has been reported that in mice in which the mGluR1 subtype of receptor had been deleted, mossy fiber LTP was observed in only 1 out of 11 animals compared to 8 out of 10 wild-type animals (Conquet *et al.*, 1994). The mGluR1 subtype of receptor can couple to PI turnover and activate protein kinase C (PKC) and one might expect that disruption of PKC activity would interfere with the functioning of this subtype of receptor. One of the most prevalent isoforms of PKC in the brain is the gamma isoform. Mice in which this isoform had been deleted have been reported to display defects in NMDA receptor-dependent LTP in the CA1 region of the

*Present address: Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812, Japan

†To whom correspondence should be addressed.

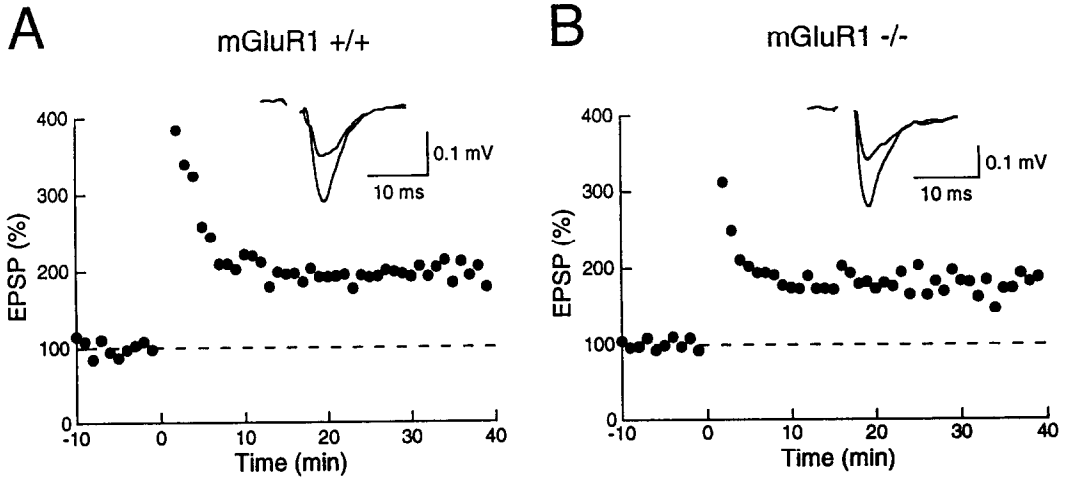

Fig. 1. Mice lacking mGluR1 display normal mossy fiber LTP. (A) A typical experiment using extracellular field recordings illustrates the time course and magnitude of mossy fiber LTP in a 6-week-old wild-type mouse. Sample superimposed traces before and after LTP induction are shown above the plot. (B) A typical experiment from a 6-week-old mGluR1 mutant mouse shows LTP of similar time course and magnitude to that of the wild-type. Each sample trace is an average of 30 individual responses. LTP was induced in the presence of DL-APV (100 μM).

hippocampus (Abeliovich *et al.*, 1993). Mossy fiber LTP, on the other hand, has not been examined in these mice. We have therefore examined mossy fiber LTP in mice lacking either the mGluR1 subtype of receptor (Aiba *et al.*, 1994) or the gamma isoform of PKC (Abeliovich *et al.*, 1993). We also performed experiments using (+)MCPG to determine if mGluRs might control the magnitude of and/or threshold for the induction of mossy fiber LTP.

METHODS

Slice preparation and extracellular field potential recordings were performed using methods similar to those described previously by this laboratory (Castillo *et al.*, 1994). For the experiments on mGluR1 mutants, the investigator was completely blind to the phenotype of the mice throughout both experimentation and analysis. Given the obvious motor impairment of the mutants, all mice were first anesthetized before being presented to the investigator. Wild-type and mutant mice were randomly presented to the investigator, and it was only after the completion of all experiments that the animals were divided into two groups for analysis. Then, after the time course of LTP for the two groups was plotted, the genotypes were revealed to the investigator. The age of these animals varied from 4 to 6 weeks and wild-type littermates served as controls. The experiments on the PKCγ mutants were not done in a blind fashion. These animals were $\sim$2 months old and wild-type littermates served as controls. For all of the experiments on mice, the genotypes were verified by PCR analysis. LTP was induced with a single, 100 Hz tetanus lasting one second in these animals. The experiments in which the effects of (+)MCPG were examined on mossy fiber LTP were all done in the guinea pig because mossy fiber responses are much easier to record and less prone to contamination than in the mouse. The responses during post-tetanic potentiation (the first minute following the tetanus) were not included in the graphs. DL-2-amino-5-phosphonovaleric acid (DL-APV) (100 μM) or D-APV (25–50 μM) (Tocris) was always present during the tetanus used to induce mossy fiber LTP. All summary data are presented as means $\pm$ SEM. (+)MCPG (Tocris) and 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylate (ACPD) (Tocris) were made up as a 100 mM stock in 100 mM NaOH. CNQX (20 μM) was applied at the end of each experiment and the remaining fiber volley was subtracted from all records.

RESULTS

Mossy fiber LTP is normal in mGluR1 mutant mice

Typical LTP experiments, one from a wild-type mouse and one from an mGluR1 mutant mouse, are shown in Fig. 1. Sample field potential records from these experiments, in which the responses before and after LTP are superimposed, are shown above the graphs. A summary graph of the results from all of the experiments is shown in Fig. 2. As can be seen from the graph, no difference could be found in the magnitude or time course of the LTP recorded in these two groups of mice. LTP was observed in 6 of 6 slices in 5 mGluR1 mutant mice, and in 7 of 7 slices in 4 wild-type mice. The average LTP, measured at 30–40 min was 234 $\pm$ 28% for the mGluR mutant mice and 222 $\pm$ 38% for the wild-type mice. These values were not significantly different (P>0.5). These results, contrary to a recent report (Conquet *et al.*, 1994), suggest that the mGluR1 subtype of receptor is not essential for mossy fiber LTP.

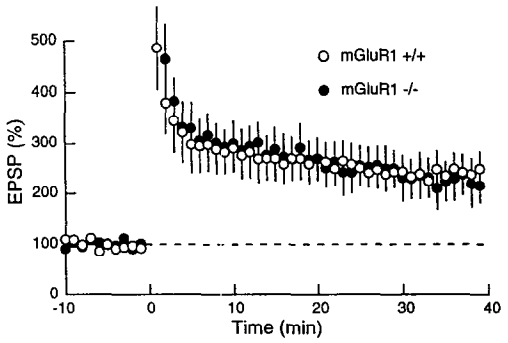

Fig. 2. Mossy fiber LTP is indistinguishable between wild-type and mGluR1 mutant mice. The graph represents a summary of experiments with 7 slices from 4 wild-type mice (○) and 6 slices from 5 mGluR1 mutant mice (●). Both the time course and magnitude of the LTP are quite similar. LTP of greater than 40%, measured at 30 min, was observed in all slices. Experiments were performed blind throughout the course of the study, on mice ranging from 4 to 6 weeks of age.

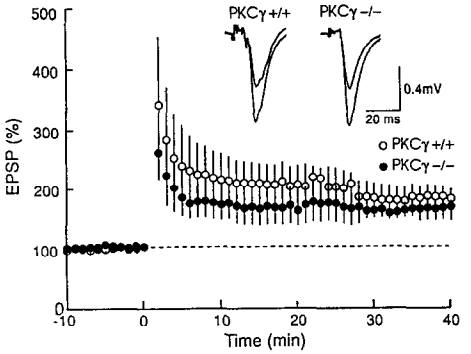

Fig. 3. Deletion of the gamma isoform of PKC has no effect on mossy fiber LTP. Normalized mossy fiber field potentials are plotted against time. The tetanus was given at time 0. In the inset, averaged records of mossy fiber responses before and about 30 min after LTP are superimposed from a PKCγ mutant mouse and a wild-type mouse. The same amount of potentiation was obtained in slices from wild-type mice (○, n=3) and from PKCγ mutant mice (●, n=3). LTP was induced in the presence of 50 μM D-APV.

Mossy fiber LTP is normal in PKCγ mutant mice

We next examined the possibility that the gamma isoform of PKC might play a role in mossy fiber LTP, either related or unrelated to mGluR activation. A summary of the experiments is shown in Fig. 3. LTP was observed in all 3 slices taken from 3 mutant mice and in all 3 slices taken from 3 wild-type mice. The average LTP measured at 30–40 min was 162±8% for the PKC mutant mice and 184±17% for the wild-type mice. These values were not significantly different (P>0.3). Since the mGluR1 subtype of receptor can activate PKC, these results complement the results with the mGluR1 mutant mice.

Pharmacological blockade of mGluRs does not affect the induction of mossy fiber LTP

Although these results suggest that neither the mGluR1 subtype of receptor nor the gamma isoform of PKC is essential for mossy fiber LTP, they do not exclude a modulatory role for metabotropic glutamate receptors. It is well established that presynaptic inhibitory metabotropic glutamate receptors are present on the terminals of mossy fibers (Lanthorn *et al.*, 1984; Manzoni *et al.*, 1995; Yamamoto *et al.*, 1983). We therefore considered the possibility that synaptically-released glutamate during a tetanus might act on these presynaptic receptors and raise the threshold for the induction of mossy fiber LTP. Such a control mechanism has been shown for synaptically released dynorphin from mossy fibers which acts on presynaptic kappa receptors to inhibit LTP (Weisskopf *et al.*, 1993). To test this possibility, we used two different stimulus protocols in guinea pig hippocampal slices. First, we used a tetanus (25 Hz lasting 1 sec) that was just above threshold for generating LTP, but well below that needed to saturate LTP. The magnitude of LTP in control conditions was compared to that observed in the presence of the mGluR antagonist (+)MCPG (0.5–1 mM) (Fig. 4). As can be seen in the figure, (+)MCPG did not enhance the magnitude of mossy fiber LTP, as might be expected if the release of glutamate during the tetanus were to activate presynaptic inhibitory mGluRs. Superimposed on this graph is a series of interleaved experiments in which saturated LTP was induced (25 Hz for 2 sec).

In the second set of experiments, the tetanus was adjusted so that it was just below the threshold for inducing LTP. An example of one of these experiments is shown in Fig. 5(A). After delivering a tetanus and verifying that LTP had not occurred, presynaptic mGluRs were activated with ACPD. (+)MCPG was then applied and shown to antagonize the action of ACPD. The same tetanus was then repeated. As can be seen, (+)MCPG did not facilitate the induction of mossy fiber LTP. At the end of the experiment, the duration of the tetanus was doubled to ensure that the tetani used in these experiments were near threshold. Considerable effort was made to ensure that the original tetanus was indeed just below threshold; specifically, all experiments in which the longer duration tetanus failed to give LTP were rejected. Sample records from this experiment are shown in Fig. 5(B), and a summary graph of all experiments is shown in Fig. 5(C).

DISCUSSION

The role of metabotropic glutamate receptors in mossy fiber LTP is controversial. Based on the use of mGluR agonists and the weak antagonist AP3, which exerted direct depressant effects, it was suggested that activation of mGluRs was involved in mossy fiber LTP (Ito and Sugiyama, 1991). More recently it was reported that the selective mGluR antagonist (+)MCPG completely blocked mossy fiber LTP (Bashir *et al.*, 1993). In a previous study, we were unable to detect any reduction in

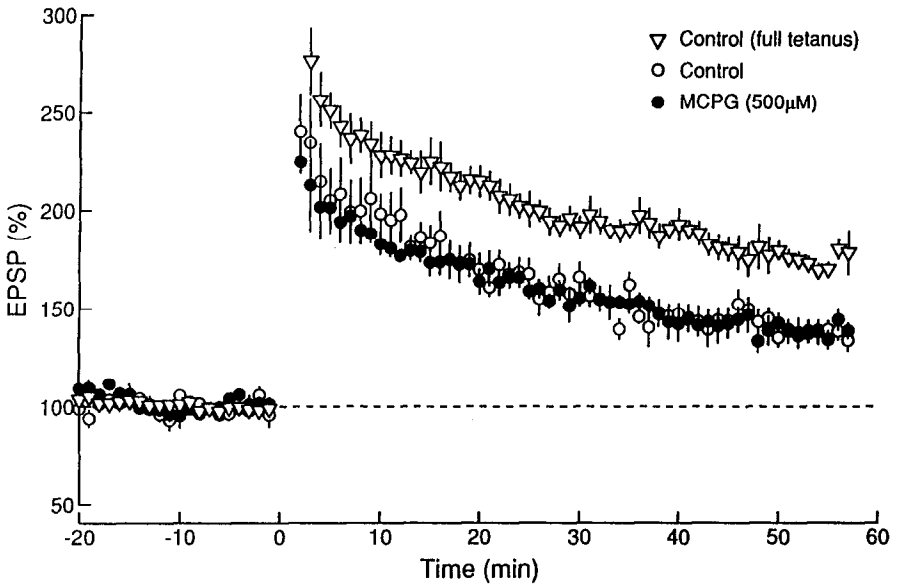

Fig. 4. (+)MCPG does not enhance the magnitude of mossy fiber LTP. Mossy fiber field potentials are plotted against time. Two slices were monitored simultaneously using two different recording chambers. One group of slices was tetanized after a 10 min application of 0.5–1.0 mM (+)MCPG (n=6, ●), and the other group served as the control (n=6, ○). The tetanus, 1 sec at 25 Hz, was just above threshold for LTP induction and was given in the presence of 25 mM D-APV. There was no enhancement in the magnitude of mossy fiber LTP in the presence of (+)MCPG. A tetanus of twice the duration (2 sec at 25 Hz), in a different set of control slices obtained during the course of these experiments, induced saturated LTP (n=6,).

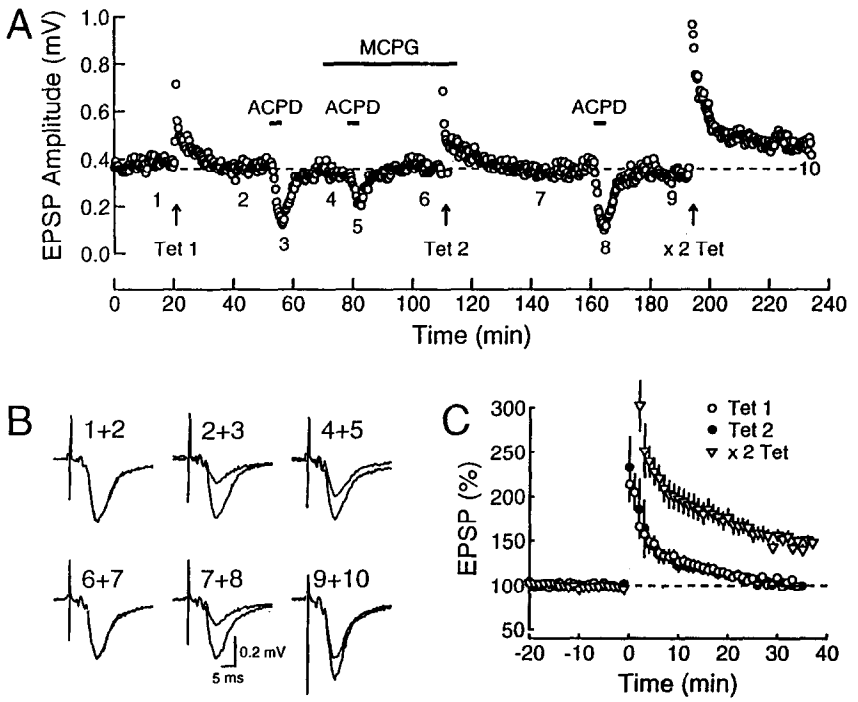

Fig. 5. (+)MCPG does not facilitate the induction of mossy fiber LTP. (A) A single representative experiment is depicted. A tetanus just below threshold for inducing LTP (0.5 sec at 25 Hz) was delivered in control Ringer's solution (Tet 1) and after 0.5 mM (+)MCPG (Tet 2). The effect of (+)MCPG was tested by antagonizing the ACPD (10 μM) inhibition of synaptic transmission. No LTP was induced in the presence of (+)MCPG, whereas a subsequent doubling of the tetanus duration (1 sec at 25 Hz) did induce LTP in the same slice (× 2 Tet). All tetani were given in the presence of 25 μM D-APV. (B) Sample records from the experiment illustrated in A. Each record is an average of 10 responses. (C) Summary graph (n=5), using the same protocol as in A.

mossy fiber LTP by (+)MCPG (Manzoni *et al.*, 1994), despite the fact that (+)MCPG clearly antagonized the presynaptic inhibitory actions of mGluR agonists (Manzoni *et al.*, 1994, 1995). In the present study, we have confirmed the inability of (+)MCPG to antagonize LTP by using tetani that are far weaker than those necessary to saturate LTP.

A recent study has reported that mossy fiber LTP is markedly impaired in mice in which the mGluR1 subtype of receptor was deleted (Conquet *et al.*, 1994). We have therefore compared the incidence and magnitude of LTP in mGluR1 mutant mice and wild-type mice, and we found no difference in the LTP between these two groups of mice. We have no obvious explanation for the discrepancy between our results and the recently published results. However, it should be pointed out that our mGluR1 mutant mice (Aiba *et al.*, 1994) and those used by Conquet and his collaborators (Conquet *et al.*, 1994) were generated independently and they are not identical with respect to the genetic manipulation applied to the mGluR1 gene and the expression of the gene product. For instance, our mGluR1 mutant mice give no detectable mGluR1 polypeptide chains (Aiba *et al.*, 1994), while a fusion protein containing part of mGluR1 is detectable in the mice of Conquet *et al.* (1994). The apparent discrepancy in the electrophysiological properties of the two mutant mice is not restricted to mossy fiber LTP; while Aiba *et al.* (1994) reported that LTP in the CA1 Schaffer collateral synapses is reduced, Conquet *et al.* (1994) reported it is normal in their mutant mice. It remains to be seen whether these discrepancies are due to the difference in the mutant strains or to the specific experimental conditions employed.

The mGluR1 subtype of receptor is known to activate PKC, and the gamma isoform of PKC is one of the most abundant isoforms in brain. We therefore searched for a possible role of this isoform in LTP by comparing LTP in wild-type and PKCγ mutant mice. We found no difference in the LTP in these two groups of mice as well.

In a final series of experiments, we tested whether mGluRs might exert an inhibitory effect on the induction of mossy fiber LTP. In these experiments, we tested if the mGluR antagonist (+)MCPG could convert a tetanus that was subthreshold for generating LTP into a suprathreshold tetanus, or if this antagonist could enhance the magnitude of LTP evoked by a tetanus that was just above threshold. In neither of these sets of experiments were we able to show that (+)MCPG influenced either the ability to obtain LTP or the magnitude of LTP.

In summary, we have used a number of approaches to determine if mGluRs play a role in the induction or expression of mossy fiber LTP. In our hands mossy fiber LTP was unaltered in mice lacking the mGluR1 subtype receptor. We have also found that mossy fiber LTP was normal in mice in which the gamma isoform of PKC had been deleted. Further experiments in which other mGluRs, which are known to be present on mossy fiber terminals (Manzoni *et al.*, 1995), are deleted would be of value. Finally, in experiments with the mGluR antagonist (+)MCPG, we were unable to find any role of mGluRs in controlling the magnitude of or threshold for the induction of mossy fiber LTP. (+)MCPG is known to block all three classes of mGluRs; however, its actions have not yet been described for all known mGluR subtypes within each of these classes. Therefore, more potent and broader spectrum antagonists would be important in either establishing or rejecting a role for mGluRs in mossy fiber LTP. The present experiments are consistent with a model for mossy fiber LTP that is dependent on calcium entry into the presynaptic terminal, but is independent of any known glutamate receptor (Castillo *et al.*, 1994).

Acknowledgement—This research was supported by the NIMH (R.A.N.) and the Howard Hughes Medical Institute at the Massachusetts Institute of Technology (S.T., A. Aiba), the Shionogi Institute for Medical Science (S.T., A. Abeliovich), and by a National Institutes of Health grant RO1 NS32925 (S.T.). R.A.N. is a member of the Keck Center for Integrative Neuroscience and the Silvio Conte Center for Neuroscience Research.

REFERENCES

Abeliovich A., Chen C., Goda Y., Silva A. J., Stevens C. F. and Tonegawa S. (1993) Modified hippocampal long-term potentiation in PKCγ-mutant mice. *Cell* **75:** 1253–1262.

Aiba A., Chen C., Herrup K., Rosenmund C., Stevens C. F. and Tonegawa S. (1994) Reduced hippocampal long-term potentiation and context-specific deficit in associative learning in mGluR1 mutant mice. *Cell* **79:** 365–375.

Bashir Z. I., Bortolotto Z. A., Davies C. H., Berretta N., Irving A. J., Seal A. J., Henley J. M., Jane D. F., Watkins J. C. and Collingridge G. L. (1993) Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. *Nature* **363:** 347–350.

Castillo P. E., Weisskopf M. G. and Nicoll R. A. (1994) The role of Ca^{2+} channels in hippocampal mossy fiber synaptic transmission and long-term potentiation. *Neuron* **12:** 261–269.

Conquet F., Bashir Z. I., Davies C. H., Daniel H., Ferraguti F., Bordi F., Franz-Bacon K., Reggiani A., Matarese V., Condé F., Collingridge G. L. and Crépel F. (1994) Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1. *Nature* **372:** 237–243.

Ito I. and Sugiyama H. (1991) Roles of glutamate receptors in long-term potentiation at hippocampal mossy fiber synapses. *NeuroReport* **2:** 333–336.

Lanthorn T. H., Ganong A. H. and Cotman C. W. (1984) 2-Amino-4-phosphonobutyrate selectively blocks mossy fiber-CA3 responses in guinea pig but not rat hippocampus. *Brain Res.* **290:** 174–178.

Manzoni O. J., Weisskopf M. G. and Nicoll R. A. (1994) MCPG antagonizes metabotropic glutamate receptors but not long-term potentiation in the hippocampus. *Eur. J. Neurosci.* **6:** 1050–1054.

Manzoni O. J., Castillo P. E. and Nicoll R. A. (1995) Pharmacology of metabotropic glutamate receptors at the

mossy fiber synapses of the guinea pig hippocampus. *Neuropharmacology*. **34:** 965–971.

Pin J.-P. and Duvosin R. (1995) The metabotropic glutamate receptors: structure and functions. *Neuropharmacology* **34:** 1–26.

Watkins J. C. and Collingridge G. L. (1994) Phenylglycine derivatives as metabotropic glutamate receptor antagonists. *Trends Pharmac. Sci.* **15:** 333–342.

Weisskopf M. G., Zalutsky R. A. and Nicoll R. A. (1993) The opioid peptide dynorphin mediates heterosynaptic depression of hippocampal mossy fiber synapses and modulates long-term potentiation. *Nature* **362:** 423–427.

Yamamoto C., Sawada S. and Takada S. (1983) Suppressing action of 2-amino-4-phosphonobutyric acid on mossy fiber-induced excitation in the guinea pig hippocampus. *Exp. Brain Res.* **51:** 128–134.