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Induction of NMDA Receptor-Dependent Long-Term Depression in Visual Cortex Does Not Require Metabotropic Glutamate Receptors

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Sawtell, Nathaniel B., Kimberly M. Huber, John C. Roder, and Mark F. Bear. Induction of NMDA receptor-dependent long-term depression in visual cortex does not require metabotropic glutamate receptors. *J. Neurophysiol.* 82: 3594–3597, 1999. We tested the role of group I mGluRs in the induction of long-term depression (LTD) in the visual cortex, using the novel mGluR antagonist LY341495 and mice lacking mGluR5, the predominant phosphoinositide (PI)-linked mGluR in the visual cortex. We find that LY341495 is a potent blocker of glutamate-stimulated PI hydrolysis in visual cortical synaptoneurosome, and that it effectively antagonizes the actions of the mGluR agonist 1S,3R-aminocyclopentane-1,3-dicarboxylic acid (ACPD) on synaptic transmission in visual cortical slices. However, LY341495 has no effect on the induction of LTD by low-frequency stimulation. Furthermore, mice lacking mGluR5 show normal NMDA receptor-dependent LTD. These results indicate that group I mGluR activation is not required for the induction of NMDA receptor-dependent LTD in the visual cortex.

INTRODUCTION

Brief monocular deprivation during early postnatal life causes a long-term depression (LTD) of synaptic transmission that renders visual cortical neurons unresponsive to stimulation of the deprived eye. One mechanism for deprivation-induced LTD in visual cortex is triggered by the residual activity arising from the deprived eye (Rittenhouse et al. 1999). Brain slice preparations have been established to understand how presynaptic activity can cause synaptic depression in visual cortex. One reliable method for inducing homosynaptic LTD in vitro is prolonged low-frequency synaptic stimulation (LFS) (Kirkwood et al. 1993). Although it is clear that activation of postsynaptic glutamate receptors is required for LFS-induced LTD in visual cortex, there are contradictory data on which receptor subtypes are involved.

Metabotropic glutamate receptors coupled to phosphoinositide (PI) metabolism (group I mGluRs) provide an attractive candidate mechanism for homosynaptic LTD (Bear and Dudek 1991). These receptors are essential for LTD in the cerebellar cortex (Linden and Connor 1993) and are highly expressed in the visual cortex during the postnatal period when visual deprivation induces LTD (Dudek and Bear 1989). Tests of mGluR involvement in visual cortical LTD have been conducted using the competitive antagonist α -methyl-4-carboxyphenylglycine (MCPG) (Haruta et al. 1994; Hensch and

Stryker 1996; Huber et al. 1998). However, interpretation of these studies is complicated by the recent finding that MCPG is an extremely weak antagonist of glutamate-stimulated PI hydrolysis in visual cortex (Huber et al. 1998). Thus the question remains whether mGluR activation is required for induction of homosynaptic LTD in visual cortex.

Here we report new tests of the hypothesis that group I mGluRs play an essential role in the induction of LTD by LFS, using a novel and potent mGluR antagonist LY341495 and mice lacking the major group I mGluR in visual cortex (mGluR5). The data show that mGluRs are not necessary for the induction of one prominent form of LTD in visual cortex.

METHODS

Phosphoinositide hydrolysis assays

Synaptoneurosome were prepared from rat visual cortices, and PI hydrolysis was measured and analyzed as described previously (Dudek et al. 1989; Huber et al. 1998). Ionotropic glutamate receptor antagonists CNQX (40 μ M) and D,L-AP5 (200 μ M) were included in the reactions to ensure that the glutamate-stimulated PI hydrolysis was due to activation of mGluRs.

Electrophysiology

Visual cortical slices were prepared as described previously (Huber et al. 1998). Slices were allowed to recover for 1–2 h at room temperature (rat) or 30°C (mouse) in artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 5 KCl, 1.25 NaH_2PO_4 , 26 NaHCO_3 , 1 MgCl_2 , 2 CaCl_2 , and 10 dextrose. ACSF was continuously saturated in 95% O_2 , 5% CO_2 . For recordings of synaptic transmission, visual cortical slices were placed in a submersion recording chamber, maintained at 30°C, and perfused with ACSF at a rate of 2 ml/min. Extracellular electrodes (filled with ACSF, 1.0 M Ω) were placed in layer II/III to monitor field potentials (FPs) evoked with a stimulating electrode (concentric bipolar tungsten) placed at the border of layer IV and upper layer V. The amplitude of the maximum negative FP was used to quantify changes in synaptic responses. Stable baseline responses were elicited at a rate of 1–4 per min, at 50–60% of the maximal response. LFS consisted of 900 pulses at 1 Hz. Data were averaged and analyzed as described previously (Huber et al. 1998). All experiments using mice were performed blind to genotype. Genotyping was done by Therion Corp.

Drug preparation

Glutamate and 1S,3R-aminocyclopentane-1,3-dicarboxylic acid (ACPD) were obtained from Tocris Cookson (St. Louis, MO). LY341495 was a generous gift from Eli Lilly. Compounds were

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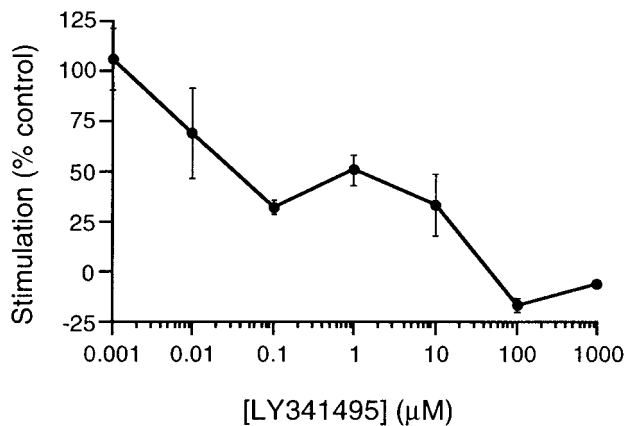


FIG. 1. LY341495 inhibits glutamate-stimulated phosphoinositide (PI) hydrolysis in visual cortical synaptoneurosomes. Effects of increasing LY341495 concentrations on PI hydrolysis stimulated by 200 μ M glutamate ($IC_{50} < 1$ μ M; $n = 4$).

dissolved in equimolar NaOH or H₂O aliquoted and stored at -20°C for no more than 1 wk.

RESULTS

The first series of experiments was designed to determine the effectiveness of LY341495 as an antagonist of group I mGluRs in the visual cortex. LY341495 has micromolar potency against quisqualate-stimulated PI hydrolysis in cell lines expressing mGluR1 and mGluR5 (Kingston et al. 1998), and against PI hydrolysis stimulated by the selective group I agonist (R,S)-3,5-dihydroxyphenylglycine (DHPG) in rat hippocampal slices (Fitzjohn et al. 1998). However, in light of

previous findings that the mGluR antagonist MCPG was effective against ACPD, but not glutamate-stimulated PI hydrolysis (Huber et al. 1998), we felt that it was crucial to verify the efficacy of the antagonist against the endogenous agonist. To address this question we examined the effects of LY341495 on glutamate-stimulated PI hydrolysis in synaptoneurosomes prepared from the visual cortex of postnatal day (P) 21–28 rats.

The rate of PI hydrolysis is determined in this assay by measuring the inositol monophosphate (IP₁) that is generated in the continuous presence of agonist (Gusovsky and Daly 1988). IP₁ accumulation was measured after a 90 min incubation of synaptoneurosomes in 200 μ M glutamate, the EC_{50} value in this preparation (Huber et al. 1998), \pm increasing concentrations of LY341495. The data confirm that LY341495 is an effective antagonist of glutamate-stimulated PI hydrolysis (Fig. 1; $IC_{50} < 1$ μ M; $n = 4$). LY341495 (100 μ M) was sufficient to completely block PI hydrolysis stimulated by 200 μ M glutamate. Therefore 100 μ M LY341495 was used in the subsequent electrophysiological experiments in slices.

Electrophysiological experiments were conducted using slices prepared from P21–30 rats, an age when glutamate-stimulated PI hydrolysis is significantly greater than in adults (Dudek et al. 1989) and within the critical period for experience-dependent visual cortical plasticity (Fagioli et al. 1994). To confirm that LY341495 is effective in visual cortical slices, we investigated the ability of the drug to block the effects of the mGluR agonist ACPD on synaptic transmission. As previously reported in hippocampal (Baskys and Malenka 1991; Selig et al. 1995) and visual cortex slices (Huber et al. 1998), brief application of ACPD (5 min; 10 μ M) rapidly and reversibly attenuated amplitudes of synaptically evoked FPs (Fig. 2).

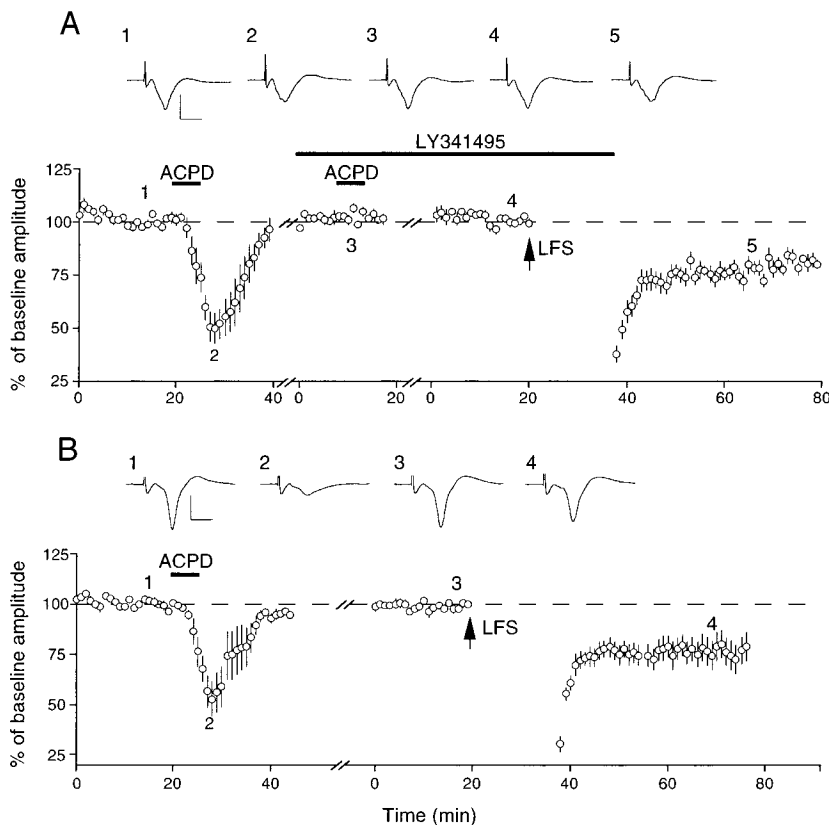
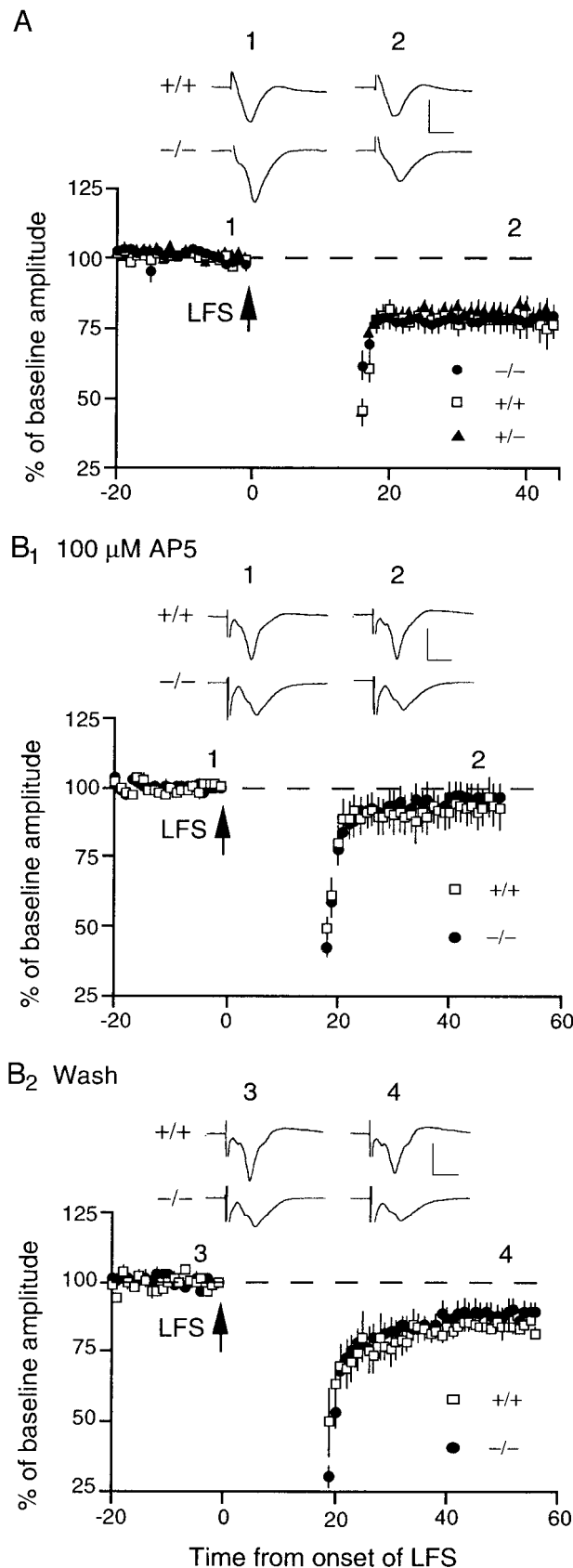


FIG. 2. LY341495 antagonizes the synaptic depression caused by 1S,3R-aminocyclopentane-1,3-dicarboxylic acid (ACPD) but does not affect long-term depression (LTD) in visual cortical slices from P21–P30 rats. *A*: application of ACPD (10 μ M; 5 min) reduced FP amplitudes ($53 \pm 8\%$ of baseline values); subsequent application of ACPD in the presence of LY341495 (100 μ M) did not ($102 \pm 2\%$ of baseline values), showing that mGluRs were antagonized by the drug. Continued application of LY341495 (100 μ M) ≥ 20 min before and during the low-frequency synaptic stimulation (LFS) did not affect the magnitude of LTD induced in the same slices. *B*: application of ACPD (10 μ M; 5 min) reduced FP amplitudes ($59 \pm 9\%$ of baseline values) in control slices. The LTD induced subsequently by LFS ($78 \pm 7\%$ of pre-LFS baseline; $n = 6$) did not differ from that evoked in the presence of LY341495. Traces here and in subsequent figures are averages of four consecutive FPs taken at the times indicated by the numbers (1–5) on the graphs. Calibration: 1 mV, 5 ms.



Preapplication of LY341495 (100 μ M) completely blocked the effects of ACPD on synaptic transmission (Fig. 2A).

In these same slices, we examined the consequences of LFS given in the presence of LY341495 (Fig. 2A). The magnitude of LTD was unaffected by the drug treatment ($78 \pm 4\%$ of pre-LFS baseline amplitude values; $n = 7$), compared with control experiments in which LFS was delivered after testing the effects of ACPD, but in the absence of LY341395 ($78 \pm 7\%$; $n = 6$; $P > 0.5$; Fig. 2B). These results indicate that LY341495 is an effective antagonist of glutamate-stimulated PI hydrolysis and ACPD-induced synaptic depression, but that it has no effect on LTD induced with LFS in visual cortex slices under our experimental conditions.

In visual cortex and hippocampus, glutamate stimulates PI hydrolysis in postsynaptic neurons via the activation of mGluR5 (Abe et al. 1992; Romano et al. 1995; Testa et al. 1994). Therefore to further test the hypothesis that postsynaptic group I mGluR activation is required for induction of homosynaptic LTD, we investigated the effects of LFS in visual cortex of mice (P21–33) with a null mutation of mGluR5 (Lu et al. 1997).

FP latency, FP half-maximal amplitude, and the stimulus intensity required to elicit half-maximal responses did not differ between wild-type (WT) mice and mGluR5 mutants (data not shown). Similarly, mutant and WT mice displayed no differences in LTD magnitude following LFS ($-/-$, $83 \pm 4\%$, $n = 14$ slices, 7 animals; $+/-$, $81 \pm 4\%$, $n = 16$ slices, 5 animals; WT, $84 \pm 9\%$, $n = 15$ slices, 7 animals; Fig. 3A).

In the rat visual cortex, LTD induction with LFS requires activation of postsynaptic NMDA receptors under our experimental conditions (Kirkwood and Bear 1994). Therefore we tested the effects of the NMDA-receptor antagonist DL-2-amino-5-phosphonovaleric acid (AP5) on LTD induction in the visual cortex of wild-type and mutant mice (Fig. 3B). In the presence of 100 μ M AP5, no significant LTD was induced by LFS regardless of genotype (WT, $93 \pm 7\%$, $n = 9$, $P > 0.1$; $-/-$, $96 \pm 5\%$, $n = 13$, $P > 0.5$). After washout of the AP5, stable LTD was induced in the same slices by a second LFS (WT, $84 \pm 1\%$, $n = 5$, $P < 0.005$; $-/-$, $89 \pm 4\%$, $n = 9$, $P < 0.05$).

Together the results confirm that LFS triggers LTD in visual cortex via activation of NMDA receptors and demonstrate that group I mGluR activation is not required for induction of this form of homosynaptic LTD.

DISCUSSION

Our data show that neither pharmacological blockade of mGluRs nor genetic ablation of mGluR5 disrupts LTD induced by LFS in slices of visual cortex. Although contrary to some earlier reports using MCPG in visual cortex (Haruta et al. 1994; Hensch and Stryker 1996), these results are consistent with a recent study in which LY341495 did not affect NMDA receptor-dependent LTD in the CA1 region of the hippocampus (Fitzjohn et al. 1998). LTD in CA1 hippocampus is also

FIG. 3. NMDA receptor-dependent LTD is induced by LFS in mutant mice lacking mGluR5. **A**: LTD magnitudes 30 min after LFS were indistinguishable in WT and mutant mice. **B₁**: LTD fails to occur in WT and mutant visual cortex when LFS is delivered in the presence of 100 μ M AP5. **B₂**: LTD can be induced in the same slices following washout (>1 h) of AP5. Data are expressed as percentage of the baseline prior to the second LFS. Calibration: 1 mV, 5 ms.

normal in mGluR5 knock out mice (Huber and Bear 1998). We believe that the controversy surrounding the role of mGluRs in synaptic plasticity has been largely attributed to the questionable efficacy of MCPG (Huber et al. 1998) and the fact that multiple forms of LTD can coexist at the same synapses (Oliet et al. 1997). The results presented here, using effective pharmacological blockade and gene knock out technology, are conclusive evidence that mGluR activation is not required for induction of at least one form of LTD in the visual cortex. We cannot rule out the possibility that a second, mGluR-dependent form of LTD also exists in visual cortex, which might be revealed under different experimental conditions. However, if such a form of LTD does exist in visual cortex, it is mechanistically distinct from the NMDA receptor-dependent form.

In a recent study, the contributions to visual cortical plasticity of group I mGluRs and the mechanisms of homosynaptic LTD were questioned on the basis of findings that infusion of MCPG failed to prevent the LTD caused by monocular deprivation in visual cortex in vivo but did block the synaptic depression caused by LFS in slices (Hensch and Stryker 1996). However, the conclusions of that study must be reevaluated in light of the evidence that 1) MCPG has little effect on glutamate-stimulated PI turnover mediated by group I mGluRs (Brabet et al. 1995; Huber et al. 1998), and 2) neither MCPG (Huber et al. 1998) nor blockade of mGluRs (present results) inhibit induction of the NMDA receptor-dependent form of LTD. In future studies it will be important to use this knowledge to dissect the individual and combined roles of homosynaptic LTD and mGluRs in visual cortical plasticity.

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