Cellular/Molecular

Developmental Switch in Synaptic Mechanisms of Hippocampal Metabotropic Glutamate Receptor-Dependent Long-Term Depression

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The presynaptic and postsynaptic properties of synapses change over the course of postnatal development. Therefore, synaptic plasticity mechanisms would be expected to adapt to these changes to facilitate alterations of synaptic strength throughout ontogeny. Here, we identified developmental changes in long-term depression (LTD) mediated by group 1 metabotropic glutamate receptors (mGluRs) and dendritic protein synthesis in hippocampal CA1 slices (mGluR-LTD). In slices prepared from adolescent rats [postnatal day 21 (P21) to P35], mGluR activation induces LTD and a long-term decrease in AMPA receptor (AMPA) surface expression, both of which require protein synthesis. In neonatal animals (P8–P15), mGluR-LTD is independent of protein synthesis and is not associated with changes in the surface expression of AMPARs. Instead, mGluR-LTD at neonatal synapses results in large decreases in presynaptic function, measured by changes in paired-pulse facilitation and the rate of blockade by the use-dependent NMDA receptor blocker (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate. Conversely, mGluR-LTD at mature synapses results in little or no change in presynaptic function, suggesting a postsynaptic mechanism of expression. The developmental switch in the synaptic mechanisms of LTD would differentially affect synapse dynamics and perhaps information processing over the course of postnatal development.

Key words: metabotropic glutamate receptor; long-term depression; protein synthesis; AMPA receptor endocytosis; hippocampus; CA1

Introduction

There is growing evidence that both the presynaptic and postsynaptic properties of synapses change over the course of postnatal development. Nascent synapses are characterized by high presynaptic release probability and few postsynaptic AMPA receptors (AMPARs). Synapse maturation is accompanied by the acquisition of AMPARs and decreases in neurotransmitter release probability (Bolshakov and Siegelbaum, 1995; Pouzat and Hestrin, 1997; Liao et al., 1999; Petralia et al., 1999; Reyes and Sakmann, 1999; Pickard et al., 2000; Wasling et al., 2004) (but see Dumas and Foster, 1995; Hsia et al., 1998). For developing synapses to remain plastic over the course of synapse maturation, plasticity mechanisms must adapt with these changing synaptic properties. Indeed, there is evidence for changes in the mechanisms of long-term potentiation during synapse development (Yasuda et al., 2003). Here, we demonstrate that both the synaptic and molecular mechanisms of synaptic long-term depression (LTD) change over the course of synapse maturation in area CA1 of the rat hippocampus. Specifically, we examined the developmental changes of LTD, which rely on group 1 metabotropic glutamate receptors (mGluRs) and dendritic protein synthesis (mGluR-LTD) (Huber et al., 2000). This work was motivated by findings that group 1 mGluR function (measured as phosphoinositide turnover) and synaptic polyribosome number peak during the period of synapse formation and maturation [approximately postnatal day 7 (P7) to P15 in the hippocampus], suggesting that mGluR-LTD plays a significant role in the plasticity of developing synapses (Steward and Falk, 1985, 1991; Nicoletti et al., 1986; Dudek et al., 1989; Palmer et al., 1990; Casabona et al., 1997).

The developmental regulation and site of expression of mGluR-LTD in the hippocampus has been controversial. Some studies suggest that mGluR-LTD is only observed at early developmental time periods in area CA1 (P7–P11) (Bolshakov and Siegelbaum, 1994; Overstreet et al., 1997). However, others indicate that mGluR-LTD is restricted to adult animals (Kemp et al., 2000). There is general agreement that mGluR-LTD is induced postsynaptically (Bolshakov and Siegelbaum, 1994; Oliet et al., 1997; Watabe et al., 2002). However, many studies of mGluR-LTD of immature neurons (P4–P11) conclude that mGluR-LTD is expressed presynaptically using both electrophysiological and optical methods (Bolshakov and Siegelbaum, 1994; Oliet et al., 1997; Fitzgerald et al., 2001; Zakharenko et al., 2002; Feinmark et al., 2003; Ramires et al., 2003).

Evidence for a postsynaptic mechanism of mGluR-LTD expression comes from studies demonstrating that the selective group 1 mGluR agonist R,S-dihydroxyphenylglycine (DHPG), which induces LTD, results in endocytosis and a persistent de-
crease in postsynaptic AMPArS and NMDA receptors (NMDARs), which requires protein synthesis (Snyder et al., 2001; Xiao et al., 2001). DHPG-induced LTD is blocked by the postsynaptic injection of endocytosis and protein synthesis inhibitors (Snyder et al., 2001; Xiao et al., 2001), suggesting a postsynaptic mechanism of expression.

Here, we find a developmental switch in the protein synthesis dependence and synaptic locus of mGluR-LTD, which occurs between the second and fourth postnatal week. This switch may occur to accommodate the changing properties of synapses and may have consequences for information processing over the course of postnatal development.

Materials and Methods

Drugs. D,L-AP-5, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten5,10-imine maleate (MK801) (Tocris Cookson, Ellisville, MO), anisomycin, and picrotoxin (Sigma, St. Louis, MO) were prepared fresh in artificial CSF (ACSF). The cannabinoid 1 receptor (CB1R) antagonist 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-[4-morpholinyl]-1H-pyrazole-3-carboxamide (AM281) and R-(-)2,3-dihydro-5-methyl-3-[(mor morphinyl)methyl]pyrrolo[1,2,3-DE]-1,4-benzoxazin-6-yl]-(1-naphthylmethyl)ethane mesylate (WIN55,212-2) (Tocris Cookson) were prepared as a 1000x stocks in DMSO, used fresh or kept as stocks at −20°C, and diluted to final concentration in ACSF.

Slices were preincubated in antagonists or inhibitors for 20–30 min before DHPG or paired-pulse low-frequency stimulation (PP-LFS). The effects of all of the pharmacological treatments on LTD were evaluated by comparing interleaved control and treated slices.

Electrophysiology. Long–Evans hooded rats were obtained from Charles River Laboratories (Wilmington, MA). Hippocampal slices (400 μm) were prepared from 8- to 35-d-old rats. Rats were anesthetized with the barbiturate pentobarbital (50 mg/kg) and decapitated soon after the disappearance of corneal reflexes. The brain was removed and dissected in ice-cold dissection buffer containing the following (in mM): 2.6 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 0.5 CaCl₂, 5 MgCl₂, and then sliced using a vibratome (VT 1000S; Leica, Nussloch, Germany) after sectioning. The slices were transferred into a reservoir chamber filled with ACSF and placed in stratum radiatum of area CA1. FPs were evoked by monophasic stimulation (durations of 2–3 ms) at 150 pulses per second (30 regions) at 20°C, and diluted to final concentration in ACSF. Slices were transferred into a reservoir chamber filled with ACSF containing the following (in mM): 2.6 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 0.5 CaCl₂, 5 MgCl₂, and then eluted with SDS–PAGE sample buffer and quantified using a BCA Protein Assay Kit (Pierce). Protein (15 μg) was removed for total protein measurements. Protein (150 μg) was then mixed with 150 μl of UltraLink immobilized NeutrAvidin beads (Pierce) for 20 h at 4°C. The beads were washed with 10 vol of RIPA buffer and then eluted with SDS–PAGE sample buffer supplemented with 50 μl dithiothreitol for 20 min at 90°C. Both total and biotinylated proteins were resolved by SDS–PAGE, transferred to nitrocellulose membranes, and probed with anti-GluR1 C-terminal antibody (1:5000; Upstate Biotechnology, Lake Placid, NY), anti-GluR2/3 N-terminal antibody (1:1000; Chemicon, Temecula, CA) or anti-a-actin antibody (1:1000; Chemicon), or anti-GluR6/7 (1:5000; Upstate Biotechnology). Immunoreactive bands were visualized by enhanced chemiluminescence, captured on autoradiography film (Eastman Kodak, Rochester, NY). Digital images were produced by densitometric scans of autoradiographs on a ScanJet 4300C (Hewlett Packard, Palo Alto, CA) and quantified using Scion (Frederick, MD) Image software. The surface/total protein ratio was calculated for each condition. When duplicate conditions were performed within one animal, the ratio values were averaged to obtain an animal average for that condition. Therefore, the n values for the biotinylation experiments (see Figs. 4–6) represent the number of rats, as opposed to slices. Significant differences between surface/total ratios of treated slices and within-animal control slices were determined using a paired r test (for ratio and p values, see Table 1). Although the raw ratio values were used for statistical comparisons, the group data are presented in Figures 4–6 as a percentage of condition control to compare across different treatment conditions.

Results

mGluR-LTD can be induced at immature synapses

We first determined whether mGluR-LTD can be induced pharmacologically and synaptic ly at immature synapses. Hippocampal slices were prepared from neonatal (P8–P15) or adolescent (P21–P35) rats. Extracellular field potential recordings were obtained in area CA1 and were elicited by Schaffer collateral stimulation. LTD was induced using either the group 1 mGluR agonist DHPG (100 μM; 5 min) or synaptic stimulation (900 pairs of stimulation pulses with a 50 ms interval delivered at 1 Hz;
Developmental switch in the protein synthesis dependence of mGluR-LTD

mGluR-LTD in adolescent rats (P21–P35) is dependent on dendritic protein synthesis and is independent of transcription (Huber et al., 2000). Because numerous synaptic polyribosomes are observed at developing synapses (Steward and Falk, 1985, 1991), we hypothesized that mGluR-LTD at neonatal synapses would also be protein synthesis dependent. Surprisingly, we find that mGluR-LTD in neonatal rats is not sensitive to the protein synthesis inhibitors (Fig. 2). In neonatal rat slices, DHPG induced significant LTD in slices pretreated with anisomycin (88 ± 2%; n = 10) (Fig. 2A), which was not different from LTD in interleaved control slices (83 ± 3%; n = 10; p = 0.21). The control DHPG-LTD values are the same data as those presented in Figure 1A and were replotted in Figure 2A for comparison with anisomycin-treated slices. In contrast, as reported previously, DHPG-induced LTD in adolescent rats was completely inhibited by anisomycin (100 ± 5%, n = 6; interleaved control slices, 84 ± 3%, n = 7; p = 0.01) (Fig. 2C). Similar results were observed with another protein synthesis inhibitor, cycloheximide (60 μM). DHPG-LTD was completely blocked by cycloheximide in adolescent rats (103 ± 2%, n = 4; control slices, 77 ± 5%, n = 4; p = 0.01) (Fig. 2D) and was unaffected in neonatal rats (80 ± 3%, n = 6; control slices, 81 ± 3%, n = 5; p = 0.79) (Fig. 2C). Likewise, LTD induced with synaptic stimulation (PP-LFS) only requires protein synthesis at mature synapses (P8–P15: control, 63 ± 4%, n = 6; anisomycin, 60 ± 5%, n = 4; P21–P35: control, 77 ± 3%, n = 18; anisomycin, 99 ± 4%, n = 16; p = 0.0001) (Fig. 3A, B). PP-LFS-induced LTD in neonatal rats was also insensitive to cycloheximide (62 ± 6%, n = 8; control slices, 64 ± 2%, n = 7; p = 0.01) (Fig. 3C) in contrast to adolescent rats (Huber et al., 2000).

Previous studies of mGluR-LTD in neonatal hippocampus have used 5 Hz synaptic stimulation (3 min) (Bolshakov and Siegelbaum, 1994; Bolshakov et al., 2000; Zakharenko et al., 2002). Therefore, we also examined the protein synthesis dependence of mGluR-LTD using this protocol. As in LTD induced with DHPG and PP-LFS at this age, 5 Hz-induced LTD was also insensitive to anisomycin (P8–P15: interleaved controls, 81 ± 5%, n = 7; anisomycin, 78 ± 4%, n = 7; p = 0.89).

These results indicate that, as synapses mature, mGluR-LTD becomes increasingly dependent on protein synthesis. In support of this idea, the ability of anisomycin to block PP-LFS-induced LTD is significantly correlated with postnatal age (r = 0.53; p < 0.002) (Fig. 3D).
Developmental changes in mGluR-induced decreases in AMPAR surface expression

In dissociated cultured neurons, activation of group 1 mGluRs results in a long-term decrease in the surface expression of AMPARs and NMDARs, which requires protein synthesis (Snyder et al., 2001; Xiao et al., 2001). These results indicate that the new proteins required for mGluR-LTD most likely function to control AMPAR endocytosis or trafficking. Therefore, mGluR-LTD at immature synapses may not be mediated by AMPAR internalization or there may be sufficient levels of protein(s) to maintain internalization in the absence of new synthesis.

To determine whether mGluR-dependent AMPAR endocytosis was developmentally regulated, we measured DHPG-induced changes in surface expression of AMPAR subunits GluR1 and GluR2/3. Previous studies of DHPG-induced AMPAR endocytosis have been performed in dissociated cultured hippocampal neurons (Snyder et al., 2001; Xiao et al., 2001). To correlate the developmental changes in mGluR-LTD with DHPG-induced AMPAR endocytosis in the same preparation, we performed these experiments in acute hippocampal slices using receptor biotinylation. We first verified that we could observe decreases in AMPAR surface expression in acute slices from adolescent rats. Slices were incubated in control ACSF or treated with DHPG (100 μM; 5 min). Surface receptors were labeled with biotin either 15 or 60 min after application of DHPG. Biotinylated receptors were precipitated, and the ratio of surface to total GluRs was determined by quantitative Western blotting. The biochemical analysis confirmed that DHPG treatment of hippocampal slices (P21–P35) results in a long-term (60 min) decrease in biotinylated (surface)/total ratio of GluR2/3 (15 min after DHPG, 81 ± 6% of control slices, p = 0.03, n = 7; 60 min after DHPG, 74 ± 6% of control slices, n = 6, p = 0.004) (Fig. 4A) and GluR1 (15 min after DHPG, 81 ± 4%, n = 9, p = 0.001; 60 min after DHPG, 80 ± 5%, n = 6, p = 0.018) (Fig. 4B; for raw ratio values, see Table 1). DHPG did not affect the total levels of GluR2/3 (15 min, 99 ± 3% of control, p = 0.87; 60 min, 103 ± 12%, p = 0.96) or GluR1 (15 min, 106 ± 13%, p = 0.4; 60 min, 101 ± 22%, p = 0.78). As in mGluR-LTD, the DHPG-induced decreases in surface expression were blocked by the broad-range mGluR antagonist LY341495 (100 μM; GluR1, 15 min after DHPG, 113 ± 12%, n = 6, p = 0.39; GluR2/3, 15 min after DHPG, 113 ± 3%, n = 5, p = 0.02) (Fig. 4C–D).

As observed previously in neuronal culture, DHPG-induced decreases in AMPAR surface expression in hippocampal slices were sensitive to the protein synthesis inhibitor anisomycin (20 μM). Slices preincubated in anisomycin displayed significantly reduced AMPAR surface expression at 15 min after DHPG treatment but not at 60 min after DHPG treatment (GluR2/3, 15 min after DHPG, 84 ± 6%, n = 8, p = 0.036; 60 min after DHPG, 102 ± 5%, n = 7, p = 0.97; GluR1, 15 min after DHPG, 84 ± 1%, n = 7, p = 0.0003; 60 min after DHPG, 94 ± 10%, p = 0.55, n = 9) (Fig. 4E,F).

To determine whether the effects of DHPG are specific to the AMPA subtype of receptors, we measured changes in surface
expression of kainate receptor subunits GluR6 and GluR7. In area CA1, kainate receptors are expressed primarily presynaptically on both excitatory and inhibitory synapses and postsynaptically on interneurons (Huettner, 2003). DHPG did not affect the surface expression of GluR6/7 (15 min after DHPG, 100 \(\mu\)M, 10% change, \(p = 0.77\), \(n = 6\); 60 min after DHPG, 93 \% change, \(p = 0.58\), \(n = 7\)) (Fig. 5A), indicating that DHPG causes a specific reduction in AMPAR surface expression. Additional control experiments confirmed that intracellular proteins, such as actin, were not biotinylated in this assay (Fig. 5B). Together, these experiments confirm that DHPG causes an mGluR- and protein synthesis-dependent long-term decrease in AMPAR surface expression in acute slices from adolescent rats.

In contrast to adolescent rats, DHPG treatment of slices from neonatal animals did not decrease the surface expression of GluR2/3 and GluR1 subunits (GluR2/3: 15 min after DHPG, 98 \% change, \(p = 0.8\), 60 min after DHPG, 93 \% change, \(p = 0.58\), \(n = 7\)); GluR1: 15 min DHPG, 99 \% change, \(p = 0.003\), \(n = 10\)) (Fig. 6A, B).

In addition to mGluRs, chemical activation of NMDARs induces LTD and internalization of AMPARs (Lee et al., 1998; Collledge et al., 2003). We performed additional experiments, treating neonatal slices with NMDA to determine whether there is a general deficit in activity-induced AMPAR internalization in neonatal slices or whether the deficit is specific for the mGluR pathway. We first confirmed that NMDA (20 \(\mu\)M, 3 min) induced LTD in CA1 of neonatal hippocampal slices (80 \% of baseline at 55–60 min after NMDA application; \(n = 5\)). Unlike DHPG treatment of neonatal slices, we observed decreases in surface expression of GluR1 and GluR2/3 at 10 min (GluR1, 82 \% change, \(p = 0.001\), \(n = 7\); GluR2/3, 74 \% change, \(p = 0.004\), \(n = 5\)).

Table 1. Raw ratio values from surface biotinylation experiments

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Average \(\pm\) SEM ratios of surface (biotinylated)/total protein from experiments presented in Figures 4–6. \(p\) values are from a paired \(t\) test comparing data from DHPG-treated (D) or NMDA-treated (N) samples with within-animal controls (C).
...in neonatal rat slices. A, Sample Western blot of total (T) and surface (S) GluR2/3. Quantitative data of the ratio of surface to total GluR2/3 in hippocampal slices taken either 15 or 60 min after NMDA application (20 μM; 3 min). B, Sample Western blot of GluR1 and quantitative data of the same samples as those used in A. C, Sample Western blot and quantitative data of the ratio of surface to total GluR2/3 in hippocampal slices taken either 10 or 60 min after NMDA application (20 μM; 3 min). D, Sample Western blot of GluR1 and quantitative data of the same samples as those used in C. Error bars represent SEM.

Figure 6. NMDAR activation, but not mGluR activation, reduces AMPAR surface expression in neonatal rat slices. A, Representative FPs elicited by paired-pulse stimulation in P8–P15 and P21–P35 hippocampal slices during baseline (1) and after 50 min of DHPG onset (2). Calibration: 0.5 mV, 20 ms. DHPG trace (2) was scaled to baseline FP1 amplitude for comparison of PPF changes within a single experiment. Note that, in the P8 rat, the second response is facilitated but is unchanged at P29. B, Group data of PPF (FP2 slope/FP1 slope as a percentage of pre-DHPG baseline) change during LTD in neonatal and adolescent animals. The asterisk indicates that PPF changes are greater in neonatal than adolescent rats (p < 0.02). C, DHPG-induced LTD of FP1 was not different among age groups. D, Representative NMDAR FPs taken at the times indicated in E and F from a P13 or P33 rat. Calibration: 0.2 or 0.1 mV (as indicated), 10 ms. E, F, Group average of decay of NMDAR FP amplitude in the presence of MK801 (10 μM) in control or after DHPG (100 μM; 5 min) treatment of neonatal (E) or adolescent (F) rat slices. Fast component (×1) is slower in DHPG-treated neonatal slices.

Presynaptic changes accompany mGluR-LTD at neonatal synapses

Previous studies of mGluR-LTD in slices from young (P4–P18) rats show strong evidence that LTD expression is mediated by a long-term decrease in presynaptic function (Bolshakov and Siegelbaum, 1994; Fitzjohn et al., 2001; Zakharenko et al., 2002; Rammes et al., 2003). Therefore, based on these studies and our findings that mGluR-dependent decreases in AMPAR surface expression are only observed in adolescent rats, we propose that there is a developmental change in the synaptic mechanisms of mGluR-LTD expression. To test this idea, we used two parameters to measure changes in presynaptic release probability during LTD, PPF, and the rate of blockade of NMDA receptor-mediated synaptic responses by MK801. These parameters were measured after DHPG-induced LTD in slices from both neonatal (P8–P15) and adolescent (P21–P35) rats. DHPG-induced LTD was examined, as opposed to synthetically induced LTD, because a similar magnitude of DHPG-induced LTD is observed across these developmental ages (Figs. 1A, 7B) and would allow comparison of the degree of PPF changes or rate of MK801 blockade across development.

Manipulations that alter presynaptic release probability, such as decreases in the Ca2+/Mg2+ ratio or adenosine, increase paired-pulse facilitation. Generally, the magnitude of PPF is inversely related to presynaptic release probability (Creager et al., 1980; Manabe et al., 1993; Debanne et al., 1996). However, preferential postsynaptic “silencing” of high- or low-release probability synapses also result in PPF changes (Poncer and Malinow, 2001). Pairs of stimulation (interstimulus interval, 50 ms) were delivered during baseline stimulation, during DHPG application, and for 1 h after DHPG. In slices from neonatal rats, PPF ratios increased during DHPG application and persisted for at least 60 min after DHPG (116 ± 2% of pre-DHPG baseline PPF values; n = 14; p < 0.0001), as reported previously (Fitzjohn et al., 2001). PPF values also increased during LTD in slices from adolescent rats (109 ± 2% of pre-DHPG baseline; n = 24; p = 0.008). However, the PPF increases in neonatal animals were significantly greater than those observed in adolescent rats (p < 0.02) (Fig. 7A). There was no difference in the magnitude of DHPG-induced LTD (P8–P15, 72 ± 2%; n = 14; P21–P35, 69 ± 2%; n = 24; p = 0.4) (Fig. 7C) or the absolute levels of PPF [P8–P15, 1.63 ± 0.05 (FP2/FP1 slope); P21–P35, 1.59 ± 0.05; p = 0.63] between these developmental ages. To further support a developmental change in the presynaptic contribution to mGluR-LTD, there is an inverse correlation of PPF changes during LTD and postnatal age (r = 0.4; p < 0.01).

As another means to measure developmental changes in presynaptic function associated with LTD, we used the irreversible, use-dependent NMDAR antagonist MK801. During repetitive presynaptic stimulation, the rate of blockade of NMDAR responses by MK801 has been used to determine presynaptic re-
lease probability ($P_r$) and detect changes in $P_r$ during long-term plasticity, such as long-term potentiation and LTD (Hessler et al., 1993; Rosenmund et al., 1993; Weisskopf and Nicoll, 1995; Kullmann et al., 1996; Xiao et al., 1997; Kaneko and Takahashi, 2004). If $P_r$ is relatively high, there is a greater number of NMDAR channels opened and a more rapid blockade of the response by MK801. We compared the rate of MK801 blockade of NMDAR-mediated FPs in control slices and those in which LTD had been induced using DHPG from both neonatal and adolescent rats. DHPG was used to induce LTD in normal ACSF. After LTD (of the AMPAR response) was established (30 min after DHPG application), a modified ACSF (see Materials and Methods) was applied to the slice to isolate the NMDAR-mediated FP, and this response was allowed to stabilize (~20–25 min). Synaptic stimulation was stopped for 10 min, and MK801 (10 μM) was applied to the slice. Synaptic stimulation (200 pulses) was resumed at 0.5 Hz in the presence of MK801. The rate of decay of the NMDAR FP amplitude by MK801 was fit by a double exponential, and time constants for the fast ($τ_1$) and slow ($τ_2$) components were obtained (Fig. 7E,F) (Rosenmund et al., 1993). These two components are thought to represent two populations of synaptic terminals with a high and low $P_r$ (Rosenmund et al., 1993). In neonatal slices, DHPG caused an increase in $τ_1$ (DHPG, $τ_1$, 49 ± 8 s; control, $τ_1$, 26 ± 3 s; n = 6; p = 0.02), consistent with a lower $P_r$ after DHPG treatment. This change was evident by the slower decay curves in DHPG-treated slices (Fig. 7E). In contrast, DHPG treatment of adolescent slices did not significantly affect $τ_1$, and the decay curves overlapped (DHPG, $τ_1$, 49 ± 8 s; control, $τ_1$, 33 ± 8 s; n = 6; p = 0.19) (Fig. 7E). There was no change in $τ_2$ at either age (neonatal: DHPG, $τ_2$, 222 ± 22 s; control, $τ_2$, 178 ± 21 s; n = 6; p = 0.15; adolescent: DHPG, $τ_2$, 188 ± 24 s; control, $τ_2$, 254 ± 35 s; n = 6; p = 0.11). There were no differences in $τ_1$ or $τ_2$ between the neonatal and adolescent groups (p = 0.45 and 0.1, respectively). These results, together with the observed PPF changes during LTD, confirm that mGluR-LTD at neonatal synapses is accompanied by decreases in presynaptic release probability (Bolshakov and Siegelbaum, 1994; Fitzjohn et al., 2001; Zakharenko et al., 2002). Our results also indicate that the magnitude of these presynaptic changes diminish as synapses mature and suggest that other, perhaps postsynaptic, mechanisms contribute to mGluR-LTD in mature synapses.

CB$_1$ receptor activation is not required for mGluR-LTD in neonatal rats

Previous work has demonstrated that the induction of mGluR-LTD at neonatal synapses requires postsynaptic Ca$^{2+}$ influx and depolarization (Bolshakov and Siegelbaum, 1994; Oliet et al., 1997). Because of the presynaptic changes associated with LTD, the existence of a retrograde messenger has been postulated. Here, we tested the involvement of potential retrograde messengers in mGluR-LTD at neonatal synapses: endocannabinoids.

Endocannabinoids are released from CA1 pyramidal neurons in response to group 1 mGluR activation and depress both excitatory and inhibitory synaptic transmission via a presynaptic CB$_1$R (Misser and Sullivan, 1999; Varma et al., 2001; Ohno-Shosaku et al., 2002). Endocannabinoids have also been implicated in LTD at inhibitory synapses in area CA1 and at excitatory synapses in the basal ganglia and neocortex (Gerdenman et al., 2002; Robbe et al., 2002; Chevaleyre and Castillo, 2003; Sjostrom et al., 2003). A previous study failed to find a role for CB$_1$R in DHPG-induced LTD in area CA1 of adolescent mice (Rouch and Nicoll, 2003). We obtained similar results in neonatal rat hippocampal slices and found no role for CB$_1$R in mGluR-LTD (Fig. 8B, C). The effects of the CB$_1$R antagonist AM281 (1 μM) were tested on mGluR-LTD in neonatal slices, which blocks mGluR and depolarization-induced endocannabinoid release (Gifford et al., 1997; Maejima et al., 2001; Ohno-Shosaku et al., 2002; Melis et al., 2004). AM281 (1 μM) had no effect on either DHPG-induced LTD (AM281, 84 ± 6%, n = 7; control, 84 ± 4%, n = 7; p = 0.94) or LTD induced with PP-LFS (AM281, 73 ± 5%, n = 8; control, 67 ± 36%, n = 10; p = 0.26). In contrast, preapplication of AM281 greatly reduced the synaptic depression induced by the CB$_1$R agonist WIN55,212-2 (2 μM; 10 min) (D’Ambra et al., 1992; Al-Hayani and Davies, 2000), indicating that the antagonist is effective in our slice preparation (WIN55,212-2, 47 ± 9% of baseline, n = 4; WIN55,212-2 plus AM281, 87 ± 2%, n = 4; p = 0.03) (Fig. 8A). These results confirm that CB$_1$R activation depresses excitatory synaptic transmission in neonatal hippocampal area CA1 (Al-Hayani and Davies, 2000) and demonstrate that CB$_1$Rs are not required for mGluR-induced LTD at this age.

Discussion

Here, we show, using both pharmacological and synaptic stimulation of group 1 mGluRs, that the synaptic mechanisms and protein synthesis dependence of mGluR-LTD change with developmental age. In neonatal synapses, mGluR-LTD does not rely on protein synthesis and is associated with large changes in PPF and the rate of MK801 blockade, suggesting a presynaptic site of LTD expression. Consistent with this idea, mGluR activation of neonatal slices does not cause a decrease in AMPAR surface expression. As synapses mature, mGluR-LTD and the associated decrease in AMPAR surface expression require new protein synthesis and result in a smaller or no change in presynaptic function.

Previous studies on the synaptic mechanisms of mGluR-LTD have yielded conflicting results. Many of the studies, which concluded there is a presynaptic site of mGluR-LTD expression, were performed in hippocampal slices from neonatal animals (P4–P18) (Bolshakov and Siegelbaum, 1994; Fitzjohn et al., 2001; Zakharenko et al., 2002; Rammes et al., 2003) (but see Watabe et al., 2002). However, the studies demonstrating a role for postsynaptic protein synthesis were performed at more mature synapses (14–21 d in vitro cultures or slices from P10–P30 rats) (Huber et
significant reduction in abrupt but gradually as the synapses mature. Furthermore, the suggestion that synaptic changes in mGluR-LTD do not occur abruptly but gradually as the synapses mature. However, the fact that there are small changes in PPF (Fig. 1B) and a nonsignificant reduction in $\tau_1$ of MK801 blockade, which occur during LTD in the adolescent group, suggest that a presynaptic expression mechanism, albeit reduced, may persist at mature synapses. However, because mGluR-LTD in adolescents is blocked by protein synthesis inhibitors, this suggests that any presynaptic contribution must also require protein synthesis. Alternatively, or in addition, PPF changes observed in the adolescent group may have a contribution from AMPAR removal at high release probability synapses (Poncer and Malinow, 2001).

There are alternative explanations for the greater PPF changes we observe during LTD at neonatal synapses. A recent study demonstrated that the baseline PPF magnitude is inversely correlated with the PPF changes observed during LTD (Santschi and Stanton, 2003). Therefore, a higher $P_0$ at neonatal synapses would be reflected by a lower PPF and could explain why there are greater increases in PPF during LTD. In our study, we find no correlation with baseline PPF and the change in PPF with LTD ($r = 0.05; p = 0.7$). The difference in our findings may be attributable to a difference in the route of LTD induction (Santschi and Stanton, 2003). Furthermore, we do not find a correlation with developmental age and the baseline PPF ($r = 0.13; p = 0.4$) or differences in the average baseline PPF values or the $\tau_1$ of MK801 blockade between neonatal and adolescent groups (results). A recent study described developmental decreases in $P_0$ at hippocampal synapses, as measured by PPF and the rate of MK801 blockade, but these changes occurred during an earlier developmental window (from P6 to P12) than our developmental switch (Wasing et al., 2004). Other studies of $P_0$ later in hippocampal development (P15–P35) report an increase in $P_0$, or no change (Dumas and Foster, 1995; Hsia et al., 1998). We think that the greater DHPG-induced changes in PPF and rate of MK801 blockade at neonatal synapses represent a greater presynaptic contribution to the LTD compared with more mature synapses.

Although we find that mGluR-LTD is independent of protein synthesis at developing synapses, our results do not address the question of whether mGluRs activate synaptic protein synthesis at this age. Instead, our data indicate that there are developmental changes in mGluR regulation of AMPAR trafficking. New proteins are required for the persistent decrease in surface AMPAR expression at mature synapses (Fig. 4E,F) (Snyder et al., 2001). Therefore, it is likely that LTD is protein synthesis independent in the neonatal slices because mGluR activation does not alter AMPAR surface expression (Fig. 6). Because NMDAR-dependent decreases in AMPAR surface expression are intact in neonatal synapses, we conclude that components of the mGluR-mediated AMPAR endocytosis process are developmentally regulated, as opposed to general AMPAR endocytosis machinery.

We measured changes in GluR surface expression and not endocytosis per se. Therefore, mGluR activation of neonatal synapses may induce endocytosis of AMPARs but also increase insertion rates so there is no net change in surface expression. In addition, with receptor biotinylation, we cannot determine whether we are measuring surface expression of synaptic or extrasynaptic receptors or both. Therefore, the developmental differences that we observe in DHPG-induced decreases in AMPAR surface expression could be attributable to a difference in the ability to detect surface changes in synaptic receptors with biotinylation. Using immunocytochemistry in dissociated neuronal culture, DHPG has been shown to reduce the number of synaptic AMPARs associated with presynaptic markers, and we would predict that similar changes are occurring in the slice (Snyder et al., 2001). However, it remains to be determined whether there is a developmental change in the effects of DHPG specifically on synaptic AMPARs. Recent work demonstrated that postsynaptic $Ca^{2+}$ increases alone, independent of NMDARs or mGluRs, are sufficient to induce "silent" synapses (presumably by removing postsynaptic AMPARs) at immature (P3–P12), but not mature (P29–P32), synapses (Xiao et al., 2004). This previous work and our current results suggest that the mechanisms that induce AMPAR removal, such as those that regulate insertion, change with synapse development (Esteban et al., 2003; Yasuda et al., 2003).

The CB$_1$R agonist WIN55,212–2 induced a small depression in the presence of 1 $\mu$M AM281 (Fig. 8A), suggesting an incomplete blockade of CB$_1$Rs. This leaves open the possibility that a minority of CB$_1$R activation can lead to full mGluR-LTD. However, we find this unlikely and think that our results are consistent with those of Rouach and Nicoll (2003) (from mature rats), who found no role for endocannabinoids in mGluR-LTD of excitatory synaptic transmission. Because endocannabinoids have been implicated in mGluR-dependent LTD of inhibitory synaptic transmission (Chevaleyre and Castillo, 2003), this suggests that mGluRs use distinct mechanisms to induce LTD of inhibitory and excitatory synaptic transmission. Another potential retrograde messenger for mGluR-LTD at immature synapses is the arachidonic acid metabolite 12-(S)hydroperoxyeicosatetraenoic acid, which is required for mGluR-LTD induced with 5 Hz stimulation (Feinmark et al., 2003). Additional experiments are required to confirm the role of arachidonic acid metabolism in DHPG and PP-LFS-induced LTD.

The developmental switch in the synaptic mechanisms of LTD may occur to accommodate the changing properties of synapses over the course of maturation. Because there are fewer surface AMPARs at immature synapses (Liao et al., 1999; Petralia et al., 1999; Pickard et al., 2000), it may be more efficacious to depress synaptic transmission at developing synapses by reducing presynaptic release probability, as opposed to endocytosis of AMPARs. The consequences of the developmental switch of LTD on hippocampal function are unclear. Because mGluR-LTD affects the short-term dynamics of nascent synapses (Fig. 7), it may differentially impact information processing and plasticity of developing and mature CA1 synapses (Fuhrmann et al., 2002).

### References


