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Metabotropic Glutamate Receptor Mediated Long-term Depression in Developing Hippocampus

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Summary—The effects of bath application of the metabotropic glutamate receptor (mGluR) agonist 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD, 10 μM) were studied at the Schaffer collateral–CA1 synapse in hippocampal slices from rats of 8–33 days postnatal age. In immature animals (8–12 days) ACPD induced a biphasic response characterized by an acute decrease in field EPSP slope (~50–60% of baseline) in the presence of the agonist, followed by long-term depression (LTD, ~75–80% of baseline) after washout. In animals older than 20 days, ACPD induced a slow onset potentiation or minimal change. Both the acute depression and LTD were blocked by the mGluR antagonist α-methyl-4-carboxyphenyl glycine (MCPG). ACPD-induced LTD was blocked by the *N*-methyl-D-aspartate receptor (NMDAR) antagonists D(−)-2-amino-5 phosphopentanoic acid (AP5) and dizocilpine maleate (MK-801), and by ethanol. Glutamic pyruvic transaminase, an enzyme that selectively metabolizes endogenous extracellular glutamate, also blocked LTD suggesting that the requisite NMDA currents were tonically activated by extracellular rather than synaptically released glutamate. ACPD-induced LTD was blocked by staurosporine, indicating a requirement for serine–threonine kinase activation, and was unaffected by the L-type voltage sensitive calcium channel blocker nitrendipine and the A1 adenosine receptor antagonist 8-cyclopentyl-1,3-dimethylxanthine (CPT). Because mGluR-mediated LTD was observed only in immature CA1, mGluRs may play a role in hippocampal development, perhaps by contributing to synapse pruning in a temporally restricted fashion. © 1997 Elsevier Science Ltd.

Keywords—Hippocampus, area CA1, synaptic plasticity, *N*-methyl-D-aspartate receptor (NMDAR), mGluR.

Metabotropic glutamate receptors (mGluRs) are a family of glutamate receptors coupled through G-proteins to second messenger cascades. Recent results suggest that mGluRs play a crucial role in synaptic plasticity in brain. For example, activation of mGluRs contributes to tetanus-induced long-term potentiation (LTP) in the adult hippocampal formation at the Schaffer collateral–CA1 (SC–CA1), mossy fiber–CA3, and perforant path–dentate gyrus synapses (Behnisch and Reymann, 1994a,b; Bashir *et al.*, 1993a; Sergueeva *et al.*, 1993; Richter-Levin *et al.*, 1994; Riedel and Reymann, 1993; Riedel *et al.*, 1994), as well as long-term depression (LTD) at the parallel fiber (PF)–Purkinje cell synapse in cerebellum (Linden and Connor, 1991; Daniel *et al.*, 1992; Hartell, 1994), and the medial perforant path–dentate gyrus synapse in adult rat dentate gyrus (O'Mara *et al.*, 1995). Anatomic support for the role of mGluRs in mediating synaptic plasticity has been provided by the restricted localization of mGluRs 1 and 5 to the postsynaptic membranes of dendritic spines in both hippocampus and cerebellum (Baude *et al.*, 1993; Gorcs *et al.*, 1993; Lujan *et al.*, 1996). Finally, mutant mice that are genetically deficient in the postsynaptic mGluR subtype mGluR1 show impaired cerebellar LTD, hippocampal (mossy fiber) LTP, eyeblink conditioning and severe motor coordination and spatial learning deficits (Conquet *et al.*, 1994; Aiba *et al.*, 1994a,b). Impairments in short-term plasticity and motor learning were observed in animals lacking the gene coding for the presynaptic mGluR subtype mGluR4 (Pekhletski *et al.*, 1996).

The mGluR family consists of eight known subtypes that can be distinguished by their characteristic pharma-

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cological properties, coupled second messengers, effectors and brain localization (Abe *et al.*, 1992; Aramori and Nakanishi, 1992; Schoepp, 1993; Tanabe *et al.*, 1993; Nakajima *et al.*, 1993; Okamoto *et al.*, 1994; Catania *et al.*, 1994; Fotuhi *et al.*, 1993, 1994; Suzdek *et al.*, 1994). They have been categorized into three groups as follows (Pin and Duvoisin, 1995): Group I mGluRs (mGluRs 1 aud 5) stimulate phosphoinositide hydrolysis and intracellular calcium signal transduction; Group II receptors (mGluRs 2 and 3) are negatively linked to the cAMP second messenger system; and Group III receptors (mGluRs 4, 6, 7 and 8) correspond to the L-AP4 subtype of receptor, of which mGluRs 4 and 7 are believed to be presynaptic autoreceptors in the brain. Recent evidence suggests that effects of mGluR activation may be developmentally regulated. For example, whereas mGluR activation by ACPD is negatively coupled to cAMP formation in adult rat hippocampus (Schoepp and Johnson, 1993a), in neonatal hippocampus a unique mGluR has been identified that is activated by ACPD, is antagonized by 2-amino-3-phosphonopropionic acid (AP3), and enhances cAMP production by an adenosine receptor-dependent mechanism (Schoepp and Johnson, 1993b). In other brain regions including cerebral cortex, hypothalamus and retina, neuronal maturation has been associated with a reduction in expression, functional activity, number and/or distribution of mGluRs (van den Pol *et al.*, 1994; Dudek and Bear, 1989; Nomura *et al.*, 1994).

In the present study, we examined the effects of mGluR activation by the broad-spectrum agonist ACPD on synaptic transmission at the SC–CA1 synapse during the first postnatal month. We found that ACPD induces both an acute depression as well as LTD. This effect is developmentally regulated, as it was seen only in animals of 8–17 days; thereafter ACPD application induced either no plastic change or LTP. Some of these results have been reported previously in abstract form (Trommer *et al.*, 1993; Overstreet *et al.*, 1994, 1995).

MATERIALS AND METHODS

Hippocampal slices from Sprague–Dawley rats (8–33 days postnatal age) were prepared as follows: animals were anesthetized with isoflorane (age >12 days only), decapitated and the brains were removed and placed in chilled artificial cerebrospinal fluid (ACSF) gassed with 95% O_2/5% CO_2 and containing (in mM): NaCl 124, KCl 3, $MgSO_4$ 1.3, $NaHPO_4$ 1.25, $CaCl_2$ 2.4, $NaHCO_3$ 24 and D-glucose 10 (pH 7.4). Slices (350–400 μm thick) were cut on a vertical tissue chopper (Stoelting), incubated at room temperature, and transferred as needed to a submersion chamber (Medical Systems Corp.) maintained at 30°C.

Field potentials were recorded in the stratum pyramidale or stratum radiatum of area CA1 in response to stimulation of the Schaffer collateral pathway. Bipolar stimulating electrodes consisted of twisted platinum iridium or stainless steel wire (25 μm). Recording electrodes were pulled from glass capillaries (OD 1.00 mm, ID 0.50 mm, Dagan Corp.) filled with 2 M NaCl, and had tip impedences of 2–6 MΩ. Constant current stimuli were delivered through stimulus isolation units (A 360, World Precision Instruments) driven by Grass S88 or S8800 stimulators, driven in turn by custom-written computer software also used for data acquisition and analysis. Responses were allowed to stabilize for 20–30 min before the start of data collection. Stimulus–response curves were constructed in response to test pulses of fixed stimulus intensities (75–500 μA) over a range of pulse widths (30–200 μsec) delivered at 0.016–0.033 Hz. Test pulses were also delivered at 0.016–0.033 Hz. Tetanic stimulation consisted of 1–2 trains of 1 sec duration at 50 or 100 Hz and 200 μsec pulse width.

Population spike amplitude (PS, mV) was measured in the stratum pyramidale and was defined as the vertical distance between the tangent connecting the two peak positivities of the EPSP and the peak of the spike. EPSP slope (mV/msec) was measured in the stratum radiatum as the maximum slope of the initial negative deflection of the evoked response. Responses were normalized by conversion to a percentage of the baseline value for each animal. In the initial somatic layer recordings, stimulus–response curves were obtained at baseline, during the last 2–5 min of ACPD administration, 30 and 60 min after washout of ACPD, and 30 min following tetanic stimulation. The evoked potentials selected for analysis were obtained at the pulse width that produced a 60–70% maximum PS amplitude in the baseline condition. In subsequent experiments responses were monitored at a single pulse width (corresponding to 60–70% maximum EPSP slope at baseline) throughout and the responses used in statistical analysis were the mean EPSP slopes approximately 30 min after washout of the drug. Recordings were performed in either the somatic or dendritic layers or both. All values given in the text are mean ± SD.

Drugs used were (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD), D(−)-2-amino-5-phosphonopentanoic acid (AP5) and (*R*) or (*R*,*S*)-α-methyl-4-carboxyphenyl glycine (MCPG) (Tocris Cookson, Bristol, U.K.); dizocilpine maleate (MK-801), 8-cyclopentyl-1,3-dimethylxanthine (CPT) and nitrendipine (Research Biochemicals International, Natick, MA, U.S.A.); glutamic pyruvic transaminase, pyruvic acid and staurosporine (Sigma, St. Louis, MO, U.S.A.). ACPD, AP5 and MK-801 were dissolved in distilled water at 1 mM and diluted in ACSF. CPT was dissolved directly in ACSF. Glutamate pyruvate transaminase was dissolved in a solution containing 2 mM pyruvic acid in ACSF. Racemic (*R*,*S*)-MCPG was dissolved directly in ACSF or in 50 μM NaOH and diluted in ACSF. (*R*)-MCPG was dissolved in 50 μM NaOH and diluted in ACSF. Staurosporine and nitrendipine were dissolved in ethanol prior to dilution in ACSF and had final ethanol concentrations of 0.018% and 0.023%, respectively.

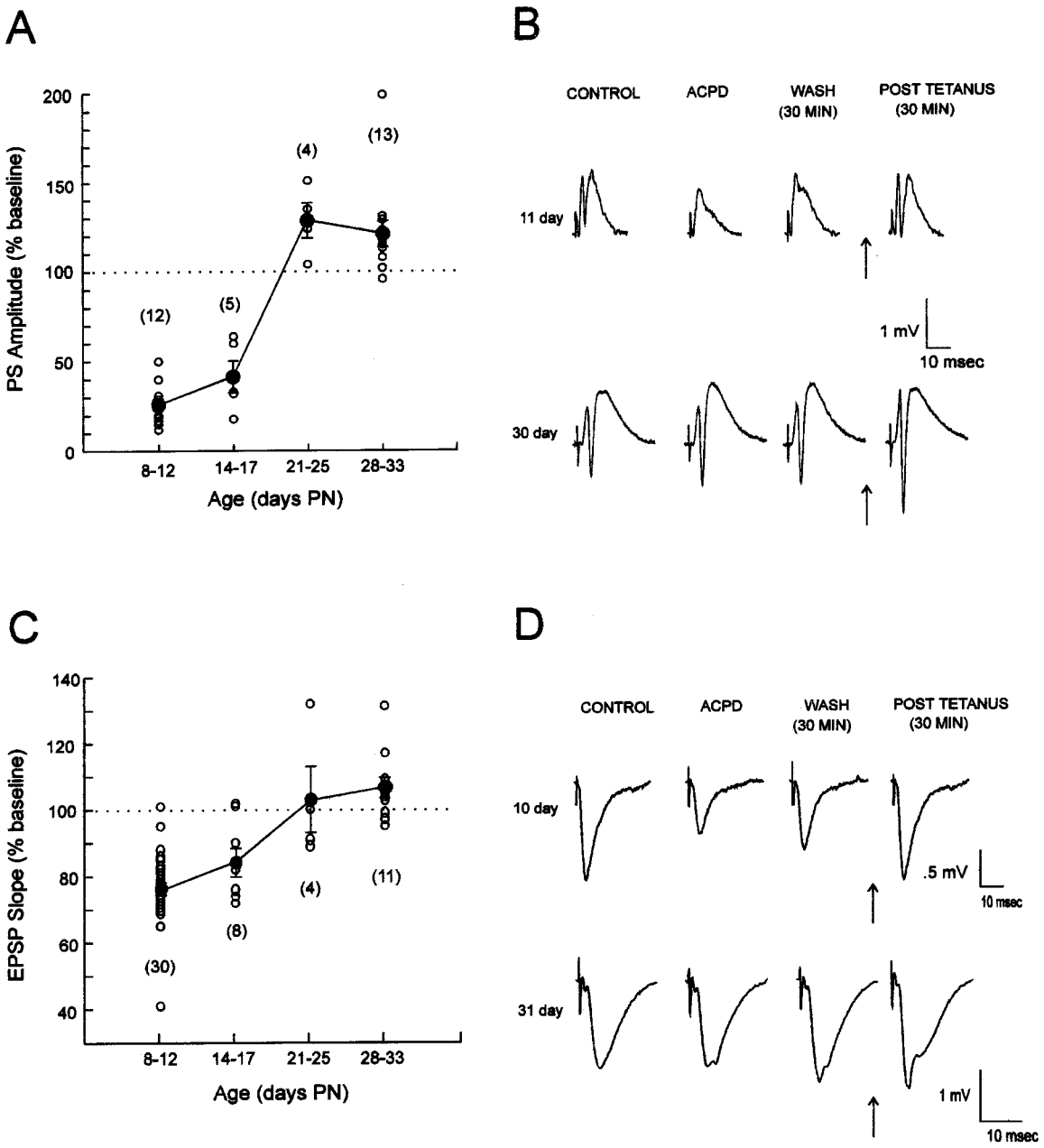

Fig. 1. The effects of mGluR activation by ACPD (10 μM) at four developmental stages. (A) Scatter plot showing the relationship between PS amplitude, measured 30–40 min after ACPD washout, and age. (○) indicate individual experiments and (●) correspond to the mean ± SE for each age group. All values are expressed as a percentage of the average baseline before ACPD application. Numbers in parentheses indicate the number of experiments per age group. (B) Examples of individual somatic recordings from an 11 day (upper) and 30 day (lower) animal obtained during baseline, during ACPD administration, 30 min after washout of ACPD, and 30 min after tetanic stimulation. (C) Scatter plot showing the relationship between EPSP slope, measured 30–40 min after ACPD washout, and age. Symbols as in (A). (D) Examples of individual dendritic recordings from a 10 day (upper) and 31 day (lower) animal obtained at the same times as in (B).

RESULTS

Developmental changes in mGluR-activated long-term plasticity

ACPD (10 μM) was bath-applied for 20 min to slices at four developmental stages: 8–12 days, 15–17 days, 21–24 days and 28–33 days. As shown in Figs 1–3, the bath application of ACPD induced both acute and long-term changes in synaptic transmission, and the direction of plastic change was highly age-dependent. As is summarized for all experiments in Fig. 1(A) (PS amplitude, $n = 34$) and Fig. 1(C) (EPSP slope, $n = 53$), the long-term plastic change (measured 30–40 min following the washout of ACPD) was LTD in animals of 8–17 days,

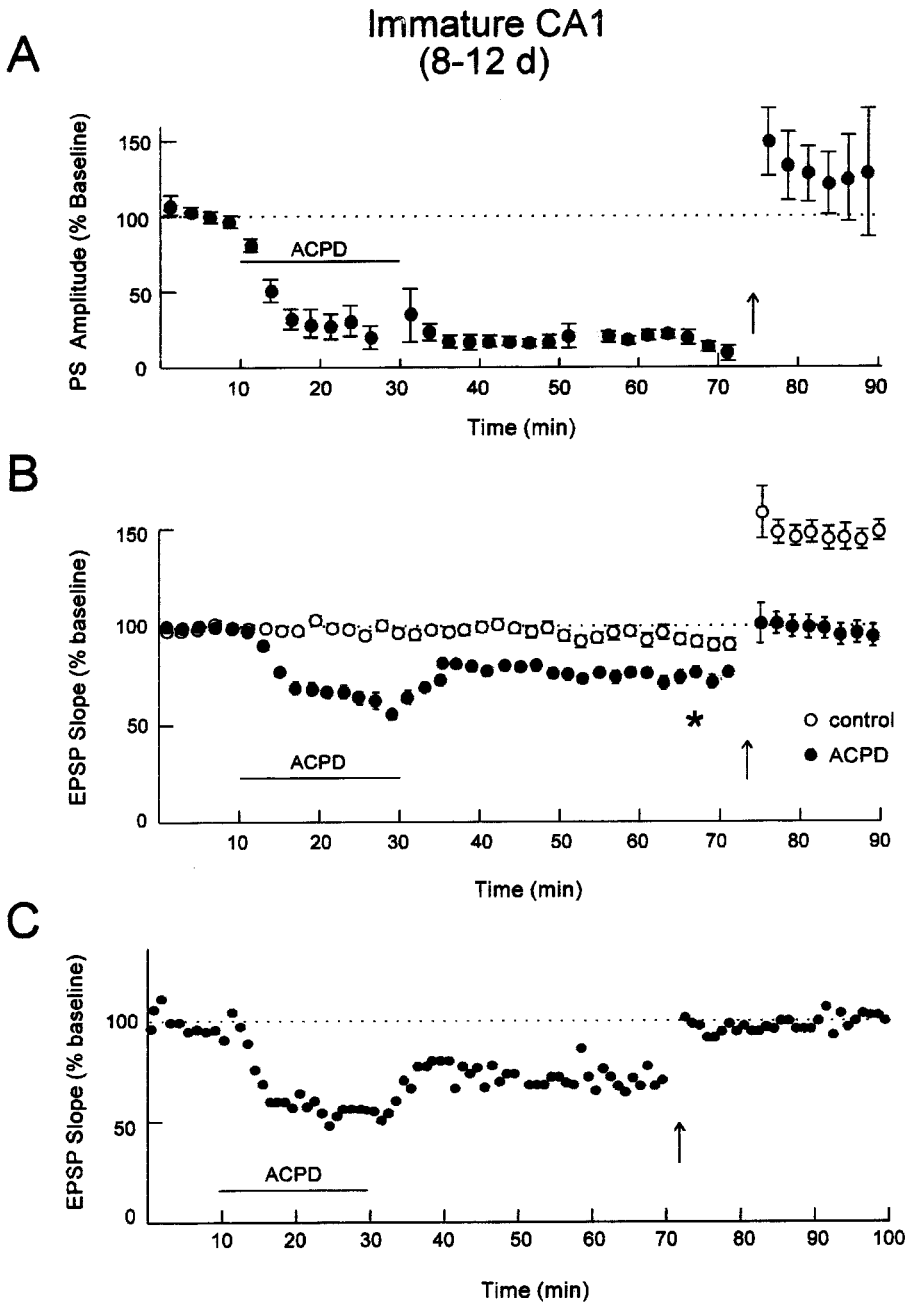

Fig. 2. ACPD induces LTD in 8–12-day animals. (A) Time course plots of PS amplitude in animals exposed to ACPD ($n = 4$, 10 μM). PS amplitude was reduced to 36 $\pm$ 29% baseline in the presence of ACPD, and further depression (18 $\pm$ 8%) was seen at 35–40 min after washout. (B) Time course plot of EPSP slope in the experimental group exposed to ACPD ($n = 13$, ●), and a control group exposed to ACSF alone ($n = 5$, ○). Asterisk (*) indicates that mean EPSP slope in experimental group (76 $\pm$ 12% baseline) differed from slope in control group (92 $\pm$ 12%, $p < 0.03$) at 35–40 min. (C) The time course of a dendritic recording in a 10 day rat. Gaps in plots correspond to the collection of stimulus–response curves. In these and all subsequent plots data were collected every 30 sec, averaged, and binned in 2 or 2.5 min intervals; vertical arrows indicate delivery of tetanic stimulation.

and LTP in animals of 21 days and older. Long-term changes were more pronounced in PS amplitude than in EPSP slope, and LTD in the younger animals was more robust and consistent than LTP in the older age groups. As illustrated in Figs 2 and 3, the direction of acute change paralleled the long-term change in the 8–12-day and 28–33-day age groups. In the intermediate ages, minimal acute drug-induced changes were observed (not shown), but long-term effects were apparent after washout.

To test whether the apparent LTD in the 8–12-day age group was in fact deterioration of the recorded potential,

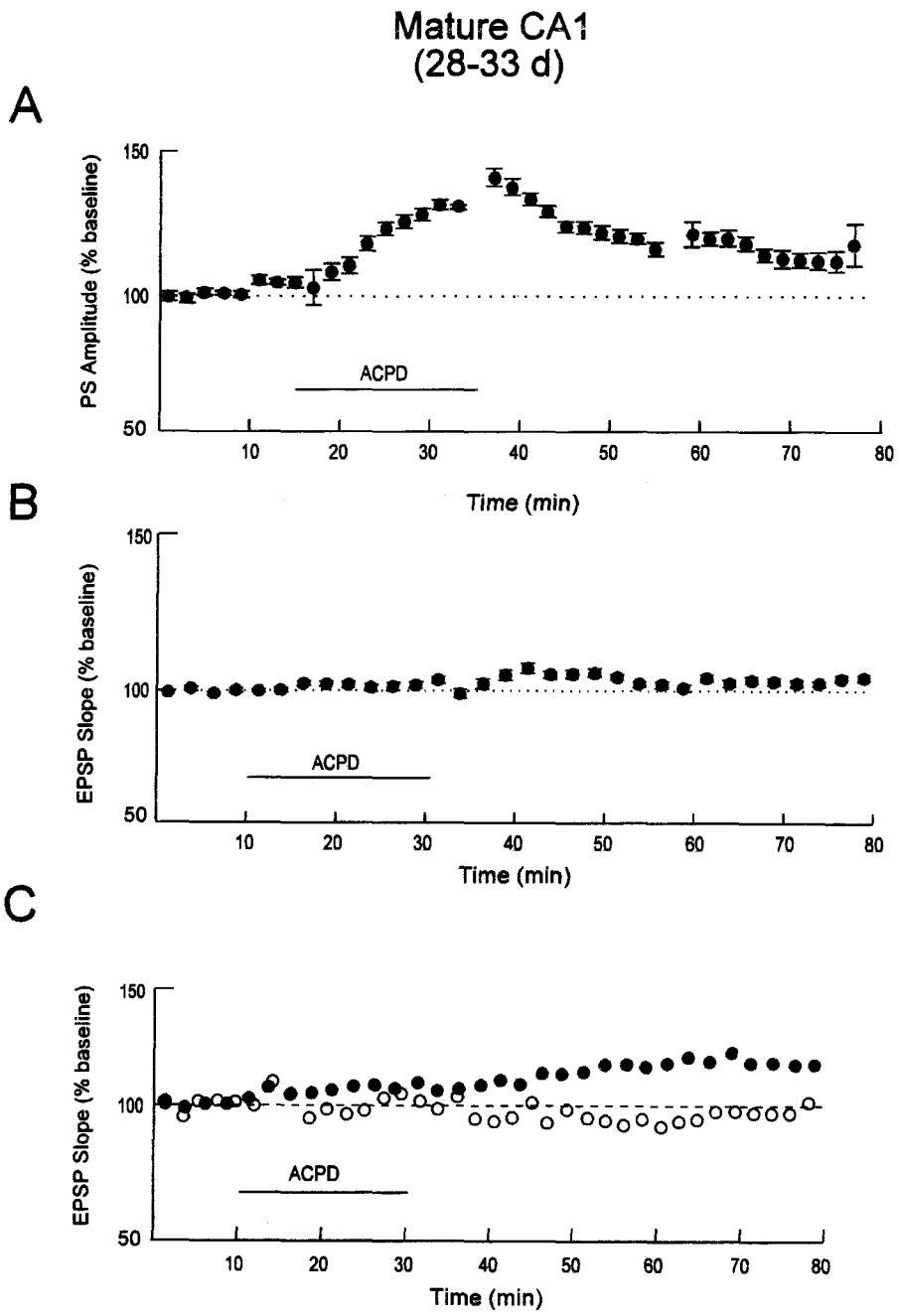

Fig. 3. ACPD effects in adult (29–33 day) animals. (A) Grouped average time course plots showing acute (131 ± 17% after 20 min exposure to ACPD) and long-term (113 ± 13% 45 min after washout) potentiation of PS amplitude ($n = 6$, 10 μM). (B) Minimal change occurred in mean EPSP slope (1 07 ± 10% at 45 min after washout, $n = 11$) in response to 20 min application of ACPD. (C) In older animals, EPSP slope showed a variable response to ACPD. Superimposed are time course plots of individual dendritic recordings from a 31 day rat (●) in which potentiation of EPSP slope (131% at 45 min) was observed, and a 29 day rat (○) in which EPSP slope was unchanged by ACPD.

baseline data were collected for 10 min ($n = 13$), 20 min ($n = 6$), or 30 min ($n = 11$) prior to ACPD application and the EPSP slope 30–40 min after washout was compared with that of control slices in which test pulses were delivered in the absence of drug application ($n = 11$). In these experiments the ACPD-induced change in EPSP slope (relative to baseline) was 75 ± 13% in the 10 min group, 77 ± 8% in the 20 min group, and 78 ± 7% in the 30 min group. None of the experimental groups differed from each other in the degree of depression, indicating that deterioration of responses with prolonged recording time did not account for the LTD. In contrast, each group differed significantly from the control group (95 ± 7%), $p < 0.01$, ANOVA with *post hoc* Tukey HSD. Time-course plots from the 10 min baseline and control groups are illustrated in Fig. 2(B). As illustrated in Fig. 2(A–C)

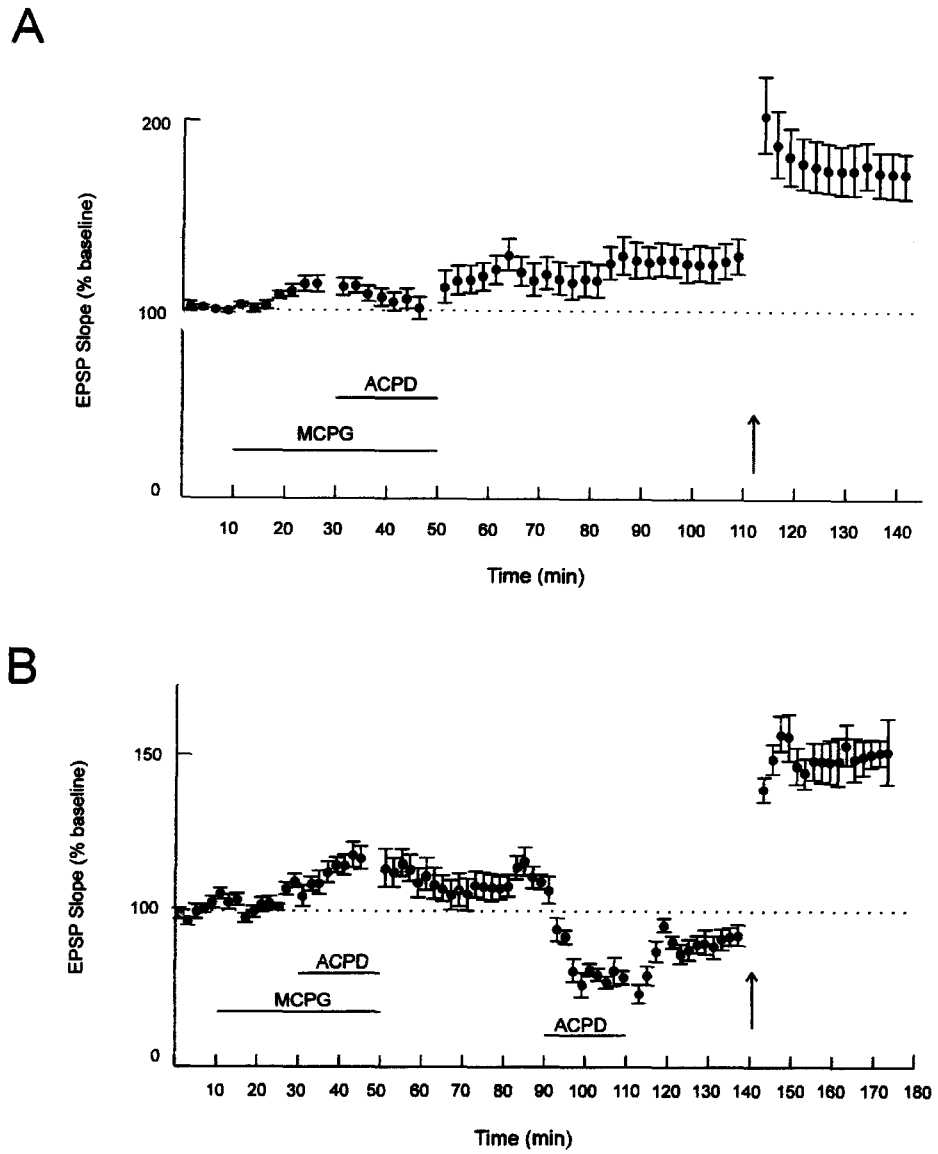

Fig. 4. Effect of blockade of mGluRs. (A) The mGluR antagonist MCPG (500 μM) blocks ACPD-induced LTD (n = 5) in immature slices (8–13 day). An increase in mean EPSP slope is seen in MCPG (111 $\pm$ 23%), in ACPD (107 $\pm$ 30%), and 60 min after wash (117 $\pm$ 48%). Subsequent tetanus induces LTP (166 $\pm$ 65% at 30 min). (B) After washout of MCPG, ACPD is capable of inducing LTD (81 $\pm$ 4% of response in ACSF just prior to second ACPD application, n = 3).

and Fig. 4(B), slice health following ACPD-induced LTD was also verified by the subsequent induction of LTP in response to tetanic stimulation.

Effects of the mGluR antagonist MCPG

In order to confirm that the ACPD-induced LTD observed in immature slices resulted from mGluR activation, the mGluR antagonist (*R,S*)-MCPG (500 μM) was applied for 20 min prior to and during the 20 min application of ACPD in 8–12-day slices (n = 5, Fig. 4(A)). In these slices both the acute depression and subsequent LTD typically induced by ACPD were blocked. Instead, a mean increase in EPSP slope was observed in MCPG alone (111 $\pm$ 23%), in the presence of both MCPG and ACPD (107 $\pm$ 30%), and after a 60 min wash (117 $\pm$ 48%). This may be due to inhibition of presynaptically-located mGluRs which depress glutamate release (Baskys and Malenka, 1991) and/or activation of MCPG-insensitive receptors. In three additional slices (Fig. 4(B)) ACPD-induced LTD was blocked by (*R*)-MCPG (500 μM); a second application of ACPD was delivered 40 min after washout of both ACPD and MCPG and induced LTD (81 $\pm$ 4% of response in ACSF just prior to the second ACPD application).

Contribution of NMDA Receptors to ACPD-induced LTD

LTP induced by the bath application of ACPD to slices from mature animals has previously been shown to be

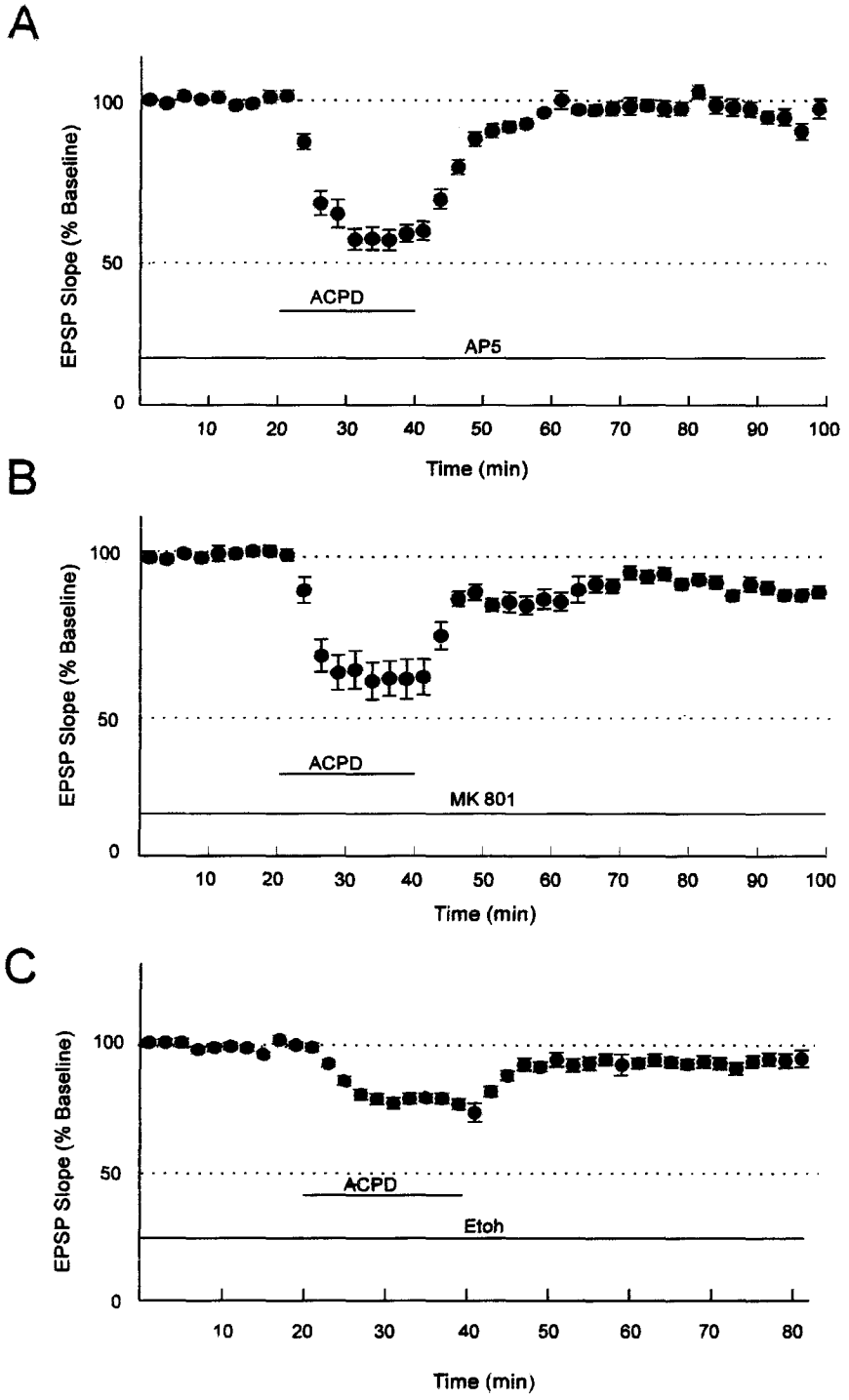

Fig. 5. ACPD-induced LTD is NMDAR-dependent. (A) In the presence of AP5 (20 μM), only a transient depression (67 $\pm$ 12%) was seen with recovery to 101 $\pm$ 10% by 30–35 min after washout (n = 5). (B) In the presence of MK801 (20 μM) similar results were obtained, with acute depression (68 $\pm$ 21%) followed by recovery (94 $\pm$ 5%, n = 4). (C) In the presence of ethanol (0.045%) ACPD also induced a transient depression of EPSP slope (79 $\pm$ 10%) followed by recovery to 93 $\pm$ 12% baseline by 30–35 min. Lower concentrations of ethanol (up to 0.023%) did not block ACPD-induced LTD (see text).

independent of *N*-methyl-D-aspartate receptors (NMDARs; Bortolotto and Collingridge, 1993). To determine whether the ACPD-induced LTD in immature CA1 requires NMDAR activation, ACPD was administered in the presence of the competitive NMDAR antagonist, AP5 (20 μM). As shown in Fig. 5(A) (n = 5), an acute depression (67 $\pm$ 12%) was observed in the presence of ACPD but recovery was seen following

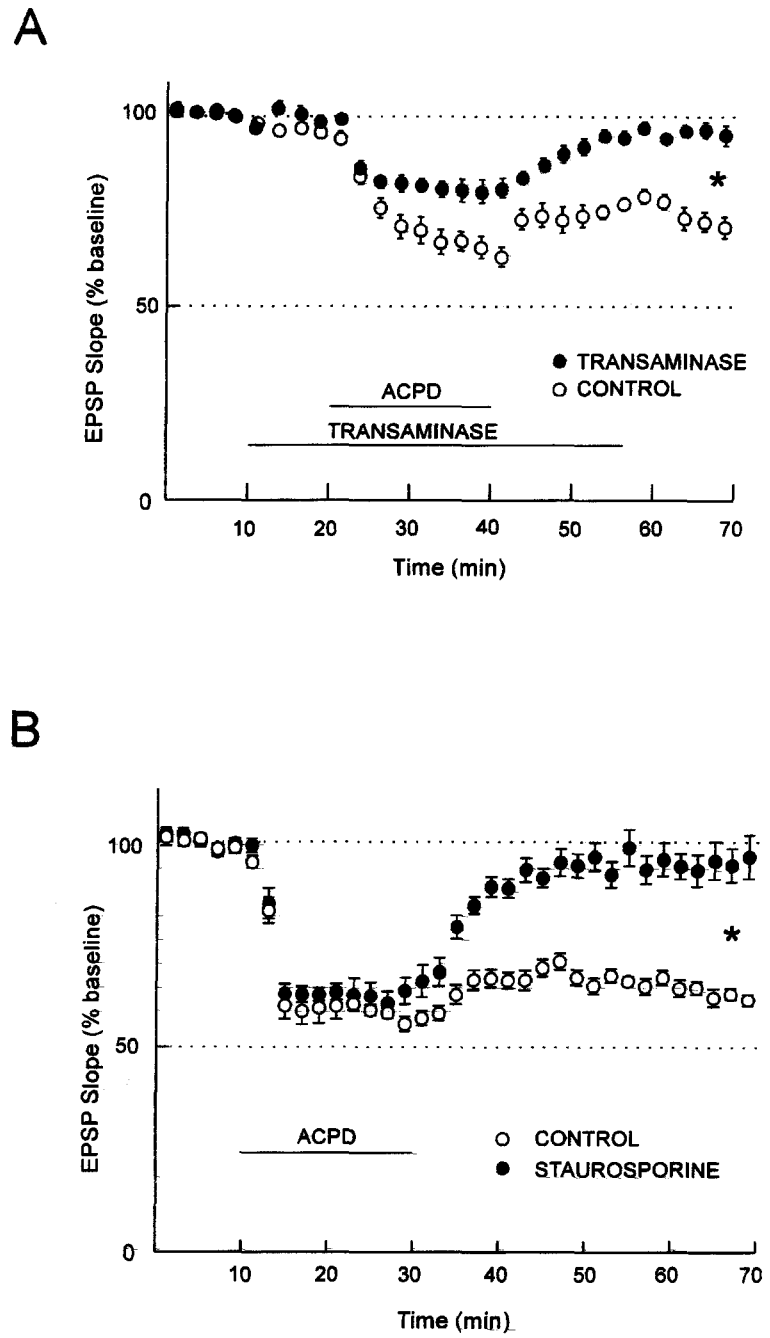

Fig. 6. (A) Effects of selective metabolism of endogenous extracellular glutamate. In the presence of the enzyme glutamic pyruvic transaminase (5 units/ml) ACPD induced an acute depression (80 ± 13%) but not LTD (recovery to 95 ± 11% baseline by 25–30 min after washout, $n = 4$, ●). Control slices ($n = 4$, ○) were exposed to ACPD alone and showed both acute depression (66 ± 12%) and LTD (71 ± 12%). (B) ACPD-induced LTD requires activation of serine–threonine kinases. In the presence of staurosporine (0.4 μM), ACPD induced acute depression (62 ± 15%) but not LTD (recovery to 99 ± 18% by 30–35 min). Control slices exhibited both acute depression (60 ± 7%) and LTD (62 ± 5%). Asterisks indicate significant differences between experimental and control slices.

washout (101 ± 10% of baseline by 30–35 min). Thus, the acute and long-term effects of ACPD can be differentiated by AP5.

Similar results were seen in the presence of the open channel NMDAR antagonist, MK-801 (20 μM, $n = 4$, Fig. 5(B)) in that an acute reversible depression (68 ± 21%) was seen during ACPD application, but this was followed by recovery (94 ± 5%) after washout.

The bath application of 0.045% ethanol also prevented ACPD-induced LTD ($n = 5$, Fig. 5(C)). An acute depression to 79 ± 10% was seen followed by recovery to 93 ± 12% by 30–35 min following ACPD washout.

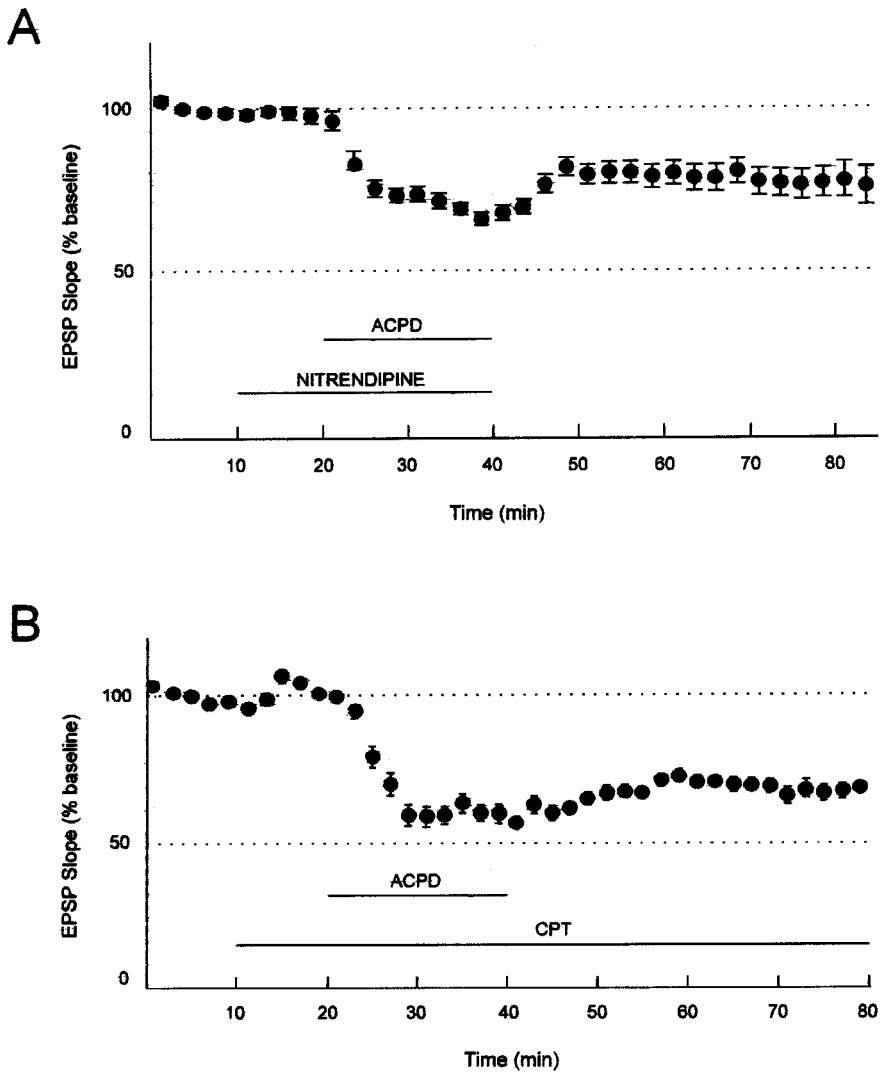

Fig. 7. ACPD-induced LTD does not depend on L-type calcium channels or A1 adenosine receptors. (A) When ACPD was applied in the presence of the L-type voltage-sensitive Ca^{2+} channel antagonist nitrendipine (5 μM) both acute depression (68 $\pm$ 9%) and LTD (77 $\pm$ 26%) were observed (n = 6). (B) Similarly, when ACPD was applied in the presence of the A1 adenosine receptor antagonist CPT (5 μM) both acute depression (60 $\pm$ 12%) and LTD (68 $\pm$ 10%) were observed (n = 5).

At this concentration, ethanol will decrease NMDAR-mediated currents (Lovinger *et al.*, 1989); lower concentrations of ethanol did not block ACPD-induced LTD either when applied alone (not shown) or when used as a solvent for CPT (0.023%, see below) or in controls for staurosporine experiments (0.018%, see below).

We then attempted to determine the source of the NMDAR-mediated current required for ACPD-induced LTD. The effects of bath application of the enzyme glutamic pyruvic transaminase (5 units/ml) were examined. This enzyme selectively metabolizes endogenous extracellular glutamate to α-ketoglutarate in the presence of elevated concentrations of pyruvic acid, and thus reversibly reduces tonic activation of NMDARs in cerebellum (Rossi and Slater, 1993). Slices (n = 4) were exposed to glutamic pyruvic transaminase and pyruvic acid (2 mM) for 10 min prior to and during 20 min ACPD application; matched control slices (n = 4) received only a 20 min application of ACPD. Experimental and interleaved control slices both displayed acute ACPD-induced depression (80 $\pm$ 13% and 66 $\pm$ 12%, respectively). However, 25–30 min following washout of ACPD, the mean EPSP slope in the glutamic pyruvic transaminase group showed recovery (95 $\pm$ 11% baseline) and differed significantly from the mean EPSP slope in the control group (71 $\pm$ 12%, $p < 0.04$, Fig. 6(A)). These experiments suggest that tonic activation of NMDARs by resting glutamate levels supplies the NMDA-mediated contribution to LTD.

Effects of blockade of serine–threonine kinases by staurosporine

Because protein kinase C (PKC) plays a role in some forms of synaptic plasticity (Bliss and Collingridge,

1993), we tested the effects of the (non-selective) serine–threonine kinase inhibitor staurosporine on ACPD-induced LTD. Experimental slices ($n = 5$) were preincubated in 0.4 μM staurosporine and interleaved with control slices from the same animal incubated in solvent (0.018% ethanol) alone. ACPD (20 μM) induced an acute depression in both staurosporine-treated (62 $\pm$ 15%) and control (60 $\pm$ 7%) slices. However, 30–35 min after the washout of ACPD, staurosporine-treated slices recovered to 99 $\pm$ 18% baseline, and differed significantly from control slices (62 $\pm$ 5%, $p < 0.05$, paired t-test). Thus, staurosporine-sensitive kinases also appear to participate in ACPD-induced LTD in immature CA1.

Effects of blockade of voltage-sensitive Ca^{2+} channels by nitrendipine

Several forms of LTD have been found to depend on L-type voltage sensitive calcium channels (L-VSCCs; Bolshakov and Siegelbaum, 1994; Christie and Abraham, 1994; Linden, 1994). To determine if ACPD-induced LTD requires activation of L-VSCCs, ACPD was applied in the presence of the antagonist nitrendipine (5 μM in 0.023% ethanol; a prior control experiment established that this concentration of ethanol did not alter synaptic transmission or block ACPD-induced LTD). As is shown in Fig. 7(A), ACPD-induced LTD was not blocked by nitrendipine ($n = 6$). A typical biphasic response was obtained, with an acute decrease in the presence of the drug (68 $\pm$ 9% of baseline) and only partial recovery in washout (to 77 $\pm$ 26% of baseline).

Effects of A1 adenosine receptor blockade by CPT

Decreased synaptic transmission in the SC–CA1 pathway, when induced by application of NMDA (Manzoni *et al.*, 1994) or cotreatment with ACPD and isoproterenol (Gereau and Conn, 1994) has been found to be mediated by adenosine receptors. To determine whether the actions of ACPD observed here were mediated in part by adenosine receptors, ACPD was applied in the presence of the A1 adenosine receptor antagonist, CPT (5 μM, $n = 5$). As is shown in Fig. 7(B), CPT did not block either the acute depression or the LTD induced by ACPD. An acute decrease in EPSP slope in the presence of ACPD (60 $\pm$ 12% of baseline) was followed by modest recovery during washout (68 $\pm$ 10% baseline at 35–40 min). In these slices no attempt was made to wash out CPT, since washout can be accomplished only slowly and with potentially unpredictable and confounding effects (Manzoni *et al.*, 1994).

DISCUSSION

The major result of our studies is that the bath application of 10 μM ACPD, an mGluR agonist, induces both acute and long-lasting depression of neurotransmission at the SC–CA1 synapse in immature animals (8–12 days postnatal age). This ACPD-induced synaptic depression is consistently biphasic in appearance, characterized by an acute decrease to ~50–60% of baseline EPSP slope in the presence of agonist and partial reversal to ~75–80% of baseline EPSP slope after washout. The transient reversible depression requires only mGluR activation, whereas the ACPD-induced LTD is dependent on co-activation of mGluRs and NMDARs as it was prevented by blockade of either receptor. ACPD-induced LTD also requires activation of staurosporine-sensitive kinases, but is independent of both L-VSCCs and A1 adenosine receptor activation.

Why should NMDAR antagonists block the LTD of synaptic transmission induced by the bath application of ACPD? We hypothesize that the requisite NMDAR component is contributed by background NMDAR-mediated currents, and that NMDAR antagonists block these background currents. The background NMDAR-mediated currents might be produced by synaptically released glutamate evoked by test stimuli and/or spontaneous miniature synaptic currents. Alternatively, tonic activation of synaptic or extrasynaptic NMDARs might arise from the background levels of glutamate present in developing brain (Komuro and Rakic, 1993; Rossi and Slater, 1993) which may be released from adjacent glia (Parpura *et al.*, 1996). Our data suggest that extracellular glutamate is responsible for the tonic activation of NMDARs since LTD was not observed in the presence of glutamic pyruvic transaminase, an enzyme that selectively metabolizes extracellular glutamate and blocks tonic NMDAR activity, without directly altering postsynaptic NMDAR sensitivity (Rossi and Slater, 1993). Although these background NMDAR-mediated currents are presumably small, there are multiple potential interactions between mGluRs and NMDARs. For example, NMDAR-mediated currents may be enhanced by mGluR activation (Aniksztejn *et al.*, 1991; Harvey and Collingridge, 1993; Kinney and Slater, 1993; O'Connor *et al.*, 1994). mGluR activation may produce depolarization of CA1 neurons (Desai and Conn, 1991), increasing the current due to tonic NMDAR activation (by relief of channel block by magnesium). Conversely, NMDAR activation can enhance the stimulation of phosphoinositol metabolism induced by mGluR activation (Irving *et al.*, 1992; Challis *et al.*, 1994). Moreover, co-activation of both mGluRs and NMDARs is required for ACPD-induced LTP of both NMDA and non-NMDA currents in dentate gyrus granule cells (O'Connor *et al.*, 1995) and cerebellar granule cells (Rossi *et al.*, 1996). Thus co-activation of NMDARs and mGluRs may result in mutual amplification of receptor function as well as an enhancement of the effects mediated by intracellular messengers.

The transient, reversible depression of SC–CA1 synaptic transmission produced by mGluR activation appears similar to that reported previously in 3–7 day (Bolshakov and Siegelbaum, 1994) and 11–30 day rat CA1 (Baskys and Malenka, 1991) and was attributed to activation of presynaptic mGluRs that reduce glutamate release. In the latter study, the activity of this presynaptic

mGluR was shown to decrease with maturation between 11 and 30 days (Baskys and Malenka, 1991). Presynaptic mGluRs that inhibit synaptic glutamate release have also been reported at the corticostriatal synapse in neostriatum (Lovinger *et al.*, 1993), amygdala (Rainnie and Shinnick-Gallagher, 1992), parallel fiber–Purkinje cell synapse in cerebellum (Glaum *et al.*, 1992; Pekhletski *et al.*, 1996) and visual cortex (Sladeczek *et al.*, 1993). In mature CA1, activation of mGluRs raised cAMP levels and depressed SC–CA1 synaptic transmission by increasing extracellular adenosine and activating adenosine receptors (Gereau and Conn, 1994); a unique neonatal mGluR that enhances cAMP production (Schoepp and Johnson, 1993b) could contribute to such an effect. However, our results argue against the involvement of extracellular adenosine (via A1 receptors) in our mGluR-induced transient reduction of EPSP slope.

Bath application of ACPD has previously been shown to induce LTD at the medial perforant path–granule cell synapse in immature (Trommer *et al.*, 1993) as well as adult dentate gyrus (O'Mara *et al.*, 1995). In contrast to our findings in CA1, ACPD-induced LTD in dentate gyrus was NMDAR-independent (O'Mara *et al.*, 1995), perhaps reflecting differential anatomic distribution and levels of expression of the mGluR subtypes within hippocampus (e.g. Shigemoto *et al.*, 1992; Ohishi *et al.*, 1993a,b; Fotuhi *et al.*, 1994; Kinzie *et al.*, 1995). Since ACPD has marked activity at mGluRs 1, 2, 3 and 5 (Watkins and Collingridge, 1994), our data do not allow us to determine whether the mGluR responsible for LTD in immature CA1 belongs to Group I or Group II. However, it has recently been found that the Group I mGluR-specific agonist (*RS*)-3,5-dihydroxyphenylglycine (DHPG) also induces LTD in immature CA1 (Fitzjohn *et al.*, 1996), thus implicating the Group I mGluRs (1 or 5). Our finding of inhibition by staurosporine is consistent with this conclusion.

ACPD-induced LTD shares some properties with LTD of SC–CA1 induced by low frequency stimulation of the SC (LFS-induced LTD) at this stage of development. Both methods of LTD induction are blocked by the NMDAR antagonist AP5 (Dudek and Bear, 1992; Mulkey and Malenka, 1992), are blocked by MCPG (Bashir *et al.*, 1993b), and are independent of L-VSCCs (Mulkey and Malenka, 1992). Despite these similarities, the overlap between LFS-induced and ACPD-induced LTD does not appear to be complete. Whereas we found that MCPG completely blocked ACPD-induced LTD, Bashir *et al.* (1993b) reported that MCPG only incompletely blocked LFS-induced LTD, reducing the magnitude of depression by an average of 54% (although LTD was completely blocked in some slices). Moreover, ACPD-induced LTD requires immature hippocampus as substrate, whereas LFS can induce LTD in adult hippocampus (Dudek and Bear, 1992) although its magnitude is greater in younger animals (Dudek and Bear, 1993). It is of interest that, at a slightly earlier stage of postnatal development (3–7 days), the mechanism of induction of LFS-induced LTD differs from that described previously: it is L-VSCC dependent and NMDAR-independent (Bolshakov and Siegelbaum, 1994).

ACPD-induced LTD also shares some features with heterosynaptic LTD as described in mature hippocampus. In this paradigm, vigorous stimulation of one input, usually sufficient to allow Ca^{2+} influx through NMDARs and produce homosynaptic LTP in the stimulated pathway, may produce LTD of a second, unstimulated (non-associative heterosynaptic LTD) or weakly and asynchronously stimulated (associative heterosynaptic LTD) input (Linden, 1994). Heterosynaptic LTD can be blocked by NMDAR antagonists (Stanton and Sejnowski, 1989; Kerr and Abraham, 1993), by L-VSCC antagonists (Christie and Abraham, 1994), by mGluR antagonists (Stanton *et al.*, 1991), or by blocking a rise in cytoplasmic Ca^{2+} by dialyzing the postsynaptic neuron (CA1) with a solution containing a Ca^{2+} chelator (Debanne *et al.*, 1994).

We found a striking dependence of ACPD-induced synaptic changes on developmental age: LTD was consistently observed at 8–12 days, whereas minimal change or LTP resulted at 29–33 days. A major unknown raised by our work is the physiologic significance of the conversion from LTD to LTP, and in particular whether the change is at the level of the receptor, second messenger systems, or effectors. It is important that the cause and mechanism of this transition be determined, since it occurs temporally as dendritic number, length, and spine density in rat CA1 reach adult levels and mature hippocampal morphology becomes apparent (Minkwitz, 1977; Pokorny and Yamamoto, 1981). It is thus tempting to speculate that the phenomenon observed here may contribute to the dynamic processes that accompany synapse maturation.

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