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Activation of group I metabotropic glutamate receptors induces long-term depression at sensory synapses in superficial spinal dorsal horn

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Abstract

Low-frequency stimulation of primary afferent Aδ-fibers can induce long-term depression of synaptic transmission in rat superficial spinal dorsal horn. Here, we have identified another form of long-term depression in superficial spinal dorsal horn neurons that is induced by specific group I but not group II metabotropic glutamate receptor (mGluR) agonists. Synaptic strength between Aδ-fibers and dorsal horn neurons was examined by intracellular recordings in a spinal cord–dorsal root slice preparation from young rat. In the presence of bicuculline and strychnine, bath application of (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD) or the specific group I mGluR agonist (*S*)-3,5-dihydroxyphenylglycine ((*S*)-3,5-DHPG) but not the specific group II mGluR agonist (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV) for 20 min produced an acute and a long-term depression of synaptic strength. Bath application of the *N*-methyl-D-aspartate receptor antagonist D-2-amino-5-phosphonovaleric acid did not affect these depressions by (1*S*,3*R*)-ACPD. After pre-incubation of slices with pertussis toxin, a G-protein inhibitor, (1*S*,3*R*)-ACPD still induced acute and long-term depressions. The phospholipase C inhibitor U73122 stereoselectively blocked the induction of long-term depression without affecting acute synaptic inhibition. This study demonstrates that, in the spinal cord, direct activation of group I mGluRs that are coupled to phospholipase C through pertussis toxin-insensitive G-proteins induces a long-term depression of synaptic strength. This may be relevant to the processing of sensory information in the spinal cord, including nociception.

Keywords: (*S*)-3,5-DHPG; (1*S*,3*R*)-ACPD; Aδ-fiber; Pertussis toxin; Phospholipase C; Spinal cord slice; Nociception

1. Introduction

Nociceptive information is encoded and conveyed by fine, thinly myelinated Aδ-fibers and unmyelinated C-fibers, which terminate predominantly on neurons in the superficial spinal dorsal horn. Glutamate or a related amino acid is used by these afferents as a fast excitatory neurotransmitter (Yoshimura and Jessell, 1990) and acts on postsynaptic ionotropic glutamate receptors of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate and/or *N*-methyl-D-aspartate (NMDA) subtypes. Recently, metabotropic glutamate receptors (mGluRs) which are coupled through G-proteins to several intracellular second messenger cascades have been identified in the superficial spinal dorsal horn (Vidnyanszky et al., 1994; Valerio et al., 1997). In the superfamily of mGluRs, group I (mGluRs 1 and 5) is believed to be coupled to phospholipase C (PLC) through G-proteins, most of which are pertussis toxin (PTX)-insensitive. The activation of the PLC–inositol-1,4,5-triphosphate (IP_3) pathway can lead to Ca^{2+} release from internal stores (Berridge, 1993). Group II (mGluRs 2 and 3) and group III mGluRs (mGluRs 4, 6, 7 and 8) are negatively coupled to adenylyl cyclase by PTX-sensitive G-proteins (Pin and Duvoisin, 1995). Neuroanatomical and immunocytochemical studies suggest that group I mGluRs are predominantly localized on postsynaptic elements, whereas group II and group III mGluRs are predominantly localized on presynaptic elements (Ohishi et al., 1993; Vidnyanszky et al., 1994; Petralia et al., 1996). Activation of spinal mGluRs may contrib-

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ute to an increase in spinal nociception and hyperalgesia (Meller et al., 1993; Neugebauer et al., 1994; Young et al., 1995). In contrast, any role for mGluRs in spinal *anti*nociceptive mechanisms has yet to be demonstrated.

At some synapses in the brain, the activation of ionotropic or metabotropic glutamate receptors has been shown to be required for some forms of long-term potentiation or long-term depression (LTD) of synaptic strength (Bliss and Collingridge, 1993; Bortolotto and Collingridge, 1993; Linden, 1994; Malenka, 1994; Overstreet et al., 1997; Cho et al., 2000).

Recent studies have identified use- and NMDA receptor-dependent LTD at synapses between fine primary afferent Aδ-fibers and spinal dorsal horn neurons (Randić et al., 1993; Sandkühler et al., 1997). Here, we have addressed the question of whether the direct activation of mGluRs is sufficient to induce long-lasting changes in synaptic strength between fine primary afferents and dorsal horn neurons.

2. Methods

Transverse slices were obtained from young (17- to 28-day-old) Sprague Dawley rats of both sexes. Under deep ether anesthesia, the lumbar spinal cord was exposed by laminectomy. The segments of the lumbosacral spinal cord (L4-S1) with long (8–15 mm) dorsal roots attached were excised. Transverse slices with one dorsal root attached were cut at 400–500 μm thickness and incubated at 33°C for at least 1 h. The incubating solution was oxygenated and consisted of (in mM): NaCl, 124; KCl, 5; KH_2PO_4, 1.2; $CaCl_2$, 2.4; $MgSO_4$, 1.3; $NaHCO_3$, 26; glucose, 10; at pH 7.4, osmolarity 310–320 mosmol/kg. A single slice was then transferred to a recording chamber (volume 1.0 ml) and was continuously perfused at a rate of 3–4 ml/min with an oxygenated recording solution at 30°C, which was identical to the incubation solution except for a lower concentration of KCl (1.9 mM).

Standard intracellular and gramicidin perforated whole-cell patch-clamp recording techniques were used to record excitatory postsynaptic potentials (EPSPs) and excitatory postsynaptic currents (EPSCs), respectively, in lamina II, which was identified as a translucent band across the superficial spinal dorsal horn. When filled with 4 M potassium acetate, the microelectrodes had a DC tip resistance between 140 and 190 MΩ. EPSPs were recorded with a high-input impedance bridge amplifier (Axoclamp 2B, Axon Instruments, Foster City, USA) and analyzed with the software package "Experimenter's WorkBench (Version 4.0)" (Data Wave Technologies Co., Longmont, CO, USA). Changes in input resistance were monitored by measuring the voltage responses of the cell to hyperpolarizing current pulses (0.05 nA, 200 ms) every 2 min.

For whole-cell recordings, patch pipettes were pulled from borosilicate glass capillaries (GC 150-15, Clark Electromedical Instruments, UK) on a horizontal puller (P-87, Sutter Instruments, Novato, CA, USA) and had resistances of 3–6 MΩ, when filled with the intracellular solution consisting of (in mM): potassium gluconate, 120; KCl, 20; $MgCl_2$, 2; Na_2ATP, 2; Na_2GTP, 0.5; EGTA, 0.5; HEPES, 20; pH 7.28, with KOH; osmolarity 310 mosmol/kg. For perforated patch recordings, the antibiotic gramicidin was used at a concentration of 50 μg/ml in a solution containing (in mM): potassium gluconate, 120; KCl, 20; $MgCl_2$, 2; HEPES, 20; pH 7.28, with KOH. Neurons were visualized with Dodt-infrared optics using a ×40, 0.80 water-immersion objective on an Olympus BX50WI upright microscope (Olympus, Japan) equipped with a video camera system (PCO, Kelheim, Germany). Recordings were made with an Axopatch 200B patch-clamp amplifier (Axon Instruments, Foster City, CA, USA) at a sampling rate of 10 kHz using a low-pass bessel filter of 2 kHz. The software program PCLAMP 8 was used for data acquisition and subsequent off-line analysis.

The dorsal root was stimulated through a suction electrode with an isolated current stimulator (A320, World Precision Instruments, Sarasota, FL, USA). The stimulus parameters necessary to activate Aδ-fibers were determined by extracellular recordings of the dorsal root volleys in a two-compartment chamber using silver wire hook electrodes as recording electrodes in oil and suction electrodes as stimulating electrodes in saline.

After the threshold for eliciting an EPSP/EPSC was determined, test pulses of 0.1 ms were given at 60 s intervals unless stated otherwise. Stimulation intensity was adjusted between 0.1 and 0.5 mA to yield EPSP amplitudes of 5 to 25 mV or EPSC amplitudes between 25 and 250 pA. Only the EPSPs/EPSCs that were presumed to be produced by excitation of Aδ-fibers were investigated further. A 10 or 20 Hz train of stimulation of seven pulses was applied to test for mono- or polysynaptic input. Two consecutive responses were averaged and synaptic strength was quantified by measuring the peak amplitude of the averaged responses. The mean amplitude of seven averaged test responses recorded prior to the agonist application served as a control. Significant changes from controls were assessed by measuring the amplitudes of seven consecutive responses at the end of the agonist application and at 20 to 30 min after washout of the agonist. Values are the mean±SEM. The non-parametric Wilcoxon rank test was used for statistical comparisons.

All drugs were dissolved in oxygenated recording solution at known concentrations. Drugs and their sources were as follows: (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD; 100 μM; Tocris, Köln, Germany); (*S*)-3,5-dihydroxyphenylglycine ((*S*)-3,5-DHPG; 100 μM; Tocris); (2*S*,2′*R*,3′*R*)-2-(2′,3′-

dicarboxycyclopropyl)glycine (DCG-IV, 5 μM; Tocris); (*S*)-4-carboxyphenylglycine ((*S*)-4CPG, 200 μM; Tocris); picrotoxin (100 μM, Tocris); bicuculline methiodide (bicuculline; 5 or 10 μM; Sigma, Deisenhofen, Germany); strychnine (2 or 4 μM; Sigma); D-2-amino-5-phosphonovaleric acid (D-AP5; 50 μM; Cambridge Research Biochemicals, Northwhich, UK); pertussis toxin (PTX; 1 or 2 μg/ml; Sigma); 1-[6-[[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione (U73122; 1 μM; Sigma); 1-[6-[[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-2,5-pyrrolidinedione (U73343; 1 μM; Sigma), gramicidin D (Sigma). They were made in stock solution and diluted in perfusing solution immediately prior to use. PTX was also dissolved in incubation solution.

3. Results

Stable intracellular recordings of up to 3 h were obtained from 88 neurons in lamina II of the spinal dorsal horn. Electrical stimulation of primary afferent Aδ-fibers in the dorsal root elicited fast EPSPs or EPSCs in all neurons included in this study. The mean resting membrane potential of all of the neurons which were included in this study was −62±2 mV (sharp electrodes) and −64±2 mV (patch-clamp electrodes), respectively. The mean input resistance was 202±13 MΩ (sharp electrodes) and 678±43 MΩ (patch electrodes), respectively, and did not change significantly during recording periods. After the threshold for eliciting an EPSP or EPSC was determined, the intensity of test stimulation of the dorsal root was adjusted between 0.1 and 0.5 mA for activating Aδ-fibers. The mean threshold for Aδ-fiber-induced EPSPs was 0.3±0.04 mA (0.1 ms pulses). When the synaptic delay was presumed to be 1 ms, the mean calculated conduction velocity of Aδ-fibers was 2±0.2 m/s. In 88 neurons tested, 69% apparently received monosynaptic inputs, as determined by constant latencies and absence of failures upon 10 or 20 Hz stimulation, the remaining 31% of the cells received polysynaptic input. Bath application of the $GABA_A$ receptor antagonists bicuculline or picrotoxin and the glycine receptor antagonist strychnine typically increased the duration of EPSPs and EPSCs and the cell excitability but had little or no effect on the amplitude and slope of the initial component of both responses (see the traces in Figs. 3–5).

3.1. Activation of mGluRs induces LTD in superficial spinal dorsal horn

To address the question of whether the activation of mGluRs is sufficient for the induction of LTD in the spinal cord, we applied (1*S*,3*R*)-ACPD, a widely used agonist for mGluRs (Cerne and Randić, 1992; Liu et al., 1993; Pin and Duvoisin, 1995). In nine cells tested, (1*S*,3*R*)-ACPD added to the perfusing solution at a concentration of 100 μM failed to induce either short-term or long-term changes in synaptic strength (Fig. 1A). At this concentration (1*S*,3*R*)-ACPD effectively activates mGluRs in slices (O'Mara et al., 1995).

Tonic GABAergic inhibition may prevent the induction of LTD by (1*S*,3*R*)-ACPD in the hippocampus (O'Mara et al., 1995). To test whether tonic inhibition also blocks LTD induction by (1*S*,3*R*)-ACPD in the spinal cord, in three cells we applied (1*S*,3*R*)-ACPD first in the absence and then in the presence of bicuculline and strychnine. In the absence of bicuculline and strychnine, bath application of (1*S*,3*R*)-ACPD had no effect on EPSP amplitudes. In the same three neurons the second application of (1*S*,3*R*)-ACPD during blockade of $GABA_A$ and glycine receptors now induced an LTD of the amplitude of the initial EPSP component (Fig. 1B). The mean amplitude of EPSPs at the end of the (1*S*,3*R*)-ACPD application was reduced to 77±6% of control ($P<0.01$). After washout of (1*S*,3*R*)-ACPD for 20 min, the mean EPSP amplitude was still depressed to 72±6% of control ($P<0.01$). In eight additional neurons, bath application of (1*S*,3*R*)-ACPD for 20 min, in the presence of bicuculline and strychnine, also induced an LTD of the amplitudes of the initial EPSP component (Fig. 2). The mean amplitude of EPSPs at the end of (1*S*,3*R*)-ACPD application was reduced to 74±3% of control ($P<0.01$). After washout of (1*S*,3*R*)-ACPD for 20 min the mean EPSP amplitude was reduced to 72±4% ($n=8$, $P<0.01$). The LTD always outlasted the recording periods of up to 140 min (Fig. 2A). These results provide direct evidence that disinhibition can unmask LTD induction by the mGluR agonist (1*S*,3*R*)-ACPD in the superficial spinal dorsal cord. In some experiments (1*S*,3*R*)-ACPD was applied in the absence of test stimulation and this also induced an LTD showing that presynaptic activity is not required for induction of this form of synaptic plasticity.

In conventional whole-cell recordings, bath application of (1*S*,3*R*)-ACPD for 20 min, in the presence of bicuculline and strychnine, had no significant effect on the amplitude of the initial EPSC component (data not shown). The mean amplitude of EPSCs at the end of (1*S*,3*R*)-ACPD application was not significantly affected (96±5% of control, $n=9$, $P>0.05$). After washout of (1*S*,3*R*)-ACPD for 20 min the mean EPSC amplitude was 94±10% ($n=4$, $P>0.05$). When the same experiments were performed with gramicidin-perforated patch recordings, bath application of (1*S*,3*R*)-ACPD for 20 min, in the presence of bicuculline and strychnine, induced an LTD of the amplitude of the initial EPSC component (data not shown). The mean amplitude of EPSCs at the end of (1*S*,3*R*)-ACPD application was reduced to 53±5% of control ($n=4$, $P<0.01$). After washout of (1*S*,3*R*)-ACPD for 15 min the mean EPSP ampli-

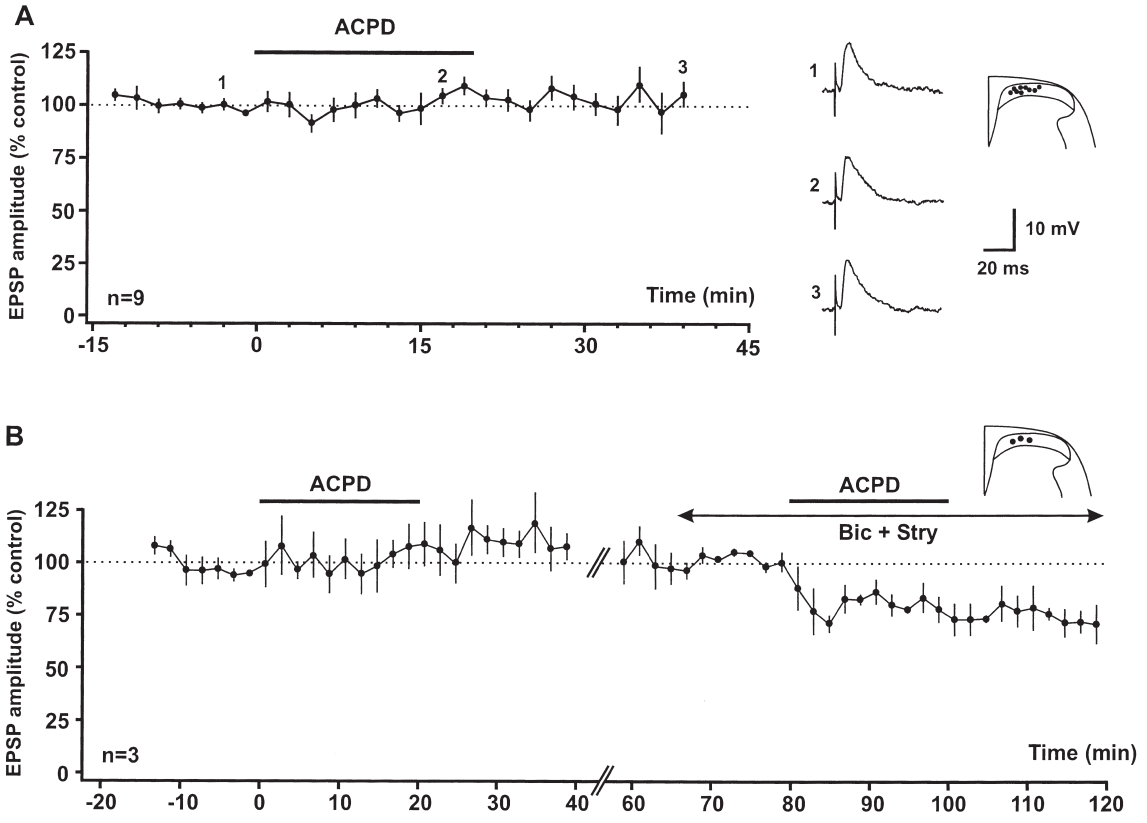

Fig. 1. Acute and long-lasting inhibition of synaptic strength by bath application of (1*S*,3*R*)-ACPD is unmasked by bicuculline and strychnine. (A) (1*S*,3*R*)-ACPD failed to affect EPSP amplitudes in all nine cells tested when added to normal recording solution for 20 min (100 μM; horizontal bar). The mean amplitude of seven consecutive EPSPs obtained in normal recording solution immediately prior to application of (1*S*,3*R*)-ACPD served as a control. Representative original EPSPs prior to application of (1*S*,3*R*)-ACPD (trace 1), at the end of (1*S*,3*R*)-ACPD application (trace 2) and after washout of (1*S*,3*R*)-ACPD (trace 3), as well as visually identified recording sites, are shown in the inset. (B) Two consecutive applications of (1*S*,3*R*)-ACPD (horizontal bars) during recording from the same dorsal horn neurons, first in the absence and then in the presence of bicuculline (Bic, 10 μM) and strychnine (Stry, 4 μM) when (1*S*,3*R*)-ACPD induced a robust LTD. The mean amplitude of seven consecutive EPSPs during application of bicuculline and strychnine (horizontal line with arrows) served as a control. The visually identified recording sites are shown in the inset.

tude was still reduced to 43±3% ($P<0.01$). These data suggest that, for the induction of LTD by the mGluR agonist (1*S*,3*R*)-ACPD in superficial spinal dorsal cord, a diffusible compound in postsynaptic neurons is a prerequisite.

3.2. (1S,3R)-ACPD-induced LTD is independent of NMDA receptor activation

LTD can be classified into NMDA receptor-dependent and NMDA receptor-independent forms (Bliss and Collingridge, 1993; Nicoll and Malenka, 1995; O'Mara et al., 1995). To assess the role of NMDA receptors in (1*S*,3*R*)-ACPD-induced LTD in the spinal cord, we added D-2-amino-5-phosphonovaleric acid (D-AP5) at 50 μM, which is sufficient to block NMDA receptor-mediated conductance, to the perfusing solution that also contained bicuculline and strychnine. In the presence of D-AP5, (1*S*,3*R*)-ACPD still induced LTD similar in magnitude to the LTD in the D-AP5-free group (Fig. 3). The mean amplitude of EPSPs at the end of (1*S*,3*R*)-ACPD application was reduced to 71±9% of control ($P<0.01$). After a 20-min washout of (1*S*,3*R*)-ACPD with a solution that contained D-AP5, bicuculline and strychnine, EPSP amplitudes were depressed to 60±11% of control (n=5, $P<0.01$). Thus, the (1*S*,3*R*)-ACPD-induced LTD in superficial spinal dorsal horn does not require activation of NMDA receptors.

3.3. PTX-sensitive G-proteins are not necessary for LTD induction by activation of mGluRs

G-proteins that are coupled to mGluRs can be divided into PTX-sensitive and PTX-insensitive subtypes based on whether the α subunits can be inhibited by PTX. To explore what kind of G-proteins are involved in the generation of (1*S*,3*R*)-ACPD-induced spinal LTD, we incubated the slices with PTX (1 or 2 μg/ml) at 37°C, for at least 2 h. This was shown to block PTX-sensitive G-proteins in the incubated slices (Bayliss et al., 1997). In

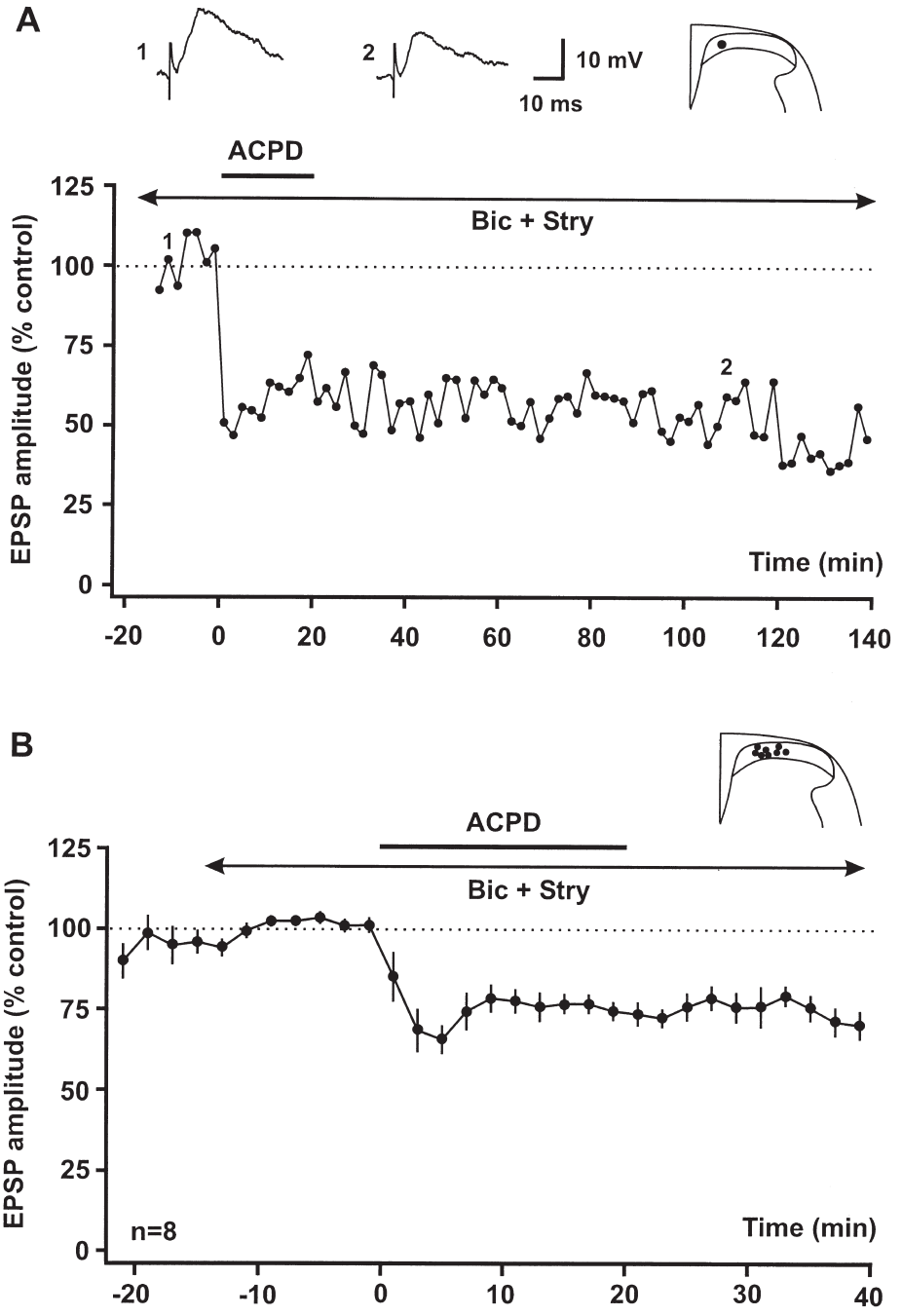

Fig. 2. Bath application of (1*S*,3*R*)-ACPD induces a robust LTD of fast excitatory synaptic transmission in the superficial spinal dorsal horn. (A) Results from one experiment. Peak amplitudes of two consecutive, averaged EPSPs are plotted versus time. (1*S*,3*R*)-ACPD was added to the superfusion solution for 20 min (100 μM; horizontal bar). Bicuculline (Bic, 10 μM) and strychnine (Stry, 4 μM) were present in the superfusion solution throughout the recording (horizontal bar with arrows). Representative EPSP recordings and the recording site in lamina II are shown in the inset. V_m=−86 mV; 20-day-old rat. (B) The mean time course of ACPD-induced LTD of eight experiments. The mean amplitude of seven consecutive EPSPs immediately prior to application of (1*S*,3*R*)-ACPD served as a control. Recording sites are shown in the inset.

all cells that were recorded in PTX-preincubated slices, (1*S*,3*R*)-ACPD still induced LTD of synaptic transmission when added to a recording solution that also contained bicuculline and strychnine (Fig. 4). The mean EPSP amplitude 20 min after onset of (1*S*,3*R*)-ACPD application was reduced to 53±9% of control ($P<0.01$), EPSP amplitudes were still depressed to 55±8% of control after washing for 20 min in a solution that contained bicuculline and strychnine (n=5, $P<0.01$). These results suggest that PTX-sensitive G-proteins are not required for the generation of ACPD-induced LTD.

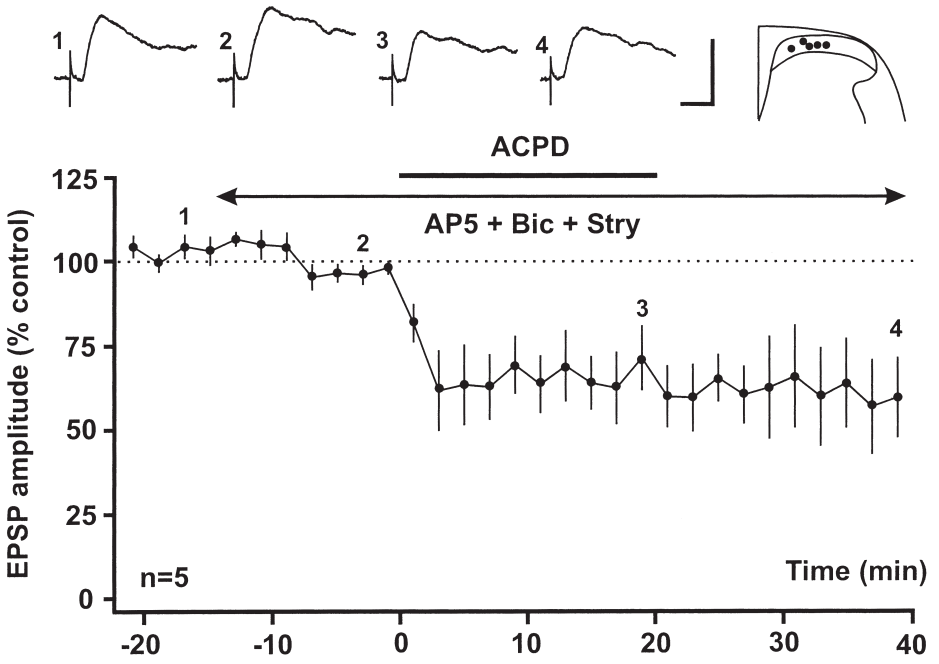

Fig. 3. Induction of LTD by (1*S*,3*R*)-ACPD does not require activation of NMDA receptors. The specific NMDA receptor antagonist D-AP5 (50 μM) was added to the superfusate (horizontal line with arrows) that also contained bicuculline (Bic, 10 μM) and strychnine (Stry, 4 μM). The mean amplitude of seven consecutive EPSPs immediately prior to application of (1*S*,3*R*)-ACPD (100 μM; horizontal bar) served as a control. Representative original EPSPs recorded from one neuron in normal recording solution (trace 1), or a solution that contained D-AP5, bicuculline and strychnine (trace 2), during (1*S*,3*R*)-ACPD application (trace 3) and after washing for 20 min (trace 4) are shown above the graph. Recording sites in lamina II are shown in the inset. Calibration bars: 10 ms/5 mV.

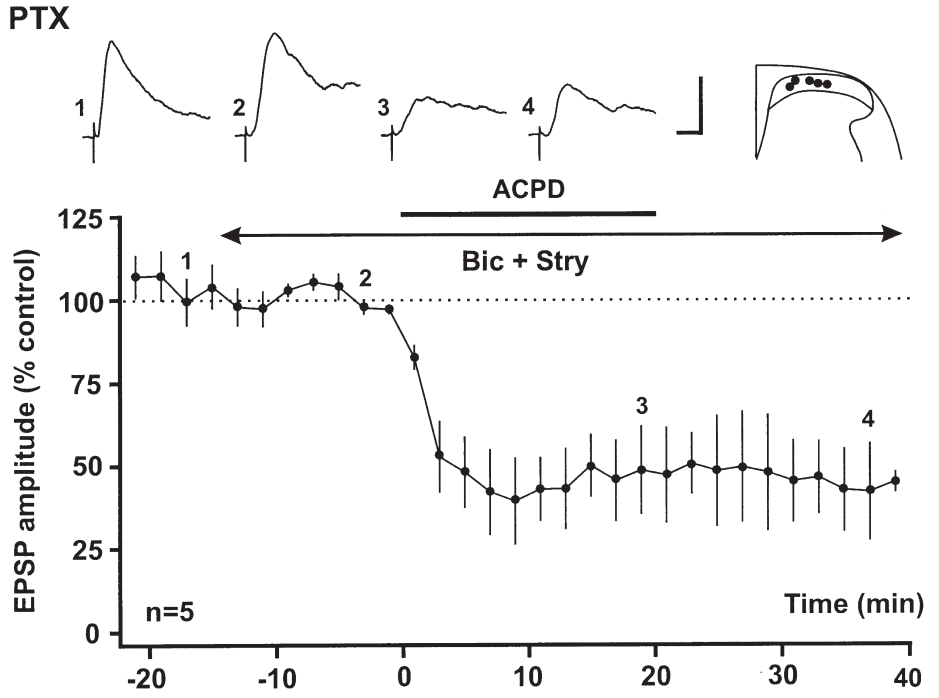

Fig. 4. Incubation of slices with pertussis toxin does not block the induction of LTD by (1*S*,3*R*)-ACPD. Slices were incubated with pertussis toxin (1 or 2 μg/ml, 37°C) for at least 2 h before recordings were begun. In the presence of bicuculline (Bic, 10 μM) and strychnine (Stry, 4 μM) (horizontal line with arrows) bath application of (1*S*,3*R*)-ACPD (upper horizontal bar) induced LTD. The mean amplitude of seven consecutive EPSPs immediately prior to application of (1*S*,3*R*)-ACPD served as a control. Representative original EPSPs in normal recording solution (trace 1), in a solution that contained bicuculline and strychnine (trace 2), during (1*S*,3*R*)-ACPD application (trace 3) and after washing for 20 min (trace 4) are shown above the graph. Recording sites are shown in the inset. Calibration bars: 20 ms/10 mV

3.4. PLC inhibitor U73122 stereoselectively blocks LTD induction in spinal cord

PTX-insensitive G-proteins may activate PLC in some cells. To assess the possible role of PLC in the generation of (1*S*,3*R*)-ACPD-induced LTD, we used the PLC inhibitor 1-[6-[[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione (U73122) and its inactive enantiomer 1-[6-[[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-2,5-pyrrolidinedione (U73343). U73122 or U73343 were applied to the perfusing solution at a concentration of 1 μM (Smith et al., 1990; Cohen et al., 1998) together with 10 μM bicuculline and 4 μM strychnine at least 15 min prior to application of (1*S*,3*R*)-ACPD. In the presence of U73122, (1*S*,3*R*)-ACPD induced an acute depression of synaptic transmission, but failed to induce LTD (Fig. 5A). At the end of (1*S*,3*R*)-ACPD application the mean amplitude of EPSPs was depressed to 61±14% of control ($P<0.01$), and after a 30-min washout period the mean amplitude of EPSPs had recovered to 101±3% of control ($n=5$, $P>0.05$). In contrast, the inactive enantiomer U73433 did not attenuate (1*S*,3*R*)-ACPD-induced LTD (Fig. 5B). The mean amplitude of EPSPs during (1*S*,3*R*)-ACPD application and at 30 min following washout of (1*S*,3*R*)-ACPD was 40±8% of control ($P<0.01$) and 46±14% of control ($P<0.01$), respectively ($n=6$). Thus, (1*S*,3*R*)-ACPD-induced LTD in the spinal dorsal horn requires activation of PLC.

3.5. LTD in superficial spinal dorsal horn is induced by activation of group I mGluRs

Since (1*S*,3*R*)-ACPD is a non-selective agonist for both group I and group II mGluRs, we next used specific group I and group II mGluR agonists. EPSCs were measured using the gramicidin-perforated patch recording technique to prevent the loss of diffusible compounds. (*S*)-3,5-DHPG (100 μM for 20 min), a specific agonist of group I mGluRs (Ito et al., 1992), was temporarily added to the superfusing solution, containing 100 μM picrotoxin and 4 μM strychnine. During (*S*)-3,5-DHPG application the mean amplitude of EPSCs was decreased to 64±4% of control ($n=6$, $P<0.01$). EPSC amplitudes were still depressed to 60±3% of control after washing for 20 min ($P<0.01$; Fig. 6). This (*S*)-3,5-DHPG-induced LTD persisted for as long as responses were recorded, up to 1.5 h after commencing washout. Application of (*S*)-3,5-DHPG had no significant effect on the resting membrane potential ($V_m=-59\pm3$ mV before and $V_{mt}=-58\pm2$ mV during superfusion with the group I agonist; $n=11$, $P>0.05$). Membrane resistance was also unaffected by (*S*)-3,5-DHPG treatment (712±65 MΩ and 709±95 MΩ, respectively). The acute and the long-lasting effects of (*S*)-3,5-DHPG on synaptic transmission could be prevented by pretreating the slices with (*S*)-4CPG (200 μM), a specific antagonist of group I mGluRs (Birse et al., 1993; Sekiyama et al., 1996), together with 100 μM picrotoxin and 4 μM strychnine at least 15 min prior to application of (*S*)-3,5-DHPG (data not shown). The mean amplitude of EPSCs during (*S*)-3,5-DHPG application was 99±3% of control under these conditions ($n=3$, $P>0.05$).

Bath application of the highly potent group II mGluR agonist DCG-IV (Ishida et al., 1993; 5 μM for 20 min) had no significant effect on synaptic transmission (Fig. 7). During application of DCG-IV, responses were 96±5% of control ($n=6$, $P>0.05$). Thus activation of group I but not group II mGluR in the spinal dorsal horn induces LTD of synaptic strength in primary afferent Aδ-fibers.

4. Discussion

The synaptic release of glutamate may have multiple effects on pre- and postsynaptic elements. Activation of either postsynaptic ionotropic (Yoshimura and Jessell, 1990) or metabotropic glutamate receptors (Batchelor et al., 1997) may trigger EPSCs. It is becoming increasingly clear that both types of glutamate receptors are also crucial for induction of long-lasting modifications of synaptic strength at different regions in the brain (Bliss and Collingridge, 1993; Linden, 1994; Malenka, 1994; Pin and Duvoisin, 1995). mGluRs may also be involved in frequency detection and temporally dispersed synaptic signal association (Batchelor and Garthwaite, 1997). Relatively little is known about the synaptic functions of mGluRs at the level of the spinal cord, where presynaptic mGluRs may depress dorsal root-evoked EPSPs of rat neonatal motoneurons (Cao et al., 1997a,b). In acutely dissociated spinal dorsal horn neurons ACPD was shown to enhance AMPA and NMDA receptor-mediated inward currents. We have reported previously that low-frequency stimulation of primary afferent Aδ-fibers could induce LTD in spinal dorsal horn in vitro and in vivo and that this form of LTD requires co-activation of group I and group II mGluRs and, in addition, activation of ionotropic NMDA receptors (Sandkühler et al., 1997; Liu et al., 1998; Chen and Sandkühler, 2000). The present study has identified a new form of synaptic plasticity in the spinal cord that is induced by activation of group I mGluRs in the absence of repetitive presynaptic activity and that does not require activation of NMDA receptors.

4.1. LTD in the spinal dorsal horn can be induced by direct activation of mGluRs

(1*S*,3*R*)-ACPD is a widely used mGluR agonist that predominantly activates group I and II but not group III mGluRs (Cerne and Randić, 1992; Liu et al., 1993; Pin

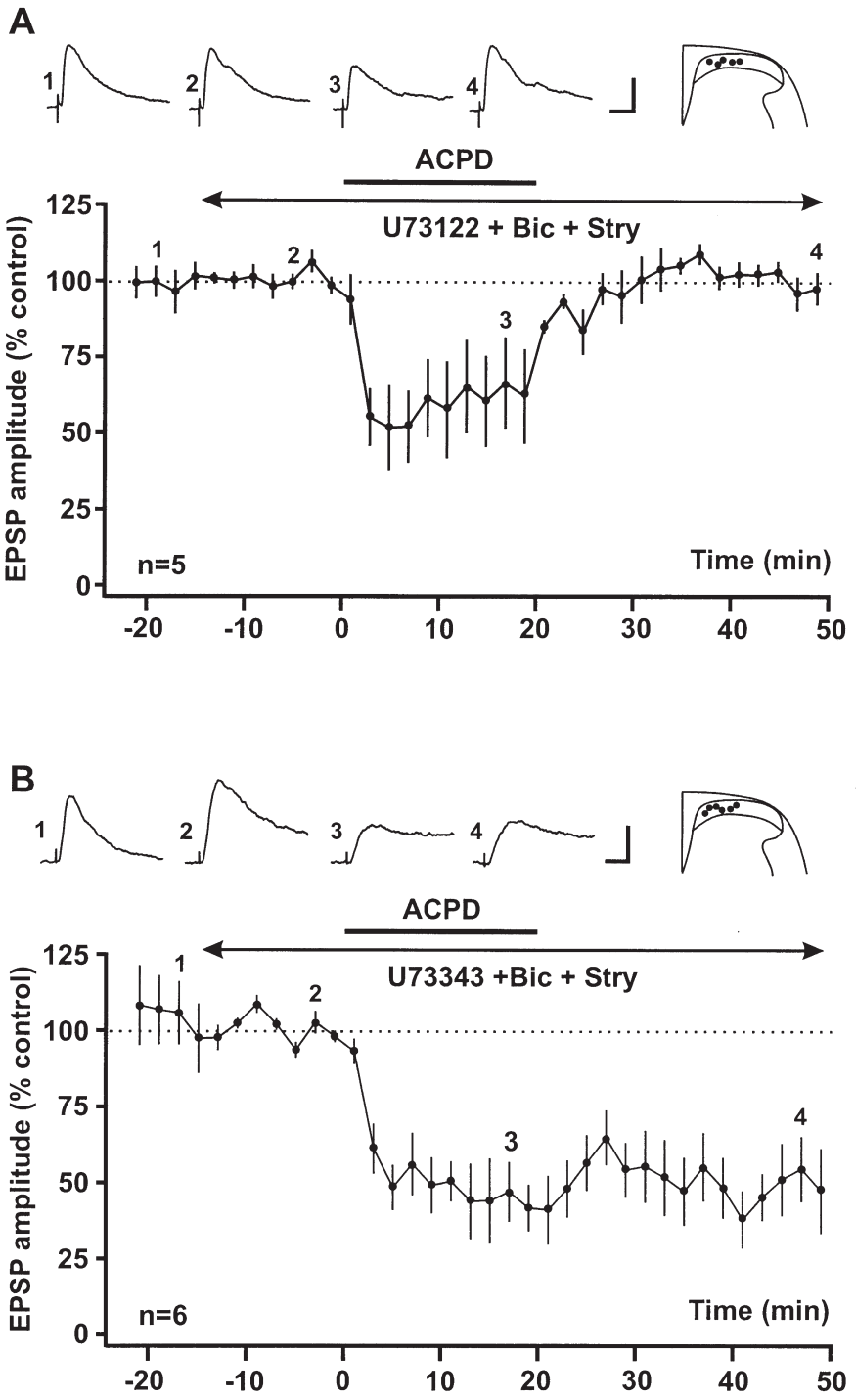

Fig. 5. Activation of PLC is required for induction of LTD by (1*S*,3*R*)-ACPD. (A) The specific PLC inhibitor U73122 (1 μM) was applied together with bicuculline (Bic, 10 μM) and strychnine (Stry, 4 μM) (horizontal line with arrows) at least 15 min before the application of (1*S*,3*R*)-ACPD. In the presence of U73122, (1*S*,3*R*)-ACPD produced an acute depression but failed to induce LTD of synaptic transmission. The mean amplitude of seven consecutive EPSPs prior to the application of (1*S*,3*R*)-ACPD served as a control. Representative original EPSPs recorded from one neuron in normal recording solution (trace 1), in a solution that contained bicuculline and strychnine (trace 2), after adding (1*S*,3*R*)-ACPD (trace 3) and after washing for 30 min (trace 4) are shown above the graph. Recording sites are shown in the inset. (B) Same experimental design as in A but with U73343, the inactive enantiomer of U73122 that failed to block the induction of LTD by (1*S*,3*R*)-ACPD.

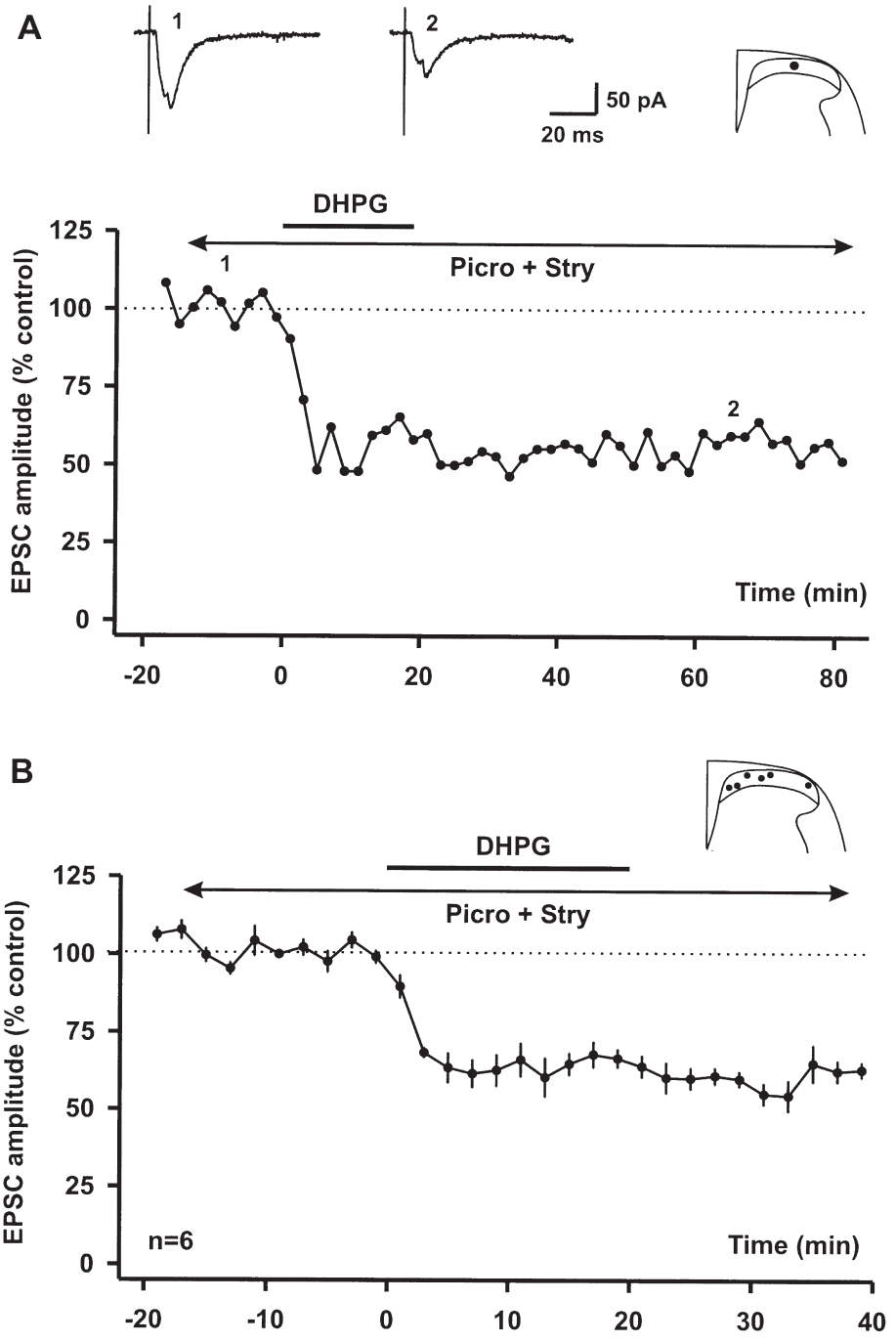

Fig. 6. Selective activation of group I mGluRs with (*S*)-3,5-DHPG induced a robust LTD. (A) Results from a typical gramicidin-perforated whole-cell patch-clamp experiment are shown. Peak amplitudes of two consecutive, averaged EPSCs are plotted versus time. (*S*)-3,5-DHPG was added to the superfusion solution for 20 min (100 μM; horizontal bar). Picrotoxin (Picro, 100 μM) and strychnine (Stry, 4 μM) were present in the superfusion solution throughout the recording. Representative original EPSC recordings and the recording site in lamina II are shown in the inset. V_m=−72 mV; 19-day-old rat. (B) The mean time course of DHPG-induced LTD in six experiments. The mean amplitude of six consecutive EPSCs immediately prior to application of (*S*)-3,5-DHPG served as a control. Neurons were voltage clamped at −75 mV. Recording sites are shown in the inset.

and Duvoisin, 1995). Activation of mGluRs by (1*S*,3*R*)-ACPD may facilitate (Behnisch and Reymann, 1993; Manahan-Vaughan, 1997; Cohen et al., 1998) or directly induce (Bortolotto and Collingridge, 1993; O'Mara et al., 1995; Overstreet et al., 1997) long-term potentiation or LTD at some synapses in the brain.

In the spinal cord, interneurons may co-release GABA and glycine as fast neurotransmitters, possibly from the

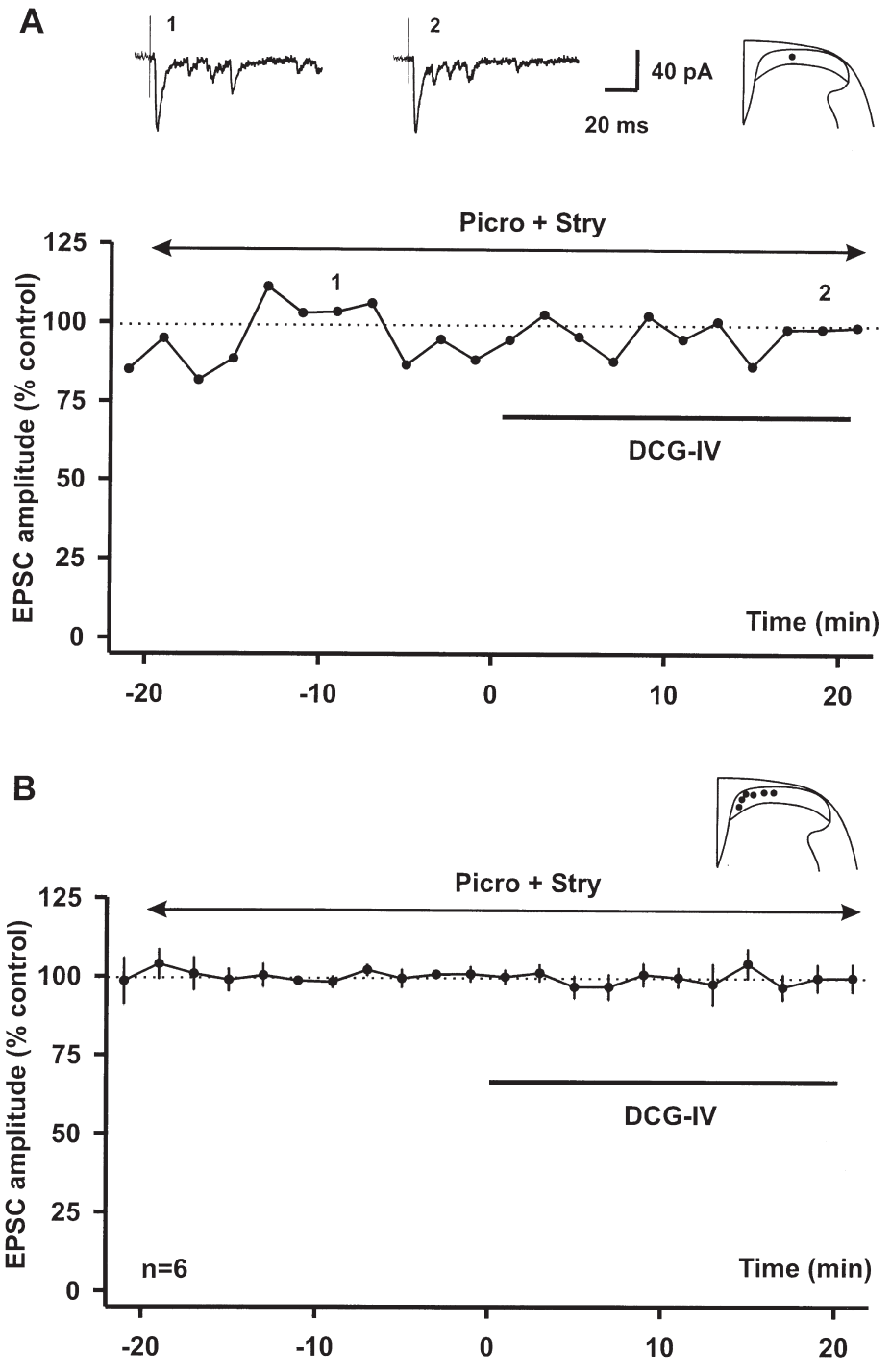

Fig. 7. Bath application of the specific group II mGluR agonist DCG-IV failed to affect synaptic transmission. (A) Results from a typical whole-cell patch-clamp experiment are shown. Peak amplitudes of two consecutive, averaged EPSCs are plotted versus time. DCG-IV was added to the superfusion solution for 20 min (5 μM; horizontal bar). Picrotoxin (Picro, 100 μM) and strychnine (Stry, 4 μM) were present in the superfusion solution throughout the recording. Representative EPSC recordings and the recording site in lamina II are shown in the inset. V_m=−62 mV; 21-day-old rat. (B) The mean time course of DCG-IV effect of six experiments. The mean amplitude of six consecutive EPSCs immediately prior to application of DCG-IV served as a control. Neurons were voltage clamped at −75 mV. Recording sites are shown in the inset.

same synaptic vesicles (Jonas et al., 1998). Only when $GABA_A$ receptors and glycine receptors were blocked, was (1*S*,3*R*)-ACPD able to induce LTD in the present study. It has been reported previously that at synapses in the hippocampus, activation of mGluRs may be sufficient to induce LTD if $GABA_A$ receptors are blocked with picrotoxin (O'Mara et al., 1995). The mechanism by which blockade of inhibitory neurotransmission facilitates (1*S*,3*R*)-ACPD-induced LTD has not been explored. At some synapses LTD induction by mGluRs requires pairing with postsynaptic depolarization (Kano and Kato, 1987; Bolshakov and Siegelbaum, 1994). Since bicuculline and strychnine increased cell excitability and the duration of EPSPs and EPSCs in the present study, we propose that disinhibition may unmask the LTD induction by increasing the excitability and depolarization of postsynaptic neurons. $GABA_A$ receptors are also present on terminals of fine primary afferents, where they mediate presynaptic inhibition. Even though it cannot be excluded it appears highly unlikely that LTD induction is facilitated by the removal of presynaptic inhibition of the primary afferents, as these afferents are not spontaneously active (with the exception of SII afferents in vivo) and were either not stimulated or stimulated at a very low frequency (0.017 Hz) in this study.

The fact that activation of group I mGluR, predominantly located on postsynaptic sites, but not activation of group II mGluR, present on presynaptic elements, induces LTD also suggests that LTD induction is postsynaptic in nature. This LTD was not accompanied by changes in membrane potential or input resistance, suggesting that release of neurotransmitters from interneurons by mGluR activation was not substantial and does not account for the LTD presently described here.

4.2. (1S,3R)-ACPD-induced LTD is NMDA receptor independent

The activation of NMDA receptors by depolarization and binding of glutamate leads to a Ca^{2+} influx that is proposed to be a crucial factor for the induction of long-term potentiation or LTD at some synapses (Bliss and Collingridge, 1993; Linden, 1994; Malenka, 1994). Here, we have identified a form of NMDA receptor-independent LTD that was induced in spinal cord by activation of mGluRs. Interestingly, similar observations were also reported in the dentate gyrus (O'Mara et al., 1995) and in the CA1 area of the hippocampus (Bortolotto and Collingridge, 1993). A moderate increase in cytosolic $[Ca^{2+}]$ that is presumed to be critical for LTD induction may have been achieved here via Ca^{2+} release from the internal stores through the PLC–IP_3 pathway. This hypothesis is supported by a report in which (1*S*,3*R*)-ACPD-induced LTD was prevented by depletion of intracellular Ca^{2+} stores (Hemart et al., 1995).

4.3. (1S,3R)-ACPD-induced LTD is mediated by PTX-insensitive G-proteins

G-proteins contain α, β and γ subunits, the latter two being tightly linked in a $\beta\gamma$ complex. α subunits bind guanine nucleotide, confer specificity in receptor–effector coupling and serve as toxin substrates. PTX leads to uncoupling of α subunits from receptors. In most cells, PLC is regulated by a PTX-insensitive G-protein. In the mGluR superfamily, principally, group I mGluRs, with the exception of mGluR1a, are coupled to PLC through PTX-insensitive G-proteins, such as Gq, whereas group II and group III mGluRs are negatively coupled to adenylyl cyclase through PTX-sensitive G-proteins, possibly the Gi family (Pin and Duvoisin, 1995). The present finding that induction of LTD by (1*S*,3*R*)-ACPD is mediated by PTX-insensitive G-proteins most likely involving group I but not group II mGluRs is in line with this hypothesis. And, indeed, (1*S*,3*R*)-ACPD is a group-I-preferring mGluR agonist (Pin and Duvoisin, 1995) and the selective group I mGluR agonist (*S*)-3,5-DHPG but not group II mGluR agonist DCG-IV induced LTD in the present study.

4.4. (1S,3R)-ACPD-induced LTD requires the activation of PLC

The present results show that LTD but not the acute suppression of synaptic transmission by (1*S*,3*R*)-ACPD can be abolished by the specific PLC inhibitor U73122. This indicates that the signal transduction pathways for acute and long-term depression are different. While the PLC pathway is required for LTD, the acute depression could result from a decrease in neurotransmitter release by activation of presynaptic mGluRs, possibly group II mGluRs that do not activate PLC (Baskys and Malenka, 1991; Dube and Marshall, 1997). The fact that ACPD failed to induce short-term depression and LTD in conventional whole-cell patch-clamp recordings but was effective in gramicidin-perforated patches suggests, however, that a diffusible mediator was required in the postsynaptic neuron both for the acute depression and for the induction of LTD.

4.5. Possible functions of mGluRs for nociception and antinociception in spinal cord

Long-term potentiation and LTD in hippocampus and cerebellum are considered to be fundamental mechanisms of learning and memory (Bliss and Collingridge, 1993; Linden, 1994; Malenka, 1994; Lisberger, 1998). In spinal cord, long-term potentiation of C-fiber-evoked field potentials that is induced by noxious stimulation or

nerve injury (Sandkühler and Liu, 1998) is considered as a synaptic mechanism underlying some forms of hyperalgesia (Randić et al., 1993; Lozier and Kendig, 1995; Pockett, 1995; Sandkühler, 1996a). The low-frequency stimulation-induced LTD of Aδ-fiber-evoked EPSPs or spinal C-fiber-evoked potentials is proposed to be relevant to antinociception following afferent stimulation such as transcutaneous electrical nerve stimulation, electroacupuncture, needle acupuncture and physical therapy (Sandkühler, 1996b). Here, a new form of synaptic LTD in spinal cord has been identified that can be induced pharmacologically by activation of mGluRs in the absence of repetitive afferent stimulation.

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