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INDUCTION OF HOMOSYNAPTIC LONG-TERM DEPRESSION AT SPINAL SYNAPSES OF SENSORY Aδ-FIBERS REQUIRES ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS

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Abstract—The synaptic strength between primary afferent Aδ-fibers, many of which convey pain-related information, and second order neurons in the spinal dorsal horn can be depressed for prolonged periods of time in a use- and *N*-methyl-D-aspartate receptor-dependent fashion. Here, we have used a transverse spinal cord slice–dorsal root preparation of young rat to characterize the nature of this form of long-term depression and the role of metabotropic glutamate receptors. Dorsal roots were bisected and intracellular recordings were made from lamina II neurons with independent excitatory synaptic inputs from both dorsal root halves. Conditioning stimulation of one dorsal root half (1 Hz, 900 pulses) induced long-term depression that was specific for the stimulated pathway, i.e. homosynaptic in nature. The induction of long-term depression was prevented by non-selective group I and group II mGluR antagonist (*S*)-α-methyl-4-carboxyphenylglycine, by selective group I receptor antagonist (*S*)-4-carboxyphenylglycine and by selective group II mGluR antagonist (*RS*)-α-methylserine-*O*-phosphate monophenyl ester. Group III mGluR antagonist (*RS*)-α-methylserine-*O*-phosphate was ineffective. Short-term depression was not affected by any of these antagonists.

Thus, a homosynaptic form of long-term depression exists at putative nociceptive synapses in the spinal dorsal horn and its induction requires the activation of both group I and II metabotropic glutamate receptors. © 2000 Elsevier Science Ltd. Published by Elsevier Science Ltd. All rights reserved.

Key words: synaptic plasticity, mGluRs, primary afferent, nociception, spinal dorsal horn, analgesia.

Glutamate or a related amino acid is the transmitter at synapses between primary afferent Aδ-fibers, including nociceptive nerve fibers, and neurons in the spinal dorsal horn.[11,48,57] Postsynaptic ionotropic glutamate receptors of the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) subtype mediate fast synaptic transmission. Inward currents through the *N*-methyl-D-aspartate receptor (NMDAR) channel contribute to the late phase of fast glutamate gated excitatory postsynaptic currents.[2,43,57] Activation of metabotropic glutamate receptors (mGluRs) that are coupled to guanosine tri phosphate-binding proteins may also trigger excitatory postsynaptic potentials (EPSPs).[4] Recently mGluRs have been identified in the spinal dorsal horn.[10,53] Based on sequence homologies, pharmacological profiles and signal transduction mechanisms, mGluRs have been classified into three groups.[40] mGluR1, 3–5 and 7 subtype mRNA was detected in the gray matter of the spinal cord with distribution being specific for the different subtypes.[10] High levels of mRNA encoding for group I mGluRs, mainly mGluR1a, mGluR5a and mGluR5b, were identified in adult rat spinal cord. mRNAs of group II mGluRs (mGluR2 and mGluR3) were found at medium levels in the spinal cord, whereas some of the group III mGluRs (mGluR4 and mGluR7) are expressed only at low levels.[53] mRNA of mGluR7 is also expressed in dorsal root ganglia.[37] Central terminals of putative nociceptive primary afferents show immunoreactivity for mGluR7.[20] Thus, a role of mGluRs for somatosensory processing in the spinal dorsal horn, including nociception, has been suggested. And indeed, activation of mGluRs in the spinal cord may lead to enhanced nociceptive neuronal discharges or hyperalgesia.[35,39,59]

We have reported that prolonged stimulation of primary afferent Aδ-fibers at a low frequency induces a robust long-term depression (LTD) of synaptic strength between Aδ-fibers and second-order neurons in the superficial spinal dorsal horn.[46] Here, we have addressed the question whether this form of LTD is synapse specific and whether mGluRs are involved.

EXPERIMENTAL PROCEDURES

Preparation of spinal cord slices

Under deep ether anesthesia, the lumbar spinal cord of 17- to 28-day-old Sprague–Dawley rats was exposed by laminectomy. All experiments conformed to guidelines approved by the Council of the International Association for the Study of Pain in December 1982. All efforts were made to minimize the number of animals used and their suffering. The L4 to S1 segments of lumbosacral spinal cord were excised with long (8–15 mm) dorsal roots attached. The distal ends of the dorsal roots were carefully split into halves in some of the experiments (see Fig. 1A). Transverse slices at 400–500 μm thickness with one bisected or intact dorsal root attached were 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 to 320 mosmol/kg. A single slice was then transferred to a recording chamber ($v = 1.0$ ml, see Fig. 1) and was continuously superfused at a rate of 3 to 4 ml/min with an oxygenated recording solution at 30°C, which was identical to

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Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; D-AP5, D-2-amino-5-phoshonovaleric acid; EPSPs, excitatory postsynaptic potentials; IP_3, inositol-1,4,5-triphosphate; LFS, low-frequency stimulation; LTD, long-term depression; mGluRs, metabotropic glutamate receptors; MSOP, (*RS*)-α-methylserine-*O*-phosphate; MSOPPE, (RS)-α-methylserine-*O*-phosphate monophenyl ester; NMDAR, *N*-methyl-D-aspartate receptor; PKC, protein kinase C; PLC, phospholipase C; PPD, paired-pulse depression; PPF, paired-pulse facilitation; (*R*)-MCPG, (*R*)-α-methyl-4-carboxyphenylglycine; (*S*)-MCPG, (*S*)-α-methyl-4-carboxyphenylglycine; (*S*)-4-CPG, (*S*)-4-carboxyphenylglycine.

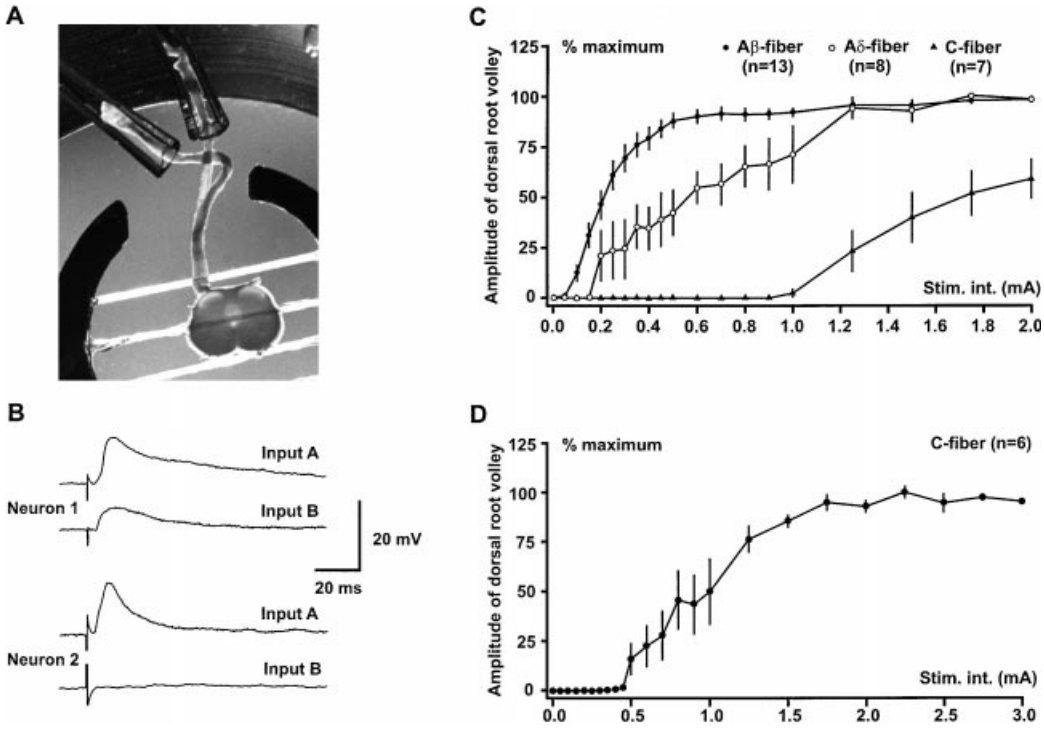

Fig. 1. Independent, convergent primary afferent input to spinal dorsal horn neurons. Some features of the experimental conditions to study homo- or heterosynaptic modulation of primary afferent neurotransmission are illustrated. (A) Photograph of a transverse lumbar spinal cord slice with one dorsal root attached. The distal part of the dorsal root was split into halves which were stimulated independently with suction electrodes. Silver wires inside and outside the suction electrodes were used for bipolar electrical stimulation but were omitted in the figure for simplification. A platinum ring and a nylon mesh were used to fix the slice at the bottom of the recording chamber. With transmitted light the area of laminae I and II is clearly visibly as a translucent band in the dorsal part of the spinal cord. (B) Representative intracellular recordings of individual (not averaged) EPSPs of two lamina II neurons are shown. In neuron 1 apparently monosynaptic EPSPs were evoked by supramaximal electrical stimulation of either half or dorsal root (inputs A and B). Neuron 2 responded only to electrical stimulation of input A but not input B, even when stimulated at supramaximal intensities (0.5 ms pulse of 3 mA). (C, D) To quantitatively determine the stimulus intensity–response relationship for recruiting primary afferent Aα/β-, Aδ-, and C-fibers in dorsal roots under the given experimental conditions, dorsal root volleys were recorded in a two-compartment chamber. The distal part of the dorsal root was placed in recording solution and stimulated by a suction electrode. The proximal end was located in paraffin oil to record dorsal root volleys with a platinum hook electrode. Stimulus–response curves are shown for pulse width of 0.1 ms (C) or 0.5 ms (D). Stim. int., stimulation intensity.

the incubation solution except for a lower concentration of potassium ions (1.9 mM).

Recording and stimulation techniques

Intracellular recordings with sharp microelectrodes were used in this study. With transmitted illumination under a dissecting microscope (Olympus, Japan), the lamina II (substantia gelatinosa) was identifiable as a translucent band across the dorsal horn (Fig. 1A). Microelectrodes were pulled from borosilicate glass tubes with an inner filament on a Flaming/Brown micropipette puller (Sutter Instrument Co., Novato, CA, U.S.A.). When filled with 4 M potassium acetate microelectrodes had d.c. tip resistances between 140 and 190 MΩ. Neurons were impaled by oscillating the capacitance compensation circuit. Recordings were made with a high-input impedance bridge amplifier (Axoclamp 2B, Axon Instruments, Foster City, CA, U.S.A.). The signal output was monitored on a digital oscilloscope (54603B, Hewlett Packard, Colorado-Springs, CO, U.S.A.), digitized by an A/D converter card (DT2821, Data Translation, Foster-City, CA, U.S.A.) and stored in a computer for off-line analysis. The software package Experimenter's WorkBench (Version 4.0, Data Wave Technologies corporation, Longmont, CO, U.S.A.) was used. A pen recorder (L120E, Linseis, Selb, Germany) was used to continuously monitor membrane potential and current injection. The input resistance was estimated from the slope of the current–voltage relationship, and changes in input resistance were monitored at 2-min intervals by measuring the voltage responses of the cell to hyperpolarizing current pulses (0.05 nA for 200 ms). Each dorsal root half was stimulated independently through a suction electrode (see Fig. 1A, B) with isolated current stimulators (Isostim A320, World Precision Instruments, Sarasota, FL, U.S.A.). The stimulus parameters necessary to activate Aδ- or C-fibers were determined quantitatively by extracellular recording of the dorsal root volleys in a two-compartment chamber using silver wire hook electrode as recording electrode in oil and suction electrode as stimulating electrode in normal recording solution.

Experimental protocols

After the threshold for eliciting an EPSP was determined for each dorsal root half, stimulation intensity was adjusted between 0.1 and 0.5 mA to yield EPSP amplitudes of 5 to 25 mV. Only the EPSPs that were evoked by Aδ-fibers were investigated further. Test pulses of 0.1 ms were given at 60 s intervals unless stated otherwise. A 10- or 20-Hz train of seven pulses was applied to test for mono- or polysynaptic input. In some experiments, paired-pulses at 50-ms intervals were applied to test for paired-pulse facilitation or paired-pulse depression.[31] After 10 to 15 stable control responses were recorded conditioning stimulation was applied to one dorsal root half or the intact dorsal root (900 pulses of 0.1 ms duration at 0.7 mA were given at 1 Hz). This low-frequency conditioning stimulation (LFS) has been shown to induce LTD in CA1 area of the hippocampus[15] and in the spinal dorsal horn.[46] Cathodal direct current (up to 0.1 nA) was passed into the cell during the course of experiment to maintain membrane hyperpolarization typically between −75 and −85 mV, as LTD induction is facilitated when the postsynaptic neuron is kept at a hyperpolarized membrane potential.[43,46]

Data analysis

Two consecutive EPSPs were averaged and synaptic strength was quantified by measuring the peak amplitude and initial slope of averaged EPSPs. The mean values of four to seven averaged, consecutive test responses recorded prior to conditioning stimulation served as controls. Significant changes from controls were assessed by comparing the values of four consecutive responses 23 to 30 min after conditioning stimulation. Measured membrane potential and input resistance

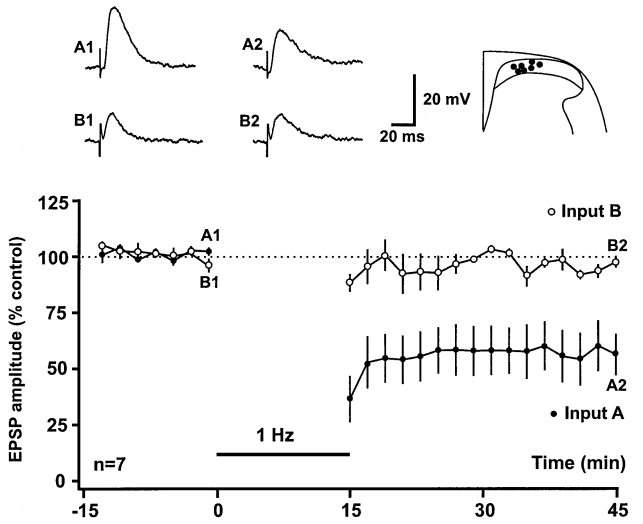

Fig. 2. Homosynaptic long-term depression at spinal synapses of primary afferent Aδ-fibers. Alternating stimulation of dorsal root halves with two suction electrodes (see Fig. 1A) was used to evoke EPSPs in spinal dorsal horn neurons with independent afferent inputs. The mean time courses of EPSP amplitudes elicited by stimulating two distinct inputs (A and B) from seven neurons are shown. Long-term depression was induced in the conditioned pathway only (A, closed circles). Synaptic strength remained unchanged in the non-conditioned pathway (B, open circles). Representative original EPSPs recorded in one neuron in response to stimulation of pathway A or B immediately before (1) and 30 min after conditioning stimulation of pathway A (2) and visually identified recording sites in lamina II of the seven cells are shown above the graph. $V_m = -70$ to -89 mV, 19- to 22-day-old rats.

were determined from original (not from averaged) recordings. All values are expressed as mean ± S.E.M. Statistical comparisons were made using the non-parametric Wilcoxon rank test. $P \leq 0.05$ was considered significant.

Application of drugs

All drugs were dissolved in oxygenated recording solution and applied to the slice by addition to the superfusing medium at known concentrations. Drugs and their sources were as follows: D-2-amino-5-phosphonovaleric acid (D-AP5, 50 μM, Cambridge Research Biochemicals, Northwhich, U.K.); (*S*)-α-methyl-4-carboxyphenylglycine ((*S*)-MCPG, 1 mM, Tocris); (*R*)-α-methyl-4-carboxyphenylglycine ((*R*)-MCPG, 1 mM, Tocris); (*S*)-4-carboxyphenylglycine ((*S*)-4-CPG, 200 μM, Tocris); (*RS*)-α-methylserine-*O*-phosphate monophenyl ester (MSOPPE, 200 μM, Tocris); (*RS*)-α-methylserine-*O*-phosphate (MSOP, 200 μM, Tocris). Drugs were dissolved in stock solution and diluted in perfusing solution to the desired concentration.

RESULTS

Afferent input to the spinal cord

The afferent input to the spinal cord that is induced by dorsal root stimulation under the given experimental conditions was assessed quantitatively. The stimulus–response curves of Aα/β-, Aδ- and C-fibers in dorsal roots of 17- to 28-day-old rats are shown in Fig. 1C and D. The mean conduction velocity of Aα/β-fibers was 17 ± 2 m/s and mean stimulation threshold was 0.1 ± 0.0 mA (0.1 ms pulses, $n = 13$). The mean stimulation threshold and conduction velocity of Aδ-fibers were 0.2 ± 0.03 mA (0.1 ms pulses) and 5 ± 1 m/s ($n = 8$), respectively. C-fibers had a mean conduction velocity of 0.7 ± 0.3 m/s ($n = 7$) and a mean stimulation threshold of 1.3 ± 0.2 mA for 0.1 ms pulses ($n = 7$) and 0.6 ± 0.1 mA for 0.5 ms pulses ($n = 6$).

Properties of primary afferent-evoked excitatory postsynaptic potentials in the superficial spinal dorsal horn

Stable intracellular recordings of up to 5 h were obtained from 93 neurons in lamina II (substantia gelatinosa) of the spinal dorsal horn. Electrical stimulation of primary afferent Aδ- or C-fibers in dorsal roots elicited fast EPSPs in all neurons included in this study. The mean resting membrane potential was −64 ± 1 mV. Mean input resistance was 188 ± 21 MΩ and did not change significantly during the recording periods of the experiments. After the threshold for eliciting an EPSP was tested, a supramaximal stimulation intensity of dorsal root was also used to activate C-fibers. In 93 successfully recorded cells, 75 cells displayed Aδ-fiber-induced EPSPs and the remaining 18 cells expressed C-fiber-induced EPSPs. Aδ-fiber-induced EPSPs had short latencies (mean 5 ± 0.2 ms) and lower thresholds (mean 0.3 ± 0.02 mA, 0.1 ms), whereas C-fiber-induced EPSPs had long latencies (mean 22 ± 0.1 ms) and higher thresholds (mean 0.6 ± 0.07 mA, 0.5 ms). When synaptic delay was presumed as 1 ms, the calculated conduction velocity of Aδ-fibers ranged from 2 to 10 m/s, and that of C-fibers from 0.3 to 1.5 m/s. Typically these values are relatively low in younger animals but within the ranges described in previous reports.[21,58] In 75 spinal lamina II neurons tested 71% of cells received apparently monosynaptic inputs, as determined by constant latencies and absence of failures upon 10- or 20-Hz stimulation. The remaining 29% of the cells received polysynaptic inputs. In the present study only the Aδ-fiber-induced monosynaptic EPSPs were investigated further. Additionally, paired-pulses were applied at 50-ms intervals to dorsal roots while recording monosynaptic EPSPs from 29 cells. Nine cells expressed paired-pulse facilitation (PPF) to 124 ± 8%; the remaining 20 cells expressed paired-pulse depression (PPD) to 74 ± 3%, showing that paired-pulses at the same frequency may induce either PPF or PPD at the synapses in the spinal dorsal horn.

Low-frequency stimulation of dorsal root induces homosynaptic long-term depression in the spinal cord

The dorsal root that was attached to the spinal slice was bisected (see Fig. 1A). Two suction electrodes were used to stimulate independent afferent nerve fibers. After penetration of a neuron, we determined whether it had independent afferent input from both dorsal root halves (Fig. 1B). No cross-excitation between dorsal roots was observed, even when stimulating at supramaximal intensities. To investigate whether LFS-induced LTD in the spinal dorsal horn is homo- or heterosynaptic in nature, conditioning stimulation was applied to one dorsal root half followed by alternate test stimulation of both halves. In all neurons tested, LTD was induced in the conditioned pathway only, and the strength of synaptic transmission in the non-conditioned pathway was not changed (Fig. 2). The mean amplitude of EPSPs 30 min after LFS in the conditioned pathway was reduced to 60 ± 12% of control ($n = 7$, $P < 0.01$), whereas it was not attenuated in the non-conditioned pathway (96 ± 3% of control, $P > 0.05$). Once induced, the LTD always lasted until the end of each experiment (up to 3 h). These results show for the first time that a homosynaptic form of LTD can be induced at spinal synapses of primary afferents.

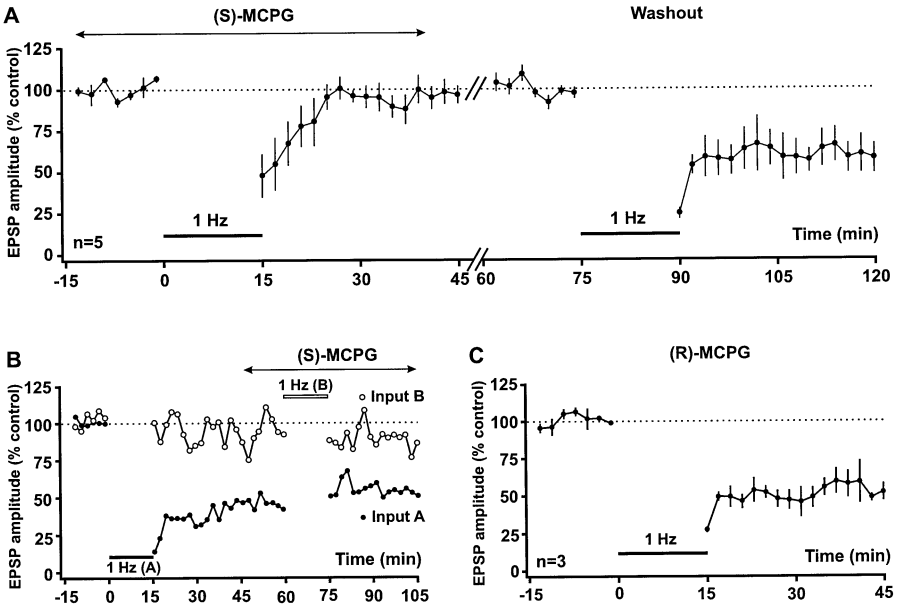

Fig. 3. (*S*)-MCPG reversibly and stereoselectively blocks the induction of LTD. Mean amplitudes of Aδ-fiber-evoked EPSPs are plotted versus time. Mean EPSP amplitudes prior to bath application of the non-selective mGluR antagonist (*S*)-MCPG served as controls. (A) The mean time course of EPSP amplitudes of five neurons before and after conditioning 1 Hz stimulation (15 min, horizontal bar) is shown. In the presence of (*S*)-MCPG (at 1 mM, arrows) conditioning stimulation induced short-term depression but failed to induce LTD in the conditioned pathway. After washout of (*S*)-MCPG, 1 Hz stimulation induced LTD in the same five cells. (B) EPSP amplitudes of one neuron in response to alternating stimulation of dorsal root halves (inputs A and B) are plotted versus time. In normal recording solution conditioning stimulation of input A (closed horizontal bar) induced homosynaptic LTD. In the same cell conditioning stimulation of input B (open horizontal bar) in the presence of (*S*)-MCPG failed to induce LTD. (C) Bath application of the inactive enantiomer (*R*)-MCPG throughout the recording periods failed to block LTD induction in all three neurons tested. $V_m = -77$ to -93 mV; 21- to 28-day-old rats.

Low-frequency stimulation-induced homosynaptic long-term depression is N-*methyl*-D-*aspartate receptor dependent*

We have previously reported that the induction of LTD by LFS required the activation of NMDAR.[46] To assay whether the LFS-induced homosynaptic LTD identified in this study is also NMDAR dependent, we used D-AP5, an NMDAR antagonist. D-AP5 was applied at a concentration of 50 μM 20 min before conditioning stimulation. In five cells tested, LFS-induced LTD was blocked or strongly reduced by D-AP5; the mean amplitude of EPSPs 30 min after LFS was $97 \pm 6\%$ of control ($P > 0.05$, data not shown). Thus, the induction of homosynaptic LTD also requires the activation of NMDAR.

Metabotropic glutamate receptors are necessary for the induction of homosynaptic long-term depression

To test whether mGluRs are involved in the induction of LFS-induced homosynaptic LTD in the spinal dorsal horn, we firstly used a non-selective antagonist at group I and group II mGluRs, (*S*)-α-methyl-4-carboxyphenylglycine ((*S*)-MCPG).[17] Twenty minutes prior to conditioning stimulation (*S*)-MCPG was added to the bath solution at a concentration of 1 mM that effectively blocks mGluRs in slices.[18] This had no detectable effect on the EPSP amplitudes ($98 \pm 6\%$ of control, $n = 5$). In all five cells tested, the induction of LTD was, however, abolished by (*S*)-MCPG (Fig. 3A). The mean amplitude of EPSPs 30 min after LFS was $96 \pm 4\%$ of control ($P > 0.05$). After washout of (*S*)-MCPG, LFS successfully evoked an LTD to $66 \pm 12\%$ of control in the same cells ($P < 0.05$). In contrast, short-term depression could still be induced in the presence of (*S*)- MCPG (Fig. 3A). Additionally, in another two cells that received two independent inputs from the bisected dorsal root halves, LFS induced LTD in the conditioned pathway in normal recording solution but failed to induce LTD when the other pathway was stimulated in the presence of (*S*)-MCPG (Fig. 3B). To test whether the blockade of LTD by (*S*)-MCPG is stereoselective, we used (*R*)-MCPG, the inactive enantiomer of (*S*)-MCPG. Bath application of (*R*)-MCPG failed to block the induction of LTD (Fig. 3C) and mean EPSP amplitude 30 min after LFS was depressed to $51 \pm 6\%$ of control ($n = 3$, $P < 0.05$). Thus, the blockade of homosynaptic LTD induction by (*S*)-MCPG is reversible and stereoselective and shows that activation of mGluRs is essential for this form of spinal LTD.

Activation of both group I and group II metabotropic glutamate receptors is required for the induction of long-term depression

In 13 control experiments the mean amplitude of EPSPs 30 min after LFS was reduced to $62 \pm 7\%$ of control in the absence of mGluR antagonists (Fig. 4A). To determine whether group I mGluRs contribute to the induction of LTD, (*S*)-4-CPG, a specific antagonist of group I mGluRs,[6,49] was applied. Bath application of (*S*)-4-CPG at 200 μM had no effect on the peak amplitudes of EPSPs before LFS ($101 \pm 9\%$ of control, $n = 5$) but abolished or strongly reduced the induction of LTD (Fig. 4B). The mean amplitude of EPSPs 30 min after LFS was $93 \pm 12\%$ of control ($n = 5$, $P > 0.05$). (*S*)-4-CPG did not affect short-term depression. Paired-pulses were applied at 50-ms intervals before and 15 min after the application of (*S*)-4-CPG in four neurons. PPD to $71 \pm 5\%$ was induced in normal recording solution and to $75 \pm 10\%$ in the presence of (*S*)-4-CPG, prompting that (*S*)-4-CPG might not interfere with presynaptic transmitter release.[31] Thus, we propose that activation of group I

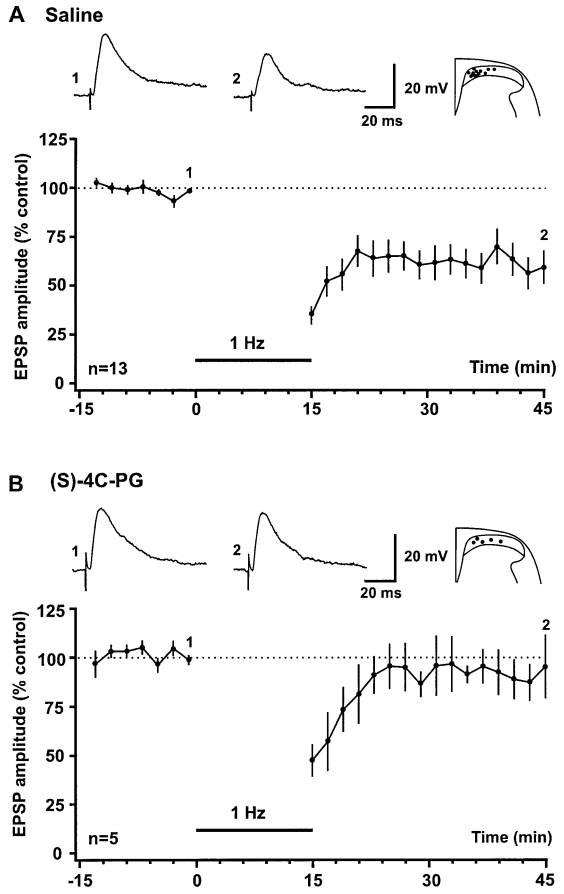

Fig. 4. Group I mGluR antagonist blocks the induction of LTD. Mean EPSP amplitudes are plotted versus time. In each neuron the mean of seven averaged responses prior to conditioning stimulation served as a control. (A) Conditioning stimulation at 1 Hz (horizontal bar) induces long-term depression of Aδ-fiber-evoked EPSPs in the conditioned pathway in all 13 neurons tested. $V_m = -75$ to -91 mV, 18- to 24-day-old rats. (B) The selective group I mGluR antagonist (*S*)-4-CPG was added to the superfusate at 200 μM throughout the recording periods and at least 20 min before 1 Hz stimulation (horizontal bar). In the presence of (*S*)-4CPG, 1 Hz stimulation induced a short-term depression lasting 6 to 10 min but failed to induce LTD. Representative original EPSPs and visually identified recording sites are shown above the graphs. $V_m = -72$ to -91 mV, 19- to 22-day-old rats.

mGluRs, probably at the postsynaptic site, is necessary for generation of LFS-induced LTD.

Because (*S*)-MCPG can block both group I and group II mGluRs, so the next step of this study was to assay the possible contribution to LTD induction by group II mGluRs. We applied (*RS*)-α-methylserine-*O*-phosphate monophenyl ester (MSOPPE), a potent antagonist at group II mGluRs,[16] which shows three times greater selectivity for group II over group III mGluRs in the spinal cord.[52] In the presence of MSOPPE at a concentration of 200 μM EPSP amplitudes were not changed ($107 \pm 15\%$ of control, $n = 5$). Conditioning LFS induced a short-term depression, but failed to induce LTD (Fig. 5). The mean EPSP amplitude 30 min after LFS was $104 \pm 8\%$ of control ($n = 5$, $P > 0.05$). In four neurons a PPD to $82 \pm 10\%$ and in two neurons a PPF to $111 \pm 4\%$ was induced under control conditions. In the presence of MSOPPE, PPD was still induced in the same four neurons (to $79 \pm 7\%$) and in two neurons PPF was observed (to $114 \pm 10\%$); since paired-pulses induced either depression or facilitation in this group of cells, however, it is difficult to propose the possible sites of the action of MSOPPE. Taken

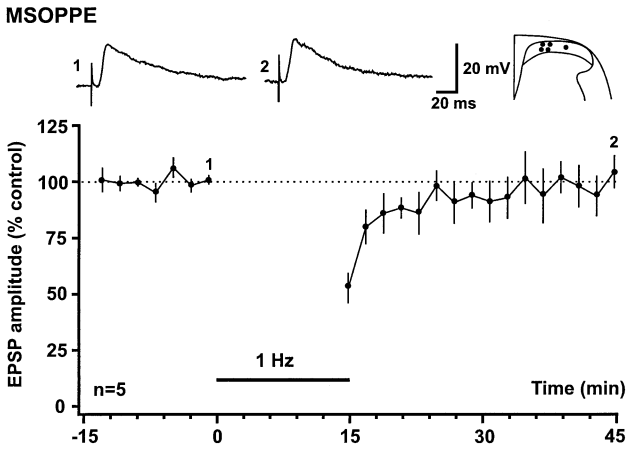

Fig. 5. Group II mGluR antagonist blocks the induction of LTD. Selective group II mGluR antagonist MSOPPE was added to the superfusate at 200 μM throughout the recording periods and at least 20 min before 1 Hz stimulation (black horizontal bar). In each neuron the seven averaged responses immediately prior to conditioning stimulation served as a control. In the presence of MSOPPE, conditioning stimulation induced a short-term depression but failed to induce LTD. Representative original EPSPs and visually identified recording sites are shown above the graph ($V_m = -80$ to -94 mV, 17- to 21-day-old rats).

together these results indicate that the activation of both group I and group II mGluRs is necessary to induce LTD in the spinal dorsal horn.

Group III metabotropic glutamate receptors are not necessary for the induction of long-term depression

To clarify whether group III mGluRs are also involved in the induction of LTD we next used (*RS*)-α-methylserine-*O*-phosphate (MSOP), which was reported as a novel specific group III mGluR antagonist, especially for the L-2-amino-4-phosphonobutyrate-sensitive presynaptic mGluRs on primary afferent terminals in the spinal cord.[52] Bath application of MSOP had no detectable effect on EPSP amplitudes ($101 \pm 4\%$ of control, $n = 5$). In the presence of MSOP (200 μM) LFS induced LTD in all five neurons tested (Fig. 6). The mean amplitude of EPSPs 30 min after LFS was reduced to $66 \pm 12\%$ of control ($n = 5$, $P < 0.01$). In two neurons paired-pulse depression was induced to $79 \pm 3\%$ of control but no depression was observed in the presence of MSOP ($92 \pm 9\%$ of control). Thus, the activation of presumably presynaptic group III mGluRs appears not to be necessary for the induction of this form of LTD in spinal cord.

DISCUSSION

Homosynaptic long-term depression of synaptic strength in superficial spinal dorsal horn

Impulses in primary afferent Aδ- or C-fibers may induce long-lasting changes in sensory processing at the level of the spinal dorsal horn. Previous reports have focused on afferent-induced increases in excitability of spinal dorsal horn neurons, including long-term potentiation of synaptic strength between primary afferents and second-order neurons in lamina II,[25,26,27,43,44] sensitization of nociceptive spinal dorsal horn neurons[22,54] and spinally mediated hyperalgesia in behaving animals.[5,33] In contrast, little is known about long-lasting inhibition in the spinal dorsal horn. Electrical high-frequency, burst-like stimulation of dorsal roots may either induce an

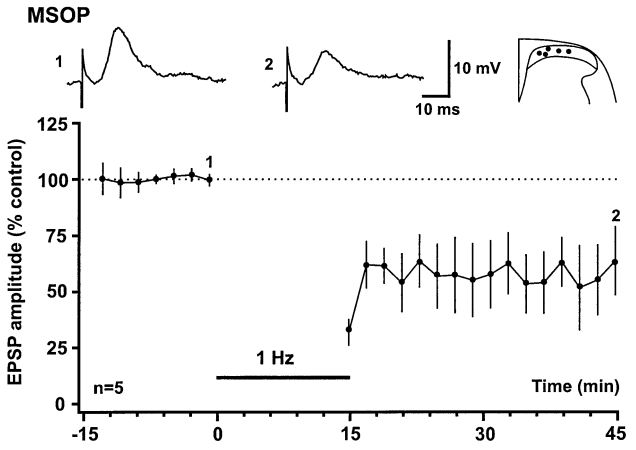

Fig. 6. Group III mGluR antagonist MSOP does not affect the induction of LTD. Selective group III mGluR antagonist MSOP was added to the superfusate at 200 μM throughout the recording periods and at least 20 min before 1 Hz stimulation (horizontal bar). In the presence of MSOP, conditioning stimulation induced LTD in all five neurons tested that was not statistically different from LTD induced in normal recording solution. Representative original EPSPs and visually identified recording sites are shown above the graph. $V_m = -73$ to -91 mV, 17- to 21-day-old rats.

LTP or an LTD at synapses of fine primary afferents and lamina II neurons. The direction of synaptic plasticity critically depends upon the activity in inhibitory systems in the spinal cord,[28] most likely reflecting the dependence of synaptic plasticity on the level of membrane potential of the postsynaptic neuron during conditioning stimulation.[1,43] A robust LTD of synaptic strength between primary afferent Aδ-fibers and lamina II neurons can be induced by low-frequency stimulation of dorsal roots.[46] Here, we show for the first time that a homosynaptic form of LTD exists at synapses in the spinal cord and that this depression requires activation of group I and group II mGluRs.

Independent, convergent inputs to lamina II neurons were stimulated in a transverse dorsal root–spinal cord preparation by splitting the attached dorsal root into halves, the distal ends of which were gently inserted into suction electrodes. LTD was induced in the conditioned pathway only while synaptic strength in the unconditioned pathway remained unaffected. Thus, this form of spinal LTD was homosynaptic in nature. We have shown previously that the induction of LTD in the superficial spinal dorsal horn requires an increase in postsynaptic $[Ca^{2+}]_i$.[46] At synapses in CA1 region of the hippocampus a rise in postsynaptic $[Ca^{2+}]_i$ for a few seconds is sufficient to induce a long-lasting change in synaptic strength.[30] If this were also the case at the presently investigated spinal synapses then the homosynaptic nature of the depression would indicate that the rise in $[Ca^{2+}]_i$ is restricted to compartments at or near the synapses that were active during conditioning stimulation. A modest and spatially restricted increase in $[Ca^{2+}]_i$ may be favored by LFS rather than by high-frequency stimulation. Alternatively, induction of homosynaptic LTD may require the coincidence of rise in postsynaptic $[Ca^{2+}]_i$ and of presynaptic activity.[19]

Homosynaptic long-term depression requires the activation of both metabotropic glutamate receptors and N-*methyl*-D-*aspartate receptors*

At some synapses activation of either ionotropic NMDARs or mGluRs is required for the induction of LTD.[38,47,55] NMDAR-dependent and mGluR-dependent forms of LTD may be mechanistically distinct forms of synaptic plasticity in that they share neither induction nor expression mechanisms.[36,38] An LTD that neither requires NMDAR nor mGluRs has also been described.[14] In the present study we have identified a new form of spinal LTD that requires both NMDAR and mGluRs for its induction. The dependence of homosynaptic LTD induction on NMDAR is very similar to that observed from the CA1 area in the hippocampus.[8] The requirement of mGluRs for LTD induction was proved not only from the same cells by adding and washout of mGluR antagonist in succession (Fig. 3A), but also from single cells that received two independent inputs, one of which showed homosynaptic LTD (Fig. 3B). The fact that (*S*)-MCPG blocked the LTD induction in the second pathway but LTD persisted in the first pathway after the drug application indicated that the main effect of (*S*)-MCPG is preventing the LTD induction but not disrupting the LTD maintenance (Fig. 3B). These electrophysiological observations about the involvement of mGluRs in LTD induction are also supported by recent molecular and histochemical evidence, which showed that most mGluR subtypes were detected on primary afferent terminals or/and the spinal dorsal horn.[10,20,37,53]

A modest increase in the intracellular Ca^{2+} is thought to be the critical factor in homosynaptic LTD induction.[23] The increase of $[Ca^{2+}]_i$ may be due to a Ca^{2+} influx through NMDAR or voltage-dependent calcium channels and/or due to Ca^{2+} release from the internal stores.[8,23,29,40] In spinal cord neurons (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid, an agonist at group I and group II mGluRs, potentiates excitatory responses to glutamate and increases both NMDAR and AMPA receptor-mediated currents.[7,12] On the other hand, group I mGluRs may activate the phospholipase C (PLC) through G-proteins; the formation of PLC not only leads to the formation of inositol-1,4,5-triphosphate (IP_3), which may trigger a release of Ca^{2+} from internal stores, but also to the formation of diacylglycerol, which may in turn activate the protein kinase C (PKC).[40] The activated PKC may then reduce the Mg^{2+}-blockade of NMDAR channels and thereby increase NMDAR-mediated currents.[13] Thus, in the present study the activation of mGluRs might be required either to facilitate an NMDA-dependent form of LTD by increasing NMDAR-mediated currents or to induce an increase of $[Ca^{2+}]_i$ via the PLC–IP_3 pathway.

The rapid development of new agonists or antagonists of mGluRs provides us a multiple choice for using different drugs. (*S*)-4-CPG, MSOPPE and MSOP were shown to be effective in the spinal cord *in vitro*[52] and *in vivo* (J. Sandkühler *et al.*, unpublished). These drugs at 200 μM concentration are specific antagonists at different groups of mGluRs.[16,40,49,52] Morphological evidence suggests that group II and group III mGluRs are predominantly localized to the presynaptic terminals, whereas group I mGluRs are localized to postsynaptic elements.[50–52] This is in line with our observation that (*S*)-4-CPG did not interfere with paired-pulse responses, and we propose that MSOPPE and (*S*)-4-CPG block the LTD induction, probably by a pre- and postsynaptic mechanism, respectively. However, more direct evidence is needed to substantiate this hypothesis. Interestingly, a requirement of co-activation of group I and group II mGluRs for LTD induction in the hippocampus in freely moving rats has been reported recently.[32]

The fact that group I mGluR selective antagonist (*S*)-4-CPG, group II mGluR preferring antagonist MSOPPE, but not

group III mGluR selective antagonist MSOP blocked the LTD induction in the present study provides a clear pharmacological profile that group I and group II mGluRs are involved in the LTD induction. This conclusion is supported by the results obtained from the hippocampus, in which LTD was proposed to be due to a coordinated pre- and postsynaptic modification[9,56] and LTD needed the activation of both group I and group II mGluRs.[32]

Potential role of long-term depression in the superficial spinal dorsal horn

Previous studies reported an up-regulation of mGluR mRNA in the spinal cord during inflammation[10] and an increase in excitability of spinal dorsal horn neurons by the activation of mGluRs.[34,35,39,59] mGluRs may also be involved in frequency detection and temporally dispersed synaptic signal association.[3] The present study has identified a new role of spinal mGluRs in sensory processing, the induction of homosynaptic LTD between primary afferent Aδ-fibers, many of which are nociceptive, and second-order neurons in lamina II. Interestingly the same mGluRs antagonists that block induction of long-term potentiation in the spinal cord by high-frequency stimulation of primary afferents (Sandkühler *et al*., unpublished) also block LFS-induced LTD. In addition, both forms of synaptic plasticity are NMDAR sensitive. This indicates that the direction of synaptic plasticity in the spinal dorsal horn is apparently not governed by different subtypes of glutamate receptors, but depends upon the pattern and the duration of conditioning stimulation, the activity of inhibitory systems in the spinal cord[28] and the level of membrane potential of the postsynaptic neuron.[43]

The long-term potentiation and LTD of the synaptic strength in hippocampus and in cerebellum are considered important cellular mechanisms in learning and memory.[8,23,24] In nociceptive pathways similar long-term changes of synaptic strength may underlie centrally-mediated hyperalgesia and afferent-induced analgesia respectively.[41,42,45]

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