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Long-term depression of C-fibre-evoked spinal field potentials by stimulation of primary afferent Aδ-fibres in the adult rat

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Abstract

Long-term potentiation (LTP) of spinal C-fibre-evoked field potentials can be induced by brief electrical stimulation of afferent C-fibres, by natural noxious stimulation of skin or by acute nerve injury. Here, we report that in urethane anaesthetized, adult rats prolonged high frequency burst stimulation of the sciatic nerve at Aδ-fibre strength produced long-term depression (LTD) of C-fibre-evoked field potentials, and also depressed the increased amplitudes of C-fibre-evoked field potentials recorded after LTP had been established (depotentiation). Electrical stimulation of Aβ-fibres failed to induce LTD or depotentiation. In spinalized rats, prolonged Aδ-fibre conditioning stimulation induced LTP rather than LTD of C-fibre-evoked field potentials. Thus, tonic descending inhibition may determine the direction of plastic changes in C-fibre-mediated synaptic transmission. Spinal application of the *N*-methyl-D-aspartic acid receptor antagonist D-APV blocked induction of LTD in intact rats and LTP in spinalized rats. The presently described LTD and the depotentiation of established LTP of C-fibre-evoked field potentials in spinal dorsal horn may underlie some forms of prolonged analgesia induced by peripheral nerve stimulation procedures.

Introduction

In many brain regions, including the spinal cord, repetitive stimulation of afferent fibres can induce long-term potentiation (LTP, Bliss & Lømo, 1973; Randić *et al.*, 1993; Liu & Sandkühler, 1997) or long-term depression (LTD, Randić *et al.*, 1993; Linden, 1994; Sandkühler *et al.*, 1997) of synaptic transmission. Homosynaptic LTP or LTD refers to a persistent increase or decrease in synaptic strength induced by activation of presynaptic fibres (Bliss & Lømo, 1973), while heterosynaptic LTP or LTD is defined as a persistent increase or decrease in synaptic strength that occurs when strong postsynaptic activity, driven by impulses in converging afferents, occurs in the absence of presynaptic activity (Lynch *et al.*, 1977; Bachoo & Polosa, 1991; Linden, 1994). Both LTP and LTD are synaptic models for information storage in the central nervous system (Bliss & Collingridge, 1993; Linden, 1994).

Intense and prolonged noxious stimulation of peripheral tissues may induce hyperalgesia, an increased response to noxious stimulation (Woolf & Thompson, 1991; Nagy *et al.*, 1993), while moderate noxious somatic or nerve stimulation may, on the other hand, produce pain relief for a long period of time (Melzack, 1975; Ishimaru *et al.*, 1995). The mechanisms underlying the prolonged bi-directional plastic changes induced by noxious stimulation are still not well understood. Previously we have shown that LTP of C-fibre evoked field potentials in the spinal dorsal horn of adult rats can be induced by high-frequency conditioning stimulation of afferent C-fibres (Liu & Sandkühler, 1995, 1997). This form of LTP is blocked by *N*-methyl-D-aspartic acid (NMDA, Liu & Sandkühler, 1995), neurokinin-1 or neurokinin-2 receptor antagonists (Liu & Sandkühler, 1997). Similarly, induction of central hyperalgesia can also be prevented by blockade of NMDA-(Woolf & Thompson, 1991; Nagy *et al.*, 1993; Ren & Dubner, 1993), neurokinin-1 (Picard *et al.*, 1993; Thompson *et al.*, 1993; Dougherty *et al.*, 1994) and neurokinin-2 receptors (Xu *et al.*, 1991; Neugebauer *et al.*, 1996). Thus, spinal LTP has been proposed as a possible mechanism of central hyperalgesia (Pockett, 1995; Randić, 1996; Sandkühler, 1996b). To date nothing is known about LTD of C-fibre-evoked field potentials. Electrophysiological studies have shown that electrical stimulation of afferent Aβ-fibres may depress nociceptive excitation of spinal dorsal horn neurons for short periods of time (Handwerker *et al.*, 1975; Chung *et al.*, 1984a,b), but this inhibition is unlikely to explain the long-lasting analgesic effects of transcutaneous electrical nerve stimulation (TENS) or acupuncture. Here, we have investigated whether conditioning stimulation of Aδ-fibres in peripheral nerves may induce long-lasting changes in C-fibre-mediated synaptic transmission in spinal dorsal horn, and the

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role of tonic descending inhibitory systems on synaptic plasticity in spinal cord was assessed.

Materials and methods

Preparation of animals

Experiments were performed on adult male Sprague–Dawley rats (250–350 g) anaesthetized with urethane (1.5 g/kg, i.p.). Surgical level of anaesthesia was verified by a stable mean arterial blood pressure and a constant heart rate during noxious stimulation. The trachea was cannulated to allow mechanical ventilation with room air. A catheter was inserted into one external jugular vein for continuous i.v. infusion of Tyrode's solution at 0.8–1 mL/h. One carotid artery was cannulated to monitor mean arterial blood pressure continuously (range 80–100 mmHg). Colorectal temperature was maintained at 37–38 °C by means of a feedback controlled heating blanket. A laminectomy was performed to expose the lumbar enlargement of the spinal cord and the dura mater was incised longitudinally. In some experiments, the C2–C3 segments were also exposed by laminectomy to allow high cervical spinal transection. The left sciatic nerve was dissected free for bipolar electrical stimulation with platinum hook electrodes. In eight experiments, the left sural nerve was exposed to record C-fibre-evoked compound action potentials (C-fibre volleys) following electrical stimulation of the sciatic nerve. All exposed nervous tissue was covered with warm paraffin oil, except for those spinal segments to be superfused (see below). All experiments were carried out in accordance with the European Community's Council Directive of 24 November 1986 (86/609/EEC).

Recordings and stimulations

Field potentials evoked by electrical stimulation of the sciatic nerve were recorded at a depth of 50–300 μm from the lumbar cord dorsum with tungsten microelectrodes (impedance 1–3 MΩ). A band width of 0.1–550 Hz was used for recording field potentials. This did not affect peak amplitudes of C-fibre-evoked field potentials as compared to DC-amplification. An A/D converter card (DT2821) was used to digitize and store data in a Pentium computer at a sampling rate of 10 kHz. Single square cathodal pulses (7–20 V, 0.5 ms, at 60-s intervals) delivered to the sciatic nerve were used as test stimuli. To induce LTD of C-fibre-evoked field potentials, four types of conditioning stimulation of A-fibres, applied to the sciatic nerve, were tested: (i) prolonged high frequency burst stimulation at Aδ-fibre intensity (10 V, 0.1 ms pulses at 100 Hz for 1 s, repeated at 0.1 Hz for 15 min, i.e. 90 tetani); (ii) brief high frequency burst stimulation [same parameters as (i) but for only 40 s, i.e. four tetani]; (iii) prolonged low-frequency stimulation (10 V, 0.1 ms at 1–2 Hz for 15 min). (iv) Aβ-fibre conditioning stimulation [same parameters as (i) but at low intensity, i.e. two times threshold for activation of Aβ-fibres (2T)].

At the end of experiments, electrolytic lesions were made at recording sites through the recording electrodes (30–40 μA, 20–25 s). Rats were killed by an overdose of pentobarbital. Coronal spinal cord sections (50 μm) were cut on a freezing microtome and stained with cresyl violet. The distance from the stimulation site on the sciatic nerve to the recording site in lumbar dorsal horn was about 11 cm.

Administration of drugs

The NMDA-receptor antagonist D(−)-2-amino-5-phosphonopentanic acid (D-APV, Research Biochemicals International, Natick, MA, USA) was dissolved in distilled water (10 mM), then diluted with artificial cerebrospinal fluid to yield a final concentration of 100 μM immediately before application to the spinal cord. For controlled superfusion, a specially synthesized silicone rubber was used to form a small well on the cord dorsum at the recording segments (see Beck *et al.*, 1995 for details).

Data analysis and statistics

The amplitudes of C-fibre-evoked field potentials were determined off-line by parameter extraction, which was implemented by Datawave system (Thornton, CO, USA). The peak amplitude of the C-fibre-evoked potential was determined as the maximal distance from the baseline. In each experiment, the amplitudes of five consecutive C-fibre-evoked field potentials, collected at 60-s intervals, were averaged. Non-parametric ANOVA (Kruskal–Wallis test) was used for statistical analysis and $P < 0.05$ was considered significant. Means are given with one standard error of the means.

Results

Induction of long-term depression of C-fibre-evoked field potentials

To determine the types of afferent fibres activated by conditioning stimulation used in this study, A-fibre and C-fibre volleys were recorded in the sural nerve of five rats following electrical stimulation of the sciatic nerve with increasing stimulation intensities (0.1 ms pulses, 0.1–28 V). The mean threshold for activation of Aβ-fibres was 150 ± 20 mV, for Aδ-fibres 1.4 ± 0.3 V and for C-fibres 15.4 ± 0.7 V. Thus, the intensity of the conditioning stimulation used in this study (10 V, 0.1 ms) activated a large fraction of A-fibres but did not recruit C-fibres.

In 13 of 15 rats tested, prolonged high-frequency burst stimulation at Aδ-fibre intensity (10 V, 0.1 ms, 100 Hz, 90 tetani) induced LTD, which lasted until the end of each recording period (1–6 h after conditioning stimulation, see Fig. 1 for an example). In the remaining two rats only short-term depression was produced, where the amplitude of C-fibre-evoked field potentials returned to control levels 30–40 min after conditioning stimulation. In six rats recordings were made for at least 2 h after prolonged high-frequency burst stimulation. The mean amplitude of C-fibre-evoked field potentials was reduced to 59 ± 11% of control at 2 h after conditioning stimulation (Fig. 2A). In three rats, C-fibre volleys in sural nerve and spinal C-fibre-evoked field potentials were simultaneously recorded. Prolonged high-frequency burst stimulation at Aδ-fibre intensity induced LTD of C-fibre-evoked field potentials but did not affect C-fibre volleys (Fig. 1). This indicates that the long-lasting decline in the amplitude of C-fibre-evoked field potentials is not due to excitability changes in the peripheral C-fibres following conditioning burst stimulation of Aδ-fibres. This is in line with our previous study showing that conditioning tetanic stimulation at C-fibre strength also did not affect excitability of C-fibres (Liu & Sandkühler, 1997).

In four of nine rats tested, brief high-frequency burst Aδ-fibre stimulation (10 V, 0.1 ms, 100 Hz, four tetani) induced LTD (to 61 ± 15% of control), which lasted until the end of recordings (1–4 h). In the remaining five rats only short-term depression was induced, lasting for 10–40 min. Prolonged low-frequency stimulation at Aδ-fibre intensity (10 V, 0.1 ms, 1–2 Hz for 15 min) was tested in 11 rats. In two rats, LTD was induced, lasting until the end of the recording period (1 h and 3.5 h). Short-term depression was induced (lasting 15 and 30 min) in another two animals; in the remaining seven rats no change of C-fibre-evoked field potentials was observed.

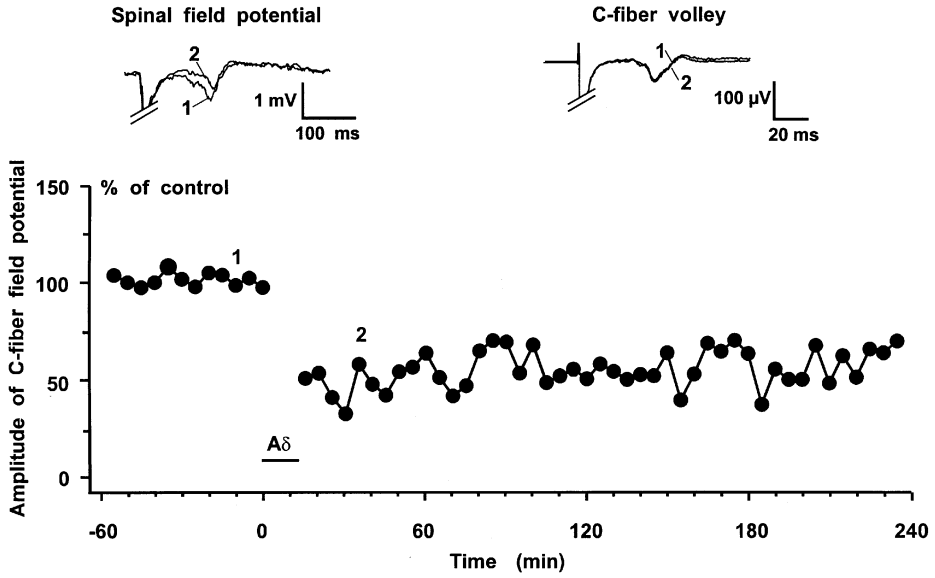

FIG. 1. Typical time course of heterosynaptic LTD of spinal C-fibre-evoked field potentials. Each data point represents the averaged peak amplitude of five consecutive spinal field potentials evoked by impulses in primary afferent C-fibres following supramaximal electrical stimulation of sciatic nerve. The horizontal bar indicates the period of conditioning stimulation of sciatic nerve at Aδ-fibre intensity (10 V, 0.1 ms pulses at 100 Hz for 1 s, repeated at 0.1 Hz for 15 min). The mean response prior to conditioning stimulation served as control. Above the graph are displayed C-fibre-evoked field potentials in the spinal dorsal horn and C-fibre volleys in sural nerve, recorded simultaneously before (1) and after (2) conditioning stimulation. C-fibre-evoked field potentials in spinal cord and C-fibre volleys in the sural nerve can be clearly distinguished from A-fibre-evoked responses, which are out of scale and are therefore truncated. In this experiment the distances from the stimulating electrode to the spinal recording site and to the sural nerve recording were 10 and 4 cm, respectively. The calculated conduction velocity in C-fibres was about 1 m/s.

LTD induced by repetitive stimulation of afferent Aδ-fibres is reversible

In five experiments after stable C-fibre-evoked field potentials were recorded for at least 40 min, prolonged high-frequency burst stimulation at intensities of 2T for activation of Aβ-fibres was delivered to the sciatic nerve. This conditioning stimulation of Aβ-fibres did not affect C-fibre-evoked field potentials in any experiment. One hour later, the same conditioning stimulation paradigm but at an intensity sufficient to excite Aδ-fibres (10 V, 0.1 ms), was given twice at 1 h intervals to saturate LTD. As before, the conditioning Aδ-fibre stimulation significantly decreased the amplitudes of C-fibre-evoked field potentials (to 71 ± 4% of control 30 min after the first Aδ-fibre stimulation, and to 47 ± 5% of control 30 min after the second Aδ-fibre stimulation). One hour after the second Aδ-fibre stimulation brief C-fibre tetani (40 V, 0.5 ms pulses at 100 Hz for 1 s, given four times at 10-s intervals), which consistently induce LTP of C-fibre-evoked field potentials in naive rats (Liu & Sandkühler, 1995, 1997), were applied (Fig. 3). This completely reversed the Aδ-fibre-induced LTD (to 117 ± 16% of control 30 min after C-fibre stimulation).

Depotentiation of established LTP by conditioning Aδ-fibre stimulation

In hippocampus established LTP can consistently be reversed by a heterosynaptic mechanism (depotentiation, see Linden, 1994 for a review). In the following experiments we tested whether LTP of C-fibre-evoked field potentials in spinal cord could be depotentiated by repetitive stimulation of Aδ-fibres. In five rats, LTP of C-fibre-evoked field potentials (to 216 ± 22% of control) was induced by stimulation of sciatic C-fibres (40 V, 0.5 ms, 100 Hz for 1 s, given 4 times at 10-s intervals). One hour later, prolonged high-frequency stimulation of Aβ-fibres at 2T was tested. This had no effect on the amplitudes of the field potentials. One hour after Aβ-fibre stimulation, prolonged high frequency burst stimulation of Aδ-fibres was applied at least twice at 1 h intervals. The first Aδ-fibre stimulation produced a small (but not statistically significant) depression in field potential amplitude (from 216 ± 22 to 187 ± 21% of control), while the second stimulation induced a substantial decrease (from 216 ± 22 to 105 ± 22% of control). Figure 4A shows an example and Fig. 4B summarizes the results from five experiments.

Descending modulation of plastic changes in synaptic transmission

The effect of tonically active descending systems on C-fibre-evoked field potentials in spinal cord was evaluated in five rats. After recording stable C-fibre-evoked field potentials for at least 40 min, 2% lidocaine (0.1 mL) was injected into the spinal segment C3 followed by cord transection at this site. This produced a persistent increase in the mean amplitude of C-fibre-evoked field potentials (Fig. 5) and decreased mean arterial blood pressure (from 80 to 100 mmHg to 60–70 mmHg). One hour after spinal transection the mean amplitude of C-fibre-evoked field potentials was 268 ± 51% of control. In five other experiments recordings of C-fibre-evoked field potentials were made in spinalized rats. Instead of inducing LTD, prolonged high frequency burst stimulation of Aδ-fibres now induced LTP of C-fibre-evoked field potentials in all five rats tested (Fig. 6A). This LTP had very short latencies (< 30 s) as compared with LTP induced in intact rats by repetitive stimulation of C-fibres (see Fig. 4A), which had latencies of 5–10 min and required 10–15 min to reach maximal potentiation. Four hours after the Aδ-fibre conditioning stimulation, the mean amplitude of C-fibre-evoked field potentials was 187 ± 19% of control (Fig. 6A).

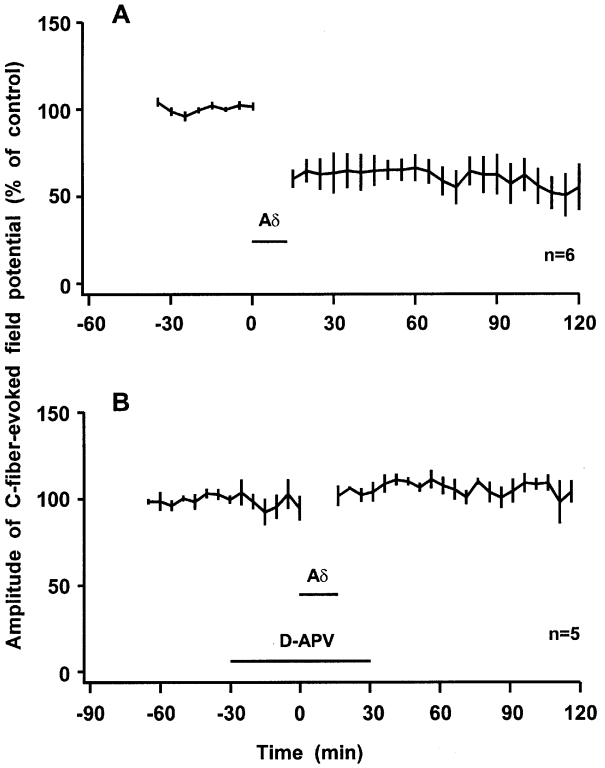

FIG. 2. Induction of LTD of C-fibre-evoked field potentials by Aδ-fibre stimulation requires activation of spinal NMDA receptors. (A) Mean time course of LTD of C-fibre-evoked field potentials in six experiments is shown. In each experiment the mean response before Aδ-fibre stimulation (from time –35–0 min) served as control. Mean amplitudes (± SEM), expressed as percentage of controls, are plotted vs. time. (B) In the five experiments, the mean amplitude recorded from time –65 to –30 min served as control. Superfusion of the spinal cord with the NMDA-receptor antagonist D-APV (100 μM, indicated by the lower horizontal bar) did not affect the baseline responses but prevented the induction of LTD by prolonged high frequency stimulation of Aδ-fibres (upper horizontal bar).

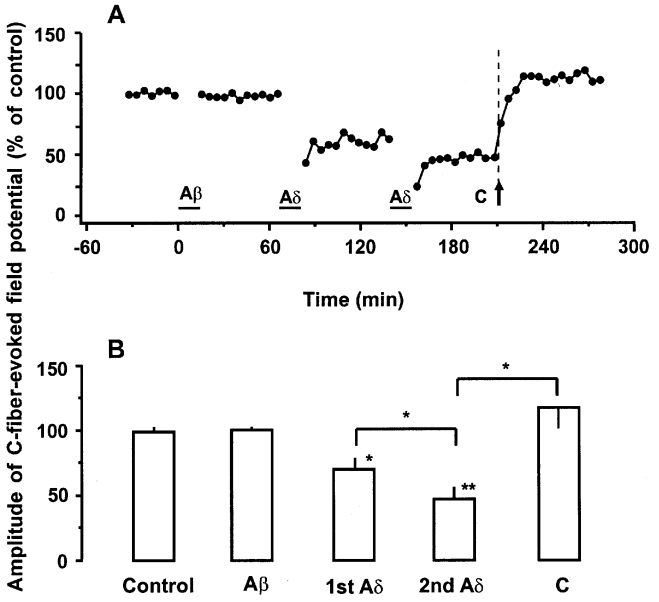

FIG. 3. Induction and reversibility of LTD of spinal C-fibre-evoked field potentials. (A) Results from one typical experiment show that prolonged high frequency burst stimulation at 2T for excitation of sciatic Aβ-fibres (300 mV, 0.1 ms) did not affect the amplitudes of C-fibre-evoked field potentials but the same conditioning stimulation paradigm at Aδ-fibre strength (10 V, 0.1 ms) induced LTD. The LTD was fully reversed by a sciatic C-fibre tetanic stimulation (40 V, 0.5 ms, at 100 Hz for 1 s, given four times at 10-s intervals, arrow). (B) shows the summary of five experiments. The mean amplitudes (± SEM) were obtained at 30 min before (control) and 30 min after each conditioning stimulation. $*P < 0.05$; $**P < 0.01$.

Involvement of NMDA receptors in LTD and LTP of C-fibre-evoked field potentials induced by Aδ-fibre stimulation

In hippocampus induction of heterosynaptic LTD may be prevented by blockade of either NMDA-receptors or voltage-gated Ca^{2+} channels (see Linden, 1994 for a review). In this study the possible role of spinal NMDA receptors in LTD in intact rats and in LTP in spinal rats was investigated by spinal superfusion of the NMDA receptor antagonist D-APV. Application of D-APV (100 μM) at the recording segments prevented the induction of LTD by subsequent prolonged high frequency burst Aδ-fibre stimulation in all five intact rats tested (Fig. 2B). In three spinalized rats the induction of LTP was also blocked by prior application of D-APV (Fig. 6B). These data indicate that both heterosynaptic LTP and LTD are NMDA-receptor-dependent.

Discussion

The characteristics of LTD and LTP of C-fibre-evoked field potentials

The characteristics of C-fibre-evoked field potentials in superficial spinal dorsal horn have been described in detail elsewhere (Schouenborg, 1984; Liu & Sandkühler, 1997). The field potentials may be generated primarily by synapses between afferent C-fibres and second order dorsal horn neurons. In this study the amplitudes of C-fibre-evoked field potentials elicited by electrical stimulation of the sciatic nerve were used as a quantitative measure of synaptic strength in afferent C-fibres.

LTP in the hippocampus is believed to underlie mechanisms of learning and memory (Bliss & Collingridge, 1993), while LTD of previously potentiated synapses may serve as a 'forgetting' mechanism (Tsumoto, 1993). Memory or memory-like mechanisms have also been proposed to be involved in some types of chronic pain (Katz & Melzack, 1990; Coderre *et al.*, 1993), but how and where the pain-related information is stored is unclear. LTP of C-fibre-evoked field potentials can be induced by conditioning electrical stimulation of C-fibres in rats with spinal cord intact and can also be induced by natural noxious stimulation in spinal rats (Liu & Sandkühler, 1995, 1997; Sandkühler & Liu, 1998). This strongly suggests that nociceptive information may be preserved at the synapses between afferent C-fibres and nociceptive neurons in the spinal dorsal horn. Here, we show that synaptic transmission in a test pathway (afferent C-fibres) is depressed for prolonged periods of time by stimulation of an independent conditioning pathway (afferent Aδ-fibres). This makes a homosynaptic mechanism highly unlikely and a heterosynaptic form of LTD is more probable. A heterosynaptic LTD would require that the test pathway and the conditioning pathway converge onto the same postsynaptic neurons and indeed it has been shown that many neurons in laminae I or II of spinal dorsal horn can be activated by both afferent A-fibres and C-fibres (Gregor & Zimmermann, 1972; Cervero *et al.*, 1976), and many neurons in this region are excited only by nociceptive afferent inputs (nociceptive specific neurons). It is also possible, however, that other neuronal circuits may be involved in this newly identified form of LTD.

It is unlikely that the long-lasting decrease in the amplitudes of

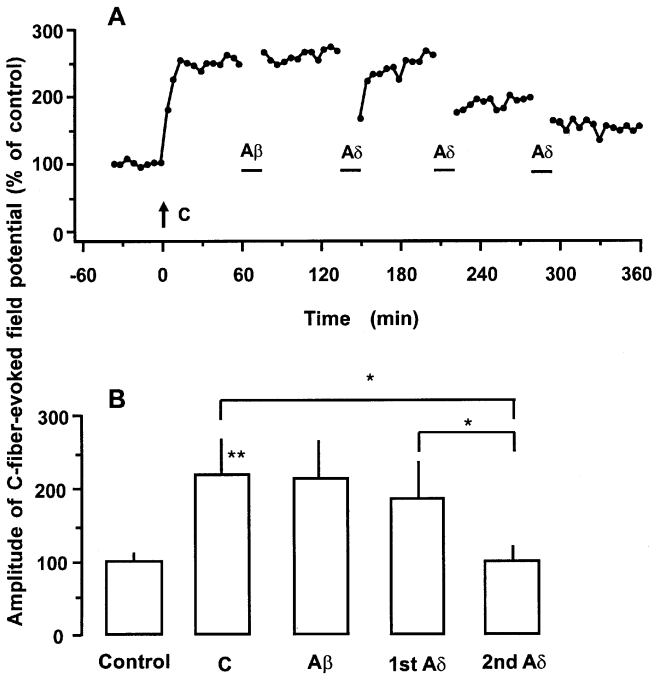

FIG. 4. Depotentiation of LTP of C-fibre-evoked field potentials by Aδ-fibre stimulation. (A) LTP of C-fibre-evoked field potentials was induced by a brief tetanic stimulation of the sciatic nerve (40 V, 0.5 ms, at 100 Hz for 1 s, given four times at 10-s intervals, arrow). Prolonged high-frequency burst stimulation at an intensity exciting Aβ-fibres (300 mV, 0.1 ms) did not affect the LTP; the same conditioning stimulation paradigm at Aδ-fibre intensity (10 V, 0.1 ms) partially reversed the LTP. (B) The summary of five experiments. The mean amplitudes (± SEM) were obtained at 30 min before (control) and 30 min after each conditioning stimulation. * $P < 0.05$; ** $P < 0.01$.

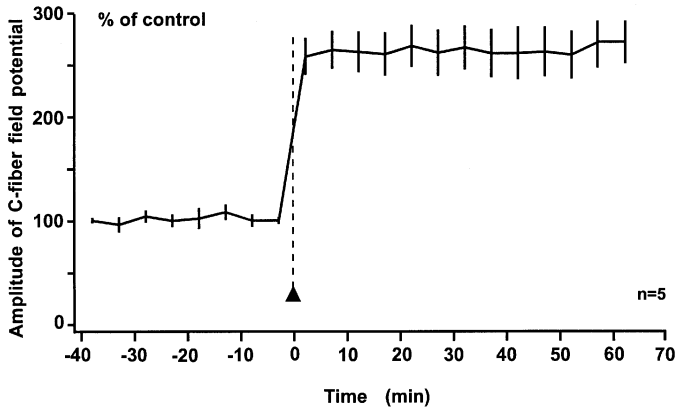

FIG. 5. Amplitudes of C-fibre-evoked field potentials are enhanced following spinalization. In each experiment the responses recorded before spinalization were averaged and served as control. Mean amplitudes (± SEM), expressed as percentage of control, are plotted vs. time. The arrowhead (at time 0 min) indicates the time of spinalization at C3 segment.

the C-fibre-evoked field potentials was due to damage of afferent C-fibres or to excitotoxic effects in the target neurons following prolonged high frequency burst stimulation, because: (i) the conditioning stimulation induced LTD but did not affect C-fibre volleys recorded simultaneously in the sural nerve, (ii) LTD was fully reversible by C-fibre tetanic stimulation and (iii) the same conditioning stimulation that induced LTD in intact rats induced LTP in rats that were spinalized.

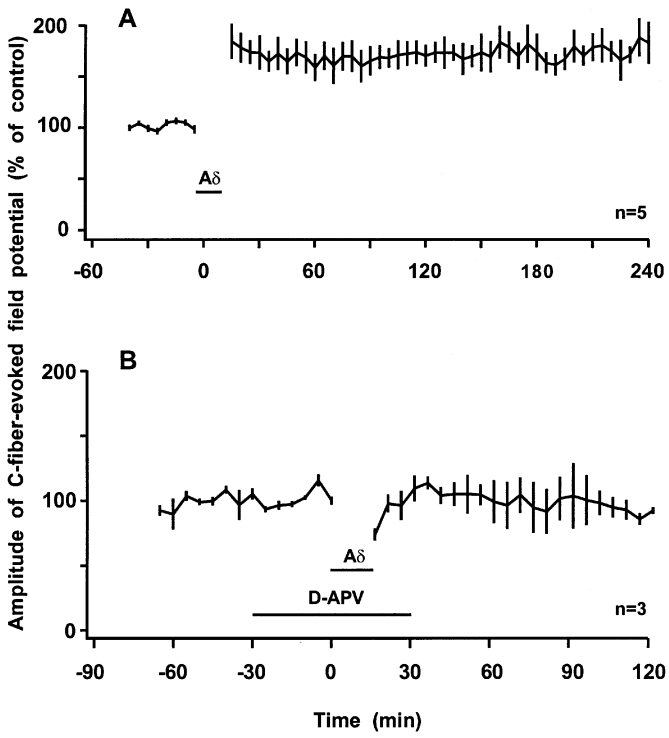

FIG. 6. In spinalized rats Aδ-fibre stimulation induces NMDA-dependent LTP of C-fibre-evoked field potentials. (A) The mean time course of LTP of C-fibre-evoked field potentials induced by prolonged high-frequency burst sciatic Aδ-fibre stimulation in five spinalized rats. In each experiment the mean response before Aδ-fibre stimulation (from time –40 to –5 min) served as control. Mean amplitudes (± SEM), expressed as percentage of controls, are plotted vs. time. (B) The induction of LTP by prolonged high-frequency Aδ-fibre stimulation is prevented by spinal superfusion with the NMDA receptor antagonist D-APV in three spinalized rats. In the three experiments, the mean amplitude recorded from time –65 to –30 min served as control. Spinal superfusion with D-APV (100 μM) is indicated by the lower horizontal bar.

The reversibility of both LTP and LTD could be due to independent mechanisms that change the amplitudes of C-fibre-evoked field potentials in opposite directions. Alternatively a true depotentiation or 'de-depression' may be involved. This would be in line with the hypothesis that phosphorylation state of some synaptic phosphoproteins would determine the long-lasting changes in synaptic strength (Lisman, 1989). At some synapses activation of protein kinases may induce an LTP and depotentiation would result from activation of the corresponding protein phosphatases.

Tonic descending systems modify the direction of synaptic plasticity in spinal dorsal horn

An interesting finding in the present study is that prolonged burst Aδ-fibre conditioning stimulation induced LTD of C-fibre-evoked field potentials in rats with intact descending pathways but LTP in spinalized animals. The mechanisms underlying this bi-directional modulation of synaptic transmission are presently unknown, but some hypotheses can be suggested. In slices of rat spinal cord identical conditioning stimulation may induce either LTP or LTD, depending on the level of postsynaptic membrane potential during tetanic stimulation (Randić *et al.*, 1993). Induction of both LTP and LTD requires elevation of Ca^{2+} concentration in postsynaptic spines (Lynch *et al.*, 1983; Malenka *et al.*, 1988; Mulkey & Malenka, 1992), but the levels of free cytosolic Ca^{2+} required for induction of LTP and

LTD are quite different (Bear & Malenka, 1994). A large surge in intracellular Ca^{2+} triggers LTP by activating Ca^{2+}-dependent protein kinases (Schwartz, 1993), while modest elevation of postsynaptic Ca^{2+} (< 1 μM) causes LTD by selectively activating protein phosphatases (Lisman, 1989; Mulkey *et al.*, 1993). Thus the magnitude of the increased Ca^{2+} levels may determine the direction of the resultant plastic changes in synaptic transmission. This is in line with the observation that both spinal LTP and LTD are NMDA-receptor-dependent (Sandkühler *et al.*, 1997; present work), and Ca^{2+} influx through NMDA-receptor channels in spinal neurons is voltage dependently blocked by Mg^{2+} (Mayer *et al.*, 1984). In intact animals spinal nociceptive neurons are subject to a powerful, pre- and postsynaptic inhibition from descending pathways (Basbaum & Fields, 1978; Martin *et al.*, 1979; Giesler *et al.*, 1981; Shah & Dostrovsky, 1982; Duggan & Morton, 1988; Willis, 1988; Sandkühler, 1996a). When postsynaptic descending inhibition is removed by spinalization, the membrane potential of spinal dorsal horn neurons may become more depolarized. The same Aδ-fibre conditioning stimulation may then induce LTP by producing a larger increase in intracellular Ca^{2+} concentrations through NMDA receptor channels as compared to animals with descending inhibition intact where a smaller increase in intracellular Ca^{2+} may be expected during conditioning stimulation, and consequently leading to LTD.

Our data show that in rats with intact spinal cord, prolonged low frequency Aδ-fibre stimulation (1 Hz, 15 min) and brief high-frequency burst Aδ-fibre stimulation (100 Hz, 4 s) induced LTD of C-fibre-evoked potentials in only 2/11 rats and in 4/9 rats, respectively. In contrast, mono-or polysynaptic Aδ-fibre-evoked EPSPs are consistently depressed following prolonged 1Hz stimulation in a spinal cord–dorsal root slice preparation of young rat (Sandkühler *et al.*, 1997) or by 100-Hz burst stimulation if the membrane potential of the postsynaptic cell is held at hyperpolarized levels (Randić *et al.*, 1993). It is well known that the induction of synaptic plasticity critically depends not only on pattern and timing of conditioning stimulation, but also on the level of membrane potential of the postsynaptic cell. Further, the stimulation parameters required for the induction of LTD may vary with different synapses and may also depend upon the age of the animals. Thus, it is not surprising that the stimulation parameters for the induction of LTD of C-fibre-evoked potentials in the present *in vivo* study with intact descending inhibition differ from those in the *in vitro* work. Possibly, the longer-lasting, high-frequency stimulation of Aδ-fibres is necessary in the present study to (i) overcome postsynaptic inhibition and (ii) to modify the strength of C-fibre synapses that were not activated during conditioning stimulation.

Synaptic mechanisms of afferent-induced spinal analgesia

Acupuncture or TENS are often used clinically to relieve acute and chronic pain in human patients (Melzack, 1975; Eriksson *et al.*, 1979; Johnson *et al.*, 1991; Ishimaru *et al.*, 1995). Stimulation for a period of 20–30 min may sometimes relieve pain for days or even for months (Melzack, 1975; Melzack *et al.*, 1983). The gate control theory (Melzack & Wall, 1965) proposed that spinal neural mechanisms can act like a gate increasing or decreasing the flow of nerve impulses from peripheral fibres to spinal cord cells projecting to the brain (large-fibre inputs tend to close the gate, small-fibre inputs tend to open the gate). This mechanism may contribute to the analgesia induced by low-intensity TENS, but it does not easily explain the long-lasting analgesic effects produced by some other forms of afferent stimulation. Stimulation of large diameter afferent A-fibres may depress nociceptive responses in the spinal cord (Handwerker *et al.*, 1975; Chung *et al.*, 1984a,b). This Aβ-fibre-mediated depression typically does not outlast the duration of conditioning stimulation for more than a few seconds or minutes and may result from activation of inhibitory, probably GABAergic, interneurons. This corresponds well to the short lasting analgesia observed during some forms of innocuous TENS that recruits only large-diameter afferent nerve fibres. In contrast, conditioning stimulation of small calibre afferent A-fibres induces a long-lasting depression of synaptic transmission in C-fibres (present work) and in Aδ-fibres that is not mediated by GABA or glycine (Sandkühler *et al.*, 1997). This may lead to the long-lasting analgesia and antihyperalgesia following (electro-) acupuncture that produces tolerable pain or following some forms of physical therapy that also activate fine primary afferents. There is evidence that TENS at high intensities (painful but tolerable) is more effective than stimulation at low intensities (Melzack, 1975; Wolf *et al.*, 1981). Recently, we have shown that cooperativity between primary afferent Aδ-fibres but not C-fibres is necessary to induce a robust LTD of synaptic transmission between afferent Aδ-fibres and neurons in lamina II in a slice preparation of young rat spinal cord (Sandkühler *et al.*, 1997). The present results extend these findings by showing that stimulation of Aδ-but not Aβ-fibres induces LTD, and depresses previously established LTP of C-fibre-evoked field potentials. Thus, conditioning Aδ-fibre stimulation may depress synaptic transmission in both Aδ-and C-fibres and this neural mechanism may underlie the strong analgesia seen in some patients following therapeutic stimulation of primary afferents.

Clinical studies have demonstrated that the efficacy of afferent stimulation may, however, be quite variable, with some patients obtaining no pain relief or even increasing levels of chronic pain (Melzack, 1975; Eriksson *et al.*, 1979; Wolf *et al.*, 1981). The present study has shown that the activity in tonic descending pathways may determine the direction of the plastic changes in synaptic strength between primary afferent C-fibres and dorsal horn neurons. It is reasonable to assume that in patients with fully intact descending inhibition, afferent stimulation may produce long-lasting relief of chronic pain. If, however, there is a deficit in descending inhibition, then the same therapeutic stimulations may have little effect. If the chronic pain is produced by severe impairment of tonic descending inhibition, then TENS might even enhance, rather than relieve, chronic pain.

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Abbreviations

D-APV	D(–)-2-amino-5-phosphonopentanic acid
EPSP	excitatory postsynaptic potential
GABA	γ-aminobutyric acid
LTP	long-term potentiation
LTD	long-term depression
NMDA	*N*-methyl-D-aspartic acid
TENS	transcutaneous electrical nerve stimulation.

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