Characterization of Long-Term Potentiation of C-Fiber–Evoked Potentials in Spinal Dorsal Horn of Adult Rat: Essential Role of NK1 and NK2 Receptors

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Liu, X.-G. and J. Sandkühler. Characterization of long-term potentiation of C-fiber–evoked potentials in spinal dorsal horn of adult rat: essential role of NK1 and NK2 receptors. *J. Neurophysiol.* 78: 1973–1982, 1997. Impulses in afferent C fibers, e.g., during peripheral trauma, may induce plastic changes in the spinal dorsal horn that are believed to contribute to some forms of hyperalgesia. The nature of lasting changes in spinal nociception are still not well understood. Here we characterized the long-term potentiation (LTP) of spinal field potentials with a negative focus in superficial spinal dorsal horn evoked by supramaximal electrical stimulation of the sciatic nerve in urethan-anesthetized adult rats. The field potentials studied in this work had high thresholds (≥7 V, 0.5 ms), long latencies (90–130 ms), and long chronaxy (1.1 ms) and were not abolished by muscle relaxation and spinalization. Thus they were evoked by afferent C fibers. In response to 1-Hz stimulation of afferent C fibers, amplitudes of C-fiber–evoked field potentials remained constant, whereas number of action potentials of some dorsal horn neurons increased progressively (wind-up). In all 25 rats tested, high-frequency, high-intensity stimulation (100 Hz, 30–40 V, 0.5 ms, 400 pulses given in 4 trains of 1-s duration at 10-s intervals) always induced LTP (to ~200% of control), which consistently lasted until the end of recording periods (4–9 h). This tetanic stimulation also significantly decreased mean threshold of C-fiber–evoked field potentials. The C-fiber volley, which was recorded simultaneously in sural nerve, was, however, not affected by the same tetanic stimulation. High-frequency, low-intensity stimulation (100 Hz, 3 V, 0.5 ms) never induced LTP in six rats tested. At an intermediate frequency, high-intensity stimulation (20 Hz, 40 V, 0.5 ms, 400 pulses given in 4 trains of 5 s at 10-s intervals) induced LTP in four out of six rats, which lasted until end of recording periods (3–6 h). In the remaining two rats, no LTP was induced. Low-frequency, high-intensity stimulation (2 Hz, 30–40 V, 0.5 ms, 400 pulses) induced LTP that lasted for 2–8 h in four out of five rats. Intravenous application of neurokinin 1 (NK1) or neurokinin 2 (NK2) receptor antagonist RP 67580 (2 mg/kg, $n = 5$) or SR 48968 (0.3 mg/kg, $n = 5$) 30 min before high-frequency, high-intensity stimulation blocked the induction of LTP in all rats tested. In contrast, the same dose of their inactive enantiomers RP 68651 ($n = 5$) or SR 48965 ($n = 5$) did not affect the induction of LTP. Spinal superfusion with RP 67580 (1 μM) from 30 min before to 30 min after high-frequency, high-intensity stimulation blocked induction of LTP in all five rats tested. Spinal application of SR 48968 (10 nM) prevented LTP in five out of seven rats. However, when spinal superfusions with RP 67580 (1 μM, $n = 3$) or SR 48968 (10 nM, $n = 3$) were started 1 h after high-frequency, high-intensity stimulation, established LTP was not affected. Thus the activation of neurokinin receptors is necessary for the induction but not for the maintenance of LTP of C-fiber–evoked field potentials in spinal dorsal horn. This model may be useful to study plastic changes in spinal cord induced by peripheral C-fiber stimulation. The LTP of C-fiber–evoked field potentials may be a mechanism underlying some forms of hyperalgesia.

INTRODUCTION

Nociceptors convey their information via fine myelinated Aδ and unmyelinated C fibers, which terminate preferentially in the superficial laminae of the spinal dorsal horn where they make synaptic contact with higher order neurons (Gobel and Falls 1979; Light and Perl 1979). After trauma of peripheral tissues, sensitization of peripheral nociceptors and plastic changes in the spinal dorsal horn are considered to contribute to hyperalgesia, i.e., an increase in responses to noxious stimuli (Coderre et al. 1993; Dubner 1991; Sandkühler 1996; Woolf 1983).

Expression of hyperalgesia requires the activation of *N*-methyl-D-aspartic acid (NMDA) receptors (Nagy et al. 1993; Ren and Dubner 1993; Woolf and Thompson 1991), neurokinin 1 (NK1) receptors (Dougherty et al. 1994; Picard et al. 1993; Thompson et al. 1993), and neurokinin 2 (NK2) receptors (Neugebauer et al. 1996; Xu et al. 1991). Blockade of any of these receptor subtypes in spinal dorsal horn abolishes or reduces both nociceptive reflexes and the enhanced responses of nociceptive spinal dorsal horn neurons to stimulation of primary afferent C fibers.

Repetitive stimulation of afferent C fibers typically at 0.3–5 Hz leads to a progressive increase in the number of action potentials in deep or superficial spinal dorsal horn neurons in response to each C-fiber stimulus (Jeftinija and Urban 1994; Mendell 1966). This has been termed ''wind-up'' and is considered to be involved in central hyperalgesia (Dubner 1991; Price et al. 1994a,b). The activation of NMDA (Price et al. 1994a; Ren 1994) or neurokinin receptors (Dougherty and Willis 1991; Thompson et al. 1994) may be involved in the wind-up. After cessation of repetitive C-fiber stimulation, the responses return to normal within few minutes (Jeftinija and Urban 1994; Price et al. 1994a; Ren 1994). Thus wind-up may only persist as long as there is a low-frequency barrage in afferent C fibers.

In a transverse slice preparation of young rat spinal cord, Randić and coworkers (1993) have shown with intracellular recordings from substantia gelatinosa neurons that primary afferent neurotransmission may either be potentiated or depressed after high-frequency stimulation (100 Hz, 3 times for 1 s) of dorsal roots. This form of long-term potentiation (LTP) and long-term depression of synaptic transmission

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was not affected by neonatal capsaicin treatment, which may destroy >50% of all afferent C fibers (Welk et al. 1984). It also has been reported that in neonatal rat spinal cord, 100-Hz conditioning stimulation of the dorsomedial white matter induces LTP and long-term depression of field potentials recorded in the intermediate gray matter (Pockett 1995), and 10-Hz stimulation of peripheral cutaneous saphenous nerve produces LTP of slow ventral root potentials (Lozier and Kendig 1995) and 1-Hz stimulation of primary afferent Aδ-fibers induces robust LTP in spinal cord substantia gelatinosa in vitro (Sandkühler et al. 1997).

We recently have provided evidence that LTP of excitatory neurotransmission in afferent C fibers exists in intact spinal cord of adult rats and that induction of this LTP requires the activation of NMDA receptors (Liu and Sandkühler 1995b). However, the LTP of C-fiber–evoked field potentials in the superficial spinal dorsal horn has not been fully characterized. Furthermore, LTP of synaptic transmission exists at many sites of the central nervous system, and an essential role of tachykinins for the induction of LTP has, to our knowledge, never been shown.

Here, we have identified and characterized LTP of C-fiber–evoked field potentials in superficial spinal dorsal horn of adult rats. The roles of NK1 and NK2 receptors for induction and maintenance of LTP were evaluated.

METHODS

Preparation of animals

Experiments were performed on male Sprague-Dawley rats (250–350 g body wt). Urethan (1.5 g/kg, given intraperitoneally) was used to induce and maintain anesthesia. Surgical level of anesthesia 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, if necessary. A catheter was inserted into one external jugular vein for continuous intravenous (iv) infusion of Tyrode's solution (see below) at a rate of 0.8–1 ml/h and for application of drugs. One carotid artery was cannulated to monitor continuously the mean arterial blood pressure, which ranged from 80 to 100 mmHg. Colorectal temperature was kept constant ~37–38°C by means of a feedback-controlled heating blanket. A laminectomy was performed to expose the lumbar enlargement of spinal cord, and the dura mater was incised longitudinally. The left sciatic nerve was dissected free for bipolar electrical stimulation with platinum hook-electrodes. In some experiments, the sural nerve was exposed ipsilaterally to record C-fiber volleys in response to electrical stimulation of sciatic nerve. All exposed nervous tissues were covered with warm paraffin oil, except for those spinal segments to be superfused (see below).

Administration of drugs

In this study, receptor antagonists were applied either intravenously or locally by controlled superfusion of spinal cord at the recording segments. To perform the controlled superfusion of spinal cord, 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). Stock solutions of nonpeptide NK1-receptor antagonist RP 67580 and its inactive enantiomer RP 68651 (Rhône-Poulenc Rorer, France) were prepared in dimethylsulfoxide (DMSO, Sigma) for iv administration (at 3 mg/ml) and for controlled superfusion (at 2.5 mM). For iv administration (2 mg/kg body wt), stock solutions were diluted in 0.3 ml Tyrode's solution [which contained (in mM) 137 NaCl, 2.7 KCl, 1.4 $CaCl_2$, 1.1 $MgCl_2$, 6.0 $NaHCO_3$, and 2.1 NaH_2PO_4]. RP 67580 and RP 68651 intended for spinal superfusion were diluted with artificial cerebrospinal fluid (ACSF) immediately before application to yield a final concentration of 1 μM. Final DMSO concentration in the diluted working solution was 0.04%. Nonpeptide NK2-receptor antagonist SR 48968 and its inactive enantiomer SR 48965 (Sanofi Recherche, France) were dissolved at 0.6 mg/ml in an aqueous solution of 0.01% Tween 80 (Sigma) for iv application and at 1 mM for controlled superfusion. Stock solutions were diluted either with ACSF to 10 or 100 nM for spinal superfusion or with 0.3 ml Tyrode's solution for iv application (0.3 mg/kg).

Electrophysiological recordings and nerve stimulation

In response to electrical stimulation of the sciatic nerve, field potentials were recorded at a depth of 50–400 μm from the dorsal surface of spinal cord with tungsten microelectrodes (impedance 1–3 MΩ), which were driven by an electronically controlled microstepping motor. A bandwidth of 0.1–550 Hz was used for recording field potentials. An A/D converter card (DT2821-F-16SE) was used to digitize and store data in a Pentium computer at a sampling rate of 10 kHz. Test stimuli (0.5 ms pulses, 10–20 V) were applied to the sciatic nerve. High-frequency (100 Hz) tetanic stimulation is most effective to induce LTP in hippocampus (Bliss and Collingridge 1993) and in spinal cord (Pockett 1995; Randić et al. 1993), whereas most afferent C fibers typically may discharge at lower frequencies when stimulated. We therefore have tested conditioning stimuli at three different frequencies and two different intensities (to recruit A fibers only or A and C fibers): high-frequency, high-intensity stimulation (100 Hz, 30–40, V, 0.5 ms, 400 pulses given in 4 trains of 1-s duration at 10-s intervals), high-frequency, low-intensity stimulation (100 Hz, 3 V, 0.5 ms, 400 pulses given in 4 trains of 1-s duration at 10-s intervals), intermediate-frequency, high-intensity stimulation (20 Hz, 40 V, 0.5 ms, 400 pulses given in 4 trains of 5 s at 10-s intervals), and low-frequency, high-intensity stimulation (2 Hz, 30–40 V, 0.5 ms, 100 or 400 pulses). In some experiments, an electrical thermometer was placed on the dorsal surface of spinal cord to measure temperature changes with time. At the end of experiments, electrolytic lesions were made at the recording sites through the recording electrodes (30–40 μA, 20–25 s). Rats were killed by an overdose of pentobarbital. Spinal cords were cut on a freezing microtome into 50-μm-thick transverse sections, which were stained with cresyl violet. Recording sites were identified and plotted onto a schematic representation of the lumbar cord. The distance from the stimulation site at the sciatic nerve to the recording site in lumbar spinal dorsal horn was ~11 cm.

Data analysis and statistics

The amplitudes of C-fiber–evoked field potentials were determined off-line by parameter extraction, which was implemented by DataWave (Colorado). The amplitude of C-fiber–evoked potential was determined as the maximal distance from the baseline (Fig. 1*A*). In some experiments, the integral of the waveform also was determined. Centered signed rank statistic and analysis of variance (Kruskal-Wallis test) were used for statistical analysis and $P < 0.05$ was considered significant. Means are given as means ± SE.

RESULTS

Properties of C-fiber–evoked field potentials

After supramaximal electrical stimulation (20–25 V, 0.5 ms) of the sciatic nerve, field potentials with different latencies were recorded in superficial spinal dorsal horn (Fig.

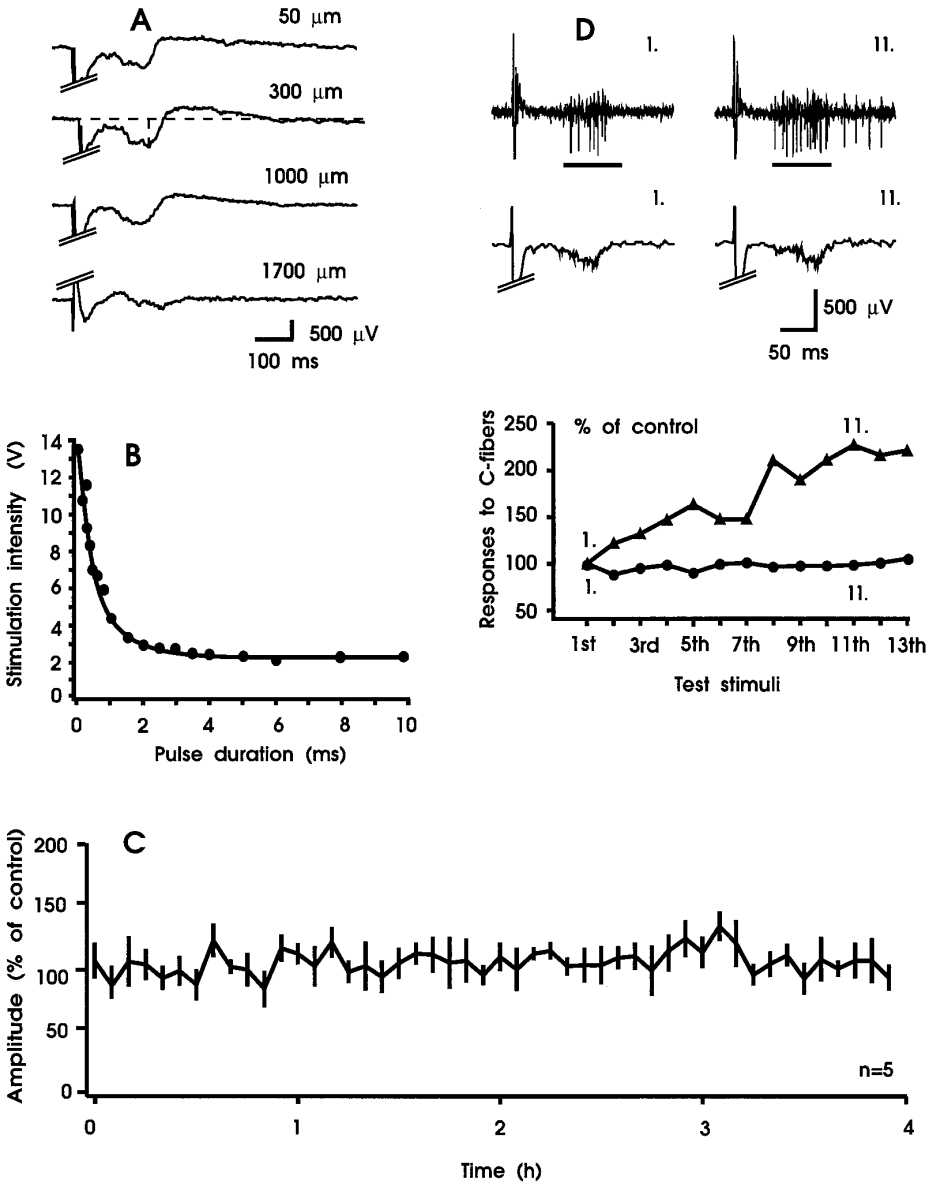

FIG. 1. Properties of C-fiber–evoked field potentials in superficial spinal dorsal horn. *A*: field potentials evoked by electrical stimulation of the sciatic nerve (20 V, 0.5 ms, at 30-s intervals) were recorded in spinal cord gray matter at different depths from the dorsal cord surface. Amplitudes of A-fiber–evoked N wave are out of scale and therefore are truncated (negativity down). – – –, the baseline and the maximal amplitude of C-fiber–evoked field potential as determined by parameter extraction software. *B*: strength-duration curve of C-fiber–evoked field potentials from 1 representative experiment is shown. Data were collected at 1-min intervals. For each pulse width (0.05–10 ms), the minimal stimulation intensity to reliably evoke a field potential was determined. In this experiment, the rheobase of the field potential was 2.2 V and chronaxy was 1.1 ms. *C*: stable C-fiber–evoked field potentials were recorded for ≥4 h at 5-min intervals. Mean amplitudes of field potentials in 5 experiments are plotted vs. time. In each experiment, responses were normalized to the mean amplitude of all potentials. Vertical bars indicate 1 SE. *D*: C-fiber–evoked action potentials in a superficial spinal dorsal horn neuron and C-fiber–evoked field potentials, which were recorded 5 min later at the same site with the same microelectrode but with different filter settings, were elicited by 13 stimuli (20 V, 0.5 ms) applied to the sciatic nerve at 1 Hz. Recording site was adjusted to yield a good signal-to-noise ratio for action potential recordings. This caused the ''noisy'' appearance of the field potential recordings. Number of action potentials (▲) underwent a wind-up but the amplitudes of C-fiber–evoked field potentials remained unchanged (●). *Top*: individual recordings shown of C-fiber–evoked action potentials (*top traces*) and C-fiber–evoked field potentials (*bottom traces*) from this experiment. Horizontal bars indicate the period of time from which the number of C-fiber–evoked action potentials were calculated.

1*A*). Only field potentials with a negative focus in superficial spinal dorsal horn, with long latencies (90–130 ms, corresponding to conduction velocities <1.2 m/s), and with high thresholds (7–13 V, 0.5 ms) were investigated further. It was verified histologically that recording sites were located at a mean depth of 400 ± 15 μm (n = 42). For the reasons listed below, the field potentials are considered to be evoked by stimulation of afferent C fibers.

In five experiments, the strength-duration relationship of C-fiber–evoked potentials was evaluated by systematically varying pulse duration (from 0.05 to 10 ms) and determining the stimulation strength (V) required to evoke the field potential. From the strength-duration curve, rheobase and the chronaxy was determined (Fig. 1*B*). Mean rheobase was 2.2 ± 0.5 V (n = 5) and mean chronaxy was 1.1 ± 0.2 ms (n = 5). These values are consistent with an activation of afferent C fibers (Li and Bak 1976).

To exclude the possibility that long latencies of the field potentials are due to activation of supraspinal loop or muscle contraction, in five experiments, stable C-fiber–evoked field potentials were recorded for ≥30 min and then 0.1 ml lidocaine (2%) was injected into the spinal cord at the C3 segment followed by surgical transection of spinal cord at the same site. After spinalization, latencies of late field potentials remained constant but amplitudes of the field potentials always increased to 170–240% of control and mean arterial blood pressure decreased from 80–100 to 60–70 mmHg (data not shown). In 15 experiments, C-fiber–evoked field potentials also could be recorded in rats that were paralyzed with pancuronium (0.5 mg/kg iv). In five other rats, stable C-fiber–evoked potentials were recorded for 30 min. The rats then were paralyzed with pancuronium (0.5 mg/kg iv) and mechanically ventilated. The amplitudes of C-fiber–evoked potentials were not changed systematically.

In 10 rats, C-fiber–evoked field potentials and C-fiber–evoked action potentials of single neurons were elicited by the same stimulation of sciatic nerve and were recorded with the same electrode and at the same site but with different

filter settings. In five experiments, action potentials were recorded first and 5 min later field potentials were recorded. In five other rats, the sequence of recordings were reversed. In two rats, this latter protocol was repeated once. Electrical stimulation of the sciatic nerve (20 V, 0.5 ms pulses given 13 times at 1 Hz) produced wind-up of the number of C-fiber–evoked action potentials in 12 neurons. In the same experiments, the amplitudes of C-fiber–evoked field potentials remained stable during the repetitive stimulation (see Fig. 1*D* for an example). This indicates that C-fiber–evoked field potentials are not the envelope of postsynaptic action potentials. One hour after conditioning stimulation with 13 pulses at 1 Hz, the amplitudes of C-fiber–evoked field potentials were not different from controls as observed in eight rats. This indicates that this stimulation protocol is not sufficient to induce LTP.

To test the stability of the amplitudes of C-fiber–evoked field potentials over time, field potentials were recorded at 5-min intervals for 4 h in five rats. The mean amplitudes of C-fiber–evoked field potentials showed no systematic change with time (Fig. 1*C*).

It was reported that the population spike amplitude recorded in hippocampal formation is correlated negatively with brain temperature (Moser et al. 1993). To test if the amplitudes of C-fiber–evoked field potentials are also temperature sensitive or change with arterial blood pressure, we have recorded simultaneously C-fiber–evoked field potentials, the temperature on the dorsal surface of spinal cord, and mean arterial blood pressure in five experiments for 90 min. The amplitudes of C-fiber–evoked field potentials were negatively correlated with the temperature on the dorsal surface of spinal cord ($r = -0.7$ and $P < 0.0001$, Spearman correlation coefficient) but they were not correlated with mean arterial blood pressure ($r = -0.052$ and $P > 0.05$). After finishing experiments, in some rats, mean arterial blood pressure was allowed to fall <30 mmHg and the amplitudes of C-fiber–evoked field potentials did not change by >5–10% of control.

LTP of C-fiber–evoked field potentials

In 56 rats, C-fiber–evoked field potentials were evoked by stimulation of the sciatic nerve with single test pulses (10–20 V, 0.5 ms, given at 5 min intervals). Stable responses for 1 h served as controls. A conditioning tetanic stimulation then was delivered to the sciatic nerve followed by single test pulses with stimulation parameters identical to controls. The efficiency of different stimulation parameters for the induction of LTP was evaluated (see METHODS). High-frequency, high-intensity stimulation (100 Hz, 30–40 V) consistently induced LTP of C-fiber–evoked potentials in 25 rats tested. In all experiments, the significant enhancement (as assessed by centered signed rank statistic) of the amplitudes of C-fiber–evoked potentials lasted until the end of the recordings periods, i.e., for 4–9 h. In eight experiments, recordings were performed for ≥7 h after high-frequency, high-intensity stimulation. The mean time course of LTP in these experiments is illustrated in Fig. 2*A*. Seven hours after tetanic stimulation, the mean potentiation was to 218% of control (range: 187–264%). To determine whether the long-term increase in the amplitudes of C-fiber–evoked field potentials was due to an increased excitability of C fibers at the stimulation site or due to the enhancement of synaptic transmission in spinal dorsal horn, C-fiber–evoked field potentials in spinal dorsal horn and C-fiber volleys in sural nerve were recorded simultaneously in three experiments. High-frequency, high-intensity tetanic stimulation induced LTP of C-fiber–evoked field potentials but did not affect the amplitudes of C-fiber volleys (Fig. 2*B*). The thresholds for spinal C-fiber–evoked field potentials decreased after high-frequency, high-intensity stimulation. Before the tetanic stimulation, mean threshold was 10.3 ± 1.0 V and decreased to 5.2 ± 0.4 V ($n = 6$, $P < 0.05$, centered signed rank test) after tetanic stimulation. Figure 3 shows stimulus-response curves before and after tetanic stimulation.

To test whether C fibers can follow electrical stimulation at 100 Hz, in four rats, C-fiber volleys evoked by stimulation of the sciatic nerve at 100 Hz were recorded in the sural nerve. Figure 4 shows results from a representative experiment. Amplitudes of C-fiber volleys decreased with increasing number of stimuli. This indicates that not all C fibers can follow 100-Hz stimulation throughout the train stimulation or that C fibers are following at lower variable rates.

High-frequency, low-intensity stimulation (100 Hz, 3 V) never induced LTP of C-fiber–evoked potentials in six rats tested. In the same rats, however, high-frequency, high-intensity stimulation did induce LTP (Fig. 5*A*). This indicates that activation of A fibers is not sufficient for the induction of LTP of C-fiber–evoked field potentials.

Intermediate-frequency, high-intensity stimulation (20 Hz, 40 V) induced LTP that lasted until end of recording periods (3–6 h after stimulation) in four out of six rats. In one rat, a short-term potentiation was induced that lasted 30 min after stimulation and in another rat a depression for 1.5 h was induced.

In three out of five experiments, low-frequency, high-intensity stimulation (2 Hz, 30–40 V) with 100 pulses induced LTP of C-fiber–evoked field potentials lasting for 3–5 h. In the remaining two rats, only short-term potentiation, which lasted <30 min, but no LTP was induced. In the same rats, low-frequency (2 Hz) stimulation was not as effective as high-frequency stimulation (100 Hz) for induction of LTP (Fig. 5*B*). Low-frequency, high-intensity stimulation (2 Hz, 30–40 V) with 400 pulses induced LTP in four out of five rats. In two experiments, LTP lasted until the end of experiments (8 h after tetanic stimulation). In the remaining two rats, LTP lasted for 2 and 4 h, respectively.

In three rats, C-fiber–evoked field potentials and temperature on the dorsal surface of spinal cord were recorded simultaneously. One hour after high-frequency, high-intensity stimulation no significant change in temperature (36.5 ± 2.5° before vs. 36.4 ± 2°C after stimulation) were found, but the amplitudes of C-fiber–evoked potentials were increased significantly (to 190 ± 15% of control).

Effects of NK1- and NK2-receptor antagonists on the LTP of C-fiber–evoked field potentials

In five rats, NK1-receptor antagonist RP 67580 was given intravenously (2 mg/kg body wt) after stable control responses were collected for 40 min. RP 67580 completely blocked induc-

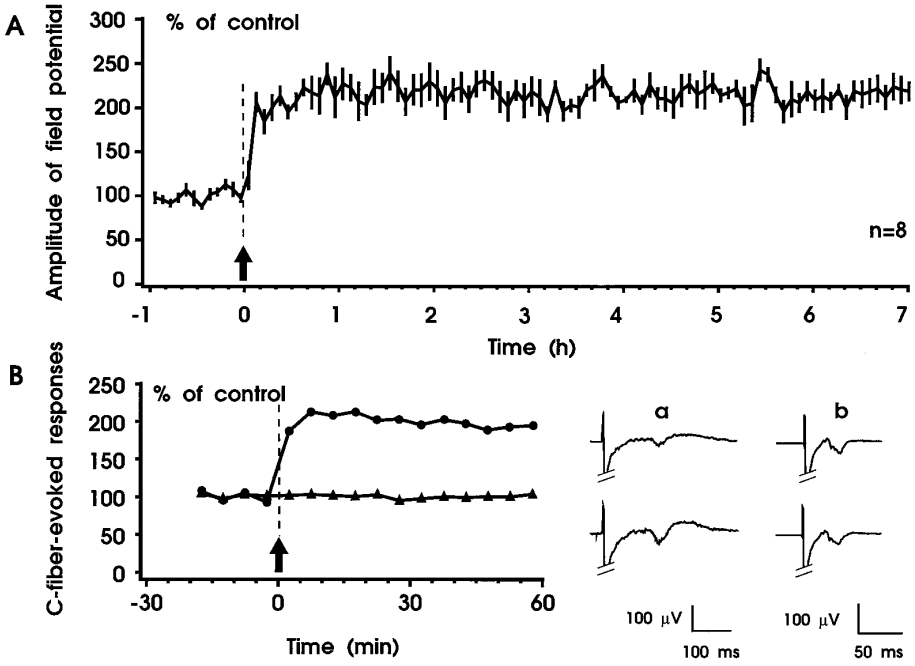

FIG. 2. Long-term potentiation (LTP) of C-fiber–evoked field potentials. *A*: mean time course of LTP of C-fiber–evoked field potentials after high-frequency, high-intensity stimulation (100 Hz, 30–40 V) of the sciatic nerve from 8 experiments is shown. →, time of tetanic stimulation. In each experiment, the mean amplitude of responses to 12 consecutive test stimuli (at times −1 to 0 h) served as controls. Mean amplitudes ± SE are expressed as percent of controls and were plotted vs. time. *B*: C-fiber–evoked field potentials (●) in spinal dorsal horn and C-fiber volleys (▲) in sural nerve were recorded simultaneously. Each data point indicates the mean amplitude of 5 consecutive field potentials recorded at 1-min intervals. High-frequency (100 Hz, 30 V) tetanic stimulation of the sciatic nerve at *time 0* (→) induced LTP of C-fiber–evoked field potentials but did not affect the amplitudes of C-fiber volleys. *Right*: original recordings of C-fiber–evoked field potentials (*a*) and C-fiber volleys (*b*) are shown; they were recorded before (*top*) and 25 min after (*bottom*) tetanic stimulation. Distance between the stimulation site at the sciatic nerve and the recording site at the sural nerve was 28 mm, and conduction velocity of C-fibers in this case was ~1 m/s.

tion of LTP without affecting the baseline responses (Fig. 6*A*). The same dose of its inactive enantiomer RP 68651 was ineffective as tested in five other rats (Fig. 6*A*). Two hours after high-frequency, high-intensity stimulation mean potentiation was to 178 ± 24% of control.

Intravenous injection of NK2-receptor antagonist SR 48968 (0.3 mg/kg) prevented induction of LTP of C-fiber–evoked field potentials in all five rats tested. The same dose of its inactive enantiomer SR 48965 did not affect the induction of LTP (to 200 ± 25% of control at 120 min, $n = 5$, Fig. 6*B*).

To test whether neurokinin receptor activation at the recording segments is required for induction of LTP, receptor antagonists were applied topically to the cord surface.

Spinal superfusion with RP 67580 at 1 μM had no effect on the baseline responses of C-fiber–evoked field potentials as tested in eight experiments. In contrast, superfusion with SR 48968 at 100 nM ($n = 3$) depressed the amplitudes of C-fiber–evoked field potentials by 60% of control but had no effect on the baseline responses at 10 nM (110 ± 20% of control, $n = 5$). In the following experiments, only the concentrations of receptor antagonists that do not affect the baseline responses therefore were used.

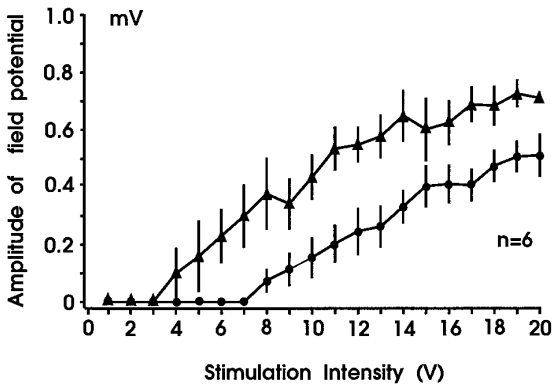

FIG. 3. Stimulus-response curves of C-fiber–evoked field potentials. Summary data from 6 experiments are illustrated. Amplitudes of C-fiber–evoked field potentials elicited by stimulation of sciatic nerve were recorded at 1-min intervals before (●) and 30–50 min after (▲) high-frequency, high-intensity stimulation (100 Hz, 30–40 V). Vertical bars indicate SE.

In five experiments, superfusions of spinal cord with RP 67580 (1 μM) were performed from 30 min before to 30 min after conditioning high-frequency, high-intensity stimulation. Figure 7*A* illustrates that RP 67580 did not affect the baseline responses but did completely block the induction of LTP in all five rats tested.

The effects of NK2-receptor antagonist SR 48968 on the induction of LTP were tested in seven rats. In five out of the seven experiments, LTP was blocked completely by superfusion of spinal cord with SR 48968 (10 nM). Figure 7*B* is a summary of these five experiments. In the remaining two experiments, SR 48968 (10 nM) failed to completely block LTP. The amplitudes of C-fiber–evoked field potentials were potentiated to 150 and 160% of control at 120 min after high-frequency, high-intensity stimulation in these two experiments. This lack of effect in the two experiments probably does not indicate a lower potency of the NK2-receptor antagonist as compared with NK1-receptor antagonist, as a very low concentration of SR 48968 (10 nM) had to be used to avoid confounding effects on baseline responses. This concentration is ~20 times of the inhibition constant ($K_i = 0.51 \pm 0.09$ nM) (Emonds-Alt et al. 1992). However, both antagonists abolished induction of LTP when given intravenously.

To evaluate whether neurokinin receptors play a role for the maintenance of LTP of C-fiber–evoked field potentials, superfusions of spinal cord with RP 67580 (1 μM) or SR 48968 (10 nM) were performed ≥1 h after induction of LTP in three rats but LTP was not affected. Figure 7*C* shows an example.

DISCUSSION

C-fiber–evoked field potentials

The field potentials studied in this work have long latencies, high thresholds, long chronaxy, and a negative focus

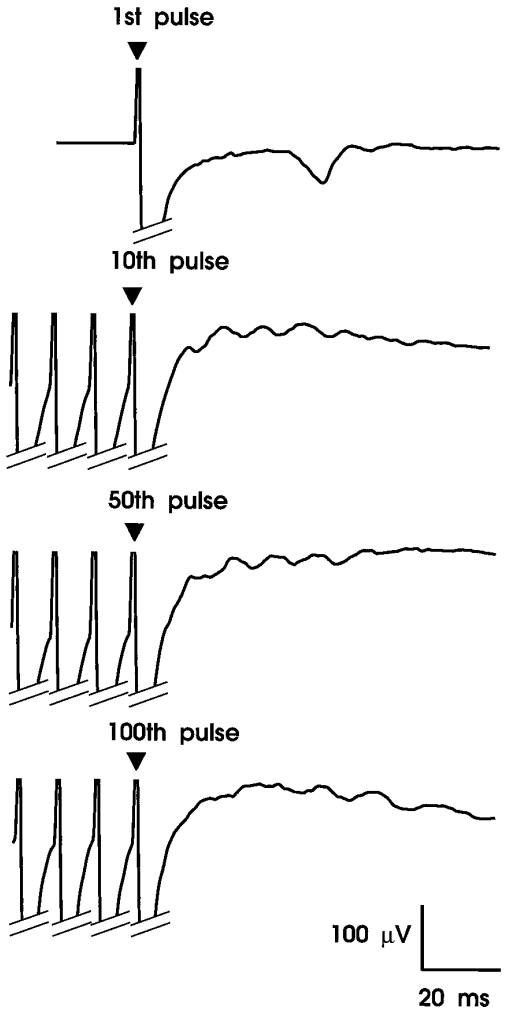

FIG. 4. C-fiber volleys follow 100 Hz electrical nerve stimulation. Distance from stimulating site at the sciatic nerve to recording site at the sural nerve was 30 mm (corresponding conduction velocity was 0.8 m/s), and the threshold of the C-fiber volleys was 9 V (0.5 ms). During 100-Hz stimulation, C-fiber volleys are masked by stimulation artifacts. Thus only the C-fiber volleys evoked by the last 4 stimuli of each train could be analyzed.

in lamina II. The late potentials were not abolished by spinalization and muscle relaxation. This suggests that the field potentials were evoked by activation of afferent C fibers. The present results are in complete agreement with the conclusion by Schouenborg (1984) that the late field potentials are generated primarily by synapses between C-afferent fibers and second-order neurons.

Induction of LTP of C-fiber–evoked field potentials

To induce LTP of C-fiber–evoked field potentials, brief bursts of 100-Hz stimulation of afferent C fibers are more effective than bursts of 20-Hz stimulation or prolonged 2-Hz stimulation. Our results show that the excitability of afferent C fibers at the stimulation site was not changed by high-frequency stimulation (100 Hz), indicating that enhancement of synaptic transmission in spinal cord is responsible for the increase in amplitudes of the field potentials.

Fitzgerald and Woolf (1981) reported that C waves recorded from dorsal root evoked by antidromic stimulation in spinal cord could follow ≤100 Hz, and our data are in line with their conclusion. Duggan and his coworkers (1995) have shown that 100-Hz burst stimulation of afferent C fibers is more effective than single-pulse stimulation at 2 Hz to release SP in spinal dorsal horn. Some C fibers are able to discharge action potentials at 100 Hz in response to natural stimulation of skin (Iggo 1960; Kress et al. 1992), and our recent results show that natural noxious skin stimulation may induce LTP of C-fiber–evoked field potentials (unpublished observation). Sandkühler and Randić (unpublished observation) found that 100-Hz stimulation of dorsal roots supramaximal for the activation of C fibers results in a strong, long-lasting depolarization of substantia gelatinosa neurons in a transverse spinal cord slice-dorsal root preparation of young rat. Present work shows that 100-Hz stimulation at C-fiber strength but not at Aβ- and Aδ-fiber strength can induce LTP. It has been shown that Ca^{2+} level in postsynaptic neurons is crucial for the induction of LTP in hippocampus (Lynch et al. 1983) and that a strong but short-lasting (1–2 s) increase in intracellular Ca^{2+} is sufficient to induce LTP (Malenka et al. 1992). High-frequency (100 Hz) stimulation of C fibers may be most powerful to produce a strong

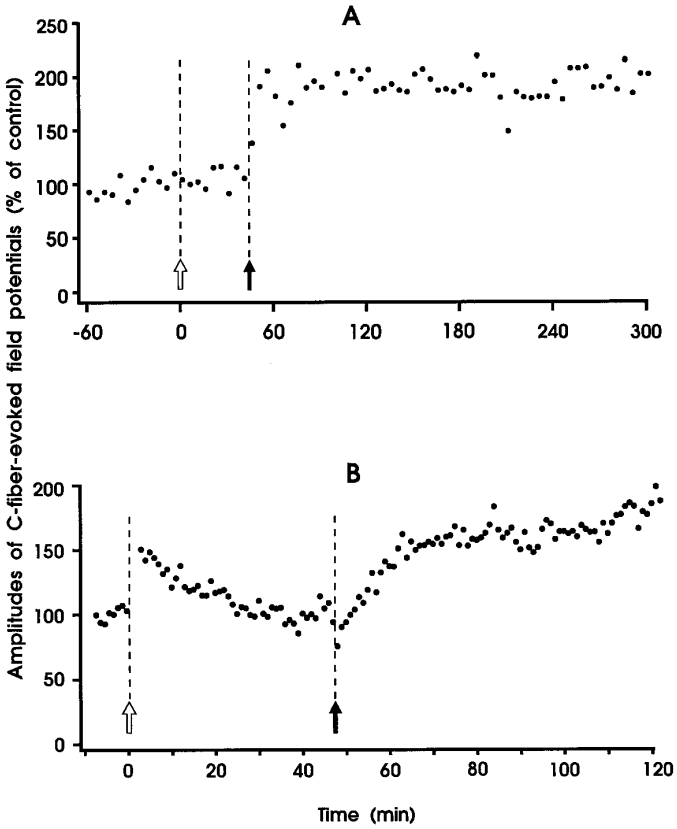

FIG. 5. Induction of LTP of C-fiber–evoked field potentials depends on stimulation parameters. Amplitudes of C-fiber–evoked field potentials in individual experiments are expressed as percent of controls and plotted vs. time. *A*: conditioning high-frequency, low-intensity stimulation (100 Hz, 3 V) at *time 0* (⇒) did not affect amplitudes of C-fiber–evoked field potentials ≤40 min after stimulation. In contrast, high-frequency, high-intensity stimulation (100 Hz, 30 V, →) did induce LTP in the same experiment. *B*: differential efficiency of low-frequency (2 Hz, 400 pulses, ⇒) at *time 0* vs. high-frequency (100 Hz, 400 pulses, →) stimulation at a high intensity (30 V) for induction of LTP in a representative experiment is shown.

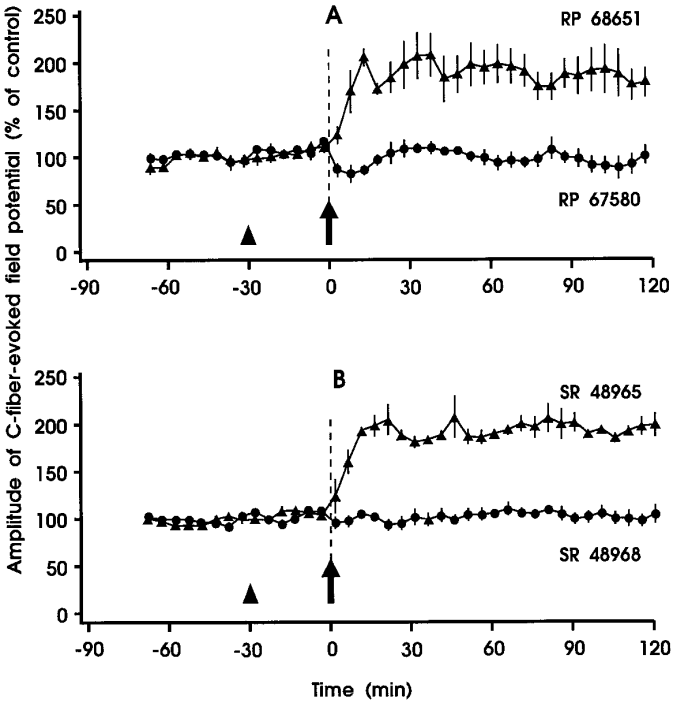

FIG. 6. Intravenous application of NK1- or NK2-receptor antagonists RP 67580 or SR 48968 blocks induction of LTP. Drugs were injected 30 min before tetanic stimulation (↑). → at *time 0* indicate application of the high-frequency, high-intensity conditioning stimulation of sciatic nerve. Each data point represents mean amplitude from 5 consecutive C-fiber–evoked field potentials collected at 1-min intervals. *A*: time course of C-fiber–evoked field potentials before and after intravenous application of RP 67580 (●, $n = 5$) or its inactive enantiomer RP 68651 (▲, $n = 5$). *B*: time course of experiments in which NK2-receptor antagonist SR 48968 (●, $n = 5$) or its inactive enantiomer SR 48965 (▲, $n = 5$) were applied.

depolarization in superficial dorsal horn neurons as compared with 20 or 2 Hz stimulation. This may be required for a strong Ca^{2+} influx into the postsynaptic cell via NMDA-receptor channels and/or voltage-dependent Ca^{2+} channels.

Role of NK1 or NK2 receptors in LTP of C-fiber–evoked field potentials

In the present work, both iv and spinal administration of the highly specific and stereoselective NK1- or NK2-receptor antagonists RP 67580 or SR 48968 (Emonds-Alt et al. 1992; Garret et al. 1991; Holzer-Petsche and Rordorf-Nikolic 1995) prevents the induction of LTP of C-fiber–evoked field potentials. When spinal superfusion with either of the antagonists was performed 1 h after tetanic stimulation, the established LTP was not reversed. We can, of course, not exclude that these receptor antagonists would have had an effect at higher concentration, which, however, also affected baseline responses. Thus activation of NK1 and NK2 receptors is essential for the induction of LTP of C-fiber–evoked field potentials in spinal dorsal horn, but it is probably not necessary for the maintenance of the LTP. This is in line with the work by Ma and Woolf (1995), who demonstrated that NK1 and NK2 receptors are involved in the induction but not the maintenance of mechanical allodynia in rat flexor motoneurons. Iontophoretic application of NK2-receptor antagonist close to the recording site in the spinal cord also may attenuate the development of spinal sensitization when used during the induction of knee-joint inflammation (Neugebauer et al. 1996). In that study, however, the antagonist also reduced responses to noxious and innocuous pressure applied to the inflamed knee joint after development of inflammation.

Strong excitation of nociceptors may lead to a release and extrasynaptic spread of neuropeptides including substance P

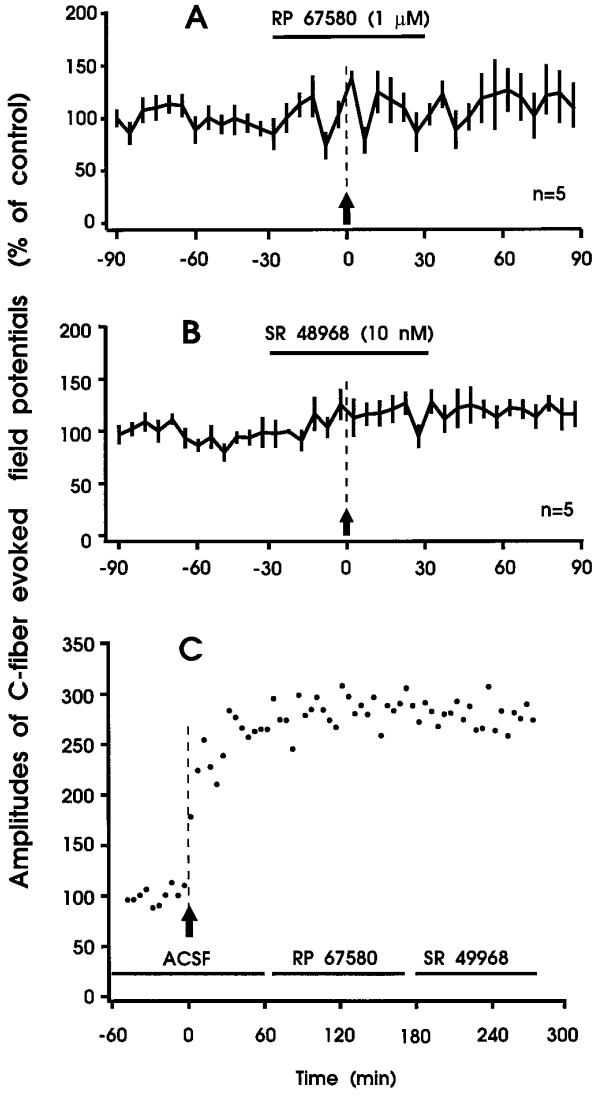

FIG. 7. Superfusion of spinal cord at the recording segments with neurokinin 1 (NK1)- or neurokinin 2 (NK2)-receptor antagonists blocks induction of LTP but does not affect its maintenance. *A* and *B*: summary data shown from experiments with spinal superfusion of RP 67580 or SR 48968. Mean amplitudes of 12 C-fiber–evoked field potentials recorded before superfusion of the spinal cord (time −90 to −30 min) served as controls. Superfusion of the cord dorsum with RP 67580 at 1 μM or SR 48968 at 10 nM started 30 min before high-frequency, high-intensity stimulation and lasted for 60 min (horizontal bars). Tetanic stimulation was given at *time 0* as indicated (→). Responses are expressed as percent of controls (±SE) and were plotted vs. time. *C*: in this representative experiment, spinal superfusions with RP 67580 and SR 48968 were performed once LTP of C-fiber–evoked field potentials was fully established (horizontal bars). Test stimuli (15 V, 0.5 ms) were given to the sciatic nerve at 1-min intervals. Each data point represents the mean amplitude from 5 consecutive C-fiber–evoked field potentials. High-frequency, high-intensity stimulation of the sciatic nerve was applied at *time 0* (→).

(SP) (Duggan et al. 1995) and neurokinin A (NKA) (Duggan et al. 1990). Binding sites for SP (NK1-receptor) are present at high concentrations in rat spinal cord (Yashpal et al. 1990), and binding sites for NKA (NK2-receptor) are also present at low density, mainly in superficial layers of spinal cord at lumbar levels (Yashpal et al. 1990). SP and glutamate are colocated in primary afferent nerve terminals in the superficial laminae of spinal cord (Bottaglia et al. 1987; Merighi et al. 1991) and are coreleased into the extracellular space in response to strong noxious stimulation (Sluka and Westlund 1993). It has been suggested that release of excitatory amino acids, including glutamate, in spinal cord is enhanced by SP (Kangrga and Randić 1990; Skilling et al. 1992) and NKA (Kangrga and Randić 1990). Spinal cord amino acid release and content is reduced by NK1-receptor blockade in an arthritis model (Sluka and Westlund 1993). SP has been found to enhance NMDA-induced currents in freshly isolated neurons of superficial spinal cord of the young rat (Randić et al. 1990) and to increase the responses of spinothalamic tract neurons of monkey to NMDA (Dougherty and Willis 1991). NMDA, NK1, and NK2 receptors also were suggested to be coactivated by nociceptive input and to act together to produce slow synaptic potentials and prolonged excitability changes in spinal dorsal horn (Nagy et al. 1993; Randić et al. 1987). It has been shown that activation of C fibers generates a prolonged synaptic potential and this potential has both a NMDA-receptor (Morris 1987) and a tachykinin-receptor mediated component (Nagy et al. 1993, 1994). Thus the role of neurokinin receptors for the induction of LTP may involve *1*) an increase in release of glutamate from afferent C fibers; *2*) an increase in NMDA-receptor-mediated currents in lamina II neurons, this would facilitate directly NMDA-receptor dependent LTP; *3*) activation of neurokinin receptors, which are colocated with glutamate receptors on many dorsal horn neurons, may be required to achieve a sufficient depolarization to remove the Mg^{2+} block of channels and to allow influx of Ca^{2+} into the cell (Mayer et al. 1994); and *4*) SP acting at NK1-receptors also may increase Ca^{2+} level in cytosol by mobilizing its release from intracellular stores (Womack et al. 1988) and by increasing Ca^{2+} influx through voltage-gated Ca^{2+} channel (Heath et al. 1994; Rusin et al. 1993). Both SP and NKA may potentiate NMDA-dependent increase in intracellular Ca^{2+} level (Rusin et al. 1993). It is well known that an increase in free cytoplasmic Ca^{2+} level is required for the induction of LTP in hippocampal neurons (Lynch et al. 1983; Malenka et al. 1988). If an increase in cytoplasmic Ca^{2+} is also essential for LTP of C-fiber–evoked field potentials, then these effects of neurokinin receptor activation may be necessary for induction of LTP.

Another form of LTP has been described in the isolated peripheral nerve spinal cord preparation of the neonatal rat (Lozier and Kendig 1995). In this model C-fiber–evoked, polysynaptic, slow ventral root, potentials can be potentiated by prolonged (60 s) tetanic stimulation of afferent C fibers at 10 Hz. Interestingly, this form of LTP was not blocked by bath application of NK1-, NK2-, and NMDA-receptor antagonists and is therefore different from the presently described LTP of C-fiber–evoked field potentials in superficial spinal dorsal horn of adult rats in vivo. It is also different from LTP of monosynaptic Aδ-fiber-evoked excitatory postsynaptic potentials (EPSPs) in substantia gelatinosa neurons in dorsal root-spinal cord slices (Randić et al. 1993). Possibly different ages of the animals used (3- to 7-day-old vs. 14- to 40-day-old or adult rats) or different stimulation parameters used for induction of LTP (10 Hz for 60 s vs. 100 Hz 3–4 trains for 1 s) are responsible for these different forms of LTP. Alternatively, potentiation of ventral root reflexes may occur at different synapses as compared with LTP in superficial spinal dorsal horn. It has been shown previously that at some synapses in CA3 LTP is independent of NMDA-receptor activation, and LTP in CA1 has also a NMDA-independent component (Grover and Teyler 1990). Activation of voltage-gated Ca^{2+} channels has been proposed as a trigger for induction of NMDA-independent LTP (Grover and Teyler 1990; Johnston et al. 1992; Lozier and Kendig 1995).

LTP, wind-up, and hyperalgesia

As a possible trigger for plastic changes in spinal nociception, wind-up has been studied intensively since it was first described by Mendell (1966). Both wind-up and LTP of C-fiber–evoked field potentials are prevented by blockade of NMDA receptors (Liu and Sandkühler 1995b; Price et al. 1994a), NK1 receptors (De Koninck and Henry 1991; Dougherty and Willis 1991; Thompson et al. 1994; present work), and NK2 receptors (Thompson et al. 1994, present work). This suggests that the initial step of wind-up and LTP might be similar. The time course of LTP and wind-up are clearly different. LTP of C-fiber–evoked field potentials lasts for $\geq$9 h. Wound-up discharges, however, return to normal discharge levels within several minutes after cessation of low-frequency stimulation (Jeftinija and Urban 1994; Price et al. 1994a; Ren 1994). Our finding that during 1-Hz stimulation of afferent C fibers, the number of action potentials in postsynaptic neurons, but not the amplitudes of the field potentials, undergo wind-up may be explained by the fact that repetitive (0.1–2 Hz) supramaximal stimulation of afferent C fibers induces a cumulative depolarization in ~85% of spinal dorsal horn neurons, but in ~15% of the neurons it produces a cumulative hyperpolarization (Jeftinija and Urban 1994). Presently described field potentials may be not able to detect these lasting changes in membrane potentials, which do not return to resting membrane potentials between the stimuli of the stimulation train (Jeftinija and Urban 1994; Sivilotti et al. 1993). Furthermore, the cumulative depolarization during repetitive stimulation of C fibers may be accompanied by either a decrease (in two-thirds of the neurons) or an increase (in one-third of the neurons) in amplitudes of fast EPSPs (Jeftinija and Urban 1994). Thus these bidirectional changes in EPSP amplitudes of different neurons may not be detected when recording C-fiber–evoked field potentials during 1-Hz stimulation of C fibers. Our data confirm that three to five stimuli of C fibers at 1 Hz are sufficient to induce wind-up, but to induce LTP, more stimuli are needed. Thus although wind-up may trigger some forms of central sensitization (Woolf 1996), it is not sufficient to induce LTP. It is most likely that a rise in intracellular Ca^{2+} produced by cumulative depolarization rather than the increase in number of action potentials of

postsynaptic neurons is responsible for induction of LTP of C-fiber–evoked field potentials. As stated above that high-intensity, repetitive stimulation of primary afferents induces cumulative depolarization in 85% of spinal dorsal horn neurons, but only in 19% of the neurons, does it induce wind-up (Jeftinija and Urban 1994). Clearly, cumulative depolarization, which may lead to a substantial increase in intracellular Ca^{2+} (McMahon et al. 1993), is more common than wind-up.

In behavioral (Gamse and Saria 1986; Ren and Dubner 1993) and in electrophysiological (Dougherty et al. 1994; Xu et al. 1992) experiments, hyperalgesia and long-term changes of spinal nociception can be induced by excitation of primary afferent C fibers. These changes require activation of NMDA (Woolf and Thompson 1991), NK1 (Liu and Sandkühler 1995a; Xu et al. 1992), and NK2 (Henry and Salter 1987; Xu et al. 1991) receptors, which are also relevant for the induction of LTP of C-fiber–evoked field potentials (Liu and Sandkühler 1995b, present work). Thus LTP of C-fiber–evoked field potentials may contribute to the phenomenon of hyperalgesia.

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