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Long-term potentiation of C-fiber-evoked potentials in the rat spinal dorsal horn is prevented by spinal *N*-methyl-D-aspartic acid receptor blockage

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Abstract

Long-term potentiation (LTP) of synaptic potentials is a fundamental mechanism of memory formation in the hippocampus. Here, we have characterized long-term changes of field potentials which were evoked in the lumbar spinal dorsal horn by supramaximai electrical stimulation of the sciatic nerve in urethane anesthetized rats. The field potentials had high thresholds (≥7 V), long latencies (90-130 ms, corresponding to conduction velocities between 1.2 and 0.85 m/s) and were not affected by spinalization (at C5–C6) or muscle relaxation (with pancuronium), i.e. the potentials were probably evoked by afferent C-fibers. Tetanic electrical stimulation (0.5 ms pulses, 30–40 V, 100 Hz, given in 4 trains of 1 s duration at 10 s intervals) of sciatic nerve induced in all 9 rats tested a LTP of amplitude of the C-fiber-evoked potential throughout recording periods which lasted between 4 and 9 h. Mean potentiation ranged from +71% to +174%. Superfusion of spinal cord with *N*-methyl-D-aspartic acid (NMDA) receptor antagonist D-(−)-4-(3-phosphonopropyl)piperazine-2-carboxylic (500 nM), which has little effect on the amplitude of C-fiber-evoked potentials, completely blocked LTP induced by tetanic stimulation in all five rats tested. Superfusion of spinal cord with NMDA (1 μM, 10 μM or 50 μM) induced LTP in only 2 out of 8 rats. This is the first report showing that LTP of C-fiber-evoked field potentials in the spinal dorsal horn in vivo may last for more than 8 h. This LTP in the spinal dorsal horn may underlie plastic changes of spinal nociception.

Keywords: Long-term potentiation; C-fiber-evoked field potential; Spinal dorsal horn; *N*-Methyl-D-aspartic acid; *N*-Methyl-D-aspartic acid antagonist, D-(−)-4-(3-phosphonopropyl)piperazine-2-carboxylic

It is well known that tissue injury may cause prolonged changes in nociception, including hyperalgesia and spontaneous pain, which may involve both sensitization of peripheral nociceptors and increased excitability in the spinal dorsal horn [6,7]. Activity-dependent synaptic plasticity was also found in other parts of the central nervous system. In the hippocampus [2], brief tetanic stimulation induces a long-lasting increase in synaptic efficiency [3,4]. This phenomenon, named long-term potentiation (LTP), has been studied extensively in the past two decades and is considered a fundamental mechanism of learning and memory formation. Considerable evidence suggests that *N*-methyl-D-aspartic acid (NMDA) receptors play an important role in both hyperalgesia and LTP [2,6,8,12]. At present, however, little is known about LTP of C-fiber-evoked potentials in the spinal dorsal horn and its possible role for nociception. In the present work, LTP of C-fiber-evoked field potentials was studied and the role of NMDA receptors was assessed.

Experiments were performed on adult male Sprague–Dawley rats (300-400 g) under urethane anesthesia (1.5 g/kg, i.p.). The carotid artery was cannulated to continuously monitor mean blood pressure which ranged between 80 and 100 mmHg. A catheter was inserted into one external jugular vein for i.v. infusion of a glucose-tyrode solution (0.8 ml/h). Colorectal temperature was kept between 37°C and 38°C by means of a feedback controlled heating blanket. Laminectomy was performed to expose the lumbar enlargement of the spinal cord. Specially synthesized silicone rubber was used to form a small well on the cord dorsum at the recording segments to allow controlled superfusion of the spinal cord with artificial cerebrospinal fluid or drugs [1]. The left sciatic nerve was dissected free for bipolar electrical stimulation. The field potentials were recorded in L4–L5 segments,

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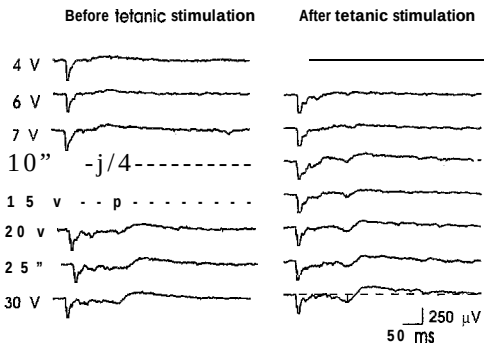

Fig. 1. Original recordings of spinal field potentials evoked by electrical stimulation of the sciatic nerve are shown from one experiment. Stimulation intensities are given on the left-hand side; pulse width was 0.5 ms. Recordings started 40 ms prior to electrical nerve stimulation which is indicated by the steep negative deflection. Amplitude of the A-fiber-evoked N-wave is out of scale and therefore truncated and the late negative deflection is the C-fiber-evoked field potential. Note that the thresholds for C-fiber-evoked potential was decreased from 10 to 6 V after tetanic stimulation and the amplitudes were increased after tetanic stimulation. In the last trace on the right, the amplitude measured from baseline (dotted horizontal) is indicated as revealed by parameter extraction software.

100–500 μm from the dorsal surface of the spinal cord with microelectrodes (impedance 1-3 MΩ). The low pass filter was adjusted to 550 Hz. An A/D converter card (DT281-F-16SE) was used. Data were digitized at a sampling rate of 10 kHz and were stored on a PC/AT computer. The amplitudes of C-fiber-evoked field potentials were determined off-line by parameter extraction, which was implemented by the Datawave system. The amplitude of C-fiber-evoked potentials was determined as the distance from baseline (see Fig. 1 bottom right). In a few experiments, it was necessary to subtract the amplitude of a late A-fiber-evoked potential. The distance from the stimulating site at the sciatic nerve to the recording site in the lumbar spinal dorsal horn was around 11 cm.

Following stimulation of the sciatic nerve with single pulses (0.5 ms, 20 V) field potentials with different latencies were recorded in the lumbar dorsal horn (Fig. 1). Histology verified that recording sites were located at 397 ± 14.3 μm (n = 42) from the dorsal surface of the spinal cord. In this study special attention was paid only to evoked potentials with long latencies (90-130 ms, corresponding to conduction velocities lower than 1.2 m/s). At the very beginning of the recordings, the potentials were not constant and had high thresholds (up to 30 V). After 10–20 test stimuli at intensities sufficient to excite C-fibers (15–20 V, 0.5 ms), stimulation threshold stabilized at a lower level ranging from 7 to 13 V and amplitudes became more constant. These field potentials were not abolished by spinalization performed at the C5–C6 segments as tested in three rats and were not affected by muscle relaxation with pancuronium (0.5 mg/kg) in all five rats tested. In six experiments the amplitudes of C-fiber-evoked field potentials recorded at a depth of 400 μm were normalized to 100%. The mean amplitude at a depth of 300 μm was 101 ± 3.7% and at a depth of 500 μm was 99.6 ± 1.9%. Thus, small changes of recording site do not affect the amplitude of C-fiber-evoked potential.

In nine rats control C-fiber-evoked potentials induced by single pulses (0.5 ms, 10–20 V, applied at 5 min intervals to sciatic nerve) were recorded for 1 h and served as controls. Then, a tetanic stimulus (0.5 ms, 30-40 V, 100 Hz, given in four trains of 1 s duration at 10 s intervals) was delivered to the sciatic nerve followed by single pulses with stimulation parameters identical to the control. Following the tetanic stimulation, a long-term enhancement of the amplitude of C-fiber-evoked potentials was observed in all 9 experiments. The mean potentiation ranged from +71% to +174% of the control. In all experiments, the significant enhancement of the amplitudes of C-fiber-evoked potentials lasted until the end of the recording periods (detected by centered signed rank statistic). In five out of the nine experiments, the recordings lasted for at least 8 h after tetanic stimulation and LTP remained constant in all five cases (Fig. 2). In addition, the thresholds for C-fiber-evoked potentials were also changed after tetanic stimulus. Before tetanic stimulation, the mean threshold was 9.7 ± 0.8 V, 1 h after tetanic stimulation, the mean threshold decreased to 5.2 ± 0.4 V (mean ± SEM, n = 6, $P < 0.05$, Fig. 1 shows an example).

To evaluate the role of NMDA receptors, the recording segments were superfused with the NMDA receptor antagonist, D-(−)-4-(3-phosphonopropyl)piperazine-2-carboxylic (D-CPP, 500 nM) for 30 min before and after the tetanic stimulation. D-CPP caused a small decrease in amplitude of C-fiber-evoked potentials. Maximal mean decrease was by 23.7 ± 13% 20 min after onset of superfusion with D-CPP. LTP was completely blocked by D-

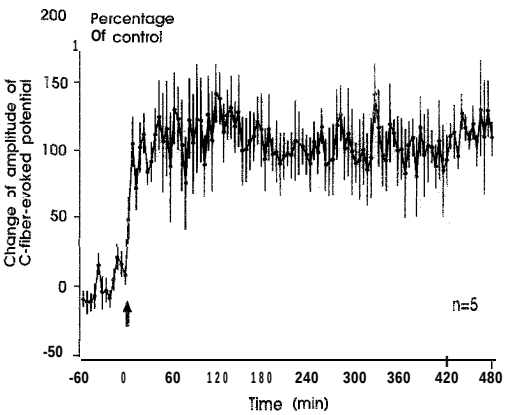

Fig. 2. The mean time course of LTP as revealed by increases in amplitudes of spinal C-fiber-evoked field potentials evoked by test pulses (20 V, 0.5 ms, applied at 5 min intervals) to the sciatic nerve of five rats. The black arrow indicates tetanic stimulation. The mean amplitude of responses to 12 test stimuli (time -60 to -5 min.) served as controls. The mean percent change from controls is plotted versus time. The vertical bars indicate one SEM.

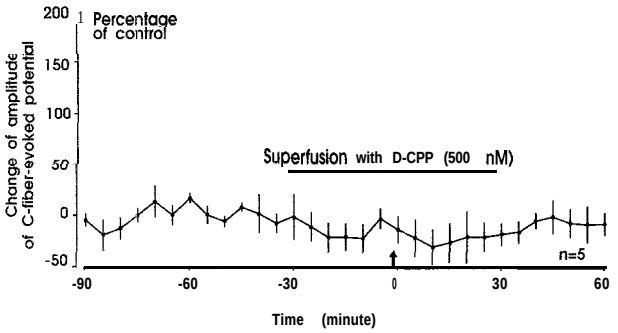

Fig. 3. Effect of the NMDA receptor antagonist D-CPP on the induction of LTP of spinal C-fiber-evoked field potentials. Amplitudes of 12 C-fiber-evoked field potentials were averaged for each of 5 rats (time -90 to -30 min) and served as controls. Superfusion of the cord dorsum with D-CPP (500 nM) started 30 min prior to tetanic stimulation (indicated by the arrow) and lasted for 60 min (black horizontal bar). The mean percent change (±SEM) from controls is plotted versus time.

CPP superfusion (500 nM) in all five rats tested (Fig. 3). In six other rats, the recording segments were superfused with NMDA at increasing concentrations (1 μM, 10 μM and 50 μM) for 30 min each, at 60 min intervals (see Fig. 4). LTP of the amplitude of C-fiber-evoked potentials was not induced in any of these six rats (Fig. 4). In two other rats, 1 μM NMDA induced enhancement of the amplitude of C-fiber-evoked potentials for 2 h. Higher concentrations were not tested in these two rats.

This is the first report showing that tetanic stimulation leads to LTP of C-fiber-evoked field potentials which can last for at least 8 h. Responses to supramaximal C-fiber stimuli were enhanced (data not shown) and the potentiation was prevented by spinal NMDA receptor blockage. This suggests that potentiation of synaptic transmission in the spinal cord but not excitability changes of afferent C-fibers was involved. This conclusion is in line with the results by Randić et al. [10] who demonstrated LTP and long-term depression of synaptic potentials in a slice preparation of the rat spinal dorsal horn. In the same neuron, LTP and long-term depression could be induced by changing the membrane potential. In our experiments the same tetanic stimulation parameters also produced LTP but never long-term depression. This suggests that under the given experimental conditions most neurons in the superficial dorsal horn are sufficiently depolarized to produce LTP.

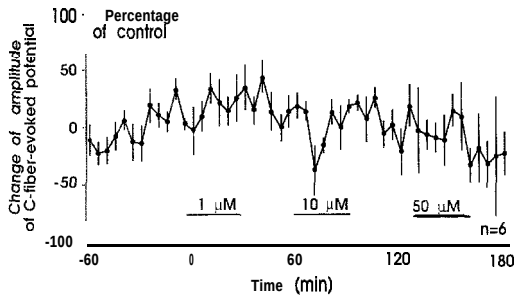

Fig. 4. Summary of effects of superfusion the spinal cord at the recording segments with different concentrations of NMDA (1 μM, 10 μM and 50 μM) on the amplitudes of spinal C-fiber-evoked field potentials. In 6 rats the mean amplitudes of C-fiber-evoked potentials prior to superfusion (time -60 to 0 min) served as controls. Mean percentage changes from controls are plotted versus time. The black horizontal lines indicate the periods of superfusions with NMDA.

The field potentials studied in this work had long latencies (90-130 ms), high thresholds (at least 7 V) and were not abolished by spinalization (at C5–C6) or by muscle relaxation, which means that the field potentials do not involve supraspinal routes and are independent of muscle contraction. Strength-duration curves showed that chronaxie of the field potential was 1.1 ms (Liu and Sandktihler, unpublished observation). This suggests that the field potentials are evoked by stimulation of afferent C-fibers. These results are in agreement with a study by Schouenborg [11] who showed field potentials in the rat lumbosacral dorsal horn which were evoked by stimulation of cutaneous C-fibers (medial sural nerve). In the same work, conditioning stimulation at low frequencies (0.1-20 Hz, 160 pulses) induced short-term potentiation of C-fiber-evoked field potential, which lasted for up to 15 min. Probably different mechanisms are involved in short-term potentiation compared to the LTP described in the present study which lasted for at least 8 h. In the hippocampus there are two types of synaptic potentiation, i.e. short-term potentiation (STP) which decays within 1 h and LTP which is sustained for much longer periods [2]. STP can be induced by low frequency tetanus (0.5 ms, 50 Hz, 1 train) [10] and LTP can be induced by high frequency tetanus, typically a train of 50-100 stimuli given at 100–400 Hz [2]. In the spinal dorsal horn, however, low frequency tetanus (2 Hz, 100 pulses, 0.5 ms, 30-40 V) also induces LTP in three out of five rats which lasts for 3 or 5 h (in three of the five rats tested and in the remaining two experiments, no LTP was induced; Liu and Sandktihler, unpublished observations). Thus, different stimulation parameters alone cannot account for different durations of potentiation (at least 8 h in the present study versus 15 min in the study by Schouenborg [11]). Possibly differences in the anesthetics used (halothane versus urethane) or in the experimental design (test stimuli given at 5 min intervals versus 20 s) may play a role.

LTP in spinal cord and in other brain regions may share similar properties. This work showed that the NMDA-receptor antagonist, D-CPP (500 nM), had little effect on the amplitude of C-fiber-evoked potentials but could completely block the induction of LTP. This result supports the view that NMDA receptors play an important role in neuronal plasticity of the spinal dorsal horn [5, 7–8] and is essential for LTP in the central nervous system [2]. The observation that superfusion with NMDA itself induced no LTP in 6/8 experiments can be explained by the theory that to trigger LTP, the membrane must be sufficiently depolarized to expel Mg^{2+} from NMDA channels at the same time that L-glutamate (or

NMDA in the superfusion experiments) has, by binding to NMDA receptors, promoted their opening [2]. NMDA alone, without sufficient depolarization of the membrane, is not sufficient to induce LTP. Only high frequency stimulation may activate many afferent fibers including Aδ and C-fibers simultaneously and possibly produce co-release of glutamate and substance P, which may lead to a strong depolarization of neurons in superficial laminae. Thus, sufficient depolarization during the tetanic stimulation may also be critical to induce LTP in the spinal dorsal horn.

The plasticity of nociception induced by strong noxious stimulations is mediated by both sensitization of peripheral nociceptors and increased excitability in the spinal cord [6]. But in some experimental models, such as inflammation of peripheral tissues, the two factors (peripheral or central changes) may be difficult to separate. In contrast, LTP of C-fiber-evoked potentials characterized in this study clearly does not involve excitation of peripheral receptors and may be a useful tool to study the mechanisms of plasticity of spinal nociception.

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