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SHORT COMMUNICATION
Induction of long-term potentiation at spinal synapses by noxious stimulation or nerve injury

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Abstract

Use-dependent long-term potentiation of synaptic strength (LTP) is an intensively studied model for learning and memory in vertebrates. Induction of LTP critically depends on the stimulation parameters of presynaptic fibres with synchronous high-frequency bursts being most effective at many central synapses. It is, however, not known whether naturally occurring discharge patterns may induce LTP and whether LTP has any biological function in sensory systems. Here we have investigated the LTP of excitatory synaptic transmission between primary afferent C-fibres, many of which are nociceptors, and neurons in rat superficial spinal dorsal horn. LTP that lasted for 4–6 h could not only be induced by electrical stimulation of sural nerve but also by natural stimulation of heat-, mechano- or chemosensitive nociceptors in the skin or by acute nerve injury. Maintenance of LTP was not affected when afferent nerves were cut 1 h or 5 min after noxious skin stimulation, indicating that an ongoing afferent barrage is not required. Natural noxious stimuli induced LTP in animals which were spinalized but were ineffective in intact animals. Thus, induction of LTP is suppressed by tonically active supraspinal descending systems. We conclude that the natural non-synchronized discharge patterns that are evoked by noxious stimulation may induce LTP and that this new form of LTP may be an underlying mechanism of afferent induced hyperalgesia.

Introduction

The processing of nociceptive information in the spinal dorsal horn may be modified for prolonged periods of time (Woolf, 1984). Strong excitation of nociceptors which typically occurs during peripheral trauma or inflammation, is followed by enhanced behavioural responses to noxious stimuli and by an increased perception of pain (LaMotte *et al.,* 1983). This hyperalgesia is believed to be mediated both by sensitization of nociceptors and by neuroplastic changes in spinal dorsal horn. The central mechanisms contributing to lasting increases in nociception are still not well understood. Recently it has been shown that strength of primary afferent neurotransmission may be potentiated or depressed following conditioning stimulation of dorsal roots in a slice preparation of the young rat spinal cord (Randić *et al.,* 1993; Sandkühler *et al.,* 1997). A robust LTP of C-fibre-evoked field potentials in the superficial spinal dorsal horn can be produced by brief, high-frequency stimulation of C-fibres in afferent nerves (Liu & Sandkühler, 1995, 1997). In spinal cord LTP has also been demonstrated in intermediate gray matter (Pockett, 1995), ventral horn (Pockett & Figurov, 1993) and with slow ventral root potentials (Lozier & Kendig, 1995). Afferent discharge pattern and timing of discharges in convergent nerve fibres is critical for the induction of long-term changes in synaptic strength (Derrick & Martinez, 1994; Linden, 1994; Tsukada *et al.,* 1994; Barr *et al.,* 1995). However, synchronous high-frequency bursts which can be triggered by tetanic electrical nerve stimulation are virtually never induced by natural noxious stimulation. Thus, it is not known whether LTP of excitatory afferent neurotransmission in Aδ- and C-fibres plays any role for spinal nociception. We now demonstrate that afferent discharge patterns which occur during natural noxious stimulation or acute nerve injury may produce a robust long-term potentiation of C-fibre-evoked field potentials in superficial spinal cord.

Material and methods

Experimental procedures are described elsewhere (Liu & Sandkühler, 1997). In brief, experiments were performed on adult male Sprague–Dawley rats of both sexes (body weight 250–350 g) which were anesthetized with a single dose of urethane (Sigma, Deisenhofen, Germany; 1.5 g/kg, intraperitoneally). A laminectomy was performed to expose the lumbar enlargement of the spinal cord. The sural nerve was dissected free in the left hind limb for bipolar electrical stimulation with platinum hook electrodes. A continuous infusion of Tyrode's solution (1-3 mL/h) was given intravenously. Rectal temperature was continuously measured and kept constant at 36–37°C. Mean arterial blood pressure was measured in a carotid artery. In some rats a second laminectomy was performed at cervical levels. Five minutes after an injection of lidocaine (Astra, Wedel, Germany; 2%) into the third cervical seg-

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ment the spinal cord was cut and the animals were mechanically ventilated. In some animals the spinal cord was superfused at the recording segment (see Beck *et al.*, 1995 for details). Experiments were terminated if mean arterial blood pressure fell below 65 mmHg after spinalization.

Supramaximal electrical stimulation of sural nerve (20–30 V, 0.5 ms) elicited C-fibre-evoked field potentials which were recorded with tungsten microelectrodes (A-M Systems, Everett, WA, USA; impedance 4–6 MΩ at 1000 Hz) at a depth of 50–350 μm in the ipsilateral superficial lumbar spinal dorsal horn (late negative deflections in Fig. 1A). These late potentials reflect currents generated at synapses between primary afferent C-fibres and neurons in the substantia gelatinosa (Schouenborg, 1984; Liu & Sandkühler, 1997). Field potentials were elicited at 60-s intervals, band-pass filtered at 0.1–550 Hz and digitized at 10 kHz. At this repetition rate responses remained stable for at least 5 h. Responses to five consecutive test stimuli were averaged off-line and the maximal amplitudes of the averaged C-fibre-evoked field potentials were determined from baseline. Mean amplitudes of 7–10 averaged responses immediately prior to conditioning stimulation served as controls, i.e. control periods lasted for 35–55 min. In each animal only one conditioning stimulus was applied. The non-parametric Wilcoxon rank sum test was used for statistical comparison, $P \leqslant 0.05$ was considered significant.

Results

Results were obtained from 49 rats. In rats with the spinal cord intact supramaximal repetitive electrical stimulation at 100 Hz for 4 s of sural nerve always resulted in LTP of C-fibre-evoked field potentials (Fig. 1A, B; Table 1). The potentiation lasted for up to 4–6 h (data not shown). Intense noxious heating of the area of skin at the ipsilateral hind paw that is innervated by sural nerve resulted in skin inflammation with blister formation but never induced LTP (Fig 1C, Table 1). As compared with electrical nerve stimulation noxious skin heating was found to be a less potent stimulus to induce LTP, probably because less small-diameter afferent nerve fibres were recruited that mostly discharge at frequencies lower than 100 Hz and with less synchrony.

When the spinal cord was transected at spinal segment C3, i.e. rostral to the recording site in the lumbar spinal cord noxious skin heating now always produced LTP (Fig. 2A, Table 1). This indicates that (i) naturally occurring discharge patterns are also capable of inducing a robust LTP and that (ii) induction of LTP can be prevented by tonically active descending pathways. Therefore, all subsequent experiments were carried out in spinalized animals.

To test whether maintenance of LTP requires an ongoing afferent barrage from the inflammed skin region, impulse conduction in sural and tibial nerves was blocked 1 h after induction of inflammation by topical application of the local anaesthetic lidocaine (2%) distal to the site of electrical nerve stimulation. Five minutes later, nerves were transected and expression of LTP was not significantly altered in these animals (Table 1). Thus, maintenance of LTP was unaffected by nerve transection.

When the sural and tibial nerves were exposed to lidocaine and transected 5 min after cessation of noxious skin heating, neither time course nor degree of LTP was affected (Table 1) indicating that full development of inflammation is not necessary for the induction of LTP and that strong excitation of heat-sensitive nociceptors is sufficient. The more gradual onset of LTP following noxious stimulation (Fig. 2A–D) as compared with the rapid onset after tetanic nerve stimulation (Fig. 1B) was similar in rats with the nerves left intact or cut 5 min after noxious skin heating. Thus, continuous discharges in afferent nerve fibres innervating the inflammed tissue are not responsible for the gradual increase in amplitudes of C-fibre-evoked field potentials. The reasons for the difference in time course are presently not known. Possibly, post-tetanic potentiation and short-term potentiation (Emptage & Carew, 1993) were induced by the highly synchronized electrical nerve stimulus but not by the natural discharge patterns encoding noxious heat.

Development of thermal and mechanical hyperalgesia involves different biochemical pathways in spinal neurons (Meller *et al.*, 1994;

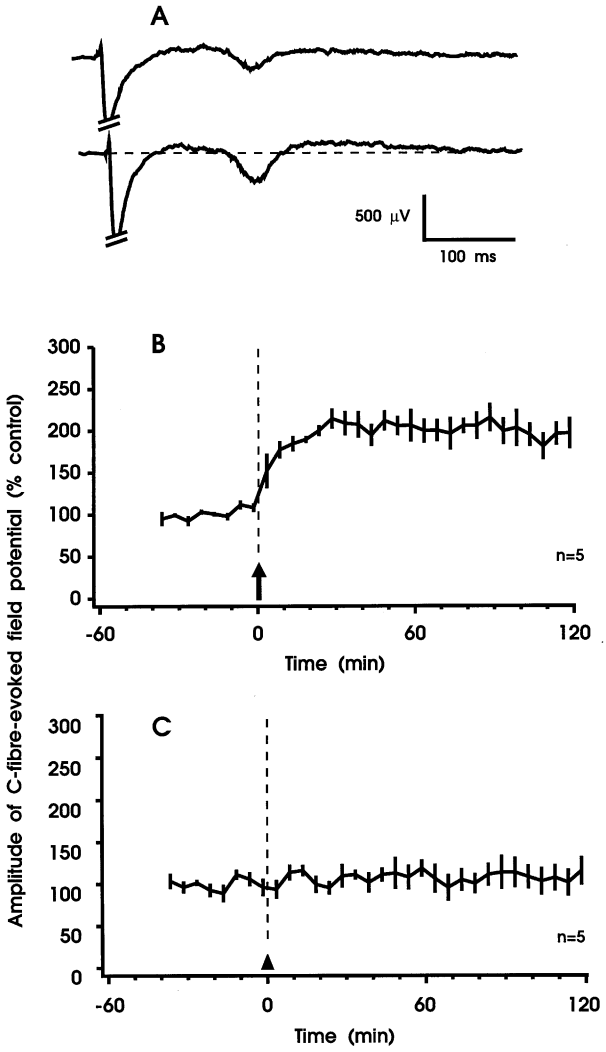

FIG. 1. Long-term potentiation of spinal C-fibre-evoked field potentials by high-frequency stimulation of sural nerve. Field potentials were elicited by supramaximal electrical stimulation of sural nerve. Traces in (A) show original (not averaged) recordings of field potentials before (upper trace) and 60 min after conditioning electrical stimulation of sural nerve with 30-V pulses of 0.5 ms duration given at 100 Hz for 1 s four times at 10-s intervals. The early, truncated negative deflection is evoked by afferent A-fibres (N-wave). The late negative deflection is evoked by primary afferent C-fibres. The dotted line indicates level of baseline which was used to determine maximal amplitude of field potentials, calibration bar: 500 μV and 100 ms. In (B) the mean amplitudes (± SEM) of averaged C-fibre-evoked field potentials in rats with the spinal cord intact are plotted versus time. Conditioning electrical stimulation of sural nerve at time zero (arrow) produced long-term potentiation of C-fibre evoked field potentials in all five rats tested. In contrast, noxious heating of the skin at the ipsilateral hindlimb including the innervation area of sural nerve (70°C four times for 30 s at 30-s intervals) at time zero (arrowhead) failed to change amplitude of C-fibre-evoked field potentials (C).

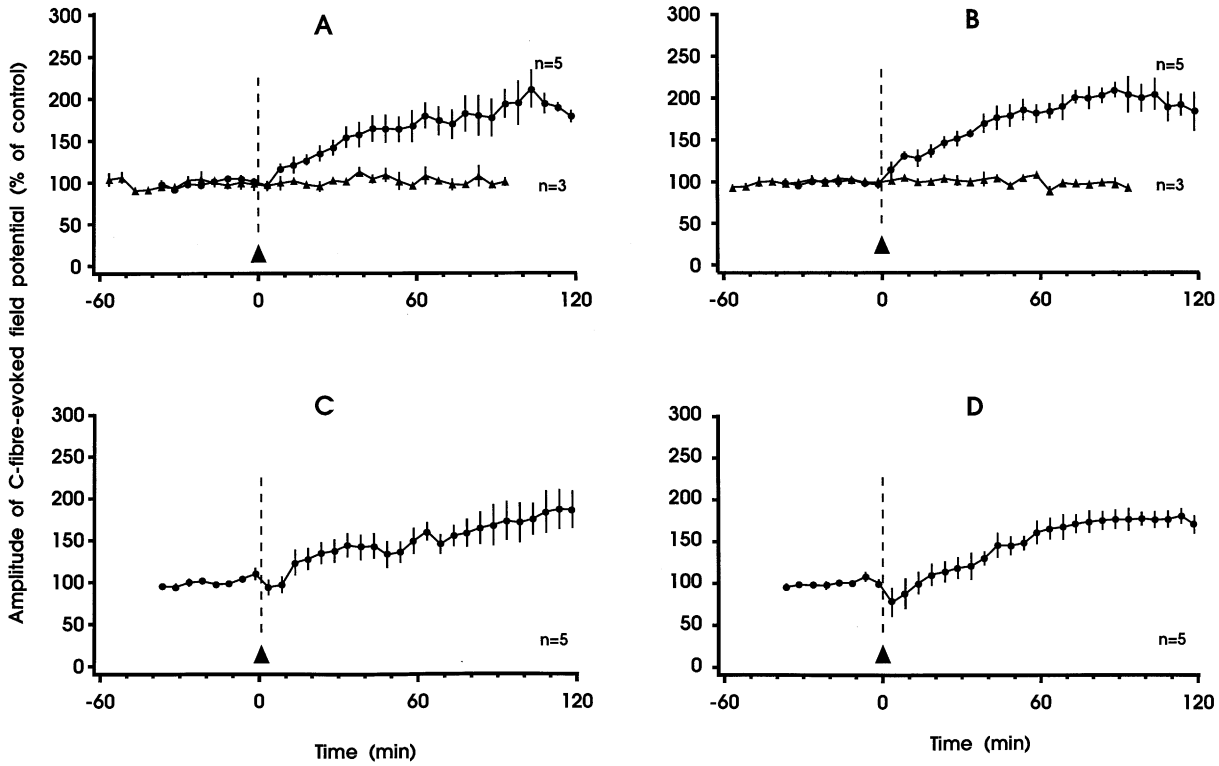

FIG. 2. Excitation of cutaneous nociceptors or acute nerve injury induce LTP in superficial spinal dorsal horn. (A) In spinalized rats conditioning noxious skin heating (70 °C four times for 30 s at 30-s intervals), noxious pinching of skin (10 times with an artery clamp (B), or subcutaneous injections of formalin (50 μL, 5%, (C) were applied at time zero (arrowheads). All noxious stimuli were applied within the innervation area of sural nerve. (D) Acute injury of sural nerve was produced by squeezing the nerve at adjacent sites (from distal to proximal, at least 3 mm distal of electrical stimulation site) with serrated forceps four times (arrowhead). Mean amplitudes (± SEM) of C-fibre-evoked field potentials are plotted versus time (●). Mean responses prior to conditioning stimulation served as controls. Topical application of D-APV at 50 μM to the dorsal surface of the spinal cord at the recording segment for 30 min beginning 15 min before onset of conditioning stimulation always abolished induction of LTP by noxious skin heating (A, arrowhead) or noxious pinching the skin (B, arrowhead) (▲). For each graph the number of experiments is given. One experiment per animal.

TABLE 1. Induction of spinal LTP by noxious stimulation or nerve injury. Summary of the effect of conditioning stimulation on amplitude of spinal C-fibre-evoked field potentials. Latency to potentiation indicates the time in minutes from onset of conditioning stimulation to the first significantly potentiated C-fibre response. The mean amplitude of C-fibre-evoked field potentials at this point of time is given in per cent of control values (± SEM). The expression of LTP was quantified by the mean amplitude of C-fibre response 100 min after onset of conditioning stimulation.

Conditioning stimulation	Spinal cord	Superfusate	Latency to potentiation		Expression of LTP		
			Time (min)	Amplitude (% control)	Time (min)	Amplitude (% control)	*n*
HFS (100 Hz)	intact	ACSF	10	177 ± 10**	100	202 ± 22**	5
Noxious heat	intact	ACSF	–	no LTP	100	108 ± 15	5
Noxious heat	spinalized	ACSF	20	126 ± 6*	100	194 ± 26**	5
Noxious heat†	spinalized	ACSF	20	156 ± 16*	100	208 ± 28*	5
Noxious heat‡	spinalized	ACSF	20	133 ± 9*	100	170 ± 16*	3
Skin squeezing	spinalized	ACSF	10	131 ± 5*	100	199 ± 17**	5
Nerve injury	spinalized	ACSF	40	129 ± 8*	100	176 ± 8**	5
S.c. formalin	spinalized	ACSF	65	161 ± 11*	100	171 ± 24*	5
Skin squeezing	spinalized	D-APV	–	no LTP	60	98 ± 2	3
Noxious heat	spinalized	D-APV	–	no LTP	60	120 ± 1	3

†Sural nerve was cut 5 min after noxious skin heating; ‡Sural nerve was cut 60 min after noxious skin heating. *Significantly greater than control $P \leqslant 0.05$; **$P \leqslant 0.02$ (Wilcoxon rank sums test).
HFS, high-frequency stimulation; s.c., subcutaneous.

Meller & Gebhart, 1994). We have therefore tested whether excitation of cutaneous high-threshold mechanoreceptors can also produce LTP. Figure 2B illustrates that excitation of high-threshold mechanoreceptors by squeezing the glabrous skin and excitation of chemosensitive nociceptors by subcutaneous formalin injection (Fig. 2C) produce robust LTP (Table 1). Thus, the ability to induce LTP may be a general characteristic of different types of nociceptors or may be due to excitation of polymodal nociceptors which respond to thermal, mechanical and to chemical stimuli.

To test whether acute nerve injury also induces LTP, the sural

nerve was squeezed four times with serrated forceps distal to the site of electrical stimulation in rats with the sural nerve intact. This nerve injury always induced LTP (Fig. 2D; Table 1).

N-Methyl-D-aspartate (NMDA)-sensitive and NMDA-insensitive forms of LTP have been described at central synapses (Nicoll & Malenka, 1995). Here, blockade of spinal NMDA receptors by topical, spinal application of 2-amino-5-phosphonopentanoic acid (D-AP5) at 50 μM always abolished induction of LTP by noxious heating or pinching the skin (Fig. 2A, B; Table 1).

Discussion

The LTP of synaptic transmission in hippocampus is an intensively studied model for learning and memory (Bliss & Lømo, 1973; Bliss & Collingridge, 1993; Malenka, 1994; Carew, 1996). It has been shown that LTP may be induced during learning (Mitsuno *et al.*, 1994), and by fear conditioning in the amygdala (McKernan & Shinnick-Gallagher, 1997; Rogan *et al*., 1997). Occlusion between learning and LTP exists (Barnes *et al.*, 1994). Pharmacological manipulations (Richter-Levin *et al.*, 1994; Riedel *et al.*, 1994) (see however Saucier & Cain, 1995) or mutations of single genes (Grant *et al.*, 1992; Silva *et al.*, 1992; Aiba *et al.*, 1994) may affect learning and LTP in a similar fashion. Sensory responses may be increased by LTP-inducing tetanic stimulations (Rogan & Le Doux, 1995). Nevertheless any biological function of LTP is still seeking final proof (Barnes, 1995) partly because it has not been shown that pattern and timing of a naturally occurring afferent barrage can induce robust LTP. Further, at some thalamocortical synapses LTP may only be induced during a critical period of early development (Crair & Malenka, 1995). Here, we have shown that in adult rats selective excitation of cutaneous nociceptors or nerve injury may result in LTP of excitatory afferent neurotransmission in C-fibres. It has been suggested that a strong postsynaptic depolarization is necessary to trigger NMDA sensitive forms of LTP (Lisman, 1989; Calabresi *et al.*, 1992; Bear and Malenka, 1994). This may be achieved by synchronized, high-frequency discharges in excitatory afferent nerve fibres. During the presently employed natural excitation of nociceptors, any synchronization of discharges can be excluded as primary afferent nerve fibres lack mechanisms for synchronization. When holding postsynaptic neurons at a relatively depolarized membrane potential afferent stimulation which previously failed to induce LTP may become effective (Artola *et al.*, 1990; Randić *et al.*, 1993). Here, a postsynaptic depolarization sufficiently strong for the induction of NMDA-sensitive form of LTP may be achieved during naturally occurring, irregular and non-synchronized afferent barrage only if animals are spinalized, as this removes any tonic descending inhibitory input from spinal dorsal horn neurons. During bath application of NMDA receptor antagonist D-AP5 basic cellular properties of dorsal horn neurons in a spinal cord–dorsal root slice preparation of young rat remained unchanged. Amplitudes of primary afferent-evoked excitatory postsynaptic potentials were only slightly reduced but decay time constants of these potentials were shortened (Sandkühler *et al.*, 1997).

Long-lasting changes in spinal nociception which can be triggered by impulses in afferent C-fibres and LTP of C-fibre-evoked field potentials both require activation of spinal NMDA receptors (Ma & Woolf, 1995a; Liu & Sandkühler, 1995), and activation of receptors for substance P (neurokinin 1 receptors) and neurokinin A (neurokinin 2 receptors) (Ma & Woolf, 1995b; Neugebauer *et al.*, 1996; Liu & Sandkühler, 1997). This further supports a role of LTP for some forms of central hyperalgesia (Sandkühler, 1996).

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Abbreviations

D-AP5	2-amino-5-phosphopentanoic acid
LTP	long-term potentiation
NMDA	*N*-methyl-D-aspartate

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