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ACTIVATION OF SPINAL *N*-METHYL-D-ASPARTATE OR NEUROKININ RECEPTORS INDUCES LONG-TERM POTENTIATION OF SPINAL C-FIBRE-EVOKED POTENTIALS

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Abstract—The use-dependent increase in synaptic strength between primary afferent C-fibres and second-order neurons in superficial spinal dorsal horn may be an important cellular mechanism underlying central hyperalgesia. This long-term potentiation can be blocked by antagonists of the *N*-methyl-D-aspartate subtype of glutamate receptor, the neurokinin 1 or the neurokinin 2 receptor. We have tested here whether activation of these receptors by superfusion of the spinal cord with corresponding agonists in the absence of presynaptic activity is sufficient to induce long-term potentiation. In urethane anaesthetized rats C-fibre-evoked field potentials were elicited in superficial laminae of lumbar spinal cord by electrical stimulation of the sciatic nerve. In rats with intact spinal cord, controlled superfusion of the spinal cord at recording segments for 60 min with *N*-methyl-D-aspartate, substance P or neurokinin A never induced long-term potentiation. Spinal superfusion with a mixture of *N*-methyl-D-aspartate, substance P and neurokinin A also failed to induce long-term potentiation in four rats tested. In spinalized rats, however, long-term potentiation was induced by either *N*-methyl-D-aspartate (at 10 μM, to 173 ± 16% of control) substance P (at 10 μM, to 176 ± 13% of control) or by neurokinin A (at 1 μM, to 198 ± .20% of control). The induction of long-term potentiation by *N*-methyl-D-aspartate, substance P or neurokinin A was blocked by intravenous application of the receptor antagonists dizocilpine maleate (0.5 mg/kg), RP67580 (2 mg/kg) or SR48968 (0.2 mg/kg), respectively.

Thus, activation of *N*-methyl-D-aspartate or neurokinin receptors may induce long-lasting plastic changes in synaptic transmission in afferent C-fibres and this effect may be prevented by tonic descending inhibition. © 1998 IBRO. Published by Elsevier Science Ltd.

Key words: pain, tachykinins, hyperalgesia, synaptic plasticity, descending inhibition, rat.

Persistent stimulation of nociceptors in peripheral tissues leads to hyperalgesia[8,16] and release of glutamate[66,68] and neuropeptides including substance P (SP)[17] and neurokinin A (NKA)[18] in superficial spinal dorsal horn. Binding sites for *N*-methyl-D-aspartate (NMDA)[22] and SP[76] (NK1 receptor) are present at high concentrations in rat spinal cord and binding sites for NKA (NK2 receptor) are also present at low density, mainly in superficial layers of lumbar spinal cord.[76] Several independent lines of evidence suggest that intrathecal injections of NMDA,[19,46,49] SP[20,47] or NKA[23] may induce hyperalgesia and spinal application of NMDA,[51,53,59] NK1[13,24,55] or NK2[75] receptor antagonists may prevent or depress hyperalgesia.

It is well established that hyperalgesia involves both sensitization of nociceptors and sensitization of neurons in spinal dorsal horn.[8,16,56,61,72] Central sensitization of spinal dorsal horn neurons by nociceptive afferent input requires the activation of NMDA receptors,[25,56,64,73] by glutamate and NK1[15,39,69,74] and NK2 receptors[75] by released europeptides. However, the cellular mechanisms of the central plastic change are still unclear. Recently we have characterized long-term potentiation (LTP) of C-fibre-evoked field potentials in superficial spinal dorsal horn induced by repetitive electrical stimulation of afferent C-fibres,[35,37] by natural noxious stimulation or by acute nerve injury.[36,62a] Very similar to hyperalgesia, this LTP can be blocked by spinal application of NMDA receptor antagonist[35] and by intravenous or spinal administration of NK1 or NK2 receptor antagonists.[37]

For induction of LTP sufficient depolarization of postsynaptic neurons is crucial[1,57] and in hippocampus a rise in postsynaptic Ca^{2+} alone is sufficient to induce LTP[42] (see Discussion). Activation of spinal NMDA, NK1 or NK2 receptors may induce prolonged postsynaptic depolarization[52] and increase Ca^{2+} in postsynaptic neurons[26,40,60,71] To further assess the role of NMDA, NK1 or NK2 receptors

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Abbreviations: DMSO, dimethylsulphoxide; LTP, long-term potentiation; MK-801, dizocilpine maleate; NK1, neurokinin 1; NK2, neurokinin 2; NKA, neurokinin A; NMDA, *N*-methyl-D-aspartate; SP, substance P.

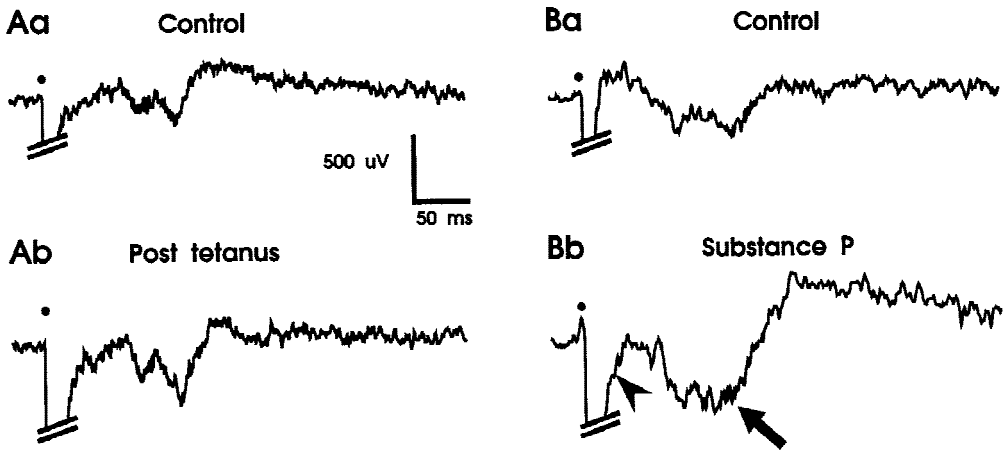

Fig. 1. Induction of LTP of C-fibre-evoked field potentials in superficial spinal dorsal horn. Traces are original recordings of field potentials in response to supramaximal stimulation of sciatic nerve (dots). Early stimulation artefacts and A-fibre-evoked potentials are truncated. Late C-fibre-evoked potentials (arrow in Bb; negativity down) were potentiated 120 min after conditioning tetanic stimulation of sciatic nerve (A) or following superfusion of spinal cord at the recording segment with SP (10 μM for 60 min, B). Putative Aδ-fibre-evoked field potentials (arrowhead in Bb) appeared to be a potentiated as well but overlapped with Aβ-fibre-evoked potentials and stimulation artefact and were therefore not analysed.

in plasticity of spinal C-fibre-mediated synaptic transmission, we tested whether spinal application of NMDA, SP or NKA is sufficient to induce LTP of C-fibre-evoked field potentials.

EXPERIMENTAL PROCEDURES

Preparation of animals

Experiments were performed on 48 adult male Sprague–Dawley rats (250–350 g body weight; Zentralinstitut Für Versuchstierzucht, Hannover, Germany). Urethane (1.5 g/kg, i.p.) was used to induce and maintain anaesthesia. 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, if necessary. A catheter was inserted into an external jugular vein for continuous i.v. infusion of Tyrode/glucose solution (0.8–1 ml/h) and for application of drugs. One carotid artery was cannulated to continuously monitor the mean arterial blood pressure which ranged from 80 to 100 mmHg. Colorectal temperature was kept between 37–38°C by means of a feedback controlled heating blanket. Two laminectomies were performed to expose the lumbar enlargement and cervical spinal cord. In 26 rats the spinal cord was surgically transected at the third cervical segment, 2–3 min after lidocaine injection (2%, 0.1 ml) at the same site. The left sciatic nerve was dissected free for bipolar electrical stimulation with platinum hook-electrodes. In five rats, the sural nerve was exposed to record C-fibre volleys. All exposed nerve tissues were covered with warm paraffin oil, except for those spinal segments to be superfused (see below).

Administration of drugs

NMDA, SP or NKA (all from Sigma) were applied by controlled superfusion of the spinal cord at the recording segments. Receptor antagonists dizocilpine maleate (MK-801; Research Biochemicals International, U.S.A.), RP67580 (Rhône-Poulenc Rorer, France) or SR48968 (Sanofi Recherche, France) were administered intravenously but not spinally to avoid unknown effects due to different diffusion kinetics of agonist and antagonists. For controlled superfusion of the spinal cord, a specially synthesized silicone rubber was used to form a small well on the cord dorsum at the recording segments. A single dose of agonist was applied to the cord for 60 min. This allowed secure penetration into the spinal dorsal horn as we have shown previously.[4] Stock solutions of NMDA (10 mM), SP (1 mM) or NKA (1 mM) were prepared in distilled water and were diluted with artifical cerebrospinal fluid immediately before application. Stock solution of non-peptide NK1 receptor antagonist RP67580 was prepared at 3 mg/ml (in dimethylsulphoxide [DMSO], Sigma) and was diluted in 0.3 ml Tyrode's solution. Final DMSO concentration in the diluted working solution was 0.04%. Non-peptide NK2 receptor antagonist SR48968 was dissolved at 0.6 mg/ml in an aqueous solution of 0.01% Tween 80 (Sigma) and the stock solution was diluted as needed in 0.3 ml Tyrode.

Stimulations and recordings

Following electrical stimulation of the sciatic nerve, field potentials with negative focus were recorded at a depth of 50–350 μm from the dorsal surface of spinal cord with tungsten microelectrodes (impedance 1–3MΩ), which were driven by an electronically controlled microstepping motor. A band-width of 0.1–550 Hz was used for recording field potentials (Fig. 1). 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. Single pulses of 0.5 ms at an intensity of 10–20 V were applied to the sciatic nerve and were used as test stimuli. In some experiments high-frequency electrical stimulation (100 Hz, 30–40 V, 0.5 ms, 400 pulses given in four trains of 1 s duration at 10 s intervals) was used to induce LTP of C-fibre-evoked field potentials (Fig. 1A). Recording sites were marked at the end of 40 experiments by electrolytic lesions (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 coronal sections which were stained with Cresyl Violet. All recording sites were histologically verified to be in lamina I or II of the spinal cord. The distance from the stimulation site at the sciatic nerve to the recording site in lumbar spinal dorsal horn was around 11 cm.

Data analysis and statistics

The amplitudes of C-fibre-evoked field potentials were determined off-line by parameter-extraction, which was implemented by Experiment's WorkBench (Version 4.0, DataWave, Colorado, U.S.A.). The amplitude of C-fibre-evoked potential was measured as the maximal distance from the baseline. In each experiments five consecutive C-fibre-evoked field potentials were averaged. Thus, amplitudes reported in this work represent the mean of five responses. ANOVA (the Kruskal–Wallis test) was used for statistical analysis and $P<0.05$ was considered significant. Mean values are given with one S.E.M.

RESULTS

Spinal application of N-*methyl*-D-*aspartate, substance P or neurokinin A induces long-term potentiation in spinalized rats*

In five rats with intact spinal cord stable C-fibre-evoked field potentials were recorded for at least 40 min and served as controls, followed by spinal superfusions with NMDA at increasing concentrations of 1 μM, 10 μM and 100 μM for 1 h each. In all rats tested NMDA failed to affect amplitudes of C-fibre-evoked field potentials (see Fig. 2A and Table 1). However, in rats which were spinalized at C3 segments spinal superfusions with NMDA at a concentration of 10 μM were sufficient to induce LTP (Fig. 2B). Thirty minutes after the onset of spinal superfusion, mean amplitudes of C-fibre-evoked field potentials increased to 127 ± 9% of control ($P<0.05$) and at 1 h mean response elevated to 173 ± 16% of control ($P<0.05$). After removal of NMDA from the spinal cord surface, LTP persisted throughout the rest of recording period (up to 6 h). The LTP induction by spinal NMDA (10 μM) was prevented when NMDA receptor antagonist MK-801 (0.5 mg/kg) was injected intravenously 10 min prior to onset of superfusion (Fig. 2C, Table 1).

Using the same experimental procedure, the roles of SP and NKA for the induction of LTP of C-fibre-evoked field potentials were evaluated. Spinal superfusions with SP at 1 μM, 10 μM or 100 μM never induced LTP as tested in five rats with intact spinal cord (Fig. 3A, Table 1). In contrast, in all five spinalized rats spinal applications of SP at a concentration of 10 μM induced LTP (Figs 1B, 3B, Table 1). Ten minutes after the onset of superfusions, mean amplitude of C-fibre-evoked field potentials significantly increased to 137 ± 15% of control ($P<0.05$) and at 1 h mean response was elevated to 176 ± 13% of control ($P<0.05$). In four spinalized rats NK1 receptor antagonist RP67580 (2 mg/kg, i.v.) was administrated 10 min prior to spinal application of SP (10 μM). This receptor blockade prevented LTP induction by SP (see Fig. 3C and Table 1).

Spinal application of NKA at 1 μM or 10 μM did not affect amplitudes of C-fibre-evoked field potentials in rats with intact spinal cord as tested in three experiments (Fig. 4A, Table 1). In five spinalized rats tested, spinal superfusions with NKA at 1 μM were sufficient to induce LTP. Thirty minutes after the onset of superfusions mean amplitude of C-fibre-evoked field potentials increased to 133 ± 5% of control ($P<0.05$) and at 1 h to 187 ± 19% of control ($P<0.05$, Fig. 4B). The LTP induction by NKA was blocked by pretreatment with NK2 receptor antagonist SR48968 (0.2 mg/kg, i.v. injected 10 min before NKA application) in all four spinalized rats tested. (Fig. 4C, Table 1).

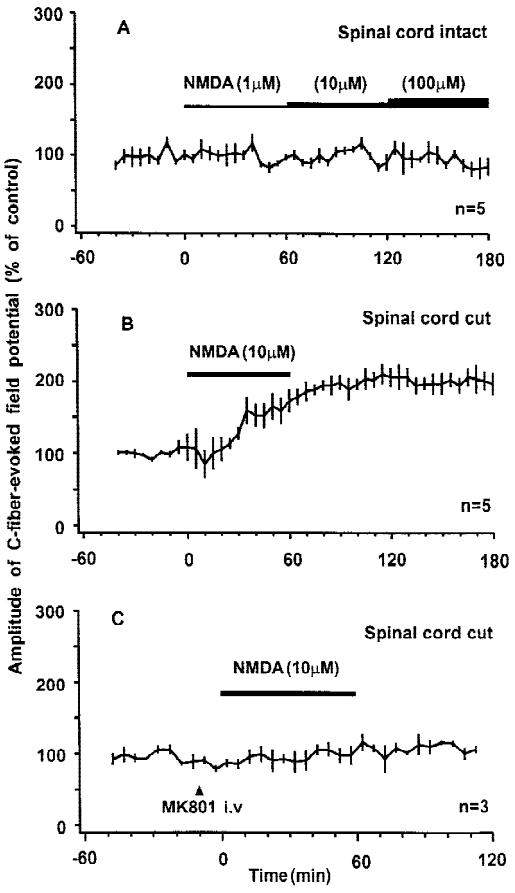

Fig. 2. Spinal superfusions with NMDA induce LTP of C-fibre-evoked field potentials in spinal rats. Mean time-courses of changes in amplitudes of C-fibre-evoked field potentials produced by spinal application of NMDA are shown. Responses prior to NMDA superfusion served as controls. Mean amplitudes (±S.E.M.) are expressed as percentage of controls and were plotted versus time. (A) Experiments were performed on rats with intact spinal cord. Spinal superfusions with NMDA at different concentrations for 60 min each did not affect the amplitudes of C-fibre-evoked field potentials. Horizontal bars with increasing width indicate periods of superfusion at different concentrations. (B) Data were obtained from rats which were spinalized at C3 segments. Spinal superfusions with NMDA at 10 μM (horizontal bar) were sufficient to induce LTP in all rats tested. After removal of NMDA from the spinal cord surface, potentiation persisted throughout the rest of the recording period. (C) In three spinal rats intravenous (i.v.) application of NMDA receptor antagonist MK-801 (0.5 mg/kg, arrowhead) 10 min before spinal superfusion with NMDA (at 10 μM for 60 min, horizontal bar) prevented induction of LTP of C-fibre-evoked field potentials.

Table 1. The effects of spinal superfusions with *N*-methyl-D-aspartate, substance P or neurokinin A on the amplitudes of C-fibre-evoked field potentials in rats with intact spinal cord or in spinalized rats

	1 μM	10 μM	100 μM	Spinal cord	*n*
NMDA	97 ± 4%	90 ± 11%	98 ± 12%	intact	5
SP	98 ± 9%	103 ± 5%	122 ± 10%	intact	5
NKA	102 ± 6%	103 ± 4%	–	intact	3
NMDA	–	173 ± 16%*	–	spinalized	5
SP	–	176 ± 13%*	–	spinalized	5
NKA	187 ± 19%*	–	–	spinalized	5
MK801+NMDA	–	103 ± 10%	–	spinalized	3
RP67580+SP	–	113 ± 9%	–	spinalized	4
SR48968+NKA	115 ± 8%	–	–	spinalized	4

Data were obtained at 60 min after the onset of spinal superfusions and are reported as mean ± S.E.M. All receptor antagonists (MK-801, RP67580 or SR48968) were injected intravenously 10 min before the onset of spinal superfusions with corresponding receptor agonists.
*$P < 0.05$.

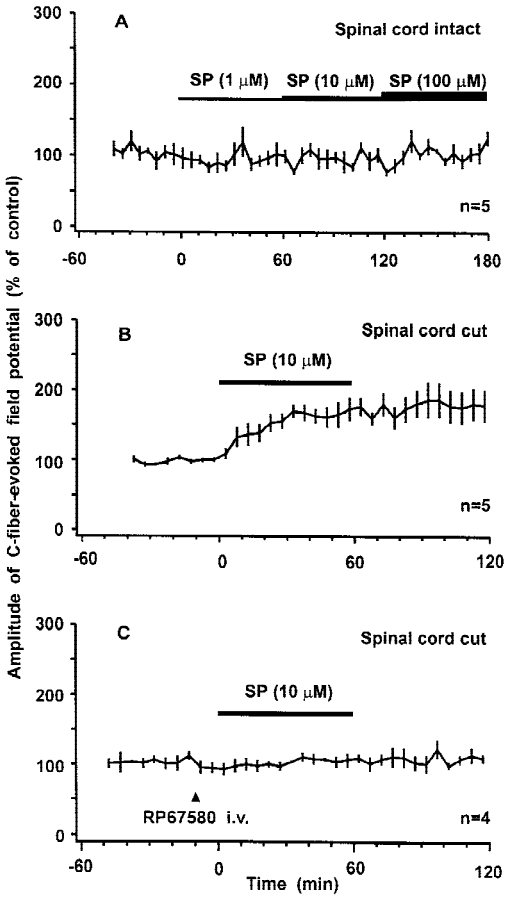

Fig. 3. Spinal superfusions with SP induce LTP in spinalized but not in intact rats. Mean time-courses of changes in amplitudes of C-fibre-evoked field potentials produced by spinal application of SP are shown. (A) In rats with intact spinal cord, spinal superfusions with SP at 1, 10 or 100 μM (indicated by horizontal bars with increasing width) never induced LTP of C-fibre-evoked field potentials. (B) In spinalized rats superfusions with SP at 10 μM (horizontal bar) was sufficient to induce LTP. (C) In four spinal rats intravenous (i.v.) application of NK1 receptor antagonist RP67580 (2 mg/kg, arrowhead) 10 min before spinal application of SP (at 10 μM for 60 min, horizontal bar) prevented induction of LTP of C-fibre-evoked field potentials.

Spinal application of a mixture of N-*methyl*-D-*aspartate, substance P and neurokinin A fail to induce long-term potentiation in rats with spinal cord intact*

We have tested the possibility that in rats with intact spinal cord induction of LTP of spinal C-fibre-evoked field potentials may require co-activation of NMDA and neurokinin receptors. In four experiments spinal superfusions with a mixture of NMDA (10 μM), SP (100 μM) and NKA (10 μM) were performed for 1 h. This also failed to induce LTP. 1 h after the onset of spinal applications mean amplitude of C-fibre-evoked field potentials was not significantly different from control (96 ± 8% of control, $P > 0.05$, data not shown).

DISCUSSION

The characteristics of the field potentials in rat spinal dorsal horn have been described in detail by Schouenborg.[65] He concluded that these field potentials may be generated primarily by synapses between primary afferent C-fibres and second-order neurons. Our recent studies[35,37] further support this conclusion. In this work the amplitudes of C-fibre-evoked field potentials recorded in lamina I or II were used as a quantitative measure of synaptic strength in primary afferent C-fibres.

Our previous work[4] has shown that following superfusion of rat dorsal spinal cord for 30 min with a single dose of [^{125}I]NKA, significant radioactivity is detected up to a depth of 1.5 mm. The highest peptide concentration was observed in laminae I and II. This distribution may be similar to the extrasynaptic spread of neuropeptides induced by noxious stimulation.[17,18] Thus, controlled superfusion of the spinal cord appears to be a suitable means to study the effects of extrasynaptic neuropeptides on the intact spinal neuronal network.

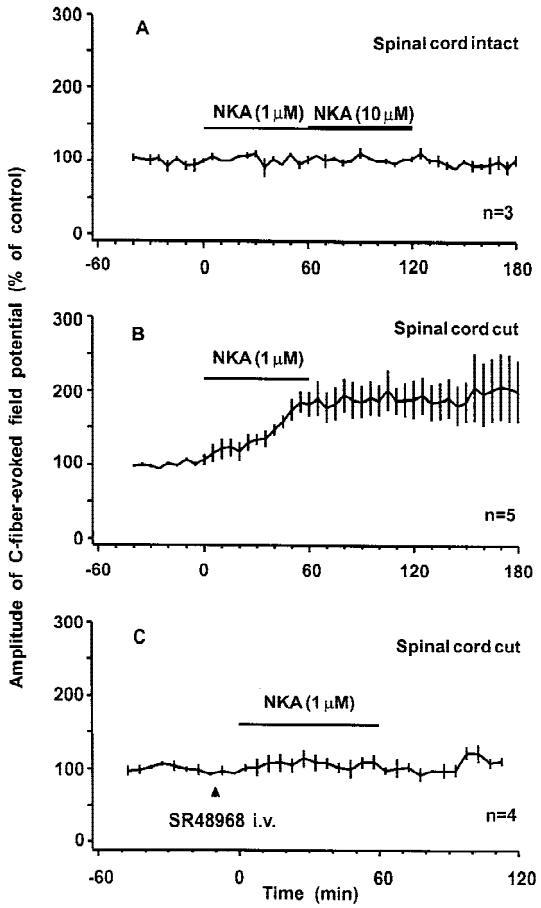

Fig. 4. Spinal superfusions with NKA induce LTP in spinalized but not intact rats. Mean time-courses of changes in amplitudes of C-fibre-evoked field potentials produced by spinal application of NKA are shown. (A) Spinal superfusions with NKA at 1 μM or 10 μM for 60 min each (horizontal bars) failed to induce LTP in rats with intact spinal cord. (B) In spinal rats superfusions at 1 μM (horizontal bar) always induced LTP. (C) In four spinal rats intravenous (i.v.) application of NK2 receptor antagonist SR48968 (0.2 mg/kg, arrowhead) 10 min before spinal superfusion with NKA (at 1 μM for 60 min, horizontal bar) blocked induction of LTP of C-fibre-evoked field potentials.

The role of N-*methyl*-D-*aspartate, neurokinin* 1 *or neurokinin* 2 *receptors for induction of long-term potentiation of C-fibre-evoked field potentials*

It has been suggested that the induction of LTP may require both the activity in presynaptic nerve terminals and depolarization of the postsynaptic neurons.[5] The activation of NMDA receptors on postsynaptic neurons by synaptically released glutamate is essential for the induction of NMDA-dependent LTP.[10,11] The postsynaptic depolarization relieves a voltage-dependent Mg^{2+} block of NMDA channel, resulting in increased calcium influx.[45,48,54] A rise in intracellular Ca^{2+} in postsynaptic neurons is crucial for the induction of LTP.[5,38] It has been recently demonstrated that the increase in intracellular Ca^{2+} in postsynaptic neurons needs to last only 1–2 s in order to induce LTP[43] and that increase in the intracellular Ca^{2+} alone is sufficent for induction of LTP.[42] Our results are in agreement with this conclusion, as spinal application of receptor agonists NMDA, SP or NKA, which increase the intracellular Ca^{2+} level in postsynaptic neurons (see below), in the absence of any presynaptic activity was shown to induce LTP. Previously, the results from *in vitro* studies have also shown that application of glutamate or NMDA is sufficient to potentiate synaptic transmission in spinal cord[57] and in hippocampus.[12,30,41] In spinal cord neurons activation of NMDA receptors depolarizes membrane potential of postsynaptic neurons[52,60] and increases cytoplasmic Ca^{2+} concentration.[40] Activation of NK1 receptors by SP may also increases Ca^{2+} level in the cytosol by mobilizing its release from intracellular stores[71] and by increasing Ca^{2+} influx through voltage-gated Ca^{2+} channel.[26,60] Both SP and NKA may enhance the release of glutamate in the spinal cord[29,66,67] and potentiate NMDA-dependent increase in intracellular Ca^{2+} level.[60] Data from *in vitro* studies have shown that in the spinal cord[57] and visual cortex[1] afferent activity leads to LTP, only when postsynaptic membrane is sufficiently depolarized. It has been extensively documented that nociceptive neurons in the spinal dorsal horn are subjected to powerful tonic inhibition from supraspinal regions.[3,62] In rats with intact spinal cord, tonic descending inhibition may prevent sufficient depolarization of spinal neurons thus stabilizing the Mg^{2+} block of NMDA receptor channels. Conversely, removal of descending inhibition may enable NMDA, SP or NKA to induce a rise in intracellular Ca^{2+} sufficient to trigger LTP. This may explain our finding that spinal application of NMDA, SP or NKA induced LTP only in spinal rats but not in rats with intact spinal cord. This study provides evidence that activation of spinal NMDA, NK1 or NK2 receptors induces plastic changes in C-fibre-mediated synaptic transmission in the spinal dorsal horn and this may be effectively blocked by tonic inhibition from descending pathways.

Hyperalgesia and long-term potentiation of C-fibre-evoked field potentials

Hyperalgesia is an increased response to noxious stimulation.[8,16] The time-courses of hyperalgesia induced by different protocols are quite different. Hyperalgesia induced by nerve injury may last for up to eight weeks[2] and that induced by intrathecal injections of NMDA,[19,32,46] SP[20,47] or NKA[23] may last a few hours or only several minutes in awake unanaesthetized animals. Our previous work[63] has also shown that intrathecal injection of NMDA fails to produce long-lasting hyperalgesia in awake, intact animals, even though this treatment induces massive expression of c-Fos protein in spinal neurons. Present work shows that spinal application of NMDA, SP or

NKA induces no LTP in rats with intact spinal cord. Thus, the transient hyperalgesia in awake, intact animals may not require LTP induction in spinal cord but may rather be due to direct excitatory effects of NMDA,[14,52] SP[6,34,58] or NKA[50,52,70] on spinal nociceptive neurons. Furthermore, it has been shown that in the spinal cord NMDA receptors are also expressed presynaptically on the terminals of SP-containing primary afferent C-fibres.[33] Activation of presynaptic NMDA receptors has been suggested to facilitate and prolong the transmission of nociceptive messages through the release of SP and glutamate from presynaptic terminals and this presynaptic mechanism may also contribute to short-lasting hyperalgesia induced by intrathecal injection of NMDA.[32]

LTP is a long-lasting enhancement of synaptic transmission which is considered as a fundamental mechanism of learning and memory,[5] and some forms of persistent pain have been referred to somatosensory memory.[8] As mentioned in the introduction, both LTP of C-fibre-evoked field potentials and central sensitization of spinal neurons are blocked by NMDA, NK1 or NK2 receptor antagonist, and LTP of C-fibre-evoked field potentials can be induced not only by electrical stimulation of afferent C-fibres but also by natural noxious stimulation or by acute nerve injury. We have, therefore, proposed that LTP of C-fibre-evoked field potentials may be one of the cellular mechanisms of central hyperalgesia.[37,61] In support of this notion, a great body of evidence has shown that LTP of synaptic transmission and central hyperalgesia share common mechanisms. Protein kinase C has been reported to be involved in both the expression of LTP in hippocampus[28] and thermal and mechanical hyperalgesia.[7,9,49,77] Nitric oxide, which is released from postsynaptic neuron by activation of NMDA receptors and reaches presynaptic terminals through the extracellular compartment,[5] may also play an important role in both the induction of LTP[44] and thermal hyperalgesia.[9,46,49] *De novo* protein synthesis is necessary for the late phase (from 3–5 h onward) of LTP in freely moving rats[31] and in hippocampal slice preparation.[21] The blockade of protein synthesis was recently shown to selectively decrease the second phase of formalin-induced behavioural hyperalgesia.[27] Taken together, convergent and independent evidence suggests that LTP of spinal C-fibre-evoked field potentials may be an important mechanism underlying long-lasting central hyperalgesia.

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REFERENCES

1. Artola A., Brocher S. and Singer W. (1990) Different voltage-dependent thresholds for inducing long-term depression and long-term potentiation in slices of rat visual cortex. *Nature* **347,** 69–72.
2. Attal N., Filliatreau G., Perrot S., Jazat F., Di Giamberrardino L. and Guilbau G. (1994) Behavioural pain related disorders and contribution of the saphenous nerve in crush and chronic constriction injury of the rat sciatic nerve. *Pain* **59,** 301–312.
3. Basbaum A. I. and Fields H. L. (1978) Endogenous pain control mechanisms: review and hypothesis. *Ann. Neurol.* **4,** 451–462.
4. Beck H., Schröck H. and Sandkühler J. (1995) Controlled superfusion of the rat spinal cord for studying non-synaptic transmission: an autoradiographic analysis. *J. Neurosci. Meth.* **58,** 193–202.
5. Bliss T. V. P. and Collingridge G. L. (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361,** 31–39.
6. Budai D. and Larson A. A. (1996) Role of substance P in the modulation of C-fibre-evoked responses of spinal dorsal horn neurons. *Brain Res.* **710,** 197–203.
7. Coderre T. J. (1992) Contribution of protein kinase C to persistent nociception following tissue injury in rat. *Neurosci. Lett.* **140,** 181–184.
8. Coderre T. J., Katz J., Vaccarino A. L. and Melzack R. (1993) Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. *Pain* **52,** 259–285.
9. Coderre T. J. and Yashpal K. (1994) Intracellular messengers contributing to persistent nociception and hyperalgesia induced by L-glutamate and substance P in the rat formalin pain model. *Eur. J. Neurosci.* **6,** 1328–1334.
10. Collingridge G. and Bliss T. (1987) NMDA receptors—their role in long-term potentiation. *Trends Neurosci.* **10,** 288–293.
11. Collingridge G. L., Kehl S. J. and McLennan H. (1983) Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *J. Physiol., Lond.* **334,** 33–46.
12. Cormier R. J., Mauk M. D. and Kelly P. T. (1993) Glutamate iontophoresis induces long-term potentiation in the absence of evoked presynaptic activity. *Neuron* **10,** 907–919.
13. Courteix C., Lavarenne J. and Eschalier A. (1993) RP-67580, a specific tachykinin NK-1 receptor antagonist, relieves chronic hyperalgesia in diabetic rats. *Eur. J. Pharmac.* **241,** 267–270.
14. Dickenson A. H. and Sullivan A. F. (1990) Differential effects of excitatory amino acid antagonists on dorsal horn nociceptive neurones in the rat. *Brain Res.* **506,** 31–39.
15. Dougherty P. M., Palecek J., Paleckova V. and Willis W. D. (1994) Neurokinin 1 and 2 antagonists attenuate the responses and NK1 antagonists prevent the sensitization of primate spinothalamic tract neurons after intradermal capsaicin. *J. Neurophysiol.* **72,** 1464–1475.
16. Dubner R. (1991) Pain and hyperalgesia following tissue injury: new mechanisms and new treatments. *Pain* **44,** 213–214.

17. Duggan A. W., Hendry I. A., Morton C. R., Hutchison W. D. and Zhao Z. Q. (1988) Cutaneous stimuli releasing immunoreactive substance P in the dorsal horn of the cat. *Brain Res.* **451,** 261–273.
18. Duggan A. W., Hope P. J., Jarrott B., Schaible H.-G. and Fleetwood-Walker S. M. (1990) Release, spread and persistence of immunoreactive neurokinin A in the dorsal horn of the cat following noxious cutaneous stimulation. Studies with antibody microprobes. *Neuroscience* **35,** 195–202.
19. Ferreira S. H. and Lorenzetti B. B. (1994) Glutamate spinal retrograde sensitization of primary sensory neurons associated with nociception. *Neuropharmacology* **33,** 1479–1485.
20. Frederickson R. C. A., Burgis V., Harrel C. E. and Edwards J. D. (1978) Dual actions of substance P on nociception: possible role of endogenous opioids. *Science* **199,** 1359–1361.
21. Frey U., Krug M., Reymann K. G. and Matthies H. (1988) Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocapal CA1 region *in vitro*. *Brain Res.* **452,** 57–65.
22. Furuyama T., Kiyama H., Sato K., Park H. T., Maeno H., Takagi H. and Tohyama M. (1993) Region-specific expression of subunits of ionotropic glutamate receptors (AMPA-type, KA-type and NMDA receptors) in the rat spinal cord with special reference to nociception. *Molec. Brain Res.* **18,** 141–151.
23. Gamse R. and Saria A. (1986) Nociceptive behavior after intrathecal injections of substance P, neurokinin A and calcitonin gene-related peptide in mice. *Neurosci. Lett.* **70,** 143–147.
24. Garret C., Carruette A., Fardin V., Moussaoui S., Peyronel J.-F., Blanchard J.-C. and Laduron P. M. (1991) Pharmacological properties of a potent and selective nonpeptide substance P antagonist. *Proc. natn. Acad. Sci. U.S.A.* **88,** 10208–10212.
25. Haley J. E., Sullivan A. F. and Dickenson A. H. (1990) Evidence for spinal *N*-methyl-D-aspartate receptor involvement in prolonged chemical nociception in the rat. *Brain Res.* **518,** 218–226.
26. Heath M. J. S., Womack M. D. and MacDermott A. B. (1994) Substance P elevates intracellular calcium in both neurons and glial cells from the dorsal horn of the spinal cord. *J. Neurophysiol.* **72,** 1192–1198.
27. Hou W. Y., Shyu B. C., Chen T. M., Shieh J. Y. and Sun W. Z. (1997) Protein synthesis inhibitor cycloheximide dose-dependently decreases formalin-induced c-Fos protein and behavioral hyperalgesia in rats. *Neurosci. Lett.* **227,** 99–102.
28. Hu G.-Y., Hvalby O., Walaas S. I., Albert K. A., Skjeflo P., Andersen P. and Greengard P. (1987) Protein kinase C injection into hippocampal pyramidal cells elicits features of long term potentiation. *Nature* **328,** 426–429.
29. Kangrga I., Larew J. S. A. and Randić M. (1990) Tachykinins and calcitonin gene-related peptide enhance release of endogenous glutamate and aspartate from the rat spinal dorsal horn slices. *J. Neurosci.* **108,** 155–160.
30. Kauer J. A., Malenka R. C. and Nicoll R. A. (1988) NMDA application potentiates synaptic transmission in the hippocampus. *Nature* **334,** 250–252.
31. Krug M., Loessner B. and Ott T. (1984) Anisomycin blocks the late phase of long-term potentiation in dentate gyrus of freely moving rats. *Brain Res. Bull.* **13,** 39–42.
32. Liu H., Mantyh P. W. and Basbaum A. I. (1997) NMDA-receptor regulation of substance P release from primary afferent nociceptors. *Nature* **386,** 721–724.
33. Liu H., Wang H., Shen M., Jan L. Y., Jan Y. N. and Basbaum A. I. (1994) Evidence for presynaptic *N*-methyl-D-aspartate autoreceptors in spinal cord dorsal horn. *Proc. natn. Acad. Sci. U.S.A.* **91,** 8383–8387.
34. Liu X.-G. and Sandkühler J. (1995) The effects of extrasynaptic substance P on nociceptive neurons in laminae I and II in rat lumbar spinal dorsal horn. *Neuroscience* **68,** 1207–1218.
35. Liu X.-G. and Sandkühler J. (1995) 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. *Neurosci. Lett.* **191,** 43–46.
36. Liu X.-G. and Sandkühler J. (1996) Long-term potentiation of spinal C-fiber-evoked potentials induced by skin inflammation or nerve injury. *IASP Abs. 8th World Congress on Pain* 40.
37. Liu X.-G. and Sandkühler J. (1997) 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,** 1973–1997.
38. Lynch G., Larson J., Kelso S., Barrionuevo G. and Schottler F. (1983) Intracellular injections of EGTA block induction of hippocampal long-term potentiation. *Nature* **305,** 719–721.
39. Ma Q. P. and Woolf C. J. (1995) Involvement of neurokinin receptors in the induction but not the maintenance of mechanical allodynia in rat flexor motoneurones. *J. Physiol., Lond.* **486,** 769–777.
40. MacDermott A. B., Mayer M. L., Westbrook G. L., Smith S. J. and Barker J. L. (1986) NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. *Nature* **321,** 519–522.
41. Malenka R. C. (1991) Postsynaptic factors control the duration of synaptic enhancement in area CA1 of the hippocampus. *Neuron* **6,** 53–60.
42. Malenka R. C., Kauer J. A., Zucker R. S. and Nicoll R. A. (1988) Postsynaptic calcium is sufficient for potentation of hippocampal synaptic transmission. *Science* **242,** 81–84.
43. Malenka R. C., Lancaster B. and Zucker R. S. (1992) Temporal limits on the rise in postsynaptic calcium required for the induction of long-term potentiation. *Neuron* **9,** 121–128.
44. Malenka R. C., Madison D. V. and Nicoll R. A. (1986) Potentiation of synaptic transmission in the hippocampus by phorbol esters. *Nature* **321,** 175–177.
45. Malenka R. C. and Nicoll R. A. (1993) NMDA-receptor-dependent synaptic plasticity: multiple forms and mechanisms. *Trends Neurosci.* **12,** 521–527.
46. Malmberg A. B. and Yaksh T. (1993) Spinal nitric oxide synthesis inhibition blocks NMDA-induced thermal hyperalgesia and produces antinociception in the formalin test in rats. *Pain* **54,** 291–300.
47. Matsumara H., Sakurada T., Hara A., Sakurada S. and Kisara K. (1985) Characterization of the hyperalgesic effect induced by intrathecal injection of substance P. *Neuropharmacology* **24,** 421–426.
48. Mayer L. M., Westbrook G. L. and Guthrie P. B. (1984) Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature* **309,** 261–263.
49. Meller S. T., Dykstra C. and Gebhart G. F. (1996) Acute thermal hyperalgesia in the rat is produced by activation of *N*-methyl-D-aspartate receptors and protein kinase C and production of nitric oxide. *Neuroscience* **71,** 327–335.
50. Munro F. E., Fleetwood Walker S. M., Parker R. M. C. and Mitchell R. (1993) The effects of neurokinin receptor antagonists on mustard oil-evoked activation of rat dorsal horn neurons. *Neuropeptides* **25,** 299–305.

51. Murray C. W., Cowan A. and Larson A. A. (1991) Neurokinin and NMDA antagonists (but not kainic acid antagonist) are antinociceptive in the mouse formalin model. *Pain* **44,** 179–185.
52. Nagy I., Maggi C. A., Dray A., Woolf C. J. and Urban L. (1993) The role of neurokinin and *N*-methyl-D-aspartate receptors in synaptic transmission from capsaicin-sensitive primary afferents in the rat spinal cord *in vitro*. *Neuroscience* **52,** 1029–1037.
53. Nasstrom J., Karlsson U. and Post C. (1992) Antinociceptive actions of different classes of excitatory amino acid receptor antagonists in mice. *Eur. J. Pharmac.* **212,** 21–29.
54. Nowak L. M., Bregestovski P., Ascher P., Herbert A. and Prochiantz A. (1984) Magnesium gates glutamate-activated channels in mouse central neurons. *Nature* **307,** 462–465.
55. Piercey M. F., Moon M. W., Blinn J. R. and Dobry-schreur P. J. K. (1986) Analgesic activities of spinal cord substance P antagonists implicate substance P as a neurotransmitter of pain sensation. *Brain Res.* **385,** 74–85.
56. Pockett S. (1995) Spinal cord synaptic plasticity and chronic pain. *Anesth. Analg.* **80,** 173–179.
57. Randić M., Jiang M. C. and Cerne R. (1993) Long-term potentiation and long-term depression of primary afferent neurotransmission in the rat spinal cord. *J. Neurosci.* **13,** 5228–5241.
58. Randić M. and Miletic V. (1977) Effect of substance P in cat dorsal horn neurones activated by noxious stimuli. *Brain Res.* **128,** 164–169.
59. Ren K. and Dubner R. (1993) NMDA receptor antagonists attenuate mechanical hyperalgesia in rats with unilateral inflammation of the hindpaw. *Neurosci. Lett.* **163,** 22–26.
60. Rusin K. I., Bleakman D., Chard P. S., Randić M. and Miller R. J. (1993) Tachykinins potentiate *N*-methyl-D-aspartate responses in acutely isolated neurons from the dorsal horn. *J. Neurochem.* **60,** 952–960.
61. Sandkühler J. (1996) Neurobiology of spinal nociception: new concepts. *Prog. Brain Res.* **110,** 207–244.
62. Sandkühler J. (1996) The organization and function of endogenous antinociceptive systems. *Prog. Neurobiol.* **50,** 49–81.
62a. Sandkühler J. and Liu X.-G. (1998) Induction of long-term potentiation at spinal synapses by noxious stimulation or nerve injury. *Eur. J. Neurosci.* (in press).
63. Sandkühler J., Treier A.-C., Liu X.-G. and Ohnimus M. (1996) The massive expression of c-Fos protein in spinal dorsal horn neurons is not followed by long-term changes in spinal nociception. *Neuroscience* **73,** 657–666.
64. Schaible H.-G., Grubb B. D., Neugebauer V. and Oppmann M. (1991) The effects of NMDA antagonists on neuronal activity in cat spinal cord evoked by acute inflammation in the knee joint. *Eur. J. Neurosci.* **3,** 981–991.
65. Schouenborg J. (1984) Functional and topographical properties of field potential evoked in rat dorsal horn by cutaneous C-fibre stimulation. *J. Physiol., Lond.* **356,** 169–192.
66. Skilling S. R., Harkness D. H. and Larson A. A. (1992) Experimental peripheral neuropathy decreases the dose of substance P required to increase excitatory amino acid release in the CSF of the rat spinal cord. *Neurosci. Lett.* **139,** 92–96.
67. Sluka K. A. and Westlund K. N. (1993) Spinal cord amino acid release and content in an arthritis model: the effects of pretreatment with non-NMDA, NMDA, and NK1 receptor antagonists. *Brain Res.* **627,** 89–103.
68. Sorkin L. S. and McAdoo D. J. (1993) Amino acids and serotonin are released into the lumbar spinal cord of the anesthetized cat following intradermal capsaicin injections. *Brain Res.* **607,** 89–98.
69. Thompson S. W. N., Dray A. and Urban L. (1994) Injury-induced plasticity of spinal reflex activity: NK1 neurokinin receptor activation and enhanced A-and C-fiber mediated responses in the rat spinal cord *in vitro*. *J. Neurosci.* **14,** 3672–3687.
70. Thompson S. W. N., Urban L. and Dray A. (1993) Contribution of NK-1 and NK-2 receptor activation to high threshold afferent fibre evoked ventral root responses in the rat spinal cord *in vitro*. *Brain Res.* **625,** 100–108.
71. Womack M. O., MacDermott A. B. and Jessell T. M. (1988) Sensory transmitters regulate intacellular calcium in dorsal horn neurons. *Nature* **334,** 351–353.
72. Woolf C. J. (1983) Evidence for a central component of post-injury pain hypersensitivity. *Nature* **306,** 686–688.
73. Woolf C. J. and Thompson S. W. N. (1991) The induction and maintenance of central sensitization is dependent on *N*-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity. *Pain* **44,** 93–299.
74. Xu X., Dalsgaard C. and Wiesenfeld Hallin Z. (1992) Intrathecal CP-96,345 blocks reflex facilitation induced in rats by substance P and C-fiber-conditioning stimulation. *Eur. J. Pharmac.* **216,** 337–344.
75. Xu X.-J., Maggi C. A. and Wiesenfeld-Hallin Z. (1991) On the role of NK-2 tachykinin receptors in the mediation of spinal reflex excitability in the rat. *Neuroscience* **44,** 483–490.
76. Yashpal K., Dam T.-V. and Quirion R. (1990) Quantitaive autoradiographic distribution of multiple neurokinin binding sites in rat spinal cord. *Brain Res.* **506,** 259–266.
77. Yashpal K., Picher G. M., Parent A., Quirion A. and Coderre T. J. (1995) Noxious thermal and chemical stimulation induce increase in 3H-phorbol 12,13-dibutyrate binding in spinal dorsal horn as well as persistent pain and hyperalgesia, which is reduced by inhibition of protein kinase C. *J. Neurosci.* **15,** 3263–3272.

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