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CHAPTER 6

Synaptic mechanisms of hyperalgesia

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Introduction

Hyperalgesia and allodynia often aggravate pain for variable periods of time after trauma, surgery or inflammation. Pain that is induced by normally non-painful stimuli (allodynia) or abnormally intense pain elicited by noxious stimuli (hyperalgesia) may be the consequence of an increased sensitivity of nociceptors (peripheral sensitization) or may be due to an increased responsiveness of neurons in the central nervous system (central sensitization). The sensitization of nociceptors is typically limited in time to the period of primary injury. Central sensitization may, however, outlast the period of noxious input to the central nervous system by hours to weeks and in some unfortunate patients the period of abnormal pain sensitivity may last even longer. The long-lasting hyperalgesia that may remain after healing of the primary injury is often difficult to treat and to prevent. Consequently much research effort has been devoted to better understand the neurobiological mechanisms underlying persistent pain and hyperalgesia (see Woolf and Mannion, 1999; Yaksh et al., 1999 for reviews). The peripheral mechanisms of hyperalgesia are discussed in other chapters of this volume. Here, we will focus on central mechanisms. Four principle mechanisms of afferent-induced central sensitization can be proposed:

(1) *Synaptic mechanisms:* These include all changes that occur after an action potential invades the presynaptic nerve terminal of primary afferent Aδ- or C-fibers up to the postsynaptic currents that are triggered by binding of the neurotransmitter to their postsynaptic receptors. Synaptic transmission may be affected by changes in the content or the release of neurotransmitter (presynaptic mechanisms), the diffusion or inactivation of neurotransmitter, the density or the binding affinity of neurotransmitter receptors, or changes in the conductance or gating behavior of ion channels in the postsynaptic membrane (postsynaptic mechanisms).

(2) *Membrane excitability:* Changes of membrane properties in postsynaptic neurons may affect the transformation of excitatory postsynaptic currents (EPSCs) to the discharge of action potentials. Examples for such alterations in nociceptive neurons are changes in input resistance or resting membrane potential, changes in threshold for action potential firing, or changes in after-hyperpolarization leading to changes in discharge frequency and/or discharge patterns.

(3) *Phenotypical changes:* These include, but are not limited to, the expression of new neurotransmitters, neuromodulators or their receptors in primary afferent nerve fibers or in spinal or supraspinal neurons following tissue injury.

(4) *Morphological reorganization:* This includes all afferent-induced morphological changes in spinal cord or brain such as sprouting of primary afferent nerve terminals, e.g. following nerve injury or apoptotic or excitotoxic cell death in spinal dorsal horn following intense noxious stimulation.

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These mechanisms may act in isolation or, what is more likely, in concert to result in a state of central sensitization. For example, a *phenotypical* switch in primary afferent Aβ-fibers following inflammation may lead to the expression of substance P in these fibers that normally do not express tachykinins (Neumann et al., 1996). The storage and the release of a tachykinin in central terminals of Aβ-fibers would be labeled a *presynaptic* change. The release of substance P from peptidergic afferents and extrasynaptic spread in spinal cord may then facilitate the release of glutamate and other amino acids (Kangrga and Randić, 1990) (*presynaptic* mechanism) and may enhance postsynaptic glutamate-receptor-gated currents (Randić et al., 1990) (*postsynaptic* mechanism). Both mechanisms would result in an increase in synaptic strength. In addition *membrane excitability* may be enhanced due to slow excitatory postsynaptic potentials evoked by substance P. This would not only increase the probability of action potential firing but may in addition lead to an excessive influx of Ca^{2+} into the postsynaptic cell through voltage-dependent NMDAR channels, calcium-permeable AMPAR channels, or voltage-gated calcium channels (see Gerber et al., 2000, this volume; Moore et al., 2000, this volume; Schaible et al., 2000 this volume) with the possible consequence of an excitotoxic cell death. Since inhibitory interneurons appear to be most sensitive to the excitotoxic effects a permanent loss of segmental inhibition in spinal cord, i.e. a *morphological reorganization* of spinal neuronal network, may result. This too could lead to a long-lasting increase in *synaptic strength* in nociceptive pathways.

Before one may propose that any of these mechanisms contribute to some forms of clinically relevant hyperalgesia and chronic pain it should be demonstrated that induction mechanisms, pharmacological characteristics, time courses, reversibility, and prevention of the proposed neurobiological changes match the corresponding clinical situation. For technical or ethical reasons this may not always be possible to test in humans. Then it is, however, mandatory to demonstrate that the neuroplastic changes that can be observed at a given level of nociceptive pathways are not being bypassed, filtered out, or compensated at subsequent stages of nociception. For example, changes that occur at the synapses between primary afferent C-fibers and second-order neurons in superficial spinal dorsal horn must not be filtered out in polysynaptic pathways to neurons in deep dorsal horn or ventral horn or to supraspinal relays including thalamus and somatosensory cortex.

Cellular and molecular long-term changes that occur at defined steps of nociception, e.g. at a given synaptic relay in spinal dorsal horn, are often most easily investigated in reduced models of nociception, e.g. in spinal cord slice preparations or in cell culture systems. To assess the relevance of neurobiological mechanisms for pain and for hyperalgesia it will, however, be necessary to also evaluate polysynaptic responses, e.g. by recording from deep dorsal horn neurons or motorneurons, to perform reflex studies in less reduced or in intact preparations, and to perform behavioral tests of nociception in drug-free animals and humans. Ideally, conditioning stimuli applied at the various organizational levels of nociception should be identical to allow comparisons. Unfortunately this is often not the case and parameters of conditioning stimulation used to induce long-term changes of nociception vary considerably between different studies. Of course, this cannot always be avoided. For example reduced preparations such as spinal cord slices and cell cultures typically lack input from nociceptors and it is then not possible to assess the impact of conditioning natural noxious stimuli that are used in behavioral studies also in vitro. Further, developmental changes of the nociceptive system must not be ignored when comparing results obtained from neonatal tissues or cells (used in some patch-clamp studies) and those from more mature animals (which are used for in vivo electrophysiology and in behavioral tests) (Fitzgerald and Jennings, 1999; see Alvares et al., 2000, this volume). Nevertheless it should be possible to test the effects of conditioning stimuli that were highly effective in reduced, neonatal preparations also in intact animals of the same developmental stage or in humans.

The present review focuses on synaptic mechanisms in superficial spinal dorsal horn that may contribute to some forms of hyperalgesia. Afferent-induced hyperalgesia may last for minutes to months and may include mechanisms such as long-term potentiation (LTP) of synaptic strength (pre- or postsynaptic mechanisms) or impairment of pre- or

postsynaptic inhibition at the first central synapse. It is likely that similar synaptic changes also occur at later stages of nociception, e.g. in deep dorsal horn, ventral horn and/or at supraspinal sites.

To assess changes in synaptic strength it is essential to record monosynaptically evoked postsynaptic currents or potentials, either with intracellular single cell recordings or as extracellular field potentials. Recordings of nociceptive signals downstream to the first central synapse, e.g. recordings of action potential firing in second- or higher order neurons, do not allow to differentiate between changes in synaptic strength, membrane excitability, or inhibitory control.

LTP of synaptic strength in primary afferent C-fibers

Changes in synaptic strength in primary afferent nerve fibers have been evaluated with in vitro and in vivo models of spinal nociception (see Moore et al., 2000, this volume). Randić and her co-workers reported that in a spinal cord slice preparation of young rat condition high frequency (100 Hz) stimulation of dorsal rootlets either induced a long-term potentiation (LTP) or a long-term depression (LTD) of monosynaptic EPSPs evoked in laminae I/II neurons by electrical stimulation of primary afferents (Randić et al., 1993). LTP could be induced in normal rats and in rats that were treated at birth with capsaicin to destroy C-fibers. This LTP of synaptic strength was prevented by NMDA-receptor blockade. Interestingly in that report, the direction of long-term changes of synaptic strength depended upon the holding level of postsynaptic membrane potential. Depolarization favored an induction of NMDA-receptor sensitive form of LTP while hyperpolarization favored induction of LTD that appeared to be independent of NMDA receptor activation. Both, the AMPA and the NMDA receptor mediated postsynaptic potentials were potentiated by 100 Hz conditioning stimulation.

LTP of synaptic strength can also be demonstrated at synapses of primary afferent C-fibers, even though it is difficult to prove that the responses are purely monosynaptic in nature. We have shown recently that in a spinal cord-dorsal root slice preparation of young rat pure C-fiber-evoked EPSPs can be potentiated by conditioning 100 Hz stimulation of dorsal root (Fig. 1). In these experiments sharp microelectrodes were used for intracellular current-clamp recordings (Fig. 1A). When we used patch-clamp recordings in the whole cell configuration, the same conditioning stimuli (current-clamp during conditioning stimulation, voltage-clamp during test stimulation) failed, however, to induce an LTP (Fig. 1B). This suggests that dialysis of the postsynaptic cell and loss of a diffusible mediator in the signal transduction pathway prevented LTP induction.

Conditioning stimulation of dorsal roots with parameters that can induce synaptic LTP (high frequency stimulation at supramaximal intensities) triggered a steep rise in $[Ca^{2+}]_i$ in nociceptive lamina II neurons (Fig. 2). This is compatible with the observation that Ca^{2+}-dependent signal transduction pathways are essential for induction of LTP (Bliss and Collingridge, 1993; Neveu and Zucker, 1996).

To test whether these long-term changes of synaptic strength in afferent C-fibers have any significance for the in vivo situation we have performed experiments in urethane anesthetized, paralyzed rats. Under these conditions supramaximal electrical stimulation of sciatic nerve consistently evoked field potentials with long latencies in superficial spinal dorsal horn. These field potentials were evoked by primary afferent C-fibers as they had high stimulation thresholds ($\geq$7 V at 0.5 ms pulse width), long latencies (90–130 ms), long chronaxie (1.1 ms), and a negative focus in superficial spinal dorsal horn. Potentials were not attenuated by muscle relaxation or spinalization rostral to the recording sites. When test stimuli were given at 1 Hz the amplitudes of C-fiber-evoked field potentials remained constant. In contrast, the number of action potentials discharged from single neurons that were recorded at the same site increased progressively with each stimulus (wind-up phenomenon). These findings suggest that the late field potentials reflect the summation of postsynaptic responses in second- order neurons to C-fiber stimulation and are not the envelope of action potential firing (Liu and Sandkühler, 1997).

Following conditioning, supramaximal stimulation of sciatic nerve at 100 Hz (for 1 s repeated three times at 10 s intervals) amplitudes of C-fiber-evoked field potentials were potentiated to about 200% of control (Fig. 3) confirming our previous

results (Liu and Sandkühler, 1995, 1997). This potentiation of the amplitudes of C-fiber-evoked field potentials reflects changes in synaptic strength rather than changes in action potential firing in second- or higher-order neurons. Potentiation was not accompanied by changes in the size of the afferent C-fiber volley. Thus, LTP was not confounded by an increase in afferent barrage (Liu and Sandkühler, 1997). Following electrical stimulation of sciatic nerve Aβ- and Aδ-fiber-evoked field potentials can clearly be differentiated from C-fiber-evoked potentials (Fig. 4). The conditioning stimulation used here induced LTP of C-fiber-evoked potential without significantly changing the size of Aβ- or Aδ-fiber-evoked potentials (Fig. 4). Thus, in the present study LTP was selectively induced at synapses of C- but not A-fibers.

The conditioning electrical nerve stimulation triggered a highly synchronized, burst-like discharge pattern in all afferent nerve fibers. This appears to be a prerequisite for LTP induction at some synapses in the brain, but it did not mimic the non-synchronous irregular discharges that are elicited in a fraction of nociceptive nerve fibers during natural noxious stimulation (see inset Fig. 5). Thus, it was questionable whether LTP of synaptic strength following conditioning electrical nerve stimulation can serve as a model for central sensitization and hyperalgesia that is induced under clinical conditions by trauma, surgery, or inflammation. To address this critical question we have recorded C-fiber-evoked field potentials in superficial spinal dorsal horn in response to electrical test stimuli applied to sural nerve. For conditioning stimulation intense noxious stimuli were used that either consisted of skin heating, mechanical trauma with serrated forceps, or subcutaneous injections of formalin within the innervation area of the nerve. In animals with spinal cord and descending inhibitory pathways in-

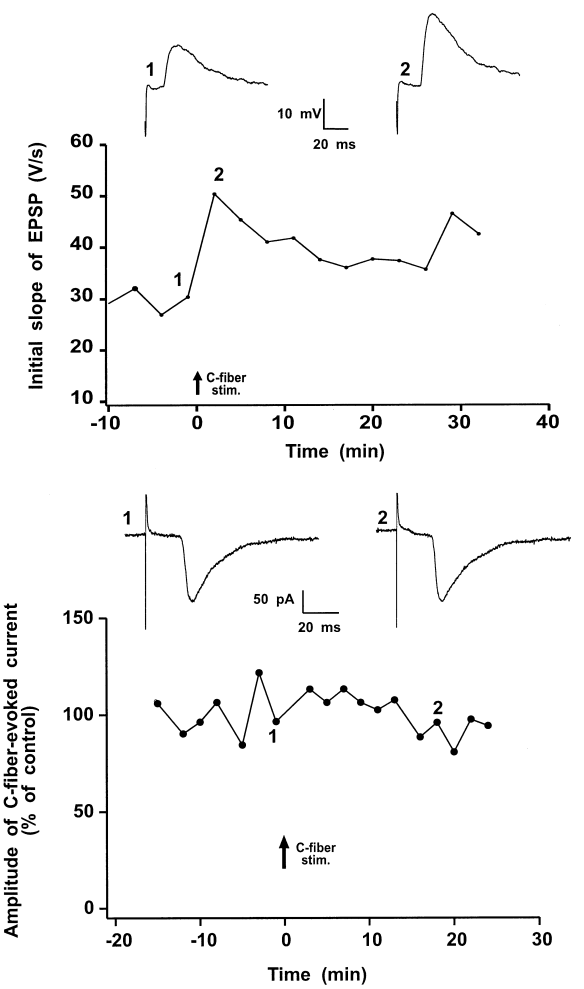


Fig. 1. LTP induction in spinal cord-dorsal root preparation of young (22-day-old) rat by conditioning stimulation of dorsal root at C-fiber strength. C-fiber-evoked responses were potentiated when recorded from lamina II neurons by intracellular microelectrodes but not by patch-clamp recordings in whole-cell configuration. (A) Excitatory postsynaptic potentials (EPSPs) were recorded from a lamina II neuron in response to supramaximal electrical stimulation of dorsal root. Conditioning stimulation of dorsal root that was identical to that used in vivo (Fig. 3) induced LTP of apparently monosynaptic C-fiber-evoked EPSPs. Initial slopes of EPSPs are plotted versus time. Original EPSP recordings are shown above the graph. (B) The same conditioning stimulation protocol as in (A) failed, however, to induce LTP of C-fiber-evoked excitatory postsynaptic currents (EPSCs) in conventional whole-cell patch-clamp recordings. Supramaximal stimulation of the attached dorsal root elicited purely C-fiber-evoked EPSCs in this lamina II neuron. Currents were recorded under voltage clamp conditions (holding potential $V_h = -75$ mV). Under these conditions conditioning stimulation had no effect on the amplitudes of the C-fiber-evoked EPSCs, suggesting that diffusible intracellular compounds are involved in the induction of long-term changes of synaptic strength. Above the graph, C-fiber-evoked EPSCs before (1) and after (2) the conditioning stimulation are displayed.

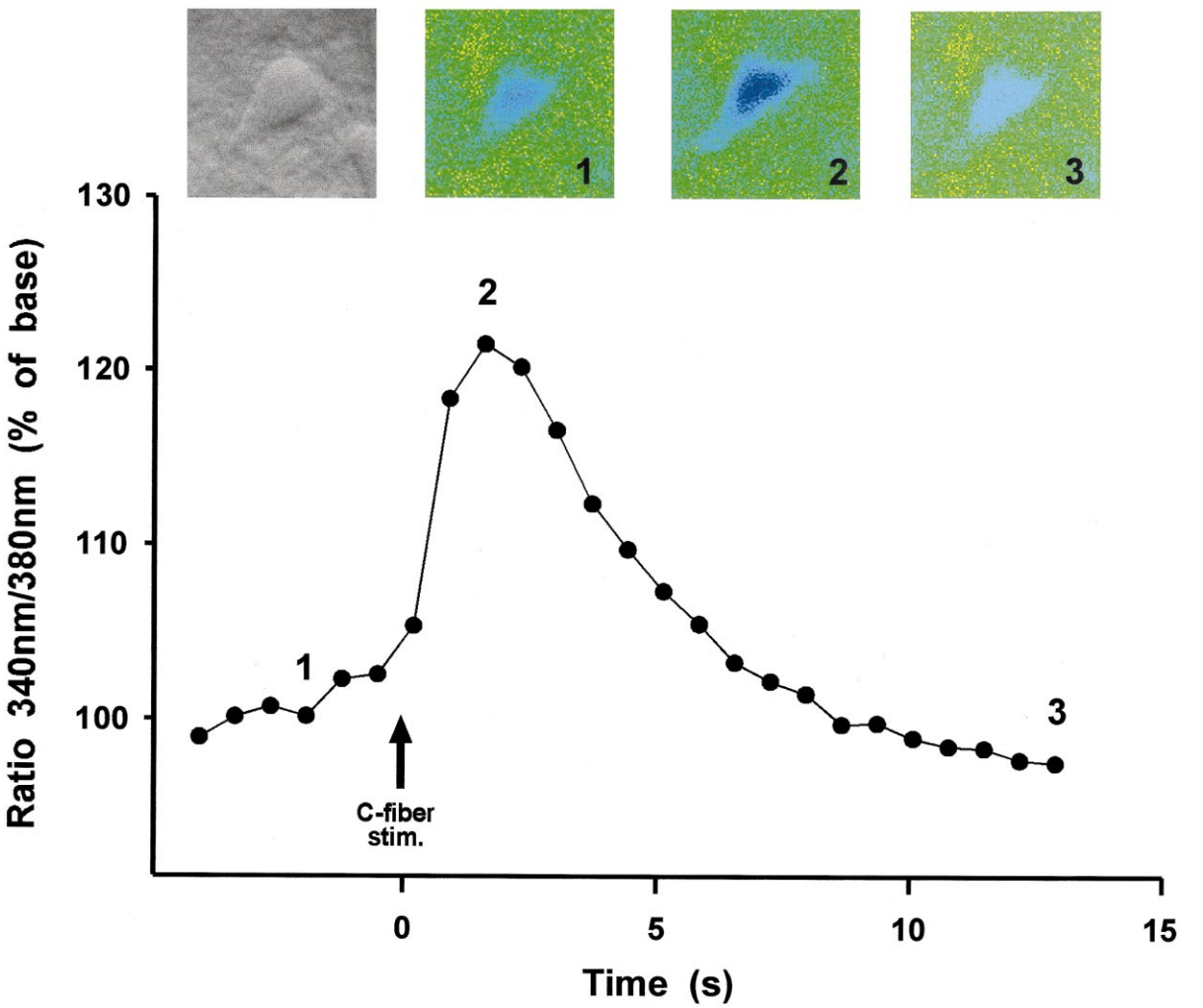


Fig. 2. In a spinal cord-dorsal root preparation of young rat a transient rise of the $[Ca^{2+}]_i$ was induced in a lamina II neuron by high frequency, supramaximal electrical stimulation of primary afferent nerve fibers (4 mA, 0.5 ms pulses given at 100 Hz for 1 s). The $[Ca^{2+}]_i$ was measured as a fluorescence ratio (percentage of base averaged from first four points, ex 340/380) in fura-2 AM loaded (10 μM) spinal cord slices. Above the graph, an infrared transmission image (left hand side) and fluorescence images of the cell are displayed, calculated from two images (ex 340 and 380 nm, respectively) before, during, and after the $[Ca^{2+}]_i$ rise.

tact, these conditioning stimuli failed to induce a long-term potentiation of synaptic strength in afferent C-fibers. In animals which were spinalized rostral to the recording site in lumbar spinal dorsal horn, the same conditioning stimuli now consistently induced an LTP of C-fiber-evoked field potentials in superficial spinal dorsal horn to 194 ± 26% of control ($n = 5$; after heat injury), to 171 ± 24% of control ($n = 5$; after s.c. formalin), or to 199 ± 17% ($n = 5$; after skin squeezing) 100 min after onset of conditioning stimulation (Fig. 5) (Sandkühler and Liu, 1998). Thus, tonic descending inhibition may prevent induction of LTP in superficial spinal dorsal horn by natural noxious stimulation. A continuous nociceptive input from the injured tissue to spinal cord was not required, neither for the induction nor for maintenance of LTP as local anesthetic block followed by nerve transection distal to the stimulation electrode 5 or 60 min after injury did not affect LTP (Sandkühler and Liu, 1998). Acute nerve

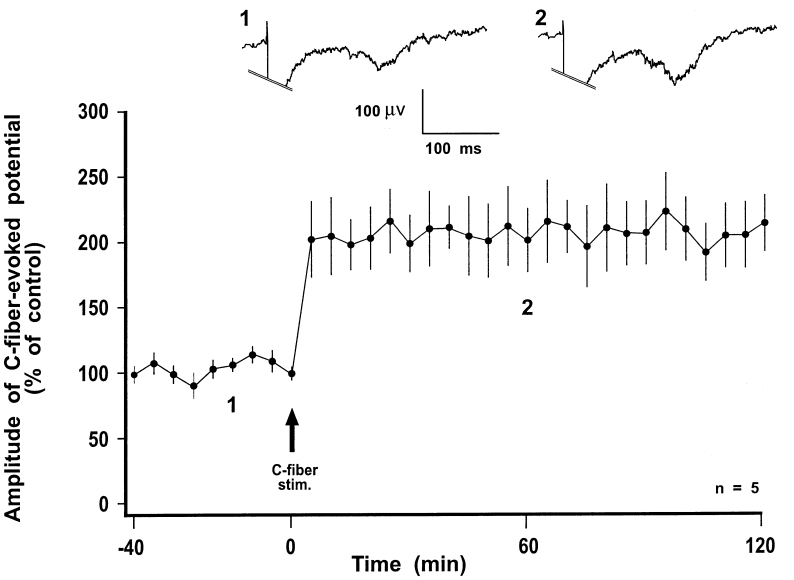


Fig. 3. Supramaximal conditioning high-frequency stimulation of primary afferent nerve fibers induces LTP of synaptic strength between C-fibers and neurons in superficial spinal dorsal horn in vivo. Mean (±SEM) amplitudes of C-fiber-evoked potentials recorded in superficial spinal dorsal horn of five urethane anesthetized adult rats are plotted versus time of the experiment. At time zero conditioning stimulation was applied to sciatic nerve at C-fiber strength (40 V, 0.5 ms pulses given at 100 Hz for 1 s four times at 10 s intervals). Conditioning stimulation induced LTP of C-fiber-evoked field potentials. Above the graph are shown original recordings of field potentials before (1) and after (2) conditioning stimulation. The late downward (negative) deflections represent C-fiber-evoked field potentials.

injury by repeated squeezing of the sciatic nerve distal to the stimulation electrode also induced LTP (to $176 \pm 8\%$ of control, $n = 5$) (Sandkühler and Liu, 1998). LTP of C-fiber-evoked field potentials induced either by conditioning electrical nerve stimulation, by nerve injury, or by natural noxious stimulation was blocked by superfusion of spinal cord at the recording segments with receptor antagonists for ionotropic NMDA receptors (Liu and Sandkühler, 1995) (Figs. 5A,B and 6A), metabotropic glutamate receptors (see also Gerber et al., 2000, this volume) (Fig. 6B), or tachykinin receptors (Liu and Sandkühler, 1997) (Fig. 6C,D). Thus co-activation of ionotropic and G-protein-coupled metabotropic glutamate receptors and tachykinin receptors is required for afferent induced LTP of C-fiber-evoked field potentials in superficial spinal dorsal horn.

Role of spinal glutamate receptors for LTP of synaptic strength in C-fibers

Glutamate or a related amino acid is the fast excitatory neurotransmitter used by all types of afferent nerve fibers including C-fibers (Yoshimura and Jessell, 1990). Single action potentials invading the presynaptic nerve terminal are sufficient to synaptically release glutamate. In second-order neurons of spinal dorsal horn functional ionotropic glutamate receptors of the NMDA subtype are expressed before functional AMPA or kainate subtypes of glutamate receptors are present. This causes the phenomenon of 'silent synapses' that fail to respond to synaptically released glutamate at resting membrane potential due to a lack of functioning AMPA and kainate receptors at early stages of development. Only when the membrane potential is strongly depolarized so that the Mg^{2+} block is removed from NMDAR channels can an NMDAR-gated current be evoked by afferent stimulation (Li and Zhuo, 1998). In fact these synapses are not 'silent' in the sense that they are unable to release glutamate but they fail to respond to this signal under normal conditions. It would therefore be more appropriate to label them 'deaf synapses'. At present it is not known whether LTP can be induced at 'deaf synapses' in spinal cord. Activation of 5-HT receptors by serotonin transforms

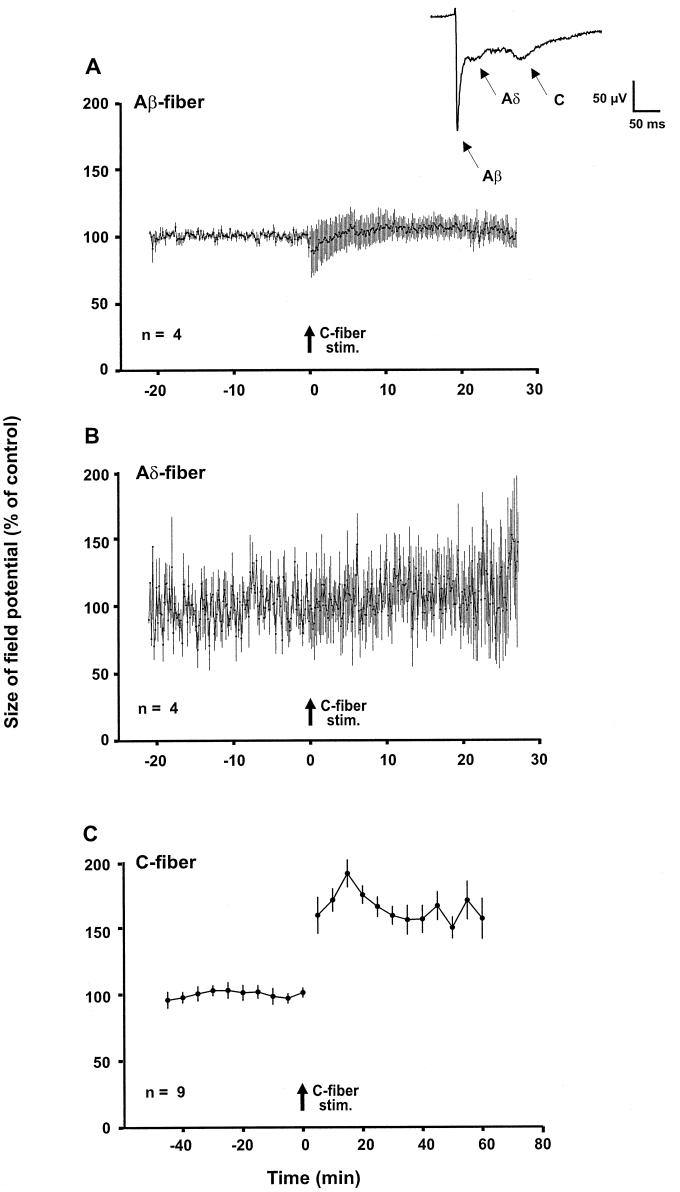


Fig. 4. Conditioning stimulation of sciatic nerve induces LTP of C-fiber- but not A-fiber-evoked spinal field potentials in vivo. In 8 adult rats field potentials were recorded in lumbar spinal dorsal horn in response to supramaximal electrical stimulation of sciatic nerve at 5 min intervals. Aβ-, Aδ-, and C-fiber-evoked potentials could be distinguished in these experiments (see traces on the right hand side). Averaged sizes of 10 field potentials evoked prior to conditioning sciatic nerve stimulation (30–50 V, 0.5 ms pulses given at 100 Hz for 1 s 4 times at 10 s intervals) served as controls. After conditioning stimulation C-fiber- but not Aβ- or Aδ-fiber-evoked potentials were potentiated for at least 2 h. Bars represent averaged sizes of field potentials 60 min after conditioning stimulation. Traces show original recordings from one experiment before and 15 min after conditioning nerve stimulation.

'deaf synapses' into active ones (see Zhuo, 2000, this volume). In more mature rats (from postnatal day 18 and in adults) where LTP has been successfully induced, the incidence of 'deaf synapses' is drastically reduced (see Moore et al., 2000, this volume).

Blockade of NMDAR abolished induction of

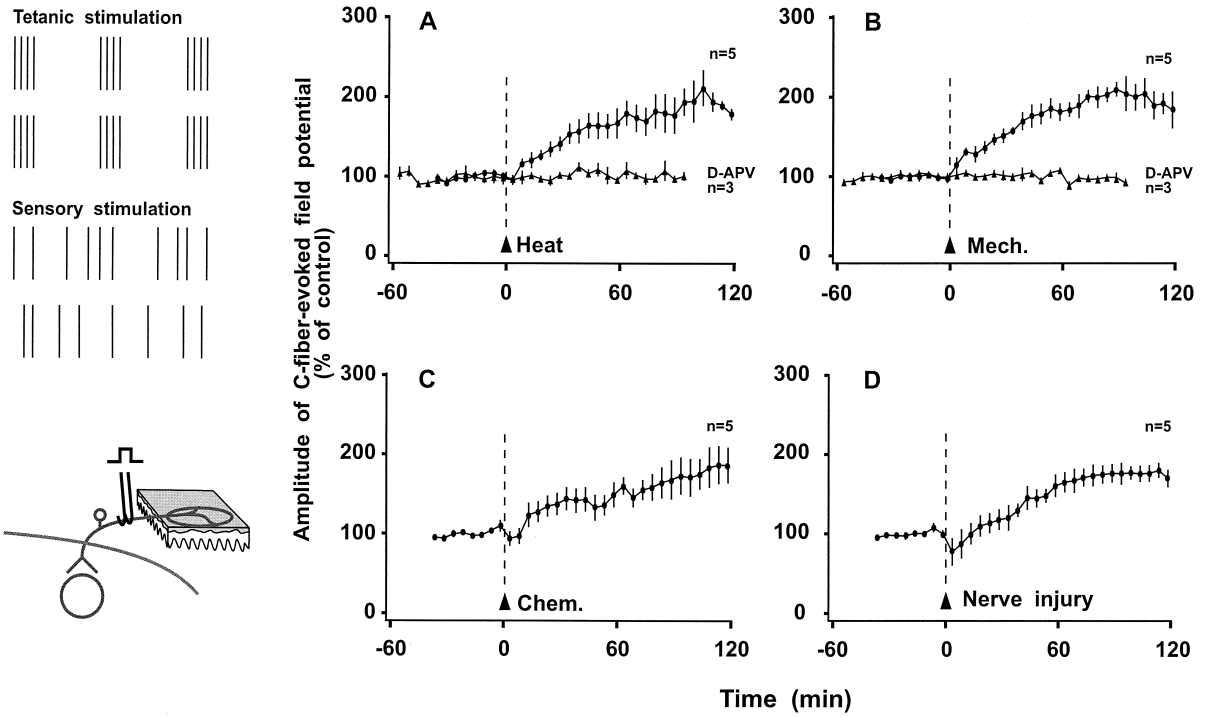


Fig. 5. Natural noxious stimulation or acute nerve injury induces LTP of C-fiber-evoked spinal field potentials in urethane anesthetized spinal rats. Test stimuli consisted of supramaximal electrical stimuli applied to sciatic nerve. Mean time courses of amplitudes of C-fiber-evoked field potentials (filled circles) are plotted versus time. At time zero (arrowheads) conditioning noxious stimuli were applied to the glabrous skin that is innervated by sciatic nerve and consisted of skin heating (A, Heat, 70ºC four times for 30 s at 30 s intervals), repetitive squeezing with an artery clamp (B, Mech.) or subcutaneous injections of formalin (C, Chem., 50 μl, 5%). In other experiments sciatic nerve was squeezed four times with forceps at 10 s intervals distal to the site of test stimulation (D, Nerve injury). These conditioning stimuli induced a slowly developing and partially reversible LTP of C-fiber-evoked potentials. In other experiments spinal cord was superfused at the recording segment with the NMDA receptor antagonist D-APV. This abolished LTP induction in all animals tested. Mean amplitudes of C-fiber-evoked field potentials in these experiments are plotted as triangles in A and B.

synaptic LTP in spinal cord in vitro and in vivo. In other regions of rat central nervous system NMDAR-sensitive and NMDAR-independent forms of LTP have been described (Nicoll and Malenka, 1995). Activation of G-protein-coupled metabotropic glutamate receptors (mGluRs) may be required for NMDAR-dependent and for NMDAR-independent forms of LTP in hippocampus (Bashir et al., 1993). Up to now eight different mGluRs have been cloned that are classified into groups I, II, and III according to sequence homologies, pharmacological profile, and signal transduction pathways involved (Pin and Duvoisin, 1995). In spinal cord LTP of C-fiber-evoked field potentials adds another flavor to the various forms of synaptic LTP. This form requires both activation of NMDAR and mGluRs. When spinal cord of urethane anesthetized, adult rats was superfused with nonspecific mGluR antagonist (+)-MCPG at 500 μM ($n = 5$; Fig. 6B) or with specific group I mGluR antagonist 4-CPG at 200 μM ($n = 5$) or group II, III mGluR antagonist M-SOPPE at 200 μM ($n = 5$) LTP induction was blocked. However, when spinal cord was superfused with selective group III mGluR antagonist M-SOP (200 μM, $n = 5$) LTP to 193 ± 23% of control was induced in five animals tested. Thus, activation of group I and group II mGluRs but not group III mGluRs is required for induction of LTP of C-fiber-evoked field potentials in superficial spinal dorsal horn (see also Gerber et al., 2000, this volume).

An influx of Ca^{2+} through activated NMDAR channels leads to a rise in $[Ca^{2+}]_i$ in spinal dorsal horn neurons. If group I mGluRs are activated the production of inositoltriphosphate via phospholipase

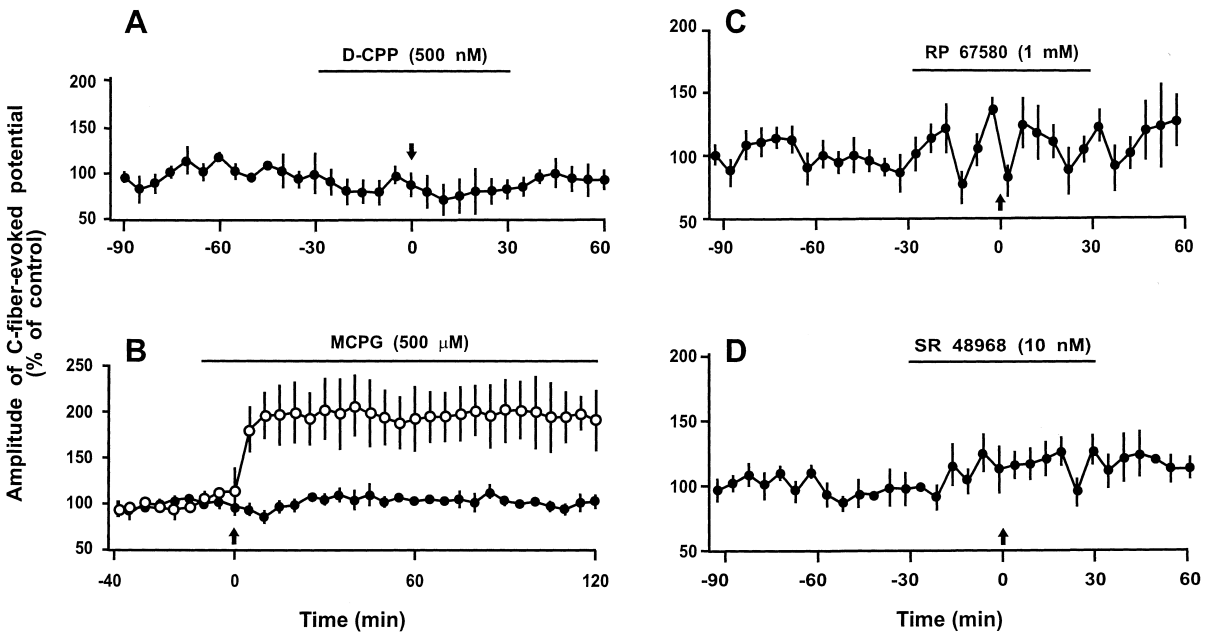


Fig. 6. Induction of LTP in superficial spinal dorsal horn in vivo requires co-activation of ionotropic and metabotropic glutamate receptors and tachykinin receptors. In spinal rats superfusion of the spinal cord at the recording segment with specific antagonists for the NMDAR (A, D-CPP 500 nM), mGluRs (B, (+)-MCPG 500 μM, closed circles; inactive enantiomer (−)-MCPG, open circles), the NK1R (C, RP 67580 1 mM), or the NK2R (D, SR 48968 10 nM) blocked induction of LTP of C-fiber-evoked field potentials by conditioning stimulation of sciatic nerve in urethane anesthetized rats. Horizontal bars indicate the period of drug application. Graphs show mean time courses of peak amplitudes of C-fiber-evoked field potentials before and after conditioning stimulation at time zero (arrows).

C activation induces Ca^{2+} release from intracellular stores. Thus, by co-activation of NMDAR and group I mGluRs an even steeper rise in $[Ca^{2+}]_i$ can be achieved. Recently it has been shown in hippocampal neurons that a transient but steep rise in postsynaptic $[Ca^{2+}]_i$ by UV-flash photolysis of caged Ca^{2+} is sufficient to induce synaptic LTP (Neveu and Zucker, 1996). A strong rise in $[Ca^{2+}]_i$ that is sufficient for LTP induction appears to be required for the activation of some calcium-sensitive protein kinases that may phosphorylate synaptic phosphoproteins (Lisman, 1989). For example the phosphorylation of AMPAR by Ca^{2+}/calmodulin-kinase II will increase single-channel conductance of existing functional AMPARs or may recruit new high-conductance-state AMPARs and thereby potentiate synaptic strength (Derkach et al., 1999). Synaptic strength may also be potentiated by rapid spine delivery and redistribution of AMPARs after synaptic NMDAR activation (Shi et al., 1999). Rise in $[Ca^{2+}]_i$ may also activate inducible nitric oxide synthase (iNOS) leading to the production of the freely diffusible gas nitric oxide (NO) with powerful actions on synaptic transmission and spinal nociception (see Meller and Gebhart, 1993 for review and Hoheisel and Mense, 2000, this volume). Activation of phospholipases and production of prostanoids in spinal neurons is another cellular cascade triggered by $[Ca^{2+}]_i$ and is believed to play an important role in long-term changes of synaptic strength and central sensitization (Yaksh et al., 1999). In addition to these post-translational changes of protein function a rise in $[Ca^{2+}]_i$ may also trigger changes at the transcriptional level by activating Ca^{2+}-dependent transcription factors (see Berthele et al., 2000, this volume). This may be important for the expression of the late phase of synaptic LTP, (see Bliss and Collingridge, 1993 for review).

Role of tachykinin receptors for induction of synaptic LTP in spinal cord

Tachykinins are stored in dense-cored vesicles of peptidergic primary afferent nerve terminals,

whereas glutamate is stored in clear vesicles. A single action potential will selectively release glutamate from the presynaptic terminals, while high frequency action potential firing is required for exocytosis of dense-cored vesicles (Peng and Zucker, 1993). Thus, tachykinins may be co-released with glutamate from peptidergic afferents upon high-intensity noxious stimulation or during burst-like discharges of peptidergic primary afferents. Low-level noxious stimulation may be ineffective or less effective in releasing tachykinins. This has been demonstrated in studies that used the internalization of the NK1 receptor as a marker of receptor activation (Allen et al., 1999; see Honoré et al., 2000, this volume), and by antibody-coated micropipettes (Duggan et al., 1987).

NK1 receptors. Superfusion of spinal cord at the recording segment with a specific antagonist at NK1 receptors (RP 67580 at 1 μM, $n = 5$) abolished induction of synaptic LTP in all animals tested (Fig. 6C), whereas the inactive enantiomer RP 68651 did not affect induction of LTP (to 178 ± 24% of control) (Liu and Sandkühler, 1997). When RP 67580 was applied one hour after conditioning stimulation, i.e. when LTP was fully expressed, blockade of NK1 receptors was ineffective (Liu and Sandkühler, 1997). These findings suggest that activation of NK1 receptors is essential for the induction but not for the maintenance of spinal LTP. Activation of NK1 receptors by substance P induces prolonged depolarization of spinal neurons (Murase and Randić, 1984) which will facilitate opening of NMDAR channels. Activation of NK1 receptors may in addition enhance NMDAR-gated currents in spinal dorsal horn neurons independent of changes in membrane potential (Randić et al., 1990). Thus, NK1R activation may facilitate induction of NMDAR-sensitive forms of synaptic LTP in superficial spinal dorsal horn. Interestingly, only a small fraction of neurons in superficial spinal dorsal horn express NK1 receptors. Intrathecal injection of substance P that is conjugated with a cell toxin leads to the internalization of the conjugate and to selective destruction of those neurons in superficial spinal dorsal horn that express the NK1R. This leads to a significant reduction of afferent-induced hyperalgesia and suggests that NK1R-expressing neurons, even though being a small group, are essential both for induction of synaptic LTP (Liu and Sandkühler, 1997) and for hyperalgesia in adult rats (Nichols et al., 1999).

NK2 receptors. Like substance P, neurokinin A is released in spinal cord upon intense noxious stimulation and may spread extra-synaptically over long distances to remote target cells that express the NK2 receptor (Duggan et al., 1990). NK2 receptors are found at low densities throughout the superficial layers of spinal dorsal horn. Blockade of spinal NK2 receptors by spinal superfusion with SR 48968 (10 μM, $n = 3$; Fig. 6D) or by intravenous injection (0.3 mg/kg, $n = 5$) stereoselectively prevented induction of LTP by conditioning electrical stimulation of sciatic nerve. Very much like substance P, neurokinin A may enhance the release of amino acids including glutamate in spinal cord (Kangrga and Randić, 1990) and may act together with substance P to produce slow synaptic currents, prolonged excitability changes (Randić et al., 1987; Nagy et al., 1993) and an increase in NMDAR-mediated rise in $[Ca^{2+}]_i$ (Rusin et al., 1993).

Thus, the co-activation of NMDARs, mGluRs, NK1R and NK2Rs appears to be required to induce a sufficient rise in $[Ca^{2+}]_i$ to trigger LTP at synapses of primary afferent C-fibers under the given experimental conditions.

Tachykinins are not the only peptides that modulate spinal nociception, especially under pathophysiological conditions galanin (see Kerr et al., 2000, this volume) and nociceptin/orphanin FQ and nocistatin (see Ito et al., 2000, this volume) may play a role in central sensitization. Nociceptin may depress, rather than potentiate synaptic transmission in primary afferent A-fibers (Liebel et al., 1997; Ruschweyh and Sandkühler, unpublished). Whether galanin is involved in long-term changes of synaptic strength in spinal cord remains to be shown.

Tonic descending control of plasticity in spinal dorsal horn

The impact of experimental conditions on induction of neuronal plasticity in spinal dorsal horn must not be underestimated. Under normal experimental conditions conditioning electrical high-frequency stimulation of all A-fibers including Aδ-fibers but without activation of C-fibers consistently failed to potentiate C-fiber-evoked field potentials but instead depressed

synaptic strength (Fig. 7A). Low-frequency, non-synchronized impulses in nociceptive afferent nerve fibers evoked by natural noxious stimulation were also ineffective (see Fig. 7C). However, when descending pathways were surgically interrupted by spinalization rostral to the recording site, high-frequency burst-like electrical stimulation at Aδ-fiber strength (Fig. 7B) or natural noxious stimulation (Fig. 7D) now induced an LTP of C-fiber-evoked potentials. The Aδ-fiber-induced LTP was blocked by spinal application of NMDAR antagonist D-APV as was LTP induction by natural noxious stimulation. Descending pathways are mainly inhibitory under the given experimental conditions of an acute experiment depressing both spontaneous and evoked activity of most nociceptive spinal dorsal horn neurons. Thus, with descending inhibition intact strong conditioning stimuli are required to induce synaptic LTP, e.g. supramaximal, high-frequency electrical stimulation of sciatic nerve. Removal of tonic descending inhibition or an insufficient activity in these systems facilitates induction of LTP and thereby weakens the protection of the spinal cord (see also Pertovaara, 2000, this volume; Svendsen et al., 2000, this volume). This could explain why seemingly identical tissue injuries may have very different effects in different patients. In a patient with an insufficient endogenous antinociception a given trauma or surgery may cause prolonged hyperalgesia, whereas the same injuries may be harmless in patients that are well protected by their antinociceptive systems.

Reversibility of synaptic LTP in superficial spinal dorsal horn

Clinical manifestations of central sensitization such as post-surgery hyperalgesia may diminish within days without special treatment, but more severe forms such as tic douloureux and phantom limb pain may persist and are very difficult to treat (see also Flor, 2000, this volume; Hasenbring, 2000, this volume). The pharmacological relief of pain is limited in time by the biological half-time of the active compound. Longer lasting analgesia can be achieved in some patients by procedures of counter-irritation including (electro-)acupuncture and transcutaneous electrical nerve stimulation (TENS). To obtain long-lasting analgesia high-intensity (low-frequency) TENS that produces tolerable pain is used (Johnson et al., 1991). We have tested whether similar forms of counter-stimulation may affect synaptic strength in C- or in Aδ-fibers.

In urethane anesthetized rats LTP of C-fiber-evoked field potentials to about 220% of control was induced by supramaximal, high-frequency stimulation of sciatic nerve. When LTP was fully expressed a second conditioning stimulus that selectively activated Aα/β-fibers failed to affect synaptic strength in C-fibers (Fig. 8). When the intensity of conditioning stimulation was raised to also recruit Aδ-fibers, then repetitive stimulation normalized previously potentiated synaptic strength in C-fibers. The duration of this depotentiation always outlasted the period of conditioning stimulation and did not vanish within the recording periods of up to 2 hours (Fig. 8) (Liu et al., 1998). The depression of synaptic strength was not limited to C-fibers and could also be demonstrated in vitro. In a spinal cord-dorsal root slice preparation of young rat conditioning low-frequency stimulation of dorsal roots at Aδ-fiber intensities induced a homosynaptic long-term depression of synaptic strength in primary afferent Aδ-fibers (Chen and Sandkühler, 2000). This LTD was NMDAR sensitive, required co-activation of group I and group II mGluRs, and was blocked by intracellular application of Ca^{2+} chelator BAPTA (see Fig. 9A). LTD induced by conditioning stimulation of Aδ-fibers was independent of activation of $GABA_A$ or glycine receptors (Sandkühler et al., 1997; Chen and Sandkühler, 2000). Another form of segmental antinociception can be achieved by conditioning stimulation of Aα/β-fibers. This induces a short-lived inhibition of C-fiber-evoked responses of spinal dorsal horn neurons involving an inhibitory interneuron and activation of $GABA_A$ receptors (Fig. 9B). The mechanisms of Aα/β-fiber-mediated antinociception are described by the classical ‘gate control theory’ (Melzack and Wall, 1965) and are being used clinically in the form of low-intensity, high-frequency TENS. The long-lasting analgesia following Aδ-fiber stimulation with high-intensity (low-frequency) TENS is, however, better explained by LTD of synaptic strength in Aδ- and in C-fibers (Fig. 9A) (Sandkühler, 2000). Thus, two independent forms of afferent-induced spinal analgesia exist that are triggered by impulses in Aα/β-fibers and Aδ-fibers.

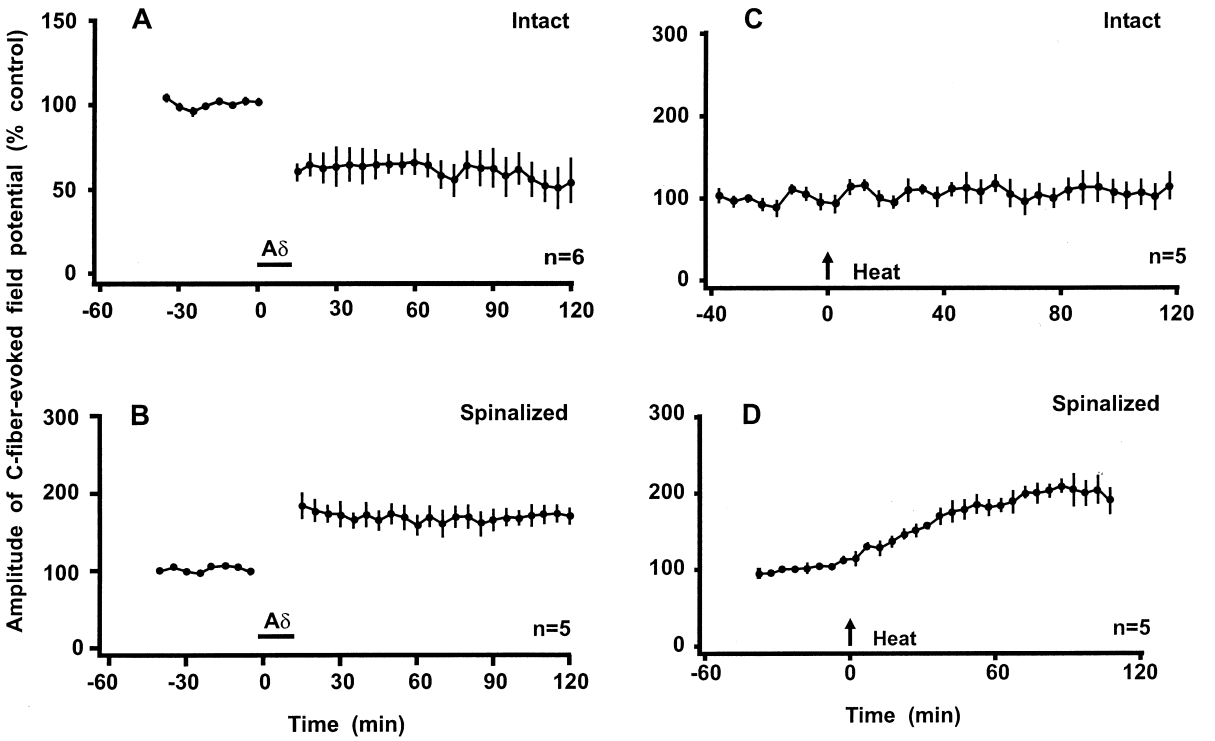
Long-term changes of nociception downstream to the first synaptic relay

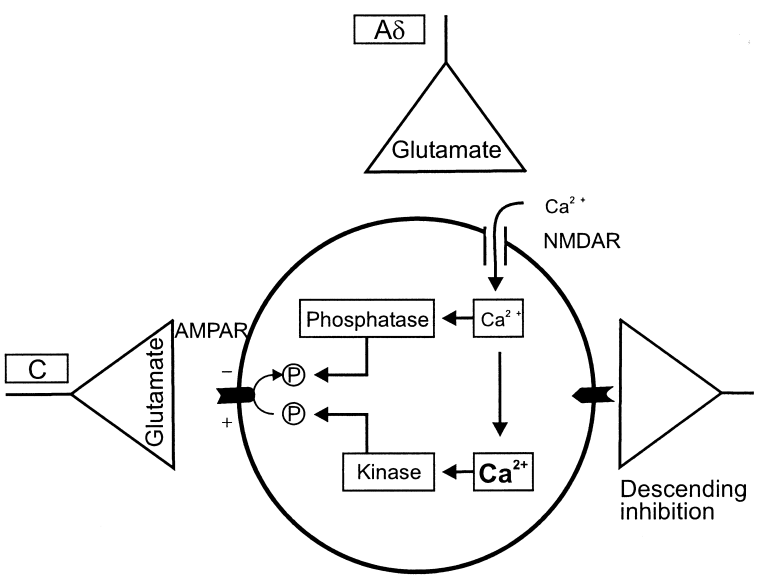
If our hypothesis is correct and synaptic LTP at the first synapse in nociceptive pathways is relevant for afferent-induced hyperalgesia and some forms of chronic pain syndromes, then it is essential to demonstrate that these long-term changes are not filtered out by subsequent processing of nociceptive information, i.e. in polysynaptic pathways to deeper layers of spinal dorsal horn, to ventral horn, or to supraspinal sites. Thus, it is important to test whether long-term facilitation of nociception can be induced at later stages of nociception by similar conditioning stimuli, with similar time courses and pharmacological profile. In fact, there is strong evidence that neuroactive drugs may have strikingly different effects at the first central synapse as compared to polysynaptic responses in nociceptive pathways (Sastry and Goh, 1983; Magnuson and Dickenson, 1991). We found that bath application of morphine, clondine, nociceptin, or mGluRs agonist ACPD had virtually no effect on the strength of the first spinal synapse of Aδ-fiber afferents but clearly blocked Aδ-fiber-evoked polysynaptic responses in spinal dorsal horn in vitro (see Fig. 10 for an example) (Ruscheweyh and Sandkühler, 2000).

Responses in deep dorsal horn. Available evidence suggests that LTP induced at the first central synapse of C-fiber afferents is not being filtered out at later stages of nociception. This was shown in a number of studies. Kjell Hole and co-workers (see Svendsen et al., 2000, this volume) recorded C-fiber-evoked discharges of single wide dynamic range (WDR) neurons in deep dorsal horn. Fortunately they performed their experiments under experimental conditions that were very similar to those used in our previous studies to induce synaptic LTP, i.e. in urethane anesthetized adult rats under muscle relaxation, conditioning stimulation consisted of repetitive high-frequency (100 Hz) stimulation of C-fibers in sciatic nerve, or by natural noxious stimulation. They found that C-fiber- but not A-fiber-evoked polysynaptic responses of WDR neurons in deeper layers of spinal dorsal horn can also be potentiated by these conditioning stimuli. The induction of long-term facilitation of C-fiber-evoked WDR neurons was NMDAR sensitive and could be more easily induced in spinalized animals similar to LTP induction of C-fiber-evoked field potentials (Svendsen et al., 1998; Rygh et al., 1999).

Responses of motoneurons. In an isolated neonatal rat spinal cord preparation tetanic train of stimuli (10 Hz for 60 s) applied to the peripheral cutaneous saphenous nerve induced an increase in a nociceptive-related slow ventral root potential (sVRP) recorded for more than one hour from a lumbar root (Lozier and Kendig, 1995). This long-term facilitation depended on C-fiber activation during conditioning stimulation and was expressed only in C-fiber-evoked, but not in A-fiber-evoked ventral root potentials. These features are reminiscent of those described for LTP of synaptic strength in C-fibers in superficial spinal dorsal horn and long-term fa-

Fig. 7. Tonic descending systems control induction of LTP of C-fiber-evoked potentials in superficial spinal dorsal horn in vivo. Supramaximal electrical stimulation of sciatic nerve was used as test stimuli. Conditioning stimulation consisted either of repetitive stimulation at Aδ-fiber strength (10 V, 0.1 ms pulses given at 100 Hz for 1 s at 10 s intervals repeated 90 times, horizontal bars in A and B). In C and D noxious radiant heating of glabrous skin (70ºC four times for 30 s at 30 s intervals) given at time zero for conditioning. (A) In animals with the spinal cord intact conditioning Aδ-fiber stimulation induced long-term depression of C-fiber-evoked field potentials. (B) In contrast, the same conditioning stimulation induced LTP rather than long-term depression in animals that were spinalized rostral to the recording site in lumbar spinal cord. It is suggested that conditioning stimulation at Aδ-fiber strength in intact animals may lead to a moderate depolarization of postsynaptic neuron and a moderate increase in postsynaptic Ca^{2+} sufficient to activate some protein phosphatases but not protein kinases, see inset (Lisman, 1989). Dephosphorylation of synaptic proteins, e.g. AMPAR channels reduces synaptic strength. In the absence of descending inhibition the same conditioning stimulation now induces a strong depolarization and a stronger increase in Ca^{2+} which may be sufficient to activate protein kinases that phosphorylate synaptic proteins and increase synaptic strength (see inset). (C) Noxious skin heating failed to change amplitudes of C-fiber-evoked field potentials in intact animals. (D) In spinalized animals the same conditioning heat stimulus induced a slowly developing LTP. It is suggested that a postsynaptic inhibition of spinal dorsal horn neuron by GABA, glycine, or opioids prevents depolarization by co-activation of AMPAR, NK1R, and NK2R that is sufficient to remove the Mg^{2+} block of NMDAR channels (see inset). Alternatively a presynaptic inhibition could reduce release of glutamate from C-fiber terminals and thereby prevent activation of NMDAR channels.





cilitation of nociceptive responses in deep dorsal horn and suggest that long-term facilitation after LTP-inducing conditioning stimuli may be present also in polysynaptic pathways to motorneurons in ventral horn. However, long-term facilitation of ventral root potentials appeared to be independent of

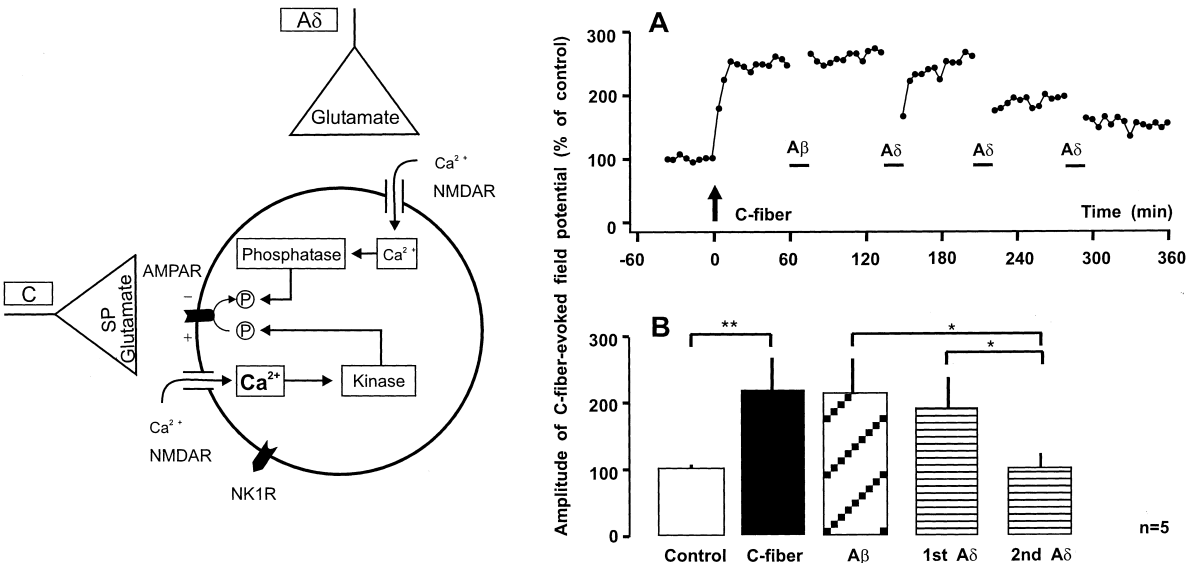


Fig. 8. Depotentiation of synaptic strength in afferent C-fibers by repetitive burst-like stimulation of primary afferent Aδ-fibers. (A) Time course of peak amplitudes of C-fiber-evoked field potentials in one representative experiment. LTP was induced to about 250% of control by supramaximal conditioning stimulation of sciatic nerve at time zero (arrow). Conditioning stimulation at Aβ-fiber intensity (0.3 V, 0.1 ms pulses given at 100 Hz for 1 s at 10 s intervals repeated 90 times, horizontal bar) failed to affect the size of C-fiber-evoked potentials. When the intensity of conditioning stimulation was raised to 10 V to also recruit Aδ-fibers but no or only few C-fibers, conditioning stimulation (horizontal bars) progressively reduced C-fiber-evoked potentials. The mean amplitudes of C-fiber-evoked field potentials following conditioning stimulation at C-fiber, Aβ-fiber or Aδ-fiber strength are shown in (B). * $P < 0.05$; ** $P < 0.01$. Possibly, LTP and LTD are the consequences of the phosphorylation state of synaptic proteins. The kind of afferent input, the pattern and the strength of afferent barrage, and the level of spinal inhibition may determine the temporal and spatial gradient of free cytosolic calcium. This may lead to a differential activation of protein kinases and phosphatases as suggested in the inset.

activation of NMDAR, NK1R, and NK2R, as bath applications of APV, RP 67580, or MEM were ineffective. Since activation of these neurotransmitter receptors is essential for LTP induction in superficial spinal dorsal horn as well as for long-term facilitation of C-fiber-evoked responses of WDR neurons in deep dorsal horn and for some forms of afferent-induced hyperalgesia, long-term facilitation of ventral root potentials apparently involves at least in part different mechanisms. Possibly the different developmental stages of the animals used (neonatal rats for ventral root potential recordings versus adult rats in the other studies), the type of nerve fibers activated during conditioning stimulation (cutaneous afferents for ventral root potential recordings versus afferents from cutaneous and deep tissues in other studies), or the stimulation parameters used were responsible for these discrepancies.

In his pioneering work Woolf (Woolf, 1984) has demonstrated that responses of flexor motoneurons in adult rats can be potentiated for several minutes by brief conditioning stimulation of sural nerve (1 Hz, 20 s at C-fiber strength). Facilitation for at least two hours was induced by cutaneous application of the chemical irritant mustard oil. Pretreatment with NMDAR antagonists MK801 or D-CCP prevented these changes. When these substances were given after long-term facilitation was fully expressed, NMDAR blockade normalized nociceptive responses. Similar effects of NMDAR blockade were reported for enhanced nociceptive responses of deep dorsal horn neurons during inflammation of the knee joint (Neugebauer et al., 1993). Thus, NMDAR activation is not only required for the induction of these long-term changes, but also for their maintenance. While the former is true also for LTP induction of synaptic strength in C-fibers, the latter may be an additional effect which is present only in polysynaptic pathways. And indeed NMDAR activation appears to be more important for signal transmission

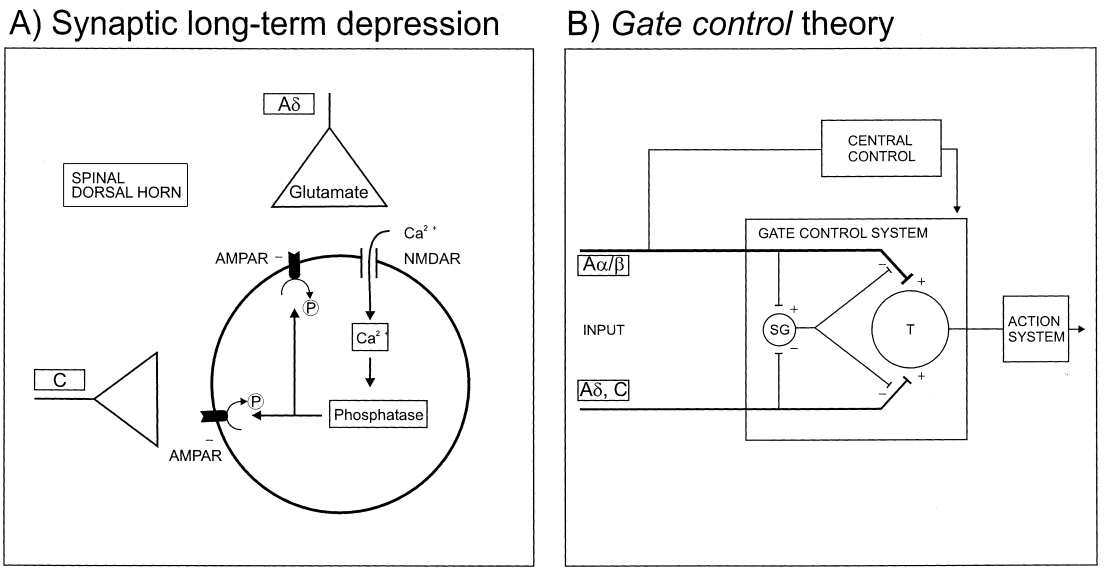


Fig. 9. Two different forms of afferent-induced inhibition of C-fiber-evoked responses exist in spinal dorsal horn. (A) Long-lasting form of inhibition can be induced by prolonged stimulation of afferent nerves at Aδ-fiber but not at Aβ-fiber strength. Under normal conditions, i.e. with spinal cord and descending inhibitory pathways intact, conditioning stimulation of Aδ-fibers leads to a moderate increase in postsynaptic Ca^{2+} that is sufficient to trigger LTD of synaptic strength, possibly by activation of protein phosphatases. Dephosphorylation of synaptic proteins at synapses with C- and Aδ-fibers leads to LTD in nociceptive pathways. This form of afferent-induced inhibition may play a role in long-lasting analgesia following high-intensity (painful) TENS, and some forms of (electro-) acupuncture and physical therapy. (B) Classical 'gate-control' theory. Stimulation of afferent nerves at Aβ-fiber intensity activates inhibitory, possibly GABAergic and/or glycinergic interneurons (SG, putatively located in substantia gelatinosa, lamina II) that produce a presynaptic or postsynaptic (not shown) inhibition of signal transmission in fine primary afferent Aδ- and C-fibers onto spinal transmission neurons (T). This form of inhibition typically ceases after conditioning stimulation is turned off and may play a role in analgesia during low-intensity afferent stimulation such as (paresthetic) TENS and vibratory stimuli (Sandkühler, 2000).

in polysynaptic pathways as compared to monosynaptic responses. Alternatively an ongoing afferent barrage might explain the continued importance of NMDARs, but this seems to be an unlikely explanation in the case of mustard-oil-induced facilitation (Woolf and Wall, 1986).

Responses in thalamus and cortex. The somatosensory discriminative component of pain is believed to be encoded by a chain of neurons that include spinothalamic tract neurons, neurons in ventrobasal complex of thalamus, and neurons in somatosensory cortex. Inflammation of peripheral tissues that is capable of inducing LTP of synaptic strength in primary afferent C-fibers and central sensitization in spinal dorsal horn may also strongly change response properties of nociceptive neurons in the ventrobasal complex of the thalamus (Guilbaud et al., 1987; Al-Chaer et al., 1996) and response properties to peripheral stimuli and laminar distribution of the different functional categories of the neurons in primary somatosensory cortex (Lamour et al., 1983; Guilbaud et al., 1993). Interestingly, the responses of nociceptive neurons in medial thalamic nuclei that might contribute to the aversive component of pain may not be affected by the same conditioning inflammatory stimuli (Dostrovsky and Guilbaud, 1990).

Taken together, the available evidence suggests that afferent-induced neuroplastic changes in superficial spinal dorsal horn may be preserved when nociceptive information is transmitted to deep dorsal and ventral horns and differentially to supraspinal relays that encode the discriminative aspect of pain.

LTP-like changes of nociception in humans

The above-mentioned studies suggest that LTP at spinal nociceptive synapses may also affect supraspinal nociception. The final proof that LTP-like phenomena are relevant for pain perception can,

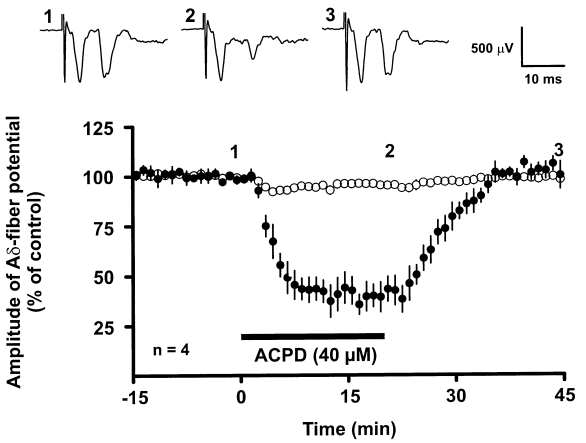


Fig. 10. Simultaneous extracellular recordings of mono- and polysynaptic Aδ-fiber-evoked field potentials were obtained from the superficial dorsal horn of transverse spinal cord slices following electrical stimulation of the attached dorsal root. Above the graph are shown original recordings of early monosynaptic and late polysynaptic Aδ-fiber-evoked field potential following the stimulation artifact. The main graph shows that bath application of the group I and II metabotropic glutamate receptor agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD, 40 μM) rapidly and strongly depressed the amplitude of the Aδ-fiber-evoked polysynaptic field potential (represented by closed circles) to $36 \pm 4\%$ of control after 20 min ($n = 4$, $p < 0.01$) while leaving the amplitude of the Aδ-fiber-evoked monosynaptic field potential (open circles) almost unaffected ($96 \pm 1\%$ of control after 20 min). These effects were completely reversible at wash-out. Field potentials are shown that were recorded immediately before application of (1*S*,3*R*)-ACPD (trace 1), after wash-in (trace 2), and after wash-out of (1*S*,3*R*)-ACPD (trace 2). Calibration bars: 500 μV, 10 ms (from Ruscheweyh and Sandkühler, 2000).

however, only be made in human subjects. We have now tested the hypothesis that conditioning high-frequency stimulation of peptidergic primary afferent nerve fibers induces long-term potentiation of pain perception in human volunteers. Pain ratings were obtained in response to punctate mechanical stimuli applied to forearm skin. Conditioning painful electrical stimulation was applied to nearby skin areas by an array of 10 punctate electrodes (100 Hz trains of 1 s duration, applied 5 times at 10 s intervals stimulation intensity was adjusted to 20–30 times the detection threshold). This induced a substantial increase in skin blood flow, determined by laser Doppler imaging, indicating that peptidergic nociceptive afferents were excited. Pain ratings increased to 200–300% of control values immediately after offset of conditioning stimulation and remained elevated for at least 40 min (Klein et al., 2000). Thus, conditioning electrical nerve stimulation with parameters that are similar to those that induce LTP at synapses of primary afferent C-fibers in vitro and in anesthetized animals induce an LTP-like hyperalgesia in humans.

Time window of central sensitization

The phenomena that are collectively referred to as central sensitization are being monitored for various periods of time ranging from minutes to hours in electrophysiological studies to a few days in most behavioral studies. In his early work Woolf has demonstrated that noxious thermal or mechanical conditioning stimuli similar to those that induce synaptic LTP in spinal cord reduced mechanical and thermal thresholds of a flexion reflex for up to six weeks in the chronic decerebrate unanesthetized rat (Woolf, 1984). It is well established that synaptic plasticity may also last for days or months, if induced under appropriate conditions, especially when induced in drug-free, unanesthetized animals (Abraham et al., 1994). This is compatible with the concept that LTP of synaptic strength between fine primary afferents and second-order neurons in superficial spinal cord may lead to very long-lasting facilitation of nociceptive reflexes and may be responsible for some forms of hyperalgesia in a clinically relevant time window. Potentiation of synaptic strength typically is a reversible, self-limiting phenomenon that may last for a few seconds (post tetanic potentiation), minutes (short-term potentiation) to hours (long-term potentiation, early phase) to weeks and months (long-term potentiation, late phase). The short-lasting potentiation that is fully reversible may play a role in central sensitization that requires an ongoing afferent barrage from nociceptive nerve fibers (Yaksh et al., 1999).

Large diameter fiber-induced pain

Some forms of persistent pain and allodynia are triggered by activation of low-threshold Aβ-fibers. These include clinical syndromes such as tic doloreux and neuropathic pain after peripheral nerve injury, and acute experimental pain in animals during blockade of segmental inhibition in spinal cord

by intrathecal application of $GABA_A$ receptor antagonists. Strengthening of polysynaptic pathways by synaptic LTP, a phenotypic switch in Aβ-fibers, or an increased membrane excitability of intercalated dorsal horn neurons may contribute to the facilitation of Aβ-fiber-mediated input to lamina II neurons in adult rats following inflammation of peripheral tissues (Baba et al., 1999). Alternatively or in addition, sprouting of Aβ-fibers and formation of new functional synapses within lamina II may be involved.

Cellular cascades leading to central sensitization

Taken together these results suggest that the following sequence of events leads to central sensitization: high-frequency impulses in fine primary afferent nerve fibers lead to the release of glutamate, substance P, and neurokinin A and other neuropeptides from their terminals in superficial spinal dorsal horn. The co-activation of AMPA, NK1, and NK2 receptors causes a prolonged depolarization of postsynaptic neurons, thereby removing an Mg^{2+}-block from NMDAR. Binding of glutamate to unblocked NMDA receptors triggers a substantial influx of Ca^{2+} into the postsynaptic neuron. A strong depolarization of postsynaptic neurons will in addition lead to a Ca^{2+} influx through voltage-gated Ca^{2+}-channels. Glutamate binding to group I metabotropic glutamate receptors triggers the phospholipase C-inositoltriphosphate pathway which leads to a release of Ca^{2+} from intracellular stores. As a consequence a strong rise in Ca^{2+} is induced in spinal dorsal horn neurons. This activates Ca^{2+}-dependant protein kinases that are known to be involved both in the induction of central sensitization and hyperalgesia and in the induction of LTP at some central synapses. Possible substrates are synaptic proteins including NMDAR and AMPAR channels. Phosphorylation of these channels leads to long-lasting increases in NMDA- and AMPA-gated currents, i.e. to LTP of synaptic strength. If these cellular cascades are also important in spinal dorsal horn neurons one might predict that any pre- or postsynaptic inhibition during conditioning stimulation would reduce or block induction of LTP an central sensitization. Indeed, in the acute preparation the level of anesthesia, choice of anesthetic used and spinal analgesia and intact descending inhibitory pathways all may interfere with induction of central sensitization and LTP. These principles underlie the concept of preemptive analgesia (see Collis et al., 1995; Katz, 1995; McQuay, 1995; Jensen and Nikolajsen, 2000, this volume; Wilder-Smith, 2000, this volume)

Preemptive analgesia

From these concepts one can predict that in human patients trauma or injury may have very different effects depending upon the balance between excitatory and inhibitory influences on nociceptive spinal dorsal horn neurons. Patients with an insufficient endogenous antinociception may be at risk for the development of post-trauma or post-surgery chronic pain, and these patients would be expected to benefit from a preemptive analgesia. In contrast, in patients with an intact endogenous antinociception small injuries or minor surgery during anesthesia may not require additional preemptive analgesia. Unfortunately at present it is not possible to routinely detect patients at risk to develop post-trauma hyperalgesia and chronic pain. The complex interaction between the strength and the duration of afferent input to spinal cord, and endogenous and exogenous pain control may explain the heterogeneous results reported for the benefits of preemptive analgesia (Collis et al., 1995; Katz, 1995; McQuay, 1995). In well controlled experimental studies and under very homogenous conditions the development of secondary hyperalgesia can, however, clearly be blocked by NMDA receptor antagonists such as ketamine or by sufficient doses of analgesics (Mikkelsen et al., 1999).

In some clinical studies preemptive analgesia was limited to the period of surgery which ignored the fact that the post surgery nociceptive input can equally well induce central sensitization. Indeed the strength of post-operative pain was found to be the best and sometimes the only indicator for chronic post-surgery pain, e.g. in breast amputees (Tasmuth et al., 1997).

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