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Long-Lasting Analgesia following TENS and Acupuncture: Spinal Mechanisms beyond Gate Control

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Stimulation of sensory nerve fibers can alleviate acute and chronic pain. The application of thermal or tolerably painful stimuli (e.g., via acupuncture needles) has a long-standing tradition in pain therapy. Shortly after the formulation of the gate control theory (Melzack and Wall 1965), a new form of afferent stimulation was successfully introduced into clinical practice: the excitation of thick, myelinated Aβ fibers, either by transcutaneous electrical nerve stimulation (TENS), by dorsal column stimulation, or by mechanical excitation of sensory nerve endings during vibratory stimuli (Wall and Sweet 1967).

Two basically distinct forms of TENS have proven clinically useful. First, stimulation at a high frequency (e.g., at 100 Hz) and low intensity evokes paresthesia but no painful sensations. This form of stimulation recruits only Aα/β fibers and typically produces an analgesia or hypoalgesia throughout the duration of stimulation. Analgesia that lasted up to 2 hours after termination of stimulation was, however, achieved in less than 20% of patients (Johnson et al. 1991). Thus, this form of paresthetic TENS is used in a patient-controlled administration for several hours every day. In animal experiments the antinociceptive effect can be blocked by the $GABA_A$-receptor antagonist bicuculline (Duggan and Foong 1985). This form of low-intensity, high-frequency, paresthetic TENS is best explained by a spinal segmental mechanism involving an inhibitory, probably GABAergic interneuron as described in the gate control theory (see Fig. 1A; Melzack and Wall 1965).

The second form of TENS requires stimulation at an intensity that produces tolerable pain that most likely involves afferent Aδ fibers. This mildly

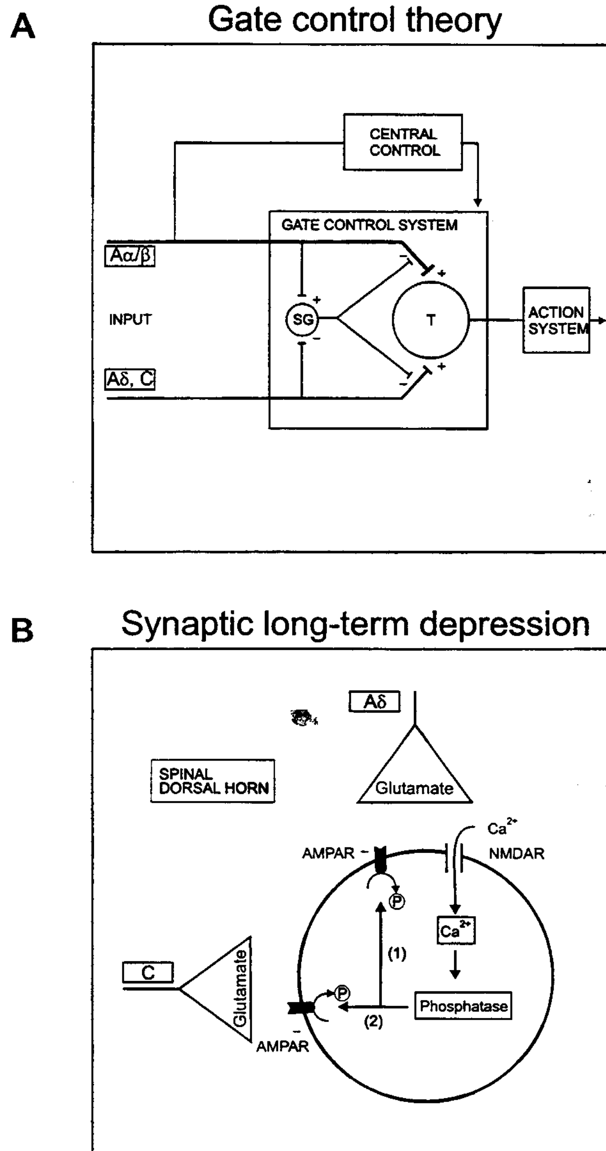

Fig. 1. Afferent-induced analgesia involves two fundamentally different mechanisms in spinal dorsal horn. (A) Conditioning stimulation of Aα/β fibers excites (+) small interneurons in substantia gelatinosa (SG) of spinal dorsal horn that exert inhibitory effects (–) on presynaptic nerve terminals of Aδ and C fibers (presynaptic inhibition). Alternatively, inhibition of spinal nociceptive projection neuron (T) may be postsynaptic (not shown) (gate control theory). (B) Conditioning stimulation of Aδ fibers induces release of glutamate from nerve terminals in superficial spinal dorsal horn that activates ionotropic glutamate receptors of the NMDA subtype (and mGluRs, not shown). This

painful TENS is tolerated if applied at relatively low frequencies (1–10 Hz) (Ishimaru et al. 1995); it is typically used for approximately 15 minutes, several times a week (Melzack 1975). This painful form of TENS (or electroacupuncture) may achieve the best analgesic effect only after repetitive stimulations over several weeks. If produced, analgesia typically outlasts the duration of conditioning stimulation by hours or days; pain reduction may be permanent in some fortunate patients (Thorsen and Lumsden 1997). This form of long-lasting analgesia, requiring mildly painful therapeutic stimulation, cannot be explained by the gate control theory.

We propose a cellular mechanism in the spinal dorsal horn that may underlie the long-lasting analgesia following painful TENS and electroacupuncture: low-frequency stimulation of Aδ fibers can induce long-term depression (LTD) of synaptic strength in fine primary afferent nerve fibers (see Fig. 1B). This form of afferent-induced spinal antinociception does not require activation of $GABA_A$ receptors but rather activation of ionotropic and metabotropic glutamate receptors.

METHODS

This section briefly reviews our methods, which are described in detail elsewhere (Sandkühler et al. 1997; Liu et al. 1998).

IN VIVO EXPERIMENTS

Adult male Sprague-Dawley rats (250–350 g) were anesthetized with urethane (1.5 g/kg, i.p.). The lumbar enlargement of the spinal cord was exposed by laminectomy. The left sciatic nerve was dissected free for bipolar electrical stimulation. Spinal field potentials were evoked by electrical stimulation of the sciatic nerve and were recorded with tungsten microelectrodes (impedance 1–3 MΩ). Single square cathodal pulses (7–20 V, 0.5 ms, at 60-second intervals) delivered to the sciatic nerve were used as test stimuli. To induce LTD of C-fiber-evoked field potentials we used conditioning stimu-

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leads to a moderate increase in free cytosolic Ca^{2+} concentration that is sufficient to activate protein phosphatases. The dephosphorylation of synaptic proteins (e.g., of ionotropic glutamate receptors of the AMPA subtype) leads to the depression of synaptic strength for prolonged periods (e.g., by reduction of postsynaptic AMPA-receptor-gated excitatory currents [–]).This LTD may be homosynaptic in nature if dephosphorylation is restricted to the synapses of Aδ fibers that were active during conditioning stimulation (1). LTD is labeled heterosynaptic when increase of Ca^{2+} and activation of phosphatases is sufficient to also affect synapses of C fibers that were not active during conditioning stimulation (2).

lation of A fibers in sciatic nerve. For controlled superfusion of the spinal cord at the recording segment, we used a specially synthesized silicone rubber to form a small well on the cord dorsum at the recording segments (Beck et al. 1995).

IN VITRO EXPERIMENTS

The L4 to S1 segments of lumbosacral spinal cord of 17- to 28-day-old rats were excised with long (8–15 mm) dorsal roots attached. We used transverse slices (400–500 μm thick) with one bisected dorsal root attached. Oxygenated recording solution (33°C) consisted of: 124 mM NaCl, 1.9 mM KCl, 1.2 mM KH_2PO_4, 2.4 mM $CaCl_2$, 1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, and 10 mM glucose, at pH 7.4, osmolarity 310–320 mosmol/kg. Intracellular recordings were made with sharp microelectrodes (170 MΩ) and a high-input impedance bridge amplifier (Axoclamp 2B, Axon Instruments). Each dorsal root half was stimulated independently through a suction electrode with isolated current stimulators. Only excitatory postsynaptic potentials (EPSPs) that were evoked by Aδ fibers were investigated further. Test pulses of 0.1 ms were given at 60-second intervals unless stated otherwise. Conditioning stimulation was applied to one dorsal root half (900 pulses of 0.1-ms duration at 0.7 mA were given at 1 Hz). Cathodal DC current (up to 0.1 nA) was passed into the cell during the course of the experiment to maintain membrane hyperpolarization typically between –75 and –85 mV (Sandkühler et al. 1997).

DRUGS

Drugs and their sources were as follows: D-2-amino-5-phosphonovaleric acid (D-AP5, 50 μM, Cambridge Research Biochemicals); bicuculline methiodide (bicuculline, 5 or 10 μM, Sigma); strychnine (2 or 4 μM, Sigma); (S)-α-methyl-4-carboxyphenylglycine ((S)-MCPG, 1 mM, Tocris); (S)-4-carboxyphenylglycine ((S)-4C-PG, 200 μM, Tocris); (RS)-α-tethylserine-O-phosphate monophenyl ester (MSOPPE, 200 μM, Tocris); (RS)-α-methylserine-O-phosphate (MSOP, 200 μM, Tocris); (1S, 3R)-1-amino-cyclopentane-1,3-dicarboxylic acid ((1S, 3R)-ACPD, 100 μM, Tocris); pertussis toxin (PTX, 1 or 2 μg/mL, Sigma).

DATA ANALYSIS

In each experiment, we averaged the amplitudes of five consecutive C-fiber-evoked field potentials, collected at 60-second intervals. We used

nonparametric ANOVA (Kruskal-Wallis test) for statistical analysis and considered $P \leq 0.05$ as significant.

Two consecutive EPSPs were averaged and synaptic strength was quantified by measuring the peak amplitude and initial slope of averaged EPSPs. The mean values of four to seven averaged, consecutive test responses recorded prior to conditioning stimulation served as controls. We assessed significant changes from controls by comparing the values of four consecutive responses 23–30 minutes after conditioning stimulation. We used the nonparametric Wilcoxon rank test for statistical comparisons and considered $P \leq 0.05$ as significant. All values were expressed as mean ± SEM.

RESULTS

LONG-TERM DEPRESSION OF SYNAPTIC STRENGTH IN FINE PRIMARY AFFERENTS

Stimulation of a dorsal root in vitro or sciatic nerve in vivo at low intensities insufficient to recruit Aδ fibers consistently failed to induce LTD of synaptic strength either in Aδ (Sandkühler et al. 1997) or in C fibers (Liu et al. 1998). Only by increasing the intensity of conditioning stimulation to also recruit Aδ fibers in dorsal roots (10 V or 0.7 mA, 0.1-ms pulses given at 1 Hz for 15 minutes) could we induce a homosynaptic LTD at synapses of Aδ fibers in vitro (Fig. 2A). When conditioning stimulation of A fibers, including Aδ fibers in sciatic nerve, was delivered in bursts (10 V, 0.1-ms pulses, 100 Hz for 1 second repeated at 0.1 Hz for 15 minutes), a putatively heterosynaptic LTD of C-fiber-evoked field potentials was induced in vivo (Fig. 2B).

Conditioning stimulation of Aδ fibers not only depressed normal synaptic transmission in Aδ and in C fibers for prolonged periods (LTD), but also normalized synaptic strength after long-term potentiation (LTP) had been induced at C-fiber synapses. A robust LTP to 216 ± 22% of control (n = 5) was induced in vivo by high-frequency, high-intensity stimulation of sciatic nerve at C-fiber strength. Two sessions of 15 minutes conditioning stimulation of Aδ fibers normalized synaptic strength (to 105 ± 22% of control) (Fig. 3) (Liu et al. 1998). LTP at synapses of C-fiber afferents is considered a synaptic mechanism of central sensitization leading to hyperalgesia (Sandkühler 1996). Thus, conditioning stimulation of Aδ fibers may not only have long-lasting analgesic effects but may also *reverse* some forms of hyperalgesia.

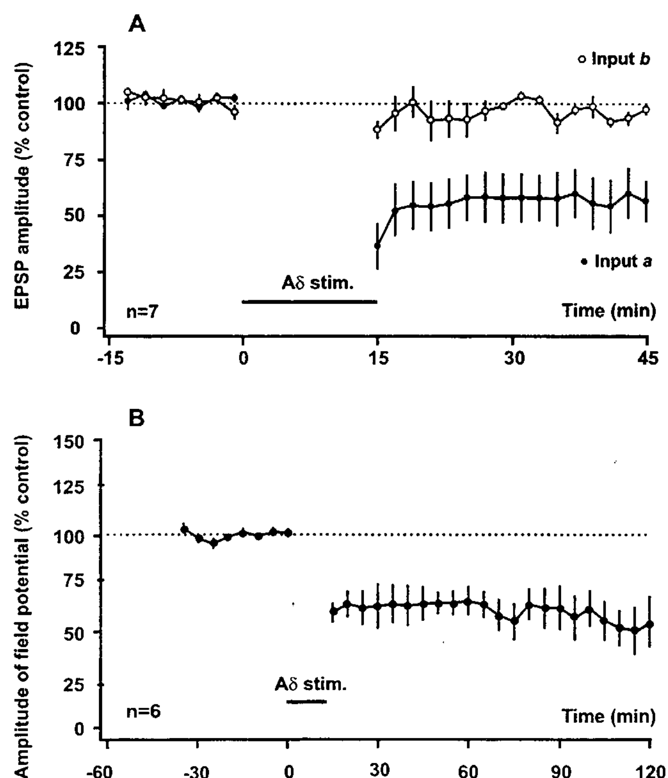

Fig. 2. Conditioning stimulation of Aδ fibers induces long-term depression (LTD) of synaptic strength in spinal dorsal horn. (A) Homosynaptic LTD at spinal synapses of afferent Aδ fibers. In a spinal cord slice preparation with one bisected dorsal root, intracellular recordings were made from neurons with monosynaptic input from both dorsal root halves (inputs *a* and *b*). EPSPs were recorded in response to inputs *a* and *b*. Conditioning low-frequency stimulation (Aδ-stim, 1 Hz, 15 minutes) was applied to one dorsal root half only (input *a*). This induced an LTD in input *a* without affecting synaptic strength in the unconditioned input *b*, i.e., LTD was homosynaptic in nature. (B) LTD at spinal synapses of afferent C fibers. In urethane-anesthetized rats, C-fiber-evoked field potentials were recorded in the superficial spinal dorsal horn in response to supramaximal electrical stimulation of sciatic nerve. Conditioning burst-like, high-frequency stimulation (Aδ-stim, 100 Hz for 1 minute repeated at 0.1 Hz for 15 minutes) of Aδ fibers in the sciatic nerve depressed synaptic strength in C fibers for prolonged periods. This LTD is probably heterosynaptic in nature. To directly prove a heterosynaptic LTD would, however, require intracellular recordings of spinal neurons with monosynaptic input from Aδ and C fibers.

TONIC DESCENDING INHIBITION MODULATES DIRECTION OF SYNAPTIC PLASTICITY IN THE SPINAL CORD

Descending systems originating at brainstem sites exert mainly inhibitory effects on nociceptive neurons in spinal dorsal horn (Fields and Basbaum

1978). The effect of conditioning Aδ-fiber stimulation critically depends upon the integrity of these descending pathways. With the spinal cord and descending pathways intact, burst-like stimulation at Aδ-fiber strength reliably induced an LTD of synaptic strength in C fibers (to 61 ± 15% of control, $n = 9$). In contrast, when the spinal cord was cut rostral to the recording site, the same conditioning stimulation now no longer produced an LTD but rather an LTP of synaptic strength in C fibers (to 187 ± 19% of control, $n = 5$). Thus, the effect of conditioning afferent stimulation depends on both the parameters of conditioning stimulation and the balance between the activity in inhibitory and in excitatory systems in spinal cord.

NEUROPHARMACOLOGY OF SPINAL LONG-TERM DEPRESSION INDUCED BY AFFERENT STIMULATION

Bath application of $GABA_A$-receptor antagonist bicuculline (5 μM) plus glycine receptor antagonist strychnine (2 μM) did not affect induction of LTD in vitro: results showed depression to 53 ± 8% of control ($n = 6$) as compared to a depression to 41 ± 10% of control ($n = 8$) in normal recording solution. Thus, neither $GABA_A$ nor glycine receptors are required for induction of LTD.

Bath application of NMDA-receptor antagonist D-AP5 (50 μM) in vitro or superfusion of spinal cord at the recording segment in vivo with D-AP5 (100 μM) abolished induction of LTD at spinal synapses of Aδ or C fibers, which suggests that these forms of spinal LTD require activation of NMDA receptors. In addition, co-activation of G-protein-coupled group I and group II mGluRs is necessary for induction of LTD at spinal synapses of Aδ fibers in vitro. Bath application of selective group I mGluR antagonist (S)-4-CPG (200 μM) or group II mGluR antagonist MSOPPE (200 μM) blocked induction of LTD (to 93 ± 12%, $n = 5$, and to 104 ± 8%, $n = 5$, respectively) by stimulation of Aδ fibers.

PHARMACOLOGICAL ACTIVATION OF LONG-TERM DEPRESSION IN THE SUPERFICIAL SPINAL DORSAL HORN

All previously described forms of LTD in the superficial spinal dorsal horn were induced by conditioning stimulation of primary afferent Aδ fibers. We have now identified a new form of spinal LTD that is induced pharmacologically by activation of mGluRs in the absence of impulses in presynaptic nerve fibers. Bath application of nonselective group I/group II mGluR agonist ACPD (100 μM) induced a robust LTD at synapses of Aδ fibers (to 72 ± 4% of control, $n = 8$) in the presence of bicuculline (5 μM) and strychnine (2 μM). This LTD was independent of NMDA-receptor

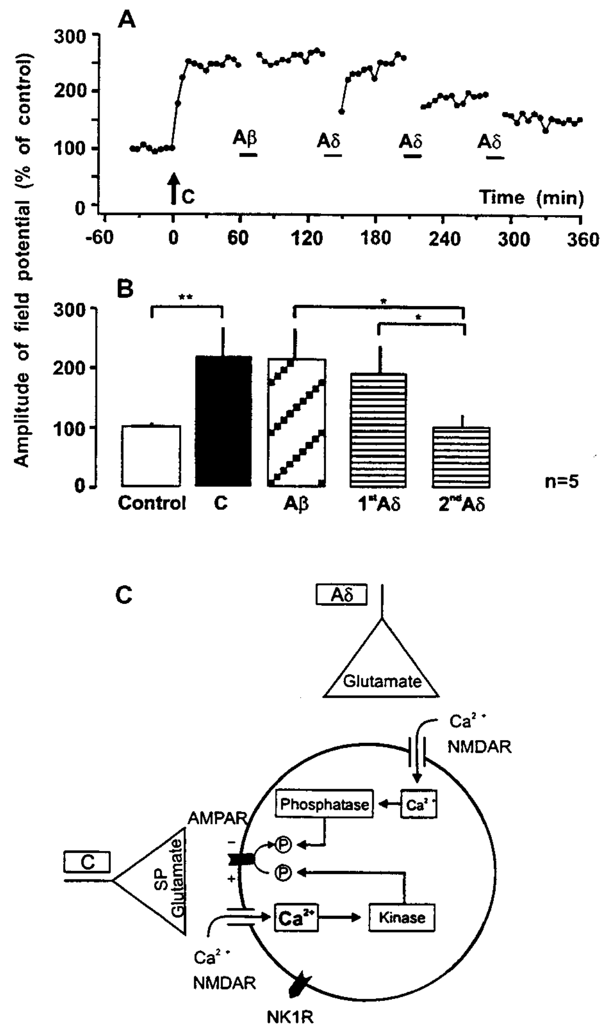

Fig. 3. Long-term potentiation (LTP) and depotentiation of synaptic strength at afferent C fibers. In urethane-anesthetized rats, C-fiber-evoked field potentials were recorded in the superficial spinal dorsal horn in response to supramaximal electrical stimulation of the sciatic nerve. Conditioning high-frequency stimulation of afferent C fibers in sciatic nerve (arrow in A) induced an LTP in this experiment to about 250% of control. Low-intensity conditioning stimulation of Aβ fibers (0.3-V, 0.1-ms pulses, 100 Hz for 1 second repeated at 0.1 Hz for 15 minutes) failed to affect synaptic strength. When stimulation intensity was raised to also recruit Aδ fibers (10 V, as above), previously potentiated synaptic strength gradually returned to normal. (B) Mean values from five experiments. (C) Putative signal transduction pathways of LTP and depotentiation in nociceptive spinal dorsal horn neurons are depicted. High-frequency conditioning stimulation of C fibers expressing substance P (SP) leads to a strong increase in $[Ca^{2+}]_i$ via activation of NMDAR and NK1R. This strong increase in $[Ca^{2+}]_i$ is sufficient to activate protein kinases that phosphorylate synaptic proteins (e.g., AMPA

activation, as bath application of D-AP5 (50 μM) failed to affect LTD (to 60 ± 11% of control, n = 5). G proteins sensitive to pertussis-toxin are not required for ACPD-induced LTD, as preincubation of spinal cord slice for at least 2 hours with pertussis toxin (1 or 2 μg/mL) did not affect induction of LTD (to 55 ± 8% of control, n = 5). Blockade of phospholipase C by U73122 but not its inactive enantiomer U73343 abolished LTD induction (101 ± 3% of control, n = 5), which suggests involvement of pertussis-toxin-insensitive G proteins that activate phospholipase C.

DISCUSSION

Clinically relevant afferent-induced analgesia can be achieved by excitation of various types of afferent nerve fibers (Aα/β, Aδ, or C fibers) or sensory nerve endings including low-threshold mechanoreceptors, nociceptors, and thermoreceptors. Paresthetic TENS will selectively recruit Aα/β fibers, while mildly painful TENS and some forms of electroacupuncture involve excitation of Aδ fibers. Vibratory stimuli selectively activate rapidly adapting mechanoreceptors, while needle acupuncture may in addition excite mechanosensitive and polymodal Aδ- and C-fiber nociceptors (reviewed by Kawakita and Gotoh 1996). Some forms of physical therapy will excite warm or cold receptors or low-threshold mechanoreceptors. From a practical and scientific point of view it is desirable to classify the various forms of treatment according to the afferent fiber type that induces the analgesia. However, at present afferent-induced analgesia is grouped according to the organizational level into segmental, propriospinal, and supraspinal descending forms.

SYNAPTIC LONG-TERM DEPRESSION OR GATE CONTROL IN SPINAL DORSAL HORN

We propose that electrical stimulation of Aα/β fibers induces a fundamentally different form of analgesia as compared to analgesia induced by excitation of all A fibers including Aδ fibers. The short-lasting analgesia produced by paresthetic TENS (i.e., at a low intensity and at high frequency) or by vibratory stimuli is best explained by mechanisms similar to those described in the gate control theory and that involve inhibitory, probably

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receptors). This will potentiate synaptic strength. In contrast, conditioning stimulation of Aδ fibers leads to a moderate increase in $[Ca^{2+}]_i$ that is insufficient for activation of protein kinases, but will activate protein phosphatases that dephosphorylate synaptic proteins and thereby normalize synaptic strength (depotentiation).

GABAergic interneurons. In contrast, the same mechanisms cannot satisfactorily explain the long-lasting analgesia that requires tolerably painful therapeutic stimulation and recruitment of Aδ fibers. This form of analgesia can be better explained by an LTD of neurotransmission in primary afferent Aδ and C fibers in the superficial spinal dorsal horn.

SOURCES OF VARIABILITY OF AFFERENT-INDUCED ANALGESIA

It is a common clinical observation that the analgesia achieved by afferent stimulation can vary considerably between and within patients. None of the present models of spinal analgesia could adequately explain this observation. We have now found that induction of putatively heterosynaptic LTD is favored by intact descending inhibition in spinal cord and that during complete interruption of descending inhibitory pathways the same conditioning stimulation that previously induced an LTD may lead to the induction of LTP (Liu et al. 1998). Thus, identical conditioning stimulation may not always have the same effects at different levels of inhibition in spinal cord. If these mechanisms also apply to pain patients, we should use *painful* TENS preferentially when a postsynaptic inhibition in superficial spinal cord favors the induction of LTD. This may be achieved by the use of a *sequential* TENS.

SEQUENTIAL TENS (PARESTHETIC TENS FOLLOWED BY PAINFUL TENS)

Sequential TENS that involves two periods of stimulation with different parameters may be most efficacious if paresthetic TENS is used first (period 1), immediately followed by painful TENS (period 2). In the first period of stimulation a gate-control-like inhibition is activated in spinal cord and in the second period an LTD is induced. TENS applied in this sequence has two beneficial effects: (1) paresthetic TENS in period 1 induces an immediate inhibition by activation of GABAergic interneurons. The postsynaptic inhibition that remains during painful TENS in period 2 favors the induction of LTD. (2) Since analgesia that is induced by paresthetic TENS is still present during the second period, the patients will tolerate higher frequencies of painful TENS. Since Aδ-fiber stimulation at higher frequencies induces a heterosynaptic LTD at Aδ- and at C-fiber terminals as opposed to homosynaptic LTD that is induced by lower frequencies of stimulation, the analgesic effect would be expected to be stronger. Sequential TENS that employs similar stimulation parameters is presently in clinical use (Ghoname et al. 1999). The rational for this approach can now be explained at the synaptic level. We might predict that not only TENS but also electro-acupuncture

and some forms of needle acupuncture could be more efficacious when applied immediately after a period of paresthetic TENS (Kawakita and Gotoh 1996).

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