Activation of Spinal D1/D5 Receptors Induces Late-Phase LTP of C-Fiber–Evoked Field Potentials in Rat Spinal Dorsal Horn

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Yang, Hong-Wei, Li-Jun Zhou, Neng-Wei Hu, Wen-Jun Xin, and Xian-Guo Liu. Activation of spinal D1/D5 receptors induces late-phase LTP of C-fiber–evoked field potentials in rat spinal dorsal horn. J Neurophysiol 94: 961–967, 2005. First published April 13, 2005; doi:10.1152/jn.01324.2004. Long-term potentiation (LTP) of C-fiber–evoked field potentials in spinal dorsal horn may be relevant to pathological pain. Our previous work has shown that the late phase of the spinal LTP is protein synthesis–dependent. Considerable evidence has accumulated that dopamine D1/D5 receptors are important for late-phase LTP in hippocampus. In this study, the role of D1/D5 receptors in LTP of C-fiber–evoked field potentials in spinal dorsal horn was evaluated in urethan-anesthetized Sprague-Dawley rats. We found the following. 1) Spinal application of SKF 38393, a D1/D5 receptor agonist, induced a slowly developed LTP of C-fiber–evoked field potentials, lasting for >10 h, and the effect was blocked by the D1/D5 antagonist SCH 23390, whereas a D2 receptor agonist (quinpirole) induced depression of C-fiber responses, lasting for 2 h. 2) The potentiation produced by D1/D5 receptor agonist occluded the late phase but not the early phase of the spinal LTP produced by tetanic stimulation. 3) SCH 23390 selectively depressed the late-phase LTP, when applied 40 min before tetanic stimulation. 4) The D1/D5 agonist-induced potentiation is blocked by the protein synthesis inhibitor anisomycin. 5) Activation of protein kinase A by spinal application of 8-Br-cAMP also induced spinal LTP, and the action occluded the potentiation induced by the D1/D5 receptor agonist. These results suggest that the spinal D1/D5 receptors participate in the protein synthesis–dependent late-phase LTP of C-fiber–evoked field potentials in spinal dorsal horn through the cAMP signaling pathway.

INTRODUCTION

Tissue or nerve injury induces pathological pain, manifested mainly as hyperalgesia, an increased response to noxious stimuli, and allodynia, a decrease in pain threshold. The striking feature of pathological pain is that it usually persists for a long time, even after the damaged tissues have healed (Coderre et al. 1993). To date, the mechanisms underlying the pathological pain are still not well understood.

Long-term potentiation (LTP) in the hippocampus has been intensively studied as a synaptic model of learning and memory (Bliss and Collingridge 1993). In recent years, it has been shown that LTP can also be induced in the synapses between afferent C-fibers and spinal dorsal horn neurons (Liu and Sandkühler 1995). Because spinal LTP can be induced by electrical stimulation of afferent C-fibers and by acute nerve injury but not by stimulation of A-fibers (Sandkühler and Liu 1998), and is prevented by blockage of spinal N-methyl-D-aspartate (NMDA) receptors (Liu and Sandkühler 1995) and neurokinin receptors (NK1 and NK2; Liu and Sandkühler 1997), it has been considered an attractive cellular model of injury-induced pathological pain (Sandkühler 2000; Willis 2002). Recent work supports this point of view by showing that activation of peptidergic cutaneous afferent C-fibers with LTP-inducing high-frequency electrical stimulation produces a long-lasting (>3 h) primary and secondary hyperalgesia and allodynia in human subjects (Klein et al. 2004).

In the hippocampus, LTP is divided into at least two phases: an early phase (1–3 h), depending on covalent modifications of existing proteins, and a late phase (>3 h), requiring new mRNA and protein synthesis (Frey et al. 1988, 1996; Krug et al. 1984; Matthies et al. 1990; Nguyen and Kandel 1996). Several lines of evidence have shown that activation of dopamine D1/D5 receptors specifically induces the late phase of LTP by stimulating a cAMP signaling pathway (Frey et al. 1993; Huang and Kandel 1994, 1995; Nguyen et al. 1994).

In the spinal dorsal horn, dopaminergic fibers are mainly from the diencephalic A11 area (Björklund and Skagerberg 1984; Hökfelt et al. 1979) and from primary afferent neurons (Weil-Fugazza et al. 1993). Dopamine D1 and D2 receptors have also been characterized in spinal dorsal horn (Dubois et al. 1986; Wamsley et al. 1989). Functionally, dopamine inhibits nociceptive spinal dorsal horn neurons (Fleetwood-Walker et al. 1988; Garraway and Hochman 2001) and produces anti-hyperalgesia or hypoalgesia (Barasi and Duggal 1985; Gao et al. 2001) by acting on D2 receptors. In contrast, the role of D1/D5 receptors in the plasticity of spinal nociception is less clear.

Our previous study revealed that protein synthesis inhibitors selectively inhibit the late-phase of LTP of C-fiber–evoked field potentials (Hu et al. 2003). In this study, the role of D1/D5 receptor and its downstream substrate, protein kinase A (PKA), in the late phase of spinal LTP was assessed.

METHODS

Surgical preparation

Experiments were performed on male Sprague-Dawley rats (250–280 g body wt). Urethan (1.5 g/kg, ip) was used to induce and maintain anesthesia. Surgical level of anesthesia was verified by the stable mean arterial blood pressure and constant heart rate during noxious stimulation. The trachea was cannulated, and the animal breathed spontaneously. One carotid artery was cannulated to continuously monitor the mean arterial blood pressure, which was maintained from 80 to 120 mmHg. A laminectomy was performed to expose the lumbar enlargement of spinal cord, and the dura mater was

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incised longitudinally. The left sciatic nerve was dissected free for bipolar electrical stimulation with platinum hook electrodes. All exposed nervous tissues were covered with warm paraffin oil, except for the spinal lumbar enlargement, onto which the drugs were applied. Colorectal temperature was kept constant (37–38°C) by means of a feedback-controlled heating blanket. At the end of the experiments, animals were killed with an overdose of urethane. The local animal care committee approved all experiments.

In some experiments, the C2–C3 segments were also exposed by laminectomy to allow high cervical spinal transection. To prevent the strong stimulation produced by spinal cord transection, 2% lidocaine (50 μl) was injected into spinal segment C3, and 8–10 min later, the spinal cord was cut at the injected site. In these experiments, animals were paralyzed with 2% gallamine triethiodide (initially, 20 mg/kg iv; maintenance, 10 mg/kg) and artificially ventilated with room air.

Electrophysiological recordings and nerve stimulation

Electrophysiological recording of C-fiber–evoked field potentials and C-fiber–evoked action potentials in the spinal dorsal horn was described previously (Liu and Sandkühler 1995, 1997). Briefly, after electrical stimulation of the sciatic nerve, C-fiber–evoked responses were recorded in the spinal dorsal horn (L4 and L5 segments) at the depth of 50–500 μm from the dorsal surface with a tungsten microelectrode (impedance, 1–2 MΩ; exposed tip diameter, 1–2 μm; World Precision Instruments), which was driven by an electronically controlled microstepping motor (Narishige Scientific Instrument Laboratory). A bandwidth of 0.1–500 Hz was used for recording field potentials. An A/D converter card (DT2821-F-16SE, Data Translation) was used to digitize and store data in a Pentium computer at a sampling rate of 10 kHz. Single square pulses (0.5-ms duration at 1-min intervals) delivered to the sciatic nerve were used as test stimuli. The strength of stimulation was adjusted to 1.5–2 times of threshold for C-fiber responses. Tetanic stimulation (0.5-ms duration, 40 V, 100 Hz, given in 4 trains of 1-s duration at 10-s intervals) was used to induce LTP (Liu and Sandkühler 1997; Pokett 1995; Randić et al. 1993). The distance from the stimulation site at the sciatic nerve to the recording site in the lumbar spinal dorsal horn was ~11 cm. Only one experiment was conducted in each animal.

Compounds and drug treatment

SKF 38393 (Sigma), SCH 23390 (Sigma), quinpirole (Sigma), or 8-Br-cAMP (Sigma) was first dissolved in 0.9% NaCl. The stock solution (20.0 mM) was subsequently diluted with 0.9% NaCl to make final concentrations of 250 μM (SKF 38393), 20 μM (SCH 23390), 100 μM (quinpirole), and 1 mM (8-Br-cAMP) immediately before administration. Anisomycin (Sigma) was first dissolved in DMSO to make a stock concentration of 50 mM, and the stock solution was subsequently diluted 250-fold with 0.9% NaCl to make a final concentration of 200 μM immediately before administration. Final DMSO concentration in the diluted working solution was 0.4%. Our previous works have shown that spinal application of 0.5% DMSO does not affect C-fiber–evoked field potentials (Hu et al. 2003; Yang et al. 2004). To directly apply the drugs on the dorsal surface of spinal cord, a small well was formed on the cord dorsum at the recording segments with 1.5% agar dissolved with 0.9% NaCl.

Data analysis

The area of C-fiber–evoked field potentials was determined off-line by parameter extraction (Fig. 1A), which was implemented by DataWave. In each experiment, responses to five consecutive test stimuli were averaged. The mean area of C-fiber–evoked field potentials before drug or saline application served as baseline. All data are expressed as means ± SE. For statistic analysis, data within animals were plotted vs. time. A: SKF 38393 (■; 250 μM) applied onto the dorsal surface of spinal cord at recording segments induced long-lasting synaptic potentiation. Spinal application of SCH 23390 (20 μM) 30 min before SKF 38393 (■) prevented LTP. Representative original recordings taken at time-points as indicated are shown (a and b). Area of C-fiber–evoked field potential as shown in b (filled with oblique lines) is determined automatically by parameter extraction. Baseline, indicated by dashed line, is determined by 2 highest points within the time range defined manually on either side of C-fiber response (arrowheads). Therefore change in size or duration of either early or late potentials had little influence on the measurement of area.

FIG. 1. A: Spinal application of SKF 38393 induces long-term potentiation (LTP) of C-fiber–evoked field potentials with a long latency. Mean response of C-fiber–evoked field potentials before drug application served as baseline. Data represent mean area of 5 consecutively recorded C-fiber–evoked field potentials. Summary data, expressed as means ± SE, are plotted vs. time. A: SKF 38393 (■; 250 μM) applied onto the dorsal surface of spinal cord at recording segments induced long-lasting synaptic potentiation. Spinal application of SCH 23390 (20 μM) 30 min before SKF 38393 (■) prevented LTP. Representative original recordings taken at time-points as indicated are shown (a and b). Area of C-fiber–evoked field potential as shown in b (filled with oblique lines) is determined automatically by parameter extraction. Baseline, indicated by dashed line, is determined by 2 highest points within the time range defined manually on either side of C-fiber response (arrowheads). Therefore change in size or duration of either early or late potentials had little influence on the measurement of area. B: spinal application of either SCH 23390 (20 μM) or saline, which was used dissolve the drugs, did not affect baseline responses of C-fiber–evoked field potentials. C: spinal application of quinpirole (100 μM), a D2 receptor agonist, induced a transient depression. Arrows indicate onset of drug applications onto the dorsal surface of spinal cord.
were compared using the nonparametric Wilcoxon signed-rank test, and those between animals were compared using a Kruskal-Wallis test. \( P < 0.05 \) was considered significant.

RESULTS

Spinal application of the D1/D5 receptor agonist but not the D2 receptor agonist induced LTP of C-fiber–evoked field potentials in the spinal dorsal horn

To study the role of D1/D5 receptors in LTP of C-fiber–evoked field potentials, a specific D1/D5 agonist SKF 38393 (250 \( \mu \text{M} \)) was applied directly onto the spinal dorsal surface at the recording segments after \( \geq 30 \text{ min} \) of stable baseline recording. As shown in Fig. 1A, the drug induced LTP of C-fiber–evoked field potentials with a long latency. At 85 min after SKF 38393, C-fiber–evoked field potentials were potentiated significantly (137.4 \( \pm \) 12.5%, \( n = 7 \), \( P < 0.05 \), Wilcoxon signed-rank test). At 195 min after SKF 38393, the potentiation reached 188.5 \( \pm \) 14.3% and remained at this level until the end of experiments (6–10 h after drug). The data suggest that activation of D1/D5 receptors is sufficient to induce LTP of C-fiber–evoked field potentials in the spinal dorsal horn.

To confirm that the action of SKF 38393 is caused by activation of D1/D5 receptors, a specific D1/D5 receptor antagonist, SCH 23390 (20 \( \mu \text{M} \)), was applied onto the spinal dorsal surface at 30 min before application of SKF 38393 in other five rats. The antagonist blocked LTP produced by SKF 38393 completely (Fig. 1A). Spinal application of SCH 23390 (20 \( \mu \text{M} \)) alone or saline, which was used to dissolve the drugs, did not affect the baseline of C-fiber responses as observed within 4 h (Fig. 1B).

In contrast, spinal application of quinpirole, a D2 receptor agonist (100 \( \mu \text{M} \)), which is negatively coupled with adenylyl cyclase (Gentleman et al. 1981; Nestler 1994), induced a depression lasting for \(< 2 \text{ h} \) (\( n = 5 \), Fig. 1C).

To test whether activation of D1/D5 receptors can induce long-lasting change in excitability of single spinal dorsal horn neurons, C-fiber–evoked field potentials and C-fiber–evoked action potentials in wide dynamic range neurons were successfully recorded for \( > 5 \text{ h} \) simultaneously with the same microelectrode in seven rats (1 unit in each rat). In accordance with previous work (Svendsen et al. 1997), C-fiber responses were calculated between 30 and 300 ms after test stimulation (conduction velocities ranged 0.36–3.6 m/s). In five of seven units, spinal application of SKF 38393 produced LTP of C-fiber–evoked action potential discharge and enlargement of cutaneous mechanoreceptive fields (see Fig. 2 for an example). In another two units, no change was observed.

Blockage of D1/D5 prevented the late phase but not early phase of the spinal LTP induced by tetanic stimulation

Our results, showing that activation of D1/D5 receptors induced LTP of C-fiber–evoked field potentials with latency as long as 85 min, suggests that late-phase but not early-phase LTP was induced. To confirm this hypothesis, in eight rats, the D1/D5 receptor antagonist SCH 23390 (20 \( \mu \text{M} \)) was applied onto the spinal dorsal surface at 40 min before tetanic stimulation. The results showed that SCH 23390 affected neither baseline synaptic response nor early phase LTP induction, but selectively depressed the late phase of spinal LTP (Fig. 3A). A significant decrease of spinal LTP was seen at 65 min after induction (143.5 \( \pm \) 10.2%, \( n = 8 \), \( P < 0.05 \), Kruskal-Wallis test, Fig. 3A) compared with 178.8 \( \pm \) 12.1% recorded in the 0.9% NaCl control group (\( n = 8 \)). At 105 min after LTP induction, the mean C-fiber response decreased to 117.8 \( \pm \).
12.9%, which is no longer different from baseline ($P < 0.05$, Wilcoxon signed-rank test). The result indicates that activation of D1/D5 receptors is necessary for induction of late-phase LTP of C-fiber–evoked field potentials in the spinal dorsal horn.

To further evaluate the role of D1/D5 receptors in the maintenance of the spinal LTP, SCH 23390 (20 μM) was administered at 30 min after LTP induction, but LTP was not affected as tested in all five rats (Fig. 3B).

**Potentiation induced by the D1/D5 agonist was blocked by inhibition of protein synthesis**

The late phase of LTP in both hippocampus (Frey et al. 1988) and spinal dorsal horn (Hu et al. 2003) requires protein synthesis. We have therefore examined whether the D1/D5 receptor agonist–induced LTP is also protein synthesis–dependent. In the presence of anisomycin (200 μM), a protein synthesis inhibitor, SKF 38393, did not produce any synaptic potentiation (Fig. 4). At 6 h after application of SKF 38393, mean area of C-fiber–evoked field potentials was 103.7 ± 12.9%, which is significantly different from that recorded in the rats treated with the D1/D5 agonist alone (185.9 ± 9.3%, $n = 7$, $P < 0.05$, Kruskal-Wallis test, Fig. 4).

**D1/D5 agonist-induced potentiation occluded late-phase but not early-phase LTP produced by tetanic stimulation**

To examine the relationship between SKF 38393-induced potentiation and LTP induced by tetanic stimulation, we performed two sets of occlusion experiments. First, we produced LTP by tetanic stimulation, and 1 h later, the intensity of test stimulation was reduced to let C-fiber responses to return to the baseline level. Thirty minutes after new baseline recordings, SKF 38393 (250 μM) induced no extra potentiation (Fig. 5A, ●). Identical results were obtained when the intensity of test stimulation was not changed after LTP induction by tetanic stimulation as tested in five other rats (Fig. 5A, ○). Second, we reversed the procedure, i.e., the D1/D5 agonist was applied before tetanic stimulation. Three hours after spinal application of SKF 38393, when LTP reached a stable level, the intensity of test stimulation was reduced to let C-fiber responses to return to the baseline level in six rats. In these experiments, tetanic stimulation induced a LTP, lasting for ~2 h. At 125 min after induction, potentiation decreased to 128.1 ± 10.2%, which is no longer different from baseline ($n = 6$, $P > 0.05$, Wilcoxon signed-rank test; Fig. 5B, ●).

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**FIG. 3.** Blockage of D1/D5 prevents the late phase but not early phase of spinal LTP induced by tetanic stimulation. Mean response of C-fiber–evoked field potentials before drug application served as baseline. Downward arrows indicate time-points at which drugs were applied onto the dorsal surface of spinal cord, and upward arrows indicate time-points when tetanic stimulation (100 Hz, 40 V, 0.5-ms duration, given in 4 trains of 1-s duration at 10-s interval) to the sciatic nerve was delivered. A: SCH 23390 (●; 20 μM, 40 min) selectively depressed late-phase LTP induced by tetanic stimulation (upward arrow). B: SCH 23390 (20 μM) had no effect on established LTP.

**FIG. 4.** Blockage of protein synthesis prevents LTP induced by a D1/D5 agonist. Protein synthesis inhibitor anisomycin (Ani, 200 μM) was applied onto the dorsal surface of spinal cord 50 min before application of SKF (250 μM). In the presence of anisomycin, SKF38393 failed to induce any potentiation, whereas anisomycin alone had no effect on baseline responses.
indicating that the tetanic stimulation induces an early-phase but not late-phase LTP. In the other five rats, the test stimulation intensity was kept constant after potentiation by SKF 38393; tetanic stimulation delivered 3.5 h after SKF 38393 induced only early-phase but not late-phase LTP (Fig. 5B, ◦).

Spinal application of an analog of cAMP also induced spinal LTP and occluded the action of SKF 38393

In the CA1 region of the hippocampus, the action of D1/D5 receptors is mediated by activation of a cAMP pathway (Huang and Kandel 1995). We next examined whether direct activation of PKA by 8-Br-cAMP can also induce LTP of C-fiber-evoked field potentials. As shown in Fig. 6, at 30 min after spinal application of 8-Br-cAMP (1 mM), C-fiber response was potentiated to 132.7 ± 6.7% of baseline (n = 5, P < 0.05, Wilcoxon signed-rank test), and at 90 min after drug application, the potentiation reached a stable level (170.8 ± 13.2%, Fig. 6). To study if the actions of 8-Br-cAMP and SKF 38393 share common mechanisms, the D1/D5 agonist SKF 38393 was applied 2.5 h after 8-Br-cAMP. In all 10 rats tested, the SKF 38393 never produced further synaptic potentiation, regardless of whether the test stimulation intensity had been reduced (n = 5) or kept constant (n = 5) after potentiation produced by 8-Br-cAMP (Fig. 6). The results of these occlusion experiments indicate that D1/D5-induced potentiation shares one or more steps in common with the cAMP pathway.

Influence of spinalization on the spinal LTP

The above results showed that activation of D1/D5 receptors in the spinal dorsal horn is necessary for induction of late-phase LTP. It has been well established that dopaminergic fibers in the spinal dorsal horn are mainly from the diencephalic A11 area (Björklund and Skagerberg 1984; Hökfelt et al. 1979) and, to a lesser extent, from primary afferent fibers (Price and Mudge 1983; Vega et al. 1991; Weil-Fugazza and Godefroy 1993; Weil-Fugazza et al. 1993). To determine whether the descending dopaminergic fibers are crucial for induction of late-phase LTP, in the next experiments, recordings were made in spinalized rats. In all six rats tested, tetanic stimulation induced LTP of C-fiber-evoked field potentials, lasting for 4 h (Fig. 7). The results indicate that the amount of dopamine released from primary afferent fibers may be enough for induction of late-phase LTP.

DISCUSSION

Our results revealed that spinal application of the D1/D5 receptor agonist induced a slow developing LTP of C-fiber-
evoked field potentials in the spinal dorsal horn, which reached to its peak around 3 h after drug application, simulating the late phase of LTP. This effect was blocked by the specific D1/D5 dopamine receptor antagonist and required new protein synthesis. Blockage of D1/D5 receptors before tetanic stimulation selectively prevented late-phase but not early-phase spinal LTP. Activation of PKA by spinal application of the cAMP analog also induced spinal LTP. The action of D1/D5 receptor agonists occluded both the cAMP-induced potentiation and tetanus-induced late phase of LTP. We therefore concluded that spinal D1/D5 receptors participate in the protein synthesis–dependent late-phase LTP of C-fiber–evoked field potentials in the spinal dorsal horn through activation of the cAMP signaling pathway.

Our results showed that spinal application of the D1/D5 receptor agonist SKF 38393 also produced a long-lasting enhancement in excitability in five of seven wide dynamic range (WDR) neurons in the spinal dorsal horn. The results suggest that the potentiation of C-fiber–evoked field potentials is associated with an increase in excitability of some but not all dorsal horn neurons. According to our results, we cannot distinguish which kinds of neurons undergoes LTP. However, a recent in vitro study with patch-clamp recording has shown that electrical stimulation of the primary afferent C-fibers induces LTP of C-fiber–evoked excitatory postsynaptic currents only in projection neurons in the spinal dorsal horn (Ikeda et al. 2003). We therefore speculate that the neurons undergoing LTP after application of the D1/D5 receptor agonist are projection ones.

It has been shown that intraplantar injection of carrageenan results in dopamine release in the lumbar spinal dorsal horn (Gao et al. 2001). There is evidence showing that, in the spinal dorsal horn, dopaminergic innervation was partly nonsynaptic (Ridet et al. 1992), suggesting that dopamine may act at least partially through volume transmission. Thus superfusion of the spinal cord with a specific D1/D5 receptor agonist or antagonist is a suitable means for evaluating the role of D1/D5 receptors in the plasticity of spinal nociception.

The concentrations of drugs used in this study appeared higher than those used in in vitro experiments. A previous work (Beck et al. 1995) has shown that, after superfusion with neurokinin A (NKA) on the dorsal surface of the spinal cord using a silicon well for 15 or 30 min, the concentration gradient of NKA in the superficial spinal cord (0–500 μm) is 25–70 times lower than that in the superfusate. In this work, the concentration of every substance is within a factor of 30 of those used in in vitro studies. Thus the concentrations of drugs in the recording site may be not higher than those used in in vitro studies. Furthermore, our results showed that pretreatment with SCH 23390, a specific antagonist of the D1/D5 receptors, blocked LTP produced by SKF 38393 completely. We do not believe that nonspecific effects of these drugs may account for these results.

Activation of D1/D5 receptors increases intracellular cAMP by stimulating adenyl cyclase, and in turn, activates PKA (Jay 2003; Nestler 1994). It has been shown that inhibition of PKA blocks both long-lasting LTP in area CA1 of hippocampal slices and the formation of long-term memory (Nguyen and Woo 2003). Activation of PKA by a cAMP agonist (Frey et al. 1993) or stimulation of adenylate cyclase by forskolin (Chavez-Noriega and Stevens 1992) elicits synaptic facilitation that occludes LTP induced by tetanic stimulation (Frey et al. 1993; Huang and Kandel 1994). The PKA pathway also plays an important role in the plasticity of spinal nociception. It has been shown that activation of the PKA by spinal application of 8-Br-cAMP (1–10 mM) produces a dose-dependent hyperalgesia and allodynia and that inhibition of PKA pathway by spinal application of adenylate cyclase inhibitor, tetraddrolfuryl adenine, or the PKA inhibitor, myrosilated protein kinase (14–22) amide, depresses the secondary hyperalgesia and alldynia produced by intradermal injection of capsaicin (Sluka 1997). Consistent with behavioral data, our previous work has shown that activation of PKA is crucial for the induction and maintenance of spinal LTP (Yang et al. 2004). We have shown that spinal application of PKA inhibitor (Rp-CPT-cAMPS) before tetanic stimulation blocks LTP induction and reverses spinal LTP when applied 15 min but not 30 min after LTP induction. In this study, we further showed that activation of D1/D5 receptors induced LTP, and the action was occluded by both cAMP-induced potentiation and tetanus-induced late-phase LTP. Taken together, we suggest that activation of PKA during or shortly after tetanic stimulation is important for late-phase LTP maintenance. These results showing that spinal application of D1/D5 receptor antagonist SCH23390 at 30 min after LTP induction did not affect the spinal LTP support this notion.

**FIG. 7.** Removing descending pathways by spinalization does not prevent induction of late-phase LTP. In spinalized rats, tetanic stimulation (0.5-ms duration, 40 V, 100 Hz, given in 4 trains of 1-s duration at 10-s interval), indicated by upward arrow, induced LTP of C-fiber–evoked field potentials, lasting for at least 4 h.
There is evidence showing that a protein synthesis inhibitor (cycloheximide) dose-dependently inhibits formalin-induced spinal c-Fos protein and tonic nociceptive responses (Hou et al. 1997). Our previous work has shown that the late phase of LTP of C-fiber-evoked field potentials in the spinal dorsal horn is protein synthesis–dependent (Hu et al. 2003). This work further showed that activation of D1/D5 receptors induced late-phase LTP. This action may contribute to hyperalgesia produced by intensive noxious stimulation or nerve injury.

REFERENCES


