Robust Changes of Afferent-Induced Excitation in the Rat Spinal Dorsal Horn After Conditioning High-Frequency Stimulation

HIROSHI IKEDA, TATSUYA ASAI, AND KAZUYUKI MURASE
Department of Human and Artificial Intelligence Systems, Fukui University, Fukui 910, Japan

Ikeda, Hiroshi, Tatsuya Asai, and Kazuyuki Murase. Robust changes of afferent-induced excitation in the rat spinal dorsal horn after conditioning high-frequency stimulation. J. Neurophysiol. 83: 2412–2420, 2000. We investigated the neuronal plasticity in the spinal dorsal horn and its relationship with spinal inhibitory networks using an optical-imaging method that detects neuronal excitation. High-intensity single-pulse stimulation of the dorsal root activating both A and C fibers evoked an optical response in the lamina II (the substantia gelatina) of the dorsal horn in transverse slices of 12- to 25-day-old rat spinal cords stained with a voltage-sensitive dye, RH-482. The optical response, reflecting the net neuronal excitation along the slice-depth, was depressed by 28% for more than 1 h after a high-frequency conditioning stimulation of A fibers in the dorsal root (3 tetani of 100 Hz for 1 s with an interval of 10 s). The depression was not induced in a perfusion solution containing an NMDA antagonist, dl-2-amino-5-phosphonovaleric acid (AP5; 30 μM). In a solution containing the inhibitory amino acid antagonists bicuculline (1 μM) and strychnine (3 μM), and also in a low Cl⁻ solution, the excitation evoked by the single-pulse stimulation was enhanced after the high-frequency stimulation by 31 and 18%, respectively. The enhanced response after conditioning was depotentiated by a low-frequency stimulation of A fibers (0.2–1 Hz for 10 min). Furthermore, once the low-frequency stimulation was applied, the high-frequency conditioning could not potentiate the excitation. Inhibitory transmissions thus regulate the mode of synaptic plasticity in the lamina II most likely at afferent terminals. The high-frequency conditioning elicits a long-term depression (LTD) of synaptic efficacy under a greater activity of inhibitory amino acids, but it results in a long-term potentiation (LTP) when inhibition is reduced. The low-frequency preconditioning inhibits the potentiation induction and maintenance by the high-frequency conditioning. These mechanisms might underlie robust changes of nociception, such as hypersensitivity after injury or inflammation and pain relief after electrical or cutaneous stimulation.

INTRODUCTION

The long-term modification of primary-afferent neurotransmission in the spinal dorsal horn is now believed to play an essential role in nociceptive plasticity (Dubner and Basbaum 1994; Randić 1996; Sandkühler 1996a,b; Treede et al. 1992; Woolf 1994). Intracellular (Jefimja and Urban 1994; Randić et al. 1993; Sandkühler 1996a,b; Sandkühler and Rankić 1997; Sandkühler et al. 1997; Woolf et al. 1998) and field-potential analysis (Dickenson et al. 1997; Liu and Sandkühler 1995) have demonstrated that brief high-frequency stimulation to the dorsal root at the intensity that activates both A and C primary-afferent fibers in the dorsal root either potentiates or depresses EPSPs evoked by C fibers in lamina II neurons for >1 h. These conventional analyses, however, have revealed the nature of only the first-order afferent synaptic transmissions to the second-order lamina II neurons (Liu and Sandkühler 1995; Schouenborg 1984). Because lamina II neurons may be excitatory or inhibitory (Willis and Coggeshall 1991; for review Ma et al. 1997; Yoshimura and Nishi 1995), it remains to be elucidated whether the conditioning stimulation leads to enhanced or suppressed expression of afferent information in the dorsal horn and how the long-term potentiation (LTP) and long-term depression (LTD) of afferent synapses contribute to it.

Heterosynaptic LTP and LTD of afferent-evoked EPSPs are also known to take place in the dorsal horn. A large portion of lamina II neurons receive glutamatergic excitatory synaptic input from both C fibers and Aδ fibers (Cervero et al. 1976; Gregor and Zimmermann 1972; Schneider and Perl 1988; Willis and Coggeshall 1991; Yoshimura and Jessell 1989). Conditioning high-frequency stimulation of Aδ fibers in the dorsal root induces LTD of C fiber-evoked field potential in anesthetized rats, whereas the same conditioning stimulation induces LTP in spinalized animals (Liu et al. 1998). We have shown that, by using an optical method in a spinal cord slice preparation, low-frequency conditioning of A fibers indeed depresses the net neuronal excitation along the slice-depth in the lamina II (Ikeda et al. 1999). We provided evidence indicating that the primary target of the plastic synapses is excitatory interneurons in the lamina II. We also demonstrated that inhibitory transmission mediated by opioids, but not by inhibitory amino acids, contributes to the LTD induction.

As a follow-up to the study on the dorsal horn plasticity induced by low-frequency conditioning, we report the properties of neuronal plasticity induced by high-frequency conditioning. By using the same optical-recording method with a voltage-sensitive dye in spinal cord slices (Ikeda et al. 1998a), we investigated the following: 1) whether or not the high-frequency conditioning stimulation known to induce plastic changes in afferent-induced EPSPs modifies the neuronal excitation in the dorsal horn, especially in the lamina II; 2) whether the plastic changes could be mediated by heterosynaptic mechanisms; 3) how inhibitory transmitters in the dorsal horn contribute to such robust changes; and 4) how low-frequency conditioning interacts with the plasticity induced by high-frequency conditioning. The present results have already appeared in abstract form (Ikeda et al. 1997, 1998b).

METHODS

The preparation, apparatus, and data processing for the optical imaging were identical to those of our previous studies (Ikeda et al. 1998b).
Preparation

Transverse slices (400–500 \(\mu\)m thick) with dorsal roots attached (5–10 mm in length) were prepared from lumbosacral enlargements of 12- to 25-day-old Sprague-Dawley rat spinal cords, which is described elsewhere (Murase and Randić 1983). A slice stained with a voltage-sensitive absorption dye, RH-482 (0.1 mg/ml, 20 min), was set in a submersion-type chamber (0.2 ml) on an inverted microscope (IMT, Olympus, Tokyo) equipped with a 150-W halogen lamp. The slice was perfused with Ringer solution containing (in mM) 124 NaCl, 5 KCl, 1.2 KH2PO4, 1.3 MgSO4, 2.4 CaCl2, 26 NaHCO3, and 10 glucose (oxygenated with 95% O2-5% CO2) at room temperature (20–24°C). The reason for using a relatively low temperature during recording was to minimize a gradual decline of neuronal excitation for a period of >3 h, especially in the presence of inhibitory amino acid antagonists.

The RH-482 (NK-3630) dye was obtained from Nippon Kanko Shikiso (Okayama, Japan) and the \(\Omega_{\text{A}}\)-2-amino-5-phosphonovarlic acid (AP5), bicuculline methiodide, strychnine hemisulfate, and naloxone were obtained from Sigma (St. Louis, MO). Each of these chemicals was dissolved in distilled water at high concentration, divided into aliquots, and kept frozen at −40°C until use. The aliquots were dissolved in the bathing solution at known concentrations during experiments, and the perfusion solution of the slice was switched to the drug-containing solution for a fixed period.

Optical recording

The light absorption change in a 0.55- or 0.83-mm2 area in the dorsal horn at a wavelength of 700 ± 32 nm was recorded by an imaging system (SD1001, Fuji Film Microdevice, Tokyo) with 128 \times 128-pixel photo sensors at a frame rate of 0.6 ms (Ikeda et al. 1993). Thirty-two single pulses were given to the dorsal root at a constant interval of 12–15 s. Starting at 10 ms before each stimulus, 128 consecutive frames of the light-absorption images were taken by the image sensor with a sampling interval of 0.6 ms. The reference frame, which was taken immediately before each series of 128 frames, was subtracted from each of the subsequent 128 frames. Thirty-two series of such difference images were averaged and stored in the system memory. We determined the initial frame by averaging the first 15 frames of the difference image and subtracting this from each of the 128 frames of the image data on a pixel-by-pixel basis to eliminate the effects of noise contained in the reference frame. The ratio image was calculated by dividing the image data by the reference frame. In most cases, the ratio image was filtered by a three-point moving average over time (see Ikeda et al. 1998a for detail).

The nominal spatial resolution was a 4.3 and 6.5 \(\mu\)m2 area per pixel (\(\mu\)m2/pixel) when a 550 and 830 \(\mu\)m2 area was viewed by the sensor with 128 × 128 pixels, respectively. However, the actual area that each pixel of the photosensor detected was larger as a result of various factors such as the optical depth of focus and the scattering of light in the slice and in the perfusate (Hopp et al. 1990). We therefore analyzed the spatial distribution of light intensity detected by the sensor when a thin opaque metal edge was placed on the slice surface. We estimated that the space constant of light diffusion was <17 and 13 \(\mu\)m (or 4 and 2 pixels) at the nominal resolution of 4.3 and 6.5 \(\mu\)m2/pixel, respectively (Ikeda et al. 1998a).

Dorsal root stimulation

The dorsal root was stimulated by a glass suction electrode. The types of primary afferent fibers activated by the electrical stimuli were identified initially by the field potentials recorded by a glass micro-electrode positioned either in the superficial dorsal horn or at the entry zone of the root, which was described previously (Ikeda et al. 1998a).

The single current-pulse stimulation of the dorsal root elicited the following optical responses in the dorsal horn (Ikeda et al. 1998a): 1) a brief (<3 ms) and small, almost undetectable, response is evoked at the entry zone of the dorsal root and occasionally in the deep dorsal horn by a 0.05-mA current pulse of 0.05 ms duration; 2) an optical response of longer duration (<100 ms) appeared in the lamina I extending to the outer part of the lamina II, the lamina III, and deeper laminae by increasing the stimulus intensity to 0.1 mA; and 3) the generation of an intense, prolonged (>200 ms) response in the superficial laminae I–III, most prominently in the lamina II with additional increases in intensity (>0.3 mA) and/or duration (>0.5 ms). The long-lasting response in the lamina II is delayed because of the latency corresponding to the slow conduction velocity of C fibers (<1 m/s). The onset of optical responses in the lamina I and the lamina III or deeper laminae elicited by any of these conditions takes place within one image frame (i.e., the latency is <0.6 ms). The conduction velocity of fibers responsible for the induction of the immediate response should be faster than 6 m/s (dorsal root length of 4 mm/0.6 ms), which corresponds to the conduction velocity of A fibers. These spatial and temporal profiles of optical responses agree fairly well with the cytoarchitectonic organization of the dorsal horn (Fitzgerald 1989; Fyéffe 1984; Sugitani 1994; Willis and Coggeshall 1991), giving additional indications of the fiber types activated by the stimuli.

The activation conditions consisted of a 0.05-mA current pulse of 0.05 ms duration for Aa\(b\)/\(b\) fibers (A fibers other than A\(a\) fibers)), a 0.1-mA current pulse of 0.05 ms duration (low-intensity stimulation) for Aa\(b\)/\(b\) fibers (all types of A fibers), and a 1.5-mA current pulse of 0.5 ms duration (high-intensity stimulation) for A and C fibers. These conditions were similar to those used in other studies (Liu and Sandkühler 1997; Randić et al. 1993; Sandkühler et al. 1997; Schneider and Perl 1988; Yoshimura and Jessell 1989).

It should be noted however that in time traces of the optical response shown in the figures in this study, the early component induced by A fibers was not always clear because signals from ~30–100 pixels of the image sensor were averaged to yield each time trace, which represented the spatial average of excitation over an area of slice. As we have reported (Ikeda et al. 1998a), the optical response induced by A fibers was small and very sparse over the slice so that at the image sensor single pixels exhibiting the response were surrounded by ones with no response. In contrast, the response induced by C fibers was intense and could be seen in all near pixels. As a result, in the time trace averaged over all pixels corresponding to an area of the slice, the component induced by A fibers became very small in comparison to that by C fibers, and it was often invisible in the time trace. An example could be seen in Fig. 1A. Immediately after the dorsal-root stimulation that activated both A and C fibers (Fig. 1, A), a small hump was induced in the optical response in the lamina III (Fig. 1, trace D) although the magnitude was about the level of noise. It was followed by a much larger C fiber-induced response, which was very much prolonged in the lamina II (Fig. 1, trace S) but shorter in duration in the lamina III (Fig. 1, trace D).

RESULTS

Optical response evoked after single dorsal root stimulation

We have reported that high-intensity single-pulse stimulation of the dorsal root (a current pulse of 1.5 mA with a duration of 0.5 ms), which activates both A and C fibers in the dorsal root, evokes an increase in the light absorption of as much as 0.2% in the dorsal horn in rat spinal cord slices stained with RH482 (Ikeda et al. 1998a). The spatiotemporal distribution and time courses of the representative optical response can be seen in Fig. 1A. The duration of optical response largely...
depended on the location (see METHODS). The response was long-lasting (>200 ms) in the superficial laminae I–III, while the duration was shorter in the deeper laminae and at the entry zone of the afferent fibers. The prolonged response in the lamina II was delayed whereas the response occurred immediately after the stimulus in the other laminae. The optical response was stable for $3 \text{ h}$; the decline in magnitude over $3 \text{ h}$ of recording was on average $4\%$. The results presented here were obtained by optical recordings performed on 101 transverse slices of 12- to 25-day-old rat spinal cords.

Robust changes after conditioning high-frequency stimulation

The excitatory optical response in the dorsal horn evoked by the high-intensity stimulation underwent robust changes for $>1 \text{ h}$ after high-frequency stimulation with low-intensity pulses that activated all types of A fibers (3 tetani of 0.1-mA current pulses with a duration of 0.05 ms at a frequency of 100 Hz for 1 s with an interval of 10 s). After the conditioning, the excitation was suppressed in the lamina II but enhanced in the deeper dorsal horn (laminae III–IV) for $>30 \text{ min}$ in the majority of the tested slices (27 of 34 slices). No change was observed in the remaining seven slices.

An example of the optical responses before and after high-frequency stimulation is illustrated with a pseudo-color in Fig. 1A. The time courses averaged over the lamina II region and the deeper dorsal horn region (areas S and D) taken every 15 min, respectively. Bottom graphs are averages of such records obtained in 4 different slices. SE of each point is indicated with a vertical bar. Numbers 1–4 on top graphs indicate records from which traces 1–4 in A were taken. Times when conditioning high-frequency stimulation was given are indicated with an arrow in each figure. Each magnitude is shown as percentage control of the spatiotemporal average over area ($30 – 100\text{ pixels}$) during a period of 12 ms (20 frames) after onset of response.

FIG. 1. Robust changes of the optical response after high-frequency conditioning to A fibers. A: optical responses were elicited by a single-pulse test stimulation (a current pulse of 1.5 mA with a duration of 0.5 ms, activating A and C fibers) to the dorsal root immediately before and at 75 min after conditioning high-frequency stimulation (3 tetani of 100 Hz for 1 s with an interval of 10 s, current pulses of 0.1 mA with a constant duration of 0.05 ms, activating A fibers) in a transverse slice stained with a voltage-sensitive dye, RH-482. Left top and bottom rows: optical responses before and after high-frequency stimulation with a pseudo-color, respectively. Time after test stimuli is indicated above each column. In schematic drawing of a transverse slice at top right, the area where images were taken is indicated by a rectangle. There are 2 lines within the rectangle, top being the border between white and gray matter and bottom that between lamina II and deeper laminae. These borders were visually identified by the transparency of slice in pictures taken during recording. Graph lines beside schematic drawing illustrate superimposed time courses of optical responses immediately before (thin lines) and 75 min after (bold lines) high-frequency stimulation at 2 different locations in the dorsal horn, lamina II and deeper dorsal horn, designated as S and D, which are indicated in schematic drawing. These responses are spatial averages recorded in all pixels present in area ($60 \text{ pixels}$). $\triangle$: time when test stimulus was given. B and C: top graphs show magnitudes of optically recorded excitation at lamina II and deeper dorsal horn (areas S and D) taken every 15 min, respectively. Bottom graphs are averages of such records obtained in 4 different slices. SE of each point is indicated with a vertical bar. Numbers 1–4 on top graphs indicate records from which traces 1–4 in A were taken. Times when conditioning high-frequency stimulation was given are indicated with an arrow in each figure. Each magnitude is shown as percentage control of the spatiotemporal average over area ($30 – 100\text{ pixels}$) during a period of 12 ms (20 frames) after onset of response.
intense excitation did not seem to be altered while the magnitude of excitation was modified throughout the lamina II by a certain ratio (Fig. 1A), although the quantitative analysis was not performed.

High-frequency conditioning with much lower intensity pulses (0.05 mA or less current pulses of 0.05-ms duration), which presumably activated only Aαβ fibers, failed to induce any robust changes. High-frequency stimulation with high-intensity pulses that activated C fibers in addition to A fibers (1.5-mA current pulses of 0.5-ms duration) produced variable results: suppression, no change, or a slight augmentation in the lamina II (n = 2, 4, and 1, respectively). This was expected, based on the result obtained in the previous intracellular study where both the LTD and LTP of afferent-evoked EPSPs were induced by the high-intensity conditioning (Randić et al. 1993), and the optical recording method used here detected the net postsynaptic events after such plastic changes at afferent synapses. Because the high-frequency stimulation with low intensity activating all types of A fibers (0.1-mA current pulse of 0.05-ms duration) was the only condition that could induce consistent effects, we proceeded to analyze the properties of robust changes induced by low-intensity high-frequency conditioning in this series of experiments.

Time course of suppression

The time courses of the magnitude of the optical responses before and after conditioning low-intensity high-frequency stimulation are shown in Fig. 1, B and C. The time courses were taken from two different areas, the lamina II and the deeper dorsal horn (laminae III–IV; S and D, respectively).

Here, the magnitude of an optical response refers to a spatio-temporal average value of the optical response. Values of ~30–100 pixels in an area where the response was strongly elicited by the high-intensity stimulation were averaged for each frame of the recorded image, yielding a spatially averaged time series of the optical response in the area. The time series was further averaged along the time axis for a period of 12 ms (20 frames) after the onset of response. The percent control values were obtained by dividing each point of the time series with the average of the values for the records taken before high-frequency stimulation and plotting it against the time.

After the high-frequency stimulation, the magnitude of optical response at the lamina II gradually decreased, reaching the maximum depression (~60% of the control) at 40–60 min and persisted at this level for a minimum of an additional 30 min (Fig. 1B, top graph). However, at the deeper dorsal horn, the excitation was enhanced gradually and reached the maximum of ~130% of control at 40 min after conditioning (Fig. 1C, top graph).

The bottom graphs in Fig. 1, B and C, illustrate the averaged time courses of the magnitude obtained in five slices. They were taken from two different areas similar to the S and D areas in the inset of Fig. 1A, respectively. The averaged percentage control values were 73 ± 3% (SE) at 60–100 min (n = 19). The variation in the control levels during the 0–30 min before the conditioning stimulus was 100 ± 3%. The values at the deeper dorsal horn were 129 ± 4% at 60–100 min (n = 19), and the values for controls were 100 ± 14%.

There was a large variability of the control response in the deeper dorsal horn among slices and we were unable to obtain

FIG. 2. Effects of high-frequency conditioning on optical response in solution containing NMDA or opioid antagonist. A: effects of NMDA antagonist 2-amino-5-phosphonovaleric acid (AP5) are illustrated. Top: shaded region in schematic drawing shows area where optical response was averaged. Averaged responses in lamina II taken at timepoints 1–3 described below are illustrated at right. Optical responses were elicited by single-pulse test stimuli every 15 min in a slice and time course of magnitude is illustrated in middle graph. First high-frequency conditioning stimulation (arrow) given during 15-min perfusion with an AP5-containing solution (30 μM; bold bar) was not effective at suppressing optical response. Second high-frequency conditioning stimulation (arrow) given after wash produced suppression. Numbers 1–3 on trace correspond to records 1–3 at top. Bottom graph shows averages and SE of results obtained in different slices. In these graphs test stimulus and high-frequency stimulation are shown with a filled triangle and an arrow, respectively. Denotations here apply to all graphs in Figs. 2–5, unless otherwise noted. B: high-frequency conditioning stimulation (arrow) given during 15-min application of naloxone-containing solution (0.5 μM; bold bar) produced a suppression in magnitude of optical responses in lamina II.
Effects of antagonists for NMDA and opioid receptors

We studied the effects of the NMDA-receptor antagonist AP5 applied at the time of the conditioning high-frequency stimulation to A fibers. As shown in Fig. 2A, the high-frequency stimulation during the perfusion of an AP5-containing solution (30 μM) did not alter the magnitude of the optical response, whereas the high-frequency stimulation given after the AP5 wash produced suppression. The presence of an opioid antagonist, naloxone, during the high-frequency stimulation did not inhibit suppression induction at low concentration (0.5 μM; Fig. 2B). This concentration has been reported to block the suppression induced by low-frequency conditioning (Ikeda et al. 1999); however, the application of naloxone at high concentration (5 μM) did inhibit the induction. The averaged percentage control values of the optical responses after the treatments with these agents obtained in different slices were 103 ± 6% for AP5 and 76 ± 9% after the removal of AP5 (n = 10), 67 ± 13% for 0.5 μM naloxone (n = 10), and 101 ± 3% for 5 μM naloxone (n = 6), at 40–60 min after low-intensity high-frequency stimulation.

Effects of antagonists for inhibitory amino acids

In the perfusate containing both GABA_A-receptor antagonist biccuculline methiodide (1 μM) and glycine-receptor antagonist strychnine hemisulfate (3 μM), the optical response in the lamina II was augmented after the low-intensity high-frequency stimulation in all of the tested slices (n = 12; Fig. 3A). The average percentage control value of the optical response after the treatments with these antagonists obtained in 12 different slices was 131 ± 4%. In the presence of one of the antagonists the conditioning stimulation elicited variable changes in the excitation in the form of slight suppression or slight enhancement (n = 8).

Effects in a low-Cl\(^{-}\) solution

Because GABA and glycine act on chloride channels in the dorsal horn neurons (Yoshimura and Nishi 1995), the effect of high-frequency stimulation was studied in a low-Cl\(^{-}\) solution in which the NaCl of the bathing Ringer solution was completely replaced with Na-glucuronate. After the perfusion of the low-Cl\(^{-}\) solution, the form of optical response induced by the test single-pulse stimulation was changed (Fig. 3B). The magnitude of the prolonged component became smaller while the initial part became more prominent and the fluctuation at the prolonged part seemed to be less. These might indicate that a stronger postsynaptic excitation was induced immediately after the C-fiber activation, but the long-lasting intermittent burst of firing and/or the prolonged increase in the excitability was less in the low-Cl\(^{-}\) solution.

The optical response in the low-Cl\(^{-}\) solution was not suppressed at all but rather augmented after the conditioning high-frequency stimulation (Fig. 3C). The averaged percentage control value of the optical response obtained in four different slices was 118 ± 16%. Under this condition, the reversal potential for Cl\(^{-}\) was raised 72 mV in accordance with the Nernst equation (58 log [[Cl\(^{-}\)]_{ext} before replacement]/[[Cl\(^{-}\)]_{ext} after replacement]) = 58 log [131.4 mM/7.4 mM]) to approximately +2 mV from the presumed original level of −70 mV (Yoshimura and Nishi 1995). In the low-Cl\(^{-}\) solution containing biccuculline (1 μM) and strychnine (3 μM), the excitation in the lamina II was enhanced after the high-frequency conditioning as well (126 ± 4%, n = 4, at 30–45 min after conditioning).

Because GABA may activate K\(^{+}\) channels in dorsal horn neurons (Kangrga et al. 1991), we also examined the effect of
high-frequency stimulation in various external K\(^+\) concentrations (1.75–12.5 mM). We found, however, that high-frequency stimulation always induced suppression of the excitation in the lamina II.

Effects of low-frequency stimulation in normal solution

Conditioning low-frequency stimulation to A fibers has been shown to suppress the excitation in the lamina II in normal external Ringer solution (Ikeda et al. 1999). We have investigated the effect of low-frequency stimulation given before high-frequency conditioning. The example can be seen in Fig. 4. The magnitude of optical response induced by single-pulse stimulation was depressed after low-frequency stimulation to A fibers (0.1-mA current pulses of 0.05-ms duration at 0.2 Hz for 10 min; bold bar in the figure). The high-frequency stimulation (arrow) given at 90 min after the low-frequency stimulation produced either no significant change in excitation or a slight suppression. The averaged percentage control values of the optical response obtained in four different slices was 79 ± 6% at 40–60 min after the low-frequency preconditioning and 75 ± 10% at 30–45 min after the high-frequency conditioning with respect to the control responses taken before the preconditioning.

Effects of low-frequency stimulation in inhibitory amino acid antagonists

We also investigated the effect of low-frequency conditioning in the presence of the antagonists for inhibitory amino acids. As described in the previous sections (e.g., Fig. 3A), the excitation in the lamina II was enhanced after high-frequency stimulation in the perfusate containing the GABA\(_A\)-receptor antagonist bicuculline methiodide (1 \(\mu\)M) and the glycine-receptor antagonist strychnine hemisulfate (3 \(\mu\)M). Once low-frequency preconditioning was introduced however, high-frequency stimulation was unable to augment the excitation, as shown in the example in Fig. 5A. The excitation depressed after low-frequency preconditioning (Fig. 5A, bold bar) was not at all enhanced (if it showed any response, it was suppressed) after the high-frequency conditioning (Fig. 5A, arrow). The averaged percentage control values of the optical response obtained in five different slices was 63 ± 10% at 45 min after the low-frequency preconditioning and 52 ± 9% at 30–45 min...
after the high-frequency conditioning, with respect to the control responses taken before the preconditioning.

The excitation enhanced by high-frequency stimulation (Fig. 5B, arrow) was returned to the original level by low-frequency stimulation (Fig. 5B, bold bar) in the solution containing antagonists. In an average of seven different slices, the excitation was enhanced to 146 ± 1% of control at 45 min after high-frequency stimulation and depressed back to 107 ± 9% at 30–45 min after low-frequency stimulation.

DISCUSSION

This study demonstrated that in the lamina II of slices of the spinal dorsal horn, the excitation elicited by afferent A- and C-fiber stimulation is suppressed robustly after conditioning high-frequency stimulation to A fibers. The conditioning high-frequency stimulation is ineffective in producing the suppression if AP5 is present in the perfusate or if preconditioning with low-frequency stimulation is introduced in advance. In the perfusate containing both bicuculline and strychnine, or in a low-CI− solution, high-frequency stimulation produces robust enhancement of the excitation in the lamina II. The low-frequency stimulation to A fibers inhibits the induction and the maintenance of the high-frequency stimulation-induced enhancement in the presence of the inhibitory amino acid antagonists.

Origin of optical response

The optical response induced by high-intensity single-pulse stimulation, which was used here as the test stimulation, primarily reflects the postsynaptic excitation evoked by C-fiber activation (Ikeda et al. 1998a). As we discussed previously (Ikeda et al. 1999), the optically recorded excitation in the lamina II is a sum of a variety of events. High-intensity single-pulse dorsal root stimulation activating afferent C fibers is known to induce a long-lasting (50 ms or longer) depolarizing response in lamina II neurons both in vivo (e.g., Mendell and Wall 1965; Wall et al. 1979) and in vitro (e.g., Gerber et al. 1991; King and Lopez-Garcia 1993; Schneider and Perl 1988; Urban and Randić 1984; Urban et al. 1994; Yoshimura and Jessell 1989) (see Willis and Coggeshall 1991 and Yoshiura 1996 for review). The response is often associated with a burst of action-potential firings and synaptic bombardment as well as an increase in membrane excitability. The low-intensity dorsal-root stimulation activating only A fibers, in contrast, produces short-lasting postsynaptic responses of ≤10 ms. The optical response evoked by the high-intensity stimulation observed in this study largely reflected such long-lasting postsynaptic neuronal activities because the optical response represents the net membrane potential change along the slice depth produced by action potentials, synaptic potentials, etc. This is in contrast to field potentials, which reflect the net synaptic current induced in the second-order neurons by the afferent stimulation (Liu and Sandkühler 1997; Schouenborg 1984).

Synaptic plasticity underlying robust changes of neuronal excitation

The long-lasting excitatory optical response in the lamina II elicited by high-intensity stimulation was suppressed for at least 1 h after high-frequency stimulation of A fibers in a majority of the tested slices. Because the conditioning stimulation was ineffective in the presence of AP5, the plastic change of transmission efficacy likely took place in glutamatergic synapses. This accords well with a previous field-potential analysis, where the conditioning stimulation is shown to produce heterosynaptic transmission of C-afferent fibers within the lamina II in anesthetized animals, and AP5 effectively inhibited the LTD induction (Liu et al. 1998).

In the perfusate containing the inhibitory amino acid antagonists bicuculline and strychnine, the neuronal excitation in the lamina II was enhanced after the high-frequency stimulation of A fibers. It is difficult to believe that a robust disinhibition of inhibitory interneurons could be responsible for the enhancement because receptors for the primary inhibitory transmitters in the region, GABA and glycine, were blocked already by the antagonists. We did not exclude the possible contribution of other putative inhibitory transmitters including serotonin, noradrenaline, adenosine, and somatostatin.

An alternative possibility is that high-frequency stimulation might induce LTD of afferent neurotransmission onto excitatory cells in the normal solution but LTP in the solution containing antagonists. The postsynaptic membrane potential has been found to determine whether the conditioning high-intensity high-frequency stimulation produces LTP or LTD of C-fiber-evoked EPSPs in lamina II cells, LTP at depolarized levels, and LTD at hyperpolarized levels (Randić et al. 1993). In the study of C-fiber-evoked field potentials in the lamina II, an LTD is induced in anesthetized rats with the low-intensity high-frequency conditioning, but an LTP occurs after the spinalization (Liu et al. 1998). This suggests that the descending inhibition mediated by GABA_A receptors regulates the mode of plasticity.

Neuronal circuitry regulating the synaptic plasticity

If the descending inhibition regulates the plasticity of afferent signal transmission via GABA_A receptors (Liu et al. 1998), the conditioning stimulation should have produced an enhancement of the excitation even in the normal solution, rather than the observed suppression, because the descending inhibition was absent in the slice preparation. However, dorsal horn neurons in vivo exhibit spontaneous EPSP and action-potential bombardments, whereas in the cells in slices these activities are absent or very infrequent (Liu and Sandkühler 1995; Schneider and Perl 1988; Woolf et al. 1988; Yoshimura and Jessell 1989). Dorsal horn neurons in slices are thus more hyperpolarized than neurons in situ (Murase and Randić 1983). It is possible that, in slices, the conditioning high-frequency stimulation would normally induce LTD, but it would produce LTP when inhibitory interneuronal connections are largely blocked and the afferent stimulation evokes large depolarizing responses in the entire dorsal horn. This hypothesis is supported by the present result that raising the reversal potential for Cl− ions prevented the induction of the suppression and instead led to robust enhancement after high-frequency stimulation. These results strongly indicated that the inhibitory amino acid-releasing activity of interneuronal elements regulates the mode of afferent synaptic plasticity by setting the membrane potential levels at the time of conditioning, that is, LTP and LTD at low levels.
and high levels, respectively (Artola et al. 1990; Pockett 1995; Randić et al. 1993), and that the regulation effectively controls the expression of sensory information in the lamina II.

High-frequency conditioning with higher intensity that additionally activated C fibers produced variable results; in some slices suppression and in others enhancement or no change of the neuronal excitation in the lamina II. In an intracellular study in a slice preparation, C fiber afferent-evoked EPSPs in lamina II cells are shown to undergo LTD or LTP with a nearly equal incidence after conditioning with a similar higher-intensity stimulation (Randić et al. 1993). By reflecting the imbalance in numbers of cells expressing LTD and LTP caused by the variability of slices, the net excitation along the slice depth recorded in this study could be suppressed or enhanced.

The excitation in the lamina III was rather enhanced after the low-intensity high-frequency conditioning, in contrast to the suppression in the lamina II. The long-term suppression of the excitation in the lamina II might be the cause for the long-term enhancement in the deeper laminae, possibly via inhibitory interneurons in the lamina II. Alternatively, afferent transmissions onto dendritic elements of deep dorsal horn neurons within the lamina II might be potentiated after the conditioning. The variability of response patterns among slices as well as low signal levels in the deeper laminae did not allow us to analyze the possible correlation of the differential effects in superficial versus deep dorsal horn in this series of experiments.

**Interaction between low-frequency and high-frequency conditioning stimulations**

The neuronal excitation in the lamina II is suppressed after low-frequency stimulation of A fibers (Ikeda et al. 1999). The suppression induction is inhibited by a low concentration of naloxone (0.5 μM) but not by the inhibitory amino acid antagonists bicuculline and strychnine, which is evidence indicating that the induction requires the activation of opioid receptors. Preconditioning with the low-frequency stimulation of A fibers inhibited the induction and maintenance of the high-frequency stimulation-induced augmentation in the inhibitory amino acid antagonists. The robust effect of the high-frequency conditioning was not inhibited by naloxone at the low concentration. A similar interaction has been reported in a field potential analysis where a burst stimulation of Aβ fibers depotentiated the high-frequency stimulation-induced potentiation (Liu et al. 1998).

It is interesting to know how the dorsal horn circuitry differentiates low- and high-frequency conditionings that are given to the same afferent fibers. Because they likely act via receptors for opiates and inhibitory amino acids, respectively, it is feasible that firing properties of enkephalin-containing neurons in the dorsal horn might be different from those of inhibitory amino acids-containing neurons. For example, the former could be sensitized gradually by low-frequency stimulation whereas the latter could fire at high frequencies without sensitization so that these conditioning stimulations might trigger a different series of events within the circuit. In support for the possibility, enkephalin-containing interneurons in the dorsal horn exhibit a characteristic morphology (for review see Willis and Coggeshall 1984), which might lead to the specific firing property.

**Robust control of nociception**

It is known that GABA and/or glycine, as well as opioids, play a large role in spinally mediated antinociception (Woolf 1994). A reduction in local segmental inhibitory mechanisms mediated by GABA and glycine receptors in the dorsal horn produces a sensitization similar to allodynia (Reeve et al. 1998; Sivilotti and Woolf 1994; Yaksh 1989). It has been suggested that the balance of inhibitory and excitatory inputs to dorsal horn neurons determines the induction of central sensitization (Dickenson et al. 1997; Woolf 1994). The present direct demonstration that inhibitory amino acids switch the mode of plasticity well explains both clinical and in vivo observations and suggests that the descending and/or segmental input to inhibitory interneurons sets the switch.

We found that a low-frequency stimulation to A fibers could reverse the high-frequency stimulation-induced potentiation and also that the high-frequency stimulation could not induce potentiation once the low-frequency stimulation was given. These findings may help to explain the pain relief resulting from A fiber stimulation introduced after (Ishimaru et al. 1995; Johnson et al. 1991; Melzack 1975; Melzack and Wall 1965) and before (Woolf and Chong 1993) painful stimulus is given.

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Address for reprint requests: K. Murase, Dept. of Human and Artificial Intelligence Systems, Fukui University, 3-9-1 Bunkyo, Fukui 910-8507, Japan.

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