High-Frequency Stimulation Together with Adrenoceptor Activation Facilitates the Maintenance of Long-Term Potentiation at Visual Cortical Inhibitory Synapses

Long-term potentiation (LTP) at inhibitory synapses of rat visual cortex requires firing of presynaptic cells for maintenance, at least at a low frequency. We examined the roles of adrenoceptors in this LTP maintenance. Although high-frequency stimulation (HFS) failed to produce LTP in normal Ca$^{2+}$ medium, it produced pathway-specific LTP with addition of noradrenaline to the medium soon after HFS. However, this LTP disappeared after washout of noradrenaline. HFS applied during noradrenaline application produced LTP persisting even after washout, indicating that HFS together with adrenoceptor activation makes the adrenergic facilitation enduring. After washout, LTP was produced further by HFS of the conditioned, but not the unconditioned, pathway by the first HFS. Pharmacological examination demonstrated that $\alpha_2$ and $\beta$, but not $\alpha_1$, receptors facilitated LTP maintenance synergistically. Bath application, but not postsynaptic loading, of either the adenylyl cyclase activator forskolin or the protein kinase C (PKC) activator phorbol ester facilitated LTP maintenance. These results suggest that adrenergic facilitation of LTP maintenance is mediated by presynaptic adrenoceptors via a subfamily of adenylyl cyclases stimulated by G$\alpha$, G$\beta$/$\gamma$, and PKC. Thus, it is likely that the activity of noradrenergic afferents takes part in the control of LTP duration at visual cortical inhibitory synapses.

Keywords: adrenergic, inhibitory synapses, LTP, maintenance, visual cortex

Introduction

Visual response properties are refined in an experience-dependent manner during postnatal development in visual cortex (Wiesel 1982; Frégnac and Imbert 1984). Noradrenergic afferents are likely to play a permissive role in this process (Kasamatsu and Pettigrew 1976; Bear and Singer 1986). Noradrenergic cells in locus ceruleus show spontaneous firing far more frequently during arousal than sleep (Aston-Jones and others 1990). During arousal, sensory stimulation produces firing in these cells (Aston-Jones and others 1990), and visual stimulation increases the release of noradrenaline in visual cortex (Marrocco and others 1987). These observations suggest that noradrenaline allows visual response properties to undergo long-lasting modification effectively when animals are looking at their visual environment attentively. The effect of noradrenaline on visual response plasticity can be mediated at least in part by modulation of long-term synaptic modification. Some papers have documented observations suggesting that adrenoceptors are involved in long-term potentiation (LTP) and long-term depression (LTD) at excitatory synapses of visual cortex (Brocher and others 1992; Kato 1993; Kirkwood and others 1999).

We found that LTP also occurs at inhibitory synapses in developing rat visual cortex (Komatsu and Iwakiri 1993) and that noradrenaline is involved in its production too (Komatsu 1996; Komatsu and Yoshimura 2000). This inhibitory LTP requires the transient elevation of Ca$^{2+}$ in postsynaptic cells in response to high-frequency stimulation (HFS) for induction, like most LTP at excitatory synapses (Komatsu 1996). This elevation likely resulted from phospholipase C (PLC) activation, inositol trisphosphate (IP$_3$) formation, and Ca$^{2+}$ release from internal Ca$^{2+}$ stores via IP$_3$ receptor activation. Noradrenaline is involved in this process through the activation of $\alpha_1$ adrenoceptors coupled with PLC.

The maintenance of this inhibitory LTP also requires neural activity, spike firing of presynaptic inhibitory cells at some frequency, which is lower than the frequency of test stimulation (0.1 Hz) usually used in LTP studies (Komatsu and Yoshimura 2000). This property has not been documented for long-term synaptic modifications other than this inhibitory LTP and N-methyl-D-aspartate receptor-independent LTP at visual cortical excitatory synapses (Liu and others 2004). According to our previous study on activity-dependent maintenance of inhibitory LTP in layer-5 cells (Komatsu and Yoshimura 2000), the maintenance is mediated by presynaptic Ca$^{2+}$ entry associated with action potentials through multiple types of Ca$^{2+}$ channels, which activates Ca$^{2+}$-dependent reactions different from those triggering transmitter release. The Ca$^{2+}$ entry is likely regulated by K$^+$ channels, presumably large-conductance Ca$^{2+}$-activated K$^+$ (BK) channels.

LTP occurred frequently at high extracellular Ca$^{2+}$ concentrations ([Ca$^{2+}$]$_o$), but rarely at normal [Ca$^{2+}$]$_o$ (Komatsu and Yoshimura 2000). When HFS was applied during a temporary reduction in [Ca$^{2+}$]$_o$ from high to normal levels, LTP occurred if high [Ca$^{2+}$]$_o$ was resumed immediately after HFS (upper diagram of Fig. 1A). On the other hand, LTP, produced at high [Ca$^{2+}$]$_o$, was abolished by a temporary reduction in [Ca$^{2+}$]$_o$, to the normal level and did not recover even after high [Ca$^{2+}$]$_o$ was resumed (lower diagram of Fig. 1A). Therefore, it is considered that the dependence of LTP on [Ca$^{2+}$]$_o$ is ascribed to the property of maintenance but not induction. The amount of Ca$^{2+}$ entry may be insufficient for LTP maintenance at normal [Ca$^{2+}$]$_o$. When noradrenaline was added to the perfusion medium during the whole recording period, LTP persisted at normal [Ca$^{2+}$]$_o$, suggesting that LTP maintenance is facilitated by adrenoceptor activation (Komatsu and Yoshimura 2000). Thus, the activity of noradrenergic afferents may regulate the duration of LTP and thereby persistence of visual responsiveness modified by visual experience.

The present study aims to characterize the adrenergic regulation of LTP maintenance at visual cortical inhibitory

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Figure 1. Noradrenaline application started soon after HFS enables the maintenance of LTP at normal [Ca\(^{2+}\)]\(_o\). (A) Schematic illustration of our previous finding indicating that LTP is induced, but not maintained, at normal [Ca\(^{2+}\)]\(_o\) (2.4 mM). The time course of changes in IPSP slope is shown schematically. The upper diagram shows that HFS applied during a temporary reduction in [Ca\(^{2+}\)]\(_o\) from high (4 mM) to normal values produces LTP when high [Ca\(^{2+}\)]\(_o\) is resumed immediately after HFS. The lower diagram illustrates that LTP produced at high [Ca\(^{2+}\)]\(_o\) is abolished by a temporary reduction in [Ca\(^{2+}\)]\(_o\) to the normal value. [Ca\(^{2+}\)]\(_o\) is 2.4 mM for the period indicated by gray, but it is 4 mM for the rest of the period. (B) Experimental arrangement of stimulating (S1 and S2) and recording electrodes (r), with the left-side figures and a dashed line indicating synapses and determine which subtypes of adrenoceptors are involved in this process and, furthermore, whether these receptors are located at pre- or postsynaptic sites. At normal [Ca\(^{2+}\)]\(_o\), HFS produced potentiation of inhibitory synaptic transmission lasting during noradrenaline application started immediately after HFS, but it disappeared after noradrenaline washout. This facilitatory effect of noradrenaline became long lasting when HFS was applied during noradrenaline application. Pharmacological analysis suggests that noradrenergic facilitation of LTP maintenance is mediated by the activation of presynaptic \(\alpha_2\) and \(\beta\) adrenoceptors in a synergistic way via cyclic AMP (cAMP) formation.

Materials and Methods

Sprague-Dawley rats at postnatal 20–29 days were deeply anesthetized with isoflurane before the whole brain was removed from the skull and immersed in an ice-cold oxygenated (95% \(\text{O}_2\) and 5% \(\text{CO}_2\)) artificial cerebrospinal fluid (ACSF) containing (in millimolar concentrations) 124 NaCl, 5 KCl, 1.5 MgSO\(_4\), 2.4 CaCl\(_2\), 1.2 KH\(_2\)PO\(_4\), 26 NaHCO\(_3\), and 10 glucose. Then, coronal slices of primary visual cortex (400-\(\mu\)m thick) were prepared using a Microslicer (DTK-1000, Dosaka, Kyoto, Japan) and kept in a recovery chamber perfused with ACSF at 33 °C. During the recording experiments, the medium, maintained at 33 °C, contained 100 \(\mu\)M N-\(\omega\)-2-amino-5-phosphonovaleric acid (APV), an NMDA receptor antagonist, and 40 \(\mu\)M 6,7-dinitroquinoxaline-2,3-dione (DNQX), a non-NMDA receptor antagonist. In some experiments, we used ACSF containing a high concentration (4 mM) of CaCl\(_2\).

Two pairs of bipolar stimulating electrodes (S1 and S2 in Fig. 1B) made of tungsten wires (diameter, 100 \(\mu\)m; interpolar distance, 2.0 mm) were placed in layer 4, separated from each other by about 0.5 mm. Layer 2–4 was surgically cut between S1 and S2 to ensure that separate groups of presynaptic fibers were activated. Test stimulation was applied alternately to S1 and S2 at intervals of 5 s. As a conditioning stimulation, HFS (50 Hz, 1 s) was applied to one of the electrodes 10 times at intervals of 10 s. The intensity of the test stimulation and HFS was adjusted to 1.5–2 and 5 times the threshold intensity, respectively, to evoke inhibitory postsynaptic potentials (IPSPs).

Intracellular recording was conducted with sharp glass pipettes containing 2 M K-methylsulfate (40–50 MΩ). In some of the experiments, the internal solution of the electrode contained 2% biocytin. For analysis, we selected cells with a stable resting membrane potential hyperpolarized more than \(-50\) mV. When the resting membrane potential was deeper than \(-60\) mV, and consequently IPSPs evoked by test stimulation were too small, the membrane potential was depolarized by current injection through the recording electrode in order to increase the amplitude of IPSPs. Input resistance was monitored throughout the experiments by injecting 0.05–0.1 nA hyperpolarizing current pulses. A bridge circuit was used to record the membrane potential while the current injection was administered through the recording electrode (Axoclamp 2A, Axon Instruments, Foster City, California). In the present study, drug application did not produce any significant changes (\(P > 0.05\)) in either the resting membrane potential or the input resistance, unless otherwise mentioned.

In experiments employing forskolin or phorbol 12,13-didecanoate (PDD), we used the blind-patch whole-cell recording method to record IPSPs. Patch pipettes (5–6 MΩ) were filled with a solution containing (in millimolar concentrations) 125 K-gluconate, 2 NaCl, 2 KCl, 3 MgCl\(_2\), 1 ethyleneglycol-bis(aminoethylether)-tetraacetic acid, 20 N\(_2\)-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 3 MgATP, 0.5 Na\(_2\)GTP, and 0.2% biocytin (pH 7.3 with KOH). For analysis, we selected cells, identified later as layer-5 pyramidal cells by histological examination, that had a high seal resistance (\(>1\) GΩ) and a series resistance <35 MΩ. The laminar location of the stimulating and recording electrodes was identified on histological sections stained with cresyl violet after the recordings, as described previously (Komatsu 1994). The procedure for biopsy staining was employed as described previously (Yoshimura and others 2005).

Data were expressed as mean ± standard error of mean (SEM), and either Student’s \(t\)-test or Welch’s test was applied. The compounds used were obtained from the following sources: APV and DNQX from Tocris (Bristol, United Kingdom); noradrenaline, methoxamine, clonidine, UK-14304, isoproterenol, rauwolscine, timolol, forskolin, PDD, and biocytin from Sigma (St. Louis, Missouri); and isoflurane from Abbott laboratories (North Chicago, Illinois).

Results

Intracellular recording studies were conducted in visual cortical slices of developing rats at postnatal 20–29 days, when LTP of IPSPs occurs frequently (Komatsu 1994). IPSPs evoked by layer-4 stimulation were recorded from layer-5 neurons under a pharmacological blockade of excitatory synaptic transmission. In most of the experiments, sharp electrodes were used to record IPSPs because stable recording was possible for longer periods compared with patch electrodes. However, in experiments in which compounds were loaded into recorded cells, we used patch electrodes instead. One of two stimulating electrodes placed in layer 4 was used to test the effect of HFS and the other served as a control (Fig. 1B). The control perfusion...
solution contained 2.4 mM of Ca\(^{2+}\), which is within the range of [Ca\(^{2+}\)]\(_o\) normally used. We employed strong HFS, which almost always produced LTP at high (4 mM) [Ca\(^{2+}\)]\(_o\) (Komatsu 1994). Some (n = 33) of the sampled cells in this study were recorded with sharp electrodes containing biocytin, and they were all identified as layer-5 pyramidal cells by a histological examination conducted later. Thus, it is likely that the sharp electrodes we used allowed us to stably record responses only from pyramidal cells for a time period long enough to analyze LTP, suggesting that the other morphologically unidentified cells were mostly pyramidal cells.

**Noradrenaline Facilitates LTP Maintenance**

Our previous study showed that LTP did not occur at normal [Ca\(^{2+}\)]\(_o\), and we concluded that HFS could induce but not maintain potentiation at normal [Ca\(^{2+}\)]\(_o\) based on analyses with changing [Ca\(^{2+}\)]\(_o\) (Komatsu and Yoshimura 2000), which is illustrated schematically in Fig. 1A. However, LTP occurred at normal [Ca\(^{2+}\)]\(_o\) when the solution contained noradrenaline (5 µM) during the whole recording period (Komatsu and Yoshimura 2000). We first tested the possibility that noradrenaline facilitates the maintenance of LTP. LTP did not occur at normal [Ca\(^{2+}\)]\(_o\), (Fig. 1C), consistent with our previous study. When we started noradrenaline application immediately after HFS, potentiation occurred in the pathway conditioned by HFS, with no changes in unconditioned pathway responses (Fig. 1D). This potentiation lasted without any obvious decline during noradrenaline application continued for 30 min. However, it gradually disappeared after starting washout of noradrenaline. Therefore, we consider that LTP persists at normal [Ca\(^{2+}\)]\(_o\), while noradrenaline application started soon after HFS is continued.

**Coapplication of Noradrenaline and HFS Produces Long-Lasting Facilitation of LTP Maintenance**

Although LTP persisted only during noradrenaline application in the experiment mentioned above, it persisted even after washout of noradrenaline when HFS was applied during noradrenaline application (Fig. 2). Thus, it is likely that the facilitatory effect of noradrenaline on LTP maintenance becomes enduring when HFS is applied during noradrenaline application. To test whether this long-lasting facilitation is specific to the synapses activated by HFS, we applied HFS again to either the conditioned or the unconditioned pathway after washout of noradrenaline. Figure 3A illustrates an example of those experiments testing this. Noradrenaline was washed out soon after HFS, which produced LTP. Forty minutes after HFS, the second HFS, applied to the pathway unconditioned by the first HFS, failed to produce LTP, indicating that noradrenaline was already washed out substantially. The third HFS, applied about 70 min after the first HFS to the pathway conditioned by the first HFS, produced further LTP.

Figure 3B,C summarizes these experiments conducted in eight cells. Forty to eighty minutes after the first HFS, HFS was applied to the initially conditioned pathway again in all the cells and, in addition, to the unconditioned pathway in five of the cells. The first HFS, applied during noradrenaline application, produced LTP in these cells (Fig. 3B). After washout of noradrenaline, LTP was always produced by HFS applied to the initially conditioned pathway (Fig. 3B), whereas it was never produced by HFS applied to the initially unconditioned pathway (Fig. 3C). With no noradrenaline application, both the first and the second HFS applied to the same pathway failed to produce LTP (Fig. 3D). Thus, we conclude that long-lasting facilitation of LTP maintenance occurs specifically at synapses activated by HFS during noradrenaline application.

**α₂ and β Adrenoceptors Are Involved in the Facilitation of LTP Maintenance**

To determine the receptors mediating the facilitatory effect of noradrenaline on LTP maintenance, HFS was applied in the presence of subtype selective adrenergic agonists, and those compounds were washed out soon after HFS (Fig. 4). The α₂-receptor agonist methoxamine, which may facilitate the induction of LTP (Komatsu 1996), affected neither baseline responses nor LTP production even at high concentrations (10 or 100 µM, Fig. 4A,E). The α₂-receptor agonist clonidine (50 µM) showed a weak facilitatory effect on LTP maintenance without affecting baseline responses (Fig. 4B,E). A similar facilitation was produced by 10 µM UK-14304 (Cambridge 1981), a more specific α₂ agonist (circles in Fig. 4E). The magnitude of LTP in the presence of either α₂ agonist was significantly greater (P < 0.001) compared with control but significantly less (P < 0.02) compared with noradrenaline (Fig. 4E).

The β-receptor agonist isoproterenol (10 µM) increased the test response significantly (P < 0.02, n = 8). The enhanced responses persisted even after washout, as shown by the control pathway responses (Fig. 4C). This lasting increase was at least in part ascribed to significant changes (P < 0.04) in the resting membrane potential and the input resistance (before, -56 ± 1.2 mV and 40 ± 3.2 MΩ, n = 8; after, -53 ± 1.6 mV and 43 ± 3.7 MΩ). HFS, which was applied after a stable baseline response was established, produced LTP in the test pathway with a magnitude slightly greater, but insignificant (P > 0.2), compared with clonidine. When these α₂ and β agonists were applied together (Fig. 4D,E), the magnitude of LTP was greater compared with either agonist alone and almost indistinguishable from that for
noradrenaline \((P > 0.9)\). Simultaneous application of these two agonists did not produce increases in test responses \((P > 0.1)\), which occurred when isoproterenol was applied alone. These results suggest that \(\alpha_2\) and \(\beta\) receptors act synergistically to enhance the maintenance of LTP.

To confirm that these receptors mediate the facilitatory effect of noradrenaline on LTP maintenance, HFS was applied in the presence of subtype-specific adrenergic antagonists together with 5 \(\mu\)M noradrenaline. Consistent with the effect of agonists, LTP was abolished by application of either 1 \(\mu\)M rauwolscine, an \(\alpha_2\)-receptor antagonist, or 20 \(\mu\)M timolol, a \(\beta\)-receptor antagonist (Fig. 5A–C). The change produced by HFS in the test responses at 40–50 min after HFS in either case was indistinguishable \((P > 0.1)\) from that in control solution (cf., Figs 4E and 5C).

LTP is maintained without noradrenaline application at high \([Ca^{2+}]_o\) (Komatsu and Yoshimura 2000). However, the activation of \(\alpha_2\) and \(\beta\) receptors by the endogenous level of noradrenaline might be sufficient for maintenance in that condition. To test this possibility, experiments were conducted in the presence of \(\alpha_2\) and \(\beta\) antagonists at high \([Ca^{2+}]_o\). In contrast to normal \([Ca^{2+}]_o\), neither \(\alpha_2\) nor \(\beta\) antagonists had any effect on LTP (Fig. 6A–C). There was no difference \((P > 0.6)\) in the magnitude of LTP in the presence of either antagonist, compared with the control value (Fig. 6E). Furthermore, LTP occurred in the simultaneous presence of very high doses of rauwolscine (5 \(\mu\)M) and timolol (100 \(\mu\)M) with a magnitude indistinguishable from the control value \((P > 0.4\); Fig. 6D,E). These results indicate that the activation of neither \(\alpha_2\) nor \(\beta\) receptors is required for the maintenance of LTP at high \([Ca^{2+}]_o\).

**Long-Lasting Facilitation of LTP Maintenance Enables HFS to Produce LTP without Activation of \(\alpha_2\) and \(\beta\) Receptors**

We then tested whether the activation of \(\alpha_2\) and \(\beta\) receptors is required for the production of LTP in the condition where LTP maintenance was facilitated long lastingly at normal \([Ca^{2+}]_o\) (Fig. 7). We applied HFS during noradrenaline application, washed out that compound immediately after HFS, and then added high doses of rauwolscine (5 \(\mu\)M) and timolol (100 \(\mu\)M) to

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**Figure 3.** Coapplication of HFS and noradrenaline enables the following HFS applied after noradrenaline washout to produce LTP. (A) An example case showing long-lasting facilitation of LTP maintenance. Noradrenaline was washed out after HFS was applied to S1. The following HFS, applied to S1 but not S2, produced LTP. The application time for HFS is indicated by arrows in the right figure, in which the initial falling slope of IPSPs for S1 (squares) and S2 pathways (triangles) was plotted against the time after the first HFS to S1. The horizontal bar (NA) shows the noradrenaline application period. (B, C) Summary of experiments illustrated in (A). In the left half of figures, IPSP slopes for S1 (squares) and S2 pathways (triangles) were plotted against the time after the first HFS to S1. Out of eight tested cells, HFS was applied to S1 in all of the cells (B) and to S2 in five cells (C) between 40 and 80 min after the first HFS to S1. In the right half of figures, IPSP slopes were plotted against the time after HFS applied to S1 (B) or S2 (C) subsequently to the first HFS. (D) Similar to (B), but the first and the second HFS were both applied to the same pathway (S1) without noradrenaline application \(n = 8\). The interval between the first and the second HFS was 40–80 min. The superimposed traces on the left show example IPSPs before and after the first HFS (top) and those before and after the second HFS (bottom).
The Location of α₂ and β Receptors Contributing to the Facilitation of LTP Maintenance

To determine whether noradrenaline exerts its facilitatory effect on the pre- or postsynaptic site, we utilized pharmacological modulation of the intracellular signal transduction pathways mediating the effect of adrenoceptor activation. In this particular experiment, responses were recorded with patch electrodes under current-clamp mode. Because the activation of β receptors is mediated by an increase in cAMP, we tested the effect of forskolin, which is a membrane-permeable adenyl cyclase activator, at normal [Ca²⁺]. Bath application of forskolin (10 μM) produced significant increases (P < 0.03) in the test response, reaching a steady level in 10–20 min, which lasted even after the termination of application. This lasting increase was at least in part ascribed to significant changes (P < 0.05) in the resting membrane potential and the input resistance (before, −58 ± 1.2 mV and 91 ± 5.0 MΩ; n = 9; after, −55 ± 1.6 mV and 96 ± 5.8 MΩ). HFS, applied at the steady level, produced LTP frequently, and the magnitude of LTP was significantly greater (P < 0.002) than that for cells recorded with patch electrodes in control solution (Fig. 8A,E). The effects of forskolin on basal responses and LTP were very similar to those of isoproterenol (cf. Fig. 4C,E), indicating that the effect of β-receptor activation is mediated by the elevation of cAMP.

The most familiar consequence of α₂-receptor activation is an inhibition of adenyl cyclases through G subunits of G proteins. However, forskolin indeed facilitated the maintenance of LTP, suggesting that the effect of α₂-receptor activation is mediated by signal transduction mechanisms other than adenyl cyclase inhibition. The activation of α₂ receptors facilitates β receptor-mediated activation of adenyl cyclase 2 through βγ subunits of G β proteins (Tang and Gilman 1991; Federman and others 1992; Lustig and others 1993; Mhaouty and others 1995). The formation of cAMP by this adenyl cyclase is also facilitated by protein kinase C (PKC) (Marjamaki and others 1997). It is likely that adenyl cyclase 4 and 7 have similar properties (Hanoune and Defer 2001). Therefore, to test whether these types of adenyl cyclases are involved in the facilitation of LTP maintenance, we examined the effect of a PKC activator. Application of phorbol ester PDD (10 μM), which is a membrane-permeable PKC activator, facilitated LTP production without any effects on the control pathway responses (Fig. 8B,E). The magnitude of LTP was significantly greater than it was in control solution (P < 0.03), but it was slightly less compared with forskolin, although the difference was insignificant (P > 0.5). These results are consistent with the hypothesis that α₂ and β receptors facilitate LTP maintenance synergistically through the activation of adenyl cyclase 2, 4, or 7.

Postsynaptic loading of either forskolin (100 μM) or PDD (100 μM) from patch electrodes did not allow HFS to produce LTP (Fig. 8C,E). To confirm that forskolin was sufficiently loaded into postsynaptic cells, the accommodation of spike firing in response to depolarizing current pulses was examined. Bath application of forskolin (10 μM) increased the number of spikes and decreased spike intervals in forskolin-unloaded cells (Fig. 9A) as was shown for hippocampal pyramidal cells (Madison and Nicoll 1986). This suppression of spike accommodation disappeared in forskolin-loaded cells (Fig. 9B). Bath application of forskolin produced a significant reduction (P < 0.02, n = 6) in spike intervals in control cells but no changes (P > 0.3, n = 7) in forskolin-loaded cells (Fig. 9C), indicating that
mediated by presynaptic facilitation the maintenance of LTP at inhibitory synapses. The present study demonstrated that noradrenaline application on LTP maintenance is mediated presynaptically.

Discussion

The present study demonstrated that noradrenaline application facilitated the maintenance of LTP at inhibitory synapses of layer-5 cells in the developing rat visual cortex. Although this facilitation was short lasting when noradrenaline was applied after HFS, it became long lasting when that application was combined with HFS. The effect of noradrenaline seems to be mediated by presynaptic α2 and β adrenoceptors in a synergistic way. HFS produces Ca2+ entry into presynaptic terminals and a resultant Ca2+ elevation, which may modulate the intracellular signal transduction following adrenoceptor activation, leading to long-lasting facilitation of maintenance specifically at the synapses activated by HFS. Previously, we demonstrated that LTP maintenance requires action potential-associated Ca2+ entry through multiple types of high-threshold (L, N, and P type) Ca2+ channels, different from those triggering transmitter release, at the presynaptic terminals, and this Ca2+ entry is likely regulated by BK channels (Komatsu and Yoshimura 2000) as illustrated schematically in Fig. 10. Thus, the modulation of these channels could contribute to the facilitation. Alternatively, the Ca2+ sensitivity of biochemical processes responsible for LTP maintenance can be reduced.

Adrenergic Facilitation of LTP Maintenance

Our previous study (Komatsu 1996) suggested that α1 adrenoceptors contribute to the induction of LTP through PLC activation, IP3 formation, and the resultant elevation of Ca2+ concentration in the postsynaptic cells (Fig. 10). On the other hand, the present study demonstrated that α2 and β receptors mediate the facilitation of LTP maintenance. The β-receptor agonist isoproterenol and adenylyl cyclase activator forskolin similarly produced the facilitation of LTP maintenance, indicating that the effect of β-receptor activation on the facilitation is mediated by the formation of cAMP. Thus, although α2-receptor activation often inhibits adenylyl cyclases via α subunits of G, proteins, this signaling pathway may not be involved in the facilitation. It is known that α2-receptor type 2 catalyzes cAMP formation in response to the activation of β receptors and Gβγ, which is facilitated by α2-receptor activation through Gβγ (Fig. 10; Tang and Gilman 1991; Federman and others 1992; Lustig and others 1993; Mhaouty and others 1995). This adenylyl cyclase is also facilitated by PKC (Marjamaki and others 1997). It is likely that adenylyl cyclase 4 and 7 also have similar properties (Hanoune and Defer 2001). Our results demonstrated a facilitation of LTP maintenance by PDD, a PKC activator, supporting the view that this type of adenylyl cyclase mediates the facilitatory action of α2 and β receptors. These three types of adenylyl cyclases are all expressed in the central nervous system (Defer and others 2000; Hanoune and Defer 2001), and, at present, it is uncertain which one is responsible for the facilitation.

In addition, we could not rule out the possibility that the effect of α2 receptors is mediated by other signaling pathways because it was suggested that α2 receptors are linked to PLC, which produces diacylglycerol that activates PKC, in some cases (Conklin and others 1992; Dorn II and others 1997). In that case, signal transduction pathways subsequent to these two adrenoceptors may converge on some of the steps to the common effector molecules or reach separate effector molecules, the modification of which is complementary for the facilitation of LTP maintenance.

Presynaptic Facilitation of LTP Maintenance

Our previous study showed that the maintenance mechanism of LTP is present at the presynaptic site (Komatsu and Yoshimura 2000). This suggests that noradrenaline exerts the facilitatory effects through adrenergic receptors located at the presynaptic site. Immunohistochemical studies suggested that α2 and β receptors are both located at postsynaptic sites and inhibitory presynaptic terminals in rat neocortex (Aoki and others 1987; Venkatesan and others 1996). Thus, to test whether presynaptic receptors contribute to this facilitation, we examined the effects of direct activation of signal transduction pathways following adrenoceptors. LTP maintenance was facilitated by bath application of forskolin and PDD, which are membrane permeable and may affect both pre- and postsynaptic sites, but not postsynaptic loading of these compounds. We confirmed that intracellular loading of forskolin sufficiently activated adenylyl cyclases of postsynaptic cells. These observations strongly support the supposition that presynaptic adrenoceptors are responsible for the facilitation.

However, it is known that α2 receptors are also located at noradrenergic, serotonergic, and cholinergic nerve terminals.

Figure 5. Effects of adrenergic antagonists applied together with noradrenaline on LTP production. (A) The α2-receptor antagonist rauwolscine (1 μM) was applied together with noradrenaline (5 μM) for the period indicated by the horizontal bar. Squares and triangles represent responses for test and control pathways, respectively. (B) Similar to (A), but for the β-receptor antagonist timolol (20 μM). The number of cells was 9 in both (A) and (B). (C) LTP magnitude, assessed at 40–50 min after HFS, for noradrenaline alone (NA), and rauwolscine (α2) and timolol (β) together with noradrenaline at normal [Ca2+]o. The asterisk indicates that the values were significantly (P < 0.05) different from those for noradrenaline.

forskolin was loaded sufficiently in our experimental condition. Therefore, it is likely that the facilitatory effect of α2 and β receptors on LTP maintenance is mediated presynaptically.
and their activation inhibits transmitter release (Moroni and others 1983; Starke and others 1989; Raiteri and others 1990; Feuerstein and others 1993; Tellez and others 1997). Thus, α₂ agonists and antagonists could affect LTP maintenance indirectly through the changes in these neuromodulatory transmitters. However, noradrenaline facilitates LTP maintenance, whereas α₂ agonists decrease noradrenaline release from the noradrenergic nerve terminals. Therefore, it is very unlikely that α₂ adrenoceptors at noradrenergic terminals contribute to LTP maintenance. Similarly, it is unlikely that α₂ receptors at serotoninergic nerve terminals are involved because serotonin also facilitates LTP production (Komatsu 1996). We could not rule out the involvement of cholinergic nerve terminals at present. In addition, α₂ and β adrenoceptors are also present in glia (Aoki and others 1987; Venkatasesan and others 1996). However, a simple interpretation of our data is that α₂ and β receptors, located both at the inhibitory presynaptic terminals, are responsible for the facilitation of LTP maintenance, which may easily explain their synergistic action.

The activation of adrenoceptors could finally upregulate L-, P-, and N-type Ca²⁺ channels and/or downregulate K⁺ channels involved in LTP maintenance (Fig. 10). It is well known that L-type Ca²⁺ channel activity in cardiac muscles is upregulated by β-receptor stimulation through cAMP-dependent protein kinase A (PKA)-mediated phosphorylation (McDonald and others 1994). A similar modulation was found in L- and P-type Ca²⁺ channels of central neurons (Gray and Johnston 1987; Kavalali and others 1997; Huang and others 1998). Noradrenaline also downregulates small-conductance Ca²⁺-activated K⁺ channels and A-type K⁺ channels, through the activation of β receptor and PKA in pyramidal cells (Madison and Nicoll 1986; Lancaster and Nicoll 1987; Foehring and others 1989; Pedarzani and Storm 1993; Hoffman and Johnston 1999). However, in these neurons noradrenaline failed to modulate BK channels (Lancaster and Nicoll 1987; Foehring and others 1989). At present, it is uncertain which channels are involved in the facilitation of LTP maintenance.

When HFS was combined with adrenoceptor activation, this facilitation became long lasting. Ca²⁺ signals resulting from HFS at the presynaptic terminals may interact with signal transduction pathways subsequent to adrenoceptor activation. Ca²⁺-dependent signaling molecules such as Ca²⁺-dependent adenylyl cyclases (Defer and others 2000; Hanoune and Defer 2001) and PKC could potentially be involved in this conversion into long-term effects.

The Site of LTP Expression

Visual cortical inhibitory LTP requires a rise of postsynaptic Ca²⁺ concentration for induction, whereas the maintenance mechanism is present at the presynaptic site (Fig. 10). Although the expression site of this LTP remains to be determined, it is thus likely that LTP requires anterograde or retrograde signaling between pre- and postsynaptic cells irrespective of the expression site. If LTP is expressed presynaptically, some information must be sent backwards from the postsynaptic cells during induction. Recent studies have suggested that
endocannabinoid and brain-derived neurotrophic factor (BDNF) could act as such retrograde messengers in long-term modifications of inhibitory synaptic transmission (Chevaleyre and Castillo 2003; Gubellini and others 2005). In an experimental condition similar to that used in this study, in which non-NMDA and NMDA receptors were pharmacologically blocked, HFS produced LTD at inhibitory synapses in CA1 pyramidal cells instead of LTP (Chevaleyre and Castillo 2003). These authors demonstrated that LTD, expressed presynaptically, requires the activation of postsynaptic metabotropic glutamate receptors, leading to the formation of endocannabinoids mediating the retrograde signal, although a rise in postsynaptic Ca\(^{2+}\) was not required for induction, which is different from visual cortical inhibitory LTP. On the other hand, BDNF was suggested to be a candidate retrograde messenger for LTP at inhibitory synapses in neonate CA3 pyramidal cells, which is expressed presynaptically and requires a rise in postsynaptic Ca\(^{2+}\) concentration for induction (Gubellini and others 2005). Thus, it is likely that these two substances are candidate retrograde messengers for visual cortical inhibitory LTP, if it is indeed expressed presynaptically.

If LTP is expressed postsynaptically, the transfer of information from the pre- to the postsynaptic cells is required during maintenance. Pharmacological blockade of GABA\(_{A}\) and GABA\(_{B}\) receptors, which abolished membrane potential changes associated with synaptic transmission in postsynaptic cells, did not affect LTP maintenance (Komatsu and Yoshimura 2000). In
in the postsynaptic cells. The maintenance of LTP requires firing of presynaptic cells at least at a low frequency and Ca$^{2+}$ entry through P-, L-, and N-type voltage-dependent Ca$^{2+}$ channels (VDCC) at the presynaptic site, which activates Ca$^{2+}$-dependent reactions different from those triggering transmitter release. The activation of $\beta$ and $\alpha_2$ adrenoceptors stimulates adenylyl cyclases via $G_s$ and $G_{bc}$, respectively, in a synergistic manner, and increases the cAMP level of the presynaptic site. An increased cAMP may upregulate Ca$^{2+}$ channels and/or downregulate K$^+$ channels, leading to increases in action potential-associated Ca$^{2+}$ entry involved in LTP maintenance, or reduce Ca$^{2+}$ sensitivity of the biochemical process responsible for maintenance. This adrenoceptor-mediated facilitation of LTP maintenance becomes long lasting when HFS is applied together with the activation of these adrenoceptors.

In addition, blockade of presynaptic L-type Ca$^{2+}$ channels, which are not involved in triggering transmitter release at most central synapses (Reuter 1996), abolished LTP maintenance without affecting control pathway responses (Komatsu and Yoshimura 2000). Thus, conventional transmitters such as GABA may not act as anterograde messengers during maintenance, and hence we favor the hypothesis of presynaptic rather than postsynaptic expression of this inhibitory LTP. Presynaptic expression may simplify the maintenance mechanism. However, we could not rule out the possibility that the anterograde signal is mediated by membrane-permeant or membrane-bound factors. Further studies are required to resolve these issues related to the site of LTP expression.

**Long-Lasting Modification of Plasticity Mechanism**

Neural activity can produce enduring modifications of synaptic transmission, contributing to long-term alteration of signal processing in the brain. In addition, activity can also modulate the capability of synaptic plasticity. Various types of alteration in synaptic plasticity subsequent to neural activity have been reported (Abraham and Bear 1996; Abraham and Tate 1997). In LTP of CA1 pyramidal cell excitatory synapses, the activation of NMDA receptors inhibits the subsequent induction of LTP (Huang and others 1992), whereas the activation of metabotropic glutamate receptors facilitates subsequent LTP induction (Bortolotto and others 1994; Cohen and Abraham 1996). These changes are likely produced by the activity of synapses undergoing modification.

Neuromodulatory afferents, those using noradrenaline, dopamine, and serotonin as transmitters, modulate synaptic plasticity by affecting cAMP and PKA, which are intimately involved in the production and regulation of plasticity through phosphorylation and synthesis of proteins (Kandel 2001; Nguyen and Woo 2003). The effects of modulatory transmitters are often long lasting, and thereby they could produce long-lasting changes in synaptic modifiability. The present study demonstrated two salient features in the adrenergic modulation of inhibitory LTP. First, noradrenaline facilitated the maintenance of LTP, although the induction mechanism was modulated in most of the cases described previously (Abraham and Tate 1997). Second, the facilitation occurred only at synapses, which were activated by HFS together with adrenoceptor activation.

**Significance of Noradrenergic Regulation of Inhibitory LTP**

The results of both our previous and present studies suggest that inhibitory LTP is strongly controlled by the activity of noradrenergic cells in locus ceruleus. Because locus ceruleus cells show spontaneous firing far less during sleep than arousal (Aston-Jones and others 1990), LTP may easily disappear during sleep. During arousal, sensory stimulation effectively produces firing of locus ceruleus cells (Aston-Jones and others 1990). It has been demonstrated that visual stimulation produces noradrenaline release in visual cortex (Marrocco and others 1987). Thus, visual cortical inhibitory synapses activated by such visual stimuli may receive a sufficient amount of noradrenaline from the nerve terminals of locus ceruleus cells during that activity and easily undergo potentiation. Once potentiated, a concomitant facilitation of the maintenance mechanism may allow the potentiation to persist for a considerably longer period by spontaneous firing of inhibitory cells even during sleep. Thus, inhibitory LTP, like plasticity of visual responsiveness, seems to be gated by noradrenergic cell activity (Kasamatsu and Pettigrew 1976; Bear and Singer 1986), supporting the hypothesis that inhibitory LTP contributes to experience-dependent maturation of visual cortical functions.

**Notes**

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**Figure 10.** Induction and maintenance mechanisms of visual cortical inhibitory LTP, proposed based on our previous and present studies. The molecules involved in induction and maintenance are indicated by blue and red colors, respectively. The induction of LTP requires the activation of GABA$_\text{A}$ receptors and adrenergic $\alpha_1$ and 5-HT$_2$ receptors, leading to IP$_3$ formation and Ca$^{2+}$ release from the internal Ca$^{2+}$ stores in the postsynaptic cells. The maintenance of LTP requires firing of presynaptic cells at least at a low frequency and Ca$^{2+}$ entry through P-, L-, and N-type voltage-dependent Ca$^{2+}$ channels (VDCC) at the presynaptic site, which activates Ca$^{2+}$-dependent reactions different from those triggering transmitter release. The activation of $\beta$ and $\alpha_2$ adrenoceptors stimulates adenylyl cyclases via $G_s$ and $G_{bc}$, respectively, in a synergistic manner, and increases the cAMP level of the presynaptic site. An increased cAMP may upregulate Ca$^{2+}$ channels and/or downregulate K$^+$ channels, leading to increases in action potential-associated Ca$^{2+}$ entry involved in LTP maintenance, or reduce Ca$^{2+}$ sensitivity of the biochemical process responsible for maintenance. This adrenoceptor-mediated facilitation of LTP maintenance becomes long lasting when HFS is applied together with the activation of these adrenoceptors.


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