Noradrenergic Regulation of Synaptic Plasticity in the Hippocampal CA1 Region

HIROSHI KATSUKI, 1 YUKITOSHI IZUMI, 1 AND CHARLES F. ZORUMSKI 1, 2
1Department of Psychiatry and 2Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110

Katsuki, Hiroshi, Yukitoshi Izumi, and Charles F. Zorumski. Noradrenergic regulation of synaptic plasticity in the hippocampal CA1 region. J. Neurophysiol. 77: 3013 ± 3020, 1997. The effects of norepinephrine (NE) and related agents on long-lasting changes in synaptic efficacy induced by several patterns of afferent stimuli were investigated in the CA1 region of rat hippocampal slices. NE (10 μM) showed little effect on the induction of long-term potentiation (LTP) triggered by theta-burst-patterned stimulation, whereas it inhibited the induction of long-term depression (LTD) triggered by 900 pulses of 1-Hz stimulation. In nontreated slices, 900 pulses of stimuli induced LTD when applied at lower frequencies (1–3 Hz), and induced LTP when applied at a higher frequency (30 Hz). NE (10 μM) caused a shift of the frequency-response relationship in the direction preferring potentiation. The effect of NE was most prominent at a stimulus frequency of 10 Hz, which induced no changes in control slices but clearly induced LTP in the presence of NE. The facilitating effect of NE on the induction of LTP by 10-Hz stimulation was blocked by the β-adrenergic receptor antagonist timolol (50 μM), but not by the α receptor antagonist phenotamine (50 μM), and was mimicked by the β-agonist isoproterenol (0.3 μM), but not by the α agonist phenylephrine (10 μM). The induction of LTD by 1-Hz stimulation was prevented by isoproterenol but not by phenylephrine, indicating that the activation of β-receptors is responsible for these effects of NE. NE (10 μM) also prevented the reversal of LTP (depotentiation) by 900 pulses of 1-Hz stimulation delivered 30 min after LTP induction. In contrast to effects on naive (nonpotentiated) synapses, the effect of NE on previously potentiated synapses was only partially mimicked by isoproterenol, but fully mimicked by coapplication of phenylephrine and isoproterenol. In addition, the effect of NE was attenuated either by phenotamine or by timolol, indicating that activation of both α and β-receptors is required. These results show that NE plays a modulatory role in the induction of hippocampal synaptic plasticity. Although β-receptor activation is essential, α1 receptor activation is also necessary in determining effects on previously potentiated synapses.

INTRODUCTION

Activity-dependent changes in synaptic efficacy are critical for the development of appropriate neural circuits and for many forms of neural plasticity. Long-term potentiation (LTP), a long-lasting increase in the efficacy of synaptic transmission induced by trains of high-frequency stimulation, has long been believed to represent a mechanism involved in information storage during learning and memory (Bear and Malenka 1994; Larkman and Jack 1995). On the other hand, repetitive stimulation of afferents at relatively low frequency results in a long-lasting decrease in synaptic efficacy, which is called long-term depression (LTD) or depotentiation, depending on whether the synapses have been previously potentiated or not. LTD and depotentiation are also thought to play important roles in how synapses function as mnemonic devices (Bear and Malenka 1994; Linden 1994).

The hippocampus receives a major adrenergic input from the locus ceruleus (Loy et al. 1980; Moore and Bloom 1979), and adrenergic receptors are present in various types of cells in the hippocampus (Nicholas et al. 1996), including principal excitatory neurons (Madison and Nicoll 1982), interneurons (Bergles et al. 1996), and glial cells (Duffy and MacVicar 1995). Adrenergic receptor activation alters the excitability and activity of hippocampal neurons through mobilization of intracellular messengers and modulation of ion channels (Madison and Nicoll 1982, 1986; Mueller et al. 1981, 1982; Segal 1982).

Because the induction of LTP and LTD is activity dependent, it is natural to consider whether adrenergic activation regulates the induction of synaptic plasticity in the hippocampus. Consistent with this view, perforant path–dentate granule cell synapses undergo long-lasting changes in synaptic efficacy by application of norepinephrine (NE) or β-adrenergic agonists (Dahl and Sarvey 1989). Stimulation of β-adrenergic receptors also enhances the expression of LTD in mossy fiber–CA3 synapses (Hopkins and Johnston 1984, 1988; Huang and Kandel 1996). In contrast, an early study indicated that NE played no role in synaptic plasticity at Schaffer collateral/commissural–CA1 synapses (Dunwiddie et al. 1982). However, recent work has provided evidence that NE modulates CA1 synaptic plasticity via β-adrenergic receptors during low-frequency synaptic stimulation (Thomas et al. 1996). In the present study we evaluated the effects of NE and related agents on the synaptic plasticity in hippocampal CA1 excitatory synapses with the use of several patterns of afferent stimuli.

METHODS

Hippocampal slices were prepared from 28- to 35-day-old male Sprague-Dawley rats with the use of standard methods. Rats were deeply anesthetized with halothane and decapitated, and the brain was removed. Hippocampi were rapidly dissected and placed in gassed (95% O2–5% CO2) standard extracellular solution containing (in mM) 124 NaCl, 3 KCl, 2.5 CaCl2, 1.3 MgSO4, 1.25 NaH2PO4, 22 NaHCO3, and 10 D-glucose. Transverse slices (500 μm thick) were cut with a McIlwain tissue chopper. Slices were then maintained in an incubation chamber for ≥1 h at 30°C in the standard solution. At the time of an experiment, individual slices were transferred to a submersion recording chamber where
they were constantly perfused with standard solution (2 ml/min) at 30°C.

Extracellular recordings were obtained from the dendritic layer of the CA1 region with the use of 5- to 10-MΩ glass electrodes filled with 2 M NaCl. A bipolar electrode was placed in stratum radiatum to stimulate the Schaffer collateral/commissural pathway. Stimuli 50 μs in duration were applied every minute. The stimulus intensity was set to evoke 40–50% of the maximal amplitude of field excitatory postsynaptic potentials (EPSPs). Different types of afferent stimulation were performed at the same relative intensity in individual slices. To induce LTP, theta-burst-patterned stimulation (TBS) was used. The paradigm consisted of 10 bursts (unless otherwise indicated) of 4 pulses at 100 Hz, applied at 5 Hz.

(−)-NE bitartrate, (−)-isoproterenol bitartrate, phentolamine methanesulfonate, timolol, 1-phenylephrine, and all other chemicals were obtained from Sigma (St. Louis, MO). Drugs were dissolved in the standard solution just before application to slices.

Field EPSPs were monitored and analyzed with the use of an IBM computer-based data acquisition system. The magnitude of potentiation or depression was expressed as the percent change in the maximal slope of EPSPs. Estimation of the reversal of LTP (depotentiation) was made by calculating the percentage of residual potentiation as follows: 100 × (averaged % increase from the baseline at 101–105 min)/(averaged % increase from the baseline at 16–20 min), where TBS was applied at time 0. Values are presented as means ± SE. Statistical significance was evaluated by analysis of variance followed by Bonferroni’s method.

**Results**

**NE has little effect on the induction of LTP by theta bursts, but inhibits the induction of LTD by low-frequency stimulation**

The effects of NE on the induction of LTP were examined with the use of a conventional stimulation paradigm. TBS, consisting of 10 bursts of 4 pulses at 100 Hz administered every 200 ms, induced robust LTP that lasted for >60 min (Fig. 1Aa, ○). Application of NE (10 μM) during TBS had no influence on the magnitude of LTP (Fig. 1Aa, ●).

Because 10 bursts of theta-patterned stimulation induce robust LTP by themselves, subtle changes in the threshold for LTP induction may not be detected by this paradigm. Therefore we tested the effect of NE with the use of fewer bursts as the conditioning stimulation. One, two, three, or five bursts of stimuli were applied in the absence or presence of 10 μM NE. Figure 1, Ab and Ac, shows the time course of potentiation induced by three and two bursts of stimuli, respectively, and Fig. 1B summarizes the results of these experiments as the percent increase in EPSP slope at 56–60 min after conditioning stimulation. We again observed no significant differences in the degree of LTP between control and NE-treated slices.

On the other hand, NE showed a clear effect on the induction of LTD. Afferent stimulation at 1 Hz for 15 min induced a long-lasting decrease in EPSP slope (decrease of 22.2 ± 3.8%, mean ± SE, from baseline 56–60 min after 1-Hz stimulation, n = 7; Fig. 2A, ○). When NE (10 μM) was perfused during the delivery of 1-Hz stimulation, the induction of LTD was inhibited. Although a short-term depression was observed within 15 min of the stimulus train, EPSP slopes showed only a 3.5 ± 6.9% decrease 56–60 min after 1-Hz stimulation (n = 6; Fig. 2A, ●).

As evident in Figs. 1A and 2A, application of NE by itself caused a small reduction in EPSPs. This effect was reversible and EPSP slopes returned to baseline levels promptly after the washout of 10 μM NE (Fig. 2B).

**Effects of NE on the frequency-response relationship of synaptic plasticity**

The above results imply that NE may effectively modulate synaptic plasticity induced by prolonged, low-frequency stimulation, rather than that induced by brief bursts of high-frequency stimulation. Previous reports demonstrate that prolonged afferent stimulation results in either LTP or LTD, depending on the frequency of stimulation (Dudek and Bear 1992). This frequency-response relationship of synaptic plasticity can be modulated by several kinds of manipulation (Coussens and Teyler 1996; Mayford et al. 1995). Recent evidence also suggests that the ability of central synapses to function as devices for information storage depends on this frequency-response relationship (Bach et al. 1995; Kirkwood et al. 1996). Therefore we examined whether NE influences the frequency-response relationship of hippocampal CA1 synapses with the use of stimulation with 900 pulses applied at various frequencies and monitoring changes in synaptic responses for 60 min thereafter. Consistent with a previous report (Dudek and Bear 1992), stimulation at lower frequencies (1 and 3 Hz) resulted in significant LTD of EPSPs, whereas stimulation at higher frequency (30 Hz) induced LTP. At 10 Hz, 900 pulses produced no lasting change in synaptic responses (Fig. 3A, ○).

In a separate group of slices, 900 pulses of afferent stimuli were applied at different frequencies in the presence of 10 μM NE. NE showed no significant effect on changes in synaptic efficacy induced by 3- and 30-Hz stimulation (Fig. 3, Aa and Ac). However, 10-Hz stimulation, which did not induce any lasting change in EPSPs under control conditions, now induced a clear potentiation that lasted for >60 min (Fig. 3, Ab and B). Changes in EPSP slope after stimulation with 900 pulses in the absence and presence of 10 μM NE are summarized in Fig. 3C. Overall, NE caused a leftward shift of the frequency-response relationship with the threshold frequency for long-lasting changes in synaptic efficacy shifting from 10 Hz to <5 Hz under NE treatment.

**β-Adrenoceptors mediate the effects of NE**

To clarify the adrenoceptor subtypes involved in the effects of NE, we examined the ability of several adrenergic agonists and antagonists to alter changes induced by 900-pulse stimulation. A stimulus frequency of 10 Hz was chosen for these experiments, because the effect of NE was most prominent at this stimulation frequency. In the first set of experiments, application of 10 μM NE and 10-Hz stimulation was performed in the presence of the α-adrenergic receptor antagonist phentolamine (50 μM) or the β-receptor antagonist timolol (50 μM). As shown in Fig. 4A (●), timolol virtually abolished the effect of NE on synaptic plasticity, although the depression of baseline EPSPs by NE persisted. In contrast, phentolamine failed to suppress the effect of NE on 10-Hz stimulation but inhibited the depression of baseline EPSPs (Fig. 4A, ○). In a second set of experiments, the effect of a potent β-selective agonist, iso-
Norepinephrine (NE) has little effect on induction of long-term potentiation (LTP) triggered by theta bursts. 

**Fig. 1.** Norepinephrine (NE) has little effect on induction of long-term potentiation (LTP) triggered by theta bursts. 

**Aa:** LTP was induced by 10 bursts of theta-burst-patterned stimulation (TBS, †) in absence (○) or presence (●) of 10 μM NE. NE was applied by bath perfusion during time indicated by bar. 

**Ab and Ac:** same experimental conditions as in Aa, except that 3 bursts (Ab) and 2 bursts (Ac) were applied at time 0. 

**B:** summary of effect of 10 μM NE on LTP induced by variable number of bursts (each consists of 4 pulses at 100 Hz) given at 5 Hz. Values represent percent changes in excitatory postsynaptic potential (EPSP) slope observed at 56 ± 60 min after burst stimulation. Values in parentheses: numbers of slices tested.

proterenol, and the α1-selective agonist phenylephrine was examined. In the presence of 0.3 μM isoproterenol, 10-Hz trains induced clear potentiation of synaptic responses (Fig. 4B), whereas phenylephrine (10 μM) failed to promote the induction of LTP consistently. In the presence of phenylephrine, only two of six slices showed an increase of >15% in EPSP slope following 10-Hz stimulation. Results from these experiments are summarized in Fig. 4C as percent changes in EPSP slope at 56–60 min after 10-Hz stimulation. Taken together, these results suggest that the effect of NE on 10-Hz stimulation is mediated primarily by β-adrenergic receptors.

We next examined the effects of subtype-selective adrenergic agonists on the induction of LTD by 1-Hz stimulation. Isoproterenol (0.3 μM), when applied during 1-Hz × 900-pulse stimulation, clearly suppressed the induction of LTD (Fig. 5, ●). Consistent with a prior report (Gereau and Conn 1994), isoproterenol by itself reversibly augmented synaptic responses (Fig. 5, open symbols). On the other hand, the α1 agonist phenylephrine did not suppress the induction of LTD. Stimulation at 1 Hz in the presence of 10 μM phenylephrine resulted in an 18.7 ± 3.1% decrease in EPSP slope 56–60 min after the 1-Hz train (n = 5).

**NE suppresses the reversal of LTP via coactivation of α1 and β-adrenoceptors**

Although 1-Hz stimulation is conventionally used to induce LTD, the effects of this stimulation paradigm can be variable, depending on experimental conditions such as age and species of animals (Barrionuevo et al. 1980; Bashir and Collingridge 1994; Fujii et al. 1991; Wagner and Alger 1995). On the other hand, 1-Hz stimulation is consistently found to depress transmission of previously potentiated synapses, referred to as LTD reversal or depotentiation (Fujii et al. 1991; Bashir and Collingridge 1994). This difference leads to the notion that the induction of LTD and depotentiation may include, or may be regulated by, different mechanisms (Wagner and Alger 1996). In the final set of experiments we examined the effect of NE on depotentiation.
LTP was induced by TBS, and 1-Hz stimulation was applied for 15 min, beginning 30 min after TBS, in the absence or presence of 10 μM NE. In the absence of drugs, 1-Hz stimulation induced a clear, although not complete, reversal of TBS-induced LTP (Fig. 6, A, left and B, ○). NE almost completely blocked the reversal of LTP by 1-Hz stimulation (Fig. 6, A, right and B, ●). Reversal of LTP was evaluated as the percentage of residual potentiation at 56–60 min after the cessation of 1-Hz stimulation, taking the potentiation at 16–20 min after the induction of LTP (just before the agonist application) as 100%. With the use of this measure, the degree of potentiation remaining after 1-Hz stimulation in the presence of 10 μM NE was nearly identical to the value when neither 1-Hz stimulation nor drugs were applied after the induction of LTP (Fig. 7).

To identify the receptor subtypes responsible for this effect of NE, we again tested the effects of phenylephrine and isoproterenol. Interestingly, isoproterenol (0.3 μM) was not as effective as NE. The suppressing effect of isoproterenol on depotentiation by 1-Hz stimulation (Figs. 6C and 7) was only partial, and did not reach statistical significance. Phenylephrine (10 μM) was not effective either. However, LTP reversal was almost completely prevented when these agonists were applied together (Figs. 6C and 7). We also investigated whether subtype selective antagonists reverse the effect of NE. Timolol (50 μM) antagonized the effects of 10 μM NE nearly completely (Fig. 7), whereas phentolamine (50 μM) attenuated the effects partially. These results suggest that the activation of both α₁ and β-receptors is required for NE to prevent the reversal of LTP.

**DISCUSSION**

In the present study we examined the effects of NE on the induction of long-term synaptic plasticity in the hippocampal CA1 region. NE had little or no effect on the induction of LTP by TBS. This was the case even when we used a modest conditioning stimulation to detect subtle changes in LTP threshold. Although these results are consistent with earlier findings of Dunwiddie et al. (1982), who reported that NE had no effect on LTP induced by a 500-Hz 10.220.32.246 train, the presence of 10 μM NE was nearly identical to the value when neither 1-Hz stimulation nor drugs were applied after the induction of LTP (Fig. 7).

Phenylephrine (10 μM) was not effective either. However, LTP reversal was almost completely prevented when these agonists were applied together (Figs. 6C and 7). We also investigated whether subtype selective antagonists reverse the effect of NE. Timolol (50 μM) antagonized the effects of 10 μM NE nearly completely (Fig. 7), whereas phentolamine (50 μM) attenuated the effects partially. These results suggest that the activation of both α₁ and β-receptors is required for NE to prevent the reversal of LTP.

**DISCUSSION**

In the present study we examined the effects of NE on the induction of long-term synaptic plasticity in the hippocampal CA1 region. NE had little or no effect on the induction of LTP by TBS. This was the case even when we used a modest conditioning stimulation to detect subtle changes in LTP threshold. Although these results are consistent with earlier findings of Dunwiddie et al. (1982), who reported that NE had no effect on LTP induced by a 500-Hz 10.220.32.246 train, the presence of 10 μM NE was nearly identical to the value when neither 1-Hz stimulation nor drugs were applied after the induction of LTP (Fig. 7).

Phenylephrine (10 μM) was not effective either. However, LTP reversal was almost completely prevented when these agonists were applied together (Figs. 6C and 7). We also investigated whether subtype selective antagonists reverse the effect of NE. Timolol (50 μM) antagonized the effects of 10 μM NE nearly completely (Fig. 7), whereas phentolamine (50 μM) attenuated the effects partially. These results suggest that the activation of both α₁ and β-receptors is required for NE to prevent the reversal of LTP.
FIG. 4. β-Adrenoceptor activation is involved in synaptic potentiation induced by 10-Hz stimulation in presence of NE. A: timolol (●, n = 6), but not phentolamine (○, n = 6), blocked effect of NE. Adrenergic antagonists (each at 50 μM) were applied during time indicated by open bar. B: effect of 0.3 μM isoproterenol (n = 5) on 10-Hz-train-induced changes in synaptic efficacy. Isoproterenol was applied during time indicated by bar. C: summary of effects of adrenergic agonists and antagonists. Values shown are % changes in EPSP slope from baseline, observed 56 ± 60 min after 10-Hz 1900-pulse train. Values in parentheses: numbers of slices tested. Single asterisk: P < 0.05 vs. no drug group. Double asterisk: P < 0.01 vs. no drug group.

A prominent effect was the inhibition of LTD induction by 1-Hz stimulation. Another obvious effect was that NE promotes the induction of LTD by stimulation at medium stimulation frequencies (5–10 Hz), resulting in a leftward shift of the frequency-response relationship. Pharmacological characterization demonstrated that the activation of β-receptors is necessary and sufficient to induce these changes. Activation of β-adrenoceptors, which are positively coupled to adenylyl cyclases, causes elevation of intracellular cyclic AMP levels. Therefore cyclic AMP production is likely to be involved in these effects of NE. In fact, application of a membrane-permeable cyclic AMP analogue inhibits the induction of LTD (Mulkey et al. 1994), and the adenylyl cyclase activator forskolin promotes the induction of LTP by 3 min of 5-Hz afferent stimulation (Thomas et al. 1996) in hippocampal CA1 synapses. Because β-receptors act via G proteins, it is also possible that noncyclic-AMP-mediated effects contribute to changes in synaptic plasticity.

Several lines of evidence indicate that intracellular levels of cyclic AMP play a key role in the induction of synaptic plasticity. This may result from the ability of cyclic AMP to alter the balance of cellular protein kinase and phosphatase activity. Cyclic AMP, via activation of protein kinase A, promotes the phosphorylation of inhibitor-1, a phosphoprotein that inhibits the activity of protein phosphatase 1 (Shenolikar and Nairn 1991). This protein-kinase-A-dependent
process appears to be a primary mechanism by which cyclic AMP inhibits the induction of LTD, because the activation of protein phosphatases is critical for the induction of LTD in naive synapses. On the basis of the effects of α₁ and β-receptor agonists and antagonists, our results suggest that activation of β-receptors alone is not sufficient and that α₁ receptor stimulation is also required for the suppression of depotentiation. Although the mechanisms of cooperation between α₁- and β-receptor-mediated events are not clear, the present results provide evidence that LTD at naive synapses and depotentiation at previously potentiated synapses are under control of different regulatory mechanisms.

In contrast to our findings, Larson et al. (1993) reported that a very high concentration (200 μM) of NE enhanced the reversal of LTP by 5-Hz × 1-min afferent stimulation. Reasons for this discrepancy are not clear, but there are a number of methodological differences between the study of Larson et al. (1993) and the present study, including the concentrations of NE. There is some evidence suggesting that LTD and depotentiation are regulated by different mechanisms. Wagner and Alger (1995) showed that γ-aminobutyric-acid-mediated inhibitory regulation is altered by prior synaptic activity such as LTP-inducing high-frequency stimulation. In light of the present results that α₁-receptor-mediated events are involved in the regulation of potentiated synapses, it is possible that the potent excitatory action of NE on hippocampal inhibitory interneurons via α₁ receptor stimulation (Bergles et al. 1996) may contribute to differential effects on potentiated versus naive synapses. Another difference between LTD and depotentiation concerns the role of intracellular Ca²⁺ stores. Depletion of intracellular Ca²⁺ stores by thapsigargin inhibits the induction of LTD but has no effect on depotentiation (Reyes and Stanton 1996), suggesting that the threshold level of intracellular Ca²⁺ for inducing long-lasting depression is altered by prior induction of LTP. This difference may also be relevant to the differential regulation of LTD and depotentiation by α₁ adrenoceptors, because α₁ receptors are linked to the activation of phospholipase C and the production of inositol trisphosphate, which mobilizes intracellular Ca²⁺ stores.

To date, little attention has been paid to the possible roles of α-adrenoceptors in the regulation of synaptic plasticity (Dahl and Survey 1989; Huang and Kandel 1996; Thomas et al. 1996). We have previously demonstrated that NE, acting on α₁ but not β-receptors, overcomes the inhibition of LTP induction by untimely activation of N-methyl-D-aspartate receptors at CA1 synapses (Izumi et al. 1992). In the present study we provide further evidence that, under certain conditions, α-adrenoceptor-mediated events are involved in the regulation of plasticity at hippocampal synapses. It is thus important to consider that NE is a more potent agonist at α₁- than β-adrenoceptors, suggesting that the effects on depotentiation observed here may be relevant to effects on synaptic plasticity mediated by endogenous adrenergic activation.
FIG. 7. Summary of effects of adrenergic agonists and antagonists on depotentiation. Values shown are percentages of residual potentiation calculated as described in METHODS. ‘No stimulation’ group did not receive 1-Hz stimulation after induction of LTP by TBS, whereas all other groups received 1-Hz stimulation for 15 min from 30 min after TBS. ‘Depotentiation’ group received no drug treatment, and other groups received drug treatment during 1-Hz stimulation as indicated. Values in parentheses: numbers of slices tested. Single asterisk: \( P < 0.05 \) vs. depotentiation group. Double asterisk: \( P < 0.01 \) vs. depotentiation group.

We thank A. Benz and J. Que for technical assistance. This work was supported by National Institute of Mental Health Research Scientist Development Award MH-00964, National Institutes of Health Grants MH-45943 and AG-11355, and a fellowship from the Banty Foundation. H. Katsuki was supported by a long-term fellowship from Human Frontier Science Program.

Address for reprint requests: C. F. Zorumski, Dept. of Psychiatry, Washington University Medical School, 4940 Children’s Place, St. Louis, MO 63110.

Received 2 December 1996; accepted in final form 11 February 1997.

REFERENCES


