Presynaptic Inhibition of Excitatory Synaptic Transmission Mediated by α Adrenergic Receptors in Area CA3 of the Rat Hippocampus *in vitro*

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We have investigated the action of norepinephrine (NE) on excitatory synaptic transmission in the hippocampus by recording from CA3 pyramidal cells in organotypic slice cultures. NE (5 μM) was found to decrease the amplitude of pharmacologically isolated EPSPs elicited with stimulation of mossy fibers or recurrent axon collaterals (mean decrease in EPSP amplitude, 44%). Desensitization was observed with repetitive applications. NE did not affect the sensitivity of CA3 cells to iontophoretically applied AMPA, and did not affect the amplitude distribution of TTX-resistant, miniature excitatory synaptic currents. These data suggest that NE acts at presynaptic receptors to decrease glutamate release. This action of NE was blocked by the α receptor antagonist phentolamine and the specific α_1 receptor antagonist prazosine, but not by the β receptor antagonist timolol or the α_2 receptor antagonist idazoxan. Inhibition of EPSPs by NE was prevented by pretreatment of cultures with pertussis toxin, indicating that G-proteins couple these receptors to their effectors. Stimulation of protein kinase C with phorbol ester blocked the action of NE on EPSPs. This effect, as well as the desensitization of NE responses, was reduced by application of the protein kinase inhibitor staurosporin.

Presynaptic inhibition of excitatory synaptic transmission, mediated by α adrenergic receptors, represents a novel modulatory action of NE in the hippocampus.

[Key words: presynaptic inhibition, EPSPs, hippocampus, norepinephrine, glutamate, desensitization]

The hippocampus receives a prominent adrenergic innervation from the locus coeruleus (Moore and Bloom, 1979). Both stimulation in the locus coeruleus, to evoke release of endogenous norepinephrine (NE), and topical application of exogenous NE have been shown to decrease the spontaneous activity of hippocampal pyramidal neurons *in vivo* (Segal and Bloom, 1974a,b; Mueller et al., 1982; Curet and de Montigny, 1988a,b; cf. Olpe et al., 1986). In contrast, NE exerts a dual action on hippocampal activity *in vitro*. NE was shown to both decrease and increase the amplitude of the population spike recorded from the stratum pyramidale. These two effects are mediated by α and β adrenergic receptors, respectively (Mueller et al., 1981; Mynlieff and Dunwiddie, 1988). Likewise, α adrenergic agonists reduce the frequency of spontaneous epileptiform discharge *in vitro*, while β adrenergic agonists have a proconvulsive action (Mueller and Dunwiddie, 1983).

The NE-induced increase in hippocampal excitability probably results from the well-characterized β receptor–mediated reduction of Ca^{2+}-dependent K^+ conductance (Madison and Nicoll, 1986). On the other hand, neither the cellular basis nor the pharmacology of the α receptor–mediated inhibitory effect of adrenergic agonists in the hippocampus has been unequivocally established. Madison and Nicoll (1986) observed a small α receptor–mediated hyperpolarization, accompanied by a decrease in input resistance, in a portion of the CA1 pyramidal cells studied. However, the variability and small size of this effect seem unlikely to underlie the consistently observed inhibition of hippocampal activity *in vivo* and *in vitro*.

Indirect evidence suggests that NE may modulate excitatory synaptic transmission in the hippocampus (Mody et al., 1983; Doze et al., 1991). We have therefore investigated the effects of NE on isolated EPSPs in hippocampal slice cultures.

Portions of these data have been presented in abstract form (Scanziani et al., 1992b).

Materials and Methods

Organotypic hippocampal slice cultures were prepared as described previously (Gähwiler, 1981). Briefly, hippocampal slices (400 μm thick) were obtained from 6–7-d-old rat pups, embedded in a chicken plasma clot on glass coverslips, and placed in test tubes containing semisynthetic medium. The cultures were then incubated on a roller drum for at least 2 weeks before experimentation, allowing for the disappearance of damaged tissue and a flattening to only one or two cells in thickness, while maintaining the normal hippocampal cytoarchitecture.

Mature cultures were placed in a recording chamber on an inverted microscope and perfused with warmed (34°C) saline containing (in mM) Na^+, 148.9; K^+, 2.7; Cl^-, 150.2; Ca^{2+}, 3.8; Mg^{2+}, 1.5; HCO_3^-, 11.6; $H_2PO_4^-$, 0.4; glucose, 5.6; and phenol red, 10 mg/liter. Microelectrodes (30–80 MΩ) were filled with 2 M KCl or 0.5 M potassium methylsulfate, and recordings were made in either current-clamp or single-electrode voltage-clamp mode (1–4 kHz switching frequency). Excitatory postsynaptic potentials were elicited with stimuli (0.1 msec) delivered via 155 mM NaCl–filled microelectrodes (3 MΩ) placed either within the dentate gyrus, or in stratum radiatum of area CA1. Stimulation intensity (−10 to −200 μA) was adjusted to elicit EPSPs of 5–10 mV in amplitude. AMPA (±-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; 10 mM at pH 8) was iontophoretically applied in some experiments using patch pipettes positioned close to the cell body of the recorded cell. Numerical data in the text and figures are presented as mean ± STD and mean ± SEM, respectively.

Spontaneous, miniature excitatory synaptic currents (mEPSCs) were recorded in whole-cell voltage clamp as described previously (Scanziani

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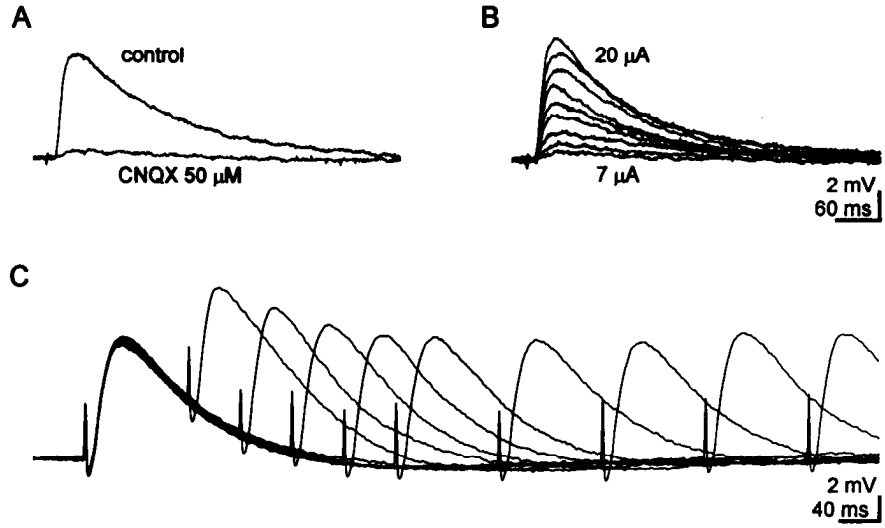

Figure 1. Isolation of monosynaptic AMPA/kainate receptor–mediated EPSPs. Responses were elicited with stimulation in the dentate gyrus in the presence of bicuculline (40 μM), CGP 35 348 (500 μM), AP5 (20 μM), and CNQX (10 μM). *A*, Increasing the concentration of the competitive antagonist CNQX to 50 μM strongly reduced the amplitude of the isolated EPSP, indicating that it was mediated by AMPA/kainate receptors. *B*, Responses of same cell to stimulation of the mossy fibers at increasing intensities. Note that the amplitude increased progressively and without obvious discontinuities. Membrane potential, −75 mV; KCl electrode. *C*, Different cell, same conditions. Nine superimposed sweeps show the response of a CA3 neuron to two paired mossy fiber stimuli, separated from each other by progressively decreasing interstimulus intervals. Note that the EPSPs were able to follow stimulation frequencies up to 10 Hz, without changes in latency or amplitude. Membrane potential, −75 mV; potassium methylsulfate electrode.

et al., 1992a). Pipettes contained (in mM) CsF, 125; HEPES, 10; and tetracesium BAPTA, 3. Tetrodotoxin (TTX; 0.5 μM) was added to the bathing solution to block action potential–dependent transmitter release, bicuculline (40 μM) to block $GABA_A$ receptors, and 50 mM sucrose to increase the frequency of mEPSCs in order to facilitate comparison of amplitude distributions. We have previously established that this procedure increases mEPSC frequency two- to threefold, without affecting mEPSC amplitude (Scanziani et al., 1992a). For statistical comparisons of mEPSC amplitude distributions, cumulative probability plots were prepared from large numbers of responses (>100) and the likelihood that two distributions were different was assessed using the Kolmogorov-Smirnov test. Two distributions were considered different if $p < 0.05$.

Drugs were applied by bath application, except for AMPA, and were obtained from the following sources: 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX), D-2-amino-5-phosphovalerate (AP5), and AMPA, Tocris Neuramin (Bristol, UK); pertussis toxin, List Biological Laboratories (Campbell, CA); TTX, Janssen Chimica (Geel, Belgium); staurosporin, Boehringer (Mannheim, Germany); BAPTA, Molecular Probes (Eugene, OR); and phorbol 12,13-dibutyrate (PDBu) and all adrenergic agonists and antagonists, Sigma (St. Louis, MO).

Results

In order to study the effect of NE on excitatory transmission independently from the NE-induced changes in inhibitory transmission (Leung and Miller, 1988; Madison and Nicoll, 1988; Doze et al., 1991), we examined the effects of NE on pharmacologically isolated EPSPs (Thompson and Gähwiler, 1992). Inhibitory synaptic transmission was blocked with the $GABA_A$ receptor antagonist bicuculline (40 μM) and the $GABA_B$ receptor antagonist CGP 35 348 (500 μM). High concentrations of the NMDA receptor antagonist AP5 (20–40 μM) and a nonsaturating concentration of the non-NMDA receptor antagonist CNQX (10 μM) were used to prevent epileptiform burst discharges. Stimulation in the dentate gyrus of hippocampal slice cultures (1/12 sec for the entire duration of the experiment) then evoked small EPSPs in CA3 cells. These EPSPs depended linearly on membrane potential (not shown), could be strongly reduced by increasing the concentration of CNQX (Fig. 1*A*), and are thus mediated by non-NMDA receptors. At membrane potentials between −60 and −80 mV, the evoked responses had an average amplitude of 7.5 ± 1.9 mV ($n = 56$). The isolated EPSPs had a constant latency and single peak over a range of stimulation intensities (Fig. 1*B*), exhibited a monophasic decay, and followed stimulation frequencies up to 10 Hz without any changes in amplitude or latency (Fig. 1*C*). These results indicate that pharmacologically isolated EPSPs result from the monosynaptic activation of many synapses, presumably formed by mossy fibers. The contribution of polysynaptic pathways is seemingly negligible, since these pathways should produce a second peak to the EPSP, with longer latency, and be unable to follow high frequencies of stimulation.

Bath application of NE (5 μM) reversibly decreased the amplitude of isolated EPSPs by 44 ± 13% (range, 13–82%, $n = 39$) (Fig. 2*A*). This action of NE was rapid in onset, and reached its maximal effect within 1–2 min. Recovery of EPSP amplitude (97 ± 13% of control amplitude) was observed 10–20 min after ceasing drug perfusion (Fig. 2*A*). NE also caused a decrease of 46 ± 15% ($n = 6$) in the amplitude of EPSPs elicited with the stimulating electrode placed in stratum radiatum of area CA1, in an attempt to activate antidromically the axons of adjacent CA3 pyramidal cells (Fig. 3). Because these EPSPs should be predominantly mediated by local recurrent collaterals (and possibly some monosynaptic CA1–CA3 projections), it is likely that all fast excitatory inputs to the CA3 cells are sensitive to NE. The following experiments were all performed on EPSPs evoked with stimulation within the dentate gyrus, however.

The effect of NE on isolated EPSPs was observed to fade with multiple applications. In experiments in which NE was applied twice, allowing for complete recovery of EPSP amplitude between applications (10–20 min), the first application reduced EPSP amplitude by 43 ± 12%, while a second application produced a significantly smaller reduction of only 23 ± 13% ($n = 12$; $p < 0.005$, paired t test) (see Fig. 7*C*).

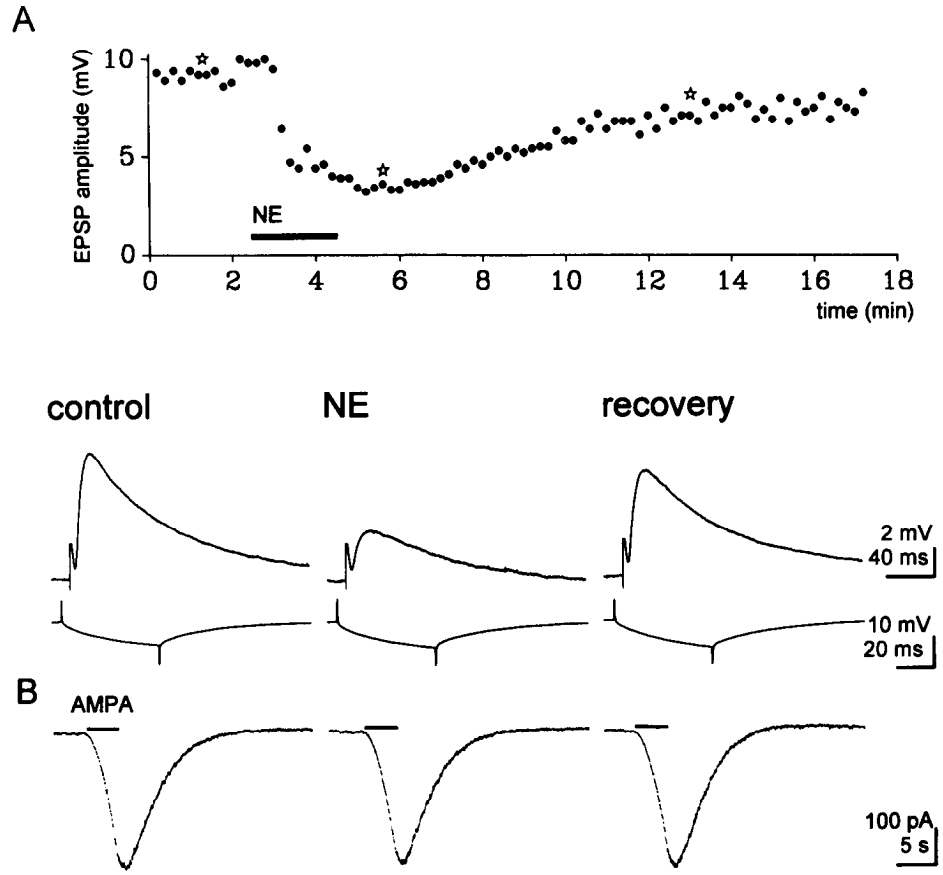

Figure 2. Effect of NE on isolated EPSPs and AMPA-induced currents. *A*, NE (5 μM) reversibly reduced the amplitude of the pharmacologically isolated EPSP recorded in a CA3 neuron after mossy fiber stimulation. Representative EPSPs from the indicated times (*stars*) are shown *below* the graph. In this and subsequent figures, each sweep represents the average of 5–10 episodes. NE had no observable effect on the passive membrane characteristics of the cell assessed by injecting hyperpolarizing current pulses (−0.125 nA, 50 msec). Membrane potential, −57 mV; potassium methylsulfate electrode. *B*, Different cell: inward current evoked by a 4 sec iontophoretic pulse of AMPA (*horizontal bars*) in a single-electrode voltage-clamped CA3 neuron in the presence of TTX (0.5 μM) and bicuculline (40 μM). Note that bath application of NE (5 μM) had no major effect on the amplitude of the inward current, suggesting that NE exerts its effects on EPSPs via presynaptic inhibition. Holding potential, −64 mV; KCl electrode.

Does norepinephrine act pre- or postsynaptically?

Decreases in EPSP amplitude may reflect decreases in the presynaptic release of glutamate or in the postsynaptic glutamate sensitivity. The linear dependence of the EPSP amplitude on membrane potential, as well as its reduction by CNQX, indicates that the glutamate receptor subtype mediating the EPSP under our conditions belongs to the AMPA/kainate class. We therefore compared the response of single-electrode voltage-clamped CA3 pyramidal cells to iontophoretically applied AMPA, before and during perfusion of NE (Fig. 2*B*). For these experiments, the cultures were continuously perfused with TTX (0.5 μM) and bicuculline (40 μM). AMPA was iontophoresed (4 sec) at regular intervals (90 sec), with the tip of the iontophoretic pipettes placed close to the recorded neurons. The amplitude of the evoked inward currents ranged from 360 to 890 pA, at holding potentials of −60 to −70 mV, and were associated with a 48 ± 5% decrease in the control input resistance ($n = 4$). Bath perfusion of CNQX (20 μM) reversibly blocked the response to AMPA. NE (5 μM), however, had no significant effect on the amplitude of the evoked AMPA currents (99 ± 3% of control, $n = 5$). Perfusion of NE in the presence of TTX (0.5 μM) and bicuculline (40 μM) was not accompanied by any change in holding current greater than ±30 pA ($n = 5$), or any change in the input resistance of the neuron (103 ± 6% of control; $n = 4$).

These results indicate that the sensitivity of pyramidal cell AMPA receptors is not affected by NE, suggesting that NE decreases EPSP amplitude by reducing the amount of excitatory transmitter release from presynaptic terminals. Nevertheless, the possibility cannot be excluded that iontophoretically applied AMPA activates many extrasynaptic receptors, which may not be under the control of the same regulatory pathways as synaptic receptors. The sensitivity of synaptic glutamate receptors can be assessed through the analysis of miniature synaptic currents. If NE were acting postsynaptically to decrease the sensitivity of pyramidal cells to glutamate, it should also reduce the amplitude of miniature excitatory synaptic currents.

Whole-cell recording from CA3 neurons, in the presence of TTX (0.5 μM) and bicuculline (40 μM), allowed the detection of

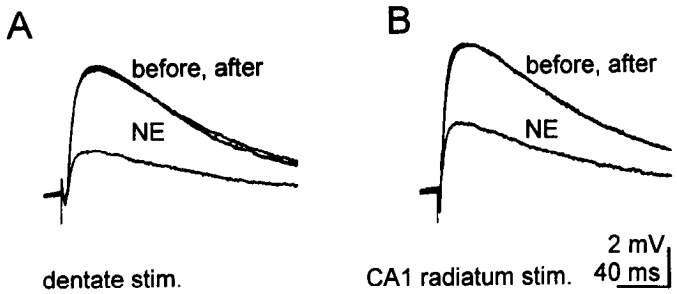

Figure 3. NE decreases the amplitude of recurrent collateral- and mossy fiber–evoked pharmacologically isolated EPSPs: response of a CA3 neuron to the stimulation of two different synaptic inputs. The stimulating electrodes were placed within the dentate gyrus for mossy fiber stimulation (*A*) and in the stratum radiatum of area CA1 to antidromically activate recurrent collaterals (*B*). Note that NE (5 μM) reduced the amplitude of both synaptic inputs to a similar extent. Membrane potential, −57 mV; potassium methylsulfate electrode.

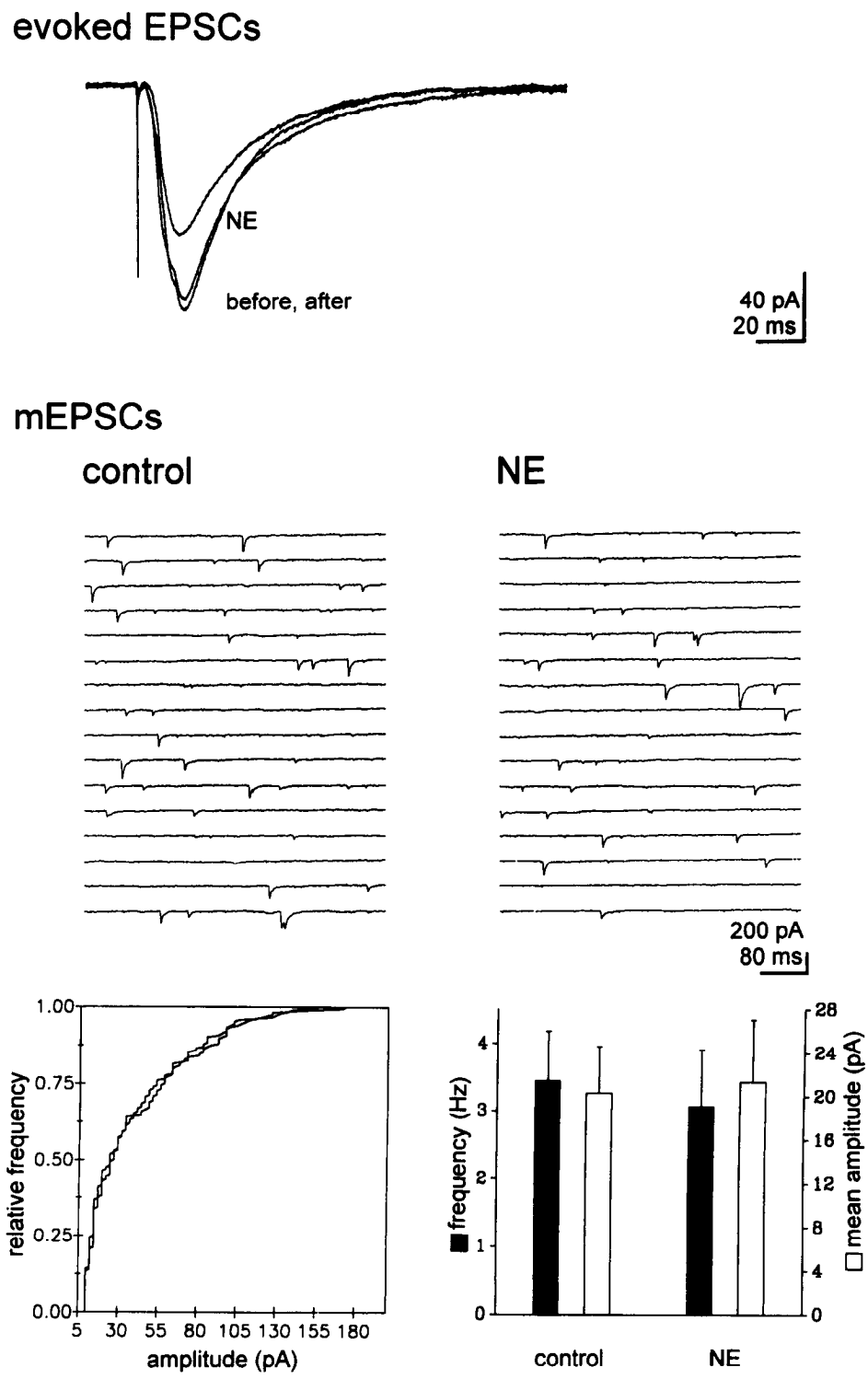

Figure 4. NE does not affect the frequency or amplitude distribution of mEPSCs. *Upper panel,* NE (5 μM) inhibited excitatory synaptic currents evoked with mossy fiber stimulation, as recorded from a whole-cell voltage-clamped CA3 neuron using a CsF-based intracellular solution to block $GABA_A$ and $GABA_B$ receptor–mediated Cl^- and K^+ conductances. Holding potential, −64 mV. *Middle panels,* Different cell: spontaneous, TTX-resistant mEPSCs recorded from a whole-cell voltage-clamped CA3 neuron in the presence of bicuculline (40 μM). Bath application of NE did not affect the frequency or amplitude of mEPSCs. Holding potential, −100 mV. *Lower panel: Left,* Same cell: amplitude distribution of the mEPSCs before and after NE application (not significantly different, $p > 0.99$, Kolmogorov-Smirnov test; number of events analyzed, 184 and 164). *Right,* Pooled data from five cells showing that NE had no effect on the mean amplitude or frequency of mEPSCs. These data are thus consistent with a presynaptic site of NE action.

spontaneous inward currents that were abolished by CNQX. The amplitude of these mEPSCs could be as large as 200 pA.

NE (5 μM) had no significant effect on either the frequency (90 ± 24% of control) or the distribution of EPSC amplitudes, as determined with the Kolmogorov-Smirnov test ($n = 5$) (Fig. 4). The lack of effect of NE cannot be ascribed to the whole-cell recording conditions, since 5 μM NE produced a 51 ± 12% decrease in the amplitude of EPSCs in CA3 neurons recorded with whole-cell clamp after mossy fiber stimulation ($n = 4$, recovery from NE = 101 ± 21% of control amplitude) (Fig. 4). This inhibition is thus very similar to the depression of EPSP amplitudes observed in intracellular microelectrode recordings. The failure to observe a change in the amplitude distribution of mEPSC following application of NE further supports the hypothesis of a presynaptic site of NE action.

Pharmacology of the presynaptic norepinephrine receptor

We used selective adrenergic agonists and antagonists to characterize the pharmacological properties of the receptors mediating the presynaptic inhibition of EPSPs by NE. Because of the

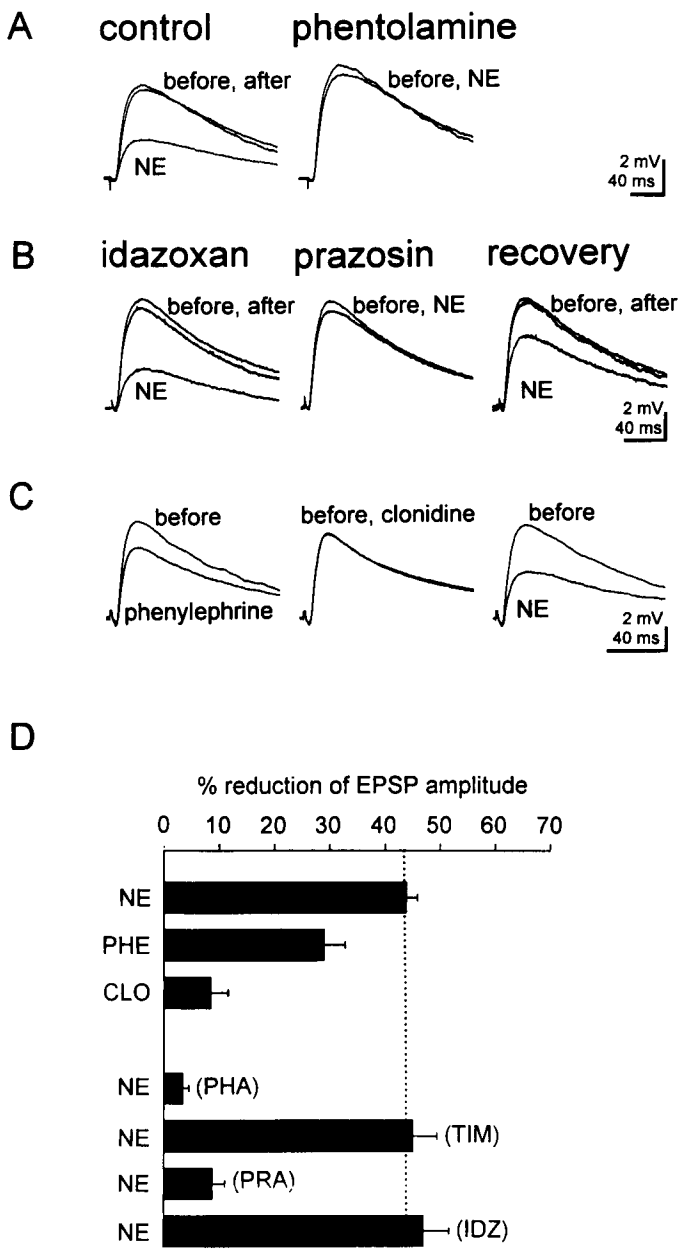

Figure 5. Pharmacological profile of the adrenergic receptor mediating the inhibition of pharmacologically isolated EPSPs: responses of CA3 cells to stimulation within the dentate gyrus. *A*, The α receptor antagonist phentolamine (50 μM) blocked the depression of the isolated EPSP produced by 5 μM NE. Membrane potential, −84 mV; KCl electrode. *B*, Different cell. NE causes a large decrease in EPSP amplitude in the presence of the specific α_2 receptor antagonist idazoxan (1 μM), whereas the effects of NE were virtually eliminated by application of the specific α_1 receptor antagonist prazosin (500 nM). Membrane potential, −70 mV; KCl electrode. *C*, Different cell. The α_1 receptor–specific agonist phenylephrine (50 μM) reduced EPSP amplitude, whereas the specific α_2 receptor agonist clonidine (5 μM) did not affect the amplitude of the EPSP. The effect of phenylephrine on EPSP amplitude was less than that of NE (5 μM) in this same cell. Membrane potential, −65 mV; potassium methylsulfate electrode. *D*, Summary of the pharmacology of noradrenergic inhibition of EPSPs. Each column shows the percentage reduction (mean + SEM) of the control EPSP amplitude for a given experimental condition. *NE*, 5 μM, n = 39; *PHE* (phenylephrine), 25–100 μM, n = 11; *CLO* (clonidine), 5 μM, n = 7; *PHA* (phentolamine) 50 μM, n = 5; *TIM* (timolol) 5 μM, n = 8; PRA (prazosin), 500 nM, n = 6; *IDZ* (idazoxan), 500 nM to 1 μM, n = 9.

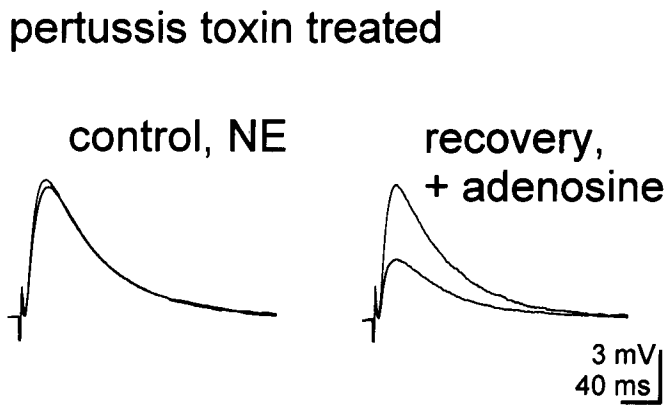

Figure 6. Pertussis toxin treatment specifically affects the presynaptic action of NE. Pretreatment of hippocampal slice cultures with pertussis toxin (500 ng/ml, 48 hr) virtually eliminated the ability of NE (5 μM) to reduce the amplitude of pharmacologically isolated EPSPs evoked with stimulation within the dentate gyrus. Adenosine, in contrast, was still able to depress the EPSP. Membrane potential, −60 mV; potassium methylsulfate electrode.

fading of successive NE responses, only the first application of agonist is included in the numerical values presented below. In the presence of the β receptor antagonist timolol (5 μM), NE (5 μM) decreased the amplitude of isolated EPSPs by 45 ± 12% (n = 8; not shown), that is, to a similar extent as in control saline. On the other hand, bath perfusion of the broad spectrum α receptor antagonist phentolamine (50 μM) virtually prevented the NE-mediated depression of the isolated EPSP (6 ± 2% reduction in amplitude, n = 5) (Fig. 5*A*). The action of NE was unaffected by the presence of the selective α_2 receptor antagonist idazoxan (1 μM) (47 ± 13% reduction in EPSP amplitude, n = 9), whereas NE depressed the isolated EPSP by only 9 ± 5% in the presence of the α_1 receptor antagonist prazosin (0.5–1 μM) (n = 6) (Fig. 5*B*). These data are summarized in Figure 5*D*.

Taken together, these results implicate presynaptic α_1 receptors in the action of NE on EPSPs. The specific α_1 agonist phenylephrine, however, was not equally effective as NE, even if applied at concentrations that take into account the 15-fold higher affinity of α_1 receptors for NE in the hippocampus (Lomasney et al., 1991). Phenylephrine (50–100 μM) reversibly reduced the isolated EPSP amplitude by only 29 ± 11% (n = 11, recovery = 94 ± 10% of control amplitude) (Fig. 5*C*), significantly (Wilcoxon test, $p < 0.01$) less than the action of 5 μM of NE (see above). On the other hand, the specific α_2 agonist clonidine (1–5 μM) had virtually no effect on EPSP amplitude (9 ± 8% reduction, n = 7; significantly less than the effect of phenylephrine, $p < 0.01$, Wilcoxon test) (Fig. 5*C*). We have not attempted to characterize further the subtype of α receptor involved in this response.

G-proteins, protein kinase C, and presynaptic α adrenergic receptors

One subtype of α_1 adrenergic receptor that has recently been cloned (α_{1A}) is abundantly expressed in the rat hippocampus and has a putative transmembrane topology similar to that of known G-protein–coupled receptors (Lomasney et al., 1991). Pertussis toxin inactivates several distinct types of G-proteins, and has been shown to prevent the action of several agonists modulating transmitter release. For these reasons, we examined the effect of pertussis toxin on NE-mediated inhibition of iso-

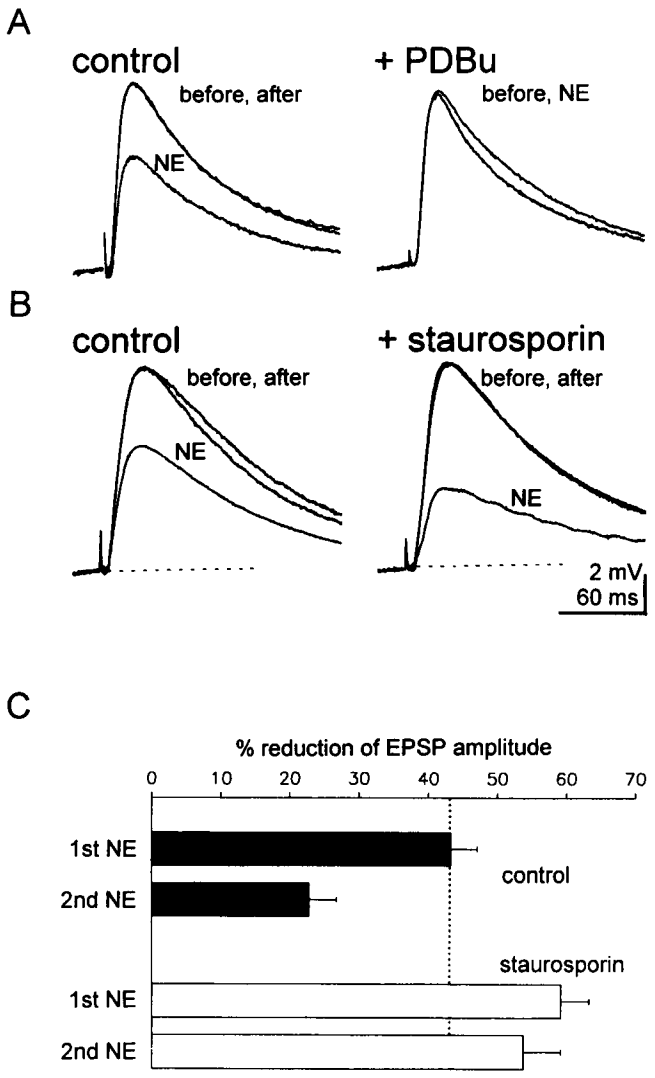

Figure 7. Influence of PKC on adrenergic presynaptic inhibition and desensitization. *A*, After perfusion of the PKC activator PDBu (200 nM, 10 min), NE (5 μM) was no longer able to reduce the amplitude of the pharmacologically isolated EPSP evoked with stimulation in the dentate gyrus. Because PDBu caused an increase of the amplitude of the EPSP, the stimulation intensity was reduced so that the EPSP returned to its original amplitude. Membrane potential, −65 mV; potassium methylsulfate electrode. *B*, Different cell, same conditions. The depression of the isolated EPSP by NE (5 μM) was potentiated by 68% after application of the protein kinase inhibitor staurosporin (1 μM). Membrane potential, −74 mV; potassium methylsulfate electrode. *C*, Pooled data from untreated cells (*solid bars*; $n = 12$) and cells treated with 1–2 μM staurosporin (*open bars*; $n = 5$). With two applications of NE, the inhibition of EPSPs was significantly less for the second application than the first. In the presence of staurosporin, however, the second response to NE was not significantly different than the first. Note also that staurosporin potentiated the presynaptic inhibition of EPSPs by NE.

lated EPSPs. After pretreatment of hippocampal slice cultures with pertussis toxin (500 ng/ml for 48 hr), the inhibitory action of NE was found to be virtually abolished (7 ± 10% reduction, $n = 10$) (Fig. 6). Pretreatment with pertussis toxin did not unspecifically impair all presynaptic modulation mechanisms, however, since 50 μM adenosine still induced a depression of the isolated EPSP (Fig. 6), in agreement with previous findings (Fredholm et al., 1989; Thompson et al., 1992).

Stimulation of protein kinase C (PKC) has been shown to inactivate skeletal muscle α_1 receptors (Leeb-Lundberg et al., 1985). Perfusion of hippocampal cultures with the PKC activator PDBu (100–500 nM) invariably led to an increase in the amplitude of isolated EPSPs of 39 ± 11% ($n = 4$; not shown). We therefore reduced the stimulation intensity in order to return the amplitude of the isolated EPSP to the control level, thus allowing a quantitative comparison of NE action. Perfusion of PDBu completely eliminated the action of NE (5 μM) on isolated EPSPs (4 ± 10% reduction, $n = 6$) (Fig. 7*A*).

After incubation of cultures for 1–2 hr with 1–2 μM of the protein kinase antagonist staurosporin, PDBu (100–500 nM) failed to block the noradrenergic depression of isolated EPSPs, indicating a specific action of the phorbol ester. In the presence of staurosporin, the decrease in EPSP amplitude elicited by NE after application of PDBu was 38 ± 10% ($n = 5$, not shown).

The effect of NE on the isolated EPSP was significantly ($p < 0.01$, Wilcoxon test) enhanced after application of staurosporin (1–2 μM) (60 ± 8% reduction in EPSP amplitude, $n = 11$) (Fig. 7*B*). This observation suggests that constitutively active protein kinases tonically modulate the efficacy of presynaptic α receptors.

Staurosporin was also found to abolish the desensitization observed with multiple NE applications. Whereas the effect of NE was 50% less in the second of two applications in control cultures, as described above, in staurosporin-treated cultures the inhibition of EPSP amplitude was 59 ± 8% for the first application and 54 ± 10% for a second application given <20 min later ($n = 5$ cells; not significantly different, $p > 0.4$, paired t test) (Fig. 7*C*).

Discussion

In the present study, we report that NE inhibits pharmacologically isolated EPSPs recorded in CA3 pyramidal cells of hippocampal slice cultures. These EPSPs were evoked in the presence of the NMDA receptor antagonist AP5, after blockade of the early and late component of the IPSP by bicuculline and CGP 35 348, respectively. A nonsaturating concentration of the non-NMDA receptor antagonist CNQX was applied in order to prevent epileptiform burst discharges. Under such conditions, the isolated EPSP had the characteristics of a monosynaptic, AMPA/kainate receptor–mediated response. EPSPs evoked with stimulation of either mossy fibers or recurrent axon collaterals of CA3 pyramidal cells were both decreased in amplitude by bath application of NE. The depression of isolated EPSPs by NE was accompanied by neither a reduction in postsynaptic sensitivity to iontophoretically applied AMPA nor a change in the amplitude distribution of mEPSCs, indicating a presynaptic site of action. We conclude that NE acts via presynaptic receptors to decrease the evoked release of transmitter from excitatory terminals of both mossy fibers and CA3 pyramidal cell recurrent collaterals.

There are conflicting reports about the action of NE on EPSPs in hippocampal slices (cf. Mody et al., 1983; Hopkins and Johnston, 1988), perhaps reflecting both desensitization of presynaptic adrenergic receptors (see below), as a result of tonic NE release from fibers damaged during the slicing procedure, and adrenergic disinhibition, which results in an indirect enhancement of EPSPs (Madison and Nicoll, 1988). Indeed, the effect of NE reported here is likely to represent the mechanism underlying the decreased excitation of interneurons responsible for this disinhibition (Doze et al., 1991).

The inhibition of EPSPs by NE was unaffected by timolol,

blocked by phentolamine and prazosin but not idazoxan, and mimicked by phenylephrine but not by clonidine. This pharmacological profile is consistent with the involvement of an α_1 adrenergic receptor. It is important to note, however, that the α_1 agonist phenylephrine was considerably less potent than NE, even with applied at a concentration that should compensate for the difference in affinity of the two agonists reported for recombinant hippocampal α_1 receptors (Lomasney et al., 1991). A similar difference in activity between NE and phenylephrine was observed in previous physiological and biochemical studies on cortical slices (Brown et al., 1984; Minneman and Johnson, 1984; Dodt et al., 1991), suggesting that phenylephrine is a partial agonist at these receptors.

Noradrenergic modulation of transmitter release has been observed in many different systems, including terminals of locus coeruleus neurons, sympathetic neurons (for review, see Starke, 1987), and mitral and granule cells of the olfactory bulb (Trombley and Shepherd, 1991). Our evidence for a presynaptic α_1 receptor–mediated action of NE is somewhat unexpected, however, since adrenergic presynaptic receptors are typically classified as α_2 (Starke, 1987). Activation of α_1 receptors in noncortical regions of the CNS usually decreases postsynaptic K^+ conductances, thus increasing neuronal excitability (e.g., North and Yoshimura, 1984; Aghajanian, 1985; McCormick and Prince, 1988). Our results are consistent, however, with the observation that the inhibition of population spike amplitude by NE in the CA1 region of the hippocampal slice is mediated by the activation of a prazosin-sensitive α adrenergic receptor (Mynlieff and Dunwiddie, 1988). Furthermore, the inhibitory effects of endogenous and exogenous NE in the hippocampus *in vivo* are also reduced by α_1 receptor antagonists (Pang and Rose, 1987; Curet and de Montigny, 1988b). Likewise, inhibition of EPSPs by NE in neocortical slices may be ascribed to α_1 receptors (Dodt et al., 1991). Considerable evidence of presynaptic inhibition mediated by α_1 receptors has been obtained in the PNS (e.g., Docherty, 1984; Story et al., 1985; Wetzel et al., 1985). Although we have not yet further characterized the subtype of α_1 receptor that mediates adrenergic presynaptic inhibition in our experiments, analysis of Northern blots and *in situ* hybridization data indicates that α_{1A} receptors are the predominant subtype expressed in the hippocampus (Lomasney et al., 1991; McCune et al., 1992).

Second messengers and presynaptic α receptors

Incubation of hippocampal cultures with pertussis toxin was found to prevent the inhibition of EPSPs by NE, indicating an involvement of a G-protein in the coupling of presynaptic α receptors to their effector mechanism. In addition, application of the PKC activator PDBu eliminated the effect of NE on EPSPs, whereas pretreatment of cultures with the protein kinase antagonist staurosporin enhanced the noradrenergic inhibition of EPSPs. This later finding further suggests that constitutively active PKC may tonically downregulate the effectiveness of NE receptors.

We have observed that multiple applications of NE lead to a pronounced decrease in its ability to inhibit excitatory synaptic transmission. Desensitization of α_1 receptor–mediated phosphatidylinositol turnover in smooth muscle has been well characterized. Exogenously applied PKC activators strongly attenuate α_1 receptor–mediated phosphatidylinositol turnover by causing phosphorylation of the α_1 receptor itself (Leeb-Lundberg et al., 1985). Interestingly, activation of α_1 receptors also induces a PKC-mediated phosphorylation of the receptor, leading to "homologous" desensitization (Leeb-Lundberg et al., 1987). The deduced amino acid sequence of the α_1 adrenergic receptor subtype expressed in the hippocampus contains potential sites for phosphorylation by PKC (Lomasney et al., 1991). If in the hippocampus, as well as in smooth muscle, activation of the α_1 adrenergic receptor promotes PKC activation, which seems likely since α receptor activation increases phosphatidylinositol turnover in the hippocampus (Janowsky et al., 1984), then the model of "homologous" desensitization may explain the reduced effect of NE with multiple applications. Indeed, we have observed that staurosporin application eliminates the desensitization observed with multiple NE applications, indicating that activation of NE receptors stimulates protein kinase and thus exerts a negative feedback on subsequent α receptor–mediated action. It should be noted that this observation does not directly demonstrate phosphorylation of receptors, as PKC may also have other targets "downstream" of the α_1 adrenergic receptor.

Although α_1 adrenergic receptors in the hippocampus and neocortex are linked to phosphoinositide hydrolysis (Brown et al., 1984; Janowsky et al., 1984; Minneman and Johnson, 1984; Schoepp et al., 1984), there is no evidence linking phosphatidylinositol turnover with inhibition of synaptic transmission, to our knowledge. In fact, phosphatidylinositol turnover leads to the generation of diacylglycerol, the physiological activator of PKC, which would be expected to increase excitatory transmission (e.g., Malenka et al., 1987). Furthermore, Dutar and Nicoll (1988) found no correlation between the effectiveness of various muscarinic agonists in stimulating phosphatidylinositol turnover and their ability to inhibit excitatory synaptic transmission in the hippocampus.

NE was found to have no effect on the frequency of spontaneous mEPSCs recorded from whole-cell voltage-clamped CA3 pyramidal neurons in the presence of TTX. In contrast, it has recently been shown that activation of presynaptic $GABA_B$ and adenosine A_1 receptors located on excitatory terminals, but not block of voltage-gated Ca^{2+} channels, can reduce the frequency of mEPSCs in the hippocampus (Scanziani et al., 1992a; Scholz and Miller, 1992), suggesting that transmitters can have a direct action on the probability that synaptic vesicles will spontaneously fuse with the presynaptic membrane. We can therefore exclude a direct action of NE on the transmitter release process. We suggest, rather, that activation of α receptors may modulate presynaptic ionic conductances.

The biphasic effect of NE on the network activity of the hippocampus may result from pharmacologically distinct α receptor–mediated inhibition and β receptor–mediated excitation (Mueller et al., 1981). Activation of β receptors has been shown to induce a long-lasting enhancement of the population spike in the CA1 region (e.g., Heginbotham and Dunwiddie, 1991) and to play a facilitatory role in the induction of long-term potentiation of mossy fiber synapses (Hopkins and Johnston, 1988). Increases in intrinsic pyramidal cell excitability following β receptor activation (Madison and Nicoll, 1986; Gray and Johnston, 1987; Dunwiddie et al., 1992) probably underlie the increased excitability of the network. The depression in the amplitude of the EPSP upon activation of presynaptic α adrenergic receptors described in this article represents a plausible mechanism responsible for the noradrenergic inhibition of hippocampal activity as described *in vivo* (Segal and Bloom, 1974a,b; Mueller et al., 1982; Neuman, 1986; Pang and Rose, 1987; Curet

and de Montigny, 1988a,b) and *in vitro* (Mody et al., 1983; Mueller and Dunwiddie, 1983; Mynlieff and Dunwiddie, 1988).

The description of opposing actions of NE on α and β receptors has some interesting functional implications for neuronal integration: α receptor–mediated presynaptic inhibition will reduce the likelihood that an EPSP will reach the threshold for action potential generation in the postsynaptic cell. Those EPSPs that do reach threshold, however, may produce a larger response, due to the β receptor–mediated increase in postsynaptic excitability. These concerted actions support the hypothesis that NE increases the signal-to-noise ratio in the hippocampus (e.g., Woodward et al., 1979).

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