Electrophysiological Correlates of Presynaptic α_2 -Receptor-Mediated Inhibition of Norepinephrine Release at Locus Coeruleus Synapses in Dentate Gyrus

Mark Washburn and Hylan C. Moises

Department of Physiology and the Neuroscience Program, The University of Michigan, Ann Arbor, Michigan 48109

Despite an abundance of evidence that presynaptic α_2 -adrenergic receptors mediate inhibitory control of the release of norepinephrine (NE) from the terminals of locus coeruleus (LC) neurons, few studies have demonstrated the physiological significance of this "autoreceptor"-mediated inhibition on NE-mediated synaptic activity within the mammalian brain. This question was addressed by examining the effects of systemic administration of α_2 -adrenergic agonists and antagonists on the ability of LC stimulation to augment the population spike recorded in the dentate gyrus in response to activation of the perforant path (PP). Extracellular field potentials were recorded in the cell body and dendritic layer of dentate gyrus following single shocks of the entorhinal cortex in halothane-anesthetized rats. Stimulation of the ipsilateral LC 35 msec prior to PP activation produced a short-term enhancement of the population spike amplitude recorded in the cell layer but did not significantly alter dendritic potentials. The effects of LC stimulation were blocked by administration of the β -adrenergic antagonist propranolol but not the α_2 -antagonist idazoxan and were abolished by pretreatment of animals with the catecholamine neurotoxin 6-hydroxydopamine. Administration of clonidine reversibly abolished the enhancement produced by LC conditioning. The effect of clonidine was dose dependent and was blocked by administration of idazoxan, which restored the LC potentiative effect. Conditioning stimulation of LC noradrenergic axons in the dorsal bundle also potentiated the PP-evoked population spike, and this effect was equally sensitive to the depressant action of clonidine. In comparison, clonidine, in the range of dosages tested, did not significantly affect the potentials evoked in the dendritic or cell layer by presentations of unconditioned PP test stimuli. We interpret these data to provide evidence for a functional impairment of LC-mediated physiological action on postsynaptic target cells as a result of presynaptic α_2 -autoreceptor-mediated feedback inhibition of NE release.

A substantial body of evidence indicates that synaptically released norepinephrine (NE) exerts feedback inhibition of its own release via interaction with presynaptic α_2 -adrenergic inhibitory

autoreceptors. This inhibitory modulation of neuronal release of NE has now been demonstrated in many areas of the CNS and in a variety of sympathetically innervated peripheral tissues (for review, see Starke, 1980; Langer, 1981). The involvement of presynaptic α_2 -adrenergic receptors in the mediation of these effects at central noradrenergic junctions is supported in large part from studies *in vitro* that have consistently demonstrated an inhibitory effect of α_2 -adrenergic agonists on the stimulation-evoked release of ^3H -NE from nerve terminal endings of locus coeruleus (LC) noradrenergic neurons in brain slice preparations of rodent cerebral cortex (Langer, 1981; Schoffeleers and Mulder, 1983). Recently, α_2 -adrenergic agonists have been reported to exert similar inhibitory effects on the calcium-dependent release of ^3H -NE elicited from rat and rabbit hippocampal slices following exposure to high extracellular potassium or electrical field stimulation (Frankhuyzen and Mulder, 1982; Jackisch et al., 1984). The additional observation in these kinds of brain slice preparations that the administration of α_2 -adrenergic antagonists alone can enhance the stimulation-evoked release of ^3H -NE has been interpreted to support an important role for presynaptic α_2 -autoreceptor-mediated inhibition in the normal physiological regulation of NE release. Nevertheless, demonstration of the physiological significance of this autoreceptor-mediated inhibition of neurotransmitter release on the functional activity of NE-mediated synapses within the mammalian brain is yet to be forthcoming.

The dentate gyrus of rat hippocampus is known to receive a dense innervation of NE-containing fibers and terminals arising largely from noradrenergic neurons whose cell bodies are located within the LC (Blackstad et al., 1967; Swanson and Hartman, 1975; Jones and Moore, 1977; Loy et al., 1980). Electrophysiological characterizations of noradrenergic action in this brain region *in vivo* (Harley et al., 1982; Neuman and Harley, 1983) and *in vitro* in the hippocampal slice (Lacaille and Harley, 1985; Stanton and Sarvey, 1987) have revealed that exogenous application of NE results in an increased excitability of granule cells to synaptic activation and that this action is mediated via the activation of β -adrenergic receptors (Lacaille and Harley, 1985). At the extracellular level this action of NE is manifested as an increase in the amplitude of the population spike recorded in the dentate gyrus cell layer in response to activation of the perforant path (PP), the major excitatory afferent to the hippocampus (Andersen et al., 1966). Release of endogenous NE via stimulation of the LC has been shown to elicit a facilitation of the synaptically evoked population spike response in dentate gyrus comparable to that produced by exogenous application of NE (Assaf et al., 1979; Harley et al., 1982; Dahl and Winsor,

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Correspondence should be addressed to Hylan C. Moises, Ph.D., at the above address.

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1985; Harley and Milway, 1986). Moreover, studies at the intracellular level have now demonstrated that the potentiative action of NE on dentate granule cell excitability is mediated postsynaptically and suggested that this effect occurs via a β -adrenergic receptor-mediated blockade of a calcium-dependent potassium conductance (Haas and Rose, 1987) and/or an increase in a voltage-sensitive calcium current (Gray and Johnston, 1987). On the basis of these findings, we reasoned that the ability of LC stimulation to enhance the amplitude of the PP-evoked dentate population spike response might provide a sensitive electrophysiological end point for assessing possible changes in NE-mediated synaptic efficacy that resulted from alterations in presynaptic release of endogenous neurotransmitter.

In the present study, the ability of conditioning stimulation of the LC to potentiate the dentate population spike was examined before and after systemic administration of agents selective for α_2 -adrenergic receptors to determine whether alterations in NE-mediated synaptic action occur as a result of α_2 -autoreceptor-mediated feedback inhibition of neurotransmitter release. The results show that administration of the α_2 -selective agonist clonidine results in a functional impairment of LC-mediated physiological action on postsynaptic target cells in the dentate gyrus and suggest that this occurs via activation of presynaptic inhibitory α_2 -autoreceptors located on the terminals of LC noradrenergic neurons. A preliminary report of some of these results has appeared elsewhere (Moises and Carlson, 1986).

Materials and Methods

Surgical preparation. Data were obtained from adult male Sprague-Dawley rats weighing 300–350 gm. Rats were initially anesthetized with 3% halothane, tracheostomized, and implanted with a catheter in the left femoral vein. Animals were then mounted in a stereotaxic apparatus and maintained at 0.75% halothane throughout the course of the experiment. Body temperature was held constant at 37°C via a servocontrol heating unit.

The skull was positioned in a 5° nose-down position to facilitate access to the LC. A craniotomy was performed to expose the dura overlying the entorhinal cortex, dentate gyrus, and LC or dorsal noradrenergic bundle (DB). Once the dura had been removed, stimulating and recording electrodes were positioned. The PP was activated by electrical stimulation via a concentric bipolar stimulating electrode positioned within the entorhinal cortex at stereotaxic coordinates 7.9 mm caudal to bregma, 4.1 mm lateral to the midline, and approximately 3.0 mm below the pial surface. A second stimulating electrode was placed in the ipsilateral LC approximately 1.1 mm posterior to lambda, 1.0 mm lateral to the midline, and 5.7 mm ventral to the pia. In experiments in which stimulation of the DB was used in place of activation of the LC, the holder was set at 15° off of vertical and the stimulating electrode positioned at 1.4 mm anterior to lambda, 0.8 mm lateral, and 6.3 mm below the pia. Upon termination of an experiment, the position of the LC or DB stimulating electrode was marked by passing a direct current of 20 μ A for 20 sec. The location of each stimulation site was verified histologically in 50 μ m frozen sections stained with cresyl violet.

Recording and stimulation. Extracellular responses were recorded in the cell body or dendritic layer of the dentate gyrus using single- or double-barreled staggered glass microelectrodes filled with 3 M NaCl and having resistances of 1–5 M Ω . Coordinates used for recording extracellular field potentials in the dentate gyrus were 4.0 mm caudal to bregma and 2.3 mm lateral of the midline, with the depth of the recording being adjusted to maximize the amplitude of the population spike and field excitatory synaptic potential (ESP) evoked by PP activation. PP stimulation consisted of a single 0.2 msec current pulse delivered to the entorhinal cortex once every 15 sec. The responses obtained at a given level of PP stimulation, delivered alone or paired with conditioning stimulation (see below), were averaged over 8 consecutive trials using a digital oscilloscope (Nicolet, Model 4094 Digital Oscilloscope) and written to floppy disk for subsequent off-line computer analysis.

Analysis of the effects of conditioning stimulation. In each experiment,

the intensity of PP stimulation was adjusted to elicit a population spike of half-maximal amplitude to ensure a clear enhancement of the response during pairings with LC or DB conditioning stimulation. Conditioning stimulation consisted of 5 current pulses of 0.2 msec duration at 300 Hz delivered 35 msec prior to PP activation. The effects of LC noradrenergic pathway activation on the PP-evoked population spike were assessed by comparing the maximal amplitudes in mV of the averaged spike responses obtained with and without LC conditioning and expressing the difference as a percent change (i.e., enhancement) of the control nonconditioned response. Population spike amplitude was calculated by taking the average of the peak spike negativity to preceding and following slow wave positivities (see Fig. 2A).

Once the control responses to PP activation alone and with conditioning were established, similar sets of averaged responses were recorded after each systemic administration of an adrenergic drug. For all drug testing paradigms the intensity of conditioning stimulation delivered to LC or DB was adjusted to elicit an increase in population spike amplitude that was approximately 50% of the maximal potentiative effect. This was done to ensure that any facilitating effect of α_2 -adrenergic antagonist administration on NE-mediated synaptic action would not be obscured on account of taking measurements at a saturating level of the functional response. The effect of drug administration on the LC- or DB-induced potentiative action was determined in a given experiment by comparing the maximal amplitudes of the conditioned PP-evoked population spike and of the unconditioned response recorded at a given dosage and expressing the difference at each sampling point as a percent enhancement of the baseline (i.e., unconditioned) response. Measurements were taken beginning 5 min after an injection of drug to allow time for equilibration and stabilization of the drug-induced effect. The average percent enhancement in population spike amplitude produced by LC or DB conditioning stimulation before and after administration of an adrenergic drug at varying levels of dosage was computed by pooling data from many experiments. These average values were then used to construct complete dose-response curves. Differences in LC- or DB-induced facilitating effects before and after administration of test substances were assessed statistically by means of analysis of variance and Student's *t* test.

Drug administration and treatment. Drugs were prepared in sterile physiological saline and injected intravenously at approximately 1 ml/min through the femoral catheter. All drugs were administered in a cumulative dosage fashion with individual concentrations being increased by approximately 3-fold increments in order to construct complete dose-response curves. The cumulative clonidine dosages that were administered ranged from 3.75 ng/kg to 12.5 μ g/kg. Cumulative idazoxan dosages were 37.5 ng/kg to 12.5 μ g/kg. Single dosages of propranolol or idazoxan were tested in some animals. Drugs were obtained from the following commercial sources: clonidine·HCl (Sigma); idazoxan·HCl (Reckitt and Colman); propranolol·HCl (Inderal, Ayerst).

Two to 3 weeks before electrophysiological recording, 4 rats were given 2 intracisternal injections, at 48 hr intervals, of 200 μ g 6-hydroxydopamine-HBr (6-OHDA, Sigma) in 50 μ l of saline containing 1 mg/ml ascorbic acid. Following electrophysiological testing, each animal was killed by decapitation and its brain removed and rapidly frozen on dry ice for later determination of catecholamine content. The endogenous hippocampal levels of NE in the 6-OHDA treated rats used here were assayed by high-performance liquid chromatography according to the method of Refshauge et al. (1974). The values obtained from the pretreated animals were compared with those from nontreated controls assayed simultaneously.

Results

LC Facilitation of PP-evoked population spike

Experiments were first carried out to establish the optimal parameters of LC conditioning stimulation to elicit enhancement of the PP-evoked population spike. Conditioning stimulation of the LC delivered 25–50 msec prior to PP activation resulted in a short-term enhancement of the dentate gyrus population spike, as shown in Figure 1. In nearly all cases, the maximal enhancement in population spike amplitude was produced when LC conditioning was delivered 35 msec before the PP test stimulus (Fig. 1). A 35 msec conditioning-PP test interval was therefore utilized in the standardized protocol devised for all drug

Figure 1. Effects of conditioning stimulation of the LC on extracellular field responses evoked in the dentate gyrus by PP stimulation. Stimulation of the LC (5 pulses of 0.2 msec duration at 300 Hz) 30–50 msec prior to PP activation enhanced population spike amplitude (filled circles) but not dendritic potentials (open circles, expressed as % baseline dendritic ESP slope). Note that the maximal enhancement in population spike amplitude was produced when LC conditioning was delivered 35 msec prior to PP activation. Points are means of averages of 8 trials each from 6–10 experiments. Bars indicate $\pm$ SEM in this and subsequent figures. *Inset* at right: Superimposed oscilloscope traces showing PP-evoked population spikes before (upper arrow) and 35 msec after (lower arrow) LC stimulation. Records shown are averaged responses of 8 trials.

interaction studies (see below) in order to facilitate comparisons between experiments. The increase in population spike amplitude produced by LC conditioning stimulation at this interval averaged $78 \pm 6.1\%$ (mean $\pm$ SEM, $n = 18$; $p < 0.005$) and ranged from 36 to 140% above the level of the baseline unconditioned response for all experiments.

Conditioning stimulation of the LC at parameters that markedly enhanced the population spike recorded in the granule cell layer typically did not affect the amplitude of the responses recorded simultaneously in the dendritic layer of the dentate gyrus (Fig. 1). Concomitant increases in the field excitatory synaptic potential (ESP) were found in less than 20% of all cases in which LC conditioning produced an enhancement of the population spike amplitude. Similarly, no appreciable effect on ESP slope or population spike latency was observed during pairing of LC conditioning with PP activation (Fig. 2). The finding of an enhancement of the population spike amplitude without change in field ESP after LC conditioning is in keeping with earlier reports (Assaf et al., 1979; Harley et al., 1982; Dahl and Winson, 1985). These data also suggest that the facilitation of the population spike response produced here by stimulation of the LC most likely reflects an increase in dentate granule cell excitability independent of a change in synaptic activation.

Table 1 summarizes the results of all experiments in which we examined the effects of conditioning stimulation of noradrenergic input to the dentate gyrus on PP-evoked population spike responses. Conditioning stimulation of the brain stem resulted in a statistically significant ($p < 0.005$) enhancement in population spike amplitude in 18 of 19 animals in which histology confirmed that the tip of the stimulating electrode was within the anatomical boundaries of the LC. Experiments were performed in an additional 13 animals in which conditioning stimulation was delivered to brain-stem sites greater than 250 μ m away from LC. There was no effect observed with conditioning stimulation of these sites in 7 of the animals. In the 6 remaining experiments, stimulation delivered outside the LC increased the amplitude of the PP-evoked population spike in

2 animals and reduced the PP-evoked spike response in 4 animals. In these cases, it was not possible to correlate observed effects with stimulation of specific brain regions outside of LC. In 9 of a final group of 11 experiments, activation of noradrenergic input to dentate gyrus via electrical stimulation of LC axons within the DB produced an enhancement ($p < 0.005$) of the PP-evoked population spike virtually indistinguishable from that obtained with LC stimulation.

Noradrenergic mediation of the LC facilitating action

Several types of experiments were performed to establish the noradrenergic nature of the LC-induced enhancement of the PP-evoked population spike. Four experiments were carried out in rats pretreated with the selective catecholamine neurotoxin 6-OHDA to determine whether an intact LC noradrenergic innervation of the dentate gyrus was required for elicitation of the enhancement. Pretreatment with the neurotoxin resulted in a marked depletion of hippocampal NE levels in each of these animals. Hippocampal levels of NE in the 6-OHDA-pretreated rats ranged from 38–52 ng/gm protein, which corresponded to greater than a 93% ($p < 0.001$) depletion of the levels found in untreated controls (592–713 ng/gm protein, $n = 4$). In 3 animals in which placement of the conditioning electrode in LC was confirmed histologically, there was no effect of conditioning stimulation on dentate population spike responses to PP activation (Table 1). Conditioning stimulation of the brain stem at a site 500 μ m medial to LC reduced the amplitude of the population spike in the remaining 6-OHDA-pretreated rat. It should be noted that an attenuation of the ability of LC stimulation to enhance the dentate gyrus population spike has also been reported after knife transections or chemolytic lesions of the dorsal noradrenergic bundle (Assaf et al., 1979; Harley et al., 1982).

The involvement of an adrenergic receptor in the mediation of the facilitating LC effect in dentate gyrus was examined by use of several pharmacological blockers known to be selective for β - or α_2 -adrenergic receptors. Systemic administration of the β -adrenergic antagonist propranolol consistently attenuated LC-

Figure 2. Comparison of the effects of LC stimulation on PP-evoked ESP and population spike responses before and after administration of clonidine in the absence and presence of idazoxan. *A*, Digitized records of extracellular field potentials recorded in the cell body (*left*) and dendritic layer (*right*) of dentate gyrus in response to PP stimulation. Each record is an average of 4 trials. The population spike amplitude was calculated as the average of amplitudes *a* and *b*. The onset latency of the population spike was defined as the time from the stimulus artifact (*single-headed arrow*) to the *asterisk*. The slopes of the extracellular ESP recorded in the cell and dendritic layer were measured as *c/c'* and *d/d'*, respectively. Stimulation of the LC 35 msec prior to PP activation significantly increased the population spike (*C*) but did not appreciably alter its latency (*D*) or the slope of the ESP recorded in the cell layer (*B*). Clonidine reduced and idazoxan restored the potentiative effect of LC stimulation on the population spike amplitude, without having any appreciable effects on either the latency of the response or the ESP. Each point represents the mean of 6 experiments. SE bars in *D* were omitted to allow for expansion of the ordinate scales. Significantly different from control at ($\square\square$) $p < 0.001$, ($\square$) $p < 0.01$, ($\square$) $p < 0.05$.

induced enhancement of the dentate population spike evoked by stimulation of the perforant path. Administration of the β -adrenergic blocker at a dose of 1.0 $\mu\text{g/kg}$ produced greater than a 55% attenuation of the LC-induced facilitating effect in 3 cases examined (Table 2). This antagonistic action of propranolol occurred with little appreciable change in amplitude of the baseline response. Complete blockade of LC action was produced by administration of higher dosages (30–100 $\mu\text{g/kg}$) of the β -antagonist, although this was accompanied by some direct attenuation of control responses.

To demonstrate the pharmacological specificity of the facilitation produced by LC stimulation, antagonism of this synergistic effect was also attempted with idazoxan, a highly selective α_2 -adrenergic receptor antagonist (Doxey et al., 1983). Administration of idazoxan up to a dosage of 3.75 $\mu\text{g/kg}$ attenuated the LC-induced enhancement of the population spike response in only 1 of 10 experiments (Table 2). In 4 cases administration

of 3.75 $\mu\text{g/kg}$ idazoxan resulted in a facilitation of the LC-induced potentiative effect (range, 18–38%; $26.5 \pm 4.3\%$ mean $\pm$ SEM), whereas in 5 cases no effect of the antagonist was observed. Idazoxan, in the range of dosages tested, did not significantly affect the amplitude of baseline responses to nonconditioned presentations of PP stimuli.

Inhibitory effects of clonidine on LC-induced enhancement of the dentate gyrus population spike

The inhibitory influence of presynaptic α_2 -adrenergic receptor activation on NE release from LC nerve terminals was examined by determining the ability of clonidine to reduce the facilitating effect of LC conditioning. Figure 3 illustrates the results of a typical experiment. In this experiment, conditioning stimulation of the LC delivered 35 msec prior to PP activation resulted in a 72% enhancement in population spike amplitude (Fig. 3, top record, left column). Administration of clonidine in increasing

Table 1. Effects of LC stimulation on perforant path-evoked dentate gyrus population spike

Conditioning stimulus	Number	Change in evoked response ^a		
		Increase	No change	Decrease
Locus coeruleus	19	18	1	0
Outside locus coeruleus	13	2	7	4
Locus coeruleus after 6-OHDA	4	0	3	1
Dorsal bundle	11	9	2	0

^a All effects of conditioning were assessed using a 35 msec conditioning-PP test interval. A change in population spike amplitude of at least 25% was required to register an effect of conditioning stimulation.

dosages produced a gradual reduction in the LC-induced facilitation without affecting the amplitude of the population spike to presentations of unconditioned PP test stimuli. At a cumulative clonidine dosage of 3.75 $\mu\text{g/kg}$, the enhancement in population spike amplitude elicited by LC stimulation was reduced to 16% over the baseline response (Fig. 3, third record, middle column). Administration of idazoxan in increasing dosages reversed the depressant action of clonidine (Fig. 3, records, right column). The potentiative effect of LC conditioning was completely restored to pre-clonidine levels following administration of idazoxan at a total dosage of 3.75 $\mu\text{g/kg}$ (Fig. 3, bottom record, right column).

The effects of clonidine on LC-induced facilitation of the PP-evoked population spike were examined this way in 13 experiments. The enhancement in population spike amplitude produced by LC stimulation in this group of experiments averaged an $80 \pm 6.6\%$ increase (mean $\pm$ SEM; range, 36–107% enhancement; $p < 0.001$) over the baseline response. Clonidine reduced the potentiative effect of LC conditioning in a dose-dependent fashion in all cases examined (Fig. 4). However, the α_2 -adrenergic agonist did not significantly ($p > 0.25$) alter the population spike response evoked by presentations of the test stimulus alone, even when administered at dosages that produced complete blockade of the LC-induced effect. Slight attenuation of the PP-evoked population spike was observed only at the highest cumulative dosage of clonidine tested (12.5 $\mu\text{g/kg}$, $n = 4$ experiments). Idazoxan reversed the depressant effect of clonidine on the LC-induced facilitating action in a dose-dependent manner in 11 of the 13 experiments. In most cases, the ability of LC stimulation to potentiate the population spike was completely restored after administration of idazoxan at a dosage of 1.25 $\mu\text{g/kg}$.

To determine the nature of the antagonistic action of idazoxan against clonidine, the depressant effect of the agonist on LC-induced population spike enhancement was assessed after administration of idazoxan at a dosage (3.75 $\mu\text{g/kg}$) that had previously been found to reverse agonist-induced effects consistently. The dose-response curves depicted in Figure 5 show the results of 10 experiments. For these experiments, the depressant effect of clonidine was expressed simply in terms of a percent inhibition of the LC-induced enhancement. Comparison of the clonidine dose-response curves generated in the absence and presence of idazoxan revealed that administration of the antagonist produced a 10-fold parallel shift to the right in the dose-response curve for clonidine without a change in maximal effect (Fig. 5). The dosages of clonidine necessary to pro-

Table 2. Effects of adrenergic drugs on LC-induced potentiation of perforant path-evoked dentate gyrus population spike

Drug administered	LC-induced increase in population spike amplitude ^a (%)		
	Predrug	After drug	Significance
Propranolol (1.0 $\mu\text{g/kg}$, i.v.) (3)	71 ± 13	16 ± 5	$p < 0.001$
Idazoxan (3.75 $\mu\text{g/kg}$, i.v.) (10)	71 ± 9	74 ± 10	n.s.
Clonidine (3.75 $\mu\text{g/kg}$, i.v.) (14)	82 ± 7	13 ± 5	$p < 0.001$

^a Values shown represent mean $\pm$ SEM of maximal LC-induced potentiation for number of experiments shown in parentheses. Differences between values obtained before and after drug administration were assessed statistically by means of 2-tailed Student's *t* test.

duce half-maximal inhibition of the LC-induced potentiation (IC_{50}) in the absence and presence of idazoxan were approximately 1 and 10 $\mu\text{g/kg}$, respectively. The increase in IC_{50} for clonidine in the presence of idazoxan was statistically significant ($p < 0.005$).

Site of clonidine-induced depression of LC-mediated enhancement

α_2 -Adrenergic receptors are known to exist on the cell bodies as well as the terminals of LC noradrenergic neurons (Young and Kuhar, 1980). The finding in earlier studies that systemically administered clonidine results in membrane hyperpolarization of LC neurons in rat (Aghajanian and Vandermaelen, 1982) raised the possibility that the reduction in LC-mediated action produced here might in part reflect a decrease in LC neuron excitability to electrical stimulation owing to an action of clonidine at somatic α_2 -adrenergic receptors. This issue was addressed in a final series of 9 experiments by determining the ability of clonidine to reduce the enhancement in the dentate gyrus population spike produced by stimulation of LC noradrenergic axons within the DB. In each case, histological analysis revealed that the stimulation site within the DB was at least 3.5 mm rostral of the anterior pole of LC. Consequently, there was little likelihood for passive spread of membrane hyperpolarization generated at somatic α_2 -adrenergic receptors to these electrotonically remote sites of axonal stimulation.

Stimulation of the DB 35 msec prior to delivery of PP test stimuli increased population spike amplitude an average of $80 \pm 14.3\%$ (mean value $\pm$ SEM of 9 experiments, $p < 0.005$), a value nearly identical to that obtained with LC conditioning (see above). Clonidine, administered in the dosage range of 12.5 ng/kg to 12.5 $\mu\text{g/kg}$, reduced the ability of DB conditioning to facilitate the amplitude of the PP-evoked population spikes. The depressant action of clonidine varied directly with the dosage of agonist and was reversed completely by systemic administration of idazoxan (Fig. 6a). A comparison of the dose-response curves for depression by clonidine of the LC- and DB-induced enhancement in population spike amplitude revealed no significant difference ($p > 0.25$, ANOVA) in agonist action against the potentiative effects obtained with axonal or somatic activation of noradrenergic input to dentate gyrus (Fig. 6b). These data strongly suggest that the depressant effect of clonidine on the LC-induced facilitation in dentate gyrus occurs largely through the activation of presynaptic α_2 -autoreceptors located on NE nerve terminals and independent of changes in LC neuron excitability per se.

Figure 3. Results of a representative experiment showing the inhibitory effect of the α_2 -adrenergic agonist clonidine on LC-induced enhancement of dentate gyrus population spike responses evoked by PP activation. Clonidine, in the dose range of 0.0125–12.5 $\mu\text{g/kg}$, reduced the potentiative effect of LC stimulation in a dose-dependent manner, without appreciably altering the population spike responses evoked by unconditioned presentations of PP test stimuli. The effects of clonidine were reversed by administration of the α_2 -adrenergic antagonist idazoxan, which at a dosage of 3.75 $\mu\text{g/kg}$ completely restored the LC potentiative effect. Panels show superimposed oscilloscope records, averaged over 8 trials, of PP-evoked population spikes before and 35 msec after (arrow) LC stimulation. Dosages of drugs shown indicate total cumulative amounts administered.

Discussion

The results presented here indicate that conditioning stimulation of the LC results in a facilitation of the PP-evoked population spike in the dentate gyrus. The activation of nonadre-

nergic afferents to the hippocampus was unlikely to account for the observed enhancement since synaptically evoked dentate gyrus population spikes were not increased by stimulation at brain-stem sites slightly outside of the LC. Moreover, the increase in population spike amplitude produced by LC condi-

Figure 4. Dose-response curves for inhibition by clonidine of LC-induced enhancement of dentate gyrus population spike responses in the absence (left) and presence (right) of idazoxan. Left, Clonidine reduced the potentiative effect of LC conditioning on population spike amplitude in a dose-dependent manner and abolished the LC effect at a total cumulative dose of 12.5 $\mu\text{g/kg}$. Right, Administration of the α_2 -selective antagonist idazoxan reversed the inhibitory effect produced by clonidine in a dose-dependent manner. Visual inspection of these curves yielded an EC_{50} for clonidine of 0.125 $\mu\text{g/kg}$ and IC_{50} for idazoxan of 0.5 $\mu\text{g/kg}$. Each point represents the mean and SEM of 13–16 experiments.

Figure 5. Dose-response curves for inhibition by clonidine of LC-induced enhancement of dentate population spikes in the absence (*closed squares*) and presence (*open squares*) of a single dose of the α_2 -selective antagonist idazoxan (3.75 μ g/kg). The maximal observed depressant effect of clonidine on the LC potentiative action is represented as 100% inhibition. Clonidine produced a dose-dependent reduction in LC-induced potentiation of dentate population spike responses in the absence and presence of idazoxan. Note that administration of the antagonist resulted in an approximately 10-fold parallel shift to the right in the clonidine dose-response curve. IC_{50} values in the absence and presence of antagonist were approximately 1 and 10 μ g/kg, respectively.

tioning was blocked selectively by administration of the specific β -adrenergic receptor antagonist propranolol but not by the α_2 -adrenergic antagonist idazoxan. Finally, destruction of the noradrenergic innervation of the dentate gyrus by pretreatment of rats with the selective catecholamine neurotoxin 6-OHDA eliminated the facilitating effect of LC conditioning. Taken together, these results suggest that the potentiative effect of LC stimulation on the PP-evoked population spike was mediated by synaptically released NE. By using this response as a physiological end point of NE-mediated synaptic action, we have been able to show that neuronal release of endogenous NE from LC nerve terminals in the dentate gyrus is subject to inhibitory feedback control via presynaptic α_2 -adrenergic receptors.

Nature of the LC facilitating effect on the PP-evoked population spike

The LC potentiative effect observed in this study has been described previously (Assaf et al., 1979; Harley et al., 1982; Dahl and Winson, 1985; Harley and Milway, 1986). The finding in earlier studies that potentiation of the PP-evoked population spike in dentate gyrus was maximal when LC stimulation was delivered 35–50 msec prior to PP activation was confirmed here. In addition, we did not observe consistent effects of LC conditioning on the granule cell layer ESP, ESP slope, or population spike latency, which is also in keeping with previous reports. In earlier studies *in vivo*, LC stimulation or iontophoretic application of NE in the granule cell layer was reported to enhance the population spike amplitude without any change in dendritic potentials (Assaf et al., 1979; Harley et al., 1982; Neuman and Harley, 1983; Dahl and Winson, 1985). Similar results have been obtained during NE superfusion in the hippocampal slice preparation (Lacaille and Harley, 1983). However, in more recent studies in the slice, NE was found to enhance the ESP slope (Lacaille and Harley, 1985) and potentiate the dendritic field

Figure 6. *a*, Dose-response curve for inhibition by clonidine of DB-induced enhancement of the PP-evoked dentate population spike. Clonidine produced a dose-dependent reduction of the DB potentiative effect and administration of idazoxan reversed the effect of clonidine. The point at far right shows the near complete reversal of the effect of 12.5 μ g/kg clonidine after administration of 0.375 μ g/kg idazoxan. Each point represents the mean and SEM of 9 experiments. *b*, Dose-response curves for inhibition by clonidine of enhancement of dentate population spikes elicited by conditioning stimulation of LC (*closed squares*) or DB (*open squares*). Note that the magnitude of potentiation elicited by DB conditioning stimulation was virtually identical to that obtained with LC conditioning (see text). Drug effects are expressed in terms of the maximal observed clonidine inhibitory effect. There was no significant difference ($p > 0.25$ ANOVA) between the ability of clonidine to reduce LC- and DB-induced potentiative effects. Error bars were omitted for reasons of clarity.

ESP (Stanton and Sarvey, 1987). Under our experimental conditions in which electrical stimulation was employed to evoke endogenous NE release, increases in population spike amplitude were associated with an increase in the dendritic ESP in less than 20% of the cases. Although the reasons for the disparity in results between studies are unclear, some of the discrepancies might be due to inherent differences that exist between *in situ* and *in vitro* preparations and/or in the methods of drug delivery.

Nevertheless, the finding here that LC conditioning consistently enhanced the population spike amplitude without affecting the field ESP can be taken as evidence that synaptic release of NE in the dentate gyrus results in an increased excitability of granule cells, independent of the degree of synaptic activation.

β -Adrenergic receptor mediation of LC-induced potentiation

In the present study, the facilitating effect of LC conditioning stimulation on the dentate population spike was attenuated by systemic administration of propranolol. It was unlikely that the antagonism by propranolol of the LC-induced facilitating action resulted from a nonspecific depressant effect on granule cell excitability since blockade of the population spike enhancement was observed largely independent of changes in the amplitude of responses elicited by presentations of unconditioned PP test stimuli. Iontophoretic administration of propranolol into the dentate gyrus *in vivo* has been reported to block an enhancement of the population spike attributable to physiological NE release (Harley and Milway, 1986). Lacaille and Harley (1983) reported that superfusion of NE in the hippocampal slice induced long-lasting enhancement of the granule cell population spike and that this effect was blocked selectively by the β -adrenergic receptor antagonist timolol. Lacaille and Harley (1985) also found that the β -selective agonist isoproterenol mimicked the action of NE but that α -adrenergic agonists and antagonists had no effect on synaptically evoked dentate gyrus population spikes. Thus, it seems reasonable to conclude that the enhancement of the PP-evoked population spike produced by activation of LC input to the dentate gyrus is mediated via activation of β -adrenergic receptors.

Recent studies at the cellular level have revealed β -adrenergic receptor-induced changes in at least 2 currents in dentate gyrus granule cells. Gray and Johnston (1987) reported that isoproterenol increased the activity of voltage-dependent calcium channels in exposed granule cells during cell-attached patch-clamp recording. On the other hand, intracellular recordings from single cells in the hippocampal slice have demonstrated that isoproterenol attenuated the calcium-activated afterhyperpolarization and accommodation of dentate gyrus granule cell firing to depolarizing current pulses (Haas and Rose, 1987). The latter effects were attributed to a β -receptor-mediated blockade of a calcium-activated potassium conductance. The functional consequences of either of these β -adrenergic actions is likely to be manifested as an increase in postsynaptic responsiveness of dentate gyrus neurons to excitatory synaptic input. Accordingly, the modulation of these conductances as a result of the activation of postsynaptic β -adrenergic receptors by synaptic release of NE could underlie the enhancement in the dentate gyrus population spike observed in this and previous studies (Assaf et al., 1979; Harley et al., 1982; Dahl and Winson, 1985; Harley and Milway, 1986).

Other kinds of data suggest that the influence of LC noradrenergic input on granule cell excitability might also be mediated at least in part indirectly, presumably as a result of alterations in the activity of GABA-containing inhibitory interneurons. Catecholamine histofluorescence (Jones and Moore, 1977; Loy et al., 1980) and immunohistochemical analyses (Swanson and Hartman, 1975) of the hippocampal noradrenergic innervation in rat have shown the heaviest concentration of noradrenergic fibers and terminals within the subcellular hilus of the dentate gyrus. Large numbers of basket-type cells populate this hilar region (Amaral, 1978), and many of these cells demonstrate

immunoreactivity for GABA and its synthesizing enzyme, glutamic acid decarboxylase (Seress and Ribak, 1983; Kosaka et al., 1984). Hilar basket cells have also been shown to form synapses on the dendrites, somata, and initial segments of axons of granule cells (Ribak and Seress, 1983; Kosaka et al., 1984). Accordingly, it seems reasonable to presume that at least some GABAergic hilar interneurons are likely to receive an innervation of LC-NE fibers and thus are strategically positioned to mediate noradrenergic effects on granule cell excitability. Although a direct action of NE on hippocampal interneurons has yet to be demonstrated, intracellular recordings from pyramidal cells in CA1 have shown that NE can reduce orthodromically and antidromically evoked inhibitory postsynaptic potentials (IPSPs) without altering direct hyperpolarizing responses to GABA (Madison and Nicoll, 1988). Interestingly, this disinhibitory action of NE did not appear to be mediated via a direct suppression of inhibitory interneurons since the frequency of spontaneous IPSPs was increased during catecholamine superfusion. Therefore, whereas ample anatomical evidence exists for potential interactions of LC noradrenergic input with GABAergic elements within the dentate gyrus, it remains to be seen whether the functional impact of such arrangements on granule cell excitability can be resolved in terms of simple disinhibition.

Inhibitory modulation of NE release by presynaptic α_2 -autoreceptors

Previous *in vitro* studies in rat and rabbit hippocampal slices have shown that administration of α_2 -adrenergic agonists inhibit the stimulus-evoked overflow of ^3H -NE elicited by high extracellular potassium or electrical field stimulation (Frankhuyzen and Mulder, 1982; Jackisch et al., 1984). α_2 -Adrenergic antagonists competitively antagonized this depressant action of the agonists (Frankhuyzen and Mulder, 1982; Jackisch et al., 1984) and when administered alone provoked an exaggerated overflow of electrically evoked ^3H -NE release (Jackisch et al., 1984). On the basis of these data, the hypothesis has been advanced that the neuronal release of NE in hippocampus is subject to tonic feedback inhibition via activation of presynaptic α_2 -autoreceptor by the endogenous neurotransmitter. A principal objective of the present study was to establish the physiological significance of this α_2 -autoreceptor-mediated feedback inhibition of neurotransmitter release on noradrenergic synaptic function. The finding that administration of clonidine produced a functional impairment of LC-mediated action in the dentate gyrus argues in favor of the participation of this feedback inhibition system in the moment-to-moment regulation of NE-mediated synaptic activity.

Several lines of evidence indicate that the reduction in LC-induced enhancement of the dentate gyrus population spike that was produced by clonidine reflects the activation of presynaptic α_2 -autoreceptors. First, the depressant effect of clonidine on the response enhancement elicited by LC stimulation displayed the requisite features of mediation via an α_2 -adrenergic receptor. This action was dose dependent, occurred at a range of systemic clonidine doses shown previously to be appropriate for eliciting α_2 -adrenergic receptor-specific electrophysiological effects (Cedarbaum and Aghajanian, 1976) and was reversed or prevented by administration of low doses of the α_2 -selective antagonist idazoxan. Second, clonidine reduced the enhancement in population spike amplitude without significantly altering extracellularly recorded field potentials evoked by PP activation alone.

Thus, it is unlikely that the depressant effect of clonidine on the LC-induced enhancement of the population spike resulted from a nonspecific depressant effect of the α_2 -agonist on granule cell excitability. Third, the enhancement in population spike amplitude produced by direct activation of LC axons within the dorsal noradrenergic bundle was equally sensitive to clonidine's depressant effect. This indicates that the reduction of the LC facilitating effect by clonidine can occur independently of changes in LC neuron excitability owing to the hyperpolarizing action of the agonist at somatic α_2 -adrenergic receptors. Finally, administration of idazoxan alone resulted in an enhancement of the LC-induced potentiation of the population spike in some cases. Taken together, these data argue strongly that the reduction of LC-induced enhancement of dentate gyrus population spikes produced by clonidine derives from an inhibition of endogenous NE release mediated by presynaptic α_2 -autoreceptors on LC nerve terminals.

Increases of 18–38% in the LC potentiative effect on dentate population spike amplitude were observed in 4 of 10 experiments after the administration of idazoxan. This experimental outcome is generally in keeping with previous observations *in vitro* that the administration of α_2 -adrenergic antagonists can increase the stimulus-evoked overflow of ^3H -NE from noradrenergic neurons in brain slice preparations (Reichenbacher et al., 1982; Schoffemeer and Mulder, 1983; Jackisch et al., 1984) and in various sympathetically innervated peripheral tissues (Fuder et al., 1984; Limberger and Starke, 1984). The facilitating effects on ^3H -NE release produced by α_2 -antagonists *in vitro* were characteristically more dramatic and more consistently observed than was the potentiative effect of idazoxan on LC-mediated action demonstrated here. However, in those latter studies in which electrical field stimulation was employed to evoke release of ^3H -NE, the enhancement by α_2 -adrenergic antagonists was shown to vary in magnitude directly with the frequency and duration of the stimulation and to be dependent on the synaptic (biophase) concentration of NE (Reichenbacher et al., 1982; Fuder et al., 1984; Limberger and Starke, 1984). Accordingly, the low incidence of facilitation in the LC potentiative effect obtained after idazoxan might reflect the attainment of only modest synaptic levels of NE with the delivery of short bursts of LC stimuli that were employed here. In any event, we interpret the finding of increases in LC potentiative action on dentate population spike amplitude after idazoxan to support a role for presynaptic α_2 -adrenergic receptors in the autoregulation of synaptic release of endogenous NE. In future studies, it will be important to define the conditions required for this enhancing effect of α_2 -adrenergic antagonists on LC-mediated action over a range of stimulation parameters and with agents of varied potency and receptor selectivity in order to establish the physiological significance of presynaptic α_2 -autoreceptor-mediated feedback inhibition in the tonic regulation of neuronal release of NE.

In summary, the present study, to our knowledge, provides the first demonstration that the activation of presynaptic α_2 -adrenergic receptors can lead to a functional impairment in noradrenergic neurotransmission at LC-mediated synapses in hippocampus. These investigations further suggest that an analysis based on LC-induced facilitation of the PP-evoked population spike in dentate gyrus may provide a useful test system for assessing changes in presynaptic α_2 -adrenergic receptor function under conditions in which alterations in receptor density are thought to occur.

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