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Antikindling Effects of Locus Coeruleus Stimulation: Mediation by Ascending Noradrenergic Projections

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Electrical stimulation of the noradrenergic locus coeruleus (LC) delays the generalization of partial seizures during amygdaloid kindling by increasing the time spent in the earliest stages of seizure development. To determine whether noradrenergic axons projecting to the midbrain and forebrain are involved in this antikindling effect, we examined the effects of lesions of the dorsal noradrenergic bundle, induced by intracerebral infusions of 6-hydroxydopamine (6-OHDA), on kindling and the antikindling action of stimulation of the LC. Stimulation of the LC during amygdaloid kindling increased the number of afterdischarges (ADs) spent in the early stages of partial seizure and decreased the number of ADs spent in later stages of generalized seizure, as has been described previously. LC-stimulated rats also displayed longer durations of AD during early stages of kindling. The antikindling effect of LC stimulation was blocked by lesions of the dorsal bundle, whereas the facilitatory effects of LC stimulation on generalization and on the duration of AD were unaffected by the lesions. These results suggest that the antikindling action of LC stimulation is mediated by the ascending projections of noradrenergic neurons, presumably through enhanced release of noradrenaline. On the other hand, the facilitatory effects of LC stimulation on the development of later stages of seizure and on the duration of AD appear to be independent of the ascending dorsal bundle.

INTRODUCTION

Kindling of seizures is facilitated by depletion of central noradrenaline (NA), induced by either infusion of 6-hydroxydopamine (6-OHDA) (4, 16) or lesions of ascending noradrenergic tracts (7). NA's antagonistic effects on kindling are restricted to the early stages of partial seizure (5, 19), and depletion of NA does not affect the duration or intensity of generalized seizures after they have been kindled (26). Collectively these findings have been taken to suggest that NA normally acts to suppress the early spread of seizure discharge and therefore that kindling in part involves an erosion of the antikindling effects of NA (4, 16). The plausibility of the NA hypothesis is strengthened by the observation that kindling can be delayed by injections of the directly acting α_2 noradrenergic agonist clonidine at doses that in general do not affect established seizures (9, 17). Because the prophylactic effects of clonidine occur after destruction of presynaptic NA axon terminals, it is likely that they are due to activation of postsynaptic α_2 receptors for NA.

Additional support for the idea that NA in the intact brain interferes with kindling is the observation (10) that electrical stimulation of the noradrenergic nucleus locus coeruleus (LC) delays progression out of the early stages of partial seizure during amygdaloid kindling. Electrical stimulation of the LC has also been reported to suppress seizures induced by the convulsant drug pentylenetetrazole (12). The noradrenergic neurons of the LC project widely throughout the neuraxis; noradrenergic axons originating in the LC innervate the forebrain, brain stem, cerebellum, and spinal cord (8). Although the anatomy of the LC is consistent with the idea that the LC might provide a kind of nonfocal antagonism of seizure activity (4), it is most likely that forebrain projections to specific sites are responsible for retarding the progression through the early stages of kindled seizures.

To clarify the source of the antikindling effects of LC stimulation, we examined the effects of stimulation after 6-OHDA-induced lesions of NA axons ascending from the LC in the dorsal tegmental bundle.

METHODS

Forty male Sprague–Dawley rats weighing about 300 g were anesthetized with sodium pentobarbital and received stereotaxic infusions of 6-OHDA bilaterally into the dorsal tegmental NA bundle in the mesencephalon. Following previously described procedures (26), we used a microsyringe to infuse 4 μg of 6-OHDA HBr (dosage

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expressed as free base) dissolved in 2 μl of sterile 0.9% saline (with 0.2 mg/ml ascorbic acid as antioxidant) into the dorsal bundle in each hemisphere. The rate of infusion was 1 μl/min, and the syringe was left in place for 1 additional min to permit diffusion of the drug and prevent backflow up the cannula track. Control rats received similar infusions of the vehicle. Coordinates for the 6-OHDA infusions were −6.0 mm from the bregma, 0.8 mm from the midline, and 5.0 mm below the dura with the skull horizontal. After completion of central infusions all rats were assigned a code number, and all subsequent surgery and testing was performed blind. A person not involved in the testing assigned rats to the various groups described below, without informing the experimenters. The code was broken at the end of the experiment, after the rats had been sacrificed.

Two weeks after infusions the rats were again anesthetized with pentobarbital and received stereotaxic implantation of electrodes into the amygdala and the LC. A monopolar stainless steel electrode was implanted into the LC and a bipolar nichrome electrode was implanted into the amygdala of the same hemisphere using coordinates previously described (10). The LC coordinates were 1.2 mm posterior to λ 1.1 mm lateral to the midline, and 5.8 mm below the dura surface with the incisor bar at −2.4 mm. A surgical screw implanted over the frontal sinus served as the reference electrode.

After a postoperative recovery period of 2 weeks, rats were assigned to one of four groups: One group comprised rats that had received infusions of 6-OHDA into the dorsal NA bundle; they received daily kindling stimulation of the amygdala after a preceding period of stimulation of the LC lasting for 20 to 30 min (DBX-LC STIM group). A second group of rats pretreated with 6-OHDA infusions into the dorsal bundle received amygdaloid stimulation without preceding stimulation of the LC (DBX group). A third group of rats pretreated with vehicle infusions into the dorsal bundle received amygdaloid stimulation after LC stimulation comparable to that in the 6-OHDA-treated group (LC STIM group), and a fourth group of rats pretreated with vehicle infusions into the dorsal bundle received amygdaloid stimulation without preceding stimulation of the LC (CONTROL group).

Amygdaloid stimulation consisted of 1-s trains of constant current biphasic square wave pulses 1.0 ms in duration at 60 Hz. Stimulation was applied three times daily at interstimulation intervals of 90 min or more. The stimulus intensity was the value found to elicit an afterdischarge (AD) of 5–15 s (10). LC stimulation consisted of 20- to 30-min trains of 0.2-ms pulses at 100 Hz, applied for 1-s periods alternating with 1-s nonstimulation periods. The intensity was determined for each rat by increasing the current until it elicited jaw and facial movements, which were indicative of the spread of current to the motor nucleus of the trigeminal nerve, and then reducing the current until motor responses ceased. The DBX and Control groups did not receive LC stimulation, but were placed in the testing chamber for periods lasting 20 to 30 min before amygdaloid stimulation. The kindling stimulus was delivered within 20 s after the offset of the LC stimulation. Behavioral seizures were classified according to the five stages defined by Racine (23). We analyzed the total number of ADs spent in each stage of seizure; for clarity of presentation, however, we shall present the data collapsed across stages 1 and 2 and stages 3 to 5 of seizure.

At the end of the experiment, histological verification of electrode placements was obtained in all rats. The rats were deeply anesthetized with pentobarbital, and anodal current was passed through electrodes to produce a lesion at the electrode tip. The rats were then perfused intracardially, the brains were frozen, and coronal sections were taken and stained with methyl green–pyronine. Biochemical verification of NA depletion was obtained in an extra group of rats comprising five 6-OHDA-treated rats and five vehicle-treated control rats prepared at the same time as the rats used in the kindling experiment. The rats were sacrificed by cervical fracture, and the brains were dissected on ice. Regional concentrations of NA were measured using high-performance liquid chromatography with electrochemical detection (11). Histology was not obtained at the site of 6-OHDA infusion since previous studies showed that this technique found no sign of nonspecific damage (6).

Data were analyzed using one-way ANOVA, and post-hoc comparisons were performed with the Bonferroni test at a level of 0.05.

RESULTS

The three major findings of the experiment were, first, depletion of NA facilitated kindling, as indicated by comparison of kindling in the Control and DBX groups. Second, stimulation of the LC delayed the emergence of generalized seizures from partial seizures, as indicated by comparison of kindling in the Control and LC STIM groups. Third, LC stimulation *failed* to delay kindling after lesions of the dorsal NA bundle, as indicated by comparison of kindling in the LC STIM and DBX-LC STIM groups.

Table 1 shows that the infusions of 6-OHDA resulted in depletion of hippocampal NA down to blank levels and depletion of hypothalamic NA to 38.9% of control. Both changes from control were highly significant. NA depletion was measured in the hippocampus because it has been shown to be an area most sensitive to 6-OHDA lesions of the dorsal NA bundle and a good indicator of forebrain depletion. The hypothalamus is sensitive to both dorsal and ventral bundle damage.

The groups differed in the overall rate of kindling of generalized seizures. The mean number of ADs required

TABLE 1

Regional Concentrations of NA, Expressed as Mean (±SEM) in ng/g Wet Weight of Tissue, in Control and 6-OHDA-Treated Rats

Region	Control group	6-OHDA-treated	Percentage depletion
Hypothalamus	1242.4 ± 112.4	483.0 ± 79.0	61.1*
Hippocampus	177.4 ± 15.6	0	100*

* Significant difference, $P < 0.001$.

TABLE 2

Mean (±SEM) Duration of AD (in seconds) in Control and 6-OHDA-Treated Rats during Phases of Amygdaloid Kindling

Group	Last three ADs in stage 1	First AD in stage 2	First AD in stage 5
Control	17.5 ± 1.6	34.3 ± 5.1	73.3 ± 5.2
DBX	25.9 ± 6.4	68.5 ± 10.2*	79.3 ± 4.2
LC STIM	34.1 ± 2.8*	64.0 ± 6.4*	62.8 ± 7.4
DBX-LC STIM	43.5 ± 7.8*	53.7 ± 3.5	63.3 ± 9.8

* Significantly different from control, $P < 0.05$.

for the development of the first stage-5 seizure in each group was DBX, 5.3 ± 1.5 (SEM); Control, 10.7 ± 1.1; DBX-LC STIM, 2.5 ± 0.7; and LC STIM, 8.3 ± 0.6. The differences between the DBX and Control groups and between the DBX-LC STIM and Control groups were significant, as was the difference between the DBX-LC STIM and the LC STIM groups (each $P < 0.01$). No other differences were significant.

Detailed analysis of the pattern of seizure development indicated that the differences between the groups in rate of kindling were in part attributable to differences in time spent in early stages of partial seizure. Figure 1 shows the mean number of ADs spent in stage 1 and 2 seizures and in stage 3 through 5 seizures (i.e., ADs in stages 3 and 4 and including the first stage-5 seizure) during kindling. With regard to the total number of ADs spent in stage 1 and 2 seizures, the DBX group required significantly fewer ADs than the Control group (means of 2.8 ± 0.9 and 5.7 ± 0.4, respectively, $P = 0.007$), whereas the LC STIM group required significantly more ADs than the Control group (means of 7.2 ± 0.5 and 5.7 ± 0.4, respectively, $P = 0.001$). The LC-STIM group required significantly more ADs than the DBX-LC STIM group (means of 7.2 ± 0.5 and 1.0 ± 0.5, respectively, $P = 0.001$), whereas the DBX-LC STIM group did not differ significantly from the DBX group (means of 1.0 ± 0.5 and 2.8 ± 0.9, respectively, $P = 0.05$). Thus, lesions of the dorsal NA bundle reduced and stimulation of the LC increased, respectively, the time spent in the early stages of partial seizure during amygdaloid kindling (5, 10). More important, LC stimulation failed to prolong partial kindling after lesion of the dorsal NA bundle.

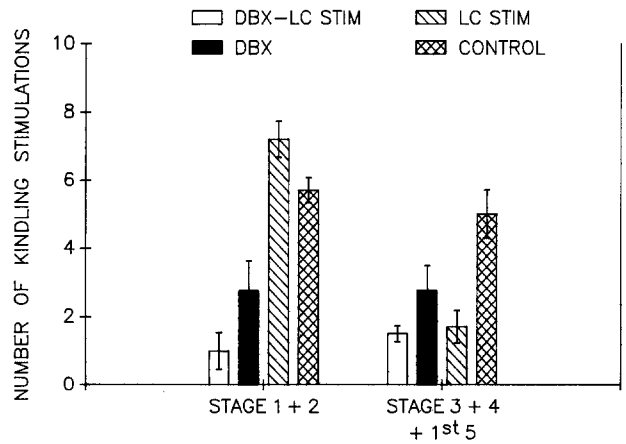

FIG. 1. Mean number of kindling stimulations required for development of different stages of seizure in NA-depleted (DBX) and control rats receiving stimulation of the LC (LC STIM). Bar graphs for each group represent, first, the number of kindling stimulations for progression through stage 1 to the last stage 2 (stage 1 + stage 2) and, second, the number of kindling stimulations for progression from the first stage 3 to the first stage 5 (stages 3 + 4 + 1st 5).

With regard to the total number of ADs spent in stage 3 through 5 seizures, the DBX group required significantly fewer ADs than the Control group (means of 2.8 ± 0.7 and 5.0 ± 0.7, respectively, $P = 0.044$), and the LC STIM group also required significantly fewer ADs than the Control group (means of 1.7 ± 0.5 and 5.0 ± 0.7, respectively, $P = 0.001$). The LC-STIM and DBX-LC STIM groups did not differ significantly (means of 1.7 ± 0.5 and 1.5 ± 0.2, respectively, $P = 1.00$); nor did the DBX-LC STIM and DBX groups (means of 1.5 ± 0.2 and 2.8 ± 0.7, respectively, $P = 0.36$). Thus, in contrast to its effects on development of partial seizures, stimulation of the LC seemed to facilitate progression through the later stages of amygdaloid kindling, as has been reported previously (10), and this occurred even after destruction of the dorsal NA bundle.

Table 2 shows the duration of ADs recorded from the stimulated amygdala. The intensity of the stimulation required to produce an AD of 5–15 s was not different in any of the groups. There was no difference between DBX and Control groups in the average duration of pooled ADs for the last three occurring in stage 1, whereas both the LC-STIM and DBX-LC STIM groups displayed significantly longer ADs than Controls in this early stage of partial seizure. The durations of the first AD in stage 2 were significantly longer in the DBX and LC STIM groups than in the Control group, but there were no significant differences between groups in duration of AD in the first stage-5 seizure.

DISCUSSION

It has been reported that 6-OHDA-induced lesions of the dorsal NA bundle accelerate amygdaloid kindling (4, 5). This acceleration was particularly evident in the tendency of kindling in lesioned rats to omit the early stages of partial seizure and progress quickly into generalization. Accelerated kindling was not associated with changes in the duration of focal AD in the earliest stages of seizure, although the duration of AD in 6-OHDA-treated rats was increased during intermediate stages of kindling. Once generalized seizures had developed, however, they were no longer or no more intense than the generalized seizures occurring in control rats (26). The facilitatory effect of intracerebral infusions of 6-OHDA on kindling was presumably a function of selective destruction of noradrenergic axons, both because such infusions generally produce neurochemically specific lesions (14, 27) and because similar effects on kindling can be produced by injections of antagonists of noradrenergic α_2 receptors (9, 17).

The results of the present experiment also replicate the observation (10) that LC stimulation delays the transition from partial seizures into generalized seizures and shortens the transition from stage 3 and 4 seizures into fully generalized seizures. Furthermore, they provide some insight into the mechanisms of this effect. We found that LC stimulation prolonged the occurrence of the early stages of partial seizure, but that the effect did not occur in 6-OHDA-treated rats. It is clear, then, that the delaying effect of LC stimulation depends on the integrity of the noradrenergic fibers innervating the forebrain and midbrain. This effect of LC stimulation might be due to stimulation-evoked release of NA in critical target sites, perhaps the amygdala or the pyriform cortex (15, 18).

It appears that the activation of NA receptors is sufficient to delay kindling. Limbic kindling is antagonized by the postsynaptic effects of injections of the α_2 agonist clonidine (9, 17), similar to the effects of LC stimulation. Neuman (20) applied electrical stimulation to the LC with parameters similar to those that we used and found that the antiepileptic effects of stimulation on penicillin-induced seizures could be reversed by either reserpine or antagonists of noradrenergic α_1 receptors. Thus it seems likely that the antikindling effects of LC stimulation are due to release of NA from the fibers of the dorsal NA bundle that ascend to the midbrain and forebrain. Further research is necessary to determine the receptor subtype at which released NA acts to delay kindling.

The duration of AD during the early stages of kindling was significantly increased in rats receiving stimulation of the LC, including those with a prior lesion of the dorsal NA bundle. LC stimulation thus produces a dissociation between duration of AD and progression out of the early stages of partial seizures. The present results indicate that this dissociation is independent of the noradrenergic innervation of the midbrain and forebrain, since it occurs even when the ascending dorsal NA bundle has been lesioned. This finding rules out the suggestion (10) that this effect of LC stimulation might be due to noradrenergic potentiation of inhibitory GABAergic activity in the substantia innominata (21).

We also found that LC stimulation significantly reduced the number of ADs spent in stages 3 and 4 of kindling (10). Thus although stimulation of the LC delayed progression of clinical seizures out of stages 1 and 2, once such progression did occur there was a compensatory acceleration through the later stages of kindling, such that the overall rate of kindling of generalized seizures did not differ significantly between the LC STIM and the Control groups. In contrast to the antikindling effects of LC stimulation, however, this facilitatory effect was not affected by lesions of the dorsal NA bundle and must therefore have been due to activation of either noradrenergic fibers spared by 6-OHDA or nonnoradrenergic neurons in or near the LC. In a previous study (10) stimulation near the LC, in the tegmental reticular formation, tended to accelerate seizure progression, even though not significantly. Lesions of the dorsal NA bundle induced by 6-OHDA spare or may even increase concentrations of NA in the hindbrain and the cerebellar cortex (14), and activation of noradrenergic pathways to either region might have been responsible for the facilitatory effect of LC stimulation. It is perhaps unlikely that noradrenergic fibers innervating the brain stem are involved, since studies of the "kindling antagonism" effect that occurs when two sites are stimulated sequentially indicate that antagonism is reduced after depletion of hindbrain and cerebellar NA (1) but not by lesions of the cerebellum alone (Burchfiel, personal communication), a finding that seems to rule out a seizure-facilitatory effect for hindbrain NA. Although it is possible to produce selective depletion of cerebellar NA (13), the effect of this intervention on kindling or on the response to stimulation of the LC has not been assessed.

There is a possibility that depletion of NA was not complete in the areas of the forebrain or the midbrain responsible for seizure propagation. If supersensitivity to NA develops in postsynaptic NA receptors, then stimulation of the LC in the NA-depleted rats could still provide activation of the now supersensitive β receptor. It has been suggested that β receptors are proconvulsant (18). This could then account for the apparent facilitation of the later stages of kindling observed with LC stimulation in the NA-depleted rats. Supersensitivity of anticonvulsant α_2 receptors might be expected to counteract the effects of β receptor activation (9), although it is unclear how widespread increased density of α_2 receptors is after depletion of NA (25). This scheme does not explain the acceleration of late kindling produced by LC

stimulation in intact rats; presumably in this situation a kindling-induced downregulation of α_2 receptors (24) would facilitate progression through the late stages of kindling. Another possibility is that LC stimulation activates nonnoradrenergic neurons in or near the LC, which could be the neural substrate for intracranial self-stimulation in that region (2, 3), and that these neurons promote the rapid generalization of clinical seizures produced by LC stimulation. One site of interest is the pedunculopontine nucleus (PPN), which might be activated by LC stimulation either directly by current spread or, perhaps more likely, indirectly via multisynaptic connections with LC. The PPN has been implicated in the triggering of limbic seizures by systemic injections of pilocarpine (22), and we are currently examining the possible involvement of the PPN in kindling.

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