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Locus Coeruleus Lesions Suppress the Seizure-Attenuating Effects of Vagus Nerve Stimulation

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Summary: *Purpose:* Although vagus nerve stimulation (VNS) is now marketed throughout most of the world as a treatment for drug-resistant epilepsy, the therapeutic mechanism of action of VNS-induced seizure suppression has not yet been established. Elucidation of this mechanism is an important first step in the development of strategies to improve VNS efficacy. Because the locus coeruleus (LC) has been implicated in the antinociceptive effects of VNS, we chemically lesioned the LC in the present study to determine if it is a critical structure involved in the anticonvulsant mechanisms of VNS.

Methods: Rats were chronically depleted of norepinephrine (NE) by a bilateral infusion of 6-hydroxydopamine (6-OHDA) into the LC. Two weeks later, they were tested with maximal electroshock (MES) to assess VNS-induced seizure suppression. In another experiment, the LC was acutely inactivated with lidocaine, and seizure suppression was tested in a similar fashion.

Results: VNS significantly reduced seizure severities of control rats. However, in animals with chronic or acute LC lesions, VNS-induced seizure suppression was attenuated.

Conclusions: Our data indicate that the LC is involved in the circuitry necessary for the anticonvulsant effects of VNS. Seizure suppression by VNS may therefore depend on the release of NE, a neuromodulator that has anticonvulsant effects. These data suggest that noradrenergic agonists might enhance VNS-induced seizure suppression. **Key Words:** Vagus nerve—Locus coeruleus—Norepinephrine—Anticonvulsant—Epilepsy.

Vagus nerve stimulation (VNS) is a novel therapy for the control of epilepsy. Clinical studies demonstrate that VNS reduces seizure frequency by >50% in 30–40% of patients with previously intractable seizures (1). In contrast to ablative neurosurgical interventions, VNS is reversible and the stimulus parameters can be titrated to increase efficacy and reduce the incidence of side effects.

The recent use of VNS in humans follows several reports that the technique successfully prevents or attenuates seizures in some animal models. Zanchetti et al. (2) and Stoica and Tudor (3,4) were among the first to experiment with the anticonvulsant properties of VNS. They showed that VNS blocked spike-wave complexes induced by cortical strychnine application in cats. Subsequently, VNS was shown to provide seizure protection in other animal models. In rats, VNS reduces seizure activity induced by maximal electroshock (MES) (5,6), pentylenetetrazol (PTZ) (5–7), and penicillin (7,8). VNS has also been reported to afford protection against PTZ- and strychnine-induced seizures in dogs (9) and from alumina-gel seizures in monkeys (10).

Although the anticonvulsant efficacy of VNS is well established, the underlying mechanisms of its action have not been elucidated. Knowledge of these mechanisms might prove useful in improving clinical efficacy or even in devising new therapies. VNS may cause widespread release of inhibitory neurotransmitters, thereby preventing seizure discharge spread (5,11), but this theory has not been empirically tested.

Studies of the circuitry involved in the antinociceptive effects of VNS have implicated the locus coeruleus (LC) as a critical component in attenuation of pain (12–14). Because LC activation can modulate seizure activity (15–18), we hypothesized that the LC may play a similar role in the anticonvulsant effects of VNS. We examined the effects of chemical LC lesions on VNS anticonvulsant efficacy in rats. To our knowledge, our results constitute the first evidence of identification of a structure mediating the anticonvulsant actions of VNS.

METHODS

Sprague Dawley female rats (Harlan Sprague Dawley, Indianapolis, IN, U.S.A.) weighing 200–300 g were

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housed individually and maintained on a normal 12/12-h light/dark schedule. Food and water to the animals were available ad libitum. All experiments conformed to the institutional guidelines for animal care and use.

Chronic LC lesions

In rats under chloral hydrate anesthesia (400 mg/kg, i.p.), a 30-gauge needle was lowered at a 15° posterior-to-anterior angle through a burr hole in the skull (0.0 mm posterior to the interaural line and 1.3 mm lateral to the midline) to a depth of 3.1 mm above the interaural line, just dorsal to the LC. 6-Hydroxy-dopamine hydrobromide (6-OHDA), 4 μg in 2 μl saline with 400 ng ascorbic acid, was microinfused into the LC over 4 min. This procedure was then repeated for the contralateral LC. Sham-lesioned control animals underwent the same surgical procedure but did not receive an infusion. A separate group of animals that did not undergo surgical manipulation at this point served as nonoperated controls.

After a 2-week recovery period, all animals received a baseline MES test to assess their ability to display hindlimb extension (HLE). HLE is the most severe manifestation of MES seizures and is commonly used as an endpoint in screening new anticonvulsants (19). The MES stimulus, consisting of 60-Hz, 125-mA alternating current, was delivered for 200 ms through saline-moistened corneal electrodes with a Wahlquist stimulator. Only animals displaying HLE were used for subsequent procedures. Two seizure parameters were assessed during the MES test: duration of tonic hindlimb flexion (HLF), defined as the time elapsed from the beginning of HLF until the beginning of HLE, and duration of HLE, defined as the beginning of HLE until the hindlimbs were 90° perpendicular to the body. Seizure severity was then determined using the duration of HLE (19) and the ratio of durations of HLE to HLF (E/F ratio) (6). A longer HLE duration or higher E/F ratio indicated a more severe seizure.

At least 24 h after the baseline MES test, the rat's left vagus nerve was exposed under chloral hydrate anesthesia. A bipolar cuff electrode was placed around the nerve in both the lesioned (6-OHDA/VNS) and previously nonoperated (NonOp/VNS) groups, and half of the sham-lesioned (Sham/VNS) animals. The leads were tunneled subcutaneously and led out an incision made in the dorsal neck region. The remaining sham-lesioned animals underwent similar surgical procedures, but were not implanted with cuff electrodes, and served as sham-lesioned, sham-implanted (Sham/Sham) controls.

Twenty-four hours later, a 20-Hz train of 800-μA, 0.5-ms biphasic pulses was delivered to the left vagus nerve through the cuff electrode (except in the Sham/Sham group, which received no VNS). Thirty seconds after the beginning of VNS, the MES stimulus was delivered to the cornea. VNS was discontinued at the completion of the MES test. Statistical significance was assessed with a repeated-measures 2 × 4 analysis of variance (ANOVA) ($\alpha = 0.05$). To determine the extent of the LC lesions, cortical and hippocampal norepinephrine (NE) levels were assayed ex vivo as described previously (20).

Acute LC inactivation

Under chloral hydrate anesthesia, 16-mm, 23-gauge stainless-steel cannulas were implanted bilaterally just dorsal to the LC, and stylets were inserted to prevent stoppage. Two weeks later, all animals underwent a baseline MES test. At least 24 h after the baseline MES test, a stimulating electrode cuff was placed around each rat's left vagus nerve.

The next day, the stylets were removed and two 30-gauge infusion needles were inserted into the guide cannulas. Either 1 μl 5% lidocaine hydrochloride in half of the animals, or 0.9% saline in the other half, was microinfused bilaterally into the LC over 2 min. Ten minutes later, animals were subjected to VNS and an MES test as already described. Forty-eight hours later, the procedure was repeated, with the reciprocal infusion substance. This allowed a within-subject comparison while counterbalancing treatment-order effects. After the final MES test, histology was performed; only animals whose cannulas were located within 1 mm of the LC were included in the data analyses. Mean HLE durations and E/F ratios were compared by a one-way, repeated-measures ANOVA ($\alpha = 0.05$).

RESULTS

Effects of chronic LC lesions on anticonvulsant actions of VNS

The 6-OHDA/VNS group was significantly depleted of NE as compared with the NonOp/VNS and Sham/VNS groups. The cortex of the 6-OHDA/VNS group (n = 13 assayed) had a mean 83.8% depletion as compared with that of the NonOp/VNS group (n = 10 assayed) and 88.9% depletion as compared with that in the Sham/VNS group (n = 13 assayed). The hippocampus was 79.4 and 83.0% depleted as compared with that of the NonOp/VNS and Sham/VNS groups, respectively. The NE depletion resulted in significantly higher baseline extension durations and E/F ratios for the 6-OHDA/VNS group as compared with controls.

The mean HLE durations for the four treatment groups are shown in Fig. 1A. HLE durations of the Sham/Sham group, in which animals underwent both operations but were not lesioned or stimulated, were not significantly reduced during the second MES test as compared with baseline values. In contrast, VNS significantly reduced HLE durations for the Sham/VNS group, which had a sham LC lesion, and the NonOp/VNS group, which did

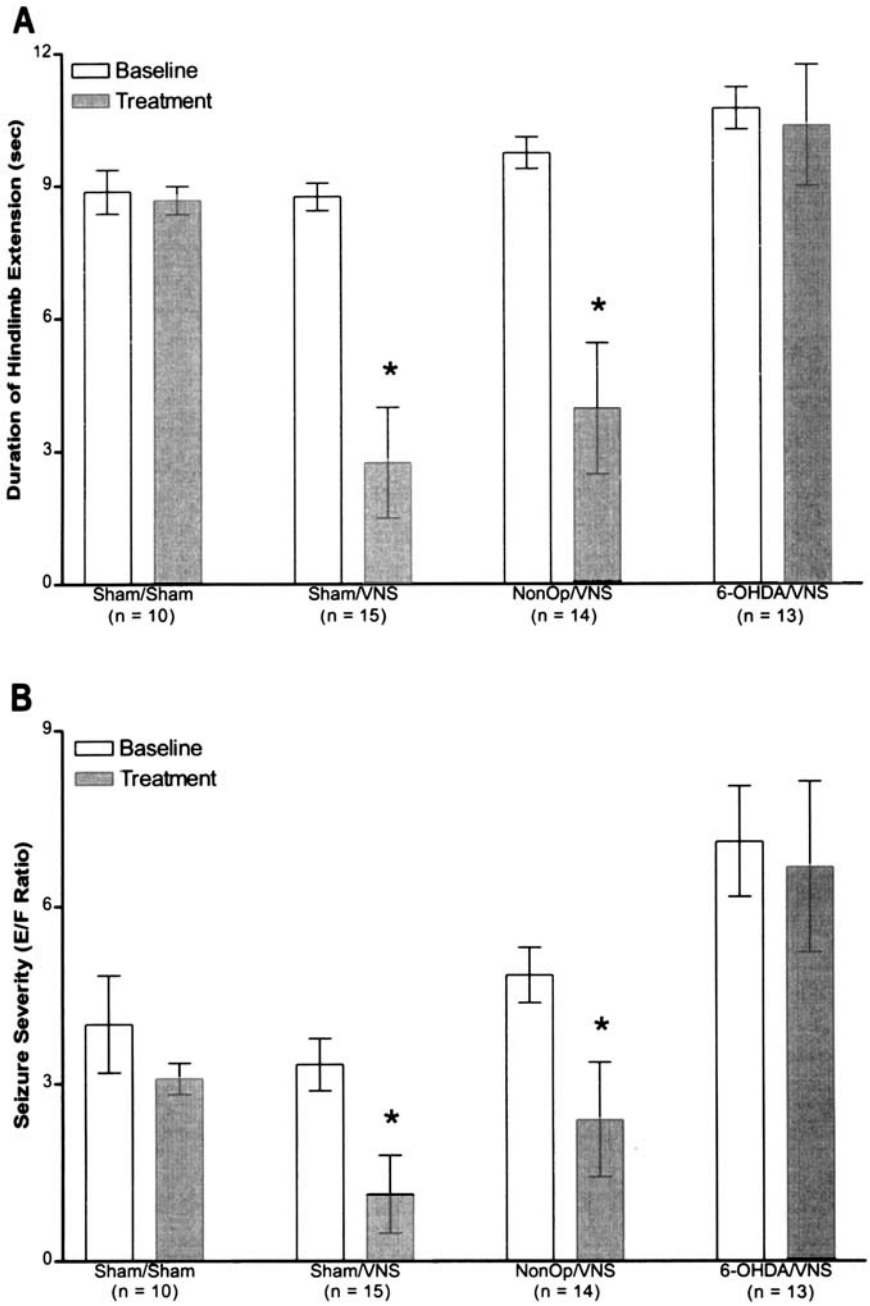

FIG. 1. Mean hindlimb extension (HLE) durations (±SE) **(A)** or hindlimb extension/flexion (E/F) ratios (±SE) **(B)** of groups receiving a sham locus coeruleus (LC) lesion and no vagus nerve stimulation (VNS) (Sham/Sham), a sham LC lesion and VNS (Sham/VNS), no LC surgery and VNS (NonOp/VNS), or an LC lesion and VNS (6-hydroxydopamine hydrobromide: 6-OHDA/VNS). Baseline measures (solid columns); measures during VNS (or 1 day after sham implantation in the Sham/Sham group) (open columns). *Seizure severity was significantly reduced from baseline values ($p < 0.05$).

not undergo the LC surgery. However, VNS did not significantly reduce the duration of HLE in animals that were substantially depleted of cortical and hippocampal NE, namely, the 6-OHDA/VNS group. Similar statistical results were obtained when E/F ratios were used as an index of seizure severity (Fig. 1B).

Effects of acute LC inactivation on anticonvulsant actions of VNS

Baseline HLE durations for the cannulated animals were similar to those already described (Fig. 2A). As expected, VNS markedly and significantly reduced HLE durations from baseline values after bilateral infusion of saline into the LC. Indeed, after VNS, only 1 of 6 saline-infused animals exhibited HLE in response to MES. In contrast, VNS no longer had a significant anticonvulsant effect on MES-induced seizures after the LC was inactivated by bilateral lidocaine infusion. The same results were obtained when E/F ratios were used (Fig. 2B).

DISCUSSION

VNS significantly reduced seizure severity in the stimulated control groups, i.e., the Sham/VNS and NonOp/VNS groups of the first experiment and the saline-infused animals in the second. These observations

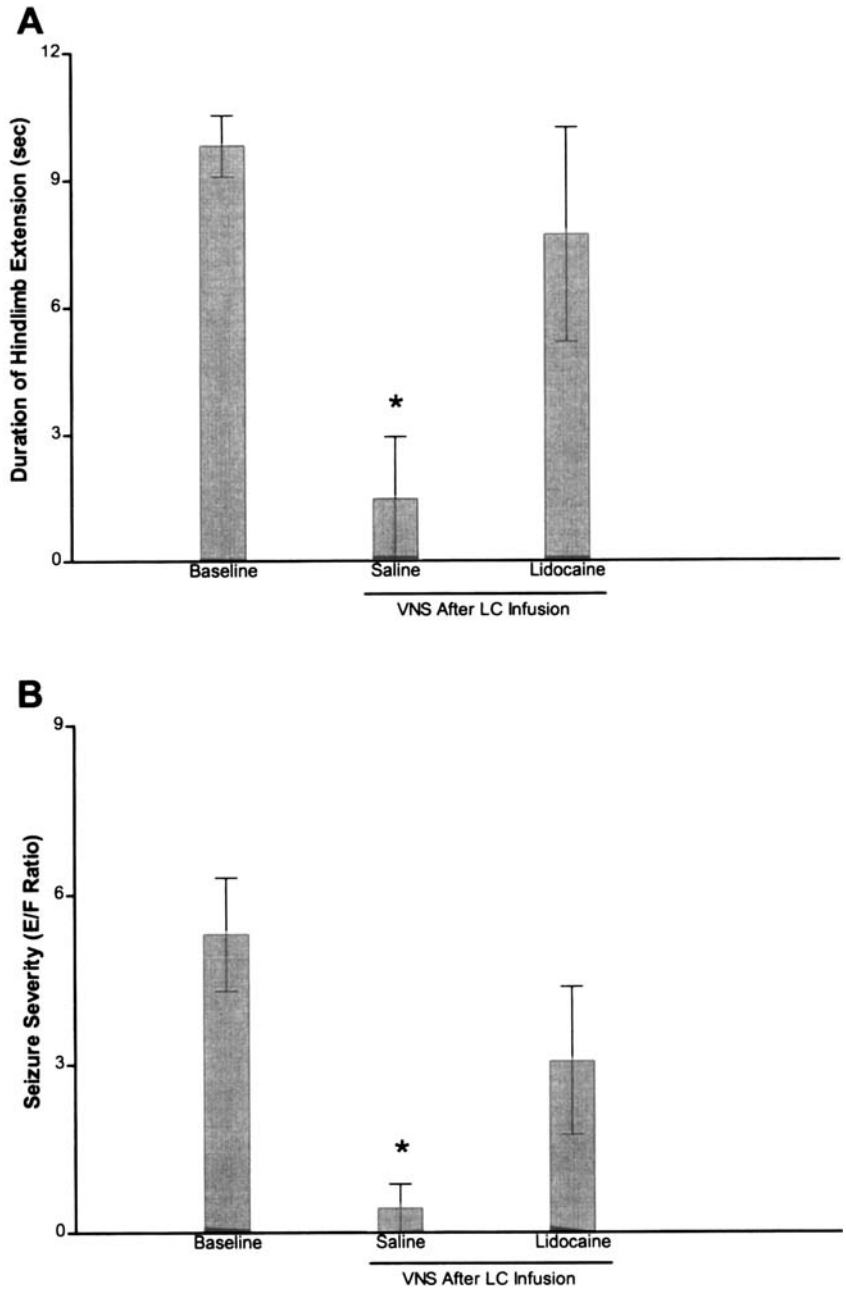

FIG. 2. Mean hindlimb extension (HLE) durations (±SE) **(A)** or hindlimb extension/flexion (E/F) ratios (±SE) **(B)** during the baseline test (Baseline), and during vagus nerve stimulation (VNS) just after administration of bilateral locus coeruleus (LC) infusions of saline (Saline) or lidocaine (Lidocaine) (n = 6). *Seizure severity was significantly reduced from baseline values ($p < 0.05$).

confirm that VNS is effective against generalized convulsive seizures (5,6). The lack of a reduction in seizure severity in the Sham/Sham group, which underwent both sham lesion and sham electrode implantation surgeries, indicates that seizure suppression in VNS-treated animals was not due simply to postsurgical stress or repeated MES seizures.

The hypothesis tested in the present study experiment was that a loss of LC function would prevent the reduction in seizure severity caused by VNS. Bilaterally lesioning the LC with the selective catecholamine neurotoxin 6-OHDA prevented the seizure-suppressing effects of VNS. Three possible explanations may account for our results. First, the reduction in VNS efficacy produced by bilateral LC lesions may be due to nonspecific chronic lesion effects, such as receptor supersensitivity at some distant site. In the second experiment, we tested this possibility by acutely inactivating the LC with lidocaine immediately before performing the MES/VNS test rather than by producing a chronic lesion. These results demonstrate that even acute LC inactivation prevents the seizure-suppressing effects of VNS, making this first possibility unlikely.

Second, chronic NE depletion resulted in a significant increase in the baseline seizure severity of the 6-OHDA/VNS group as compared with the control groups. NE

depletion increases seizure susceptibility and severity. For example, intracisternally administered 6-OHDA prolonged the duration of MES-induced HLE (21). Moreover, rats depleted of NE are more susceptible to seizures induced by an infusion of *N*-methyl-D-aspartate (NMDA) into the inferior colliculus than are nondepleted controls (20). Accelerated kindling rates have also been demonstrated after NE depletion (22–24), as have increased seizure severities in genetically epilepsy-prone rats (25). NE depletion in this experiment may therefore have produced a seizure so severe that it could not be prevented by VNS.

To test this second possibility, we gave a separate group of rats a series of three MES tests, each 48 h apart. This treatment has been shown to increase seizure severity in a kindlinglike fashion (26). Mean E/F ratios (±SE) increased daily as expected (4.15 ± 0.56 on day 1, 4.81 ± 0.60 on day 2, and 5.62 ± 0.51 on day 3). Rats then underwent implantation of cuff electrodes on day 4 and, 24 h later, received VNS and MES as already described. If LC lesions reduce the effectiveness of VNS solely because of increased seizure severity, repeated MES sessions should mimic this effect. Our data, however, did not support this hypothesis. VNS significantly reduced MES seizure severity (mean E/F ratio of 3.91 ± 1.14) as compared with the last MES test ($p < 0.05$), demonstrating that VNS retained efficacy despite an increase in severity such as that resulting from chronic NE depletion.

The final possibility, and the one we believe most parsimoniously accounts for our present results, is that lesioning the LC blocks the anticonvulsant effects of VNS by preventing VNS-induced NE release either globally or in some specific brain site. NE exerts anticonvulsant effects in numerous seizure models. Treatments that increase NE availability or release appear to protect animals from MES seizures (27). For instance, direct LC stimulation, the main source of the brain's NE, can suppress epileptiform activity in animals after PTZ (15) and penicillin application (16) and can retard amygdala kindling (17). In humans, LC stimulation prevents electrographic seizure discharges (18). Moreover, depletion of NE increases seizure severity and susceptibility in a wide variety of seizure models (19).

In recent years, a positive link between VNS and the LC was demonstrated in studies by Gieroba and Blessing (28) and Naritoku et al. (29) in which VNS caused an increase in *fos,* an immediate early gene expressed during periods of increased neuronal activity, in the LC of rabbits and rats, respectively. In an electrophysiological study, we observed a substantial VNS-induced firing rate increase in a population of LC neurons (30). Sole et al. (31) showed that acute myocardial infarction, which activates vagal afferents, causes an increase in NE turnover in the LC. These studies provide evidence that VNS activates the LC and causes NE release. Other studies have demonstrated that the LC mediates the antinociceptive effects of VNS—when the LC is inactivated, VNS no longer causes antinociception (12–14). The results of these studies are consistent with our evidence that the LC mediates VNS-induced seizure suppression.

The mechanism by which the LC exerts seizure suppression in the MES model is believed to involve descending spinal cord connections (19). For instance, selective depletion of NE in the spinal cord results in an increased HLE duration in mice after MES (32). Seizure suppression by VNS may therefore involve a similar descending noradrenergic mechanism, analogous to that which produces antinociception (13). However, forebrain structures may be similarly affected since suppression of PTZ-induced clonus is frequently observed during VNS (unpublished observations) (5).

A role for the LC in preventing seizures after VNS may have potential pharmacological benefits in clinical practice. Drug therapies that activate the LC or potentiate the effects of NE may increase the efficacy of VNS. Such therapies may allow a reduction in the amplitude and/or frequency of stimulation, thereby decreasing VNS side effects and improving patients' quality of life.

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