Epileptiform activity extinguished by amygdala infusion of the neurotoxin ibotenate in a rat model of temporal lobe epilepsy

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Object. The long-term antiseizure effects of local convection-enhanced infusion of the excitotoxin ibotenate were examined in a rat model of temporal lobe epilepsy.

Methods. A single injection of kainate, an epileptogenic excitatory amino acid, into the left amygdala elicited chronic spontaneous recurrent seizure activity for at least 36 days after the injection. Two weeks after the injection, infusion of ibotenate, a nonepileptogenic excitatory amino acid that is an axon-sparing neuronal cell toxin, into the left amygdala and piriform lobe induced immediate and permanent extinction of electrical and behavioral seizure activity.

Conclusions. Lesioning of an epileptic focus by convective distribution of ibotenate can produce an enduring suppression of seizure activity, indicating a chemical neurosurgical approach for epilepsy therapy.

Key Words • ibotenate • epilepsy • convection • amygdala • rat

Abbreviations used in this paper: CNS = central nervous system; EEG = electroencephalographic; NIH = National Institutes of Health; NMDA = N-methyl-D-aspartate; SD = standard deviation.

We examined whether administration of ibotenate can suppress electrical and behavioral seizure activity in the kainate epilepsy model. Kainate is a powerful convulsant that, similar to ibotenate, is a glutamate receptor agonist. Unlike ibotenate, however, kainate does not interact with NMDA receptors. Intraamygdala administration of kainate causes an acute episode of status epilepticus that lasts 1 or 2 days followed by a silent phase that extends over 1 to 3 weeks. Many kainate-treated animals exhibit spiking activity in amygdala EEG recordings and spontaneous recurrent behavioral seizures. Our results indicate that ibotenate infusion can largely obliterate a kainate-induced seizure focus.

Materials and Methods

Male Sprague–Dawley rats, each weighing between 290 and 400 g, were obtained from the NIH animal program. All procedures were conducted in strict compliance with the NIH Guide for Care and Use of Laboratory Animals under a protocol approved by the NIH Animal Use Committee.

A left basolateral amygdala “chemitrode” was stereotactically implanted (anteroposterior −2.1 mm, lateral 4.2 mm, and dorsoventral −7.3 mm with respect to the amygdala) into each animal for infusion and depth EEG recording; a right amygdala electrode was implanted to obtain depth EEG recording alone. A screw electrode was placed in the skull over the right frontal cortex to obtain EEG recording with respect to a second screw electrode (as ground), which was placed over the left cerebellar cortex. Animals received injections of 1 μg kainic acid into the left amygdala on the 1st day of the study. When the rats began to exhibit recurrent spontaneous seizure activity, we began to record EEG activity daily for 1 hour and continued this until the end of the study. The EEG signal was acquired at a sampling rate of 100 Hz by using a software-programmable 32-channel
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Fig. 1. Graphs demonstrating that recurrent spontaneous epileptiform events in rats with kainate-induced amygdaloid seizures are suppressed by infusion of ibotenate. Kainate injection into the left amygdala on Day 1 induces frequent, recurrent, spontaneous electrical epileptiform events that diminish in number only slightly and not significantly for 36 days following the injection. Ibotenate infusion into the left (ipsilateral) amygdala on Day 15 (arrowhead) produces long-term suppression of epileptiform activity, which is recorded by both ipsilateral (left) and contralateral (right) amygdala depth EEG electrodes. Data points represent the means ± standard errors of the means (SEMs) of data from five animals.

Results

Only kainate-injected animals in which we documented spontaneous recurrent epileptiform events on the basis of EEG recordings and episodic behavioral seizures were studied. Twenty-four animals were injected with kainate. Three were eliminated from the study because of hardware failure. Five animals died from status epilepticus after kainate injection on the 1st day of the study. Ten (62.5%) of the remaining 16 rats displayed spontaneous recurrent epileptiform events. The electrical and behavioral manifestations of delayed seizures occurring after kainate injection were similar to those described previously. All rats included in the study exhibited bursts of high-frequency epileptiform activity, which were associated with transient behavioral arrest (tonic immobility). In addition, one third of the animals also exhibited prolonged 1- to 5-minute discharges that were associated invariably with Stage 3 or greater behavioral seizures (forelimb clonus, rearing, and falling). Epileptiform activity occurred sporadically during the first 2 weeks after kainate injection. By Day 15, the rate of occurrence of high-voltage spontaneous epileptiform events (defined in Materials and Methods) stabilized at a rate of two to three per second.

As illustrated in Fig. 1 left, ibotenate infusion into the left amygdala on Day 15 caused a sustained suppression in the mean frequency of spontaneous events recorded from the left amygdala electrode. There was no change in the mean frequency of events in animals that had been infused with vehicle. For the ipsilateral side the average percentage of reduction in epileptiform events after treatment was 85% (SD 11%) in the five rats in the ibotenate-treated group, where-
as in the vehicle-treated group there was an average increase of 139% (SD 211%). Two rats in which there were increases of 434% and 242%, respectively, largely influenced the results in the vehicle-only group. The permutation test yielded a two-sided probability value of 0.016 for the hypothesis that there would be no difference in the percentage of reduction. Testing (again performed using permutation tests) of group differences in seizure counts on Days 8, 14, 22, and 36 yielded respective probability values of 0.79, 0.45, 0.02, and 0.07 for the hypothesis that there would be no difference in seizure counts between groups. The insufficiency of the data obtained on Days 8 and 14 suggests that the two groups were similar before ibotenate administration.

Ibotenate infusion was also associated with a marked decrease in the frequency of events recorded from the contralateral amygdala electrode (Fig. 1 right). The extent of the decrease, however, was less than that measured on the ipsilateral side. The average percentage of reduction in epileptiform event counts after infusion was 72% in the ibotenate-treated group (SD 42%), whereas there was an average increase of 1% (SD 61%) in the vehicle-treated rats (p = 0.05 for percentage change). Tests of seizure counts on Days 8, 14, 22, and 36 yielded respective probability values of 0.79, 0.83, 0.24, and 0.59. The insufficiency of findings on Days 22 and 36 may appear to contradict the overall significance shown with the percentage change test; however, the paradox is resolved by noting that post hoc tests are used to examine counts only at a fixed point in time, whereas the percentage change is used to consider effects over time within each rat. Although high pre- and posttreatment counts were measured in two ibotenate-treated rats, both showed a large percentage of reduction in the frequency of events. This pattern contributes to insignificant differences at a point in time, although there are significant differences in the percentage of change.

In addition to reducing the frequency of electrical epileptiform discharges, infusion of ibotenate also suppressed spontaneous behavioral seizure activity (Fig. 2). There was a marked reduction in the frequency of behavioral arrests and seizures on the day following infusion in ibotenate-treated animals, but not in vehicle-treated control animals. The reduction in seizures persisted for the duration of the observation period. Thus, in the ibotenate-treated animals, the mean number of behavioral events during 1-hour recording sessions dropped from 5.2 ± 1.2 on Day 14 (1 day before ibotenate infusion) to 1.2 ± 0.9 on Day 36 (21 days after ibotenate infusion). In contrast, there was no significant change in the number of behavioral arrests and seizures in control animals that received vehicle infusions (4.8 ± 1.1 on Day 14; 4.6 ± 0.7 on Day 36).

Additional measures of the level of behavioral impairment in kainate-treated animals are weight and appearance. Animals in the vehicle-treated control group were emaciated for their ages, whereas those treated with ibotenate had normal weights for their ages. Figure 3 shows the mean weights of animals in the vehicle- and ibotenate-treated groups. Both groups maintained approximately equal weights during the time period before ibotenate infusion. Rats that received ibotenate, however, displayed significantly greater weights 3 weeks after infusion (ibotenate-treated group 501.4 ± 24.2 g; vehicle-treated group 399.6 ± 5.7 g; p < 0.01), indicating that increased seizure activity in the vehicle-treated animals was associated with failure to thrive. Other behavioral effects such as maze running ability or memory were not assessed.

In general, histological examination of specimens from animals infused with kainic acid and ibotenate showed a greater degree and a wider distribution of cell loss in the temporal lobe (particularly the piriform cortex) of the infused side than those in specimens from animals that received kainate alone. No attempt, however, was made to
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d quantify the extent of cell loss contributed by kainate and ibotenate in the same region.

Discussion

These experiments demonstrate that a single intraamygdala infusion with the excitotoxin ibotenate can permanently obliterate a seizure focus induced by kainate, resulting in suppression of electrical and behavioral seizure activity. We hypothesize that the effects of ibotenate on seizure activity are caused by destruction of hyperexcitable neurons in the seizure focus. Chemical eradication of epileptic neurons suppresses abnormal firing at the focus site in the ipsilateral amygdala and also reduces spread of epileptic activity to distal sites, as demonstrated by diminished epileptiform activity in the contralateral amygdala and a reduction in behavioral seizure activity. Ibotenate is a neuron-specific cell toxin that creates axon-sparing lesions in part through activation of NMDA-type Ca<sup>2+</sup>-permeable ionotropic glutamate receptors. Nevertheless, ibotenate is a more potent cell toxin than NMDA itself, possibly because of its additional actions on metabotropic glutamate receptors that can trigger intracellular Ca<sup>2+</sup> release. Ibotenate is unique among excitatory amino acids in that it is not epileptogenic. Although the precise differences between ibotenate and other excitatory amino acids that account for this lack of epileptogenic activity are not known with certainty, it has been noted that ibotenate undergoes enzyme-catalyzed decarboxylation to the primary amine muscimol. The resulting muscimol, a potent γ-aminobutyric acid A receptor agonist, could limit the magnitude and spread of excitation produced by ibotenate. In any case, our studies confirm that large doses of ibotenate can be infused into limbic structures of the rat brain without causing seizures or death.

Convection-Enhanced Delivery

Many neurological disorders, including epilepsy, might be best treated by selective inhibition or augmentation of neuronal circuits. Although a number of effective therapeutic compounds exist for treating such pathological disorders in small animal models, the clinical use of these agents has not been possible because of the inability to deliver them in a selective and homogeneous manner in clinically significant volumes to the CNS by using conventional delivery methods. Specifically, the systemic delivery of therapeutic compounds to the CNS is not anatomically selective, is limited by the blood–brain barrier, and may be associated with systemic toxicity. Regional methods for CNS drug delivery include intraventricular infusion and implanted polymers. Because both these techniques rely on diffusion for distribution of various molecules in clinically significant volumes within the brain and does so with relative independence of the molecular weight, size, concentration, and diffusivity of the therapeutic agent. Moreover, the “square-wave” distribution pattern afforded by convection-enhanced delivery allows for precise homogeneous delivery of the desired infusate concentration to specific brain regions.

Because of the unique properties of convection-enhanced delivery, its use in the excitotoxic ablation of selected brain regions for the treatment of medically intractable epilepsy may have benefits over conventional surgical treatment techniques. First, because ibotenate lesioning is specific to neurons and spares white matter, inadvertent damage to surrounding white matter tracts can be avoided. Second, the arrest of seizure activity in this model of epilepsy appears to be predicated only on the selective destruction of neuronal perikarya and not on the damage or interruption of white matter tracts in the resection region. This suggests that only the selective destruction of neurons is necessary to affect a successful outcome. Therefore, it may be possible to eliminate the need for extensive anatomical resection and its attendant risks. Third, in this animal study the ibotenate-induced lesions were less extensive than the corresponding regions that are resected during temporal lobectomy performed in humans. Ibotenate infusions created lesions in the amygdala and surrounding structures, including the piriform cortex, endopiriform nucleus, and perirhinal cortex. These structures are confined within the parahippocampal gyrus of the human brain. Human temporal lobe resections typically involve various combinations of lateral structures (superior, middle, and inferior temporal gyri) and parahippocampal structures (hippocampus, parahippocampal gyrus, and amygdala). Thus, the new approach described here eliminates the destruction of the lateral temporal neocortex to achieve obliteration of an epileptic focus within parahippocampal gyrus and amygdala. Therefore, the convection-enhanced delivery of surrogate tracers that can be imaged with computerized tomography or magnetic resonance imaging could be used to define the extent of the expected lesion precisely before final ablation (Nguyen, et al., and Lonsor, et al., unpublished data). Similarly, infusion with reversible inhibitors of neuronal activity, such as local anesthetic agents or γ-aminobutyric acid A receptor agonists (such as muscimol) might permit the surgeon to verify the functional effects of the expected lesion.

Conclusions

The present results indicate that targeted local delivery of neuron-specific neurotoxins may be an alternative to resective neurosurgery in the treatment of localization-related epilepsies. Because the basis of human focal epilepsies at present is poorly understood, however, it remains to be determined whether an approach that is successful in ameliorating an induced epileptogenic focus in an animal model...
can also be of use in the treatment of the spontaneously arising foci underlying partial seizure disorders in humans.

References


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