Grafted noradrenergic neurons suppress seizure development in kindling-induced epilepsy
(neuronal transplantation/locus coeruleus)

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ABSTRACT Norepinephrine-rich cell suspensions, prepared from the locus coeruleus region of rat fetuses, were grafted bilaterally into the hippocampus of rats made hypersensitive to hippocampal kindling by a neurotoxic lesion of the central catecholamine system. The animals with grafts showed a marked suppression of the onset and progression of kindling-induced epilepsy, and this effect was correlated with the degree of graft-derived noradrenergic innervation of the host hippocampal formation. We conclude that grafted neurons can modulate the excitability of epileptic brain regions.

Kindling is one of the most extensively studied animal models of epilepsy. It refers to a process whereby repeated administration of an initially subconvulsive electrical stimulus results in progressive intensification of stimulus-induced seizure activity, culminating in a generalized seizure (1, 2). Kindling triggered by stimulation in the limbic system has been proposed to be analogous to complex partial epilepsy (also called temporal-lobe epilepsy) (3, 4), which is the most frequent type of epilepsy in adult humans (5). The ascending noradrenergic projection system originating in the locus coeruleus seems to provide a tonic inhibitory or suppressive action on the development of kindling, and damage to this system has been shown to greatly facilitate the kindling process (6-9). A similar seizure suppressant action of the central noradrenergic system has been found in other experimental epilepsy models (10).

Intracerebral grafts of fetal catecholaminergic brain stem neurons have the capacity to restore transmitter turnover and release in a denervated brain region (11-14), and there is electrophysiological data to indicate that they can reinstate tonic inhibitory neurotransmission in the area reinervated by the grafted neurons (15). This raises the possibility that implantation of norepinephrine-rich grafts could be employed as a tool to modify the excitability of a brain region sufficiently to dampen the development or spread of seizure activity. We have tested this hypothesis in rats made hypersensitive to hippocampal kindling by a neurotoxic lesion of the central catecholamine system.

METHODS Eighteen adult male Sprague-Dawley rats (ALAB, Stockholm) were given an intraventricular injection of 6-hydroxydopamine (250 μg of free base). This treatment is known to permanently deplete the forebrain, including the hippocampal formation, of >95% of its norepinephrine content (14, 16, 17). Six rats received an intraventricular vehicle injection and served as controls. Two weeks later, grafts prepared from the pontine locus coeruleus region of 13- to 14-day rat fetuses were implanted as a cell suspension bilaterally into the hippocampal formation in 11 of the 6-hydroxydopamine-treated rats. The locus coeruleus region was dissected as an ~1-mm³ piece from each side of the rhombencephalon as described in ref. 14. At four surgical sessions, the tissue from 9 to 22 fetuses was collected in 0.6% glucose/saline in room temperature and mechanically dissociated in 50–150 μl of glucose/saline. A total of four 1.5-μl deposits were made in each hippocampus at two cannula penetrations using the following coordinates (toothbar at 0): (i) 3.8 mm caudal to bregma, 3.5 mm lateral to midline, and 3 mm ventral to dura; (ii) 5.3 mm caudal to bregma, 0.75 mm lateral to midline, and injected along the long axis of the hippocampus, angled 40° from the vertex (in the coronal plane), with deposits made 7.9 mm, 5.4 mm, and 2.5 mm from the dura. The remaining seven 6-hydroxydopamine-treated animals received similar vehicle injections. After 6 to 11 months, a sufficiently long time for the grafts to reinervate the host hippocampus (14), bipolar stainless steel electrodes (o.d., 0.25 mm) were implanted bilaterally in the hippocampus (toothbar at 0, 5.3 mm caudal to bregma, 5 mm lateral to midline, and 5.3 mm ventral to dura). Unipolar recording electrodes were implanted bilaterally in the amygdala (toothbar at 0, 2.8 mm caudal to bregma, 5 mm lateral to midline, and 7.2 mm ventral to dura) and positioned bilaterally on the frontal cortex. Two weeks after electrode implantation kindling was induced by means of an uninterrupted sequence of up to 45 daily stimulations in the left hippocampus (2-sec trains of 1-msec pulses at 60 Hz and 40–120 μA), with simultaneous electroencephalogram (EEG) registration and video recordings of the concomitant behavioral manifestations. The stimulation current for each rat was set at 10% above the threshold for inducing after-discharge. There was no significant difference (P > 0.05) in after-discharge threshold between control, lesioned, and grafted animals. Animals were considered fully kindled when they had exhibited three grade-5 seizures. Seizures were rated using the severity scale of Racine (18), modified to include falling on the back without prior rearing as grade 5. The seizures were rated as follows: Grades: 1, facial clonus; 2, as grade 1 with neck clonus; 3, as grade 2 with forelimb clonus; 4, as grade 3 with trunk clonus and rearing; 5, as grade 4 with hindlimb and tail clonus and falling. After kindling, all brains were analyzed by fluorescence histochemistry (19).

RESULTS AND COMMENTS Consistent with reports (20–22), the development of seizures was considerably faster in the norepinephrine-depleted rats than in the control rats (Fig. 1). Both the onset and the progression of kindling were affected. Thus, the first grade-1

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seizure occurred after a mean of 4 days in 6-hydroxydopamine-treated rats as opposed to 14 days in controls. The first grade-5 seizure was seen after 8 days in lesioned rats as opposed to a mean of 31 days in controls. Progression from grade 1 to grade 5 took 5 ± 2 days (mean ± SEM) in lesioned rats and 16 ± 4 days in controls (significant difference at P < 0.05; one-way analysis of variance (ANOVA) with post hoc Newman–Keuls’ test). +, Grafted rats were significantly different from lesioned rats at P < 0.01, except for the first grade 4 where P < 0.05; *, grafted rats were significantly different from controls at P < 0.05 except for the first grade 3 where P < 0.01.

The fluorescence histochemical analysis revealed a nearly complete removal of the forebrain noradrenergic innervation in the 6-hydroxydopamine-lesioned rats with only a few individual noradrenergic fibers remaining in the hippocampal formation. In the grafted rats there was a clear graft-derived noradrenergic fiber ingrowth in all subfields—CA1, CA3, and dentate gyrus—of the dorsal two-thirds of the hippocampal formation, with a laminar distribution that was similar to that of normal locus coeruleus noradrenergic afferents. In contrast, only few scattered noradrenergic fibers were observed in nongrafted forebrain areas. The extent and density of the graft-derived noradrenergic innervation in the hippocampus varied markedly, however, between the individual animals, as summarized in Table 1. The two grafted rats that kindled quickest (rats 8 and 9 in Table 1) had small grafts and little or no noradrenergic reinnervation on either side (Fig. 2A). The cell count revealed <100 surviving noradrenergic neurons on each side in these two cases. Four of the remaining seven rats (two could not be quantified due to poor histofluorescence reaction, but had large surviving grafts bilaterally; cf. Table 1) had moderate-to-rich bilateral noradrenergic reinnervation in the hippocampal formation, as illustrated for rat 4 in Fig. 2B. Kindling was markedly retarded in all four rats as compared to in the nongrafted lesioned rats. Over 200 surviving noradrenergic neurons were found on each side. In the third remaining rats (rats 3, 6, and 7) good reinnervation was only found in the stimulated hippocampus, but either sparse or no reinnervation contralaterally. Interestingly, the kindling rate was as slow in these rats as in the ones with good bilateral reinnervation. In all animals the tip of the stimulating electrode was located in the CA1/CA3 region of the dorso-caudal hippocampus (as illustrated by the black spots in Fig. 2). In most grafted animals the electrode was in the periphery of the hippocampal area reinnervated by the grafts. In all but

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**Table 1. Noradrenergic reinnervation density and cell numbers in hippocampus compared to number of stimulations to first grade-5 seizure in the individual grafted animals**

<table>
<thead>
<tr>
<th>Rat</th>
<th>Stimulated side</th>
<th>Contralateral side</th>
<th>Stimulated side</th>
<th>Contralateral side</th>
<th>Stimulations to first grade-5 seizure, no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.0</td>
<td>3.0</td>
<td>1713</td>
<td>1114</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>3.0</td>
<td>2.5</td>
<td>582</td>
<td>390</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>2.7</td>
<td>0.9</td>
<td>342</td>
<td>209</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>2.5</td>
<td>2.9</td>
<td>271</td>
<td>226</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>2.4</td>
<td>2.4</td>
<td>276</td>
<td>230</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>2.1</td>
<td>0</td>
<td>115</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>7</td>
<td>2.0</td>
<td>0.9</td>
<td>296</td>
<td>120</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>1.5</td>
<td>0.8</td>
<td>96</td>
<td>61</td>
<td>11</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td>←</td>
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<tr>
<td>11</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td>←</td>
</tr>
</tbody>
</table>

The degree of reinnervation in the dentate gyrus and the CA1 and CA3 regions of the dorso-caudal hippocampus was scored blindly at four coronal levels. The scale was as follows: 0, no fibers; 1, significant but sparse innervation; 2, moderately dense innervation (40–60% of normal); 3, near-normal innervation or suspected hyperinnervation. Intermediary scores were also employed. The total reinnervation score on each side was calculated as the mean of the 12 values obtained.

Number of grafted noradrenergic cells in and directly outside the hippocampus was corrected for double counting errors according to Abercrombie (23).

*Noradrenergic innervation was visible but no scoring was possible due to poor histofluorescence reaction.

*Noradrenergic neurons were present but their number could not be quantified due to poor histofluorescence reaction. Large grafts were visible in and directly outside the hippocampus.
two of the grafted animals (rats 8 and 9), there was a significant noradrenergic fiber network around the electrode tip.

These observations suggest that the graft-induced suppression of the development of kindling-induced seizures is clearly related to the degree of noradrenergic neuron survival and reinnervation of the host hippocampal formation. Furthermore, reinnervation of the hippocampus on the stimulated side may be of primary importance. This impression is supported by a correlation analysis (Fig. 3), showing that the number of stimulations to first grade-5 seizure was significantly correlated to the degree of reinnervation of the stimulated hippocampus (Kendall rank correlation; $P < 0.05$). Rate of kindling development was not correlated to the
This indicates that reinstatement of noradrenergic terminals in the norepinephrine-depleted animals is sufficient to suppress the development of seizures in the noradrenergic-depleted animals. This is consistent with the observation of McIntyre of the facilitation of amygdala kindling after regional norepinephrine depletion (by local 6-hydroxydopamine injection) around the kindling site (26).

The present study corroborates findings (6–9, 20–26) that the central noradrenergic system normally retards the development of kindling-induced seizures, and the results demonstrate that this suppressive action can be exerted also by grafted locus coeruleus neurons. Intracerebral implantation of inhibitory neurons may, therefore, provide an experimental strategy to control the generation or spread of seizure activity.

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Fig. 3. Relationship between transplant-induced noradrenergic (NA) reinnervation in the hippocampus on the stimulation side and the number of stimulations needed to induce the first grade-5 seizure in grafted rats 1–9. Dots within squares refer to rats 8 and 4 in Fig. 2. Number of stimulations to reach first grade-5 seizure in the controls and lesioned rats are shown for comparison (means ± SEM).

degree of reinnervation of the contralateral hippocampus or to the total reinnervation on the two sides combined.

DISCUSSION

The results show that the increased susceptibility to hippocampal kindling seen in the 6-hydroxydopamine-lesioned rats can be at least partly normalized by intrahippocampal grafts obtained from the fetal locus coeruleus region. Although other mechanisms could hypothetically contribute to this effect, the importance of noradrenergic neurons can be inferred from several considerations. (i) Studies (24, 25) have shown that the facilitation of kindling seen in the 6-hydroxydopamine-lesioned rats is due to the removal of an inhibitory noradrenergic system. (ii) Biochemical studies of norepinephrine turnover and metabolism (14) indicate that locus coeruleus grafts are capable of restoring near-normal norepinephrine neurotransmission in the reinnervated target, and electrophysiological recordings in rats with intrahippocampal locus coeruleus grafts (15) have provided evidence for the formation of normal inhibitory noradrenergic synapses from the grafted neurons onto neuronal elements in the reinnervated host hippocampus. (iii) The dampening effect of the locus coeruleus grafts on the kindling rate in the 6-hydroxydopamine-pretreated rats was at least grossly correlated with noradrenergic neuron survival and outgrowth into the stimulated host hippocampus. A significant effect was seen only in rats with grafts containing >100–200 surviving noradrenergic neurons on the stimulated side—i.e., a number sufficient to produce a fairly extensive new noradrenergic terminal network in parts of the host hippocampal formation.

Whereas the intraventricular 6-hydroxydopamine lesion causes an extensive noradrenergic denervation throughout the central nervous system (16, 17), the noradrenergic reinnervation produced by the locus coeruleus grafts was confined to parts of the hippocampal formation, uni- or bilaterally. This indicates that reinstatement of noradrenergic trans-