Changes in gamma-aminobutyric acid and somatostatin in epileptic cortex associated with low-grade gliomas

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The role of specific neuronal populations in epileptic foci was studied by comparing epileptic and nonepileptic cortex removed from patients with low-grade gliomas. Epileptic and nearby (within 1 to 2 cm) nonepileptic temporal lobe neocortex was identified using electrocorticography. Cortical specimens taken from four patients identified as epileptic and nonepileptic were all void of tumor infiltration. Somatostatin- and γ-aminobutyric acid (GABAergic)-immunoreactive neurons were identified and counted. Although there was no significant difference in the overall cell count, the authors found a significant decrease in both somatostatin- and GABAergic-immunoreactive neurons (74% and 51%, respectively) in the epileptic cortex compared to that in nonepileptic cortex from the same patient. It is suggested that these findings demonstrate changes in neuronal subpopulations that may account for the onset and propagation of epileptiform activity in patients with low-grade gliomas.

Key Words • epilepsy • immunoreactive neuron • gamma-aminobutyric acid • somatostatin • low-grade glioma

The majority of patients (50% to 90%) with supratentorial low-grade gliomas initially present with seizures. Intraoperative electrocorticography in these patients reveals epileptic foci separate from the tumor nidus. Histologically, these epileptic foci are commonly devoid of tumor infiltration. To understand the neurochemical basis of tumor-associated epileptiform activity, resected tissue from both epileptogenic and nonepileptogenic areas in patients undergoing electrocorticography during glioma surgery have been analyzed. Our investigation has concentrated on changes in γ-aminobutyric acid (GABA)- and somatostatin-immunoreactive neurons from these tissue samples of neocortex.

Both human and animal studies of epileptic foci have identified changes in cell numbers and in specific populations of neurons and neurotransmitter substances that may lead to hyperexcitability. These changes include decreases in GABAergic-immunoreactive neurons and changes in modulatory neuropeptides such as somatostatin and neuropeptide Y. Initial studies of the neuronal reorganization of human hippocampal epileptic foci associated with mesial temporal lobe sclerosis have shown a selective loss of somatostatin- and neuropeptide Y-immunoreactive neurons with no appreciable change in the population of GABAergic interneurons. In those studies, the hippocampus from epileptic patients were compared to "control" hippocampi from other epileptic patients with temporal lobe lesions or tumors not involving the hippocampus, and to nonpathological hippocampi obtained at autopsy from patients with no history of seizures. The tumor-associated hippocampi were much like the control hippocampi and did not demonstrate any significant changes in the numbers of somatostatin- or GABA-immunoreactive neurons. The relationship of tumor-associated hippocampi to the patient's epilepsy was unknown. It is not clear whether the somatostatin and neuropeptide Y cell losses are related to epilepsy per se or are part of the specific pathology of mesial temporal sclerosis. Studies of the relationship of anterior (epileptic) to posterior (nonepileptic) human hippocampi have shown no changes in GABAergic neurons even though there is a significant reduction of principal cells involved in the mesial sclerosis.

Animal models of experimental epilepsy have demonstrated an almost complete loss of somatostatin-immunoreactive neurons. However, these same models have shown both decreases and no change in the number of GABAergic-immunoreactive neurons.
In the present study, we have demonstrated that in epileptic patients with low-grade gliomas there is a concomitant decrease in somatostatin- and GABAergic-immunoreactive neurons in the nontumor epileptic cortex compared to adjacent nontumor nonepileptic cortex from the same patient. Therefore, this study establishes a relationship between decreases in these cell types and their respective neurotransmitters to epileptic activity.

**Clinical Material and Methods**

**Patient Population**

Four patients with seizure disorders were suspected of having low-grade temporal lobe gliomas based on computerized tomography and magnetic resonance imaging criteria. Two of the four patients were operated on under local anesthesia and the other two under general anesthesia. Intraoperatively, ultrasonography was used to localize the tumor and electrocorticography identified the seizure foci by the presence of interictal epileptiform abnormalities. In all cases, epileptiform areas were located adjacent to the tumor. A nonepileptic cortical site (control) was resected with the tumor within 1 to 2 cm of the epileptic focus (Fig. 1). In three of the four cases, the epileptic and nonepileptic cortex was taken from the same gyrus; in the fourth patient, samples were taken from the middle and inferior temporal gyri. The epileptic and nonepileptic tissue specimens were submitted for neuropathological examination.

The remaining tissue was sliced into sections 3 mm thick and placed in paraformaldehyde (3.5%) and glutaraldehyde (0.1%) phosphate buffer (pH 7.4) at 4°C. Neuropathological evaluation of the epileptic and nonepileptic tissue specimens found no evidence of tumor infiltration or gliosis in all four patients. In all of these cases, the tumor was identified as a low-grade astrocytic glioma.

**Immunohistochemical Analysis**

The techniques used for immunocytochemical staining have been described previously, but are briefly detailed here. Following paraformaldehyde-glutaraldehyde fixation on a shaker for 16 to 24 hours, the tissue was infiltrated with 30% sucrose in 0.1 M phosphate buffer for 16 to 24 hours at 4°C. Frozen sections 35 μm thick were cut and sorted for Nissl or immunocytochemical staining. Sections for immunocytochemistry were washed in 0.1 M phosphate buffer with 0.01% NaN₃, for 6 to 16 hours at 4°C, followed by pretreatment for endogenous peroxidase activity with 0.5% H₂O₂ in 0.1 M Tris buffer (pH 7.4) for 2 to 3 hours. Sections were rinsed in 0.1 M Tris buffer-saline, then preincubated.
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**TABLE 1**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Nissl (all cells)</th>
<th>Somatostatin</th>
<th>GABA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonepileptic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>270 ± 13</td>
<td>19.4 ± 1.1</td>
<td>27.7 ± 2.1</td>
</tr>
<tr>
<td>2</td>
<td>296 ± 10</td>
<td>16.6 ± 1.4</td>
<td>24.4 ± 1.8</td>
</tr>
<tr>
<td>3</td>
<td>278 ± 5</td>
<td>23.9 ± 1.4</td>
<td>28.8 ± 1.0</td>
</tr>
<tr>
<td>4</td>
<td>297 ± 12</td>
<td>13.2 ± 1.7</td>
<td>21.4 ± 1.7</td>
</tr>
</tbody>
</table>

* Cell counts expressed as means ± standard error of the means of 25 800-sq mm sections. Data are given for nonepileptic and epileptic cortex.

**Results**

**Cell Counts**

Cell counts of the Nissl-stained section were performed to establish both the total number of cells (Table 1) and the number of cells between 6 and 45 μm in diameter. More than 96% of the total cells were between 6 and 45 μm in diameter. In both the overall cell count and the count of the small cells, there was no significant difference between the epileptic and nonepileptic cortex. A subtle infiltration of small, atypical (tumor) cells would likely increase the total cell count for the epileptic cortex, whereas gliosis could decrease the total cell count. These differences were not seen. The number of GABAergic- and somatostatin-immunoreactive cells accounted for only 2% to 10% of the total cells and 5% to 13% of the cells between 6 and 45 μm in diameter, so changes in the number of immunoreactive cells would not be expected to have a significant effect on the overall cell counts.

**Morphological Changes**

Morphological comparisons between the epileptic and nonepileptic GABAergic- and somatostatin-immunoreactive cells demonstrated no significant difference in cell size, shape, or dendritic and axonal staining. One of the two morphological types of somatostatin-immunoreactive cells from epileptic cortical tissue is shown in Fig. 2A. A somatostatin neuron from nonepileptic cortex tissue is shown in Fig. 2B. At lower-power views, small round somatostatin neurons are shown in Fig. 3A and B. The GABAergic neurons were primarily of one cell type (small and round), whether from epileptic or nonepileptic cortex (Fig. 2C and D).

**Changes in Somatostatin-Immunoreactive Neurons**

The most dramatic finding of this study was the almost total loss of somatostatin-immunoreactive neurons in the epileptic foci compared to nonepileptic control tissue (mean decrease 74%, range 69% to 83%). The filters of the imaging system were set to select labeled cells 6 to 30 μm in diameter, and then a threshold was set for all measurements made on the epileptic and nonepileptic sections from that patient. Cells

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* Imaging system manufactured by Analytical Imaging Concepts, Irvine, California.
FIG. 2. Photomicrographs showing the morphology of cell staining for nonepileptic and epileptic somatostatin and GABAergic neurons. Somatostatin-immunoreactive cells demonstrate staining of soma and dendritic arbors from epileptic (A) and nonepileptic (B) cortical tissue. GABAergic-immunoreactive cells are shown from epileptic (C) and nonepileptic (D) cortical samples. No significant differences in morphology were identified except for the slight predominance of larger somatostatin cell bodies in the epileptic samples. Scale bar = 25 μm.

smaller than 6 μm or larger than 30 μm were not selected.

A low-power view of the region between 300 and 900 μm below the pial surface is shown in Fig. 3. The typical band of somatostatin-immunoreactive cells in the middle layers is shown in the nonepileptic cortex (Fig. 3A); in sharp contrast is the decrease in the somatostatin cells in the epileptic cortex (Fig. 3B). This decrease was typical of the changes found in the somatostatin cells in the epileptic cortex in all four patients (Table 1). The choice of nonepileptic cortical sections was random because the epileptic focus was identified first, then the nonepileptic site was chosen based on the nearest area of cortex to be included in the resection exhibiting no evidence of epileptiform activity by electrocorticography.

Changes in GABA-Immunoreactive Neurons

The number of GABAergic neurons in the epileptic cortex was also decreased compared to that in the nonepileptic controls (Table 1). Although all four patients showed a significant decrease in GABAergic neurons in the epileptic cortex compared to nonepileptic cortex, the changes were more variable (mean decrease 51%, range 25% to 68%). The variability also did not correlate with the changes in somatostatin; the largest decrease in GABAergic neurons was not in the patients with the largest decrease in somatostatin cells. Photomicrographs of nonepileptic and epileptic sections at low power are shown in Fig. 4A and B, respectively.

All of the cortical tissue samples (epileptic and nonepileptic) were taken from the anterior temporal lobe in either the superior, middle, or superior portion of the inferior temporal gyrus. The fact that the cell counts from the different regions, both epileptic and nonepileptic, were not significantly different among patients suggests that there should be no large differences among the samples. The total number of GABAergic neurons was also similar in three of the four patients studied. The magnitude of decrease in the epileptic cortex of
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FIG. 3. Photomicrographs showing low-power view of somatostatin-immunoreactive neurons in nonepileptic (A) and epileptic (B) cortex. The pial surface is toward the top and the white matter toward the bottom of the pictures. The top is approximately 250 µm below the pial surface. There is a dramatic decrease in cell count in the middle layers (II, III, and IV) of neocortex in the epileptic (B) compared to the nonepileptic (A) cortex. Scale bar = 50 µm.

FIG. 4. Photomicrographs showing low-power view of GABAergic neurons in nonepileptic (A) and epileptic (B) cortex. The pial surface is toward the top and the white matter toward the bottom of the pictures. The top is approximately 250 µm below the pial surface. There is a significant decrease in cell count in the epileptic (B) compared to the nonepileptic (A) cortex. This decrease did not appear to be confined to the middle layers like that of the somatostatin epileptic changes. Scale bar = 50 µm.

the remaining patient (Case 4), who had a slightly different GABAergic cell count in the nonepileptic control tissue, was similar to that of the other three patients.

Discussion

We have shown significant decreases of certain immunoreactive neurons (somatostatin and GABAergic) in epileptic foci in patients with low-grade gliomas compared to nonepileptic control cortex from the same patients. Other investigators have demonstrated in patients with mesial temporal sclerosis that epileptic hippocampal tissue shows a reduction in somatostatin-immunoreactive neurons, as well as in the total number of cells. Unfortunately, in these studies, electrocorticography was not carried out in the control patients with tumors so the extent of epileptic activity in those hippocampal tumor specimens is unknown. Furthermore, the tumor-associated epileptic hippocampi were not taken from patients with only low-grade gliomas but also from patients with hamartomas, vascular lesions, mixed oligoastrocytomas, and anaplastic astrocytomas. Our findings, although different from those of the other studies, may be more meaningful with regard to changes that occur at epileptic foci specifically in patients with low-grade gliomas because we have identified and studied the epileptic lateral neocortex adjacent to the nidus of the low-grade glioma. These decreases in the interneuron populations containing somatostatin and GABA are closely associated with the interictal activity found near low-grade gliomas.

The pathophysiology of epileptic cortex associated with low-grade gliomas is quite unlike that seen in

J. Neurosurg. / Volume 77 / August, 1992
mesial temporal lobe epilepsy, especially with regard to the cell loss and lack of GABA changes in the latter cases. However, there are similarities between the mesial temporal lobe and low-grade glioma epileptic tissue, especially regarding the reduction in somatostatin immunoreactivity.

**Morphological Changes**

A number of studies have shown that somatostatin tends to co-localize with GABA in interneurons. Although in this study we had no direct method of distinguishing co-localization, the cell types in Figs. 2C and D and 3A appear quite similar and may certainly be considered candidates for cells that might co-localize GABA and somatostatin. The neocortical somatostatin cell types in Fig. 2A and B are clearly quite different in shape and dendritic pattern from the GABAergic- and somatostatin-immunoreactive neurons of Figs. 2C and D and 3A. However, normal hippocampal interneurons in the stratum oriens/aldaeus border of the CA1 region also show a co-localization of somatostatin and GABA in neurons with a morphology much like those pictured in Fig. 2A and B.

**Changes in Somatostatin-Immunoreactive Neurons**

Decreases in somatostatin immunoreactivity are found in two very different types of epilepsy. First, mesial temporal sclerosis is presumably due to an early insult with gradual cell loss, gliosis, and the development of epileptic activity. Second, low-grade gliomas develop as a slow-growing lesion with no apparent cell loss or significant tumor invasion into the adjacent epileptic tissue, suggesting that the loss of these neurons may be an indicator of a region with hyperexcitability. Recent radioimmunoassay studies have also shown a significant decrease in the quantity of somatostatin in the temporal lobe neocortex associated with temporal lobe lesions (B Strowbridge, et al., unpublished data). These findings all point to an association between decreases in somatostatin immunoreactivity and hyperexcitability.

There remains the possibility that the loss of immunoreactivity is not concomitant with a cell loss. Studies on cultured somatostatin-immunoreactive striatal neurons have demonstrated that these neurons are very sensitive to excitatory amino acids and that exposure to N-methyl-D-aspartate (NMDA), a specific class of glutamate receptor agonist thought to be involved in epileptiform activity, causes depletion of somatostatin. However, in recent studies of the epileptic hippocampi (dentine hilus) from patients with mesial temporal sclerosis, a concomitant loss of somatostatin neurons and the messenger ribonucleic acid for somatostatin was found. These findings suggest that the somatostatin neurons have been either damaged or destroyed, or have changed their phenotype due to a suppression of somatostatin gene expression. Thus, the loss of somatostatin immunoreactivity in the low-grade glioma epileptic foci is likely due to a depletion of somatostatin immunoreactivity associated with an actual loss of somatostatin neurons.

The loss of somatostatin neurons raises the question of the physiological and anatomical significance of such an occurrence. If somatostatin as a neurotransmitter causes hyperpolarizations (inhibition), then the loss of these neurons may lead to hyperexcitability; however, if somatostatin primarily causes depolarizations (excitation), then the loss of somatostatin neurons may actually inhibit hyperexcitability. Animal models of epilepsy, especially kindling, have found that somatostatin depletion actually inhibits kindled seizures. Even with in vitro studies, a confusing picture emerges regarding the physiological action(s) of somatostatin since somatostatin applied to hippocampal neurons causes both excitation and inhibition.

Therefore, the overall effect of depletion of somatostatin in the epileptic foci may be hyperexcitability, but the final answer is far from clear and awaits further study. One alternative possibility is that the loss of somatostatin synaptic activity allows for the sprouting of neighboring collaterals, and an overall increase in new excitatory synapses may lead to epileptiform activity. This hypothesis is supported by recent findings that there is an increase in the number of NMDA and quisqualic acid receptors in epileptic regions of the hippocampus.

**Changes in GABA-Immunoreactive Neurons**

One of the major hypotheses advanced to explain the development of an epileptic focus is that of an imbalance between excitation and inhibition. This proposes that decreases in inhibitory activity may lead to hyperexcitability and potentially epileptic activity. Animal models of epilepsy (especially those involving alumina gel lesions) have frequently revealed a decrease of up to 50% in the number and amount of GABAergic interneurons. Except for the late overall cell loss found in alumina gel models, the structural changes occurring in low-grade gliomas are similar to those found in alumina gel animal models of epilepsy.

In contrast, a kindling model of rat hippocampal epilepsy demonstrated a significant change in somatostatin but no change in GABA. Studies of glutamate decarboxylase-imunoreactive neurons in human epileptic hippocampi found decreases in the principal cells of 50% to 90% with associated increases in GABAergic terminals. The study by Babb, et al., is one of the few to compare tissue samples from the same patient, and it did not show significant decreases in GABAergic terminals in epileptic hippocampal tissue.

Other studies evaluating hippocampal tissue from patients with lesions in the temporal lobe did not find changes in the GABAergic neuron population. This may be due to the fact that the epileptic foci in these cases may not have included the hippocampus. Mesial sclerotic epileptic hippocampi show a loss of somatostatin immunoreactivity but no changes in GABAergic
staining. However, the mesial sclerotic hippocampi do show a decrease in the amount of inhibition by in vitro electrophysiological studies. One of these studies found that the tumor-associated hippocampus was similar to that of controls (reportedly with no history of seizures) obtained at autopsy. These types of studies emphasize the need for careful selection of both cortical tissue that represents the epileptic focus and nonepilepti
cmic control tissue. The finding in these same studies that the mesial temporal sclerotic epileptic hippocampi show no change in GABAergic immunoreactivity supports the possibility that the mesial temporal lobe epileptic focus is a different entity from the epileptic focus associated with low-grade gliomas. It may be that the hippocampal epileptic focus develops in a different manner than the neocortical epileptic focus, or that the early insult associated with mesial hippocampal epileptic foci leads to different effects on specific neuronal populations than the slow growth of low-grade gliomas.

Low-Grade Gliomas and Epileptic Foci

The low-grade glioma epileptic foci partially resemble the mesial epileptic hippocampal foci in that there is a significant decrease in the amount of somatostatin immuno
geractivity; however, the amount of cell loss is considerable in the mesial sclerosis tissue compared to our findings associated with low-grade gliomas. Recent studies of epileptic cortex associated with lesions in the temporal lobe have also shown a decrease in somato
statin measured by radioimmunoassay and a correlated loss of inhibition. The confusing picture of somato
tatin’s physiological role needs to be further clarified before the true meaning of the consistent decreases in somatostatin immunoreactivity at different types of epileptic foci can be identified. For now, the loss of somatostatin neurons at epileptic foci can be used as a marker for hyperexcitability. Whether these decreases in somatostatin immunoreactivity represent the loss of a subset of GABA/somatostatin interneurons or just a loss of immunoreactivity remains to be elucidated.

There are no clear studies into the changes of somato
tatin immunoreactivity at the borders of alumin
gel-induced chronic epileptic foci, but the changes in GABAergic neurons and glutamic acid decarboxylase (an enzyme involved in the synthesis of GABA) are very similar to those found in low-grade gliomas. As other investigators have pointed out, this type of human epilepsy study demonstrates the importance of choosing animal epilepsy models carefully. Our study also emphasizes the similarities (changes in somatostatin) and differences (changes in GABA) that can be found even among epileptic cortex in human studies. Since epilepsy is a multifactorial disease process, it will not be surprising to find that the mechanisms leading to the development of an epileptic focus may have multiple etiologies.

Acknowledgments

The authors thank Roger Tootell for the use of his imaging analysis system and Mary Gross for her histological expertise.

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This research was supported by the American Association of Neurological Surgeons Research Foundation and Grass Foundation Morison Fellowships to Dr. Haglund; an American Cancer Society Career Development Award and National Institutes of Health (NIH) Grant NS K08-01253-01 to Dr. Berger; and NIH Grants NS 17111 and NS 20482 to Dr. Ojemann.

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