Neurochemical Profile of Glioneuronal Lesions from Patients with Pharmacoresistant Focal Epilepsies

HELMUT K. WOLF, TORSTEN BIRKHOHLZ, JÖRG WELLMER, INGMAR BLÜMCKE, TORSTEN PIETSCHE, AND OTMAR D. WIESTLER

Abstract. Gangliogliomas, dysembryoplastic neuroepithelial tumors (DNT) and glioneuronal malformations are frequently encountered in patients with pharmacoresistant focal epilepsies. In order to characterize the neurochemical profile of these neoplastic and malformative glioneuronal lesions, we have examined the presence of the α subunit of the GABA_A receptor, the N-methyl-D-aspartate receptor subunit 1 (NR1), glutamate decarboxylase, tyrosine hydroxylase, somatostatin, parvalbumin, and calretinin in 60 gangliogliomas, 11 DNT, 10 tuberous sclerosis-like lesions and 17 non-tuberculous sclerosis-like glioneuronal malformations. All DNT and tuberous sclerosis-like lesions, 59 gangliogliomas (98%), and 13 non-tuberculous sclerosis-like hamartias (76%) were positive for at least one of the markers. Despite a great variation between and within the different entities, the neurochemical profile was generally reminiscent of normal neocortex: glutamate decarboxylase, GABA_A receptor and NR1 which are common in neocortical neurons were present in the great majority of the lesions and often showed high labeling indices. There were three tuberous sclerosis-like lesions (30%) that contained both NR1 and glutamate decarboxylase immunoreactive giant cells in addition to well-differentiated ganglion cells. This supports the idea that at least some of these giant cells are of neuronal origin. The oligodendroglial-like cells of DNT and glioneuronal hamartias did not show immunoreactivity for any of the markers. The very high incidence of gangliogial lesions in patients with chronic focal epilepsies and the presence of neurotransmitter-producing enzymes, neurotransmitter receptors, neuropeptides, and calcium-binding proteins in many of these lesions suggests that they may play an active role in the pathogenesis of epileptic seizures.

Key Words: Epilepsy; Immunohistochemistry; Malformation; Neurotransmitter; Pathology; Tumor.

INTRODUCTION

Neoplastic and non-neoplastic lesions composed of highly differentiated ganglion cells and glial elements are frequently encountered in patients with medically intractable epilepsies. In a recent survey of 279 consecutive surgical specimens from patients with chronic pharmacoresistant epilepsy, gangliogliomas represented 46% of all brain tumors and glioneuronal malformations were present in 20.6% of all surgical specimens (1). Another distinct glioneuronal lesion which occurs almost exclusively in association with chronic focal epilepsies is the dysembryoplastic neuroepithelial tumor (DNT; 2–5). It seems unlikely that the high incidence of these otherwise unusual glioneuronal tumors and malformations in epilepsy patients is purely coincidental. Therefore, the question arises whether the neuronal component of these lesions participates in the generation of seizures through its neurochemical or electrical activity. Here we examine the presence of the α subunit of the GABA_A receptor, the N-methyl-D-aspartate receptor subunit 1 (NR1), glutamate decarboxylase, tyrosine hydroxylase, somatostatin, parvalbumin, and calretinin in a large series of glioneuronal tumors and malformations. These molecules have been associated with the pathogenesis of both experimental and human focal epilepsies.

Glutamate is the principal excitatory neurotransmitter in the brain, and rapid excitatory signal transduction in the central nervous system (CNS) is largely mediated through glutamate receptors. One subtype of glutamate receptor, the N-methyl-D-aspartate (NMDA) receptor, has recently received considerable attention due to its potential involvement in excitation–induced neuronal death and kindling, an animal model of human temporal lobe epilepsy (6, 7). NR1 is an essential subunit of native and functionally active NMDA receptors and shows a rather ubiquitous expression in the CNS (for review see 8).

Glutamate decarboxylase is the main rate-limiting enzyme for the synthesis of GABA, the major inhibitory transmitter of the CNS. The GABA_A-benzodiazepine receptor is present in approximately one-third of all brain synapses (9) and the α subunit of the GABA_A receptor is ubiquitously expressed throughout the brain (10). Based on the effectiveness of various ligands to the GABA_A-benzodiazepine receptor in the treatment of human epilepsies and on a number of experimental seizure models, alterations of GABAergic neurotransmission have been implicated in the pathogenesis of focal epileptic seizures (for review see 11–16).

Tyrosine hydroxylase is the rate-limiting enzyme in the synthesis of catecholamines. Neurons with immunoreactivity for tyrosine hydroxylase are increased in the hippocampus of patients with temporal lobe epilepsy and Ammon’s horn sclerosis (17). Significant increases of tyrosine hydroxylase activities have been documented in...
spiking human cortex as compared to non-spiking cortex (18, 19).

Parvalbumin and calretinin are calcium-binding proteins which are expressed in certain neuronal subpopulations (for review see 20, 21). A loss of parvalbumin has been observed in human temporal lobe epilepsy (22) and it has been suggested that calcium-binding proteins may provide protection from excitotoxic cell death (23, 24).

Somatostatin is a cyclic peptide and serves as a neurotransmitter or neuromodulator (for review see 25). Increased somatostatin immunoreactivity has been found in the hippocampus and other brain regions following amygdala kindling, and decreased numbers of somatostatin immunoreactive neurons have been identified in human epileptic cortex surrounding low grade gliomas (26–28).

While gangliogliomas and DNT are well-defined entities, the terminology of malformative lesions as they occur in patients with seizure disorders is not generally agreed upon (29). Our previously introduced nomenclature for these lesions has been maintained in the present study (1, 30–32). Some malformative cortical glioneuronal lesions reveal a striking histological resemblance to cortical tubers of tuberous sclerosis even in the absence of extracerebral stigmata for this phacomatosis. We have designated these tuberous sclerosis-like glioneuronal malformations. In addition, there are microscopic glioneuronal lesions that presumably result from a defect in cell migration during development and have no resemblance to tuberous sclerosis. These are designated as non-tuberous sclerosis-like glioneuronal hamartias. In contrast to hamartomas, glioneuronal hamartias do not produce grossly visible masses. Detailed histopathological evaluations of gangliogliomas, DNT and glioneuronal malformations have recently been reported elsewhere (32, 33).

Our main goal was to provide data which may help to elucidate the pathogenesis of focal seizures in patients with gangliogliomas or malformations. In addition, from the point of developmental neuropathology, it appears intriguing to ask whether the neurochemical characterization may provide insights into the origin and evolution of ganglioglial lesions.

MATERIALS AND METHODS

All slides, tissue blocks and pathology reports from surgical specimens of patients with gangliogliomas and other glioneuronal lesions operated at the University of Bonn Medical Center between March 1968 and September 1994 were retrieved from the files of the Department of Neuropathology. The great majority of these cases have been evaluated previously with respect to their histopathological features, proliferative activity and clinical presentation (32–34). However, some cases from the previous studies had to be excluded because of insufficient amounts of remaining tissue. On the other hand, a few recent cases were added to the study. All specimens had been fixed in formalin at room temperature for 8 hours (h) to 3 days and routinely processed into paraffin. Hematoxylin and cosin (H&E), Nissl, and combined H&E- luxol fast blue stains as well as immunohistochemical reactions for glial fibrillary acidic protein (GFAP), synaptophysin, neurofilament protein and neuron-specific enolase were carried out to document the glioneuronal nature of the lesion. These routine immunohistochemical reactions were performed with commercial primary antibodies, secondary biotinylated antibodies and the avidin-biotin-peroxidase complex method with diaminobenzidine as chromagen according to the instructions of the manufacturer (DAKO, Glostrup, Denmark). All cases were reviewed by two neuropathologists to confirm the diagnoses.

Immunohistochemistry

The sections were cut at 4 microns, mounted on slides coated with 3-aminopropyltriethoxysilane (Fisher Scientific, Pittsburgh, PA), air-dried in an incubator at 40°C and heated on a 60°C hot plate for a few seconds until the paraffin melted. After deparaffinization and rinses in 100% and 95% ethanol, the slides were incubated in 2% hydrogen peroxide diluted in methanol for 15 minutes (min), rehydrated in 95% ethanol and rinsed in phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA). PBS with 1% BSA was also used for the subsequent washes and dilutions unless otherwise specified.

For NR1, GABA receptor, tyrosine hydroxylase, and parvalbumin immunohistochemistry, the slides were then placed into a 0.01 molar citrate buffer at pH 6.0 and heated in a microwave oven at 650 watts and 425 watts as detailed in Table 1. The buffer solution was boiling during the microwave treatment. The slides were allowed to cool for 15 min in this buffer solution at room temperature and subsequently washed in PBS. For somatostatin staining, instead of microwave treatment, the slides were incubated in a 0.1% solution of trypsin (E. Merck, Darmstadt, Germany) and 0.1% calcium chloride at pH 7.4 and room temperature for 2 min. For calretinin and glutamate decarboxylase immunohistochemistry, neither microwave nor proteolytic pretreatment was necessary. At this point, preincubation with 5% of the appropriate blocking medium diluted in PBS was performed according to Table 1. This was succeeded by incubation with the primary antibody at the dilutions, temperatures and durations specified in Table 1. The sections were then incubated with the appropriate biotinylated secondary antibodies (goat-anti-rabbit Immunoglobulin from Vector Laboratories, Burlingame, CA; swine-anti-rabbit Immunoglobulin or rabbit-anti-mouse Immunoglobulin from DAKO) diluted 1:200 in PBS at 42°C for 20 min. This was followed by incubation with avidin biotinylated horseradish peroxidase complex (Vectastain Elite ABC Kit, Vector Laboratories) for 20 min at 42°C according to the instructions of the manufacturer. All steps were followed by appropriate washes in PBS. Finally, the sections were developed in a substrate solution of 0.05% diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in 0.05 M Tris-HCl, pH 7.6, washed, lightly counterstained with hematoxylin, dehydrated in ethanol and mounted. All reagents for immunohistochemistry were purchased from DAKO unless otherwise specified. Negative control sections were prepared by substitution of the primary antibodies with equivalent dilutions of normal rabbit IgG or nonimmune mouse IgG. As positive
<table>
<thead>
<tr>
<th>Antigen</th>
<th>Microwave or protease treatment</th>
<th>Blocking</th>
<th>Primary antibody</th>
<th>Secondary antibody</th>
<th>Source of primary antibody</th>
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<tr>
<td>Glutamate decarboxylase</td>
<td>Not required</td>
<td>Normal swine serum; 1:20; 3% non-fat dry milk; 24 h; 42°C</td>
<td>Polyclonal rabbit K2; 1:500; 24 h; 4°C</td>
<td>Biotinylated swine anti-rabbit Ig</td>
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<td>Parvalbumin</td>
<td>Sodium citrate, pH 6.0 7'650 watts 5'425 watts</td>
<td>Normal rabbit serum; 1:20; 3% non-fat dry milk; 24 h; 42°C</td>
<td>Mouse clone PV 235; 1:500; 24 h; 4°C</td>
<td>Biotinylated rabbit anti-mouse Ig</td>
<td>Sigma Chemie GmbH, Deisenhofen, Germany</td>
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<tr>
<td>Calretinin</td>
<td>Not required</td>
<td>Normal goat serum; 1:100; 20'; 42°C</td>
<td>Polyclonal rabbit CR 7696; 1:5,000; 16 h; 22°C</td>
<td>Biotinylated goat anti-rabbit Ig</td>
<td>Swant, Bellinzona, Switzerland</td>
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<td>Tyrosine hydroxylase</td>
<td>Sodium citrate, pH 6.0 7'650 watts 5'425 watts</td>
<td>Normal rabbit serum; 1:200; 20'; 42°C</td>
<td>Mouse clone TH LNC; 1:200; 16 h; 42°C</td>
<td>Biotinylated rabbit anti-mouse Ig</td>
<td>Chemicon Inc., Temecula, PA</td>
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<tr>
<td>Somatostatin</td>
<td>Trypsin solution</td>
<td>Normal rabbit serum; 1:200; 3% non-fat dry milk; 24 h; 42°C</td>
<td>Rat clone MAB354; 1:200; 24 h; 4°C</td>
<td>Biotinylated rabbit anti-rat Ig</td>
<td>Chemicon Inc., Temecula, PA</td>
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<td>α₁ subunit of the GABA&lt;sub&gt;a&lt;/sub&gt; receptor</td>
<td>Sodium citrate, pH 6.0 7'650 watts 5'425 watts</td>
<td>Normal rabbit serum; 1:20; 3% non-fat dry milk; 24 h; 42°C</td>
<td>Mouse clone bd 24; 1:1,000; 24 h; 4°C</td>
<td>Biotinylated rabbit anti-mouse Ig</td>
<td>Boehringer Mannheim, Mannheim, Germany</td>
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<tr>
<td>NMDA receptor subunit 1</td>
<td>Sodium citrate, pH 6.0 10'650 watts</td>
<td>Normal rabbit serum; 1:20; 3% non-fat dry milk; 24 h; 42°C</td>
<td>Mouse clone 54.1; 1:500; 24 h; 4°C</td>
<td>Biotinylated rabbit anti-mouse Ig</td>
<td>Pharmingen, San Diego, CA</td>
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Ig = immunoglobulin.
Fig. 1. Immunoreactivity of the neuronal component in glioneuronal lesions for the \(N\)-methyl-D-aspartate receptor subunit 1 (NR1), the \(\alpha\) subunit of the GABA\(_A\) receptor (GABAR), glutamate decarboxylase (GAD), calretinin (CR), parvalbumin (PV), somatostatin (SOM), and tyrosine hydroxylase (TH); NOS = not otherwise specified. The distinct column patterns represent fractions of immunoreactive neurons.

RESULTS

There were 60 gangliogliomas, 10 tuberous sclerosis-like lesions, 17 non-tuberous sclerosis-like hamartias and 11 DNT. Fifty-four of 60 patients with gangliogliomas (90%) and all patients with glioneuronal malformations and DNT had a history of chronic pharmacoresistant epilepsy.

The great majority of the lesions had neuronal immunoreactivity for at least one of the seven markers examined. All tuberous sclerosis-like lesions and DNT, 59 of 60 gangliogliomas (98%), and 13 of 17 (76%) of the non-tuberous sclerosis-like hamartias were positive for at least one of the markers. In all types of lesions was the neuronal staining pattern similar to that of normal ganglion cells as seen in control specimens and as described in the literature (Fig. 2). Antibodies to glutamate decarboxylase, somatostatin, parvalbumin, and calretinin labeled the neuronal cytoplasm whereas NR1 and GABA\(_A\) receptor immunoreactivity was present both within the cytoplasm and at the neuronal membrane. In addition to their cytoplasmic and cell membrane staining, NR1 and GABA\(_A\) receptor were also localized in the fibrillar matrix of glioneuronal lesions similar to their expression in the neuropil of normal gray matter. The cytoplasmic staining intensity for the GABA\(_A\) receptor was generally lower than the linear signal at the cell membrane (Fig. 2B). In contrast, in NR1-positive neurons, it was frequently difficult to detect a distinct membranous staining pattern because of the intense cytoplasmic immunoreactivity for NR1. Some neurons with no cytoplasmic immunoreactivity for glutamate decarboxylase revealed a distinctly granular perineuronal staining pattern for glutamate decarboxylase consistent with the presence of GABAergic axon terminals (Fig. 2G). The same pattern was occasionally observed with antibodies to parvalbumin and calretinin.

There was good inter-observer agreement in the semi-quantitative assignment of neuronal labeling. The neuronal labeling indices for the various markers are shown in Figure 1. While gangliogliomas and tuberous sclerosis-like lesions showed frequent isolated neuronal processes with immunoreactivity for parvalbumin and calretinin, these structures were only rarely labeled in non-tuberous sclerosis-like hamartias and DNT.

Seventy-five percent or more of the gangliogliomas were immunoreactive for glutamate decarboxylase, GABA\(_A\) receptor or NR1. In striking contrast, there was no or only little labeling for somatostatin and tyrosine hydroxylase. Of the 11 gangliogliomas with somatostatin immunoreactivity, ten were positive for glutamate decarboxylase and nine for the GABA\(_A\) receptor. The series of gangliogliomas included 54 WHO grade I gangliogliomas, four WHO grade II gangliogliomas and two anaplastic gangliogliomas (WHO grade III). There were no apparent differences between the marker profiles of gan-
neurochemical profile of glioneuronal lesions

Gliogliomas with different histopathological grades or of gliogliomas from patients with (n = 54) and without (n = 6) a history of chronic epilepsies.

All tuberous sclerosis-like lesions contained NR1, GABA_A receptor, glutamate decarboxylase (Fig. 2G) and somatostatin immunoreactive neurons. Ninety percent of the lesions were immunoreactive for parvalbumin and calcineurin and 80% had tyrosine hydroxylase-positive neurons. Because the nature of the bizarre balloon cells has not been established, it was of interest to determine the marker profile of this cell population separately. In six of ten cases (60%), some balloon cells showed cytoplasmic positivity for one or more neuronal markers (Fig 2H). The staining of balloon cells was less intense than that of adjacent well-differentiated ganglion cells of the same lesion. Three cases (30%) contained both NR1 and glutamate decarboxylase immunoreactive balloon cells. Parvalbumin was the only marker that was never detected in balloon cells.

In DNT, immunoreactive neurons for NR1, GABA_A receptor, and glutamate decarboxylase were frequently encountered. In contrast, there was little labeling for parvalbumin. Somatostatin, tyrosine hydroxylase and calcineurin showed intermediate levels of expression. Oligodendrogia-like cells with small round nuclei and clear cytoplasm, which are common in non-tubereous sclerosis-like hamartias and are a prominent feature in DNT, did not express any of the neuronal markers.

DISCUSSION

The presence of neurotransmitter receptors, neurotransmitter-producing enzymes, neuropeptides, and calcium-binding proteins in the great majority of glioneuronal tumors and malformations indicates that ganglion cells in these lesions have a high level of neurochemical differentiation. This correlates well with their structural maturity as indicated by abundant cytoplasm, well-formed Nissl substance, and numerous neuronal processes. It appears that at least a significant number of these neurons have the structural and chemical capability to participate in interneuronal signal transduction. The variation of the neurochemical profile between and within the different entities is reminiscent of the structural diversity of the abnormal ganglion cells as indicated by the range in size, shape and disfigurement of the individual neurons and the wide differences in the neuronal densities. Despite this variability, the neurochemical profile of the different entities was generally reminiscent of normal neocortex: glutamate decarboxylase, GABA_A receptor, and NR1 which are very common in neocortical neurons were present in the great majority of the lesions and often showed high labeling indices. Similarly, tyrosine hydroxylase which is present in less than 0.1% of neocortical neurons (35) was relatively infrequent in gangliogial lesions. Somatostatin, calcineurin and parvalbumin generally showed intermediate levels of expression. There were some exceptions to the general rule, however. Parvalbumin, which is present in approximately 10% of neocortical neurons (21), showed significantly higher labeling indices in many tuberous sclerosis-like malformations and tyrosine hydroxylase was much more frequent in DNT than it is in the cerebral cortex.

On average, the non-tubereous sclerosis-like hamartias contained fewer immunoreactive neurons than the other entities. Most likely this reflects the fact that these lesions are small and often only a few lesional neurons are represented on each section (32).

Many neurochemical markers characterize specific neuronal subpopulations of the normal neocortex. For example, glutamate decarboxylase labels aspiny nonpyramidal cells (11, 22, 36), parvalbumin is a marker for smooth stellate cells, large basket cells, and chandelier cells, and calcineurin is mainly localized in bipolar GABAergic interneurons of the deeper cortical layers (20, 21). The disfigurement of abnormal ganglion cells in glioneuronal lesions and their seemingly haphazard placement within the lesional matrix precludes a classification of these neurons according to the criteria that have been developed for the normal neocortex.

The neoplastic nature of gangliogliomas is generally recognized. In contrast, DNT are considered as neoplasms by some (2) and as malformative lesions by others (37). In all entities examined in this study, there was a marked heterogeneity of neuronal labeling within individual lesions. A similar degree of heterogeneity with respect to immunoreactivity for neuroendocrine markers has previously been noted by Takahashi et al (38) in a series of five ganglion cell tumors. This suggests that the neuronal component does not represent the result of a clonal proliferation as would be expected in a truly neoplastic process but rather indicates a malformative nature of the ganglion cell populations. This conclusion is consistent with the absence of notable proliferative activity in the neuronal component of gangliogliomas (33). The fact that there was no decrease of neuronal labeling in gangliogliomas of intermediate and high histopathological grades (WHO grades II and III) as compared to low grade gangliogliomas (WHO grade I) indicates that these ganglion cells maintain their level of differentiation and probably do not participate in the malignant transformation of these tumors.

Neuronal immunoreactivity for somatostatin (38, 39) and tyrosine hydroxylase has previously been shown in a small number of ganglion cell tumors (38, 40–42). The presence of tyrosine hydroxylase immunoreactive neurons and dense-core granules in ganglion cell tumors of the CNS has been suggested to indicate a possible derivation from autonomic nervous tissue (38, 40, 43). However, tyrosine hydroxylase also occurs in a small fraction of neocortical neurons (35). In addition, many ganglio-
gliomas express the $\alpha_1$ subunit of the GABA$_A$ receptor and NR1, which are typical for the CNS. Thus, our results suggest that these neurons are derived from the central rather than the autonomic nervous system.

Epileptic seizures are a common manifestation of tuberous sclerosis, and cortical lesions with giant dysplastic neurons and glial cells are the well-established structural correlates in these patients (44). In the present study, ten patients had cortical lesions with the histopathological features of cortical tubers. However, only one of these patients presented with additional extracerebral manifestations of this phacomatosis. It is unknown if the isolated cerebral changes represent a *forme fruste* of tuberous sclerosis (44–46) or if they are pathogenetically unrelated. A recent review has placed these lesions in the spectrum of cortical dysplasia (47). Giant bizarre balloon cells with multiple nuclei and abundant homogeneous and weakly eosinophilic cytoplasm devoid of Nissl substance are typical for tuberous sclerosis-like malformations. The question of whether these balloon cells are neuronal or glial in nature has been controversial (48–50). Co-localization of GFAP and synaptophysin has been demonstrated in some balloon cells of tuberous sclerosis-like lesions from patients with chronic epilepsies (46, 51). Seventy percent of the tuberous sclerosis-like lesions in the present study were negative for all of the neuronal markers examined. However, 30% of the cases contained balloon cells that were immunoreactive for NR1 and glutamate decarboxylase. This supports the idea that at least some of the tuberous sclerosis cells are of neuronal origin.
neuroepithelial tumor. A parvalbumin immunoreactive ganglion cell is located in a loosely fibrillar tumor matrix (Nomarski differential interference contrast, ×300). F: Non-tuberoous sclerosis-like glioneuronal hamartia with scattered NR1 immunoreactive neurons. Note that the oligodendroglia-like component is not immunoreactive (×300). G: Tuberoous sclerosis-like lesion. There are large dysplastic neurons with numerous perinuclear vacuoles and small puncta immunoreactive for glutamate decarboxylase. These puncta indicate axon terminals of GABAergic neurons. In addition, there is a small neuron (arrow) with striking cytoplasmic immunoreactivity for glutamate decarboxylase (×300). H: Tuberoous sclerosis-like lesion. Three balloon cells show cytoplasmic staining for NR1. This suggests that these cells are of neuronal differentiation (×480).

Oligodendroglia-like cells occur in DNT and non-tuberoous sclerosis-like hamartias. A recent ultrastructural and immunohistochemical examination of DNT has shown evidence for glial or neuronal differentiation in some and a complete lack of neuronal or glial markers in other subsets of these cells (37). An ultrastructural characterization of oligodendroglia-like cells in glioneuronal hamartias has not yet been performed due to the very small size of these lesions. The lack of any immunoreactivity for neuronal markers in the present study and the absence of neuron-specific enolase and synaptophysin in these cells (52) suggest that the oligodendroglia-like cells in hamartias and DNT represent non-neural cells.

In humans, the pathogenesis of focal epileptic seizures is not well understood. There are two principal routes by which focal lesions may induce seizures. First, any lesion may disrupt the normal architecture and conduction pathways of the brain and generate seizures through deafferentation of circumscribed cortical areas or through their direct physical effects such as stretching of cortical tissue, reduction of the extracellular space, altered ion homeostasis, or inefficient clearing of released neurotransmitters which may then result in epileptogenic activity through increased excitation, disinhibition or hypersynchrony of neuronal discharges (for review see 53–55). This is certainly the most likely scenario in patients with electrically and neurochemically inert focal lesions such as vascular malformations, gliomas, abscesses, or post-traumatic scars (for review see 53–56). In the case of glioneuronal tumors and malformations a second possibility exists. It
is conceivable that the population of highly differentiated ganglion cells of these lesions actively participates in the generation of seizures in addition to the nonspecific effects described above. This could be accomplished through the release of neurotransmitters or neuromodulators into the adjacent brain tissue or through the electrical activity of their neuronal population. The very high incidence of otherwise unusual glioneuronal lesions in patients with chronic focal epilepsies and the presence of neurotransmitter-producing enzymes, neurotransmitter receptors, neuropeptides, and calcium-binding proteins in many of these lesions suggests that the neuronal population of these lesions plays an active role in the pathogenesis of epileptic seizures. The fact that six of the 60 gangliogliomas in the present series were not associated with chronic epilepsies demonstrates that epileptogenesis is a complex process which depends on a variety of parameters such as the size and location of a structural lesion in addition to its cellular and neurochemical composition.

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Received March 6, 1995
Revision received May 9, 1995
Accepted May 10, 1995