Gliarial Cell Nuclear Hypertrophy in Complex Partial Seizures


Abstract. The white matter of resected temporal lobes from patients with intractable complex partial seizures shows increased cellularity which appears to be related to glia and neurons. This study, using quantitative methods, defines an increase in glial cell numbers and a significant increase in glial nuclear size within a defined area of white matter in the lateral temporal lobe. Evaluation was made on specimens from 10 patients with complex partial seizures compared with two patients with non-epileptic brain lesions and five autopsy patients with no neurologic disease. The importance of recognizing these alterations in glias and the possible relevance to the pathogenesis of epilepsy are discussed.

Key Words: Astrocyte; Gliarial hypertrophy; Lobectomy; Seizure pathology.

INTRODUCTION

Intractable complex partial seizures originating in the medial temporal lobes have been found at surgery to be associated with one of two major medial temporal lobe abnormalities, Ammon's horn sclerosis or gangliogliomas (1, 2). A few patients have nonspecific changes (e.g. end folium sclerosis). The puzzle of the pathogenesis of a single clinical seizure disorder by at least two seemingly opposite disease processes, one degenerative and the other proliferative, prompted us to compare all of the resected tissue from the medial and lateral temporal lobe tissues resected from patients with Ammon's horn sclerosis, patients with gangliogliomas, and patients with nonspecific changes. We found that all patients had similar microscopic structural abnormalities: increased cellularity of the white matter, heterotopic grey matter, increased numbers of neurons within the white matter of the lateral temporal lobe, foci of disordered layering of lateral temporal cortex, and malaligned granule cells in the dentate gyrus (4). Not every abnormality was present in every case, but they were observed more often in the brains from the seizure patients than in the brains from autopsy or surgical non-seizure controls. Many of these microscopic structural abnormalities have been recorded by others (5-8).

The purpose of this study was to study quantitatively the first of these microscopic changes, i.e. increased cellularity of the white matter. This change was always present in the epilepsy cases. The numbers of glial cells and the size of their nuclei which were present in a defined area of the white matter of the lateral temporal lobes of epileptic patients with Ammon's horn sclerosis, gangliomas, and nonspecific changes were evaluated and compared with similar tissue from non-epileptic patients studied in the same way.

MATERIALS AND METHODS

The resected lateral temporal lobes from 10 patients with intractable complex partial seizures, unresponsive to medical therapy, were examined. The ages of the patients ranged from 8 to 54 years (average 32.2 years). These patients were representative of patients with a diagnosis of Ammon's horn sclerosis, ganglioglioma and nonspecific changes. Additional details concerning these patients are given in Table 1. Control tissue was studied from autopsy tissue and from neurosurgical tissue resected from non-epileptic patients. The ages of the control patients ranged from 10 to 52 years (average age 28.9 years). Details concerning the control patients are given in Table 2.

The preparation of resected temporal lobes at our institution has been published (3). All of the tissue was examined. The lateral temporal lobe resection (the largest measuring in the range of 5 x 4 x 3 cm) was drawn or photographed and fixed in 10% formalin or 4% paraformaldehyde for 1-3 days. It was then divided into sequential blocks and labeled serially from anterior to posterior. There were as many as seven blocks of lateral temporal lobe from each patient. (In the cases studied, the numbers of blocks ranged from four to seven. The surgical control cases had much less tissue available.) A slide from each block was studied quantitatively in the following way. A selected area of white matter in the lateral temporal lobe, one high power field away from the cortical-white matter junction at the crest of the first gyrus, was photographed at 400X and prints were made after enlarging them five times.

The photograph of the selected subcortical white matter in each of the sections from the lateral temporal cortex for each case and control was analyzed using a Sigma Scan Scientific measurement program which employs a personal computer and digitizing tablet (Fandel Scientfic reel digitizer with pen body [digital paintbrush]) to make two dimensional measurements. The number of glial cell nuclei in each photograph was counted. The area of each nucleus within the white matter was measured in square microns, excluding those nuclei which were incomplete at the margin of the photographs. The nuclei identified as belonging to endothelial cells were not measured. The numbers...
of neurons were tabulated separately. Figure 1 is an example of a photograph of one area measured at one section from the lateral temporal cortex of a patient with intractable epilepsy compared with a similar (Fig. 2) area from a non-epileptic control patient. The average diameter of the glial cells was calculated for each photograph, and the average diameter for each case was plotted for each patient for each block (see Table 3).

The numbers and the sizes of glial cell nuclei in the areas of white matter studied from the patients with complex partial seizures were compared with the numbers and sizes of glial cell nuclei in control non-epileptic white matter. The results were submitted to Student's t-test. Representative slides from the lateral temporal cortex of patients with complex partial seizures and a diagnosis of Ammon's horn sclerosis, ganglioglioma, or nonspecific changes, and which did not contain any focal lesions, and a comparable slide from a control brain were stained using the peroxidase-anti-peroxidase methods with anti-glial fibillary acidic protein (GFAP) (DAKO Corporation, Carpinteria, CA), anti-myelin basic protein, anti-vimentin, anti-synaptophysin, and anti-galactocerebrosides (all from Boehringer Mannheim, Indianapolis, IN).

RESULTS

It was usually not possible, either on the microscopic slides or in the photographs, to positively identify the nuclei in the white matter as being astrocytic or oligodendrogial (see Figs. 1, 2). The astrocytes had no identifiable cytoplasm on the hematoxylin and eosin stain and were not typical of the "reactive astrocyte." However, in the epilepsy cases GFAP staining did reveal many of the cells as having positive-staining stellate cytoplasm and fibers, in the pattern of the reactive astrocyte. In the control cases there was almost no staining of the white matter with GFAP. Vimentin was negative in the patients and in the controls. Anti-myelin basic protein and anti-galactocerebrosides were positive in the epilepsy cases and in the control cases. Both of these reactions gave a diffuse pattern of staining so that it was not possible to characterize individual cells. Anti-synaptophysin did not stain the white matter.

The number of glial cells in the small area of brain selected for study was increased. The average number of glial cell nuclei in the area of the photograph in the control cases was 50, with a range of 39–61. The average number of glial nuclei in the area of the photograph of the epilepsy cases was 63, with a range of 50–98 nuclei. The differences between these numbers are not greatly significant.

The sizes of the glial cell nuclear area in each section were significantly increased in the white matter of the patients with epilepsy compared with those of the controls (p ≥ 0.002). These differences are demonstrated in the graph (Fig. 3) depicting the average glial cell nuclear area for all glial cells in each of the sections in each of the consecutive blocks available for the patients and the control tissues.

DISCUSSION

This quantitative evaluation of the apparent increase in the cellularity of the white matter in the temporal lobes of the epileptic patients defined an alteration in the glia which we consider to be a significant finding in our evaluation of tissue changes in epilepsy. The numbers of glial cells were not increased significantly, but there was a significant change in the size of glial nuclei. Many of these cells had prominent GFAP-positive fibers and were reactive astrocytes. The "control" brain is not normal brain but is not associated with a seizure disorder, being chosen from autopsy cases of non-neurologic conditions and from surgical tissues adjacent to lesions in non-epileptic disease. Thus, the concern that there may be artifactual swelling and shrinkage of the nuclei in the autopsy controls was controlled for in this study because the glia in the surgical controls, which had been fixed the same as the epilepsy cases, had nuclear areas similar to the autopsy control cases. We therefore consider that enlargement of the glial nuclei in epilepsy is indicative of a form of gliosis which is an intrinsic change in epileptic brain.

The definition of this gliosis in the white matter of epileptic brain requires some consideration. In normal white matter there are the traditionally named "fibrous astrocytes," possibly type 2 astrocytes (9), which accompany axons and blood vessels and form a loose meshwork which is poorly defined by the stains and reactions which
Fig. 1. Photograph of one section from the lateral temporal lobe resection from a patient with intractable epilepsy illustrating the appearance of the glial cells in the selected area of white matter.

Fig. 2. Photograph of one section from the lateral temporal lobe from a control patient illustrating the appearance of the glial cells in the selected area of white matter.
GLIAL CELL NUCLEAR HYPERTROPHY

TABLE 3
Range of Glial Cell Nuclear Areas in Each Case
(in micron squared)

<table>
<thead>
<tr>
<th>Controls</th>
<th>Epilepsy cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 47–59</td>
<td>1. 70–108</td>
</tr>
<tr>
<td>2. 48–70</td>
<td>2. 88–106</td>
</tr>
<tr>
<td>3. 48–73</td>
<td>3. 77–88</td>
</tr>
<tr>
<td>4. 45–54</td>
<td>4. 55–80</td>
</tr>
<tr>
<td>5. 44–57</td>
<td>5. 71–80</td>
</tr>
<tr>
<td>6. 58–66</td>
<td>6. 74–92</td>
</tr>
<tr>
<td>7. 36–43</td>
<td>7. 80–106</td>
</tr>
<tr>
<td></td>
<td>8. 64–77</td>
</tr>
<tr>
<td></td>
<td>9. 55–101</td>
</tr>
<tr>
<td></td>
<td>10. 64–100</td>
</tr>
</tbody>
</table>

Each case had 4–7 photographs of white matter analyzed. Each photograph of control cases contained an average of 50 nuclei (39–61). Each photograph of the epilepsy cases contained an average of 63 cells (50–98).

identify glial cell fibers in "reactive astrocytes": the Holzer, PTAH and anti-GFAP preparations. The astrocytes’ responses to injury have been characterized as degenerative, hyperplastic and hypertrophic (10). In the epileptic brain we have observed both hyperplasia and hypertrophy of glial cells. The increase in the numbers of glial cells (hyperplasia) at the site in the brain which was chosen for our quantitative studies (chosen because it represented a consistent site at which we could make comparable samples) was not significant. However, it has been our impression that the deeper white matter is more cellular than our selected gyril white matter. Indeed, in some cases the cellularity is so obviously increased that it resembles a neoplasm. We consider that diagnosis of low grade astrocytoma should be excluded if there is a history of longstanding epilepsy and if there is no mass lesion or area of localized enhancement on imaging studies.

Our study did define a very significant hypertrophy in the size of the glial nuclei. Isolated nuclear alteration is seen in astrocytes in metabolic disorders and in the developing brains of infants. Metabolic factors are undoubtedly important in relationship to the altered nuclear size in epilepsy. We were unable to stain these cells with vimentin, so that the glia probably not actively proliferating or immature astrocytes (10). Some of these enlarged glial nuclei may be oligodendroglial cell nuclei. They do not all stain with GFAP, and the anti-galactocerebroside defines some of them to be oligodendrocytes. Studies are underway with various glial cell markers in an effort to better characterize these cells.

Metabolic factors involved in nuclear hypertrophy are numerous and may be present in an epileptic brain (11). In experimental neuronal hyperactivity, glial cells remove K from extracellular space and become swollen (12). Glotzner (13) observed increased glial potassium trans-

port and glial hypertrophy in aluminum hydroxide-induced epileptic foci. There is controversy concerning the competence of glial cells as spatial buffers in epilepsy (14–16); however, there are other explanations for glial nuclear hypertrophy. In culture systems, astrocyte morphology is controlled by beta adrenergic receptors and astrocytes become hypertrophic when exposed to isoproterenol (17). In epilepsy there is an increase in brain catechols (18, 19) near regions of spiking foci and a possible source of catechols in aberrant tyrosine hydroxylase-immunoreactive neurons of the hippocampus and subiculum (20). Eng (10) has postulated that neuronal death can stimulate astrocytes to hypertrophy and in Ammon’s horn sclerosis there is ongoing neuronal loss (21).

The changes in astrocyte morphology have been studied in several animal models of epilepsy. Munoz et al (22) did not define any changes in the density of cell bodies of fibrous astrocytes or their processes in several areas of brain from an epileptic chicken. Their study was performed using immunocytochemistry and the nuclear size was not commented upon. However, enlarged astrocytes have been observed in brains of rats that had seizures induced by implantation of iron chloride salts into one hippocampus (23). In these animals the cortical glial cells on the opposite side of the brain showed a significant cellular enlargement, suggesting, as in our cases, that epilepsy may induce changes in astrocytes remote from the origin of the seizures.

The astrocyte in the epileptic brain may be altered in
both appearance and function. In the normal brain the astrocyte, in addition to its structural role, acts as an ion buffer, regulates neurotransmitters and growth factors, processes antigens, and may influence the caliber of vessels (24–29).

In epilepsy, some of the characteristics of normal astrocytes have been observed to be altered or expressed in ways that could promote epileptic activity of neurons. For example Na+ and K+-ATPase have been shown to be less active in the epileptic brain than in normal brain (30, 31). The activity of glutamine synthetase is decreased in models of chronic epilepsy (32, 33). There are decreased numbers of benzodiazepine receptors on the astrocytes cultured from genetically epilepsy-prone rats (34). The astrocyte is a source of neuronal growth factor (35) which may induce the sprouting of hippocampal mossy fibers forming aberrant, and possibly epileptogenic, synapses on granule cells (36). Thus, it is possible to involve “gliosis” in the pathogenesis of epilepsy. The mechanisms could involve failed spatial monitoring of K+, failure of availability of glutamine for the production of GABA (because of altered GABA uptake and deficient glutamine synthetase in astrocytes), or the evolution of epileptogenic synapses. On the other hand, as mentioned above, there is evidence that astrocytes may function better than normal in terms of their special buffering activity (13).

This study of glial cells in the white matter adjacent to focal epileptic lesions in patients emphasizes important questions which epileptologists ask about the role of gliosis in epileptic foci. Is gliosis a useful response to the elevation of K ion in the extracellular space near seizure-nergic neurons, or does it signify functional impairment which contributes to the epileptogenesis? These questions also concern the neuropathologist as we attempt to understand the stimuli involved in gliosis and the functional connotations of subtle morphologic changes.

ACKNOWLEDGMENTS

The authors acknowledge Dr. David Shine who allowed us to use his quantitating programs and analysis systems, Dr. Gregory Buffone who performed statistical analyses, Laurie Lees who prepared the photographs for analysis, Barbara Antalifly who did the histology preparations, and Sally Wood who prepared the manuscript.

REFERENCES

28. Tower DB. Development of knowledge about astrocytes since Virchow. In: Norenberg MD, Hertz L, Schousboe A, eds. The bio-


Downloaded from http://jneuro.pubs.oxfordjournals.org/ by guest on October 8, 2016
34. Dusis I, Norenenberg LOB, Norenenberg MD. The benzodiazepine receptor in cultured astrocytes from genetic epilepsy-prone rats. Brain Res 1990;531:318–21

Received December 3, 1993
Revision received April 12, 1994
Accepted April 13, 1994