Mesial temporal lobe epilepsy: a proton magnetic resonance spectroscopy study and a histopathological analysis

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Object. Proton magnetic resonance (MR) spectroscopy imaging of the ratio of N-acetylaspartate (NAA) to creatine (Cr) has proved efficacious as a localizing tool in demonstrating the metabolic changes associated with temporal lobe epilepsy. To analyze the significance of these MR spectroscopy findings further, the authors explored the relationship between regional alterations in the NAA/Cr ratio in hippocampi measured preoperatively and histopathological findings in hippocampi resected in patients with intractable mesial temporal lobe epilepsy (MTLE).

Methods. Twelve patients in whom the diagnosis of MTLE had been made and 12 healthy volunteers with no known history of neurological disease underwent high-resolution 1H MR spectroscopy imaging of NAA and Cr (0.64 cm3 nominal voxel resolution) in five voxels spanning the anteroposterior length of the hippocampus. The authors correlated the NAA/Cr ratio with neuropathological findings in resected hippocampi, specifically glial fibrillary acidic protein (GFAP) immunoreactivity and pyramidal neuronal loss. A linear regression analysis of the ipsilateral NAA/Cr ratio revealed a statistically significant relation to the extent of hippocampal neuronal loss in only the CA2 sector (correlation coefficient [r] = −0.66, p < 0.03). The ipsilateral NAA/Cr ratio displayed significant regressions with GFAP immunoreactivity from all the CA sectors (r values ranged from −0.69 and p < 0.01 for the CA4 sector to −0.88 and p < 0.001 for the CA2 sector) except for the CA1. The extent of neuronal cell loss in every hippocampal subfield (r = 0.71−0.74, p < 0.007), except the CA2 (p = 0.08), correlated to the extent of neuronal cell loss in the dentate gyrus. There was no significant relationship between the duration or frequency of seizures and the mean ipsilateral NAA/Cr ratio; however, the mean density of GFAP-immunopositive cells correlated with seizure frequency (p < 0.03).

Conclusions. The NAA/Cr ratio may not measure the full extent of hippocampal neuronal cell loss. The significant association of the NAA/Cr ratio with the GFAP immunoreactivity of most CA sectors indicates that the NAA/Cr ratio may provide a more accurate measurement of recent neuronal injury caused by epileptic activity. The coupling between neuronal impairment and astroglial GFAP expression may indicate the close association between neuronal and glial dysfunction in patients with epilepsy.

Key Words • magnetic resonance spectroscopy • glial fibrillary acidic protein • epilepsy • N-acetylaspartate • hippocampal sclerosis

In the past decade, high-resolution 1H MR spectroscopy has proved efficacious in lateralizing neurochemical abnormalities in the temporal lobes of candidates for epilepsy surgery.8,10–14,17,18,20,21,26,28,31,35–37,39,42–44,46,47,50,53,55,59,61 This imaging modality allows the in vivo measurement of brain metabolites including NAA and total Cr. The use of the NAA/Cr ratio as a measure of both the bioenergetic status of the hippocampus and the extent of neuronal injury has arisen from the observation that decreases in NAA can occur with the dysfunction of neuronal mitochondria32 and with neuronal loss,39 whereas Cr is found in high concentrations in glia.62,63 Conversely, the reversibility of contralateral hippocampal deficits in the NAA/Cr ratio after resection of isilateral epileptogenic medial temporal lobe structures30,36,66 has demonstrated that a reduction in the NAA/Cr ratio is not due to neuronal loss alone.

An examination of NAA content may provide a non-invasive and well-localized evaluation of neuronal function. Given the potential role of bioenergetic insufficiency in modulating seizure activity, it is important to understand the biological basis underlying these MR spectroscopy findings. In this study, we will discuss correlations between the histopathology of MTLE and noninvasive evaluations of the NAA/Cr ratio performed using MR spectroscopy. A better understanding of the clinical correlates of abnormal findings on MR spectroscopy studies may increase the application of this imaging modality in the preoperative examination of candidates for epilepsy surgery and enhance our understanding of underlying mechanisms and networks in epileptogenesis.

Clinical Material and Methods

Patient Population

Twelve patients in whom the diagnosis of unilateral MTLE had been made who underwent preoperative exami-
nation before anteromedial temporal lobectomy and amygdalohippocampectomy were studied. The clinical characteristics and preoperative findings in these patients are summarized in Table 1. There were five men and seven women in the study with a mean age of 39.12 years (mean ± standard deviation). The mean duration of epilepsy in these patients was 28.10 years, and the mean number of seizures per month was four. All patients underwent comprehensive preoperative examinations; the only sign of a structural abnormality was hippocampal atrophy, which appeared on preoperative MR images in 11 patients. One patient in whom there was no finding of this abnormality on MR images underwent an intracranial study performed using strips, grid, and depth electrodes. Her seizures were localized to one hippocampus. In nine patients there was a history of febrile seizures before the age of 2 years, in one patient a history of encephalitis at the age of 2 years, and in the last two patients there were no identifiable epilepsy risk factors. All patients had experienced at least one episode of secondary generalization of their seizures. None of these patients had undergone intracranial surgery before undergoing MR spectroscopy. All but two patients were free from seizures after they underwent anteromedial temporal lobectomy and amygdalohippocampectomy. Twelve healthy volunteers in a similar age range (six men and six women with a mean age of 32 ± 10 years) who had no history of neurological disorders were included in this study. The Institutional Review Board approved the study and all patients consented to their participation.

Methods of 1H MR Spectroscopy

Spectroscopy data were acquired along the planum temporale (10-mm thickness) with the aid of a Varian Inova 4-tesla whole-body MR system. To exclude large lipid resonances from extracranial fat, the acquired volume was restricted to an 80 × 100-mm rectangle within the plane by using adiabatic refocusing pulses. The data were further localized by applying 24phase encodes over a field of view of 192 × 192 mm, resulting in a nominal voxel measuring 0.64 cm³. Water suppression was achieved using a broadband semiselective excitation pulse in conjunction with a delays-alternating-with-nutations-for-tailored-excitation suppression pulse. Relying on anatomical images as a reference, we selected voxels spanning both hippocampi in the posteroanterior direction. The hippocampal pixels lateral to the aqueduct were initially designated Location 3; two additional locations were chosen posteriorly (Locations 1 and 2) and two anteriorly (Locations 4 and 5) to provide a consistent regional assessment (Figs. 1 and 2). The spectra

<table>
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<tr>
<th>Case No.</th>
<th>Age (yrs), Sex</th>
<th>Epilepsy Risk Factors</th>
<th>Epilepsy Duration (yrs)</th>
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<th>Origin of Seizures Based on Imaging Studies</th>
<th>Pathological Findings</th>
<th>Outcome†</th>
<th>Follow Up (mos)</th>
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<td>FS</td>
<td>23</td>
<td>1</td>
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<td>rt temporal</td>
<td>NA</td>
<td>not localized</td>
<td>1A 12.0</td>
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* EEG = electroencephalography; Enceph = encephalitis; FS = febrile seizure; HS = hippocampal sclerosis; NA = not performed/not available; PET = positron emission tomography; SPECT = single-photon emission computerized tomography; UK = unknown.
† Based on Engel’s classification of seizure outcome: 1A = free from seizures and auras; 3A = worthwhile seizure reduction; 4A = no appreciable change.
from these locations were then fit into the spectral domain and the NAA/Cr ratio was determined by dividing the resonance areas.

**Surgical Technique**

All patients underwent a standard anteromedial temporal lobectomy as described previously. Briefly, a frontotemporal craniotomy was performed in the standard fashion and the lateral temporal neocortex was exposed. The superior temporal gyrus was spared and 3 to 3.5 cm of the middle and inferior temporal gyri, as measured from the temporal pole, were resected to expose the temporal horn of the lateral ventricle. The exposure of the hippocampus was then extended by dissection of the occipitotemporal fasciculus and gentle lateral retraction of the temporal neocortex. The amygdala, hippocampus, and parahippocampal gyrus were removed en bloc. The resection of the hippocampus was terminated at the point at which the structure curved around the brainstem.

**Neuropathological Methods**

We selected a coronal section of the midbody of the hippocampus for histopathological morphometry. The tissue was fixed in formalin overnight at room temperature, after which it was dehydrated with graded alcohol, impregnated with xylene, and embedded in paraffin. To count the neurons, 5-μm-thick deparaffinized sections were stained with hematoxylin and eosin, Luxol fast blue, and/or Nissl stain. The neurons were counted in a manner previously described, after which the total numbers of neurons were adjusted using the Abercrombie formula with a value of 12.2 μm for the nuclear diameter of pyramidal neurons and a value of 9.2 μm for that of granular neurons. Reference counts were obtained from an autopsy control population consisting of 26 cadaveric specimens.

An immunohistochemical analysis of 5-μm-thick tissue sections was performed in a moisture chamber. The deparaffinized sections were treated with 3% H₂O₂ for 3 minutes and covered with 2% normal goat serum for 30 minutes after they had been washed with TBS. The sections were incubated with mouse anti-GFAP antibody (dilution 1:1000, Dako Corp., Carpinteria, CA) at 4°C overnight, rinsed with TBS, and incubated with biotinylated anti-mouse immunoglobulin G antibody for 1 hour. The tissue sections were again rinsed with TBS, followed by incubation with streptavidin–peroxidase complex for 1 hour. The immunoreactivity of the tissue was evaluated after treatment with diaminobenzidine, a substrate.

To count the total number of GFAP-immunopositive glia, multiple thin parallel lines were drawn perpendicular to the horizontal axis along the floor of the temporal horn of the lateral ventricle. The interval between two adjacent parallel lines was 1250 μm and the lines covered the entire coronal section of the hippocampus. The number of GFAP-immunopositive glia within a 100 × 200–250 μm unit area were counted using an ocular grid that was calibrated with a stage micrometer. The unit area was consecutively moved along the perpendicular line so that we could count the number of glia in each unit area. The mean number of glia per unit area was adjusted by applying the Abercrombie formula. The final cell density was expressed as the mean number of glia per cubic millimeter. The GFAP antibody staining detects the intermediate filament expressed in reactive astrocytes and may therefore measure the astrocytes’ reaction to pathological conditions of neurons associated with epilepsy (Fig. 3).

**Statistical Analysis**

We analyzed NAA/Cr ratios from patients and healthy volunteers by performing an analysis of variance to determine the presence of significant differences between the two groups. This method was also used to detect regional variations in NAA/Cr ratios along the hippocampus within each group. The neuropathological and MR spectroscopy data were compared using a linear regression analysis. We did not correct the significance level for multiple comparisons.

We investigated the relationships between the data on the ipsilateral hippocampal NAA/Cr ratio and the patient age, duration of epilepsy, and seizure frequency by using a Spearman rank correlation.

FIG. 2. Axial MR image demonstrating Locations 1 to 5 (in ascending order from the bottom of the figure) along the long axis of the hippocampus and the corresponding patient’s spectra.

FIG. 3. Coronal section of the midbody of the hippocampus demonstrating a dark discoloration due to GFAP immunopositivity.
Results

Relationships Between Clinical Factors and the 'H MR Spectroscopy and Histopathological Data

There was no significant association between the duration or frequency of seizures and the mean neuronal loss or the mean ipsilateral NAA/Cr ratio. The mean density of GFAP-positive cells in the hippocampus correlated with the frequency of patients' seizures (p < 0.03) but not the duration of their epilepsy. Two patients continued to have seizures after they had undergone anteromedial temporal lobectomy. On the MR spectroscopy images obtained in one of these patients there was evidence of a bilateral reduction of the hippocampal NAA/Cr ratio. In the other patient the lateral NAA/Cr ratio correlated with the extent of hippocampal neuronal loss only in the CA2 subfield (r = −0.66, p < 0.03) (Fig. 5).

Discussion

Mesial temporal lobe epilepsy is often associated with various degrees of neuronal loss and gliosis in the dentate gyrus, hippocampal formation (Ammon horn), and subiculum. Noninvasive in vivo evaluation of the metabolites and histological alterations involved in the pathophysiology of MTLE in humans may provide insights into the mechanisms of epileptogenesis. Proton MR spectroscopy imaging provides region-specific maps of the chemical content of the brain. N-acetylaspartate is represented by the dominant peak 'H MR spectra recorded in the normal adult brain. The inclusion of these potentially epileptogenic parahippocampal structures may affect the MR spectroscopy measurements but not the GFAP immunoreactivity measurements. Nevertheless, the linear regression relationship between the measurements of GFAP immunoreactivity and MR spectra most likely remains the same. The high-spatial-resolution spectroscopy imaging

<table>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
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<tr>
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<td>1.16 ± 0.29</td>
<td>0.93 ± 0.21</td>
<td>0.87 ± 0.13</td>
<td>0.90 ± 0.12</td>
<td>0.94 ± 0.25</td>
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<tr>
<td>contralat</td>
<td>1.22 ± 0.24</td>
<td>1.03 ± 0.17</td>
<td>1.17 ± 0.27</td>
<td>1.02 ± 0.23</td>
<td>1.08 ± 0.28</td>
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<tr>
<td>healthy volunteers</td>
<td>1.57 ± 0.29</td>
<td>1.38 ± 0.14</td>
<td>1.24 ± 0.17</td>
<td>1.36 ± 0.20</td>
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Sources of Error

Before proceeding to the discussion of neuropathological and imaging data, we will briefly describe the potential sources of error in this work. Magnetic resonance spectroscopy measurements are influenced by the inclusion of adjacent medial temporal structures including the entorhinal cortex and subiculum. The inclusion of these potentially epileptogenic parahippocampal structures may affect the MR spectroscopy measurements but not the GFAP immunoreactivity measurements. Nevertheless, the linear regression relationship between the measurements of GFAP immunoreactivity and MR spectra most likely remains the same. The high-spatial-resolution spectroscopy imaging
used in this study (0.64-cm³ voxels), in comparison with more conventional single-voxel studies (4–8 cm³), is critical in minimizing partial volume effects. Pathologically, absolute counts of neurons and glia may be misleading because of changes in reference volume caused by tissue shrinkage, swelling, and surgical manipulation. Thus, the measurements of neurons in this study were made as fractions, and all data (numbers of neurons and GFAP immunoreactivity) were compared with those of reference tissues from nonepilepsy autopsy controls. The use of monoclonal mouse antibodies to measure GFAP immunoreactivity allows a better recognition of cell bodies against the background glial processes, especially in the CA1 sector.

Regional Heterogeneity in the Ipsilateral and Contralateral Hippocampi Revealed on ¹H MR Spectroscopy

The bilateral reduction of the NAA/Cr ratio demonstrates the prevalence of bilateral metabolic changes in patients with presumably unilateral temporal lobe epilepsy. The anterior–posterior gradient of proton metabolites along the long axis of the hippocampus is consistent with the work of Vermathen and colleagues. This gradient may be accounted for by the marked neuronal injury and bioenergetic deficiency reported in the anterior part of the hippocampus compared with that in posterior regions.

Histopathological and Clinical Data Analysis

The proposed basic network operating in the hippocampus is a trisynaptic circuit (entorhinal cortex → dentate gyrus → CA3 sector → CA1 sector). This circuitry may explain the marked neuronal dropout and gliosis observed in the CA1 and CA3 sectors and in the granular layer of the dentate gyrus in patients with hippocampal sclerosis. Although the association between early-life insults such as febrile seizures and hippocampal damage remains controversial, the CA3 and CA1 sectors and the dentate gyrus may be most severely damaged, with partial sparing of the CA2 subfield early in the pathogenesis of epilepsy. Our neuropathological data on neuronal loss revealed a significant relationship between the dentate gyrus and all sectors except the CA2. This observation is consistent with the trisynaptic pathway and demonstrates the consistency of the present patient group.
Histopathological Findings in Relation to the Preoperative 1H MR Spectral Data

If the changes in GFAP immunoreactivity represent recent neuronal hyperactivity and injury rather than neuronal dropout, the strong relationships between the NAA/Cr ratio and GFAP immunoreactivity in three of the four hippocampal subfields indicates that the NAA/Cr ratio can be used as a noninvasive evaluation of recent seizure activity. Given the potential role of NAA in the evaluation of the bioenergetic status of neurons, GFAP immunoreactivity may be related to neuronal energetic dysfunction—that is, this metabolic aberrancy may be specific to neuronal hyperactivity and injury. Taking this view to a practical level, the relationship between the NAA/Cr ratio and GFAP immunoreactivity is also consistent with the finding of the postoperative reversibility of contralateral hippocampal abnormalities in the NAA/Cr ratio among patients who become free from seizures after surgery.10,38,56,66 Once the ipsilateral focus is successfully resected, regions in the propagation pathway may be relieved of epileptic activity, allowing the abnormalities in GFAP immunoreactivity and the NAA/Cr ratio to resolve.

If the NAA/Cr ratio reflects the expression of GFAP and, ultimately, neuronal impairment, a significant correlation between the NAA/Cr ratio and the extent of neuronal loss in only the CA2 sector is not surprising. This observation is consistent with the hypothesis that the CA2 sector may demonstrate maximal sensitivity to additional neuronal injury. The relationship may support the presence of ongoing neuronal injury in the CA2 sector, which may be due to mitochondrial damage and which results ultimately in the loss of neurons.

Given the interrelationships between astrocytes and neurons in neurotransmission and energy metabolism, our observed correlation between astrocytic GFAP expression and the NAA/Cr ratio as a measure of neuronal mitochondrial functionality may raise questions regarding the role of energetic or functional sufficiency of either cell type in epilepsy. Neuronal injury may be the initial cause of GFAP expression. This does not, however, preclude the possibility that gliosis can produce pathological effects by interfering with the function of neuronal networks or by altering the buffering capacity of astrocytes.22,24 In this progressive situation, the degree of neuronal injury or death would also be expected to be more severe. Nonetheless, whether neurons and/or astrocytes are dysfunctional in patients with epilepsy, it is clear that the in vivo metabolic measurements of the NAA/Cr ratio are highly informative in the evaluation of a dynamic pathophysiologic dysfunction associated with epileptic activity.

Conclusions

The reduction in the NAA/Cr ratio and the amplification of GFAP immunoreactivity were correlated with a reduction in neuronal density only in the CA2 sector of the hippocampus. In contrast, decreases in the NAA/Cr ratio were linearly correlated with increases in GFAP immunoreactivity across most CA sectors. Therefore, the ratio of NAA to Cr may primarily reflect a recent or ongoing injury to neurons, which stimulates reactive gliosis, as opposed to an initial loss of neurons due to the precipitating injury. These
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findings emphasize the important role of astrocytes in regulating the response of neurons following epileptic activity.

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References


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