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INVESTIGATION OF BRAIN METABOLITES AND CORTICAL GLUCOSE METABOLISM IN HUMAN PARTIAL EPILEPSY

PhD theses

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INTRODUCTION AND STUDY BACKGROUND

Epilepsy is one of the most common, serious neurological conditions and around 50 million people in the world have epilepsy. Imaging plays an important role in the evaluation of patients with epilepsy and seizure disorders which have a cortical origin. Structural and functional studies including magnetic resonance imaging (MRI), proton magnetic resonance

spectroscopy (^{1}H MRS), and 2-deoxy-2[F-18]fluoro-D-glucose positron emission tomography (FDG PET) are used to identify the epileptogenic zone and to confirm the ictal focus based on EEG recordings.

Brain metabolites, neurotransmitters and glucose metabolism in epilepsy

N-acetyl aspartate (NAA), creatine/phosphocreatine (Cr), cholin compounds (Cho) and glutamate/glutamine/γ-aminobutyric acid [GABA] complex (Glx) detected by ^{1}H MRS are the principal signals of interest in epilepsy studies. NAA is the second most abundant amino acid in the human central nervous system, and it appears to exhibit neurotransmitter activity acting on glutamate receptors. NAA is localized in mitochondrium fractions and in cytoplasm of the neuron, and primarily formed in neurons from acetyl-CoA and aspartate. Cr and Cho are found both in neurons and glial cells, but they are present at much higher concentrations in oligodendrocytes and astrocytes than in neurons. Creatine is converted to phosphocreatine through the enzyme creatine kinase. Phosphocreatine is a high-energy phosphate, which is critical for maintaining cellular energy dependent systems. The Cho resonance contains contributions from a number of mobile choline compounds. These membrane-bound compounds are generally not MR-visible; however, in disease processes which result in membrane breakdown the formerly bound cholin is released into free cholin pool and becomes MR-visible.

Glutamate is the main excitatory neurotransmitter in the human brain and is believed to play an important role in the initiation, spread, and maintenance of epileptic activity. Glutamate receptors are located on glial and neuronal cells. Increases in the density of NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid), and kainate receptors, and increased number of glutamate receptor subunits have been described in epilepsy. The functions of glutamate metabotropic and NMDA receptors are modified in epilepsy, and alterations in glutamate transporters also potentially can contribute to epileptogenesis. The role of glutamate in the mechanism of seizure is supported by antiepileptogenic pharmacological compounds which can decrease glutamate release acting on presynaptic terminals, while others,

used in human practice, reduce the extracellular glutamate concentration by sodium channel inactivation. Furthermore, antagonists of NMDA and AMPA receptors are anticonvulsants in animal models of epilepsy. ^{1}H MRS detects mainly the intracellular Glx pool (containing predominantly glutamate and glutamine), and these are present in both neuronal and in glial cells.

FDG PET is used to demonstrate the regional cerebral glucose metabolism and it is often applied for the localization of epileptogenic brain regions. Regional hypometabolism identifies the epileptogenic lobe in about 80-90 % of children with refractory epilepsy who exhibit focal FDG PET abnormalities. In contrast, children with recent-onset epilepsy appear to have less frequent and less profound metabolic abnormalities than those with chronic epilepsy.

Relation between N-acetyl compounds (NA) and glutamate/glutamine metabolism

Similar to NAA, the peptide neurotransmitter N-acetyl aspartyl glutamate (NAAG), a derivative of NAA, is also localized in neurons and synthesized from NAA and glutamate. The recently identified cellular separation of the two anabolic and two catabolic enzymes in the NAA and NAAG cycles points to a three-cell compartmentalization involving neurons (NAA and NAAG synthases), astrocytes (NAAG peptidase), and oligodendrocytes (aspartoacyclase). NAAG, released by neurons, is cleaved at the cell surface of astrocytes into NAA and glutamate. NAA is hydrolyzed in oligodendrocytes, and the end products may serve message to neurons. Glutamate is taken up by astrocytes, and it is recycled to neurons via the glutamate-glutamine conversion.

NAAG may have two major roles following the synaptic release: activation of type 3 metabotropic glutamate receptors, and, based on its rapid turnover, delivery and production of glutamate. NAA also shows neurotransmitter activity; however, the relatively slow rate of NAA turnover suggests that its major role is as substrate for the formation of NAAG. In pathological states, alterations in the levels of NAAG and in the activity of NAAG peptidase have been found decreasing or increasing the availability of NAA and glutamate in brain synapses. Both NAA and NAAG concentrations can be determined by ^{1}H magnetic resonance spectroscopy (^{1}H MRS) *in*

vivo, since the resonance of NA at 2.02 pars per million (ppm) chemical shift value contains overlapping resonances including NAA and NAAG.

Coupling between glutamate and glucose metabolism

A recently articulated hypothesis suggests that the majority of the signal derived from 2-deoxy-2[F-18]fluoro-glucose uptake measured with PET is due to glutamate stimulation of glucose uptake by astrocytes. The hypothesis regarding glutamate and glucose coupling is based upon the observation that glutamate stimulated 2-deoxyglucose uptake and phosphorylation by astrocytes in primary culture. Further data to support possibly this theory are derived from [C-13]MRS studies in which cortical rates of oxidative glucose metabolism and glutamate neurotransmitter cycling were measured in rats under different degrees of anesthesia. An approximate 1:1 stoichiometry was reported between glutamate cycling and glucose metabolism, with glutamatergic synaptic activity accounting for over 80% of total glucose oxidation under conditions of mild anesthesia. All of the data supporting this hypothesis, however, were derived either from cultured astrocytes or anesthetized rodents, and therefore, the applicability of these findings to humans remains to be established.

STUDY GOALS

The goals of my investigations were:

1., to detect the altered signal intensity of brain metabolites in the epileptogenic region in the unstimulated interictal and stimulated ictal/periictal states in human partial epilepsy patients by ^{1}H MRS,

2., to determine the brain glucose utilization in the same cortical regions which were used for ^{1}H MRS studies in the same ictal states using FDG PET,

3., to investigate the relationship between the cortical tissue concentrations of N-acetyl compounds and glutamate/glutamine based on associations between NAAG and glutamate neurotransmission,

4., to test the hypothesis of coupling between glutamate and glucose metabolism in human epileptic and non-epileptic cortex by comparing regional values of glucose metabolism from FDG PET studies with ^{1}H MRS measurements of Glx tissue concentration.

PATIENTS AND METHODS

Subjects

Eleven patients (five females and six males, mean age 7.5 years, age range 0.3-20 years) with medically intractable partial epilepsy were included in the study. As part of the presurgical evaluation, all patients underwent MRI, quantitative single voxel ^{1}H MRS, FDG PET and prolonged video-EEG recordings with scalp and sphenoidal electrodes. Intraoperative electrocorticography was performed in two patients, and three patients underwent chronic intracranial EEG monitoring. Ictal EEG recordings were obtained in all patients. Patients were selected for the study for whom both scans were obtained for the same ictal state (i.e. either both

scans were interictal [n=7] or both scans were ictal/periictal [n=4]) and for whom the antiepileptic treatment was identical during both scans.

PET scanning protocol

Patients were fasted for four hours prior to the PET studies. Scalp EEG electrodes were placed according to the International 10-20 system, and EEG was monitored throughout the tracer uptake period. A venous line was established for injection of FDG (0.143 mCi/kg) produced using a Siemens RDS-11 cyclotron (Knoxville, Tennessee, USA).

FDG PET studies were performed using a CTI/Siemens EXACT/HR whole body positron tomograph (Knoxville, Tennessee, USA). This scanner has a 15 cm field of view and generates 47 image planes with a slice thickness of 3.125 mm. The reconstructed image in-plane resolution obtained is 6.5 $\pm$ 0.35 mm at full-width-at-half-maximum (FWHM) and 6.0 $\pm$ 0.49 mm in the axial direction for the FDG PET (reconstruction parameters: Shepp-Logan filter with 1.1 cycles/cm cutoff frequency and Hanning filter with 0.20 cycles/pixel cutoff frequency).

MRI/MRS data acquisition

MRI and MRS exams were performed on a GE 1.5 Tesla Signa 5.7 unit (GE Medical Systems, Milwaukee, Wisconsin, USA). Multiplanar MRI sequences were obtained in all cases including a 124 slice (1.5mm) T1 weighted spoiled gradient echo (SPGR), fluid-attenuated inversion-recovery (FLAIR) and a 21 plane axial T2 weighted sequences.

During ^{1}H MRS examination a Stimulated Echo Acquisition Mode (STEAM) pulse sequence was used to acquire spectra using the following acquisition parameters and also included unsuppressed water reference scans for neurochemical quantitation: an echo time of 30 msec, a modulation time of 13.7 msec, a repetition time of 2 sec, 8 step phase cycle, 2048 points, a spectral width of 2500 Hz, and 128 averages for a total acquisition time of approximately 5 minutes. *In vivo* spectra were acquired from approximately 8cc volumes of interest (VOIs) in the region of the seizure focus and symmetrically on the contralateral side such that they contained almost entirely gray matter and did not contain structural lesions, if present.

MRI/PET coregistration

Coregistration of FDG PET and MRI image volumes was performed using a multi-purpose three-dimensional registration technique (MPItool). The PET image volume was coregistered with the axial MRI image volume using MPItool and a new image volume was created with 21 image planes corresponding to the original MRI image planes. Brain regions of interest (ROIs) for the position of the MRS voxels were registered on the axial T2 MRI images and then transferred to the coregistered FDG PET images.

Visual PET image analysis

The PET scans were evaluated visually, and areas that were designated as having abnormal glucose metabolism were recorded.

Quantitative PET image analysis

Regional values for tracer concentration were obtained by taking the average value for all planes in which the voxel was located. Regional values were then normalized to the concentration for the normal hemisphere (contralateral to the seizure focus), yielding a ratio of relative regional cerebral glucose utilization (rCGU). For the assessment of asymmetries in glucose metabolism, an asymmetry index (AI) was used:

$$AI\ (\%) = (A_C - A_I)/[(A_C + A_I)/2] \times 100\ (\%)$$

where A_I and A_C are the radioactivity concentrations (μCi/ml) for the defined brain regions on the side of the seizure focus and contralateral to the focus, respectively.

Quantitative MRS analysis

Compounds, which were identified in short echo ^{1}H MRS human brain studies, included NA, Glx, Cr, Cho, and myo-Inositol (mI); however, the mI concentration values were not evaluated in this study. The area under each of the resonances is proportional to the concentration of the specific neurochemical compound. Individual peak areas were fit using time domain analysis software, and the concentrations of each compound are reported in arbitrary quantitative units as a ratio to brain water concentration ($x10^4$/water). The chemical shift values are 2.02 ppm for NA, 2.3 for the Glx complex, 3.03 ppm for Cr, 3.22 for Cho, and 3.56 for mI based on literature values. The resonance of NA contains overlapping resonances including

predominantly NAA, as well as NAAG in smaller proportion. The Glx resonance typically contains approximately equal amounts of glutamate and glutamine, with a broader underlying macromolecule component and a relatively small contribution from GABA. However, with the acquisition parameters used in this study, the relative MR visibility of neurochemicals in the Glx resonance is more heavily weighted toward glutamate than glutamine because of relaxation and modulation effects (conservatively, a 60-to-40 ratio).

Statistical analysis

A Pearson's correlation was used to assess the correlation between the absolute values for NA and Glx values, as well as for Glx and relative rCGU values.

RESULTS

Tissue concentrations of brain metabolites

Decreased NA and Glx concentrations on the side of the seizure focus relative to the contralateral side were found in all seven patients with interictal studies. Four patients with ictal/periictal studies had higher NA and Glx concentrations on the side of the epileptic focus.

The NA and Glx quantitative values showed correct focus (as determined by intracranial and/or scalp EEG) lateralization in each case, while it was shown by the NA/Cr, and the NA/Cho metabolite ratios only in eight patients. The lateralization value of these ratios was correct in all cases when the metabolites were registered from brain regions with at least 11% FDG PET asymmetry.

Regional brain glucose utilization

Various portions of the cortex and subcortical structures appeared normal by visual analysis; however, other parts of the brain showed abnormal glucose metabolism. The PET images revealed decreased regional cortical glucose metabolism on the side of seizure focus in all

patients with interictal study, while PET images, obtained during ictal/periictal state, showed increased regional glucose metabolism corresponding to the side of EEG focus.

Quantitative analysis of rCGU for the region of the seizure focus showed a broad range of values, varying from a decrease of 23.8% to an increase of 16.9% compared to the contralateral homotopic normal region. Decreased glucose metabolism was found in the seizure focus in all patients examined in the interictal states, while increased metabolism was observed in patients who had ictal/periictal studies. The rCGU values showed correct focus lateralization in all examined patients including both the interictal and ictal/periictal studies.

Relation between NA and Glx concentrations

A significant correlation was found in the comparison of tissue concentration of NA with concentration of Glx using ROIs in the epileptic cortex ($r=0.60$, $p=0.048$), while using contralateral homotopic ROIs, there was a tendency for statistical significant correlation ($r=0.58$, $p=0.061$).

Relation between rCGU values and Glx concentrations

Significant correlations were found in the comparison of glucose metabolism with tissue concentration of glutamate/glutamine using ROIs in the epileptic cortex ($r=0.67$, $p=0.021$), using contralateral homotopic ROIs ($r=0.60$, $p=0.047$), and using the combined ROIs from focus and non-focus regions ($r=0.64$, $p=0.0009$).

DISCUSSION

In this study, brain metabolite concentrations of NA, Cr, Cho, Glx and cortical glucose metabolism were investigated in humans under normal and pathological conditions by ^{1}H MRS and FDG PET in symmetrical brain regions. In order to compare the focus side to the non-focus side, one of the basic requirements of this study was to identify VOIs that contain predominantly

gray matter, since brain metabolite concentrations, as well as glucose metabolism are different in gray and white matter.

^{1}H MRS measurements in focal epilepsy

The interictal results are consistent with the findings of previous MRS studies performed in patients with temporal and extratemporal lobe epilepsy showing reduction of NAA signal and decreased NAA/Cho and NAA/Cr ratios ipsilateral to the focus. In pathological states, decreases in the levels of NAA, NAAG, and in the activity of NAAG peptidase have been found correlating with neuronal loss. Normalization of decreased NAA after epilepsy surgery has been shown including both the ipsi and contralateral side. Thus, the reversible decrease of NAA concentration may represent decreased production due to neuronal and/or mitochondrial dysfunction, or degradation of NAA following neuronal membrane disruption. In contrast, larger diversity has been shown in NAA levels by ictal and postictal MRS studies, probably due to the differences in timing of scanning and acquisition after the seizure onset. In human studies, the values of NAA/Chol and NAA/Cr ratios were decreased in the ipsilateral temporal lobe; however, the majority of observations were made in the postictal period. In animal models, increased NA/Cr ratios were found in ictal state, while significant NAA changes were not observed postictally. The increased NA/Cr ratios have been considered to be a reflection of NAA synthesis increases, since negligible change in brain Cr levels has been shown under different pathologic conditions.

Our data showing decreased Glx concentration interictally and increased concentration with seizure activity are consistent with previous findings. Lower Glx concentration has been shown ipsilateral to seizure onset in patients with temporal lobe epilepsy and hippocampal sclerosis as compared to normal controls. Enhanced glutamate release, contributing to the initiation of seizure activity, has been reported in microdialysis studies during seizures in patients with partial epilepsy, as well as in some animal models. Increased glutamate/glutamine level was also measured with MRS after status epilepticus in one patient with focal epilepsy. These data suggest that Glx concentration is lower in epileptogenic brain regions than in normal

tissue, but may increase with ictal activity. The mechanism for a rapid increase in total tissue Glx during seizure activity, however, is unclear. The increase may be the result of increased flux through the glutamate/glutamine synthesis cycle. Another possible mechanism, which might contribute to a rapid increase in total tissue glutamate, could be the release of glutamate through the cleavage of NAAG. NAAG is released by neuronal depolarization and is converted to NAA and glutamate by glutamate carboxypeptidase II, an enzyme present on the extracellular surface of glia and neurons. Alterations in NAAG and the carboxypeptidase activity have been reported in several animal models of epilepsy. In genetically epilepsy-prone rats, the activity of this membrane bound enzyme is increased in several brain regions, which increases the availability of NAA and glutamate in certain synapses of the brain. Kindling-induced increased NAAG level was observed in the entorhinal cortex. Furthermore, the rapid turnover of NAAG shows that the conversion of NAA to NAAG can be an important modulator of synaptic activity.

FDG PET measurements in focal epilepsy

The characteristic of an epileptogenic focus, studied interictally in lesional and nonlesional neocortical epilepsy, is an area surrounded by large areas of reduced glucose metabolism which is usually larger than the pathological abnormality. The most likely reason for the large region of reduced metabolism is the inhibition or deafferentation of neurons around an epileptogenic focus. Partial seizures are associated with an increase in regional cerebral glucose metabolism in the region of the epileptogenic focus. Hypermetabolic areas have also been found in children with partial seizures, who did not have overt seizures and in patients with cryptogenic temporal lobe epilepsy who had never recieved antiepileptic drugs. The biochemical basis of interictal and ictal hypermetabolism is probably related to increased energy consumption by an active epileptogenic focus.

Lateralization of seizure focus

^{1}H spectroscopic imaging of brain metabolites and FDG PET have been proven to be a sensitive indicator for the lateralization of seizure foci in focal epilepsies; however, previous studies showed different concordance between the distribution of ^{1}H MRS, FDG PET and EEG abnormalities. The sensitivity of ^{1}H MRS in lateralization of unilateral temporal and extratemporal lobe epilepsy varied between 55-88% showing bilateral abnormalities in less than 50% of the patients. These results were based on calculations of NAA/Cr, NAA/Cho and NAA/(Cr+Cho) metabolite ratios. Interictal FDG PET studies in patients with longstanding epilepsy predicted the side of the focus in almost all cases, though patients with recent-onset epilepsy appeared to have less intense metabolic abnormalities. In this study, the lateralization of epilepsy foci was precisely confirmed by quantitative values of NA, Glx and rCGU, while on the basis of the NA/Cr and NA/Cho metabolite ratios just partly. The lateralization value of metabolite ratios was higher in those patients having had voxels localized to areas with more intense abnormalities in cortical glucose metabolism. Interictally, the decreased metabolite ratios can be due to decreases of NA concentrations and/or increases of Cr and Cho concentrations. Intensive metabolic abnormalities are associated with profound neuronal dysfunction causing changes in the signal intensity of NAA. The concentrations of Cr and Cho appear to be much higher in oligodendrocytes and astrocytes than in neurons, thus the increased signal from these compounds may reflect gliosis or consistent with reactive astrocytosis. Conversely, there are several interictal studies with no significant changes in Cr and Cho levels. According to these observations, it is very likely that in those cases when gliosis or reactive astrocytosis are not present in epileptogenic brain tissue, the seizure focus lateralization is more precise by quantitative values than metabolite ratios.

Relation between NA and Glx tissue concentrations

NAA and glutamate could be derived from many possible sources including the synaptic release of NAAG. NAAG has been linked to seizure disorder by kindling-induced increases of NAAG concentrations in entorhinal cortex. These increases proved to be persistent for at least one week. In conditions associated with increased excitability, the activity of NAAG peptidase can be elevated increasing the availability of NAA and glutamate in certain synapses of the brain leading to an excess of NAA uptake to oligodendrocytes and glutamate uptake into astrocytes. Further consequences of these processes are NAA hydrolysis and glutamine synthesis. Thus, it is likely that change in the balance of synthesis/release/catabolism of NAAG may play an important role in epileptic activity. In addition, conditions which increase the level of acetyl-CoA may significantly increase the synthesis of NAA. Increases in NAA levels may associate with increases in glutamate levels, since it appears that most of the NAA-derived [^{15}N]aspartate undergoes transamination resulting in the formation of [^{15}N]glutamate.

Relation between glucose metabolism and glutamate/glutamine concentrations

By combining FDG PET with ^{1}H MRS, it is possible to study the relationship between cortical glucose metabolism and glutamate/glutamine concentration in humans under normal and different ictal conditions. Using this approach, we found a positive linear correlation between cortical glucose utilization and the glutamate/glutamine concentration. Glutamate uptake into the astrocytes by a glutamate transporter resulted in a concomitant stimulation of glucose uptake via a mechanism involving activation of the Na+/K+ ATPase, followed by an increase in lactate efflux from the astrocytes. Thus, this mechanism accounts for the coupling between neuronal activity and energy metabolism.

SUMMARY AND NOVEL FINDINGS

In conclusion, both ^{1}H MRS and FDG PET proved to be a useful diagnostic tool in the evaluation of partial epilepsies *in vivo*. To my best knowledge, this is the first investigation in which brain metabolites and glucose metabolism were studied in the same EEG defined epileptic foci during the same ictal sates. The cortical seizure focus can be characterized by lower NA and Glx concentrations, in addition to decreased glucose metabolism interictally, and by higher metabolite levels and increased glucose utilization in ictal state. The NA and Glx quantitative values of ^{1}H MRS studies and the rCGU values of FDG PET studies showed higher sensitivity in focus lateralization than the NA/Cr and NA/Cho metabolite ratios. Furthermore, these results demonstrate a significant relationship between tissue glutamate/glutamine and NAA/NAAG concentrations in the epileptic cortex. Finally, the calculated data support the coupling of glucose metabolism to glutamate metabolism in the cerebral cortex, and extend previous findings to humans under normal and pathological conditions.

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