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Hippocampal extracellular GABA correlates with metabolism in human epilepsy

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Abstract

As the major inhibitory neurotransmitter in human brain, GABA is an important modulator of hyperexcitability in epilepsy patients. Given the high energetic cost of neurotransmission and synaptic activity, GABA concentrations may be hypothesized to correlate with metabolic function. We studied human epilepsy patients undergoing intracranial EEG monitoring for seizure localization to examine microdialysis measures of extracellular GABA (ecGABA), pre-operative MR spectroscopic measures of neuronal mitochondrial function (NAA/Cr), and wherever possible, neuropathology and hippocampal volumetry. Two groups undergoing intracranial monitoring for seizure localization were studied: surgically treated hippocampal epilepsy (MTLE) and neocortical (non-hippocampal seizure onset) epilepsy. All data are hippocampal and thus these groups allow comparisons between the epileptogenic and non-epileptogenic regions. ecGABA was measured using in vivo microdialysis performed during intracranial monitoring. Pre-operative in vivo MR spectroscopic imaging was performed to measure the ratio of N-acetyl aspartate (NAA) to creatine. Standard methods for neuropathology and hippocampal volumetry were used. In the neocortical group, increased ecGABA correlated with greater NAA/Cr ($R=+0.70$, $p<0.015$, $n=12$) while in the MTLE group, increased ecGABA linked with decreased NAA/Cr ($R=-0.94$, $p<0.001$, $n=8$). In MTLE, ecGABA (increased) and NAA/Cr (decreased) correlated with increased glial cell numbers ($R=+0.71$, $p<0.01$, $n=12$, $R=-0.76$ $p<0.03$ respectively). No relationship was seen between ecGABA and hippocampal volumes in either group. In epilepsy, ecGABA increases occur across a range of metabolic function. Outside the seizure focus, ecGABA and NAA/Cr increase together; in contrast, within the seizure focus, ecGABA increases with declining mitochondrial function.

Keywords

Epilepsy; GABA; NAA; Microdialysis; Hippocampus; Metabolism

Introduction

Several studies have shown there is a link between GABA neurotransmission with mitochondrial and metabolic function. For example, data from a rat Huntington's model have shown that application of 3-nitropropionic acid (a toxin of the mitochondrial succinate dehydrogenase) results in neuronal depolarization and up to 30% increased spontaneous GABA release (Saulle et al. 2004). Similarly, a model of mild to moderate oxidative stress in the mouse hippocampus has shown increases in basal GABA release, ranging from 30 to more than 550% (Saransaari and Oja 1997). These observations are of particular interest for epilepsy. In epilepsy, GABA is thought to be a key component underlying the abnormal hyperexcitability; clinically, abnormal metabolic imaging is sufficiently accurate to be used for seizure localization. Given this and the known high energetic cost of neurotransmission and synaptic activity (Atwell and Laughlin 2001), we anticipated that GABA neurotransmission and metabolic function would be correlated. This may be especially important given the recent in vitro work that has suggested GABA function may be either anti- or proconvulsant (Woo et al. 2002; Palma et al. 2006).

We studied human epilepsy patients to examine the coupling between extracellular GABA (ecGABA) as measured with in vivo microdialysis with mitochondrial function as assessed by MR spectroscopic (MRS) imaging. All patients were undergoing intracranial monitoring for the clinical purpose of epilepsy localization. Prior the intracranial monitoring, patients (as outpatients) were studied with MR spectroscopic imaging and hippocampal volumetry. During the intracranial monitoring period, patients were evaluated with microdialysis to determine extracellular GABA levels, these (inter-ictal) data acquired while patients were awake. All data were acquired prior any therapeutic surgical intervention. In those patients who underwent resective surgical therapy, we also evaluate neuropathology. The interictal ecGABA measurements were measured by quantitative zero flow microdialysis, which estimates the true basal concentrations of ECF neurochemicals such as glutamate, glutamine and GABA (Cavus et al. 2005). The MR spectroscopic imaging measures of N-acetyl aspartate (NAA) and ratios of NAA to creatine (NAA/Cr) have been extensively used to assess the metabolic state of the neuronal–glial unit. In vitro studies of NAA find that it is synthesized specifically in neuronal mitochondria, and correlates with neuronal mitochondrial ATP synthesis (Patel and Clark 1979). Given that creatine levels are high in astrocytes and relatively well preserved in epilepsy (Mueller et al. 2004), abnormal values of NAA/Cr are frequently used in epilepsy to localize and lateralize the seizure focus (Hetherington et al. 1995; Cendes et al. 1997). These data, particularly those acquired from regions at the seizure focus vs. outside of the seizure focus, allow an evaluation of the pathophysiological relationships of GABA.

Methods

Patients

Patients (age 18–45) from the Yale University Epilepsy Surgery Program undergoing intracranial EEG (icEEG) monitoring for surgical evaluation were invited to participate in the MR imaging and microdialysis protocols approved by the Yale Human Investigations Committee (HIC). Patients ($n=24$ total, Tables 1 and 2) with hippocampal probes were included in this analysis. All these patients had hippocampal depth probes to study hippocampal involvement in their seizures. Of the 24 patients, there were $n=12$ (6F) neocortical epilepsy patients and $n=12$ (5F) hippocampal epilepsy patients (Table 1). Of the $n=12$ neocortical epilepsy patients, 11 were ultimately determined to be of temporal neocortical origin, one of occipital origin (Table 2). These classifications were assigned after the intracranial study. In the neocortical epilepsy patients, the evaluated hippocampus was

ipsilateral to the hemisphere of neocortical onset. These two groups allow comparison of measurements between the epileptogenic ipsilateral hippocampus (MTLE group) vs. the non-epileptogenic hippocampus (non-MTLE group). Patients taking valproate were excluded because of its known effect on brain GABA levels (Owens and Nemeroff 2003). In all of the 12 resected hippocampal patients, post-surgical neuropathology was available for review. Seven patients were described as hippocampal sclerosis and five as “paradoxical” hippocampal epilepsy with neuronal loss less than 40%. MRS data were also collected from $n=42$ age matched (age 18–50) healthy controls (19 F).

All patients were imaged within 3 months prior to the intracranial monitoring procedures. All intracranial procedures (icEEG and microdialysis acquisitions) were performed during a 5–10 day session during which time seizures were evaluated clinically. If clinically indicated (i.e., seizures were successfully localized), resection was performed either immediately after completion of the intracranial monitoring session, or more commonly, approximately 4–6 weeks later in a second surgical procedure. Patients identified to have hippocampal onset of their spontaneous seizures underwent resection of the epileptogenic medial temporal lobe and en block specimens were evaluated for neuropathology (Kim et al. 1990).

Microdialysis and HPLC

The microdialysis procedures are described in detail by Cavus et al. 2005 and Cavus et al. 2008. Briefly, microdialysis probes (CMA North Chelmsford, MA) are inserted into the depth electrodes (Spencer probe, Ad-Tech Instrument Co., Racine, WI) such that the microdialysis and EEG electrode probes are embodied in a single unit. This combined probe is implanted in the hippocampus using a stereotactic neuro-navigation device (BrainLAB, Westchester, IL). A MRI/CT scan was performed 24 h after implantation to verify probe placement. Following 1–2 days of recovery in the Neurosurgical Intensive Care Unit, patients were transferred to the Epilepsy Unit for continuous icEEG and audiovisual monitoring. Anti-epileptic drugs (AEDs) were clinically withdrawn usually within 3–5 days post-operatively. Patients were monitored for 7–14 days until an adequate number of seizures had been recorded for localization. To avoid acute probe insertion effects, microdialysis studies were initiated 3–5 days after implantation. Where there were two probes in a single hippocampus ($n=11$ patients), the average value was used. Data were acquired while patients remained on medications similar to the pre-operative imaging studies. To minimize effects from ictal activity, behavioral stimuli or food intake on the microdialysate measurements, studies were conducted in the afternoon when patients were quietly resting, >6 h from any intracranially recorded seizure activity, and at least 2 h following any food intake. In our experience the probes remain safe and provided reliable measurements for up to 2 weeks.

The interictal levels of GABA were measured using quantitative microdialysis. The duration of a typical dialysis study was 6 h. Sterile artificial CSF was infused using portable CMA107 syringe pumps (CMA, North Chelmsford, MA) at a rate of 2.0 $\mu\text{l}/\text{min}$ for 1 h to reach steady state. GABA was analyzed using a standard of 2-amino-n-valeric acid and derivatized 2 μl samples on a BAS, ODS column. Chromatograms were generally complete in 22 min. Basal concentrations of GABA were estimated using the zero-flow method: progressively slower flow rates were used to collect 20 μl dialysate samples, stored at -80°C for HPLC analysis. A regression analysis with a 2nd polynomial order was then projected to a flow of zero giving the basal GABA concentration (Cavus et al. 2008). Given the non-gaussian distribution of measured GABA levels, a log transform was performed.

Magnetic resonance spectroscopy (MRS)

¹H spectroscopic imaging: All data were acquired using a whole body 4T Varian Inova imaging spectrometer with a volume ¹H TEM coil. Scout images were acquired with an inversion recovery gradient echo. The triply obliqued hippocampal slice was defined along the planum temporale prescribed from an off-sagittal slice. After scout imaging, 3D localized shimming was performed over the bilateral hippocampi using first, second and third order shims. The spectroscopic image was optimized to collect data on N-acetyl aspartate (NAA), creatine (Cr) and choline using a 3D localized adiabatic LASER sequence with two dimensions of phase encoding, with TR/TE 2 s/72 ms (Pan and Takahashi 2005). The spectroscopic image had a nominal voxel size of 0.64 cc (24 × 24 encodes, FOV 192 × 192 mm, acquisition time 19 min).

Data analysis—To provide reproducible volume selection along the hippocampal plane between subjects, a semi-automated single voxel selection and reconstruction routine was used. The data were analyzed by tracing the boundaries of the hippocampi, calculating a midline, and then reconstructing four pixels along the length of the hippocampus centered over the mid-line. The positions were selected by centering pixel #2 at the level of the aqueduct and reconstructing two pixels anterior and one pixel posterior. The spatial reconstruction utilized a cosine filter to confine the point-spread function with an effective sampling volume of 1 cc. The ¹H data were fit in the spectral domain with the resonance areas determined as ratios. Because the location of microdialysis probes varied slightly between subjects, to provide a consistent MR comparison, the spectroscopic data were averaged over hippocampal voxels #1 through #4.

Neuronal and glial cell neuropathology

Coronal sections of the hippocampus taken at the midposterior level were used for quantitative morphometry. After resection, tissue was immediately placed into 10% buffered formalin and paraffin embedded. Paraffin sections, 6 mm thick, were stained with hematoxylin and eosin, Luxol fast blue or Nissl stain. Neuronal counts were performed as previously described (Kim et al. 1990) and were adjusted using Abercrombie 1946 formula utilizing 12.2 μm for the nuclear diameter of pyramidal neurons, and 9.2 μm for granular neurons. Reference counts were taken from an autopsy control population (*n*=26), mean age 38 years, who all died of non-neurological causes. For glial counting, multiple thin parallel lines were drawn perpendicularly to the horizontal axis along the floor of the temporal horn of the lateral ventricle at an interval of 1,250 μm to cover the whole coronal section of the hippocampus. Both astrocytes and oligodendrocytes within a 100 μm × 200 μm unit area were counted. The unit area was consecutively placed along the perpendicular line, as counting glia in each unit area is completed. The average glial number/unit area was adjusted with Abercrombie's formula. The final glial density was expressed as mean glial number/cubic millimeter.

Hippocampal volumetry

T1 gradient echo weighted imaging using an inversion recovery preparation was used to acquire high contrast images at 1.5 mm isotropic resolution. Off-midline sagittal imaging was used to acquire triply obliqued images through the bilateral hippocampi to visualize the planum temporale. Volumetry was performed using coronal images taken perpendicular through planum temporale according to the published methods (Watson et al. 1992; Jack et al. 1989) so as to include the tail, body and pes hippocampus, subiculum and dentate. The volumetry was performed such that those pixels defined as being within the tracing are included in the final measurements. Using these methods, our healthy control hippocampal measurements were 2.69±0.32 cc (left hippocampus) and 2.84±0.33 cc (right hippocampus),

which supports the generally observed view that the right is larger than the left hippocampus and is in relatively good agreement with published literature (Jack et al. 1989).

Statistics

Group differences were evaluated using a two-tailed *t*-test and multiple comparisons corrections applied where needed. Because of non-normal distribution of EC GABA concentrations in comparison to the normal distributions seen in the imaging and neuropathology data, GABA data were analyzed after log transformation. Correlations were performed using a Pearson analysis using $p < 0.05$ as the criterion for statistical significance.

Results

The mean concentration of EC GABA in the surgically treated MTLE group was 375 ± 454 nM (ipsilateral hippocampus, $n=12$). In the neocortical epilepsy group (non-epileptogenic hippocampus, $n=12$), this was 542 ± 553 nM. These values were not significantly different.

Figure 1 displays typical scout images showing the loci and reconstructed spectra from the hippocampus. The mean value of the ipsilateral hippocampal NAA/Cr obtained in the MTLE group ($n=8$ patients) was 0.87 ± 0.07 , significantly different from healthy controls ($n=42$, combined left and right hippocampi, $p < 0.001$) at 1.27 ± 0.12 . This can be compared to the hippocampal measurements from the neocortical epilepsy group ($n=12$ patients), NAA/Cr at 1.25 ± 0.30 . The MTLE group NAA/Cr value was significantly different from both the control and neocortical groups at $p < 0.02$ (Holm-Sidak multiple comparisons corrected) while there was no difference between the control and neocortical groups.

In the MTLE group, increasing ecGABA strongly correlated with *decreasing* NAA/Cr, $R = -0.94$, $p < 0.001$ (Fig. 2). In the non-MTLE group, increasing ecGABA correlated with *increasing* NAA/Cr, $R = +0.70$, $p < 0.015$. Thus ecGABA links well with mitochondrial function whether inside or outside of the seizure focus (MTLE and neocortical epilepsy groups respectively). Although the values of ecGABA were comparable between the two groups, comparison of the 95% confidence intervals of the regression data revealed that their relationships with mitochondrial function are significantly different.

In the MTLE group where neuropathology was available, a significant positive relationship was seen between increasing glial counts and increasing ecGABA, $R = +0.71$, $p < 0.01$ (Fig. 3). Consistent with this and the very strong (negative) ecGABA relationship with NAA/Cr, increased glial counts also correlated with declining NAA/Cr, $R = -0.76$, $p < 0.03$ (data not shown). No significant relationship was found between neuronal numbers and GABA. Also, no significant relationship between hippocampal volumetry and ecGABA or NAA/Cr was seen for either the MTLE or the neocortical epilepsy group. Thus in the MTLE group, ecGABA and glial numbers increased together, while NAA/Cr declined.

Discussion

Basal levels of ecGABA

The microdialysis measurements of ecGABA most likely represent a balanced integration of neurotransmitter levels and activities, reflecting extra-synaptic GABA levels, resulting from release, re-uptake and diffusion including synaptic spillover and non-vesicular neuronal release (Del Arco et al. 2003). Simultaneous icEEG recordings and microdialysis data have shown specific and dynamic changes in the measured neurochemicals synchronous with seizures (During and Spencer 1993; Wilson et al. 1996), strongly arguing that the dialysis membranes evaluate electrically active tissue and the neurotransmitter changes are reflective

of pathologic events in the seizure focus. Our measures of basal ecGABA are in relatively good agreement with published rodent data (Kennedy et al. 2002).

ecGABA, mitochondrial function and injury in MTLE

In the surgically treated MTLE patients, there appears to be an injury-linked process resulting in significant relationships between increasing glial numbers, increasing ecGABA and declining NAA/Cr. The negative ecGABA and NAA/Cr relationship is unusually tight, with $R=-0.94$, $p<0.001$ ($R^2=0.88$). Statistically, this means that the variability in either of these parameters is well reflected in the variability of the other, i.e., that within the seizure onset zone (presumed injury zone), extracellular GABA and mitochondrial function are largely representing parallel or identical processes. As a correlation, it is not possible to exactly ascertain the causal relationships between ecGABA, mitochondrial function and injury. However there are numerous neurochemical and neurophysiological injury studies that specifically link metabolic function with GABA. For example, Nguyen and Picklo 2002 suggested that injury-produced lipid peroxidation products inhibit the TCA cycle enzyme succinic semialdehyde dehydrogenase (SSAD, which metabolically degrades GABA). This would result in both decreased TCA cycle flow and decreased GABA clearance.

Alternatively, electrophysiological data have suggested that in the context of injury and dysfunctional chloride transport, ecGABA can become excitatory (Woo et al. 2002) and can further propagate seizure-linked injury. While these data cannot specify which or several of these injurious processes may be ongoing in the seizure onset zone, it is evident that the relationship of ecGABA with NAA/Cr within the ipsilateral hippocampus is clearly distinctive in comparison to ecGABA correlations in the non-MTLE group.

ecGABA with NAA/Cr in non-MTLE

In the neocortical epilepsy patients and outside of the seizure onset zone, the relationship between ecGABA and NAA/Cr, is also significant but positive with $R=+0.70$ $p<0.015$ ($R^2=0.49$). This correlation shows an increasing ecGABA with increasing NAA/Cr and is significantly different from the MTLE correlation. In these neocortical patients, the hippocampus being studied was ipsilateral to the cortical seizure onset region and thus may be a site of proximal propagation. Nonetheless, as it is not the seizure focus, these data remain conceptually similar to the results of Petroff et al. 2001, who found that well outside the seizure focus (studying the occipital lobe), patients with better seizure control have higher tissue GABA levels. Similar to the above, these correlational data do not permit the determination of the specific relationship between ecGABA and mitochondrial function. However as these data were made in relatively healthier tissue, the physiological relationships that link these parameters are likely to be different in comparison to that seen in the seizure focus. For example, a potentially common source for increased ecGABA even in healthy brain has been suggested from the readily reversible GABA transporter GAT-1 (Richerson and Wu 2003).

Implications for GABA

Overall, these data are consistent with Petroff et al. 2001, who argued that GABAergic medications may be therapeutic by suppressing seizure spread, rather than reducing excitability at the focus. This might be a viable hypothesis since increased GABA outside of the seizure onset zone correlates with increased (better) mitochondrial function, and is also found in patients with better seizure control. On a practical level, these data also suggest that the GABA/NAA ratio may be helpful to specifically identify the seizure onset zone. For example, NAA/Cr is known to be decreased in several disorders (e.g., degenerative or demyelinating diseases, Ackl et al. 2005; Coulthard et al. 2006; Tedeschi et al. 2002), but the elevation of GABA in the presence of depressed NAA/Cr may be specific to the seizure onset zone.

Finally, the present data are consistent with extensive animal literature linking GABA with mitochondrial function. However the distinctly different correlations seen in these two patient groups raise the possibility that there are at least two regimes, which simplistically described may be characterizing severe injury in the seizure onset zone, versus lesser injury in regions of seizure propagation. More work will be needed to better understand these two groups in terms of their mitochondrial differences and inhibitory and excitatory neurotransmission.

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Fig. 1. Scouts showing (*left panel*) the location of study taken along the planum temporale and (*right panel*) the loci of the hippocampal voxels. For the correlational analyses, the same (*right or left*) hippocampus from which microdialysis data were acquired was also used for NAA/Cr measurements, as an average of the hippocampal voxels loci 1–4

Fig. 2.

Plot showing correlation between log basal ecGABA with hippocampal NAA/Cr from (*filled circles*) MTLE patients and (*open circles*) from neocortical epilepsy patients. Individual regression lines are shown for each group with 95% confidence intervals. For the MTLE group, $R=-0.94$, $p<0.001$; for the neocortical non-MTLE group $R=+0.70$, $p<0.015$

Fig. 3.
Plot showing correlation between log basal ecGABA with hippocampal glial count. $R=+0.79$, $p<0.02$

Table 1

Patient clinical characteristics, MTLE group

Hippocampal epilepsy	Gender	Age	HS or PTLE	Outcome at 1 year, ILAE scale	AED use	EC GABA ⁺ (nM)
1	M	23	PTLE	1	DPH,TPM,GBP	138
2	F	52	PTLE	1	DPH	31
3	M	40	HS	1	LTG, CLZ	872.2
4	M	44	HS	1	ZNS	410
5	F	32	HS	1	CBZ	1640.1
6	M	17	HS	4	OXC,LTG,LEV	144.9
7	F	46	PTLE	4	LEV, DPH	161.4
8	M	25	HS	1	CBZ,DPH,ZNS	199
9	M	50	HS	1	LEV, OXC, GBP	151
10	F	38	PTLE	1	TPM	161
11	F	49	PTLE	1	LEV	223
12	M	45	HS	1	LTG,FBM	371

HS, hippocampal sclerosis, defined by >40% neuronal loss (Kim et al. 1990); PTLE, paradoxical temporal lobe epilepsy

⁺Measurements obtained from ipsilateral hippocampus

Table 2

Patient clinical characteristics, neocortical group

Neo-cortical epilepsy	Gender	Age	Epileptic focus	Surgical procedure, if any	Surgical pathology, if any	Outcome at 1 yr, IL/AE scale	AED use	EC GABA (nM)
1	F	35	R Lat Temporal	Lateral temporal lobectomy incl Heschel's g	Mild gliosis	2	DPH	849.2
2	M	22	R Inf Temporal	Inferior temporal lobe to fusiform g	Scattered heterotopic neurons in molecular layer, reactive gliosis	3	LTG, CBZ	201
3	F	39	R Temporal-Parietal	Post temp lobe resection, MST supramarginal g; and inf. parietal lobule	Focal atrophy and gliosis	1 (3 post-op seizures)	LTG, CBZ, PB	566.6
4	M	32	R Temporal-Occipital	Temporal anterior occipital resection and Hippocampectomy	Glial scarring in cortex with ulegyria; hippocampal sclerosis.	1	LTG	47
5	M	28	L Temporal-Parietal, Mid-frontal	Resection of post temporal parietal encephalomalacia, subtotal resection mid-frontal seizure focus and MST	Reactive astrocytes with increased cellularity	4	LTG, LEV, DPH	302
6	F	21	L Occipital	None	N/A		CBZ	150.4
7	F	27	L Temporal	None	N/A		LTG, CBZ	1290.3
8	F	39	L Temporal	None	N/A		LEV	476
9	M	47	L Temporal	Excisional topectomy	Non-specific	2	DPH, TPM	47.8
10	M	36	L Temporal-Parietal	Temporal pole and temporal-parietal MST	Laminar cortical neurons, but partly appears thick, rare heterotopic neurons.	1	CBZ, LEV	573.0
11	M	44	R Temporal	None	N/A		CBZ, ZNS	145.1
12	F	30	L Temporal	Lateral temporal and hippocampus, sparing amygdala	Gliosis with hippocampal sclerosis	1	OXC, LTG	1801

AED abbreviations:

CBZ carbamazepine; CLZ clonazepam; DPH phenytoin; GBP gabapentin; LTG lamotrigine; LEV levitracetam; OXC oxcarbazepine; PB phenobarbital, TPM topiramate; ZNS zonisamide

ILAE scale: 1: seizure free (no auras); 2: auras only; 3: 1–3 seizure days per year; 4: <50% original seizure frequency; 5: no change in seizure frequency; 6: >50% worsening of seizure frequency (classes 4–6 referenced to pre-operative seizure frequency).