Correlations between granule cell physiology and bioenergetics in human temporal lobe epilepsy

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Summary
Human temporal lobe epilepsy (TLE) is associated with bioenergetic abnormalities including decreased phosphocreatine (PCr) normalized to ATP. The physiological consequences of these metabolic alterations have not been established. We hypothesized that impaired bioenergetics would correlate with alterations in physiological functions under conditions that strongly activate neural metabolism. We correlated several physiological variables obtained from epileptic human dentate granule cells studied in slices with hippocampal PCr/ATP measured using in vivo magnetic resonance spectroscopy. The physiological variables included: the ability to fire multiple action potentials in response to single stimuli, the inhibitory postsynaptic potential (IPSP) conductance and the responses to a 10 Hz, 10 s stimulus train. We noted a significant negative correlation between the ability to fire multiple spikes in response to single synaptic stimulation and PCr/ATP (P < 0.03) and a positive correlation between the IPSP conductance and PCr/ATP (P < 0.05). Finally, there was a strong correlation between PCr/ATP and the recovery of the membrane potential following a stimulus train (P < 0.01), with low PCr/ATP being associated with prolonged recovery times. These data suggest that the bioenergetic impairment seen in this tissue is associated with specific changes in excitatory and inhibitory neuronal responses to synchronized synaptic inputs.

Keywords: dentate gyrus; phosphocreatine; 13P-MRS; slice physiology; temporal lobe epilepsy

Abbreviations: PCr = phosphocreatine; GABA = \( \gamma \)-amino-butyric acid; GIPSP = IPSP conductance; IPSP = inhibitory postsynaptic potential; \([K^+]_o\) = extracellular potassium concentration; MRS = magnetic resonance spectroscopy; MTS = medial temporal lobe sclerosis; MTLE = medial temporal lobe epilepsy


Introduction
Phosphocreatine (PCr) transports high-energy phosphate within intracellular domains and thus energetically buffers tissues with a high metabolic rate to minimize changes in intracellular ADP and magnesium levels during periods of increased activity (Wallimann and Hemmer, 1994; Wyss and Kaddurah-Daouk, 2000; Ellington, 2001). Given the high metabolic rate of brain tissue, PCr is critical in ensuring that ATP levels are maintained at sodium-potassium ATPases by shuttling high-energy phosphate from mitochondria to the plasma membrane. This may be especially important for interneurons that exhibit high levels of action potential firing and must bear the associated metabolic cost (e.g. Attwell and Laughlin, 2001; Boero et al., 2003; Lennie, 2003). Alterations in PCr/ATP ratios are known to alter brain excitability, function and behaviour in humans (Kekelidze et al., 2001; Rango et al., 2001; Jost et al., 2002; Streijger et al., 2004), although ex vivo measurements of PCr are difficult because of its high lability. However, in vivo measurements of PCr and ATP can be reliably acquired with recent developments of magnetic resonance spectroscopy (MRS) studies (Kemp, 2000; Kekelidze et al., 2001; Rango et al., 2001).

Evidence for a link between energetics and physiological function comes from studies of temporal lobe epilepsy (MTLE) in which lowered PCr/ATP levels are highly predictive of the site of seizure onset (Kuzniecky et al., 2001; Hetherington et al., 2002, 2004). Moreover, following temporal lobectomy, there is an improvement in the contralateral energetics (Hugg et al., 1996; Vermathen et al., 2002). Thus, alterations in bioenergetics are part of the spectrum of changes that characterize MTLE, along with cell loss and synaptic reorganization.
Methods

MRS studies

These studies were carried out using hippocampi resected from 14 patients with intractable unilateral MTLE for whom we had both in vivo 31P spectroscopic data and in vitro slice recordings. 31P spectroscopic images were acquired at 4T using a Varian INOVA whole-body system and a 13 × 13 × 13 spherical sampling scheme and field of view of 240 × 240 × 240 mm. Data were collected using a 0.5-s recycle time, a 37° excitation pulse and six averages per encoding step. The total acquisition time was 46 min. To minimize synaptic activity in epileptic human tissue have not been evaluated.

Therefore, in this study we tested the hypothesis that there would be a relationship between the electrophysiological characteristics of resected MTLE hippocampi and the preoperative hippocampal PCr/ATP values. Specifically, we hypothesized that at low stimulus frequencies, the changes in synaptic connectivity seen in MTLE would dominate the underlying bioenergetic deficiencies would be seen when the tissue is forced to become metabolically active. We chose to study the dentate gyrus since it is the best-preserved region of the epileptic human hippocampus and provides the best-studied major synaptic input into the rest of the hippocampal formation.

Slice studies

The hippocampus was resected en bloc and immediately dissected into 5-mm sections orthogonal to the long axis of the structure (Spencer, 1991). We received tissue from the mid-body of the hippocampus. The tissue was placed in ice-cold, oxygenated artificial cerebrospinal fluid (ACSF) and transported to the laboratory; the delay between the tissue resection and the preparation of the slices was ~10 min. Hippocampal slices (400 μm) were prepared using a vibratome and were kept at ~4°C during the slicing procedure. Subsequently, the slices were maintained at 33–34°C in an interface slice chamber (Fine Science Tools, Foster City, CA, USA) (Williamson et al., 1995, 1999). The tissue was exposed to an environment oxygenated with 95% O2/5% CO2 and bathed with ACSF containing (in mM): NaCl 124, KCl 3, MgSO4 2, NaH2PO4 1.2, NaHCO3 26, CaCl2 2.0 and dextrose 10 (pH 7.4). All tissue was resected for clinical reasons and these experiments were approved by the Yale University Human Investigation Committee. The MRS studies were approved by the Albert Einstein College of Medicine Committee on Clinical Investigations.

Intracellular recordings were performed using sharp, 4 M K+-acetate filled microelectrodes. Only cells with resting membrane potentials more hyperpolarized than ~65 mV and input resistances greater than 40 MΩ were included in the analysis. All data were obtained using an Axoclamp 2B amplifier (Axon Instruments) and Axograph software on a Macintosh computer. Axograph and Igor Pro (Wavemetrics, Inc.) were used for data analysis. Synaptic stimulation was delivered to the outer molecular layer using a twisted bipolar electrode.

Three measures were used to assess tissue excitability: the ability to produce multiple spikes in response to single stimuli (bursting); the strength of inhibition [inhibitory postsynaptic potential (IPSP) conductance (GIPSP)] and the frequency of spontaneous presynaptic excitatory activity. The bursting and GIPSP measures were examined using low rates of stimulation (0.1–0.2 Hz). The bursting score was obtained by adding the number of action potentials produced by three successive stimuli delivered at one, two and three times the intensity needed to evoke a single action potential. These studies were carried out at holding potentials between ~70 and ~80 mV with injected current (see Fig. 2). The strength of fast synaptic inhibition was assessed by measuring the fast GIPSP. Stimuli were delivered at several membrane potentials and GIPSP was defined as the difference between the membrane conductance (slope of the I–V curve) measured prior to the stimulus and at the peak of the fast IPSP (Luhmann and Prince, 1991; Williamson et al., 1999) (see Fig. 3). In those cells where an IPSP could not be clearly visualized, the conductance was measured 22 ms following the stimulus artefact. Finally, we measured the frequency of baseline activity by measuring the frequency of spontaneous excitatory postsynaptic potentials (events ≥2 mV amplitude with a rapid rise time and a slower decay) over a 20-s time period. These events were measured at approximately ~70 mV to reduce the possibility of counting spontaneous IPSPs. Our previous studies did not reveal (e.g. de Lanerolle et al., 2003). MRS studies have also demonstrated that N-acetyl aspartate, a metabolite that reflects neuronal mitochondrial function, is reduced in MTLE, supporting the concept of energetic failure in the epileptic temporal lobe because this reduction cannot be completely attributed to the loss of neurons (Kuzniecky et al., 2001). Indeed, work from many groups suggests that bioenergetic impairment is widespread in many types of human epilepsy and may reflect a basic pathophysiological mechanism (Pan et al., 1999; Savic et al., 2004). Thus, while a significant bioenergetic component is associated with the aetiology of seizure disorders, it remains unclear whether this reflects the general damage associated with seizures or is a causative factor, and how these energetic changes affect cellular function.

Several recent studies have used high-resolution respirometry to demonstrate mitochondrial dysfunction in the CA3 region of human epileptic tissue (Kunz et al., 2000; Kunz, 2002), which correlate with the hypometabolism seen in fluorodeoxyglucose (FDG)-PET studies (Vielhaber et al., 2003b). Similar findings have been made using the pilocarpine rat model of MTLE (Kundin et al., 2002). While the relatively well-preserved dentate gyrus was not found to have significant alterations in mitochondrial metabolism when examined under resting conditions (Kovacs et al., 2001), the dynamics of oxidative metabolism during periods of synaptic activity in epileptic human tissue have not been evaluated.

The region sampled is shown in Fig. 1A. The time domain data were filtered with a 10–25 Hz exponential to Gaussian conversion and a 250 Hz convolution difference prior to Fourier transformation. The data were analysed using spectral domain fitting with Gaussian line shapes for the PCr, and the α-,β-,γ-ATP resonances. Additional methodological details are given in Hetherington et al. (2002). The area ratios of PCr/ATP were normalized to those of control subjects.
a significant difference in the IPSP reversal potential between the medial temporal lobe sclerosis (MTS) and non-MTS tissue (Patrylo et al., 1999).

In order to challenge the tissue metabolically, we examined neuronal responses (with the membrane potential held at −70 to −75 mV with injected current) to trains of stimuli (10 Hz, 10 s) that were delivered at an intensity twice that needed to evoke a single action potential. We examined the time needed for the membrane potential to return to baseline following the train; the data are presented as the rate of recovery, in mV/s. Although the recovery tended to be exponential, our preliminary studies indicated that there is less membrane potential-dependent variability in the mV/s calculation than in the time constant; therefore, this value should provide a better comparison between patients. In addition, we determined the time at which 50% of the membrane potential had recovered. Train data were obtained from a total of 23 cells from all 14 patients in which a shift >2 mV from baseline was obtained. A shift of this amplitude allowed for an accurate determination of the recovery rate.

Data analysis
All physiological data from a given patient (2–4 cells/patient) were averaged to obtain representative values for each patient. All data are presented as mean ± SEM. Statistical significance for grouped data was determined using the Mann–Whitney test; correlative studies were done using a Spearman rank correlation test using Statview software. Significance was set at $P < 0.05$.

Results
Patient data
The majority of cases were MTLE patients ($n = 11$) whose hippocampi demonstrated typical pathology for sclerosis. This included evidence for mossy fibre sprouting into the inner third of the molecular layer as demonstrated either by Timm stain ($n = 8$) or by dynorphin immunohistochemistry ($n = 11$)
Fig. 2 Inverse correlation between PCr/ATP levels and hyperexcitability in human dentate granule cells. Note that in A1, increasing stimulus intensity [measured relative to action potential threshold (T)] elicited a single spike (membrane potential = −72 mV). In contrast, in A2, increasing the stimulus intensity produced a three- and then a five-spike burst of action potentials (membrane potential = −76 mV). B shows that there is a significant inverse correlation between the extent of synaptic bursting and the PCr/ATP levels measured in vivo (P < 0.03). Action potentials in both A1 and A2 have been truncated for clarity. Data in A1 and A2 are from different patients.

Fig. 3 Positive correlation between GIPSP and PCr/ATP. A and B show examples of strong (A) and weak (B) IPSPs that followed synaptic stimulation in human dentate granule cells. Note that in A, a robust IPSP could be evoked, while B shows an example of a cell in which only a small IPSP could be evoked. The holding potentials are shown for each trace. The solid symbols indicate the time at which the IPSP conductance was measured, while the open symbols show where the membrane conductance measurement was taken. The insets show the most depolarized trace in isolation. C shows the conductance plots for the fast IPSPs (solid symbols) and for the membrane conductance (open symbols) in the cells shown in A and B; note that the slope is much steeper for the cell shown in A. The GIPSP values plotted in D are the mean differences between the slope of the GIPSP and the membrane conductance for the 14 patients. D shows that there is a significant correlation between the PCr/ATP levels and the mean GIPSP for each patient (P < 0.05). Action potentials in both A and B have been truncated for clarity. Data in A and B are from different patients.
lated relative to autopsy controls). The mean $Z/C_0$ for the dentate granule cell counts was 6.2 (calculated relative to autopsy controls) within these 11 MTS patients, the mean $Z/C_0$ was 6.2 (calculated relative to autopsy controls) with cell counts between groups, the $Z/C_0$ being calculated as the difference between the counts for each region in a given patient and the mean of the autopsy $Z/C_0$-score being calculated as the difference between the counts in total neuronal numbers relative to autopsy controls.

The mean age of these patients was 39.0 ± 3.8 years, and they had a mean seizure duration of 31.9 ± 3.4 years. The majority of the patients had typical (MTLE) with cell loss throughout the hippocampus and extensive synaptic reorganization. These changes are not seen in the paradoxical temporal lobe epilepsy (PTLE) group. In one patient (No. 12) the only significant pathology was cell loss in CA1. Anti-epileptic drug (AED) regimens are abbreviated as follows: cbz = carbamazepine; diamox = acetazolamide; klonopin = clonazepam; ltg = lamotrigine; ph = phenytoin; vpa = valproic acid; tpm = topiramate; dzp = diazepam; gpn = gabapentin; lev = levetiracetam. The cell counts were performed as described by Kim et al. (1990). The value presented here reflects the percentage change in total neuronal numbers relative to autopsy controls.

(de Lanerolle et al., 1992; Mathern et al., 1995, 1999); and evidence for interneuronal reorganization and a pattern of cell loss consistent with MTS (n = 11) (Kim et al., 1990). Z-scores were used to compare the cell counts between groups, the $Z/C_0$ being calculated as the difference between the counts for each region in a given patient and the mean of the autopsy controls divided by the standard deviation of the control group. Within these 11 MTS patients, the mean $Z/C_0$ for the dentate granule cell counts was $-4.27 ± 1.2$ (calculated relative to autopsy controls). The mean $Z/C_0$ score was $-2.6 ± 0.62$ for the remaining three patients ($P < 0.05$). The three non-MTS patients did not have evidence of mossy fibre sprouting (as assessed by both Timm and dynorphin staining) (for a detailed discussion of the pathological changes seen in these different cell groups, see de Lanerolle et al., 2003). Patient data, including the anticonvulsant regimen at the time of surgery, are shown in Table 1.

### MRS studies

The normalized hippocampal PCr/ATP values for the 14 patients included here ranged between 0.82 and 1.01 (mean $0.91 ± 0.01$). Examples of $^{31}$P spectra from the hippocampus of a patient and a healthy control volunteer are shown in Fig. 1B. The spectrum from the patient demonstrates the decline in PCr/ATP ratio that is evident in comparison with the control.

### Cellular excitability

We were able to obtain recordings that fit our inclusion criteria from between 2–7 cells per patient, for a total of 54 cells. Approximately 15% of the cells studied from the MTS hippocampi and 10% from non-MTS hippocampi had acceptable input resistances but were depolarized above $-65$ mV, suggestive of an energetic deficit rather than cell injury. This was not significant, however. The studies used to assess cellular excitability were done using low stimulus frequencies (0.1–0.2 Hz) to limit possible plasticity. As previously described (Williamson et al., 1995; de Lanerolle et al., 2003), the majority of cells from MTS patients fired multiple action potentials in response to single stimuli to the outer molecular layer; this type of response was rarely seen in tissue without extensive cell loss and synaptic reorganization. Examples of these synaptic responses are shown in Fig. 2A1 and A2. Within the population studied here, the mean burst score was $4.0 ± 0.43$ (range 1.66–8.5). A significant inverse correlation was noted between the number of action potentials induced by orthodromic stimulation and PCr/ATP levels ($P < 0.02$; Fig. 2B).

The studies examining the responses to single, low-frequency synaptic stimulation involve the synchronous activation of bundles of axons and a superthreshold response in the postsynaptic neurons. All of these processes are metabolically demanding and would be expected to correlate with a measure of bioenergetic potential. As an internal control, the frequency of spontaneous excitatory activity was compared with the PCr/ATP levels. We have previously shown that there is a higher frequency of these events in MTS tissue as compared with tissue from patients without significant cell loss (Williamson et al., 1995) and with the presence of mossy fibre sprouting (de Lanerolle et al., 1997). However, there was no correlation between this variable and PCr/ATP (data not shown). These data suggest that only those measures of excitability that are associated with a high energy demand correlate with the in vivo bioenergetic measures.

### Table 1 Patient data

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gender</th>
<th>Age at surgery (years)</th>
<th>Duration of epilepsy (years)</th>
<th>AED therapy</th>
<th>Pathology</th>
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<tr>
<td>1</td>
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<td>30.9</td>
<td>cbz, ltg, diamox</td>
<td>MTLE</td>
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<td>18.0</td>
<td>cbz</td>
<td>MTLE</td>
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<tr>
<td>3</td>
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<td>17.3</td>
<td>16.8</td>
<td>ltg, cbz, ph</td>
<td>MTLE</td>
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<tr>
<td>4</td>
<td>M</td>
<td>41.2</td>
<td>40.2</td>
<td>ph</td>
<td>MTLE</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>32.0</td>
<td>23.0</td>
<td>vpa, ltg, klonopin</td>
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</tr>
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<td>6</td>
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<td>46.4</td>
<td>tpm</td>
<td>MTLE</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>39.8</td>
<td>37.8</td>
<td>gpn, ltg, lev</td>
<td>MTLE</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>51.8</td>
<td>49.8</td>
<td>cbz, lev</td>
<td>MTLE</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>51.3</td>
<td>42.3</td>
<td>cbz, tpm, dzp</td>
<td>MTL</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>22.3</td>
<td>17.3</td>
<td>cbz, vpa</td>
<td>MTL</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
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<td>32</td>
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<tr>
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<td>F</td>
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<td>ph</td>
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<td>13</td>
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<td>gpn, tpm, ph</td>
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<td>60.4</td>
<td>52.4</td>
<td>lev, ltg</td>
<td>PTLE</td>
</tr>
</tbody>
</table>

The mean age of these patients was 39.0 ± 3.8 years, and they had a mean seizure duration of 31.9 ± 3.4 years. The majority of the patients had typical (MTLE) with cell loss throughout the hippocampus and extensive synaptic reorganization. These changes are not seen in the paradoxical temporal lobe epilepsy (PTLE) group. In one patient (No. 12) the only significant pathology was cell loss in CA1. Anti-epileptic drug (AED) regimens are abbreviated as follows: cbz = carbamazepine; diamox = acetazolamide; klonopin = clonazepam; ltg = lamotrigine; ph = phenytoin; vpa = valproic acid; tpm = topiramate; dzp = diazepam; gpn = gabapentin; lev = levetiracetam. The cell counts were performed as described by Kim et al. (1990). The value presented here reflects the percentage change in total neuronal numbers relative to autopsy controls.

### Notes

1. The normalized hippocampal PCr/ATP values for the 14 patients included here ranged between 0.82 and 1.01 (mean 0.91 ± 0.01). Examples of $^{31}$P spectra from the hippocampus of a patient and a healthy control volunteer are shown in Fig. 1B. The spectrum from the patient demonstrates the decline in PCr/ATP ratio that is evident in comparison with the control.

2. The studies examining the responses to single, low-frequency synaptic stimulation involve the synchronous activation of bundles of axons and a superthreshold response in the postsynaptic neurons. All of these processes are metabolically demanding and would be expected to correlate with a measure of bioenergetic potential. As an internal control, the frequency of spontaneous excitatory activity was compared with the PCr/ATP levels. We have previously shown that there is a higher frequency of these events in MTS tissue as compared with tissue from patients without significant cell loss (Williamson et al., 1995) and with the presence of mossy fibre sprouting (de Lanerolle et al., 1997). However, there was no correlation between this variable and PCr/ATP (data not shown). These data suggest that only those measures of excitability that are associated with a high energy demand correlate with the in vivo bioenergetic measures.
Extent of inhibition relates to energetic state

A recent study demonstrated that mitochondrial creatine kinase is highly enriched in inhibitory hippocampal interneurons compared with principal neurons in the normal adult rat. Moreover, this enzyme can be upregulated by seizures (Boero et al., 2003). Therefore, we tested the hypothesis that the strength of synaptic inhibition would be positively correlated with the PCr/ATP levels of epileptic humans in which there are distinct patterns of interneuronal loss and synaptic reorganization within the dentate gyrus (de Lanerolle et al., 1992; Matherne et al., 1995, 1999; Magloczky et al., 2000). Within the patient group studied, the mean polysynaptic IPSP conductance was 29.1 ± 5.6 ms (range 9.5–73.0 ms), which was concurrent with the range we have previously noted in granule cells from tissue resected from MTS patients (Williamson et al., 1999). As shown in Fig. 3, significantly higher IPSP conductances were noted in those patients with higher (more normal) levels of PCr/ATP (P < 0.05).

The correlations between these stimulus variables and the PCr/ATP may not have a common basis in hyperexcitability produced by disinhibition, as we did not observe a significant correlation between the IPSP conductance and burst scores. This question was examined both on a patient-by-patient (P = 0.13) and a cell-by-cell (P = 0.45) basis.

Stimulation-induced increases in metabolic demand

In the second series of experiments, the tissue was challenged using a 10 Hz, 10 s train with the recovery of neuronal membrane potential following the train measured. These prolonged trains should induce a significant metabolic load (Kann et al., 2003) and do not induce observable synaptic plasticity in the human dentate gyrus (A. Williamson, P. R. Patrylo, S. H. Lee and D. D. Spencer, unpublished results), and thus allowed us to indirectly evaluate the Na⁺/K⁺ ATPase function through activation of this enzyme during restoration of the membrane potential (Thompson and Prince, 1986; Xiong and Stringer, 2000). Although the Na⁺/K⁺ ATPase is a relatively slow ion pump compared with the ion channels involved in synaptic transmission, it uses much of the ATP generated in the brain to maintain the ionic gradients needed for neural functioning (Attwell and Laughlin, 2001). In addition, in the MTS tissue the extracellular potassium concentration ([K⁺]₀) may also be elevated following the train to a greater degree than in non-MTS tissue, owing to activity in the recurrent mossy fibre axons. Based on these data, we hypothesized that the PCr levels would also be associated with slower events as well as with the responses to synaptic stimulation.

The trains were delivered from a holding potential between −70 and −80 mV. In the majority of cells studied, the membrane potential depolarized during the train (17 of 23 cells; mean 7.05 ± 1.25 mV, range 2.1–13.9 mV). In the remainder of neurons, there was a hyperpolarization (six of 23; mean −4.6 ± 1.09 mV; range −2.3 to −8.3 mV). The recovery rates were not significantly different between hyperpolarizing and depolarizing responses; therefore, we used the absolute value for analysis. This change in membrane potential produced by the 10 Hz trains probably predominately reflects changes in the gradients for both Na⁺ and K⁺ rather than Cl⁻, as the bulk of γ-aminobutyric acid (GABAₐ) mediated conductance is inactivated by these trains (e.g. McCarren and Alger, 1985). The rate of recovery ranged from 0.122 to 3.2 mV/s (mean 1.01 ± 0.21 mV/s) by cell, and from 0.195 to 2.99 mV/s (mean 1.25 ± 0.19 mV/s) by patient.

Examples of the recovery of the membrane potential following a train are shown in Fig. 4A. Within the patient population studied, a highly significant correlation was noted between the rate of recovery and the PCr/ATP (P < 0.005) (Fig. 4B). This correlation was also seen when the time to 50% recovery of the membrane potential was correlated with the PCr/ATP (P < 0.02) (see arrows in Fig. 4B; data not shown). These data reveal that the more normal the levels of PCr/ATP, the more rapid the rate of recovery of membrane potential following a 10 Hz, 10 s train, and that patients with very poor energetics exhibited very slow recovery times in comparison.

We have used a consecutive series of patients in which both the PCr/ATP data and sufficient physiological data were available. As noted in Table 1, this group included both typical MTS patients as well as those with minimal cell loss in the dentate gyrus. In order to verify that the correlations observed between the physiological data and the MRS data were not skewed by the presence of the different pathologies, we examined the MTS group in isolation. The four correlations described above remained significant when the data from the MTS tissue were analysed in isolation (PCr versus burst, P < 0.05; versus GIPSP, P < 0.02; versus recovery time, P < 0.005; versus 50% recovery time, P < 0.05). Similarly, when the three non-MTS patients were examined, the data showed a similar trend, but the sample size was too small for a meaningful analysis.

Discussion

There is increasing recognition that hypometabolism, along with specific patterns of cell loss and synaptic reorganization, is a common feature of human MTLE. However, the cellular correlates to the hypometabolism seen in FDG-PET (Casse et al., 2002) or using 31P-MRS have not been established. The data reported here provide the first evidence for a relationship between in vivo bioenergetics and specific electrophysiological alterations in epileptic human tissue.

As described previously, each of the activities intrinsic to brain function may be ascribed an energetic cost (Attwell and Laughlin, 2001; Lennie, 2003). Those activities such as ionic gradient restoration and synaptic transmission would be expected to be ‘high’ cost activities, and therefore dependent on available PCr/ATP levels. Our data indicate that several distinct aspects of neuronal excitability are correlated with the PCr/ATP levels in this tissue, further supporting this broad hypothesis for epileptic tissue.
It is important to note that the responses to single electrical stimuli were correlated with the PCr/ATP, while a measure of baseline excitability (spontaneous activity) was not. These data are consistent with the hypothesis that the levels of PCr in brain are linked with the ability to maintain normal synaptic functions both in response to single stimuli, which will produce a limited metabolic load, as well as to trains of stimuli, which will potently activate mitochondrial respiration (Kann et al., 2003). Our data are consistent with the hypothesis that baseline, asynchronous activity does not require high PCr turnover, but that evoked stimulation, which activates numerous presynaptic and postsynaptic elements, does.

Moreover, we did note a highly significant correlation between PCr/ATP and the rate at which the membrane potential recovered following the train as well as the time needed for the membrane potential to recover to 50% of the amplitude. We posit that there is sufficient oxidative metabolic capacity in the dentate to support synaptic transmission, but that the lowered PCr levels limit the ability of the tissue to restore ionic gradients efficiently during repetitive stimulation. Ion gradients are restored by a wide variety of mechanisms including pumps and channels (Lux et al., 1986), both of which are altered in epileptic tissue. There are profound changes in the distribution and function of the Na\(^+\)/K\(^+\) ATPase in epileptic human tissue (Grisar et al., 1992; Brines et al., 1995). Brines et al. (1995) demonstrated that there is an increase in functional Na\(^+\)/K\(^+\) ATPase in epileptic human tissue compared with controls (normalized to total protein), but a decrease in cytochrome oxidase. That study did not, however, distinguish between neuronal and glial ATPase isoforms. This is consistent with our data showing that there is a decrease in the available energy needed to drive the pumps, but not a loss in the total number of ATPase molecules. Additionally, the ability of the glial ATPase to respond to elevations in [K\(^+\)]\(_o\) appears to be impaired in epileptic human tissue (Grisar and Delgado-Escueta, 1986; Grisar et al., 1992), which may explain the prolongation of the post-stimulus membrane potential seen here, especially in highly gliotic tissue.

Alternatively, a component of prolonged recovery of the post-stimulus membrane potential may be mediated through alterations in the regulation of [K\(^+\)]\(_o\) by Ba\(^{2+}\)-sensitive K\(^+\) channels (e.g. Gabriel et al., 1998a). However, studies using
K⁺-selective electrodes in human and epileptic CA1 found that there was a profound loss of Ba²⁺-sensitive K⁺ regulation in the most damaged region of the epileptic human hippocampus, but not the dentate (Gabriel et al., 1998a,b). These data are consistent with those of Bordey and Spencer (2002) demonstrating significant differences in glial properties between the CA1 and dentate regions in tissue resected from these patients.

Taken together, these data suggest that while the recovery of the membrane potential can reflect multiple variables (e.g. K⁺ channels, Na⁺/K⁺ ATPase), there is likely to be a profound role for changes in the efficacy of the Na⁺/K⁺ ATPase due to the availability of adequate energy reserves. It is important to note, however, that both neurons and glia are involved in the maintenance of the extracellular milieu, especially K⁺ buffering (Walz, 2000), and both cell types use PCr. We therefore cannot determine which cell type is primarily responsible for these physiological changes. This proposed inability of the dentate to maintain [K⁺]o during periods of synaptic activation due to metabolic failure may be a critical factor in allowing seizures to spread from the dentate gyrus to the very metabolically impaired CA3 region (Kunz et al., 2000; Elger and Kunz, 2003; Vielhaber et al., 2003b).

**Energetics relates to inhibitory function**

This latter correlation was unexpected, as the metabolic demands of inhibition may be expected to be swamped by that of glutamatergic excitation given that principal cells predominate. It is notable that we did not observe the expected correlation between the bursting score and GIPSP, which suggests that these measures of excitability are not directly linked. The fact that both variables correlate with PCr/ATP supports the hypothesis that both abnormal excitatory and inhibitory physiological responses depend on altered energetics. Several lines of data indicate, however, that the energetics of synaptic inhibition may be distinct from that of glutamatergic transmission. First, metabolic modelling studies have suggested that synaptic inhibition is energetically demanding, primarily because many interneurons can fire at frequencies up to 200 Hz and thus need higher levels of ATP to maintain the appropriate ionic gradients for neuronal function (Attwell and Laughlin, 2001). Secondly, the bulk of neuronal glutamate uptake is carried out by interneurons (e.g. Matthews and Laughlin, 2001). Secondly, the bulk of neuronal glutamate uptake is carried out by interneurons (e.g. Matthews and Laughlin, 2001). Moreover, in epileptic tissue specific groups in inhibitory hilar neurons are lost, while other groups undergo extensive synaptic reorganization (e.g. Mathern et al., 1995; Magloczky et al., 2000). The basket cells, however, tend to be preserved (Babb et al., 1989), but there may be changes in their function (e.g. Sloviter et al., 2003). The metabolic consequences of these changes have not been addressed.

We have shown previously that there are decreases in both polysynaptic and monosynaptic inhibition in patients with MTS (Williamson et al., 1999) relative to the paradoxical MTLE group. Similar findings were described in a rat model of MTLE (Kobayashi and Buckmaster, 2003). The IPSP conductances measured in the present group of patients is intermediate to those reported for the MTS and paradoxical MTLE groups (Williamson et al., 1999), reflecting the mix in pathologies represented (see Table 1). Our data, showing a positive correlation between the strength of polysynaptic inhibition and PCr/ATP values, suggest that some component of synaptic inhibition is coupled to readily available energy supplies. This may reflect the ability of interneurons to maintain adequate levels of GABA or possibly their membrane potential given the high firing rate of specific populations of interneurons.

Therefore, taken together with our data showing a strong correlation between evoked inhibition and PCr/ATP levels, synaptic inhibition may account for a greater proportion of neural metabolism than cell counts alone would predict. It is important to note that we observed all four of the physiology–MRS correlations within the whole patient population, as well as for the MTS patients alone. Therefore, these correlations may represent a general feature of brain metabolism.

**Possible confounds**

The levels of ATP in the brain remain stable during all levels of neural activity and even a modest drop in ATP levels is associated with altered consciousness. Therefore, in order to maintain these stable ATP concentrations, PCr and, consequently, ADP levels fluctuate with metabolic demand (reviewed in Kemp, 2000). Therefore, by normalizing to ATP, the PCr levels can be examined more independently of cell number, although the differences between ATP levels in neurons and glia (Urenjak et al., 1993) are a potentially confounding factor given the neuronal loss and concomitant gliosis that is observed in MTLE hippocampi. Although it has not been clearly established whether reactive glia have different levels of PCr compared with resting or non-reactive astrocytes in epileptic tissue, the gliosis associated with ischaemia does not appear to alter PCr/Cr levels (Konaka et al., 2003).

Thus although the energetic changes of different cell types cannot be separated, these in vivo measurements reflect an integration of the energetic state of all these cell types. Given the (neuronal) electrophysiological correlates seen with the present data, we postulate that neuronal activities may govern the integrated tissue energetic state and/or that the energetic contributions from other cell types are within noise or do not effectively bias the overall state.
As shown in Table 1, these patients were on a variety of anticonvulsant regimens. Some of these compounds are known to affect metabolic function, including barbiturates and topiramate (Marzatico et al., 1987; Vullo et al., 2004), and could thus confound the results. However, no difference was noted in the relationship between the PCr/ATP and the physiological variables when comparing the data from the seven patients taking neither barbiturates nor topiramate with those who were. These data suggest that the drug regimen of these patients does not appear to be a significant variable at this level of analysis.

It is important to note that these correlations are being made at very different levels of anatomical detail, in that the physiology is based on the responses from single neurons while the PCr/ATP measures are made from large ensembles of cells. There is the assumption, therefore, that the physiological responses of the recorded cells are typical of the larger population and that comparable changes are seen in the hippocampus proper (as compared with the dentate gyrus).

It is possible that these correlations between PCr/ATP and the physiological variables studied here are a reflection of a shared pathology and not of a shared mechanism. We do not believe this to be the case. These hippocampal PCr/ATP levels do not correlate with quantitative neuronal counts from the hippocampus (J. Pan and J. Kim, personal communication), either on a region-by-region basis or when a composite measure was used to assess the overall degree of sclerosis. Therefore, PCr/ATP is not a reflection of cell loss, but of a more dynamic regulation of neural function. Similarly, none of the physiological data correlated directly with the degree of granule cell loss. While our previous work has shown that different types of pathologies, e.g. MTS versus patients without cell loss and reorganization (de Lanerolle et al., 2003), are associated with distinct physiological alterations, this was not seen on a patient-by-patient basis in this study. Given that we noted no significant divergence of the PCr/ATP in relation to the physiological data from the three non-MTS patients (based on cell count data), we propose that the dynamic aspects of hippocampal function exist along a broad continuum and that the severity of the disorder is related to multiple factors, including bioenergetics.

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