

Chapter 2

Metabolism and Epilepsy

This chapter will examine some aspects of the metabolic (especially energy metabolism) and pharmacologic responses to epileptic seizures. This involves animal models as well as imaging studies in patients. Some results are in conflict, and this is probably linked to the usual differences in methodology, and in definitions and data interpretation.

What's certain is that in seizure activity, there is significant electrical discharge of groups of neurons sufficient to usually result in an uncontrolled motor response. This in turn is associated with a change in cerebral energy metabolism and changes in synaptic function.

Methodological techniques include two basic mechanisms. The first includes directly measuring metabolites and neurotransmitters, mostly in experimental animals. The second method is to estimate energy metabolism using 2-deoxy-glucose PET, and magnetic resonance spectroscopy, both suitable for measuring glutamate turnover. Imaging methods have the advantage of being able to evaluate many cerebral areas simultaneously.

Dozens of studies have been performed looking at the direct measurement of energy metabolites in brains of seizing laboratory animals. There have been studies showing little change in adenosine triphosphate (ATP) or phosphocreatine (PCr), however many studies have shown dramatic alteration in energy metabolites.

One key is to be mindful of the concept that many metabolic encephalopathies have highly regional cerebral effects, and that includes seizures. Studies in which effects on energy metabolism of seizures gained by analyzing whole brain are much less meaningful when compared to regional studies.

Glutamate, the most prominent excitatory neurotransmitter in brain, has turnover which is tightly coupled to glucose metabolism (Magistretti and Pellerin 1996).

The rapid removal of both glutamate and GABA from the synapse is an energy (ATP) requiring process, explaining the coupling between neurotransmitter and glucose metabolism. Compensatory mechanisms such as increased cerebral blood flow during seizures provide increased oxygen and glucose to the stressed brain.

Transporters which serve to remove glutamate and GABA from the synapse are an astrocyte product, and the astrocytes are in close proximity to the synapse. In the

Relationship between ATP and PCr

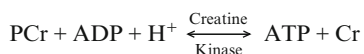


Fig. 2.1 Relationship between ATP-PCr. Adapted from Collins, R., Posner, J., and Plum, F. *Am. J. Physiol.* 218: p. 944, 1970

first few days after birth, the brain is more resistant to metabolic stress because overall utilization of high energy phosphates is lower due to lower relative activity.

As mentioned earlier, glutamate is the primary excitatory neurotransmitter, and GABA is the key inhibitory neurotransmitter. The balance between these two neurotransmitters is thought to be important in maintaining ion channel homeostasis. An increase in synaptic glutamate or a decrease in inhibitory GABA is seen as conducive to seizures. This simple concept underlies the mechanism of action of the majority of all antiepileptic drugs (AEDs).

Early studies of energy metabolism were undertaken to define characteristics of the seizures in relation to changes in high energy phosphates before, during, and after the seizure. Maximal electroshock was utilized as a stimulus for seizure production because of the consistency of the response and ease of producing it. One early study (Collins et al. 1970) looked at mice with electroshock-induced tonic-clonic seizures as regards cerebral energy metabolism.

The question asked was how do mice in tonic-clonic seizures compare when paralyzed and unparalyzed. The unparalyzed mice were not only seizing, but were hypoxic due to inadequate pulmonary ventilation. Mice were shocked through ear electrodes at a level which produced tonic-clonic seizures. Two groups received ventilation of either room air or 100% oxygen. Unparalyzed mice received nothing except the electroshock. Mice were sacrificed in liquid Freon, then the whole brains were dissected and saved frozen for analysis.

Results showed the mouse response to the stimulus consisted of a 15–20 s period of tonic-clonic extension followed by a 30–45 s period of clonic jerks. Postictal depression followed the ictal phase. Respiration ceased for the initial 20 s in unventilated paralyzed mice. The only seizure sign was a body twitch during delivery of the stimulus.

High energy phosphates ATP and PCr dropped significantly from control values in the unparalyzed group of mice. The decrease was greater than 50% in unparalyzed mice, and glucose was similarly decreased. After 25 s, values began to return to normal. By contrast, the paralyzed ventilated room air mice had depletions of only 37% for PCr, and 25% for ATP. Those mice ventilated with 100% oxygen had unchanged cerebral metabolites (see Fig. 2.1).

The authors comment that their results show that if the electroshock mouse model has an adequate oxygen supply, there is a capacity to balance a three- to fourfold increase in electroshock-induced energy demand. The ability of the brain to maintain energy reserves is dependent on a supply of 100% oxygen.

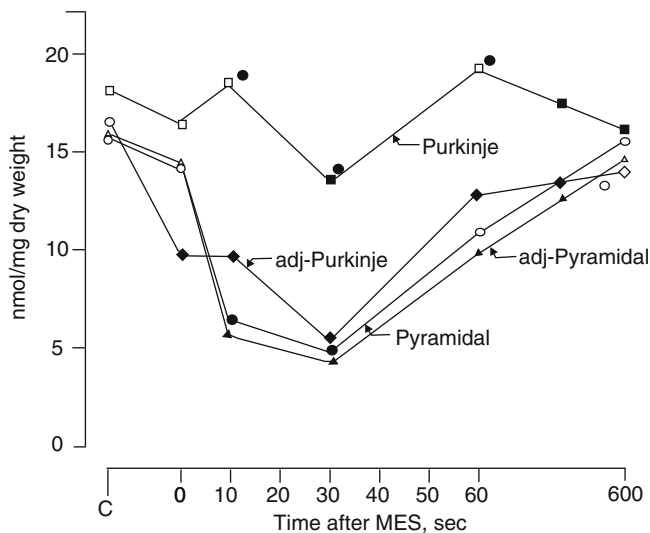


Fig. 2.2 Changes in ATP concentrations in cell bodies of cortex and cerebellum in mice after maximal electroshock. Redrawn from McCandless, D., et al. P.N.A.S. 76: p. 1484

In another report, the effects of maximal electroshock on energy metabolism were examined in single neural cells from the cortex and cerebellum of mice (McCandless et al. 1979). In this study, maximal electroshock was administered to mice via corneal electrodes, frozen at various times after electroshock, and the cortical/cerebellar samples freeze dried and cellular samples prepared and analyzed by elegant methods previously described (Lowry and Passonneau 1972).

Results showed that glucose, PCr, and ATP were decreased in cortical pyramidal neurons, Purkinje cells in the cerebellum, and adjacent neuropil in cortex and cerebellum following electric shock. The largest decrease in metabolism was seen in mice 30 s after maximal electroshock, and at 10 min later, there was an increase in metabolites.

A differential effect was seen in ATP values in Pukinje cells of the cerebellum. In this case, the ATP values were less affected in the cerebellar Purkinje cells than other metabolite levels. It seems that the electroshock is attenuated before it reaches the Purkinje cells. This is in keeping with the concept that the excitable state cannot be sustained in the cerebellum. This difference has not been described before and is only noted by examining single cells (see Fig. 2.2).

Purkinje cells represent the output of the cerebellum, and are inhibitory. The delayed response from the Purkinje cells suggests that when it does become obvious, the inhibitory output modulates the seizure activity coming from pyramidal cells. Thus, the output of the cerebellum is initially low. If the cerebellar output was increased immediately following maximal electroshock, the duration/severity of the seizure response might be lessened. The sparing effect is therefore deleterious to extracerebellar paroxysmal activity.

The 2-deoxy-glucose method (Sokoloff et al. 1977), used for neuroimaging, has been extensively employed to evaluate cerebral activity in an epilepsy mouse model, the E1 mouse. Following seizures in the E1 mouse induced by positional change, 2-deoxy-glucose was highest in brain 5 min after administration. The entire cortex in seizure mice showed a dense radiolabel. The entire hippocampus was similarly darkened by the presence of the isotope.

The results of the 2-deoxy-glucose study represent the total serum level of isotope deposition over the 40 min of the exposure. This includes both the seizure time period and partial recovery. Lightly labeled areas such as thalamic nuclei may represent hypometabolism or decreased circulatory activity. These results are somewhat dissimilar when compared to electroshock and this is due to different mechanisms.

The effects of status epilepticus on cerebral cortical energy metabolism have been described in primates (McCandless et al. 1986). Many studies have focused on single seizures and energy metabolism, while only a few have looked at status epilepticus (Meldrum 1983). Status is of special interest due to the supposed severe cellular damage which can result (Meldrum et al. 1973). The use of primates facilitates translation.

In this study, *Cynomolgus fascicularis* monkeys were immobilized with ketamine and after ether anesthesia, a craniotomy was performed, exposing the cerebral cortex. EEG electrodes were placed on the cortex. A sharpened spoon was used to remove small motor cortex samples prior to bicuculline administration, 20 min after onset of seizures, and 2 h after the previous seizure (2 h 20 min).

Tissue samples were prepared for metabolite analysis as previously described (McCandless et al. 1979). Samples were prepared for electron microscopy by fixation in glutaraldehyde and postfixation in osmium tetroxide. Blood samples were removed for measuring glucose.

Results showed that bicuculline produced sustained epileptiform activity on EEG recordings. At 2 h 20 min, the EEG was characterized by slow, low voltage activity, and occasional spikes. EEG results were normal in appearance until 2 h 20 min, at which time endoplasmic reticulum was dilated, mitochondria were swollen, vesicles were seen, and the cytoplasm was watery and dispersed.

The effects of bicuculline-induced seizures on energy metabolites were pronounced. Glucose samples from the motor cortex were essentially not changed, but PCr was statistically significantly decreased in the two pyramidal cell layers (outer small pyramidal cells and inner large pyramidal cells), and in adjacent white matter 20 min after bicuculline. In contrast, ATP was maintained at normal levels at 20 min, whereas 2 h later, ATP was decreased in all layers of the motor cortex except white matter. Phosphocreatine had returned to normal at 2 h 20 min (see Figs. 2.3 and 2.4).

Many previous studies have examined energy metabolism in experimental models of seizures. Most studies that looked show regional changes in high energy phosphates. But, just like regional changes varying from site to site, so are differences seen between cortical layers from the same site. This emphasizes the importance of measuring labile metabolites in samples as small as possible. The present study shows this still holds true in prolonged status epilepticus.

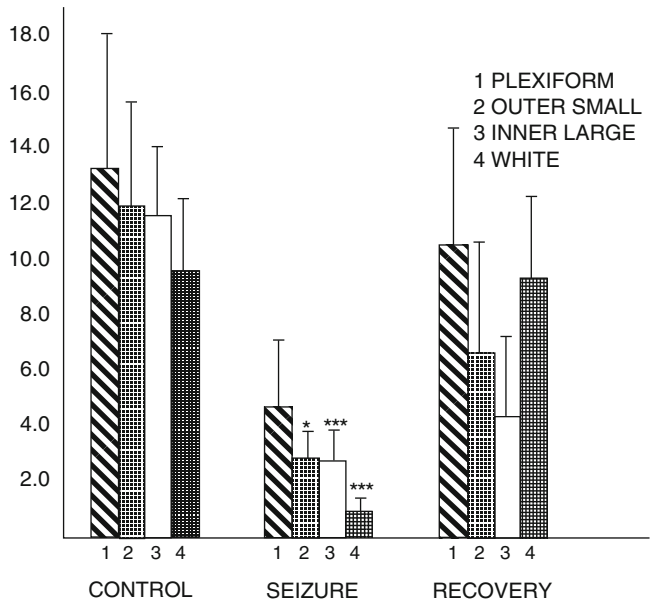


Fig. 2.3 Effect of bicuculline-induced seizures and recovery on regional motor cortex phosphocreatine. Data expressed as nmoles/mg dry weight. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.005$. McCandless, D., et al. Am J. Physiol. 251: p. 778, 1986

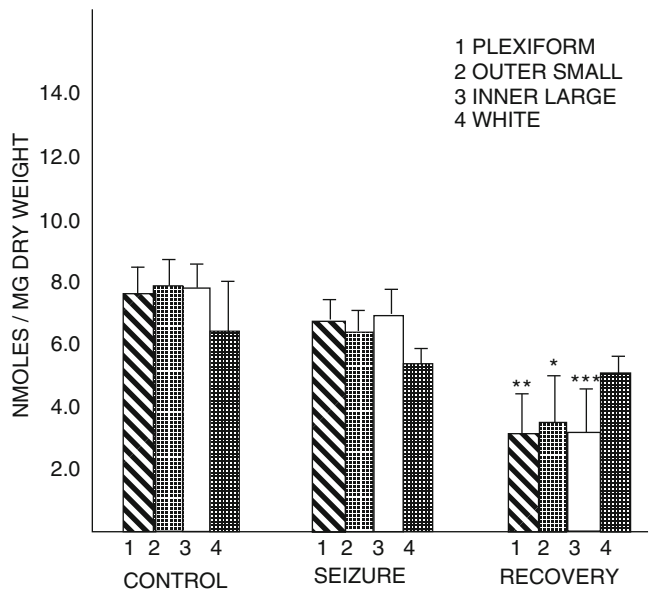


Fig. 2.4 Effect of bicuculline-induced seizures and recovery on regional motor cortex ATP. Layers and significance as in Fig. 2.3. Redrawn from McCandless, D., et al. Am J. Physiol. 251: p. 778, 1986

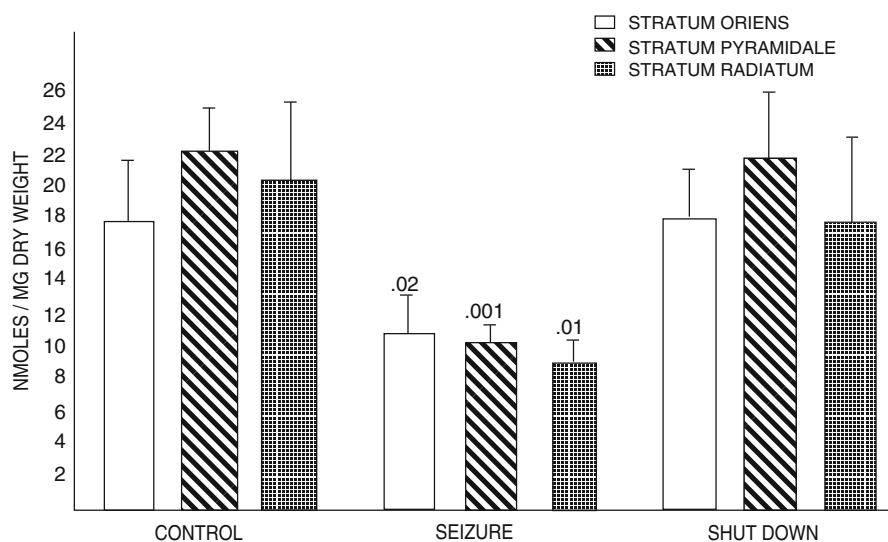


Fig. 2.5 Hippocampal CA1 phosphocreatine during and after seizure activity. Data (mean plus/minus SEM) expressed as nmoles/mg dry weight. Redrawn from DeFrance, J. and McCandless, D. M.B.D. 6: p. 89, 1991

The E.M. changes seen in the present study in mitochondria are in keeping with changes in energy metabolism. These changes are similar to that seen in ischemic cell damage. The possibility that repeated motor cortex sampling might have contributed was examined in a series of rats in which cortical samples were removed over a 2 h 20 min period and metabolism analyzed. There were no significant changes in energy metabolites in this series of rats treated exactly like the primates, except for bicuculline. It is likely single cell analysis might be more revealing than the layer results. Data on metabolite turnover would also be of interest.

The hippocampus displays a postictal depression period in which further seizures cannot be elicited. The hippocampus plays an important role in memory, and so hippocampal postictal depression may underlie postictal amnesia (Penfield 1958). The present study (DeFrance and McCandless 1991) was undertaken to look at energy metabolism in rat brain hippocampus following electrical stimulation.

Results showed that during the electrically induced seizure activity, glucose, ATP, and phosphocreatine were all decreased by about 40% in all three hippocampal CA1 layers. Within 60 s after the seizure, metabolite levels had returned toward normal. At a time when the hippocampus (CA1) was unresponsive, metabolites were at control levels. This suggests the physiological shutdown of the hippocampus was not associated with perturbed energy metabolism. Whatever is the basis of the postictal refractory state, depleted energy metabolites are not the mechanism. These results do not rule out the possibility that some group of single cells is not involved. Testing this would require single cell analysis (see Figs. 2.5 and 2.6).

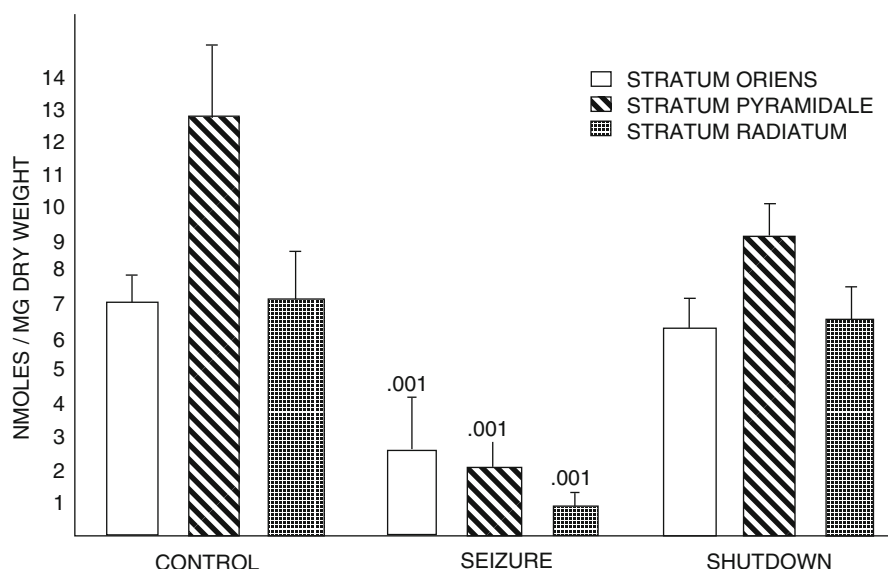


Fig. 2.6 Hippocampal ATP CA1 during and after seizure activity. Data (mean plus/minus SEM) expressed as nmoles/mg dry weight. Redrawn from DeFrance, J. and McCandless, D. M.B.D. 6: p. 89, 1991

Further evidence as regards energy metabolism and seizures can be derived from results of treatment with the ketogenic diet (Bough 2008). The ketogenic diet (and the modified Atkins diet) are very high in fat, low in carbohydrates, and adequate in all other requirements (see Chap. 30). The diet results in increased levels of ketone bodies, which are utilized to affect the depletion of glucose as a result of low carbohydrate intake.

Microarrays are used to identify patterns of gene expression in rats on a ketogenic diet for 3 weeks. Results show 384 transcripts were upregulated. The most frequent group of differentially expressed genes was those associated with energy metabolism, and 21 encoded genes were involved in oxidative phosphorylation (Bough et al. 2006).

Examination of mitochondria by electron microscopy showed a 46% increase in mitochondrial density in the ketogenic diet rats as compared to control animals. When actual energy metabolites were measured, ketogenic diet rat hippocampus showed little change in ATP, ADP, or AMP, however PCr was elevated. This is in keeping with the concept that the ketone bodies act to “supercharge” the energy capabilities of cerebral tissue. Glutamate was also elevated, but most brain glutamate is used as an energy substrate.

The mechanism of action of the ketogenic diet on seizure control is not clear, but metabolic perturbations may contribute significantly to synaptic instability. The author speculates that the E.M. result showing a 50% increase in mitochondrial density suggests an increase in ATP production. This excess energy is stored in the

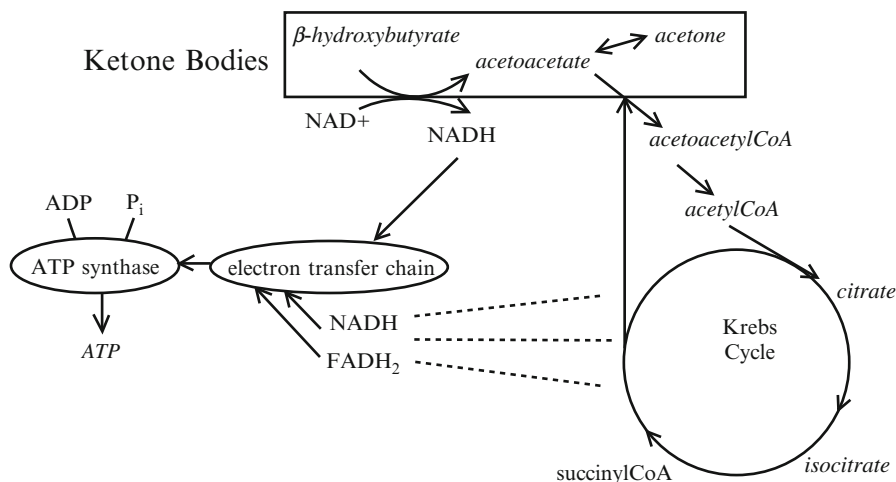


Fig. 2.7 Ketone body metabolism. Adapted from Massino, S., et. al. *Curr. Neuropharmacol.* 7: p. 260, 2009

form of PCr until needed as ATP. This acts to stabilize the brain electrically leading to a lower sensitivity to seizures. This has also been seen in humans (Pan et al. 1999), (see Fig. 2.7).

Further data supports the concept that positive energy balance, produced by the ketogenic diet can benefit GABA. The creatine kinase enzyme is found mostly in GABAergic neurons (Boero et al. 2003). In this case, the ketogenic diet enhancement of energy metabolism could in turn act to stabilize postsynaptic GABA_A receptors. There may also be an effect on glutamate such that its levels are lowered.

The author concludes saying much data exist supporting the idea that the ketogenic diet “dramatically” enhances cerebral energy metabolism leading to increased energy stores, and a stabilization of brain in terms of potential seizure activity. The author states the ketogenic diet is efficacious due to its ability to compensate for other deficits in epileptic foci.

Glycogen may also play a role in seizures in both animal models and in human epilepsies (Cloix et al. 2008). During periods of hypometabolism in epileptic foci, glycogen from astrocytes, or glycogenolysis can be a source of potential energy. Brain biopsies of hippocampus from human epileptic patients have been noted to be high relative to other brain areas (Dalsgaard et al. 2007).

Methionine sulfoximine (MSO) is a compound which resembles glutamate, and may actually cause seizures by acting at the synapse in a way similar to that of glutamate. In this way MSO could induce seizures. MSO has been administered IP as an inducer of seizures in experimental models of epilepsy for over 50 years. Seizures in mice and rats are generalized tonic-clonic seizures. The time frame is a preictal period of several hours, followed by continuous seizures from 24 to 48 h. This is followed by a postictal period.

The preseizure period is characterized by an accumulation of intracerebral glycogen, which in turn signals no correlation with the convulsive period, or a consequence of seizure activity. Accumulation of glycogen is usually localized to astrocytes in the cerebral cortex and cerebellum. With MSO administration, there is a significant accumulation in the cortex and cerebellum of mice and rats. Seizure latency and glycogen accumulation are attributable to genetic factors.

Another paper from the same group (Cloix and Hvéor 2009) examines various aspects of neurotransmission and epilepsy/energy metabolism. Glutamate and GABA are the most common neurotransmitters in the CNS, with glutamate being an excitatory neurotransmitter and GABA being inhibitory. The ionotropic receptors associated with glutamate are AMPA, NMDA, and kainate receptors. As regards GABA receptors, the two are GABA_A and GABA_B. The former facilitates inward movement of chloride ions, the latter the exit of K ions; both yield an inhibitory action. It is a disequilibrium of these two neurotransmitters which can produce seizures.

Cortical pyramidal cells (large and small) project axons into other brain sites and the spinal cord. These cells are excitatory and neurotransmission is via glutamate. Axons of cortical basket cells contact other cortical neurons and are inhibitory using GABA as a neurotransmitter. These neurons represent the main contributions to EEGs. The EEGs are contributed to by millions of neurons, mostly cortical pyramidal output.

The EEG is composed of by alpha, theta, and delta waves, and is a result of synchronization of groups of neurons (Liberson 1989). Synchronization occurs in seizures and large spikes and waves occur. Synchronization etiology is unclear, as in the “all or none” timing. How thousands of neurons accomplish this feat at the same time requires more of an explanation than decreased inhibition. This area needs more study.

The relationship between astrocytes and neurons no doubt involves a role for astrocytes in the supply of energy to neurons. This occurs especially when the energy potential is not met by blood supply. Two explanations may occur: one is that the astrocyte supplies the neurons with lactate to enter the TCA cycle and the other explanation suggests the astrocytes supply glucose to the neurons.

Glucose enters brain from blood, crossing the BBB in so doing. Three main GLUT proteins facilitate this transport, GLUT1, GLUT2, and GLUT3. GLUT3 is thought to have a higher affinity for glucose as well as a higher capacity for glucose transport. In addition to transport into neurons, it may be that some glucose is transported to astrocytes, then into neurons. Both processes may be occurring simultaneously. Lactate is transported out of cells by MCT transporters.

Adenosine is a neuromodulator with a wide range of actions in the CNS. It is involved in neurochemical responses and morbidity in traumatic brain injury (TBI) (Lusardi 2009), as well as many other neurological perturbations (Boison 2008a). In terms of TBI, epilepsy is common sequelae which has a significant latency. Seizures related to TBI can materialize many years later, even decades, after a TBI.

Recovery from TBI is frequent, especially in terms of gross motor function and intellectual domain, but various sequelae/comorbidities may remain. These include migraine, anxiety, and chronic pain, as well as epilepsy. Adenosine modulation is a key feature of these TBI-related comorbidities.

Posttraumatic epilepsy is a common long latency after effect of TBI. This can occur in both pediatric and adult TBI patients. That the A1 receptor is involved in seizure suppression is shown by results in which an A1 receptor knockout mouse is highly susceptible to status epilepticus. Even in cases in which there are no overt seizures, epileptiform activity is demonstrable. Studies implicate adenosine dysregulation in epileptogenesis (Boison 2008b).

A group of inborn errors of creatine metabolism have been recently described (Schulze 2003). Creatine transport deficiency is one of several of these inborn errors. The diagnosis of this disorder is made by incubating fibroblasts, measuring creatine uptake, and analysis of the SLC6A8 gene *in vitro*. The phenotype in males includes mental retardation, language delay, autism, and epilepsy.

In a recent retrospective study (Fons et al. 2009), clinical data, seizure types, treatment, etc., were reviewed in seven patients. Results showed an age range from 11 years to 40 years old. All had moderate to severe mental retardation, and six of seven had epilepsy. No genotype–phenotype correlations were obvious.

All patients showed slow EEG background activity, without specific paroxysmal activity. Brain levels of creatine were not associated with epilepsy severity. Imaging studies failed to show any association with brain structural lesions. The authors state that any patient with mental retardation, language disorder, autism, and seizures should be tested for creatine deficiency.

Creatine plays a significant role in forming phosphocreatine, an energy yielding compound. This is a critical role in cerebral energy metabolism. This is another example of an energy metabolism alteration which would appear to directly produce seizures and epileptiform activity.

Another recent paper related to energy metabolism and adenosine has appeared (Masino et al. 2009). Adenosine serves at least two key functions in brain: first as a neuromodulator, and second as a vital component of ATP. In terms of epilepsy and ATP, the efficacious ketogenic diet used to treat refractory epilepsies, has as a mechanism, the providing of ketone bodies which feed the TCA cycle, thereby increasing ATP and stabilizing neurons. This in turn decreases seizures and acts to protect neurons (Masino and Geiger 2008).

Adenosine is a neuromodulator, and the receptor A1 is inhibitory. It is located at least in the hippocampi and cerebral cortices, and when increased, can serve to prevent seizures. In a sense, adenosine as a modulator of brain activity, acts at a “higher” level much as does neuropeptide Y, or ketogenic mechanisms.

The effects of both adenosine and the ketogenic diet on brain metabolism are rather different than that of classical AEDs. Thus, their use in AED refractory epilepsy cases has shown significant beneficial effects. *In vitro*, ketone bodies increase ATP/ADP ratios, and the ketogenic diet has a similar effect *in vivo* on energy metabolite ratios in specific brain regions. Hippocampal CA3 neurons can in fact actually regulate their own excitability when glucose is low, and ATP is in sufficient concentration, or higher.

The clinical significance of this can hardly be understated. Evidence is well established that ketone bodies, adenosine, creatine, etc., can decrease seizures and offer cellular protection, and stabilize neurons such that epileptiform activity stops.

Many pediatric patients have a highly significant decrease in seizure frequency or outright cessation of seizures which can be long lasting. This effect is not limited to pediatric patients (Bodenant et al. 2008).

The authors of this paper conclude saying that “.....the metabolic relationship among adenosine, a ketogenic diet, and epilepsy could open major new therapeutic applications and avoid peripheral side effects in a way that has eluded receptor-based strategies.” This entire area warrants much additional study and translational trials.

A clinical technical problem of significance is the physical resolution of MRI. This problem has been addressed using 18F-FCWAY (18F-trans-4-fluoro-N-2-(4-(2-methoxyphenyl) piperazin-1-Y)ethyl-N-(2 pyridyl) cyclohexane carboxamide PET. This acts to improve resolution such that more specific 5-HT receptor binding is seen.

In this study, the authors (Liew et al. 2009) noted that 30% or more patients with refractory to AEDs temporal lobe epilepsy show no lesions on MRI examination. Foci localization is difficult in these patients, and 18F-FDG PET may not be a viable option. The present study was performed in order to evaluate 18F-FCWAY in refractory epilepsy cases. The goal was localization of otherwise obscure foci in refractory temporal lobe epilepsy cases.

Twelve patients were studied. All had medically refractive temporal lobe epilepsy, and all had MRIs which had resolution insufficient to permit identification of structural abnormalities. Results using 18F-FCWAY showed in 11 of 12 patients an identifiable lateralized epileptogenic area of focus. Nine patients were resected, and five of these were seizure free at one year postoperatively.

The authors comment that both 18F-FCWAY-PET and 18F-FDG-PET were helpful in seeking epileptogenic zones in nonlesioned (by MRI) temporal lobe epilepsy. 18F-FDG-PET could have shown potentially misleading images in two cases. 18F-FCWAY-PET may be a more accurate method than 18F-Fdg-PET.

About one third of all epilepsy patients are refractory to AEDs, and become theoretical candidates for surgery. Many of these are not actually good surgical candidates for a variety of reasons such as multiple foci, and thus better nonsurgical therapies are essential. Recent data has suggested an amplification of glutamate activity may be conducive to the seizure state. The present paper examines this hypothesis (Eid et al. 2008).

It is suspected that excessive extracellular glutamate may contribute to seizure activity and an imbalance in levels of GABA/glutamate. In humans, extracellular hippocampal glutamate increases sixfold during seizures, and this lasts several minutes after the seizure ends. A slowing of the glutamate/glutamine cycle is thought to be the cause of the increased extracellular glutamate.

Glutamate does not cross the BBB readily, so most is synthesized from glucose in astrocytes. The first cell type in this scheme is astrocytes, therefore there are several routes possible, including the TCA cycle. Alpha ketoglutarate is one metabolite associated with the TCA cycle, and from which glutamate is formed.

Glutamate in the astrocyte is converted to glutamine (enzyme: glutamine synthetase) then transported to neurons where it again becomes glutamate which can

enter extracellular space. Glutamine synthetase is a key enzyme in the glutamate/glutamine cycle. The authors of this paper speculate that an alteration of glutamine synthetase can slow the formation of glutamine, thereby causing excess glutamate, conducive to seizure activity. A loss of astrocytic glutamate transporters could have a similar effect, however the loss of glutamine synthetase is a more likely explanation (Petroff et al. 2002).

A chronic animal model of hippocampal glutamine synthetase deficiency has been developed by daily injections of MSO into the hippocampus. This model is associated with spontaneous recurrent seizures and lowered hippocampal glutamine synthetase activity. The hypothesis is that the loss of hippocampal glutamine synthetase is intimately involved in the pathology of mesial temporal lobe epilepsy.

The mechanism for the glutamine synthetase deficiency may be related to a downregulation due to neuron loss in the hippocampus. This has been shown previously (Derouiche et al. 1993). The glutamate/glutamine cycle can be studied using human tissue from patients undergoing temporal lobe resection for epilepsy (Eid et al. 2004).

Results from the above-mentioned studies of four mesial temporal lobe epilepsy patients showed normal glutamate/glutamine cycling in the neocortex and hippocampus. However, when challenged with ammonia, an increased cycling occurred in the cortex, but not in the hippocampus. This finding could be significant even though there are decreased numbers of neurons in the hippocampi of mesial temporal lobe epileptic patients. Studies have shown that astrocytes can be a source of extracellular glutamate (Volterra and Meldolesi 2005). Astrocytes do not have glutamine synthetase in the hippocampi of temporal lobe epileptic patients, therefore accumulation of astrocytic glutamate may impair clearance of the compound from extracellular space. These data, although suggestive, require further investigation.

Another recent paper examines aspects of glutamine synthetase activity (Bidmon et al. 2008). The study authors note that astrocyte specific glutamine synthetase is intimately involved in glutamate recycling as well as GABA metabolism. It is proposed that glutamine synthetase activity alteration is associated with epilepsy, but the exact mechanisms are not clear.

In this study, Wistar rats were maintained in a cage in which seizure activity was measured. Three groups of rats were defined: one group received saline, another group received MSO, and a third received the convulsant pentylenetetrazole. Groups 1 and 3 were sacrificed at 14 days, and group 2 was sacrificed 24 h after treatment.

Results showed rats receiving 2 weeks of pentylenetetrazole injections had an upregulation of heat shock protein-27 (HSP-27) in cortical and hippocampal astrocytes. MSO-treated rats showed significant glutamine synthetase inhibition and HSP-27 induction. Repeated pentylenetetrazole injection induced seizures in all rats, but the phenotype was not identical. There was a small decrease in glutamine synthetase activity after 2 weeks, but a more significant decrease after MSO treatment.

MSO treatment resulted in a more uniform and widespread HSP-27 induction compared to that induced by pentylenetetrazole injections for 14 days. The MSO group showed a significant decrease in glutamine synthetase immunoreactivity in

the cortex and hippocampus. There were subregion differences in hippocampal and cortical areas.

Results further showed repeated seizures elicited a stress response in astrocytes, but adversely affected glutamine synthetase by tyrosine nitration and inhibition. The effects were localized to epileptic circuitry and amygdala related structures. The changes were most significant in the entorhinal cortex and dentate gyrus.

Differences between the pentylenetetrazole model and the MSO model include the concept that the pentylenetetrazole model induces seizures in seconds by affecting GABA neurotransmission. MSO, however, is a partial inhibitor of a precursor of GABA and glutamate. Concomitant ammonia increases are not associated with seizures. MSO-induced seizures may be caused by other features such as cerebral energy metabolism and neurotransmitter changes (Cloix and Hevor 1998).

The authors state their findings suggest binding of MSO to the active site of glutamine synthetase and its phosphorylation blocks the enzyme conformation, decreasing activity. Nitration probably has no adverse effect.

The data do support findings of Steffens (Steffens et al. 2005) showing no activity changes in glutamine synthetase in the temporal cortex of human resected tissue. Thus, in the pentylenetetrazole model the authors of the present paper were unable to find any reduction in glutamine synthetase activity. The implication is that the pentylenetetrazole model of tonic-clonic seizures may be excellent for the study of early processes in borderline nonsclerotic tissue. Many questions remain to be resolved in this interesting area.



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