Bioenergetic Mechanisms of Seizure Control

Richard Kovács1*, Zoltan Gerevich1, Alon Friedman2,3, Jakub Otáhal4, Ofer Prager2, Siegrun Gabriel1 and Nikolaus Berndt5,6

1 Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institut für Neuropsychiologie, Berlin, Germany, 2 Departments of Physiology and Cell Biology, Cognitive and Brain Sciences, The Zlotowski Center for Neuroscience, Ben-Gurion University of the Negev, Beersheba, Israel, 3 Department of Medical Neuroscience, Faculty of Medicine, Dalhousie University, Halifax, NS, Canada, 4 Institute of Physiology, Czech Academy of Sciences, Prague, Czechia, 5 Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institut für Biochemie, Berlin, Germany, 6 Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institute for Computational and Imaging Science in Cardiovascular Medicine, Berlin, Germany

Epilepsy is characterized by the regular occurrence of seizures, which follow a stereotypical sequence of alterations in the electroencephalogram. Seizures are typically a self-limiting phenomenon, concluding finally in the cessation of hypersynchronous activity and followed by a state of decreased neuronal excitability which might underlie the cognitive and psychological symptoms the patients experience in the wake of seizures. Many efforts have been devoted to understand how seizures spontaneously stop in hope to exploit this knowledge in anticonvulsant or neuroprotective therapies. Besides the alterations in ion-channels, transmitters and neuromodulators, the successive build up of disturbances in energy metabolism have been suggested as a mechanism for seizure termination. Energy metabolism and substrate supply of the brain are tightly regulated by different mechanisms called neurometabolic and neurovascular coupling. Here we summarize the current knowledge whether these mechanisms are sufficient to cover the energy demand of hypersynchronous activity and whether a mismatch between energy need and supply could contribute to seizure control.

**Keywords:** seizure, lactate, adenosine, neurometabolic coupling, neurovascular coupling, pericyte

**INTRODUCTION**

Epilepsy is a common neurological disease characterized by the manifestation of unprovoked seizures (Fisher et al., 2014). An epileptic seizure is “a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain” (Fisher et al., 2005). Seizures are represented by a stereotypic sequence of electroencephalographic events and associated clinical symptoms. Seizure termination can be described as a homeostatic self-limitation of the hypersynchronous activity, representing not a mere cessation but a characteristic change in the excitability of the nervous tissue. These changes evolve successively and may already start during the course of the seizure before the activity would terminate (Essig and Flanary, 1966). The current review focus on the contribution of altered energy metabolism to seizure control. For reviews on other anticonvulsant mechanisms mediated by intrinsic ion-channel modifications, changes in functional, network and synaptic properties of the neurons and effects of neuromodulators see Löscher and Köhling (2010) and Zubler et al. (2014).
Seizures represent an extraordinary burden on the energy metabolism of the brain as restoration of the transmembrane ion gradients is mediated by energy consuming pump mechanisms. Thus, it is tempting to speculate that the metabolic changes that develop slowly during the course of a seizure might finally lead to the cessation of the hypersynchronous activity. Metabolic alterations may include altered substrate and oxygen availability, accumulation of metabolic intermediates and byproducts (such as adenosine, lactate and CO$_2$) which in turn leads to activation of adenosine receptors, extracellular acidosis and opening of K$_{ATP}$ channels due to local shortage of ATP. These changes build up consecutively during the course of a seizure establishing conditions that not only contribute to the cessation of the activity but could potentially alter the normal neuronal functioning in the postictal phase. Conversely, disturbed energy metabolism could by itself favor seizure initiation by altering the ability of the tissue to recover transmembrane ion gradients following neuronal activity, as it is seen in mitochondrial encephalopathies (Kang et al., 2013; Zsurka and Kunz, 2015) or in mesial temporal lobe epilepsy (Rowley and Patel, 2013; Boison and Steinhäuser, 2018). Intercritical hypoperfusion (Guillon et al., 1998; Suh et al., 2005; Geneslaw et al., 2011) and hypometabolism (Hetherington et al., 1995; Cendes et al., 1997; Hong et al., 2002) have been demonstrated in a wide range of epileptic syndromes including temporal lobe epilepsy, generalized childhood absence epilepsy and status epilepticus. Although seizure-induced cell loss and sclerotic modification of the tissue could partially explain the hypometabolic state, there is also evidence for dysfunction of the neurometabolic coupling (Kann et al., 2005) and oxidative damage of respiratory enzymes (Kunz et al., 2000; Vielhaber et al., 2003; Folbergrová and Kunz, 2012; Rowley and Patel, 2013). Lasting deficiencies in energy metabolism might underlie the frequent observation that “seizures-beget-seizures” (Ben-Ari, 2001; Farrell et al., 2017b) by favoring the initiation of subsequent hypersynchronous activity (Zsurka and Kunz, 2015). Mismatch in the neurometabolic coupling might be responsible for seizure-associated cell loss via either free radical dependent or hypoxic mechanisms (Ingvar, 1986; Frantseva et al., 2000; Kovács et al., 2002; Rowley and Patel, 2013). Metabolic disturbances might also contribute to the development of pharmaco-resistance, i.e., the incapability to prevent seizures with two or more antiepileptic therapy regimens directly or indirectly following sclerotic transformation of the tissue (Kovács and Heinemann, 2014). Thus, it is fundamental to understand the potentially pro- or anticonvulsant consequences of the seizure-associated changes in energy metabolism.

In the following we provide a short overview of the literature about the alterations of neurometabolic coupling during seizures and how these could contribute to seizure control. We discuss possible therapeutic targets which exploit metabolism-related mechanisms (2-deoxy-D-glucose (2DG), pyruvate, fructose 1,6 bisphosphate, ketogenic diet, CO$_2$ inhalation, carbonic anhydrase inhibitors, adenosine 1 agonists). The last part is devoted to the mechanisms of seizure-induced neurovascular decoupling, which might be responsible for postictal hypometabolism and have been suggested to be associated with cognitive disturbances. We apologize that we cannot extensively discuss all aspects of seizure termination and regulation of neuronal energy metabolism as well as the anti-seizure mechanisms associated to caloric restriction and ketogenic diet.

**NEUROMETABOLIC COUPLING DURING ACUTE SEIZURES AND IN CHRONIC EPILEPTIC TISSUE**

Neuronal activity results in local changes in transmembrane ion-gradients, such as the increase in extracellular [K$^+$] which is immediately countered by a concurrent increase in Na-K-ATPase activity (Lux et al., 1986). In order to keep pace with the ATP-demand of the ion-pump activity as well as that of the transport processes related to synaptic signaling (Liotta et al., 2012; Hall et al., 2012), energy metabolism has to adopt to the different neuronal activity states (Ames, 2000; Heinemann et al., 2002; Berndt et al., 2015). This neurometabolic coupling could operate via “pull” and “push” mechanisms, i.e., either as a consequence of alterations in ATP or other energy metabolism intermediates, or in a feed forward manner by activity dependent changes in intracellular and intramitochondrial Ca$^{2+}$, K$^+$ and nitric oxide (NO) concentrations (Szewczyk et al., 2006; Denton, 2009). During physiological and intercritical activity changes, in overall intracellular ATP concentration are expected to be rather small (see Seizure Associated Alterations in Energy Metabolism Intermediates and pH). Yet, an increase in ATP utilization by the Na-K-ATPase leads to a concomitant increase in ADP and AMP concentration, as the adenylate kinase maintains a fast equilibrium between the adenine nucleotides. Assuming an ATP level of 3mM and an ADP level of 0.3 mM, even a minor decrease in ATP by 10% would decrease the ATP/ADP ratio from 10 to 4.5 and increase AMP levels more than fourfold. These changes in adenylates have immediate consequences for the regulation of energy metabolism (Figure 1). First, the adenine nucleotide translocator is activated leading to a decrease in mitochondrial ATP and mitochondrial membrane potential ($\Psi$) as the exchange of ATP (3 negative charges) for ADP (2 negative charges) is an electrogenic process. The shift in cytosolic and mitochondrial ATP/ADP ratio and the decrease in $\Psi$ activate F0F1-ATPase, further draining the $\Psi$. As a consequence, complexes IV, III, and I of the ETC are activated by associated shifts in the electron carriers, cytochrome C and ubiquinone (QH$_2$). This activation results in the initial oxidation shift in mitochondrial NADH, which can be observed by NADH-autofluorescence (Kovács et al., 2001; Schuchmann et al., 2001; Berndt et al., 2015). The decrease in mitochondrial NADH activates the regulatory dehydrogenases of the tricarboxylic acid cycle as well as the pyruvate dehydrogenase reaction, thereby channeling pyruvate into the tricarboxylic acid cycle. At the same time, the glycolytic pathway is activated by the decreased ATP/ADP ratio and the increase in AMP at the level of lactate (Berndt et al., 2015).
FIGURE 1 | Schematic representation of central neuronal energy metabolism (adapted from Bedner et al., 2015). Proton dependent processes are presented in magenta and calcium dependent processes are presented in blue. Glycolysis degrades glucose beginning with hexokinase (hk) dependent phosphorylation to glucose-6-phosphate ending with pyruvate kinase (pk) dependent formation of pyruvate. Pyruvate can be either transformed to lactate by the lactate dehydrogenase (LDH) and be exported from the cell by monocarboxylate transporter (MCT) (anaerobic glycolysis) or taken up into the mitochondria via proton symport by pyruvate transporter (PyrT) with subsequent conversion to Acetyl-Coa by pyruvate dehydrogenase (PDH) for oxidation to CO2 in the citric acid cycle (CAC) (aerobic glycolysis). The CAC cycle, starting with the formation of citrate (Cit) from oxaloacetate (OA) and acetyl-CoA (ACoA) by citrate synthase (CS) and proceeding via the formation of isocitrate (IsoCit) by aconitase (ACN), α-ketoglutarate (αKG) by isocitrate dehydrogenase (IDH), succinyl-CoA (SucCoA) by α-ketoglutarate dehydrogenase, succinate (Suc) by succinyl-CoA synthetase, fumarate (Fum) by fumarate dehydrogenase, malate (Mal) by malate dehydrogenase (MDH), forms NADH and FADH2 used by the respiratory chain (RC). Complex I-IV of the RC are proton pumps generating the proton motive force that determine the rate of proton-assisted ion transport of Na+, K+, Ca2+ and phosphate (P) across the inner mitochondrial membrane, the rate of the adenine nucleotide transporter (ANT) exchanging mitochondrial ATP against cytosolic ADP and the rate of ATP synthesis by the FO1-ATPase (FO1). Shuttling of electrons (NAD-bound hydrogen) between the cytosol and the mitochondrial matrix is catalyzed by the Malate-Aspartate shuttle, comprising the malate/α-ketoglutarate carrier (MAC), the mitochondrial and cytosolic aspartate amino transferase (AAT) and the proton driven antiport of glutamate (Glu) and aspartate (Asp) by the aspartate/glutamate carrier (AGC). Besides protons, calcium plays a key role in neuronal energy metabolism as it controls the key mitochondrial dehydrogenase PDH, IDH and KGDH and the rate of electron shuttling by AGC (ARALAR). Calcium uptake into the mitochondrion proceeds in a calcium dependent manner by MCU thereby coupling neuronal excitation with neuronal energy metabolism.

The “push” mechanisms, such as the mitochondrial Ca2+-cycle, are complementary in regulating energy metabolism (Kovács et al., 2005; Wei et al., 2011; Berndt et al., 2015; Nicholls, 2017; Kannurpatti, 2017). Neuronal firing and synaptic activity results in localized transient cytoplasmic Ca2+ increases, which are sequestered by mitochondria via the mitochondrial Ca2+ uniporter (MCU) channel complex leading to increased matrix Ca2+ concentration. Matrix Ca2+ plays a key regulatory role as it can enhance ETC in a potassium dependent manner (Szewczyk et al., 2006), increase aspartate malate shuttle and
pyruvate supply (Gellerich et al., 2012; Rueda et al., 2014) and it is a positive modulator of three key dehydrogenases of the tricarboxylic acid cycle (TCA, McCormack et al., 1990; Denton, 2009), thereby leading to the enhanced formation of reducing equivalents (Figure 1). Typical biphasic changes in the redox state of flavin and adenine dinucleotides (FADH2 and NADH) can be observed following physiological stimulus in vivo (Kasischke et al., 2004) and during seizure-like events in brain slices consisting of an initial oxidation and a lasting reduction which outlasts the hypersynchronous activity (Schuchmann et al., 1999; Kovács et al., 2001). In this latter preparation enhanced Ca2+ cycling across the mitochondrial membrane during seizures has been shown to be responsible for the dissipation of ψ (Kovács et al., 2005), although the contribution of the mitochondrial permeability transition induced by high mitochondrial Ca2+ load cannot be completely excluded (Kovac et al., 2012). While mitochondrial depolarization by definition decreases the proton motive force (composed of Δψ and ΔpH), it could also represent one way to enhance respiration (Malinska et al., 2010). Due to the fixed stoichiometry of the F0F1-ATPase of three protons per generation of one ATP, the decrease in proton motive force will have no energetic efficiency consequences as long as the energy of the three protons exceed the formation enthalpy of ATP. However, the ψ dependence of the F0F1-ATPase activity leads to a strong activation and thereby increased ATP formation (see above). Thus, seizure termination in vitro seems to occur in the presence of enhanced energy metabolism, when neither the availability of the reducing equivalents nor oxygen could represent a limitation for the electrical activity (Schoknecht et al., 2017). However, in case of frequently recurring seizure-like events, which would resemble status epilepticus in vivo, signs of metabolic impairment develop in a free radical dependent manner (Kovács et al., 2002; Malinska et al., 2010; Zsurka and Kunz, 2015). Under these conditions seizure termination is impaired and the electrical activity transforms from seizure-like events to pharmacoresistant late recurrent discharges, indicating that the slowly developing energy crisis is rather pro-epileptic (Schuchmann et al., 1999; Ivanov et al., 2015). Seizure-associated enhancement of mitochondrial free radical formation might play an important role in the development of metabolic disturbances both in vitro (Frantseva et al., 2000; Kovács et al., 2002) and in vivo (Kunz et al., 2000; Vielhaber et al., 2003; Rowley and Patel, 2013). In addition, the seizure associated increase in NO formation was found primarily pro-epileptic as blockade of the neuronal NO synthase decreased severity of kainic acid induced seizures in vivo (Beamer et al., 2012) and delayed seizure onset in vitro (Schuchmann et al., 2002; Kovács et al., 2009). Unfortunately, it is not possible to unequivocally determine the contribution of NO-mediated inhibition of the electron transport chain complexes to the pro-epileptogenic effect (Ledo et al., 2010), as NO influences neuronal excitability and cerebral blood flow in many different ways.

Most of the aforementioned findings were obtained in experiments with acutely induced epileptiform activity in tissue samples of otherwise healthy animals. However, in chronic epileptic tissue the metabolic consequences of prior activity might influence the effects of seizures on energy metabolism. Indeed, signs of metabolic impairment were evident in sclerotic hippocampal tissue from epilepsy patients as well as in the pilocarpine model of epilepsy, which may correspond to the hypoperfusion and hypometabolism in these areas (Kunz et al., 2000; Vielhaber et al., 2003; Kann et al., 2005). In conclusion, although metabolism related factors (see below) might contribute to seizure termination, this can also happen in the presence of intact neurometabolic coupling and - at least in vitro - no obvious restriction of glucose and oxygen supply (Figure 2). On the other hand, metabolic disturbances during frequently recurring seizures or in chronic epileptic tissue rather favor than limit the occurrence of hypersynchronous activity (Otáhal et al., 2014, and citations therein).

**SEIZURE ASSOCIATED ALTERATIONS IN ENERGY METABOLISM INTERMEDIATES AND pH**

Recurrent seizures represent an extraordinary metabolic burden, leading to increased tissue lactate and decreased phosphocreatine, glucose and glycogen content (Folbergrová et al., 2000). One plausible explanation for self-limitation of the hypersynchronous activity would be an exhaustion of the high energy phosphate and/or the glucose reserves. Neuronal metabolism is fueled almost exclusively by glucose as substrate, but the role of glucose availability in seizure development and termination is controversial. The glucose transporter GLUT1 plays an essential role in capillary to brain glucose transport and mutations in the SLC2A1 gene that encodes GLUT1 lead to deficiency of the transporter and cause several types of generalized epilepsy (Hildebrand et al., 2014). Epilepsy patients with diagnosed GLUT1 deficiency highly benefit from ketogenic diet. Severe hypoglycaemia by itself is known to induce seizures and moderate hypoglycemia decreased the threshold of seizure induction by fluorothyl in vivo (Kirchner et al., 2006). This could be brought about by the enhanced excitability as a consequence of altered Na-K-ATPase activity. Alternatively, an imbalance of excitatory and inhibitory neuron tonus has been also suggested, as the latter cells are more dependent on oxidative metabolism (Pumain et al., 2008). On the contrary, hypoglycaemia exerted anticonvulsant effects in vitro on low-Mg2+ induced seizures (Kirchner et al., 2006). With respect to another immediate energy resource, phosphocreatine, it was shown that brain-type creatine kinase deficient transgenic mice were less prone to develop seizures under pentylenetetrazole. In these animals initial discharges developed into a depression, which suggests that an energy crisis might actually contribute to seizure termination (Streijger et al., 2010). However, the evidence for an energy crisis in terms of ATP depletion is rather limited in other animal models of epilepsy. ATP and high energy phosphate levels are kept close to normal even during recurrent seizures (status epilepticus) lasting for hours as was shown in an NMR study of kainate-induced status (Meric et al., 1994). By modeling seizure associated changes in energy metabolism intermediates and cerebral metabolic rate of oxygen consumption (CMRO2) in a brain slice preparation
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FIGURE 2 | Summary scheme of seizure-associated changes in cellular energy metabolism and cerebral blood. (A) Seizure-associated changes in transmembrane ion gradients ultimately activate neuronal energy metabolism in order to counterbalance the ATP-need of the ion pump activity. In brain slices kept under constant carbogen gassing conditions seizure-associated enhancement of respiration can be measured as a drop in pO$_2$ whereas a shift in the fluorescence of NADH represents the metabolism-dependent redox shift of this electron carrier (fp: local field potential). Whereas the respiration slowly returns to the pre-seizure values following cessation of the activity, the reducing shift in the NADH/NAD$^+$ ratio outlast the changes in respiration. This mismatch might favor the late enhancement of reactive oxygen species (ROS) formation, as represented here by the oxidation of the mitochondrially targeted ethidium derivative, MitoSox in the same preparation. Seizures are associated with reversible arteriolar and capillary vasodilation, the latter likely mediated by contractile pericytes, and a consequent overshooting pO$_2$ response. (B) There are considerable differences in the seizure-associated neurometabolic and neurovascular responses between early and late events during status epilepticus, as well as in healthy and chronic epileptic tissue (arrows indicate the direction of the change of individual parameters). Seizure-associated overshooting NADH reduction eroded during recurrent seizures in vitro and it was almost absent in chronic epileptic tissue from epilepsy patients, due to the free radical dependent damage of mitochondrial dehydrogenases. On the other hand, mitochondrial injury might in turn enhance subsequent formation of reactive oxygen species (ROS) in a vicious cycle. Seizures resulted in lactate release both in control and chronic epileptic tissue samples, whereas in the latter even interictal lactate levels were increased likely reflecting altered expression pattern of monocarboxylate transporters and energy metabolism of glial cells. Seizure-associated enhancement of respiration and lactate release result in a metabolic acidosis, which – along with the enhanced adenosine levels- represents a strong anticonvulsant mechanism even in chronic epileptic tissue. Despite the induction of massive CBF increase by seizures vascular responsiveness was found to be decreased following status epilepticus, and changes in pericytic reactivity are observable already few minutes after seizure onset. This could be brought by oxidative stress of these cells as described in the no reflow phenomenon after hypoxia-reperfusion. Although seizure-associated increase in nitric oxide (NO) formation contributes to the hyperemia, in the presence of superoxide NO is turned into peroxynitrite with deleterious consequences on microcirculation. Postictal hyperresponsiveness of the microvasculature has been speculated to underlie cognitive disturbances and epilepsy comorbidities, whereas damage of the blood-brain barrier (BBB), albumin leakage and the subsequent transformation of the nervous tissue were shown to be pro-epileptogenic. (Hippocampal pyramidal cell and capillary pericytes are just for illustration purposes, some of the traces in A are adopted from Malinska et al., 2010).

Schoknecht and colleges showed that a fivefold increase in CMRO$_2$ was able to compensate for the enhanced energy demand of seizures whereas the decrease in overall cytosolic ATP content remained relatively small (Schoknecht et al., 2017). Nevertheless, in certain metabolic compartments – such as the axonal/presynaptic domain of fast spiking interneurons - larger local ATP consumption could be hypothesized (Hu et al., 2018).

In addition to the increased blood flow and the consequent increase in glucose supply, astrocytic glycogen reserves are also mobilized during seizures (Folbergrová et al., 2000). Astrocytes form an electrically and metabolically coupled network of cells (Boisson and Steinhäuser, 2018), which is capable to transport glucose to the most active areas during seizure-like events in vitro (Rouach et al., 2008). Impairment of gap junctional coupling and connexin expression of astrocytes is often observed in chronic epileptic tissue (Bedner et al., 2015). Whether these changes contribute to the control of seizures remains controversial as both, pro- and anticonvulsant effects of the uncoupled network are possible, via impaired potassium, glutamate buffering and...
nutrient redistribution, respectively (Boison and Steinhäuser, 2018; and citations therein).

In addition to enhanced oxidative metabolism, surplus glucose can be turned into lactate by anaerobic metabolism. Interstitial lactate accumulation during seizures is a general finding in patients, animals as well as in in vitro models of epilepsy (Calabrese et al., 1991; Meric et al., 1994; Doğan et al., 2017). In epilepsy patients interictal lactate levels were found to be elevated, suggesting altered energy metabolism and lactate turnover in general (Cavus et al., 2005). Although lactate is effectively removed into the bloodstream, it has been long speculated that lactate might serve as an alternative substrate for energy metabolism of neurons under conditions of increased demand. Despite the controversy on the validity of astrocytic-neuronal lactate shuttle (ANLS) (Pellerin and Magistretti, 2012; Diener, 2017), lactate seems to play a role in synaptic transmission and plasticity in the presence of ample glucose and might support energy demanding network oscillations in the gamma range (Galow et al., 2014; Nagase et al., 2014). Monocarboxylate transporters (MCT) mediate lactate uptake and release, depending on the transporter affinity and the concentration gradients, although lactate release via ion channels has been also described (Pierre and Pellerin, 2005; Sotelo-Hitschfeld et al., 2015). MCT4 is the major MCT isoform present with particularly high density at the astrocytic endfeet enwrapping the cerebral microvasculature (Pierre and Pellerin, 2005), hinting that MCT4 facilitates the removal of lactate into the circulation. In addition to the perivascular endfoot, the high affinity MCT2 isoform is preferentially localized on post-synaptic structures (Bergersen et al., 2005; Bergersen, 2007) suggesting that this isoform might be responsible for the metabolic support of synaptic signaling (Galeffi et al., 2007; Nagase et al., 2014). The low affinity isoform MCT1 is expressed in astrocytes, oligodendrocytes and capillary endothelial cells and it is critically involved in oligodendrocyte development and maintenance of axon functions (Lee et al., 2012).

We have recently shown that intrinsic lactate supports synaptic signaling in rat hippocampal slices and it contributes to energy demand of the restoration of transmembrane ion gradients (Angamo et al., 2016). Inhibition of the MCT2-mediated lactate transport by α-cyano-4-hydroxycinnamic acid (4CIN) led to a decrease in postsynaptic action potential generation and this effect was dependent on the activation of K<sub>ATP</sub> channels, indicative of local energy shortage. Indeed, MCT inhibition reduced basal and stimulus-dependent CMRO<sub>2</sub> and induced marked alterations in the redox state of mitochondrial FADH<sub>2</sub> (Angamo et al., 2016). With respect to hypersynchronous activity we found a strong inhibition of interictal- and seizure-like events in presence of 4CIN in different in vitro models of epilepsy. Interestingly, while K<sub>ATP</sub> channels contributed to the suppression of synaptic activity, the anticonvulsant effect of MCT inhibitors could not be blocked by glibenclamide, which is in line with the assumption that seizure-associated overall increase in ATP consumption is counterbalanced by the enhanced oxidative metabolism (Schoknecht et al., 2017). The anticonvulsant effect of MCT inhibitors was present both, in brain slices from control rats and also in chronic epileptic tissue from epilepsy surgery. This suggests that lactate support of energy metabolism during seizures is a general phenomenon and it is not affected by the observed changes in MCT expression in chronic epileptic tissue (Lauritzen et al., 2012; Angamo et al., 2017). The anticonvulsant effect of lactate uptake inhibitors complements the study of Sada and colleagues, demonstrating that inhibition of the lactate dehydrogenase (LDH) with a stiripentol analog suppressed seizures in the pilocarpine model in vivo (Sada et al., 2015).

The suppression of seizure-like activity by MCT inhibitors was not mediated by extracellular lactate accumulation and subsequent acidosis but rather by activation of adenosine receptors following adenosine release upon enhanced neuronal activity in the presence of substrate restriction. Application of the adenosine 1 (A1) receptor antagonist DPCPX could reverse completely the antiseizure effect of 4CIN (Angamo et al., 2017) indicating the critical role of these receptors in seizure termination. Indeed, endogenous adenosine is known to be released during seizures and A1 receptors are mainly responsible for the control of neuronal excitability by adenosine (Dunwiddie, 1980; Fedele et al., 2006; Lovatt et al., 2012). Microdialysis studies in patients with temporal lobe epilepsy have also shown that adenosine levels rise as a consequence of seizures and the subsequent seizure termination is mediated by the activation of A1 receptors (During and Spencer, 1992; Van Gompel et al., 2014). A1 receptors are Gi/o protein coupled receptors which reduce the release of glutamate by inhibiting voltage-gated calcium channels (Kuroda, 1978; Mogul et al., 1993) and decrease postsynaptic excitability of neurons by enhancing G-protein coupled inwardly rectifying potassium conductances (Haas and Greene, 1984; Trussell and Jackson, 1987). Adenosine is thought to act as a neuromodulator involved in the homeostatic balance between inhibition and excitation, whereby A1 receptors may be responsible for reduced excitability and synaptic transmission subsequently resulting in a dampened network activity (Cunha, 2001; Dias et al., 2013). In fact, A1 receptors were found to decrease the power of gamma oscillations in hippocampal brain slices (Pietersen et al., 2009; Schulz et al., 2012). Activation of A1 receptors has in general a strong anticonvulsant efficacy in both rodent and human brain slices (Szybala et al., 2009; Klaft et al., 2012, 2016). A1 agonists were even capable to prevent the transition to status epilepticus-like recurrent discharges in vitro (Avsar and Empson, 2004) suggesting that status epilepticus may result from impaired adenosine receptor-dependent seizure termination mechanisms (Young and Draganow, 1994; Koczanek et al., 2006; Hamil et al., 2012).

Notably, inhibiting glycolysis by application of 2DG is able to arrest seizures in different in vivo models (Stafstrom et al., 2008, 2009; Shao and Stafstrom, 2017). However, a more intricate set of effects is hinted by the findings that acute exposure to 2DG could either elevate or decrease the seizure threshold, depending on the epilepsy model (6 Hz stimulation versus kainic acid, PTZ, electroshock and amygdala kindling induced seizures; Gasior et al., 2010), whereas chronic application of 2DG initiated epileptogenesis (Samokhina et al., 2017), likely mimicking the effect of mild hypoglycaemia.
Thus, Gasior and colleagues concluded that the proconvulsant action might be related to restriction of glucose uptake and subsequent hypoglycaemia while anticonvulsant effects could work via the inhibition of glycolysis. Under these conditions, glucose metabolism is shunted via the pentose phosphate cycle, which has been suggested to be anticonvulsant per se (Lian et al., 2007). Remarkably, in this study bypassing the block of the glycolytic pathway by the administration of extrinsic lactate abolished the anticonvulsant activity of 2DG in the pilocarpine model of epilepsy. This finding further reinforces the role of intrinsic lactate as possible energy resource during seizures and not a mere byproduct, which has to be removed into the bloodstream. Alterations in the expression pattern of different MCT subtypes in chronic epileptic tissue are in favor of lactate retention (Lauritzen et al., 2012), which might explain the increased interictal lactate levels, despite of the hypometabolism (Cavus et al., 2005).

The acute anticonvulsant effects of the ketogenic diet might be also related to the fact that ketone bodies represent a bypass of glycolysis, supporting the role of lactate in seizure control (Hartman and Stafstrom, 2013). However, mimicking the ketogenic diet in vitro failed to prevent the occurrence of seizures (Samoilova et al., 2010). On the long term both, chronic 2DG supply and ketogenic diet seem to work by changing gene expression and the whole machinery of energy metabolism (Bough and Rho, 2007). Similar long term changes might underlie the fact that chronic metabolic support by pyruvate administration can be actually anticonvulsant in three different epilepsy models (Popova et al., 2017). As these alterations are not expected to contribute to seizure termination under normal dietary conditions, the reader is referred to excellent recent reviews on this topic.

Although the effect of MCT inhibitors was not mediated by changes in pH, the acidosis occurring during seizures is in turn capable to reduce neuronal excitability (Lux et al., 1986; Xiong et al., 2000; Wemmie et al., 2013) and by itself can exert strong anticonvulsant effects (Caspers and Speckmann, 1972). Extracellular acidosis emerges from the enhanced respiration resulting in H2CO3 formation in addition to the release of lactate (Mookerjee et al., 2015). Besides its intracellular effects (Wei et al., 2011) metabolic acidosis can alter excitability by many different ways (Sinning and Hübner, 2013), i.e., by activation of ASIC channels on interneurons (Ziemann et al., 2008) negative modulation of the N-methyl-D-aspartate (NMDA) receptor currents (Tang et al., 1990), inhibition of presynaptic voltage gated Ca2+ currents (Wemmie et al., 2013) as well as facilitation of ecto-ATPases and increasing adenosine release (Dulla et al., 2005).

Nevertheless, intracellular acidosis could directly influence GABAergic transmission as the GABA receptor channel is permeable to HCO3− (Pavlov et al., 2013; Ruusuvuori and Kaila, 2014). Indeed, inhibitors of brain carbonic anhydrases, which actively control pH balance by catalyzing the interconversion of carbon dioxide to bicarbonate (HCO3−) and a proton (H+), possess anticonvulsant effects (Reiss and Oles, 1996; Mishra et al., 2018).

Thus, while manipulation of substrate availability and metabolic pathways could be pro and anticonvulsive, acidosis and adenosine represent two powerful and interdependent negative feedback mechanisms for intrinsic seizure termination by signalizing excessive metabolic activity before shortage of the substrates would appear (Dulla et al., 2005; Tolner et al., 2011).

**SEIZURE-ASSOCIATED CHANGES IN CMRO2 AND CEREBRAL BLOOD FLOW**

Besides the potentially catastrophic events such as disturbances in cerebral blood flow or phenomena like spreading depolarization (Dreier, 2011), seizures represent a metabolic burden which likely pushes brain energy metabolism to its limits. This assumption is supported by the finding that energy demanding physiological type of network activity, such as carbachol induced lasting gamma oscillations in the brain slice preparation, result in a similar increase in CMRO2 as observed during seizure-like activity in the same tissue (Huchzermeyer et al., 2008; Kann et al., 2011). While the enhancement of CMRO2 is sufficient to keep transmembrane ion-gradients constant during gamma oscillations, seizure-like events result in massive changes in ion homeostasis (Huchzermeyer et al., 2013). Thus, it is tempting to speculate that ion transport capacity— and the corresponding ATP demand— is covered by the increase in the CMRO2 under physiological network activity. Any further increase in ion-fluxes exceeding pump capacity would result in altered transmembrane gradients, without further induction of CMRO2 increase.

Seizure-associated increases in CMRO2 in vivo can be calculated from the simultaneous determination of local tissue pO2 and cerebral blood flow (CBF, Gjedde, 2005; Thomsen et al., 2009) or by using fMRI as CBF, CMRO2 and CBV are the dominant physiologic parameters that modulate the BOLD signal (Kida et al., 2000). However, these calculations has to take into account the local variability of the signals and the fact that their relation can be changed by the seizure itself. Thus under bicuculline induced lasting ictal activity a positive BOLD was observed in the cortex and a negative BOLD signal in the hippocampus, despite of the increase in CBV and neuronal activity in both regions, suggesting a local mismatch between CMRO2 and CBF (Schridde et al., 2008).

The brain slice preparation offers a simpler solution, because in the absence of blood flow, and permanent carbogen gassing (95%O2 5%CO2) of the tissue, local pO2 depends solely on the rate of respiration and the diffusion distance from the surface (Liotta et al., 2012; Huchzermeyer et al., 2013). By recording basal and seizure-associated changes in pO2 and extracellular potassium/sodium concentration it was possible to calculate ATP consumption, ATP levels, CMRO2 and to predict the electron flow via the TCA enzymes and electron carrier nucleotides, FADH2 and NAD (Schoknecht et al., 2017). The transformation from interictal to seizure-like events was associated with a fivefold increase in oxygen consumption, whereas the corresponding increase in ATP demand was significantly higher, indicating that coupling ratio (ATP/O2) and thereby the efficiency of oxidative metabolism is improved during seizure-like events. Notably, at
maximum CMRO₂, tissue pO₂ as well as the reducing equivalents for the ETC were not rate limiting for the energy metabolism, suggesting that shortage of these factors is not a necessary prerequisite for seizure termination (Foster et al., 2005; Ivanov et al., 2015). Although these data were obtained in vitro, the results on CMRO₂ and pO₂ distribution can be extrapolated to the in vivo situation by knowing the intercapillary distance and the seizure-associated changes in local cerebral blood flow response.

In general, based on the close relationship between neuronal activity and vascular response (i.e., neurovascular coupling), seizures induce reversible vasodilation and disproportionately high increases in the blood flow, resulting in an overshooting supply of oxygenated hemoglobin, underlying the seizure-associated BOLD response (Tyvaert et al., 2009; Thornton et al., 2010). The first hypotheses of functional hyperemia assumed that increased energy consumption by active neurons induce vasodilation directly by altering the concentration of extracellular potassium, oxygen, protons and different metabolites (Nilsson et al., 1978). Alternatively, neuronal activity-dependent increase of extracellular glutamate can induce the release of vasoactive substances such as NO leading to subsequent smooth muscle cell relaxation (Lauritzen, 2005; Busija et al., 2007). Astrocytes, the anatomical intermediaries between neurons and blood vessels, are important mediators of neurovascular coupling by releasing either vasodilator or vasoconstrictor agents in addition to potassium signaling at the astrocytic end-feet (Zonta et al., 2003; Filosa et al., 2006; Gordon et al., 2008; Petzold and Murthy, 2011; Filosa and Iddings, 2013). Ictal increases in parenchymal lactate concentration also contribute to the regulation of cerebral blood flow. In the presence of restricted oxygen availability and high astrocytic calcium concentrations astrocytic lactate release is maximized. The subsequent lactate accumulation attenuates transporter-mediated uptake of prostaglandin E2 (PGE2) from the extracellular space leading to subsequent vasodilation (Gordon et al., 2008). Thus, the vascular effect of lactate has to be kept in mind when considering the anticonvulsant effects of lactate uptake or glycolysis inhibitors under in vitro conditions. Finally, seizure-associated increases in extracellular adenosine have also vasodilatory effects by inhibiting constriction of arteriolar smooth muscle cells via adenosine receptors (Gordon et al., 2008).

The overshooting blood flow response to seizures led to the ‘unorthodox’ hypothesis that ictogenesis is actually a restorative process to increase supply in underperfused areas (Doman and Pelligra, 2003). Nevertheless, more recent results show that BOLD signal is preceded by a local pO₂ dip and temporary reduction of the blood flow was documented in the tissue surrounding the epileptic focus as well as in the contralateral hemisphere (Zhao et al., 2011; Ma et al., 2013; Harris et al., 2014), indicating that the underperfusion per se is not an initiator of ictogenesis.

Status epilepticus and even a single seizure is associated with neuronal injury which in turn might favor secondary ictogenesis (Heinemann, 2004). The first clear proof of selective neuronal loss following status epilepticus appeared early in the 18th century, describing a sequence of cellular changes observed with Nissl staining in the brains of seven patients dying during the course of status epilepticus (Clark and Prout, 1903). The idea that ischemic processes could explain the cell loss in status epilepticus was developed about 100 years ago but questioned steadily based on the presence of massive hyperaemia and hyperoxygenation (Pfleger, 1880; Meldrum, 2002). Alternatively Pinard et al. (1984) suggested that not hypoxia but rather the release of endogenous substances and excitotoxic cascades mediate an excessive rise in [Ca²⁺]i resulting in cell death. Theoretically, a relative hypoperfusion of the tissue might occur also with intact neurovascular coupling if we presume that the diffusion-limited oxygen delivery can not keep up with a local increases in CMRO₂. This kind of hypoperfusion might underlie the local negative BOLD signal indicating a mismatch between CBF and CMRO₂ (Schridde et al., 2008). While such changes would remain undetected with the conventional doppler flowmetry (Geneslaw et al., 2011), doppler-based functional ultrasound imaging modality could reveal seizure-associated relative hypoperfusion at the microcircuity level (Urban et al., 2017). Oxygen transport from the capillary in the tissue is driven by the oxygen gradient within the tissue. Thus an increase in CMRO₂ would require an equal increase in the oxygen gradients. Taking into account that during high energy demand a significant part of the tissue exhibits pO₂ below 50% compared to the capillary value (Schneider et al., 2017), the increase in capillary pO₂ observed as a positive BOLD signal can still mean hypoxic pO₂ at certain places within the brain parenchim. Such phenomenon was observed during spreading depolarization in the neocortex where monitoring the tissue redox potential revealed hypoperfused “islets” between capillaries (Takano et al., 2007). Similar hypoperfused areas might be present at the immediate vicinity of seizure focus, indicating altered blood distribution rather than disturbances of neurovascular coupling (Zhao et al., 2009). On the other hand, status epilepticus was shown to induce damage and to severely alter the neurovascular unit, including dysfunction of the blood-brain-barrier (BBB, Abbott and Friedman, 2012; Heinemann et al., 2012), which might also affect the functionality of neurovascular coupling and substrate supply during recurrent seizures. Thus interictal hyperperfusion is not a sole consequence of cell loss and impaired neuronal energy metabolism but it might also represent disturbances of neurovascular coupling at the microcirculation level (see next chapter).

**UNCOUPLING OF BLOOD FLOW AND ICTAL EVENTS AND POSTICTAL DISTURBANCES OF METABOLIC SUPPLY**

Neurovascular coupling necessitates information flow between neurons, astrocytes, endothelial cells and the contractile elements of the vasculature, i.e., the smooth muscle cells in arterioles and pericytes in capillaries (Petzold and Murthy, 2011; Hall et al., 2014). Disturbances in NVC has been associated with several pathologies, including stroke (Yemisci et al., 2009), hypertension (Dunn and Nelson, 2014), Alzheimer’s disease...
or endomicroscopy, the difficulty with monitoring of pericytic
the brain tissue and only accessible to multiphoton imaging
seizures. Besides the fact that capillaries are buried deep in
of metabolic- and neurovascular-abnormalities associated with
importance of examining the time course and development
of subsequent seizures. Altogether, these studies highlight the
contribute to the development of local under-supply in case
conventional methods of blood flow monitoring and could
changes in capillary perfusion would remain undetected with
hypoxia-reperfusion, epileptic seizures are also associated with increased formation
of oxygen and nitrogen centered free radicals, both in vivo
hyperglycemia and insulin resistance, are common features of chronic metabolic diseases (Kovács et al., 2002,
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(Keating et al., 2014). Manipulating substrate availability, preventing
glycolysis, lactate uptake as well as enhancing pentose phosphate
shunt might have both acute pro- or anticonvulsant effects,
whereas chronic dietary changes can exert seizure control
via epigenetic mechanisms. Thus targeting negative feedback
mechanisms involved in metabolism dependent regulation of
excitability as well as supporting cellular bioenergetics and
protecting neurovascular coupling may represent promising
therapeutic approaches in the treatment of epilepsy.

CONCLUDING REMARKS

In conclusion, substantial evidence suggests that seizure
termination can occur in the presence of intact neurometabolic
coupling and overshooting neurovascular responses without
immediate limitation on the metabolism. On the other hand,
enhanced energy metabolism exerts a strong negative feedback
on neuronal excitability, mostly via adenosine and pH changes.
Free radical-dependent damage of mitochondrial enzymes and
the subsequent disturbances in energy metabolism observed in
chronic epileptic tissue were found to be rather proconvulsive
(Figure 2). Manipulating substrate availability, preventing
glycolysis, lactate uptake as well as enhancing pentose phosphate
shunt might have both acute pro- or anticonvulsant effects,
whereas chronic dietary changes can exert seizure control
via epigenetic mechanisms. Thus targeting negative feedback
mechanisms involved in metabolism dependent regulation of
excitability as well as supporting cellular bioenergetics and
protecting neurovascular coupling may represent promising
therapeutic approaches in the treatment of epilepsy.

AUTHOR CONTRIBUTIONS

RK, NB, OP, and ZG reviewed the bibliography and wrote the
first draft of the manuscript. AF, SG, and JO added substantial
comments after critical reading of the text.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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