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Invited review

Energy depletion in seizures: Anaplerosis as a strategy for future therapies

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ABSTRACT

Seizure activity can lead to energy failure and neuronal injury, resulting in neurological and cognitive sequelae. Moreover, mutations affecting genes encoding for proteins that maintain energy homeostasis within the cell often result in an epileptic phenotype, implying that energy failure can contribute to epileptogenesis. Indeed, there is evidence to indicate that the efficacy of the ketogenic diet, a treatment for refractory epilepsy, can be partly explained by its effect on increasing energetic substrates.

The ATP level, reflecting the energy level of a cell, is maintained by the potential gradient across the mitochondrial membrane. This potential gradient is maintained by $NADH/H^+$ equivalents, produced by reactions within the tricarboxylic acid cycle (TCA-cycle). Anaplerosis, the replenishment of TCA-cycle substrates, therefore represents an appealing strategy to address energy failure such as occurs in seizures. There is accumulating evidence that pyruvate, a classical anaplerotic substrate, has seizure suppressive effects and protects against seizure induced cell death. This review summarizes the evidence for the contribution of TCA cycle deficits in generating seizures. We highlight the role for TCA substrate supplementation in protecting against seizures and seizure induced cell death, and propose that these are important targets for future translational research addressing energy depletion in seizures.

This article is part of the Special Issue entitled 'New Targets and Approaches to the Treatment of Epilepsy'.

1. Introduction

There is a longstanding belief that seizure activity leads to energy failure and consequently neuronal injury, which is responsible for the clinical sequelae of seizures. Indeed, early experimental studies, measuring ATP content in brain tissue and decreases in ATP preceding epileptiform activity, have identified a pivotal role of energy failure in seizures (Sacktor et al., 1966; King et al., 1967; Sanders et al., 1970). Intracellular ATP depletion in seizures has since been confirmed in several studies (Duffy et al., 1975; Dale and Frenguelli, 2009; Wasterlain et al., 2010; Kovac et al., 2012). Mitochondria are the main source of ATP within a cell. ATP synthesis largely depends on the mitochondrial chain (MRC), which is formed of five large multi-subunit complexes. There is evidence that human epileptic tissue is deficient in complex one and there is cumulating evidence that mitochondrial dysfunction is a key feature in epileptic phenotypes (Kunz et al., 2000; Kunz, 2002; Kudin et al., 2009; Zsurka and Kunz, 2010). These complexes provide a potential gradient across the mitochondrial membrane by translocation of protons from the mitochondrial matrix into the intermembrane space (Nicholls and Budd, 2000). In addition to ATP production, mitochondria are major calcium buffers. Calcium influx is a hallmark of neuronal excitability and thus epileptic activity, and cyclic calcium rises through ligand gated and voltage gated ion channels have been observed during epileptiform activity. We have shown that these events induce an intracellular cascade which leads to cell death during prolonged seizure activity (Kovac et al., 2012). ATP synthesis and calcium buffering during epileptiform activity are both dependent on the voltage gradient across the mitochondrial membrane. Using live cell imaging techniques we have shown that calcium induced mitochondrial membrane potential depolarisation during seizures impairs energy metabolism specifically in neurons (Kovac et al., 2012; Fig. 1). Moreover, the maintenance of ion gradients across the neuronal plasma membrane is ATP dependant, further highlighting the need for a high potential gradient across the mitochondrial membrane to maintain cell viability (Fig. 1). This potential gradient is mainly maintained by $NADH/H^+$ equivalents. $NADH/H^+$ is produced by reactions within the tricarboxylic acid cycle (TCA-cycle; Fig. 2) and also in the glycolysis reaction. In order to improve cell survival during seizures, ATP supplementation would seem a reasonable strategy; however, the average half-life of

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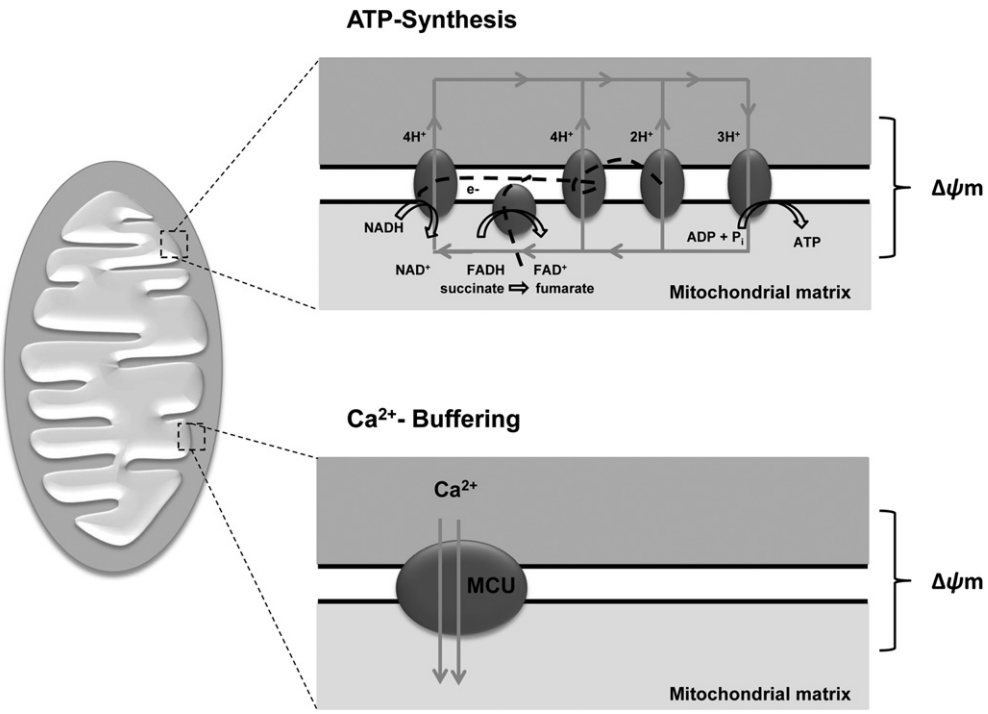

Fig. 1. Production of ATP and calcium buffering at the inner mitochondrial membrane. The upper panel shows the inner mitochondrial membrane with the electron transport chain (complex I–V) involved in producing a transmembrane proton gradient as a result of the redox reactions. In the final reaction ATP is formed at the ATP synthetase (complex V). The lower panel shows calcium buffering at the inner mitochondrial membrane through the mitochondrial calcium uniporter (MCU). Note that both ATP production and calcium buffering are dependent on the mitochondrial membrane potential ($\Delta\psi m$).

the 3 mM of ATP in the brain is 3 s. A different approach is to supplement compounds that will increase ATP production. Glucose breakdown leads to pyruvate, one of the main anaplerotic substrates of the TCA cycle. However, glucose administration could lead to tissue acidosis by breakdown to lactic acid (Parsons et al., 2002) and has detrimental non-nutritional side effects on neurons (for a comprehensive review see Tomlinson and Gardiner, 2008). It is not surprising that high glucose levels in acute neurological disease correlate with a worse outcome (Béjot et al., 2012). Moreover the glycolytic capacity of neurons is very small when compared to astrocytes as they lack the glycolysis-promoting enzyme 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase, isoform 3 (PFKFB3; Bolaños et al., 2010)

Therefore an appealing alternative strategy to maintain mitochondrial transmembrane gradient and so prevent energy failure during epileptiform activity is the replenishment of energetic substrates by providing pyruvate and TCA cycle intermediates leading to anaplerosis, defined as *de novo* formation of intermediates of the TCA cycle. This would lead to increased intracellular energy levels during epileptiform activity and provide an appealing target to prevent neuronal damage and seizure induced cell death as well as to reduce seizure activity.

Beside a pivotal role in the provision of NADPH energy equivalents, TCA cycle intermediates are intimately linked to neurotransmission, because alpha-ketoglutarate is the precursor for the most prevalent neurotransmitters glutamate and GABA in the brain. Anaplerosis substitutes for the loss of alpha ketoglutarate derivates as glutamate and GABA during high levels of neuronal activity. Whereas previously it had been assumed that this replenishment occurs in astrocytes, it has been shown that neurons are the main source of alpha ketoglutarate (Hassel and Brâthe, 2000; Hassel and Bråthe, 2000). Previous studies have shown that seizures and status epilepticus lead to activation of caspases and consequent Poly(ADP-ribose) polymerase (PARP) activation, an enzyme that is involved in the DNA repair machinery of the cell (Ekdahl et al., 2001, 2002). PARP consumes nicotinamide adenine dinucleotide therefore decreasing the substrate availability for mitochondrial respiration and further highlighting the need for anaplerotic substrates (Abramov et al., 2004; Abramov and Duchen, 2010). Entry of amino acids across the blood brain barrier is small (Miller and Oldendorf, 1986) and therefore carboxylation, mainly of pyruvate, is traditionally seen as the major anaplerotic pathway in the brain. However, all substrates of the TCA cycle are capable of replenishing the system and there are reports of TCA cycle substrates other than pyruvate being used to protect against seizure induced cell death or to prevent seizures.

We here aim to review the rationale for the use of anaplerotic substrates in seizures and epilepsy. These mechanisms are also shared with those underlying the ketogenic diet. We will summarize the evidence and previous experience from both experimental animal and human studies supporting the use of anaplerotic TCA cycle substrates in seizures and in epilepsy and outline mutations affecting levels of these substrates. This review will provide targets for future translational research addressing energy depletion in seizures.

In order to review the literature about the use of anapleurotic substrates in epilepsy and seizures we screened all PubMed indexed literature for the search terms “epilepsy” and “seizures” and the metabolic substrate. This search strategy also identified whether mutations in the enzymes of this substrate were linked to an epileptic phenotype. Pyruvate and the TCA cycle are central to cell metabolism and TCA cycle defects have long been thought to be incompatible with life (Chayen, 1993). However, although extremely rare, a few cases with mutations affecting pyruvate regulating enzymes and TCA cycle enzymes have been reported in the literature (Rustin et al., 1997; Naito, 2001; De Meirleir, 2002). Such cases provide additional insights into the impact of loss of TCA cycle substrates on seizure susceptibility. There are only occasional