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Purines and the Anti-Epileptic Actions of Ketogenic Diets

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Abstract: Ketogenic diets are high in fat and low in carbohydrates and represent a well-established and effective treatment alternative to anti-epileptic drugs. Ketogenic diets are used for the management of a variety of difficult-to-treat or intractable seizure disorders, especially pediatric refractory epilepsy. However, it has been shown that this dietary therapy can reduce seizures in people of all ages, and ketogenic diets are being applied to other prevalent medical conditions such as diabetes. Although used effectively to treat epilepsy for nearly 90 years, the mechanism(s) by which ketogenic diets work to reduce seizures remain ill-understood. One mechanism receiving increased attention is based on findings that ketogenic diets increase the brain energy molecule ATP, and may also increase the levels and actions of the related endogenous inhibitory neuromodulator adenosine. ATP and adenosine have both been identified as important modulators of seizures; seizures increase the actions of these purines, these purines regulate epileptic activity in brain, adenosine receptor antagonists are pro-convulsant, and adenosinergic mechanisms have been implicated previously in the actions of approved anti-epileptic therapeutics. Here we will review recent literature and describe findings that shed light on mechanistic relationships between ketogenic diets and the purines ATP and adenosine. These emerging mechanisms hold great promise for the effective therapeutic management of epileptic seizures and other neurological conditions.

Keywords: Adenosine, ATP, brain, epilepsy, metabolism, seizures.

INTRODUCTION

Epilepsy is a prevalent brain disorder characterized by a predisposition to generate seizures due to excessive or asynchronous neuronal activity in brain [1]. The incidence of epilepsy is particularly high in young children (≤ 5 years old), in young adults especially those engaged in combat, and in those over the age of 65 [2, 3]. As a neurological disorder, epilepsy is poorly classified as it has multiple known and unknown genetic and environmental causes, manifestations, seizure types, and identifiable syndromes; an onset of epilepsy associated with head trauma, central nervous system infections, and tumors may occur at any age [3]. The incidence and prevalence of epilepsy have been increasing because of combat-associated head injuries and an aging population, and this trend is predicted to continue [4, 5]. Thus, new therapeutic strategies are needed, and effective interventions could benefit a broad and growing population.

Antiepileptic drugs (AEDs) are used typically and widely for the treatment of epilepsy. The aim of AED treatment is to control seizures while minimizing adverse side-effects; unfortunately AEDs are effective for only 60–70% of epileptic individuals, and undesirable side-effects are not uncommon [6-8]. In ancient times, epilepsy was treated through the dietary intervention of starvation, which forces a switch from

glucose-based to ketone-based metabolism. This simple but extreme treatment led to the hypothesis and its clinical confirmation that maintaining ketone-based metabolism by limiting carbohydrates, while still ingesting adequate calories, could control seizures [9]. Currently a major alternative and non-pharmacological treatment for epilepsy is adhering to a high-fat, low-carbohydrate ketogenic diet (KD), and over many years its efficacy has been confirmed in retrospective, prospective, and multi-center studies [10-13]. Since its inception, KD has been employed to maintain ketosis and control epileptic seizures primarily in children who do not respond well to AEDs [14, 15].

KETOGENIC DIETS AND EPILEPSY: APPLICATIONS, BENEFITS AND LIMITATIONS

The KD requires strict compliance to maintain anticonvulsant effects. It limits the consumption of carbohydrates persistently and sufficiently such that the carbohydrate content does not provide enough glucose to serve as an available energy source. Without adequate glucose, the brain is forced metabolically to switch from mostly glycolytic pathways to ketolytic mechanisms whereby ketones are produced (mainly in the liver) and used as the main fuel to generate ATP for cellular metabolism.

The KD ratio prescribed clinically is a specific ratio of fat to protein plus carbohydrate. A typical KD contains the ratio of 4:1 (fat: protein + carbohydrates). Approximately 90% of dietary calories are derived from fat whereas $< 5\%$ comes from carbohydrates [16, 17]. Proteins are added to the diet to meet requirements depending on the individual's age

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and size. In some treatment protocols, fluids and calories are restricted - total caloric intake is targeted to 75% of the recommendation for the individual's age; however there is no evidence that forced caloric restriction or fluid restriction is necessary for the clinical benefit to be observed [18].

As noted, an analogous switch to ketone-based sources of cellular energy occurs during fasting/starvation. Without imposing unhealthy fasting/starvation approaches, adherence to a KD can provide adequate calories and energy while protecting against generation of epileptic seizures. The diet reduces seizure frequency by greater than 90% for one-third of children on the diet and by greater than 50% for an additional one-third of children [19, 20]. It appears that a KD is more or less as effective in controlling seizure activity in adults as it is in treating children with epilepsy, but there have been comparatively few studies on adults [21, 22]. One reason why a KD is more commonly applied in and perceived as successful in pediatric epilepsy is that it is easier to initiate, control food intake and maintain this strict diet in children. Most pediatric patients that respond well to the KD are weaned from the diet after two years because the anti-convulsant effect achieved is often permanent [23, 24].

Although the KD is highly effective, it does have its shortcomings; main criticisms include it being a diet that is restrictive and unpalatable. In addition, it can cause constipation, kidney stones, and, depending on the saturated fat composition of the diet, cardiovascular complications [25-28]. A low-carbohydrate diet with a healthier fat composition might reduce complications [29]. More research is clearly needed in this area to determine more accurately side effects and long term consequences.

To date, the key mechanisms underlying the success of a KD in reducing seizures are unclear [30, 31]. Proposed mechanisms include increased GABA, increased mitochondrial gene expression, direct effects of ketones on neurons, pH changes, and altered ion channels - particularly K^+ channels. This knowledge gap has limited the use of KDs for epilepsy and potentially other disorders, as well as stymied efforts to develop drugs that could take advantage of the anti-convulsant mechanisms mobilized by a KD [32]. Despite its efficacy, KD treatment has inherent drawbacks and limitations and a better mechanistic understanding of its actions could lead to better diet-based approaches including those with fewer restrictions and complications. Importantly, an understanding of underlying mechanisms should result in new directions for basic and clinical research [33].

Here we focus on the relationship between adhering to a KD and increased brain levels of ATP and adenosine as a new insight into epilepsy treatment. The purines ATP and adenosine link metabolism to neuronal activity [34], and cell bioenergetics may be a key regulator of seizure activity [35, 36]. Importantly, adenosine is an endogenous anticonvulsant [37-39] which is effective in numerous seizure models, including acute and chronic electroconvulsive and pharmacological paradigms [40-44], and is also effective in pharmacoresistant kainate-induced seizures [45-47].

Adenosine acting at the adenosine A_1 receptor (A_1R) subtype could be the final key mechanism by which adhering to a KD prevents seizures [48, 49]. Although more research is needed, to date there are a few seizure models where both

adenosine and a KD have been shown to be effective, including electrical kindling of the amygdala in rats [42-44, 50], acute bicuculline injection [41, 51, 52], pentylenetetrazol kindling in mice [53-55], and the epileptogenic phase after kainate treatment [45-47, 56, 57]. Overall, a purine-targeting metabolic strategy - ultimately increasing the actions of the anticonvulsant molecule adenosine - has great potential to treat a wide variety of epileptic seizures.

EPILEPSY AND ENERGY METABOLISM

During seizures neurons undergo prolonged depolarization that leads to large fluxes of ions across plasma membranes. As a result there is intracellular accumulation of sodium and calcium ions, and an increased requirement to expend large amounts of energy (mostly ATP) to re-establish the interior milieu. ATP is necessary to maintain resting membrane potentials, and depletion of intracellular ATP in an effort to export accumulated ions can lead to bioenergetic crisis and/or redox catastrophe. Sodium-potassium-ATPase is largely responsible for maintaining these ionic gradients [58]; under basal conditions it has been estimated that ATPase consumes 25-40% of the brain's energy [59], and after seizure activity ATPase likely consumes an even greater percentage of brain energy to restore the resting membrane potential. Decreased levels of ATP can reduce the cell's energy charge and its ability to maintain homeostasis and normal neuronal function and signaling, and, during an acute energy demand such as a seizure, the need for ATP can very readily and rapidly supersede supply. Thus, adequate intracellular ATP is central to controlling cellular energy, seizure generation, and neuronal membrane polarity.

Compared to the large and constant levels of ATP inside the cell, extracellular ATP is relatively insignificant and transient. A net dephosphorylation of ATP to ADP, AMP and adenosine occurs extremely rapidly (the $t_{1/2}$ of ATP in the extracellular space is about 200 ms [60]). Together, ATP and its core molecule adenosine are intricately involved in maintaining cellular energy levels and in controlling neuronal excitability via cell-surface receptors. Intracellular adenosine is especially important because it is a very sensitive measure of the balance between cellular energy supplies and energy demand. Its actions to reduce neuronal activity and energy demand and re-establish equity between supply and demand via feedback on cell-surface receptors is a concept central to adenosine being considered a retaliatory metabolite [61]. Extracellular adenosine is a powerful endogenous anticonvulsant, with great potential for treating all types of epileptic seizures. Traditionally, adenosine has been recognized as the brain's own anticonvulsant molecule and a powerful endogenous neuromodulator: tonic levels are present throughout the extracellular space, it is released from cells during seizures, ischemia, and hypoxia [62-64], its actions can also be increased by stimuli such as changes in temperature and pH [65-70], and it may be more sensitive to these changes during conditions of increased excitability [71].

Adenosine is released during seizures, and adenosine through its activation of high-affinity A_1R s contributes to termination of seizures by reducing neuronal excitability [45, 72-74]. Activation of A_1R s pre- and postsynaptically decreases excitatory neurotransmitter release [75] and hyperpolarizes postsynaptic membrane potentials by promoting po-

tassium conductance [76]. The brain in general and the seizure-prone hippocampus in particular contain high levels of A₁Rs. There is abundant evidence that the powerful anticonvulsant role of endogenous adenosine occurs through this receptor subtype [77-80]. For example the ictal EEG events of bicuculline-injected rats were suppressed by focal application of adenosine [78] and A₁R agonists suppress even pharmacoresistant epilepsy of kainate injected mice [45]. Conversely, antagonists of A₁Rs are pro-convulsant [81]. Thus, adenosine is similar to the KD in that both are effective at reducing seizures that are refractory to other treatments. Unfortunately, drug-based strategies that directly target adenosine A₁Rs generally face vexing pharmacological obstacles including peripheral side effects such as reduced heart rate and blood pressure [82]. More recently, localized adenosine based-therapies show significant promise but are necessarily invasive and best suited to well-localized, focal seizures [80, 83].

Extracellular ATP is a major source of extracellular adenosine [84, 85], and, as noted, is metabolized rapidly to adenosine [60]; these enzymatic reactions are catalyzed by a variety of ecto-ATPases and 5'-nucleotidases [60]. Thus, increased levels of either purine in seizure-prone brain areas containing A₁Rs could help to either prevent or to terminate seizures. At this time it is neither well-understood how a KD differentially affects purinergic mechanisms and metabolism in various cell types in the central nervous system, nor has it been determined what roles different cell types might play in a KD's anticonvulsant benefits. Nevertheless, it has been established that ATP and adenosine in the central nervous system can originate from neurons themselves as well as from other cell types, most notably from astrocytes. Astrocytes are abundant, and particularly complex in humans [86, 87], and purines released from astrocytes influence synaptic transmission. Astrocytes release ATP through hemichannels as well as from neurosecretory vesicles; extracellular adenosine derived from astrocytic ATP influences neuronal excitability, plasticity, and sleep [88-90]. However, astrocyte involvement in the relationship between purines and epilepsy is complicated by findings that astrogliosis occurs in epilepsy and brain injury, astrocytes transport adenosine into the cell interior, adenosine kinase very actively catalyzes the phosphorylation of adenosine to AMP within astrocytes, and increased levels of adenosine kinase decreases the extracellular levels and actions (including anticonvulsant actions) of adenosine [91].

PURINES AND THE KETOGENIC DIET

Many hypotheses have been advanced and tested in ongoing attempts to determine mechanisms responsible for the antiepileptic effects of a KD. Acidosis, dehydration and ketosis were the first mechanisms to be considered when the KD was first introduced as a metabolic strategy which could mimic fasting-induced seizure protection [15]. None of these initial mechanisms appeared sufficient to explain clinical observations, and subsequently, investigators attributed the protective actions of a KD to alterations in levels of neurotransmitters (including GABA, norepinephrine and glutamate), glucose restriction, activation of ATP-sensitive K⁺ channels, circulating factors including polyunsaturated fatty acids and their downstream effectors, decreased neuronal excitability, transformations of cerebral energy metabolism

and increased purines [15, 30, 48]. Identifying critical anticonvulsant mechanisms underlying the success of KD therapy remains a topic of intense research, and changes in gene expression, altered energy metabolism, and changes in the levels and actions of purines are very strong candidates [48, 49, 92-94].

For over 30 years it has been recognized that KD can lead to increased levels of ATP in brain [92] and it also increases the ratio of phosphocreatine to ATP [95]. More recently we used methods able to provide precise and accurate measures of brain energy metabolites in brain and reported that rats fed a calorie-restricted KD for three weeks had increased ratios of phosphocreatine to creatine in hippocampus [94]; such increases suggest that alternative sources of high energy phosphate are available and might thereby protect against ATP utilization and depletion. Subsequently, we showed that KD increased levels of ATP in brains of KD-fed animals.¹

Some clinical reports suggest that a KD can offer seizure protection promptly [96, 97], and some research shows that acute ketone treatment can be directly anticonvulsant [98]. At least two reports show that ketones or an in vitro analogue of ketogenic metabolism increases hyperpolarization via postsynaptic ATP-sensitive K⁺ channels [99, 100]. However, many reports note that a KD can take several days or weeks before becoming maximally effective [eg. 101]. Accordingly, it was hypothesized that this delay was due to alterations in gene expression generally and more specifically due to alterations in genes involved in the control of energy metabolism [94]. Using gene microarray technology, normal 37-41 day old rats placed on a KD for 21 days were found to have upregulated genes for oxidative phosphorylation. In agreement with this, mitochondrial biogenesis was increased, and, as noted above, brain levels of phosphocreatine relative to creatine were increased [94]. Thus, improved brain bioenergetic status might play an important role in the anti-seizure effects of KD.

Measured increases in cell bioenergetics in conjunction with the gene array data suggest strongly that brain energy metabolism in general and purines in particular might be mechanisms central to the therapeutic effects of the KD. Increasingly, recent research and proposed hypotheses about the KD suggest that higher levels of both ATP and adenosine underlie some of the anticonvulsant success as well as emerging applications for a KD and analogous ketogenic metabolic strategies [33, 48, 49, 100]. Augmenting extracellular adenosine offers both pre- and postsynaptic inhibitory mechanisms as well as established clinical potential beyond epilepsy including brain injury, pain and sleep disorders [33, 88]. Furthermore, understanding the mechanisms underlying the success of KD for epilepsy would be a major breakthrough, as this metabolism-based therapy can alleviate seizures even in cases of intractable epilepsy.

Clinical observations that a KD can be effective in intractable epilepsy suggest that although the spectrum of mechanisms underlying the success of KD therapy could overlap, they must be distinct from those targeted primarily by AEDs.

¹Masino SA, Gockel JA, Wasser CD, Pomeroy LT, Wagener JF, Gawryluk JW, Geiger JD. The relationship among ATP, adenosine and a ketogenic diet. *Soc Neurosci Abstr.* 2007; 33:595.12.

Research in animal models is very clear that adenosine is a powerful global and local seizure-suppressor, and an adenosine or A₁R deficiency alone is sufficient to trigger seizure activity [47, 102]. To date, a variety of adenosinergic mechanisms have been implicated in the actions of AEDs including AEDs interacting with A₁Rs and increasing their anti-epileptic effectiveness, and AEDs binding to A₁Rs directly [103-105]. When considering all of the intra- and extracellular regulatory mechanisms for controlling the levels and actions of purines in brain, and the respective contributions of neurons and astrocytes, the greatest net benefit of the anticonvulsant effects of purines would be achieved by 1) maintaining overall cell energy levels, 2) at least maintaining (if not increasing) cellular levels of ATP, and 3) increasing levels of extracellular adenosine, either directly or as a consequence of increased extracellular ATP and its dephosphorylation to adenosine.

In addition to evidence that a KD *in vivo* may change ATP and adenosine, increases in these purines have been implicated in cellular consequences of ketogenic metabolism both *in vitro* and *in vivo*. For example, moderate hypoglycemia or glycolytic inhibition can both increase adenosine [106-108] and offer anticonvulsant benefits [109, 110]. Recent electrophysiological evidence shows that sufficient or high intracellular ATP and lowered extracellular glucose mobilizes an autocrine inhibition *via* A₁Rs [100]. Diverse findings that a KD or ketogenic metabolic strategies work through purines, and particularly through adenosine, does not negate or eliminate other metabolic changes precipitated by this metabolic switch. Rather, these findings may help simultaneously pinpoint specific and critical mechanisms and identify a new strategy for adenosine regulation, opening new avenues for the treatment of seizure disorders and a broad array of other diseases.

SUMMARY

Traditionally, the KD is used mainly in children. While it has limitations, KD therapy may be more effective in the management of childhood seizures than currently available AEDs [111], and can offer permanent benefits [112]. The KD might also slow the progression of this disorder and thus be antiepileptogenic [56, 113]. Certainly more research is needed in this area. Unfortunately, until the mechanism(s) by which the KD provides protection are better delineated, this knowledge gap makes it difficult to fashion an even better and more widely accessible therapeutic approach. Nevertheless, with respect to the relationship among KDs and purines, a better insight into how this metabolic switch influences purines could yield new approaches to purine-based therapies which have shown potential for treating a diverse array of acute and chronic conditions.

ACKNOWLEDGEMENTS

The authors acknowledge grant funding from the National Institutes of Health, CHDI and the National Science Foundation.

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Received: December 28, 2009

Revised: January 13, 2010

Accepted: February 16, 2010

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