



Adenosine: A Fundamental Factor Formed From Fatty Feasts for Fighting Fits?

A Ketogenic Diet Suppresses Seizures in Mice Through Adenosine A₁ Receptors.

Masino SA, Li T, Theofilas P, Sandau US, Ruskin DN, Fredholm BB, Geiger JD, Aronica E, Boison D. *J Clin Invest* 2011;121(7):2679–2683.

A ketogenic diet (KD) is a high-fat, low-carbohydrate metabolic regimen; its effectiveness in the treatment of refractory epilepsy suggests that the mechanisms underlying its anticonvulsive effects differ from those targeted by conventional antiepileptic drugs. Recently, KD and analogous metabolic strategies have shown therapeutic promise in other neurologic disorders, such as reducing brain injury, pain, and inflammation. Here, we have shown that KD can reduce seizures in mice by increasing activation of adenosine A₁ receptors (A₁Rs). When transgenic mice with spontaneous seizures caused by deficiency in adenosine metabolism or signaling were fed KD, seizures were nearly abolished if mice had intact A₁Rs, were reduced if mice expressed reduced A₁Rs, and were unaltered if mice lacked A₁Rs. Seizures were restored by injecting either glucose (metabolic reversal) or an A₁R antagonist (pharmacologic reversal). Western blot analysis demonstrated that the KD reduced adenosine kinase, the major adenosine-metabolizing enzyme. Importantly, hippocampal tissue resected from patients with medically intractable epilepsy demonstrated increased adenosine kinase. We therefore conclude that adenosine deficiency may be relevant to human epilepsy and that KD can reduce seizures by increasing A₁R-mediated inhibition.

Commentary

Adenosine is a purine nucleoside that modulates many physiologic processes, playing pivotal roles in biochemical pathways, signal transduction cascades, and inhibitory neurotransmission, and it even mediates cellular protection in the CNS. Two primary receptors bind adenosine, the ubiquitous inhibitory adenosine A₁ receptor (A₁R), and the less abundant excitatory A_{2A}Rs. The most recognizable role of adenosine is that involving the stimulant caffeine, which counteracts the inhibitory effects of adenosine. Due to its broad inhibitory action, adenosine has long been thought of as an endogenous anticonvulsant, and hence enhancement of purinergic activity would be expected to counter seizure activity (1).

However, despite compelling laboratory evidence (2, 3), there is as yet no clinical evidence demonstrating that adenosine induces direct anticonvulsant effects. Further, none of the currently utilized anticonvulsant medications are believed to exert their clinical effects through modulation of purinergic neurotransmission. Interestingly, however, allopurinol (a structural isomer of hypoxanthine and a naturally occurring purine in the body used primarily to treat hyperuricemia) has been shown recently in a double-blind, placebo-controlled trial to provide anticonvulsant effects in humans when used

adjunctively to treat medically refractory epilepsy (4). Further, a deficit in A₁R expression has been observed in surgically resected specimens from patients with temporal lobe epilepsy (5), although it is difficult to determine whether such changes are causally linked to seizure genesis or a consequence of the epileptic state (6).

Nevertheless, the concept that adenosine may be important to anticonvulsant activity has been strengthened over the past few years by a series of intriguing research studies and hypothesis papers linking this important substrate to the ketogenic diet (KD) (1, 7, 8). Masino and colleagues reasoned that adenosine could be a critical player in KD action because of the following observations: 1) adenosine is a powerful endogenous CNS depressant whose levels are influenced by dietary manipulation; 2) deletion of A₁Rs and enhanced adenosine clearance by increases in adenosine kinase (ADK)—the major adenosine-metabolizing enzyme—both cause spontaneous electrographic seizures in hippocampus (9); 3) adenosine represents the core structural moiety for the all-important energy molecule ATP, which among other actions maintains ionic gradients across the neuronal cell membrane (i.e., through Na⁺-K⁺-ATPase activity); and 4) the anticonvulsant KD has been shown to raise levels of energy substrates in animals (10).

Earlier, these investigators found that a component feature of KD treatment—that is, glucose restriction—led to ATP release through pannexin hemichannels localized to CA3 hippocampal neurons (8). The increased extracellular ATP, upon rapid degradation by ectonucleotidases to adenosine, resulted



in activation of A_1 Rs on CA3 neurons. In turn, A_1 R activation led to opening of plasmalemmal ATP-sensitive (K_{ATP}) channels, which cause membrane hyperpolarization. Although other investigators have proposed that ketone bodies produced by the liver during KD treatment might directly augment K_{ATP} channel activity (11), there is as yet no reconciliation of the fact that K_{ATP} channels are inhibited by high ATP/ADP ratios and that the KD actually increases ATP levels. In contrast, Masino and colleagues described a novel form of autocrine regulation precipitated by decreased glucose availability—a mechanism involving adenosine (and still invoking K_{ATP} channels) but whose activation relied on release of ATP into the extracellular space and binding to A_1 Rs prior to K_{ATP} channel activation (8).

In extending this line of investigation, these researchers utilized a powerful gene-dosing strategy involving three lines of transgenic mice that all exhibit spontaneous electrographic seizures as a result of deficient adenosine signaling (9): A_1 R heterozygous (+/-) mice, A_1 R knock-out (-/-) mice, and ADK overexpressing (*Adk-Tg*) mice. Three weeks of KD treatment resulted in a 50% reduction in electrographic seizures in A_1 R +/- mice, no significant changes in seizure frequency in A_1 R -/- mice, and nearly complete elimination of seizures in *Adk-Tg* mice. As a control indicator of dietary effects, there were no significant differences in levels of β -hydroxybutyrate, which was increased among the KD-treated groups, and no differences in the cohorts that were treated with a standard rodent diet, indicating that, irrespective of genotype, metabolic responses were similar. Further, Masino and colleagues found that glucose injection—which would counter ketosis—in *Adk-Tg* and A_1 R +/- mice that exhibited reductions in seizure activity after KD treatment increased seizure frequency within 30 to 90 minutes, paralleling the human observation that acute carbohydrate ingestion can immediately counter seizure control in patients previously controlled on the KD.

To more directly test the hypothesis that A_1 Rs are involved in KD effects, these researchers found that administration of a nonconvulsive dose of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), a selective A_1 R antagonist, led to restoration of seizure frequency to baseline levels in both *Adk-Tg* and A_1 R +/- mice. Finally, using Western blots, they showed that KD treatment led to a down-regulation of ADK expression. Taken together, the evidence provided in this research study further support the hypothesis that adenosine deficiency can lead to a hyperexcitable state and that the anticonvulsant effects of the KD may be explained in part by an increase in A_1 R-mediated inhibition.

Although this study is compelling from the vantage point of using several genetically engineered mice, targeted pharmacology, and molecular biological techniques, there are two major caveats to consider. First, the major endpoint for this and prior studies by this research group was the assessment of subclinical seizure activity; electroclinical seizure activity was not documented with video-EEG recordings. However, it is increasingly recognized that electrographic—and indeed even interictal—EEG activity may have profound effects on epileptogenesis, and that the traditional clinical bias that seizures are only valid if there is documented electro-clinical validation may be overly simplistic and perhaps erroneous. The second caveat is that brain levels of adenosine, especially in seizure-prone areas such as hippocampus and neocortex, were not

determined. So, although it appears that A_1 R receptors may be critically involved in KD action, it remains unclear whether the KD actually enhances adenosine levels, perhaps as a consequence of increased ATP production and subsequent degradation (10). This limitation should be tempered by the fact that adenosine levels are extremely difficult to measure *in vivo*.

Clearly, despite several decades of research into the therapeutic potential of exploiting molecular targets of purinergic neurotransmission, it is only recently that adenosine has been, in a sense, “rediscovered.” And, these insights have come largely from mechanistic studies of the KD (12). Adenosine may be even more pleiotropic in its actions in the CNS, as there is emerging evidence that it profoundly affects inflammation (13) and synaptic plasticity, the latter, in part, by enhancing thrombospondin-1 expression in astrocytes (14). The studies by Masino and colleagues—although focusing primarily on the metabolic changes that accompany KD treatment—should also be viewed in the context of medically refractory epilepsy, in that the efficacy of enhanced purinergic neurotransmission (and indeed neurometabolic approaches in general) may represent a useful strategy for the treatment of epileptic conditions that do not respond to our current and expanding armamentarium of anticonvulsant medications (12). So, although newer agents hold some degree of promise because of primary actions on targets such as voltage-gated potassium channels or synaptic vesicle-binding proteins, a reconsideration of a fundamental player in bioenergetics might prove fruitful in translational epilepsy research.

by Jong M. Rho, MD

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