HOW DOES ALTERED METABOLISM LEAD TO SEIZURE CONTROL? PARTIALLY FILLING THE KNOWLEDGE GAP

Metabolic Autocrine Regulation of Neurons Involves Cooperation Among Pannexin Hemichannels, Adenosine Receptors, and KATP Channels. Kawamura M, Jr., Ruskin DN, Masino SA. *J Neurosci* 2010;30(11):3886–3895. Metabolic perturbations that decrease or limit blood glucose—such as fasting or adhering to a ketogenic diet—reduce epileptic seizures significantly. To date, the critical links between altered metabolism and decreased neuronal activity remain unknown. More generally, metabolic changes accompany numerous CNS disorders, and the purines ATP and its core molecule adenosine are poised to translate cell energy into altered neuronal activity. Here we show that nonpathological changes in metabolism induce a purinergic autoregulation of hippocampal CA3 pyramidal neuron excitability. During conditions of sufficient intracellular ATP, reducing extracellular glucose induces pannexin-1 hemichannel-mediated ATP release directly from CA3 neurons. This extracellular ATP is dephosphorylated to adenosine, activates neuronal adenosine A_1 receptors, and, unexpectedly, hyperpolarizes neuronal membrane potential via ATP-sensitive K^+ channels. Together, these data delineate an autocrine regulation of neuronal excitability via ATP and adenosine in a seizure-prone subregion of the hippocampus and offer new mechanistic insight into the relationship between decreased glucose and increased seizure threshold. By establishing neuronal ATP release via pannexin hemichannels, and hippocampal adenosine A_1 receptors coupled to ATP-sensitive K^+ channels, we reveal detailed information regarding the relationship between metabolism and neuronal activity and new strategies for adenosine-based therapies in the CNS.

COMMENTARY

Although severe hypoglycemia is a well-known precipitant of seizures in both human and animal models, there is mounting clinical and experimental evidence that a more modest reduction in blood glucose can be highly effective in controlling intractable epilepsy (1,2). The best clinical examples linking glucose restriction with seizure control come from decades of clinical experience with the ketogenic diet (1) and more recently, from studies utilizing a low glycemic index treatment in pediatric patients with medically refractory epilepsy (2). Intriguingly, calorie restriction, which shares the common feature of limited glucose with both low glycemic index treatment and the ketogenic diet, has been shown to retard aging in organisms from yeast to nonhuman primates (3) and exerts neuroprotective activity (4). While the anticonvulsant (and neuroprotective) mechanisms underlying glucose restriction remain unclear, prior animal studies have shown that pharmacological attenuation of glycolytic flux, with either 2-deoxy-D-glucose (2-DG, an inhibitor of phosphoglucose isomerase) or fructose-1,6-bisphosphate (which diverts substrates to the hexose monophosphate shunt [also known as the pentose phosphate shunt or PPP]), significantly retards seizure progression in the rat kindling model of temporal lobe epilepsy (5) and blocks acute seizure provocation in a number of established animal models (6), respectively. As a caveat, it should be noted that 2-DG has recently been found to exert proconvulsant effects in other acute seizure models, specifically the mouse electroshock, intravenous pentylenetetrazol, and intravenous kainic acid tests (7). Thus, it would appear that 2-DG, per se, may yield opposing effects depending on the net effects of glucose uptake, glycolytic activity, and shunting to the PPP.

So how might inhibition of glycolysis prevent seizure activity? It has long been speculated that glucose restriction could limit a neuron's ability to reach (and maintain) the high levels of synaptic activity necessary for sustaining seizure activity. In other words, a deficiency of energy reserves would be akin to a fire extinguishing itself due to lack of oxygen. However, it is unlikely that such a change in bioenergetics could account for the seizure protection conferred by reduced glucose. There is little evidence that the triggering or termination of seizures under ordinary conditions is a consequence of energy failure, although it is well recognized that hypometabolism exists interictally in epileptic foci (8) and that MR spectroscopic studies of patients with epilepsy show significant decreases in bioenergetic substrates in epileptogenic zones (9).

A more plausible explanation may lie in the intricacies of purinergic signaling within the CNS (10,11). ATP and adenosine are critical mediators that play fundamental roles in cellular energetics and intercellular communication, and as such, are prime candidates linking metabolism with neuronal activity.

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Moreover, because of their important role in modulating cellular homeostasis, especially in the face of stress or injury, these purines may provide neuroprotective actions, possibly during the course of epileptogenesis (12).

Against this burgeoning scientific backdrop, Kawamura and colleagues evaluated the electrophysiologic effects of reduced glucose—importantly, under conditions of adequate or enhanced intracellular ATP levels that are similar to the ketogenic diet (13)—in CA3 hippocampal pyramidal neurons, using whole-cell recording techniques. They chose to examine pyramidal neurons in CA3, since this is a highly seizure prone area of the brain. Upon reducing glucose to 3 mM, the investigators observed a significant but reversible hyperpolarization (an outward current) in CA3 pyramidal neurons and a concomitant decrease in input resistance—effects that were critically and bidirectionally determined by the concentration of intracellular ATP. Further, they showed the hyperpolarizing current induced by reduced glucose was abrogated in adenosine receptor-1 (A_1R) knockout hippocampal slices, thereby invoking adenosine receptor activation as a critical step in explaining their initial electrophysiological observations.

Postsynaptic activation of A_1Rs traditionally has been associated with G-protein-coupled inwardly rectifying K^+ channels in CA1 pyramidal neurons, so the investigators reasoned that CA3 pyramidal neurons might exhibit the same phenomenon. Certainly, based on the reversal potential and shape of the *I–V* (current–voltage) relationship of the low glucose-induced outward current, this seemed plausible. However, they were surprised to find indirect evidence that the reduced extracellular glucose-induced outward current might be mediated by the opening of postsynaptic ATP-sensitive potassium (K_{ATP}) channels linked to the initial activation of A_1Rs, as demonstrated by blockade of the outward current by 500 μM tolbutamide. While the authors made this intriguing connection to K_{ATP} channels, they did not reconcile the fact that intracellular ATP levels were maintained at normal levels, and it is well known that K_{ATP} channels are activated when ATP levels are low. Thus, the precise manner in which low extracellular glucose and A_1R activation could be coupled to K_{ATP} channels remains unclear.

To extend these findings, Kawamura and colleagues asked how extracellular ATP might be released and then rapidly degraded by ectonucleotidases into adenosine under their experimental conditions of low glucose. Although several mechanisms for adenosine release have been described in the literature (10), one target of major interest is the gap junction pannexin family of large-pore channels (or hemichannels), which can conduct molecules smaller than approximately 1 kD and have been implicated as a mechanism of direct ATP release from neurons (14). Thus, to determine whether CA3 pyramidal neurons were responsible for directly releasing ATP, Kawamura et al. applied both selective and nonselective gap junction hemichannel antagonists and found that both presynaptic and postsynaptic A_1R activation occurred subsequent to ATP released by hemichannels under conditions of reduced extracellular glucose. They showed that the specific pannexin hemichannel inhibitor ^{10}panx significantly blocked or prevented the outward current induced by low extracellular glucose. Together, their results revealed a novel mechanism of metabolic autocrine regulation of CA3 neurons involving close co-operativity among pannexin hemichannels, adenosine receptors, and possibly K_{ATP} channels.

Overall, this is a highly novel study, as it strongly implicates pannexin-1 hemichannels as a mechanistic target of reduced glucose. How exactly pannexin-1 channels become activated in response to restricted glycolytic flux remains uncertain and is an important question to pursue in the future. The report by Kawamura and colleagues is also intriguing because it is the first demonstration that postsynaptic A_1 receptors could potentially couple to K_{ATP} channels in hippocampal CA3 pyramidal neurons, again providing fertile grounds for further investigation.

Given the similarities between the experimental conditions employed by Kawamura et al. and the metabolic changes observed during ketogenic diet treatment in patients with epilepsy—specifically, an increase in bioenergetic reserves (14) and reduced blood glucose (1)—it is tempting to invoke this intriguing metabolic interplay as the mechanistic basis of the ketogenic diet. Specifically, with respect to implicating K_{ATP} channels, it should be further noted that their data support the observations of Ma et al. (15), who showed that ketones bodies, elaborated by the ketogenic diet, can attenuate the intrinsic firing of GABAergic pars reticulata neurons in the substantia nigra (a putative subcortical seizure gate), through the opening of K_{ATP} channels. However, Kawamura and colleagues bring greater mechanistic relevance to seizure genesis than do Ma et al., as the findings were made in CA3, which, as mentioned, is more widely viewed as a seizure-susceptible area of the brain.

In assessing the significance of this study, there are a couple of additional important caveats. First, the experimental findings of Kawamura and colleagues were observed in normal (not epileptic) and adult (not pediatric) rat brain. Whether or not their findings translate to the pathological or clinical condition remains unclear. Second, the authors used a nonpathological and physiologically relevant condition (i.e., ATP was maintained at adequate or enhanced bioenergetic levels) to show that reducing glucose has an inhibitory effect on CA3 pyramidal neurons. While adequate ATP may be observed during ketogenic diet treatment, the finding is in stark contrast to human epilepsy data derived from detailed magnetic resonance spectroscopic studies demonstrating deficits in bioenergetic substrates in the epileptic hippocampus (10). Finally, how do the experimental data provided by Kawamura et al.

relate to the phenomenon of hypoglycemia-induced seizures? Perhaps the severity of hypoglycemia, with diminished output from glycogenolysis, might lead to severely reduced intracellular ATP levels, such that even activation of K_{ATP} channels under these conditions could not overcome the destabilization of the cellular membrane potential that results from the impairment of critical pumps, such as the Na^+,K^+-ATPase.

Notwithstanding the usual concerns raised when detailed investigations of this type are conducted on normal nonepileptic neurons, this compelling study by Kawamura and colleagues provides yet another molecular target that may play a pivotal role in reducing seizures under conditions of reduced glycolytic flux and helps fill an important gap in understanding how metabolic changes or adaptations yield profound effects on neuronal excitability and integrity.

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