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Role of adenosine in status epilepticus: A potential new target?

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Summary

The homeostatic bioenergetic network regulator adenosine is an endogenous anticonvulsant of the brain playing critical roles in seizure termination and postictal refractoriness. Adenosine homeostasis in the adult brain is largely under the control of metabolic clearance through adenosine kinase (ADK), expressed predominantly in astrocytes. The role of adenosine in status epilepticus (SE) appears to be a double-edged sword. We demonstrated that the severity of an SE clearly depends on the expression levels of ADK. A genetic knockdown of ADK prevented SE in a mouse model, whereas transgenic overexpression of the enzyme aggravated the SE. Thus, ADK inhibition or adenosine augmentation might be a therapeutic strategy to terminate or attenuate an SE. On the other hand, SE triggers a surge of endogenous adenosine, which may initiate secondary events leading to epileptogenesis. Two new findings point into this direction: *(i)* Elevated adenosine triggers changes in the epigenome; and *(ii)* SE triggers transient changes in ADK expression, which have been linked to neurogenesis. While the ADK/adenosine system is an attractive target for the attenuation of an SE, the same system may also trigger downstream events related to epileptogenesis.

Keywords

Adenosine; Adenosine kinase; Adenosine augmentation therapy; Epigenetics; Neurogenesis

The purine ribonucleoside adenosine is an endogenous homeostatic regulator of network activity (Boison et al., 2011; Diógenes et al., 2012) and adenosine deficiency has been identified as a pathological hallmark of the epileptic brain (Aronica et al., 2013). Consequently, adenosine augmentation therapies (AATs) constitute an effective strategy to suppress induced and spontaneous seizures, even those that are refractory to conventional antiepileptic drugs (Boison, 2009). In the adult brain the ambient concentration of adenosine is under the control of metabolic clearance through astrocytes (Boison, 2012). Whereas adenosine can be released from neurons or can be derived from the extracellular cleavage of ATP, the astrocyte-based enzyme adenosine kinase (ADK) eliminates adenosine via phosphorylation to AMP. Since astrocytes express two types of equilibrative nucleoside transporters, it is the metabolic clearance through ADK that drives the flux of adenosine into astrocytes, which thereby form a sink for the metabolic clearance of adenosine. Thus, the levels and activity of astrocytic ADK control adenosine homeostasis in the brain: High

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levels of astrocytic ADK correspond to low levels of tissue adenosine, whereas low levels of ADK lead to an increase in adenosine (Shen et al., 2011).

Adenosine controls neuronal network function through multiple mechanisms. Adenosine is the endogenous ligand of four types of G protein coupled adenosine receptors designated A_1R, $A_{2A}R$, $A_{2B}R$, and A_3R (Fredholm et al., 2011). Activation of the A_1R provides immediate anti-seizure effects through *(i)* the presynaptic inhibition of glutamate release, and *(ii)* the stabilization of the postsynaptic membrane potential (Dunwiddie & Masino, 2001). Consequently, ADK inhibitor-induced increases in adenosine, or direct A_1R agonists, provided potent seizure control in a multitude of model systems including kindled seizures and spontaneous recurrent seizures in post SE models of epilepsy (Boison, 2009). In addition to the activation of adenosine receptors, adenosine has evolutionary ancient, receptor independent roles. Adenosine is directly linked to mitochondrial bioenergetics (Boison et al., 2011) and is an obligatory end product of transmethylation reactions, which also include DNA methylation (Boison et al., 2002). These findings suggest a novel role of adenosine as epigenetic regulator (Boison, 2012; 2013). In status epilepticus (SE), three aspects of the ADK/adenosine system warrant further discussion:

1. Susceptibility to SE: As key regulator of endogenous adenosine, the expression levels of ADK determine the brain's susceptibility to an SE. Using mice with genetically altered expression levels of ADK in the brain and a model of focal onset SE triggered by the intraamygdaloid injection of kainic acid (KA), we demonstrated that the global brain-wide overexpression of ADK to levels 140% above normal, led to an aggravation of the SE phenotype as evidenced by increased seizure activity, spread of neuronal injury throughout the hippocampal formation and lethal outcome under conditions, where wild-type animals survived and showed only focal CA3- selective neuronal injury. Conversely, mice with a reduction of hippocampal ADK to levels of 60% of control were completely resistant to the intraamygdaloid KA-induced SE. Therefore, ADK expression levels determine susceptibility to SE and seizure spread (Li et al., 2008). Furthermore, the transplantation of human mesenchymal stem cells engineered to release adenosine, based on lentiviral expression of a micro-RNA targeting ADK, suppressed focal onset SE and limited neuronal injury when transplanted into the infrahippocampal fissure of mice one week prior to the intraamygdaloid injection of kainic acid (Ren et al., 2007).

2. Epigenetic consequences of an SE: Like a variety of injuries to the brain, SE triggers a massive surge in adenosine (micromolar concentrations as opposed to baseline concentrations in the 25 to 300 nmolar range) which is linked to ATP breakdown and the transient downregulation of ADK in astrocytes (Clark et al., 1997; Gouder et al., 2004). We propose that the SE-induced adenosine surge can trigger downstream mechanisms involved in epileptogenesis. New data from our laboratory show that increased adenosine or the downregulation of ADK block DNA methylation in the brain and that this activity leads to a hypomethylated state of the DNA, which in turn might permit the expression of genes that drive epileptogenesis (Williams-Karnesky et al., 2013). This novel epigenetic effect of adenosine is directly linked to the transmethylation pathway and the inhibition of methyltransferase enzymes by S-adenosylhomocysteine, a metabolite that increases, when the metabolic clearance of adenosine is reduced by lower ADK activity (Boison et al., 2002). These findings suggest that an SE may trigger changes in the epigenome through an adenosine-dependent mechanism.

3. Neurogenesis: SE is known to trigger neurogenesis in the dentate gyrus of the hippocampus and increased neurogenesis may affect the development of epilepsy

(Parent & Lowenstein, 2002; Parent, 2007). While the short cytoplasmic isoform of ADK is almost exclusively expressed in astrocytes and responsible for the regulation of the tissue concentration of adenosine (Studer et al., 2006; Li et al., 2008), ADK exists in a second, long isoform, which is expressed in the nuclei of astrocytes and dentate granular neurons and thought to be involved in cell-autonomous regulatory mechanisms related to cellular differentiation and plasticity (Studer et al., 2006). In pancreas and heart muscle, the nuclear isoform of ADK has been shown to prevent cell proliferation, whereas ADK inhibition promoted cell proliferation (Fassett et al., 2011; Annes et al., 2012). Our data show that ADK in the nucleus of granular neurons of the dentate gyrus is transiently downregulated following an intrahippocampal KA-induced SE (Gouder et al., 2004) and this transient time window of ADK reduction matches the time-window during which neurogenesis takes place. We thus propose that nuclear ADK in granular neurons plays a role as ‘gate-keeper’ for neurogenesis and that SE triggers neurogenesis through a mechanism that involves the transient downregulation of the nuclear isoform of ADK. To address this hypothesis, we generated a new line of mice that lacks the nuclear isoform of ADK in granular neurons of the dentate gyrus. Intrahippocampal KA-induced SE enhanced neurogenesis > 2-fold as compared to normal littermate controls. Those data suggest that SE may contribute to the regulation of neurogenesis through dynamic expression changes of the nuclear isoform of ADK in dentate granular neurons.

Is the adenosine system a new target for SE? It depends. As we have previously shown, life-style choices, including diet, influence the expression levels of ADK in the brain. Thus, a ketogenic diet was shown to reduce ADK expression in the brain in line with seizure suppression (Masino et al., 2011). Other life-style choices, such as exercise, have likewise been shown to enhance adenosine levels in the brain (Dworak et al., 2007). Together, these findings suggest that life-style choices can influence the brain’s excitability and thereby the susceptibility to SE. As a potent anticonvulsant strategy with effectiveness in a model of pharmacoresistant epilepsy (Gouder et al., 2003), adenosine augmentation might be a useful therapeutic strategy to terminate refractory and potentially life threatening SE. Under those conditions, the systemic use of adenosine augmenting agents might be justifiable.

A second area of therapeutic interest is the prevention of post-SE epileptogenesis. As discussed here epigenetic factors and neurogenesis might be involved and linked to an SE-associated surge in adenosine. The nuclear isoform of ADK might be a potential target to influence epigenetic mechanisms and neurogenesis in future attempts to prevent epileptogenesis. The therapeutic modulation of ADK expression, e.g. by gene therapy (Boison, 2010), might be a useful approach to regulate the tissue concentration of adenosine, but also to regulate epigenetic factors and neurogenesis. More knowledge is needed to decide, which isoform of ADK to target, and in which cell types, and in which direction (up- or downregulation). Eventually, the key for successful antiepileptogenic interventions will lie in regional, cell-type, and compartmental specificity of therapeutic approaches. In summary, the adenosine/ADK system is of therapeutic interest not only for the prevention or termination of SE, but also to influence pathological downstream events triggered by an SE.

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