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Adenosine dysfunction in epilepsy

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Abstract

Extracellular levels of the brain's endogenous anticonvulsant and neuroprotectant adenosine largely depend on an astrocyte-based adenosine cycle, comprised of ATP release, rapid degradation of ATP into adenosine, and metabolic reuptake of adenosine through equilibrative nucleoside transporters and phosphorylation by adenosine kinase (ADK). Changes in ADK expression and activity therefore rapidly translate into changes of extracellular adenosine, which exerts its potent anticonvulsive and neuroprotective effects by activation of pre- and postsynaptic adenosine A₁ receptors. Increases in ADK increase neuronal excitability, whereas decreases in ADK render the brain resistant to seizures and injury. Importantly, ADK was found to be overexpressed and associated with astrogliosis and spontaneous seizures in rodent models of epilepsy, as well as in human specimen resected from patients with hippocampal sclerosis and temporal lobe epilepsy. Several lines of evidence indicate that overexpression of astroglial ADK and adenosine deficiency are pathological hallmarks of the epileptic brain. Consequently, adenosine augmentation therapies constitute a powerful approach for seizure prevention, which is effective in models of epilepsy that are resistant to conventional antiepileptic drugs. The adenosine kinase hypothesis of epileptogenesis suggests that adenosine dysfunction in epilepsy undergoes a biphasic response: An acute surge of adenosine that can be triggered by any type of injury might contribute to the development of astrogliosis via adenosine receptor –dependent and –independent mechanisms. Astrogliosis in turn is associated with overexpression of ADK, which was shown to be sufficient to trigger spontaneous recurrent electrographic seizures. Thus, ADK emerges as a promising target for the prediction and prevention of epilepsy.

Introduction

The purine ribonucleoside adenosine is a primordial metabolite that likely played important roles in the origin of life on Earth (Oro 1961). As constituent of the energy metabolite adenosine-5'-triphosphate (ATP) and of RNA, adenosine is uniquely suited to adjust cellular activity (RNA as a primary source for new protein synthesis) to available energy (ATP). Adenosine has therefore been described as a "retaliatory metabolite" that protects cells against the effects of excessive energy deficits, an important role to maintain energy homeostasis in most organ systems including brain (Newby et al. 1985). Early work from Bertil Fredholm and Thomas Dunwiddie has first recognized adenosine as an endogenous modulator of neuronal excitability (Dunwiddie 1980; Fredholm and Hedqvist 1980).

Today the role of adenosine as endogenous anticonvulsant and neuroprotectant is well recognized (Boison et al. 2010; Fredholm et al. 2005a; Ribeiro 2005; Stone et al. 2009). Importantly, microdialysis studies performed in human patients with temporal lobe epilepsy have shown that endogenous adenosine levels rise as a consequence of seizures and – consequently – seizure termination is thought to be mediated by the seizure-induced release of endogenous adenosine (During and Spencer 1992). Adenosine provides potent seizure

control in all experimental model systems studied including a model of pharmacoresistant epilepsy (Gouder et al. 2003). The anticonvulsant effects of adenosine are largely mediated by activation of pre- and postsynaptic $G_{i/o}$ protein coupled adenosine A_1 receptors (A_1 Rs), which induce presynaptic inhibition by reducing the inward flows of calcium, and which reduce excitability of the postsynaptic membrane by enhancing postsynaptic outflow of potassium through G-protein coupled inwardly rectifying potassium channels (Fredholm et al. 2005a). In addition to the global inhibitory tone provided by A_1 R activation, adenosine further fine-tunes neuromodulation through all four types of adenosine receptors (A_1 , A_{2A} , A_{2B} , A_3), which interact, at least in part, by heterodimerization with other G protein coupled receptors, and thereby affect all major neurotransmitter and neurotrophin systems (Sebastiao and Ribeiro 2009). Therefore, *endogenous* adenosine is uniquely able to control neuronal excitability on multiple levels, and – consequently – any pathological disruption of adenosine homeostasis is likely to affect network excitability (Fig. 1).

Astrocyte-based adenosine cycle

Astrocytes play a key role in regulating adenosine homeostasis by releasing ATP as the major physiological precursor of adenosine. Elegant studies from Phil Haydon's lab have employed transgenic mice with an inducible astrocyte-selective dominant negative mutation of soluble N-ethylmaleimide-sensitive factor attachment protein receptor (dnSNARE-mice) to study the contribution of astrocytic ATP release to the regulation of synaptic transmission (Pascual et al. 2005). Importantly, in the mutant animals the adenosine-dependent tonic suppression of synaptic transmission was removed, providing the first direct evidence that astrocytes are the source of synaptic adenosine that regulates the tonic suppression of neuronal networks (Pascual et al. 2005). Apart from the vesicular release of ATP, astrocytes can also release ATP more directly via hemichannels (Halassa and Haydon 2010; Kang et al. 2008). Once in the extracellular space, ATP is rapidly dephosphorylated into adenosine via a series of ectonucleotidases. In contrast to classical neurotransmitters, the physiological activity of adenosine is not terminated by energy-driven transport systems. Instead, astrocytes express two types of equilibrative nucleoside transporters that permit rapid equilibration of intra- and extracellular levels of adenosine (Baldwin et al. 2004). Influx of adenosine into the astrocyte is driven by metabolic clearance through the intracellular enzyme adenosine kinase (ADK; EC 2.7.1.20), a phosphotransferase that converts adenosine into adenosine-5'-monophosphate (AMP). Importantly, the intracellular levels of adenine nucleotides are about 100,000-fold higher than levels of the nucleoside (i.e. adenosine) (Fredholm et al. 2005a); thereby, changes in ADK activity will affect preferentially the levels of ambient adenosine without affecting the pool of the energy metabolites AMP, ADP, and ATP. Due to the existence of a substrate cycle between adenosine and AMP, even minor changes in ADK activity rapidly translate into major changes in the tone of ambient adenosine. A recent study using online quantification of adenosine in hippocampal slice preparations with adenosine microelectrode biosensors documented that basal synaptic adenosine levels are largely regulated by astrocytic ADK (Etherington et al. 2009). Whereas astrocytic ATP (independent of ADK) represents the major source for synaptic adenosine, its reuptake is controlled by intracellular astrocytic ADK; thus the tone of ambient adenosine is controlled by an astrocyte-based adenosine-cycle.

Adenosine kinase (ADK) – an astrocyte-based enzyme

In the adult brain ADK is predominantly expressed in astrocytes (Studer et al. 2006). Two alternatively spliced isoforms of ADK exist, ADK-long and ADK-short, which are expressed within the nucleus and cytoplasm, respectively (Cui et al. 2009). Interestingly, during early brain development in rodents (Studer et al. 2006) and humans (unpublished data) ADK expression undergoes a coordinated switch from primarily neuronal expression

(nuclear isoform) to primarily astrocytic expression (nuclear and cytoplasmic isoform), with the exception of neurons from the olfactory bulb, which maintain cell division and high levels of ADK expression into adulthood (Gouder et al. 2004). The early developmental expression of ADK within the nucleus, but not the cytoplasm, of immature neurons and maintenance of nuclear ADK expression in adult astrocytes (Studer et al. 2006) suggests a specific function of nuclear ADK related to gene regulation, development, or cellular plasticity. It is striking to note that only those cells that are newly born or still capable to divide, in particular immature neurons, adult olfactory neurons, and astrocytes, express the nuclear isoform of ADK. In contrast, the cytoplasmic isoform of ADK appears to be involved primarily in the regulation of the adenosine tone (see below for details), which in adult brain is maintained within the 25 to 250 nM range (Fredholm et al. 2005a). Thus, ADK appears to be an enzyme with multiple functions (Studer et al. 2006). In particular, putative functions of ADK related to gene regulation and cellular plasticity might play crucial roles in pathological conditions, such as epilepsy, that involve long-lasting changes in network plasticity. ADK is essential, since deletion of the gene leads to early postnatal mortality due to reduction of body temperature, prolonged periods of apnea, and fatal hepatic steatosis (Boison et al. 2002).

ADK – a key regulator of synaptic adenosine

An alternative pathway for metabolic adenosine clearance is based on deamination of adenosine into inosine via the metabolic enzyme adenosine deaminase (ADA). However, K_m values for adenosine in rat brain are much lower for ADK (~2 μ M) than those for ADA (~17 μ M) (Phillips and Newsholme 1979) suggesting that ADK is the key enzyme for metabolic adenosine clearance under physiological baseline conditions, whereas the contribution of ADA might become relevant only under conditions of stress, which lead to increased extracellular adenosine concentrations (Latini and Pedata 2001). In contrast to the hypothalamus where ADA is highly expressed and thought to be implicated in sleep regulation, the hippocampus is the brain region with the lowest expression and activity levels of ADA (Geiger and Nagy 1986). Several lines of direct experimental evidence now demonstrate that ADK is the key regulator of hippocampal adenosine. Data derived from hippocampal slices demonstrated that pharmacological inhibition of ADK but not of ADA increased endogenous adenosine and depressed neuronal activity (Pak et al. 1994). Using a combination of electrophysiology and adenosine biosensor technology in an *in vitro* hippocampal slice model of electrically-evoked epileptiform activity, it was shown that pharmacological inhibition of ADK led to an increase in synaptic adenosine, which in turn suppressed glutamatergic excitatory synaptic transmission in an A_1R -dependent manner under both baseline and Mg^{2+} -free (ictogenic) conditions (Etherington et al. 2009). Under conditions of ADK inhibition, seizure activity induced by high-frequency stimulation was significantly reduced (Etherington et al. 2009). These findings indicate that ADK, expressed only in astrocytes of the adult hippocampus, controls synaptic adenosine homeostasis under both baseline and pathological conditions. Proof, that changes in ADK expression effect basal adenosine levels likewise *in vivo* was recently achieved using adenosine biosensors in mice with genetically engineered changes in ADK expression in brain. Triple-mutant fb-Adk-def mice underexpress ADK in the entire telencephalon, but to overexpress ADK in all other brain areas (Li et al. 2008). Adenosine microelectrode biosensors implanted into cerebral cortex (65% of wild-type ADK) or striatum (164% of wild-type ADK) of fb-Adk-def mice revealed significant increases (163% of wild-type cortex) and decreases (50% of wild-type striatum) of adenosine levels (Shen et al. 2011). These data demonstrate an inverse relationship between ADK expression and adenosine levels *in vivo* and imply that pathological increases in ADK results in adenosine deficiency.

Overexpression of ADK and adenosine deficiency in epilepsy

Astrogliosis is a pathological hallmark of the epileptic brain. Due to the astrocyte-specific expression profile of ADK we hypothesized that astrogliosis might disrupt homeostatic functions of the ADK-adenosine system, which might have direct implications to the epileptic phenotypic spectrum. Overexpression of ADK in hypertrophied astrocytes was first identified in a mouse model of mesial temporal lobe epilepsy (MTLE) in which status epilepticus (SE) triggered by a unilateral intrahippocampal injection of the excitotoxin kainic acid (KA) resulted over a time span of 4 weeks in ipsilateral neuronal cell loss in area CA1, mossy fiber sprouting, granule cell dispersion, hippocampal sclerosis, and spontaneous recurrent seizures (Gouder et al. 2004) (Fig. 2). Interestingly, ADK expression followed a biphasic kinetic following the KA injection: acute downregulation of ADK during the first 24 hours following the injury was followed by gradual overexpression of the enzyme during the progression of epileptogenesis (Gouder et al. 2004). Eight weeks following the KA-injection, the epileptogenic hippocampus was characterized by a 177% increase in the enzymatic activity of ADK suggesting a relationship among increased ADK immunoreactivity, enhanced metabolic adenosine clearance, and seizures (Gouder et al. 2004). Importantly, seizures that are resistant to conventional antiepileptic drugs in this model, were completely suppressed after treating the animals with the ADK inhibitor 5-iodotubercidin (ITU; 3.1 mg/kg, i.p.), a therapeutic effect that was prevented when ITU was combined with the A₁R antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 1mg/kg i.p.) (Gouder et al. 2004). Although astrogliosis and overexpression of ADK correlated with spontaneous seizures in this model of MTLE, a causal relationship between astrogliosis and ictogenesis could not be established in this complex model of epilepsy, which, like human MTLE, is characterized by a multitude of histopathological alterations.

To isolate the contribution of astrogliosis and associated dysfunction of adenosine homeostasis to seizure generation in epilepsy we developed and characterized a minimalistic mouse model of focal epileptogenesis that is comprised of focal astrogliosis, associated overexpression of ADK, and spontaneous electrographic seizures, but without any confounding histopathological alterations commonly seen in MTLE such as neuronal cell loss, mossy fiber sprouting, or granule cell dispersion. In this model a single unilateral injection of KA into the basolateral amygdala triggered focal onset SE that was terminated after 30 minutes with lorazepam. As a result of this manipulation acute neuronal cell loss was restricted to the ipsilateral amygdala and CA3 (Li et al. 2011; Li et al. 2008). Importantly, within three weeks astrogliosis, overexpression of ADK, and spontaneous electrographic seizures at a rate of around four seizures per hour resulted at the sites of the original injuries, i.e. restricted to the ipsilateral amygdala and CA3 (Li et al. 2011; Li et al. 2008). These data demonstrated a tight spatial association of astrogliosis, overexpression of ADK and spontaneous recurrent seizures. In a time-course experiment, onset of seizures at around 12 days following the KA-injection was also associated with the onset of astrogliotic overexpression of ADK suggesting not only a spatial but also a tight temporal association of astrogliosis, overexpression of ADK and spontaneous recurrent seizures (Li et al. 2007a). To identify the focal origin of seizures and to study mechanisms of seizure spread in this model, we recently performed synchronous electroencephalographic (EEG) - recordings from multiple intracranial leads. Specifically, we recorded simultaneously from the ipsilateral amygdala, CA3, CA1, dentate gyrus, and the cerebral cortex (Li et al. 2011). Spontaneous seizures were restricted to the ipsilateral amygdala and CA3. Importantly, seizures between these two foci occurred independently and were not synchronized based on the focal nature of adenosine deficiency (Li et al. 2011). These focal seizures appeared stable over time; however, they rapidly synchronized and generalized once adenosine signaling was globally impaired in brain. Thus, astrogliosis, associated dysfunction in adenosine signaling, and

focal onset of subclinical seizures might constitute an early event in the epileptogenic cascade (Li et al. 2011).

Overexpression of ADK appears to be a general pathological hallmark of the epileptic brain. It seems to be independent of the model used and was recently described following the electrical induction of SE in rats (Aronica et al. 2011). Most importantly, robust overexpression of ADK and association with astrogliosis was found in epileptogenic tissue from human patients with hippocampal sclerosis that underwent surgical resection of their hippocampus as a treatment of their epilepsy (Aronica et al. 2011; Masino et al. 2011b). These findings suggest that overexpression of ADK is also a prominent feature of human epilepsy and further imply that consequential adenosine deficiency is at least a contributing factor to seizure generation in human epilepsy.

Overexpression of ADK is sufficient to trigger seizures

The evidence outlined above demonstrates a tight association of astrogliosis, overexpression of ADK, and ictogenesis. However, to ascribe a specific role of increased ADK (i.e. decreased adenosine) for ictogenesis, it is necessary to molecularly dissect overexpression of ADK from the underlying astrogliosis. Several lines of evidence now suggest that overexpression of ADK *per se* and in the absence of astrogliosis or any other epileptogenic event sufficiently triggers electrographic seizures. Thus, transgenic Adk-tg mice with a brain-wide 1.4-fold overexpression of the cytoplasmic isoform of ADK on top of an ADK-deficient background (to eliminate the endogenous regulatory mechanisms of ADK expression) experience spontaneous recurrent hippocampal seizures at a rate, frequency, and duration comparable to spontaneous seizures in the mouse model of intra-amygdala KA-induced focal epilepsy (Li et al. 2007a; Li et al. 2008). More direct evidence for the crucial role of ADK for seizure generation was recently obtained in a viral gene expression approach. Adeno associated virus serotype 8 (AAV8) was used to overexpress a cDNA of the cytoplasmic isoform of ADK under the control of a truncated GFAP promoter as a molecular strategy to selectively overexpress ADK in astrocytes (Theofilas et al. 2011). Four weeks following the intrahippocampal injection of the ADK-overexpressing AAV8, robust electrographic seizure activity was recorded from the AAV8-injected hippocampus (Theofilas et al. 2011). Importantly, overexpression of the cytoplasmic isoform of ADK in astrocytes was sufficient to trigger the seizures. Conversely, the knockdown of ADK in Adk-tg mice using an AAV8-based antisense approach robustly abrogated the spontaneous seizures within the injected hippocampus (Theofilas et al. 2011). Together, the transgenic and viral approaches described above demonstrate that overexpression of ADK as such and in the absence of astrogliosis is sufficient to trigger spontaneous electrographic seizures. However, what are the causal relationships between astrogliosis, overexpression of ADK, and ictogenesis?

Astrogliosis and ADK

Overexpression of ADK always seems to be associated with astrogliosis, even in non-related pathologies such as Alzheimer's disease and amyotrophic lateral sclerosis (unpublished data). It is unlikely that overexpression of ADK is the cause for astrogliosis, since the AAV8-based overexpression of ADK in astrocytes did not trigger astrogliosis (Theofilas et al. 2011). However, the temporal coincidence of astrogliosis and overexpression of ADK (Li et al. 2007a) suggests that the two might be triggered by common mechanisms.

ADK and seizures

The evidence described above clearly demonstrates that overexpression of ADK can directly cause electrographic seizures. However, the reverse assumption that seizures might cause

overexpression of ADK requires some thought: It is known that seizures trigger the release of *endogenous* adenosine (During and Spencer 1992), which is expected to require higher demands on metabolic clearance. Therefore it is tempting to speculate that the seizure-associated release of adenosine might also contribute to trigger astrogliosis and overexpression of ADK as a means to enhance metabolic clearance of adenosine. This could in fact lead to a self-propagating vicious cycle in which seizures induce overexpression of ADK, which in turn promotes further seizure activity. Based on current data however, this scenario seems to be unlikely. In the rat kindling model electrically induced seizures do not seem to trigger astrogliosis or overexpression of ADK (unpublished data). Moreover, in the mouse model of focal epileptogenesis, the degree of ADK overexpression appears to be stable over time at least for several weeks (Li et al. 2011). In addition, A₁R knockout mice, which experience spontaneous recurrent electrographic seizures that are quantitatively and qualitatively equivalent to seizures recorded from Adk-tg mice or from epileptic mice following the intraamygdaloid injection of KA, do not display any signs of overexpression of ADK (Li et al. 2007a; Masino et al. 2011b).

Overexpression of ADK in epilepsy: a likely explanation for comorbidities

Clinically, comorbid cognitive impairments are among the most debilitating and persistent concerns of chronic epilepsy, with a comorbidity rate reaching up to 64% compared to a maximal of 27% among non-epilepsy controls (Hermann et al. 2008). In particular, episodic memory impairment is now recognized as a key feature of MTLE and cognitive impairment has been described as “the most problematic of the comorbidities of epilepsy” (Bell et al. 2011).

As outlined above, overexpression of ADK – and resulting adenosine deficiency – in sclerotic tissue of the epileptic brain can be a direct cause for seizures. Studies performed in ADK-overexpressing Adk-tg mice not only revealed spontaneous electrographic seizures, but also comorbidities commonly associated with epilepsy (Yee et al. 2007). In comparison to non-transgenic control mice, Adk-tg mice were characterized by devastating deficits on both the working and reference memory versions of the Morris water maze spatial memory tests, and by pronounced and selective deficits in the acquisition and expression of a conditioned response to a discrete conditioning stimulus as assessed in the conditioned freezing paradigm (Yee et al. 2007). Thus, adenosine deficiency in Adk-tg mice was not only associated with spontaneous seizures but also with significant comorbid impairment. Adenosine deficiency in epilepsy is also likely to affect psychomotor control by insufficient activation of A_{2A} receptors (Shen et al. 2008), sleep regulation (Palchykova et al. 2010), and might contribute to depression (Hanson 2009). Therefore it is tempting to conclude that glial disruption of adenosine homeostasis in epilepsy is not only associated with epileptic seizures but also with several comorbidities that are part of the epileptic syndrome. This conclusion might provide a more holistic view of the complexity of the disease.

Seizure suppression by therapeutic adenosine augmentation

If adenosine deficiency is a pathological hallmark of epilepsy, then therapeutic adenosine augmentation should be an effective tool to control epileptic seizures. Indeed, systemic augmentation of adenosine signaling by either A₁ receptor agonists or by ADK-inhibitors effectively suppress seizures in a wide range of epilepsy models including those that are resistant to conventional AEDs (Benarroch 2008; Gouder et al. 2003; McGaraughty et al. 2005). Unfortunately, systemic augmentation of adenosine signaling is not a therapeutic option due to widespread, mainly cardiovascular, side effects, and due to liver toxicity of ADK disruption (Boison et al. 2002; Fredholm et al. 2005a). Therefore, focal adenosine augmentation approaches, with the aim to reconstruct adenosine homeostasis within an

epileptogenic brain area, become a therapeutic necessity. Focal adenosine augmentation to suppress epileptic seizures was first successfully performed in the rat kindling model of epilepsy using intraventricular implants of baby hamster kidney fibroblasts that were engineered to release adenosine and that, prior to transplantation, were encapsulated into a semipermeable polymer membrane to prevent immune rejection (Huber et al. 2001). In this approach recipients of the adenosine releasing cells were completely protected from generalized kindled seizures, whereas recipients of control cells continued to display their pre-implantation seizure profile (Huber et al. 2001). Importantly, focal adenosine augmentation, in contrast to systemic adenosine augmentation, was not associated with any sedative side effects (Güttinger et al. 2005). Unfortunately, duration of seizure control (~ 12 days) was limited by a reduced life expectancy of the encapsulated cells (Huber et al. 2001). Focal adenosine augmentation as a strategy for seizure control was subsequently validated in an independent laboratory using a strategy of intracranial injection of adenosine to prevent epileptiform events in a rat model (Anschel et al. 2004). Currently, four different approaches are being pursued to make therapeutic use of adenosine augmentation:

Adenosine releasing silk

Silk is an FDA-approved biocompatible biopolymer that can be engineered for controlled focal drug release. In two recent studies, infrahippocampal implants of silk that were engineered to release defined daily doses of up to 1mg adenosine provided complete control of seizures in the kindled rat for the duration of the adenosine release (1mg adenosine per day for 10 days) (Szybala et al. 2009), but also dose-dependently suppressed kindling epileptogenesis when transplanted prior to the onset of kindling (Szybala et al. 2009; Wilz et al. 2008).

Adenosine releasing stem cells

Adenosine releasing mouse embryonic stem cells and human mesenchymal stem cells were engineered to release adenosine based on genetic disruption of the endogenous *Adk* gene or by expressing a miRNA directed against *Adk* via a lentiviral vector (Fedele et al. 2004; Ren et al. 2007). Progeny of adenosine releasing stem cells potently suppressed kindling epileptogenesis after transplantation into the infrahippocampal fissure (Li et al. 2007b). More importantly, when adenosine releasing stem cells were transplanted into the infrahippocampal fissure of mice 24 hours after the intraamygdaloid injection of KA, three weeks later astrogliosis was significantly attenuated, ADK expression levels were normalized, and not a single seizure was recorded in >100 hours of EEG recordings. In contrast, recipients of control cells, or sham control animals displayed profound astrogliosis, overexpression of ADK and ~4 seizures per hour in area CA3 ipsilateral to the injection of KA (Li et al. 2008). These findings support a novel antiepileptogenic effect of focal adenosine delivery.

Gene therapy

As outlined above, glial expression of an *Adk*-antisense construct in the hippocampus of *Adk*-tg mice via an AAV8-based gene expression system completely abrogated seizures in this model system (Theofilas et al. 2011). Thus ADK is a therapeutic target for antisense-based gene therapy approaches.

Ketogenic diet

Ketogenic diets have successfully been used for >80 years to suppress seizures in patients with epilepsy including those with refractory seizures, although the underlying anticonvulsive mechanisms were incompletely understood (Kossoff and Rho 2009). We recently demonstrated that a ketogenic diet reduces the expression of ADK in mice (Masino

et al. 2011b). While the diet effectively suppressed seizures in adenosine deficient Adk-tg mice, it failed to suppress seizures in A₁R knockout mice (Masino et al. 2011b). These findings demonstrate that dietary manipulations can be used to enhance adenosine signaling and A₁R activation in the brain.

The ADK hypothesis of epileptogenesis

The data discussed above suggest that astrogliosis and changes in adenosine homeostasis are intricately linked to both epileptogenesis and subsequent ictogenesis. The ADK hypothesis of epileptogenesis is based on biphasic changes in adenosine homeostasis during disease progression (Boison 2008) and the following sequence of events is proposed and supported by experimental data as indicated: Acute injury to the brain, e.g. brain trauma (Clark et al. 1997), stroke (Pignataro et al. 2008), or SE (Gouder et al. 2004) leads to an immediate and acute surge in adenosine at micromolar levels that is further potentiated by acute downregulation of ADK. In models of stroke and SE, ADK levels drop down to minimum levels at two to three hours following the injury and gradually recover to normal within 24 hours (Gouder et al. 2004; Pignataro et al. 2008). The acute adenosine response makes physiological sense, as it significantly enhances the neuroprotective capacity of the brain. Under conditions of impaired adenosine signaling the brain is indeed more susceptible to aggravated neuronal injury and lethal outcome following traumatic brain injury, stroke, or SE (Fedele et al. 2006; Kochanek et al. 2006; Li et al. 2008; Pignataro et al. 2007). While beneficial acutely following an initial epileptogenesis precipitating injury, the same surge of adenosine might also be a trigger for subsequent epileptogenesis. Several adenosine receptor mediated downstream effects might contribute to epileptogenesis, among which increased signaling via the A_{2A} receptor appears to be crucial:

Brain insults lead to an increase in A_{2A}R signaling

The expression of A_{2A}Rs in glial cells appears generally to be induced following brain insults. In one study, LPS treatment led to a robust induction of A_{2A}R mRNA and protein in primary cultures of glial cells with a peak at 48 hours after the treatment (Saura et al. 2005). Likewise, A_{2A}R expression was induced in microglial cells and astrocytes of mouse substantia nigra 24 hours after intoxication with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

Stimulation of astrogliosis

Astrocyte proliferation is in part regulated by adenosine receptors expressed on the astrocyte membrane. In particular, activation of A_{2A}Rs by extracellular adenosine increased astrocyte proliferation and activation, whereas inhibition of the A_{2A}R prevented brain derived neurotrophic factor (BDNF)-induced astrogliosis (Brambilla et al. 2003; Hindley et al. 1994).

Immunomodulation

Adenosine modulates important functions of the brain immune system (Hasko et al. 2005). It is well known that inflammatory responses, microglial activation, and changes in the blood brain barrier contribute to epileptogenesis (Ravizza et al. 2008; Uva et al. 2008; Vezzani et al. 2008). Adenosine is known to stimulate the proliferation of naïve microglial cells via simultaneous activation of A₁ and A_{2A} receptors (Gebicke-Haerter et al. 1996). Additional pro-inflammatory effects of adenosine are mediated via A_{2A} receptor dependent upregulation of cyclooxygenase 2 (COX-2) and the release of prostaglandin E₂ (PGE₂) (Fiebich et al. 1996).

As outlined above, once astrogliosis and overexpression of ADK are established, spontaneous recurrent electrographic seizures result (i.e. contribution to ictogenesis). Indeed, overexpression of ADK as such is sufficient to trigger those seizures. It is important to note that the ADK-associated seizures are frequent (usually around 4 seizures per hour), relatively short in duration (around 20 seconds), but electrographic and subclinical in nature; ADK-associated seizures in our model of focal epileptogenesis can only be recorded with intracranial electrodes, co-localize with overexpressed ADK, and under normal conditions these seizures appear to be stable over both space and time, likely because the adenosine system in the vicinity of the epileptogenic focus is still intact (Li et al. 2007a; Li et al. 2011; Li et al. 2008). In support of this notion, we have previously demonstrated that A₁R activation is necessary to keep an epileptogenic focus localized and to prevent seizure spread (Fedele et al. 2006). Consequently, injection of animals that express focal ADK-associated seizures with a non-convulsive dose of the A₁R antagonist DPCPX can turn those pre-existing electrographic seizures into clinical grade convulsive seizures (Li et al. 2011). As indicated above, multiple pathological events can trigger focal astrogliosis; such triggers can range from minor traumatic injuries or micro-strokes to pathologies with known involvement of astrogliosis, such as Alzheimer's disease, autism, or amyotrophic lateral sclerosis (Rosengren et al. 1992; Schiffer and Fiano 2004; Van Eldik and Griffin 1994). Could it be that 'silent seizures' linked to astrogliosis and focal adenosine deficiency, are fairly widespread – if not common? Unless those seizures have a clinical manifestation, they would not necessarily be detected. However, those pre-existing subclinical seizures might still constitute a risk factor for subsequent seizure generalization and development of epilepsy. Of note, patients with Alzheimer's disease frequently suffer from episodes of sudden severe confusion that can best be explained by the occurrence of such 'silent seizures' in deep brain structures. In addition, epileptic (clinical) seizures constitute a well-known comorbidity of Alzheimer's disease. Thus, focal ADK-associated seizures might well be a substrate for future seizure generalization and might constitute a first step in the epileptogenic cascade, i.e. a condition comprised of astrogliosis, overexpression of ADK and spontaneous electrographic seizures. A secondary event or a secondary "hit" might be required leading to network rewiring, or failure of other endogenous antiepileptogenic systems might become involved. In this regard it is important to note that A₁R downregulation was found both in animal models with spontaneous recurrent convulsive seizures as well as in temporal lobe resections from patients with intractable epilepsy (Ekonomou et al. 2000; Glass et al. 1996). These findings suggest that downregulation of A₁Rs in chronic epilepsy might be a necessary prerequisite for seizure spread and generalization.

Adenosine receptor-independent effects of adenosine

While adenosine receptor dependent pathways of adenosine are well characterized (Fredholm et al. 2005b; Fredholm et al. 2011; Sebastiao and Ribeiro 2009; Stone et al. 2009) and have extensively been explored within the context of epilepsy (Benarroch 2008; Boison 2005; Jacobson and Gao 2006) it is important to point out that adenosine has additional adenosine receptor independent functions that have not yet been explored within the context of epilepsy. Of note are:

Transmethylation and epigenetics

Adenosine is an obligatory biochemical endproduct of all transmethylation reactions (Mato et al. 2008). It results from the cleavage of *S*-adenosylhomocysteine (SAH) into adenosine and homocysteine via the bidirectional enzyme SAH-hydrolase. If adenosine is not constantly removed via ADK, the equilibrium of this reaction is shifted towards SAH formation (Boison et al. 2002). Increased SAH potently inhibits transmethylation, which also includes DNA- or histone methylation. As a primordial biochemical regulator,

adenosine could thus have direct influence on epigenetic functions, which are thought to be involved in epileptogenesis, and which form the basis of the ‘methylation hypothesis’, suggesting that seizures by themselves can induce epigenetic chromatin modifications, thereby aggravating the epileptogenic condition (Kobow and Blumcke 2011). Interestingly, the nuclear isoform of ADK undergoes tightly controlled developmental expression switches during early postnatal brain development (Studer et al. 2006), suggesting a direct involvement of the enzyme in gene regulation.

Mitochondrial bioenergetics

As a ‘retaliatory metabolite’ adenosine is tightly linked to its metabolic precursors AMP, ADP, and ATP. It is important to note that there is no direct biochemical biosynthetic pathway for the synthesis of adenosine. Purine biosynthesis results in 5'-inosine-monophosphate (IMP), which is then transformed into AMP. Thereby, adenosine is intricately linked to its phosphorylated precursors and the energy state of the cell. Mitochondrial bioenergetics and the mitochondrial capacity to produce ATP as the major precursor of adenosine thereby critically influence homeostatic functions of the adenosine system (Masino and Geiger 2008; Masino et al. 2011a). Conversely, adenosine homeostasis and ADK expression influence mitochondrial function, which is suggested by a severe mitochondrial pathology in *Adk*-knockout mice (Boison et al. 2002). Mitochondrial dysfunction in turn has been implicated in epileptogenesis (DiMauro et al. 2002; Kunz 2002). In particular, mitochondrial complex I deficiency has been identified in the epileptic focus of patients with temporal lobe epilepsy. Given the mitochondrial pathology of *Adk*-knockout mice it is tempting to speculate that an acute injury-induced surge of adenosine might lead to mitochondrial impairment as an additional contributing factor to epileptogenesis.

Additional biochemical pathways

Deamination of adenosine via ADA leads to the formation of inosine, which is further degraded into hypoxanthine; both compounds have moderate anticonvulsant activity (Lewin and Bleck 1985). Recent studies in a tissue culture system have shown that adenosine augmentation inhibits tissue factor induction via activation of the phosphoinositide 3-kinase/Akt signaling pathway. Those effects were maintained in cell types that did not express the A₁ or the A_{2A} receptor (Zhang et al. 2010). The authors of this study proposed an adenosine receptor independent pathway as explanation for their results, however the underlying biochemical mechanism was not elucidated and a possible contribution of A_{2B} or A₃ receptors was not excluded.

Given the multiple activities of adenosine as an upstream regulator, controlling not only basic biochemistry but also specific receptor-mediated functions of relevance for epilepsy, adenosine is a prime candidate to modulate network activity in epilepsy in the broader sense of ‘homeostatic bioenergetic network regulation’. Since the epilepsy syndrome is a complex disorder of network dysfunction it is highly likely that disruption of adenosine homeostasis – linked to glial dysfunction in epilepsy – affects pathogenetic processes on multiple levels via a multitude of adenosine receptor dependent and independent effects.

Conclusions and outlook

Overexpression of ADK in astrocytes has been identified as a direct molecular link to neuronal hyperexcitability in epilepsy. Consequently, therapeutic adenosine augmentation might uniquely be suited to reconstruct homeostatic functions of the adenosine system. As upstream regulator – in contrast to AEDs that act on selective and neuronal downstream targets – adenosine may affect epilepsy as a syndrome on multiple different levels via

adenosine receptor dependent pathways, but also through biochemical, epigenetic and bioenergetic mechanisms. Thus, reconstruction of adenosine homeostasis might influence epilepsy in a more holistic way (Fig. 3); new data discussed here suggest disease modifying and antiepileptogenic effects of focal adenosine augmentation that go far beyond mere seizure control. Questions that need to be addressed in future work include: (i) Identification of the mechanisms by which adenosine can affect network changes and disease progression in epilepsy; a focus on adenosine receptor independent effects of adenosine may yield promising results. (ii) Development of diagnostic tools to quantify ADK expression in the living brain. The development of PET or SPECT ligands directed against ADK could be used to monitor longitudinal changes in ADK expression during disease progression and could also be used as diagnostic tool to identify areas of ADK-associated neuronal hyperexcitability. (iii) Finally, clinical trials should be initiated to test safety and feasibility of focal adenosine augmentation for epilepsy. Adenosine is already FDA-approved and routinely used in clinical applications for the treatment of supraventricular tachycardia. First clinical studies could be realized by combining focal adenosine infusion with presurgical invasive diagnostics using intracranial EEG-recording electrodes coupled with cannula for the infusion of adenosine. Those studies should yield valuable safety, efficacy, and dose-response data. Given the short half-life of adenosine (~minutes in brain), adenosine infusion is considered to be safe.

Evolution might have provided us with a primordial metabolite (adenosine) that appears to be a ‘master switch’ regulating key homeostatic functions of any given cell. Understanding those mechanisms in health and disease will eventually allow us to develop entirely new conceptual strategies to treat and cure a complex comorbid syndrome such as epilepsy in a broader sense that goes far beyond mere symptom suppression. This ambitious goal will require critical rethinking and abandonment of current dogmas.

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References

- Anschel DJ, Ortega EL, Kraus AC, Fisher RS. Focally injected adenosine prevents seizures in the rat. *Exp Neurol*. 2004; 190(2):544–7. [PubMed: 15530893]
- Aronica E, Zurolo E, Iyer A, de Groot M, Anink J, Carbonell C, van Vliet EA, Baayen JC, Boison D, Gorter JA. Upregulation of adenosine kinase in astrocytes in experimental and human temporal lobe epilepsy. *Epilepsia*. 2011; 52(9):1645–1655. [PubMed: 21635241]
- Baldwin SA, Beal PR, Yao SY, King AE, Cass CE, Young JD. The equilibrative nucleoside transporter family, SLC29. *Pflugers Arch*. 2004; 447(5):735–43. [PubMed: 12838422]
- Bell B, Lin JJ, Seidenberg M, Hermann B. The neurobiology of cognitive disorders in temporal lobe epilepsy. *Nat Rev Neurol*. 2011; 7(3):154–64. [PubMed: 21304484]
- Benarroch EE. Adenosine and its receptors: multiple modulatory functions and potential therapeutic targets for neurologic disease. *Neurology*. 2008; 70(3):231–6. [PubMed: 18195269]
- Boison D. Adenosine and epilepsy: from therapeutic rationale to new therapeutic strategies. *Neuroscientist*. 2005; 11(1):25–36. [PubMed: 15632276]
- Boison D. The adenosine kinase hypothesis of epileptogenesis. *Progress in Neurobiology*. 2008; 84(3): 249–262. [PubMed: 18249058]
- Boison D, Chen JF, Fredholm BB. Adenosine signalling and function in glial cells. *Cell Death Differ*. 2010; 17:1071–1082. [PubMed: 19763139]
- Boison D, Scheurer L, Zumsteg V, Rulicke T, Litynski P, Fowler B, Brandner S, Mohler H. Neonatal hepatic steatosis by disruption of the adenosine kinase gene. *Proc Natl Acad Sci USA*. 2002; 99(10):6985–6990. [PubMed: 11997462]

- Brambilla R, Cottini L, Fumagalli M, Ceruti S, Abbracchio MP. Blockade of A2A adenosine receptors prevents basic fibroblast growth factor-induced reactive astrogliosis in rat striatal primary astrocytes. *Glia*. 2003; 43(2):190–4. [PubMed: 12838511]
- Clark RS, Carcillo JA, Kochanek PM, Obrist WD, Jackson EK, Mi Z, Wisniewski SR, Bell MJ, Marion DW. Cerebrospinal fluid adenosine concentration and uncoupling of cerebral blood flow and oxidative metabolism after severe head injury in humans. *Neurosurgery*. 1997; 41(6):1284–1292. discussion 1292–1293. [PubMed: 9402580]
- Cui XA, Singh B, Park J, Gupta RS. Subcellular localization of adenosine kinase in mammalian cells: The long isoform of AdK is localized in the nucleus. *Biochem Biophys Res Commun*. 2009; 388(1):46–50. [PubMed: 19635462]
- DiMauro S, Andreu AL, De Vivo DC. Mitochondrial disorders. *J Child Neurol*. 2002; 17(Suppl 3): 3S35–45. discussion 3S46–7. [PubMed: 12597054]
- Dunwiddie TV. Endogenously released adenosine regulates excitability in the in vitro hippocampus. *Epilepsia*. 1980; 21(5):541–8. [PubMed: 7418669]
- During MJ, Spencer DD. Adenosine: a potential mediator of seizure arrest and postictal refractoriness. *Ann Neurol*. 1992; 32(5):618–24. [PubMed: 1449242]
- Ekonomou A, Sperk G, Kostopoulos G, Angelatou F. Reduction of A1 adenosine receptors in rat hippocampus after kainic acid-induced limbic seizures. *Neurosci Lett*. 2000; 284(1–2):49–52. [PubMed: 10771159]
- Etherington LA, Patterson GE, Meechan L, Boison D, Irving AJ, Dale N, Frenguelli B. Astrocytic adenosine kinase regulates basal synaptic adenosine levels and seizure activity but not activity-dependent adenosine release in the hippocampus. *Neuropharmacology*. 2009; 56(2):429–437. [PubMed: 18957298]
- Fedele DE, Koch P, Brüstle O, Scheurer L, Simpson EM, Mohler H, Boison D. Engineering embryonic stem cell derived glia for adenosine delivery. *Neurosci Lett*. 2004; 370(2–3):160–165. [PubMed: 15488315]
- Fedele DE, Li T, Lan JQ, Fredholm BB, Boison D. Adenosine A₁ receptors are crucial in keeping an epileptic focus localized. *Exp Neurol*. 2006; 200(1):184–190. [PubMed: 16750195]
- Fiebich BL, Biber K, Lieb K, van Calker D, Berger M, Bauer J, Gebicke-Haerter PJ. Cyclooxygenase-2 expression in rat microglia is induced by adenosine A2A-receptors. *Glia*. 1996; 18(2):152–60. [PubMed: 8913778]
- Fredholm BB, Chen JF, Cunha RA, Svenningsson P, Vaugeois JM. Adenosine and brain function. *Int Rev Neurobiol*. 2005a; 63:191–270. [PubMed: 15797469]
- Fredholm BB, Chen JF, Masino SA, Vaugeois JM. Actions of adenosine at its receptors in the CNS: Insights from knockouts and drugs. *Annu Rev Pharmacol Toxicol*. 2005b; 45:385–412. [PubMed: 15822182]
- Fredholm BB, Hedqvist P. Modulation of neurotransmission by purine nucleotides and nucleosides. *Biochem Pharmacol*. 1980; 29(12):1635–43. [PubMed: 6250541]
- Fredholm BB, Ijzerman AP, Jacobson KA, Linden J, Muller CE. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors—an update. *Pharmacol Rev*. 2011; 63(1):1–34. [PubMed: 21303899]
- Gebicke-Haerter PJ, Christoffel F, Timmer J, Northoff H, Berger M, Van Calker D. Both adenosine A1- and A2-receptors are required to stimulate microglial proliferation. *Neurochem Int*. 1996; 29(1):37–42. [PubMed: 8808787]
- Geiger JD, Nagy JI. Distribution of adenosine deaminase activity in rat brain and spinal cord. *J Neurosci*. 1986; 6(9):2707–14. [PubMed: 3746429]
- Glass M, Faull RL, Bullock JY, Jansen K, Mee EW, Walker EB, Synek BJ, Dragunow M. Loss of A1 adenosine receptors in human temporal lobe epilepsy. *Brain Res*. 1996; 710(1–2):56–68. [PubMed: 8963679]
- Gouder N, Fritschy JM, Boison D. Seizure suppression by adenosine A₁ receptor activation in a mouse model of pharmacoresistant epilepsy. *Epilepsia*. 2003; 44(7):877–885. [PubMed: 12823569]
- Gouder N, Scheurer L, Fritschy J-M, Boison D. Overexpression of adenosine kinase in epileptic hippocampus contributes to epileptogenesis. *J Neurosci*. 2004; 24(3):692–701. [PubMed: 14736855]

- Güttinger M, Padrun V, Pralong W, Boison D. Seizure suppression and lack of adenosine A₁ receptor desensitization after focal long-term delivery of adenosine by encapsulated myoblasts. *Exp Neurol*. 2005; 193:53–64. [PubMed: 15817264]
- Halassa MM, Haydon PG. Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. *Annu Rev Physiol*. 2010; 72:335–55. [PubMed: 20148679]
- Hanson EA. Can adenosine substitute for SAM-e as an adjunct in the treatment of depression? *Med Hypotheses*. 2009; 73(5):857–9. [PubMed: 19482439]
- Hasko G, Pacher P, Vizi ES, Illes P. Adenosine receptor signaling in the brain immune system. *Trends Pharmacol Sci*. 2005; 26(10):511–6. [PubMed: 16125796]
- Hermann B, Seidenberg M, Jones J. The neurobehavioural comorbidities of epilepsy: can a natural history be developed? *Lancet Neurol*. 2008; 7(2):151–60. [PubMed: 18207113]
- Hindley S, Herman MA, Rathbone MP. Stimulation of reactive astrogliosis in vivo by extracellular adenosine diphosphate or an adenosine A₂ receptor agonist. *J Neurosci Res*. 1994; 38(4):399–406. [PubMed: 7932872]
- Huber A, Padrun V, Deglon N, Aebischer P, Mohler H, Boison D. Grafts of adenosine-releasing cells suppress seizures in kindling epilepsy. *Proc Natl Acad Sci USA*. 2001; 98(13):7611–6. [PubMed: 11404469]
- Jacobson KA, Gao ZG. Adenosine receptors as therapeutic targets. *Nat Rev Drug Discov*. 2006; 5(3):247–64. [PubMed: 16518376]
- Kang J, Kang N, Lovatt D, Torres A, Zhao Z, Lin J, Nedergaard M. Connexin 43 hemichannels are permeable to ATP. *J Neurosci*. 2008; 28(18):4702–11. [PubMed: 18448647]
- Kobow K, Blumcke I. The methylation hypothesis: do epigenetic chromatin modifications play a role in epileptogenesis? *Epilepsia*. 2011; 52(Suppl 4):15–9. [PubMed: 21732935]
- Kochanek PM, Vagni VA, Janesko KL, Washington CB, Crumrine PK, Garman RH, Jenkins LW, Clark RS, Homanics GE, Dixon CE, et al. Adenosine A₁ receptor knockout mice develop lethal status epilepticus after experimental traumatic brain injury. *J Cereb Blood Flow Metab*. 2006; 26:565–575. [PubMed: 16121125]
- Kossoff EH, Rho JM. Ketogenic diets: evidence for short- and long-term efficacy. *Neurotherapeutics*. 2009; 6(2):406–14. [PubMed: 19332337]
- Kunz WS. The role of mitochondria in epileptogenesis. *Curr Opin Neurol*. 2002; 15(2):179–84. [PubMed: 11923632]
- Latini S, Pedata F. Adenosine in the central nervous system: release mechanisms and extracellular concentrations. *J Neurochem*. 2001; 79(3):463–84. [PubMed: 11701750]
- Lewin E, Bleck V. Effect of inosine on seizures induced with pentylentetrazole, bicuculline, or picrotoxin. *Epilepsia*. 1985; 26(3):258–61. [PubMed: 3924588]
- Li T, Lan JQ, Fredholm BB, Simon RP, Boison D. Adenosine dysfunction in astrogliosis: cause for seizure generation? *Neuron Glia Biology*. 2007a; 3:353–366. [PubMed: 18634566]
- Li T, Lytle N, Lan J-Q, Sandau US, Boison D. Local disruption of glial adenosine homeostasis in mice associates with focal electrographic seizures: a first step in epileptogenesis? *Glia*. 2011 in press.
- Li T, Ren G, Lusardi T, Wilz A, Lan JQ, Iwasato T, Itohara S, Simon RP, Boison D. Adenosine kinase is a target for the prediction and prevention of epileptogenesis in mice. *J Clin Inv*. 2008; 118(2):571–582.
- Li T, Steinbeck JA, Lusardi T, Koch P, Lan JQ, Wilz A, Segschneider M, Simon RP, Brustle O, Boison D. Suppression of kindling epileptogenesis by adenosine releasing stem cell-derived brain implants. *Brain*. 2007b; 130(Pt 5):1276–88. [PubMed: 17472985]
- Masino SA, Geiger JD. Are purines mediators of the anticonvulsant/neuroprotective effects of ketogenic diets? *Trends in Neurosciences*. 2008; 31(6):273–278. [PubMed: 18471903]
- Masino SA, Kawamura M, Ruskin DN, Geiger JD, Boison D. Purines and Neuronal Excitability: Links to the Ketogenic Diet. *Epilepsy Res*. 2011a in press.
- Masino SA, Li T, Theofilas P, Sandau US, Ruskin DN, Fredholm BB, Geiger JD, Aronica E, Boison D. A ketogenic diet suppresses seizures in mice through adenosine A₁ receptors. *J Clin Inv*. 2011b; 121(7):2679–2683.

- Mato JM, Martinez-Chantar ML, Lu SC. Methionine metabolism and liver disease. *Annual Review of Nutrition*. 2008; 28:273–293.
- McGaraughty S, Cowart M, Jarvis MF, Berman RF. Anticonvulsant and antinociceptive actions of novel adenosine kinase inhibitors. *Curr Top Med Chem*. 2005; 5(1):43–58. [PubMed: 15638777]
- Newby AC, Worku Y, Holmquist CA. Adenosine formation. Evidence for a direct biochemical link with energy metabolism. *Adv Myocardiol*. 1985; 6:273–84. [PubMed: 2986260]
- Oro J. Mechanism of synthesis of adenine from hydrogen cyanide under possible primitive earth conditions. *Nature*. 1961; 191:1193–4. [PubMed: 13731264]
- Pak MA, Haas HL, Decking UKM, Schrader J. Inhibition of adenosine kinase increases endogenous adenosine and depresses neuronal activity in hippocampal slices. *Neuropharmacol*. 1994; 33:1049–1053.
- Palchikova S, Winsky-Sommerer R, Shen H-Y, Boison D, Gerling A, Tobler I. Manipulation of adenosine kinase affects sleep regulation in mice. *J Neurosci*. 2010 in press.
- Pascual O, Casper KB, Kubera C, Zhang J, Revilla-Sanchez R, Sul JY, Takano H, Moss SJ, McCarthy K, Haydon PG. Astrocytic purinergic signaling coordinates synaptic networks. *Science*. 2005; 310(5745):113–6. [PubMed: 16210541]
- Phillips E, Newsholme EA. Maximum activities, properties and distribution of 5' nucleotidase, adenosine kinase and adenosine deaminase in rat and human brain. *J Neurochem*. 1979; 33(2): 553–8. [PubMed: 224144]
- Pignatario G, Maysami S, Studer FE, Wilz A, Simon RP, Boison D. Downregulation of hippocampal adenosine kinase after focal ischemia as potential endogenous neuroprotective mechanism. *J Cereb Blood Flow Metab*. 2008; 28:17–23. [PubMed: 17457365]
- Pignatario G, Simon RP, Boison D. Transgenic overexpression of adenosine kinase aggravates cell death in ischemia. *J Cereb Blood Flow Metab*. 2007; 27(1):1–5. [PubMed: 16685255]
- Ravizza T, Gagliardi B, Noe F, Boer K, Aronica E, Vezzani A. Innate and adaptive immunity during epileptogenesis and spontaneous seizures: evidence from experimental models and human temporal lobe epilepsy. *Neurobiol Dis*. 2008; 29(1):142–60. [PubMed: 17931873]
- Ren G, Li T, Lan JQ, Wilz A, Simon RP, Boison D. Lentiviral RNAi-induced downregulation of adenosine kinase in human mesenchymal stem cell grafts: a novel perspective for seizure control. *Exp Neurol*. 2007; 208:26–37. [PubMed: 17716659]
- Ribeiro JA. What can adenosine neuromodulation do for neuroprotection? *Curr Drug Targets CNS Neurol Disord*. 2005; 4(4):325–9. [PubMed: 16101552]
- Rosengren LE, Ahlsen G, Belfrage M, Gillberg C, Haglid KG, Hamberger A. A sensitive ELISA for glial fibrillary acidic protein: application in CSF of children. *J Neurosci Methods*. 1992; 44(2–3): 113–9. [PubMed: 1474847]
- Saura J, Angulo E, Ejarque A, Casado V, Tusell JM, Moratalla R, Chen JF, Schwarzschild MA, Lluís C, Franco R, et al. Adenosine A2A receptor stimulation potentiates nitric oxide release by activated microglia. *J Neurochem*. 2005; 95(4):919–29. [PubMed: 16092928]
- Schiffer D, Fiano V. Astroglialosis in ALS: possible interpretations according to pathogenetic hypotheses. *Amyotroph Lateral Scler Other Motor Neuron Disord*. 2004; 5(1):22–5. [PubMed: 15204020]
- Sebastiao AM, Ribeiro JA. Adenosine receptors and the central nervous system. *Handb Exp Pharmacol*. 2009; (193):471–534. [PubMed: 19639292]
- Shen H-Y, Coelho JE, Ohtsuka N, Canas PM, Day Y-J, Huang Q-Y, Rebola N, Yu L, Boison D, Cunha RA, et al. A critical role of the adenosine A2A receptor in extra-striatal neurons in modulating psychomotor activity as revealed by opposite phenotypes of striatum- and forebrain-A2A receptor knockouts. *J Neurosci*. 2008; 28(12):2970–2975. [PubMed: 18354001]
- Shen HY, Lusardi TA, Williams-Karnesky RL, Lan JQ, Poulsen DJ, Boison D. Adenosine kinase determines the degree of brain injury after ischemic stroke in mice. *J Cereb Blood Flow Metab*. 2011; 31(7):1648–1659. [PubMed: 21427729]
- Stone TW, Ceruti S, Abbracchio MP. Adenosine receptors and neurological disease: neuroprotection and neurodegeneration. *Handb Exp Pharmacol*. 2009; 193:535–87. [PubMed: 19639293]

- Studer FE, Fedele DE, Marowsky A, Schwerdel C, Wernli K, Vogt K, Fritschy JM, Boison D. Shift of adenosine kinase expression from neurons to astrocytes during postnatal development suggests dual functionality of the enzyme. *Neuroscience*. 2006; 142(1):125–37. [PubMed: 16859834]
- Szybala C, Pritchard EM, Wilz A, Kaplan DL, Boison D. Antiepileptic effects of silk-polymer based adenosine release in kindled rats. *Exp Neurol*. 2009; 219:126–135. [PubMed: 19460372]
- Theofilas P, Brar S, Stewart K-A, Shen H-Y, Sandau US, Poulsen DJ, Boison D. Adenosine kinase as a target for therapeutic antisense strategies in epilepsy. *Epilepsia*. 2011; 52(3):589–601. [PubMed: 21275977]
- Uva L, Librizzi L, Marchi N, Noe F, Bongiovanni R, Vezzani A, Janigro D, de Curtis M. Acute induction of epileptiform discharges by pilocarpine in the in vitro isolated guinea-pig brain requires enhancement of blood-brain barrier permeability. *Neuroscience*. 2008; 151(1):303–12. [PubMed: 18082973]
- Van Eldik LJ, Griffin WS. S100 beta expression in Alzheimer's disease: relation to neuropathology in brain regions. *Biochim Biophys Acta*. 1994; 1223(3):398–403. [PubMed: 7918676]
- Vezzani A, Ravizza T, Balosso S, Aronica E. Glia as a source of cytokines: implications for neuronal excitability and survival. *Epilepsia*. 2008; 49(Suppl 2):24–32. [PubMed: 18226169]
- Wilz A, Pritchard EM, Li T, Lan JQ, Kaplan DL, Boison D. Silk polymer-based adenosine release: Therapeutic potential for epilepsy. *Biomaterials*. 2008; 29(26):3609–3616. [PubMed: 18514814]
- Yee BK, Singer P, Chen JF, Feldon J, Boison D. Transgenic overexpression of adenosine kinase in brain leads to multiple learning impairments and altered sensitivity to psychomimetic drugs. *Eur J Neurosci*. 2007; 26:3237–3252. [PubMed: 18005073]
- Zhang W, Wang J, Wang H, Tang R, Belcher JD, Viollet B, Geng JG, Zhang C, Wu C, Slungaard A, et al. Acadesine inhibits tissue factor induction and thrombus formation by activating the phosphoinositide 3-kinase/Akt signaling pathway. *Arterioscler Thromb Vasc Biol*. 2010; 30(5):1000–6. [PubMed: 20185792]

Disruption of network homeostasis in epilepsy

Figure 1.

Disruption of network homeostasis in epilepsy. Epilepsy is a comorbid syndrome comprised not only of spontaneous recurrent seizures, but also of cognitive impairment, depression, psychiatric conditions, and changes in sleep patterns. Neuronal dysfunction is only part of the epilepsy syndrome and downstream of glial dysfunction, which includes adenosine deficiency, and which is influenced by changes in the blood brain barrier, inflammatory processes, and bioenergetic and epigenetic factors. It is therefore not surprising, that antiepileptic drugs that exclusively act on neuronal downstream targets fail to restore network homeostasis in epilepsy.

Figure 2.

Overexpression of ADK in the KA-treated hippocampus of a mouse. Representative images of the hippocampal formation (A, B) and dentate gyrus (C, D, areas magnified from insets shown in A and B) from coronal brain sections of a control mouse (A, C, $n = 4$) and an epileptic mouse (B, D, $n = 4$) four weeks after the intrahippocampal injection of KA after staining for ADK immunoreactivity (immunoperoxidase method) show profound overexpression of ADK in astrocytes of the epileptic hippocampus. Note the lack of ADK expression in dispersed granular neurons (located between arrow heads in B) and prominent expression in hypertrophied astrocytes (see arrows in D) of the epileptic dentate gyrus. Specific areas of the Ammon's horn are indicated as CA1, CA2, and CA3. DG identifies the dentate gyrus. Scale bars: A, B = 1 mm; C, D = 250 μm .

Towards a 'holistic' treatment approach for epilepsy

Figure 3.

Towards a 'holistic' treatment approach for epilepsy. Adenosine can affect epilepsy and its comorbidities on different levels: Activation of A₁ receptors provides potent seizure suppression and neuroprotection. In addition adenosine can modulate glial function and contribute to bioenergetic and epigenetic functions that all have antiepileptogenic potential.