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REVIEW

Adenosine augmentation therapies (AATs) for epilepsy: Prospect of cell and gene therapies

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Summary Deficiencies in the brain's own adenosine-based seizure control system contribute to seizure generation. Consequently, reconstitution of adenosinergic neuromodulation constitutes a rational approach for seizure control. This review will critically discuss focal adenosine augmentation strategies and their potential for antiepileptic and disease modifying therapy. Due to systemic side effects of adenosine focal adenosine augmentation — ideally targeted to an epileptic focus — becomes a therapeutic necessity. This has experimentally been achieved in kindled seizure models as well as in post-status epilepticus models of spontaneous recurrent seizures using three different therapeutic strategies that will be discussed here: (i) polymer-based brain implants that were loaded with adenosine; (ii) brain implants comprised of cells engineered to release adenosine and embedded in a cell-encapsulation device; (iii) direct transplantation of stem cells engineered to release adenosine. To meet the therapeutic goal of focal adenosine augmentation, genetic disruption of the adenosine metabolizing enzyme adenosine kinase (ADK) in rodent and human cells was used as a molecular strategy to induce adenosine release from cellular brain implants, which demonstrated antiepileptic and neuroprotective properties. New developments and therapeutic challenges in using AATs for epilepsy therapy will critically be evaluated.

Contents

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Introduction

The key roles of purines in neurotransmission and neuromodulation were first recognized by Burnstock in 1972 with the identification of 5′-adenosine-triphosphate (ATP) as a novel neurotransmitter, a finding that led to the concept of purinergic neurotransmission (Burnstock, 1972). Subsequently, the release of the endogenous purine ribonucleoside adenosine, a degradation product of ATP, was shown to regulate hippocampal excitability *in vitro* (Dunwiddie, 1980; Dunwiddie and Hoffer, 1980). A few years later it was demonstrated that adenosine and its analogues modulated amygdala kindling in rats and adenosine was proposed to be the brain's endogenous anticonvulsant (Dragunow and Goddard, 1984; Dragunow et al., 1985; Dragunow, 1986). The crucial role of adenosinergic neuromodulation in the control of seizure activity is now well established and has recently been reviewed (Boison, 2005). In addition, adenosine is involved in one of several endogenous mechanisms of the brain that have evolved to terminate seizures (Lado and Moshe, 2008).

Adenosine exerts its neuromodulatory functions by binding to four known adenosine receptor subtypes (A_1R, $A_{2A}R$, $A_{2B}R$, A_3R) that all belong to the family of seven-membrane-spanning G-protein coupled receptors (Fredholm et al., 2001, 2005, 2007). Binding of adenosine to the high affinity A_1R, which is prominently expressed at pre- and postsynaptic sites within the hippocampal formation, leads to decreased neuronal transmission and reduced excitability that are largely based on inhibition of presynaptic transmitter release and stabilization of the postsynaptic membrane potential through increased potassium efflux via G protein-coupled inwardly rectifying potassium (GIRK) channels (Sebastiao and Ribeiro, 2000). The A_1R-mediated functions are largely responsible for the anticonvulsant and neuroprotective activity of adenosine. Thus, A_1R knockout mice experience spontaneous hippocampal seizures (Li et al., 2007a) and are hypersensitive to status epilepticus- or trauma-induced brain injury (Fedele et al., 2006; Kochanek et al., 2006). While the A_1R is thought to set a global inhibitory environment within the brain and to provide heterosynaptic depression, the stimulatory $A_{2A}R$ on postsynaptic locations is thought to be preferentially activated by high frequency stimulation and thus is ideally suited to potentiate selected synaptic transmission within a globally inhibited network (Cunha, 2008). In contrast to the well characterized role of the A_1R in epilepsy, A_{2A} receptor activation in epilepsy appears to have both proconvulsant as well as anticonvulsant characteristics depending on the context of activation (Boison, 2005, 2007b). Whereas A_1Rs and $A_{2A}Rs$ are primarily responsible for the central effects of adenosine (Ribeiro et al., 2003), the low affinity and low abundance $A_{2B}Rs$ and A_3Rs are currently not considered as therapeutic targets for epilepsy (Boison, 2005, 2007b). Functional receptor—receptor interactions of A_1Rs and different types of metabotropic and ionotropic receptors allow a further complexity in adenosinergic neuromodulation (Sichardt and Nieber, 2007).

Synaptic levels of adenosine in adult brain are largely regulated by an astrocyte-based adenosine-cycle (Boison, 2008c), and conversely, adenosine plays important roles for astrocyte physiology (Bjorklund et al., 2008). Synaptic adenosine largely originates from extracellular breakdown of ATP (Dunwiddie et al., 1997; Ziganshin et al., 1994; Zimmermann, 2000), which in turn is derived from vesicular release from astrocytes or neurons (Fields and Burnstock, 2006; Halassa et al., 2007; Haydon and Carmignoto, 2006; Pascual et al., 2005). Alternatively, adenosine as such can directly be released from astrocytes (Frenguelli et al., 2007; Martin et al., 2007). Under physiological conditions, extra- and intracellular levels of adenosine are rapidly equilibrated via distinct families of nucleoside transporters (Baldwin et al., 2004; Gray et al., 2004). Intracellularly, adenosine is rapidly phosphorylated into 5′-adenosine-monophosphate (AMP) via adenosine kinase (ADK; EC 2.7.1.20), an evolutionary conserved member of the ribokinase family of proteins (Park and Gupta, 2008). Due to the high metabolic activity of ADK and the existence of equilibrative transport systems for adenosine, synaptic levels of adenosine are thought to be controlled by intracellular metabolism of adenosine via ADK that assumes the role of a metabolic reuptake system for adenosine; in contrast to classical neurotransmitters, which all have their specific re-uptake transporters, a comparable transporter-controlled re-uptake system for adenosine appears to be lacking (Boison, 2006). It is important to note that in adult brain ADK is almost exclusively expressed in astrocytes (Studer et al., 2006).

Adenosine augmentation: therapeutic rationale

Based on the failure of traditional neuron-centered pharmacotherapy in about one third of patients with epilepsy, the exploitation of non-neuronal and non-chemical synaptic signalling pathways may offer alternatives for epilepsy therapy (Szente, 2008). Several lines of evidence suggest that astrocyte dysfunction and deficiencies in endogenous adenosinergic neuromodulation contribute to seizure generation. In healthy adult brain, physiological adenosine concentrations (25—250 nM) are kept in the range of the