



A Scaffold as a Platform for New Therapies?

Neurabin Scaffolding of Adenosine Receptor and RGS4 Regulates Anti-Seizure Effect of Endogenous Adenosine.

Chen Y, Liu Y, Cottingham C, McMahon L, Jiao K, Greengard P, Wang Q. *J Neurosci* 2012;32:2683–2695.

Endogenous adenosine is an essential protective agent against neural damage by various insults to the brain. However, the therapeutic potential of adenosine receptor-directed ligands for neuroprotection is offset by side effects in peripheral tissues and organs. An increase in adenosine receptor responsiveness to endogenous adenosine would enhance neuroprotection while avoiding the confounding effects of exogenous ligands. Here we report novel regulation of adenosine-evoked responses by a neural tissue-specific protein, neurabin. Neurabin attenuated adenosine A₁ receptor (A₁R) signaling by assembling a complex between the A₁R and the regulator of G-protein signaling 4 (RGS4), a protein known to turn off G-protein signaling. Inactivation of the neurabin gene enhanced A₁R signaling and promoted the protective effect of adenosine against excitotoxic seizure and neuronal death in mice. Furthermore, administration of a small molecule inhibitor of RGS4 significantly attenuated seizure severity in mice. Notably, the dose of kainate capable of inducing an ~50% rate of death in wild-type (WT) mice did not affect neurabin-null mice or WT mice cotreated with an RGS4 inhibitor. The enhanced anti-seizure and neuroprotective effect achieved by disruption of the A₁R/neurabin/RGS4 complex is elicited by the on-site and on demand release of endogenous adenosine, and does not require administration of A₁R ligands. These data identify neurabin-RGS4 as a novel tissue-selective regulatory mechanism for fine-tuning adenosine receptor function in the nervous system. Moreover, these findings implicate the A₁R/neurabin/RGS4 complex as a valid therapeutic target for specifically manipulating the neuroprotective effects of endogenous adenosine.

Commentary

The brain's own endogenous neuroprotectant and anticonvulsant adenosine is one of the most potent antiepileptic substances known, with demonstrated effectiveness in pharmacoresistant epilepsy (1). Adenosine's acute protective effects are largely based on activation of presynaptic and postsynaptic adenosine A₁ receptors (A₁Rs), which are coupled to inhibitory G-proteins (2). Activation of these receptors leads to presynaptic inhibition and stabilization of the postsynaptic membrane potential, two potent mechanisms limiting neuronal excitability and excitotoxicity (3). Consequently, therapeutic augmentation of A₁R signaling holds significant potential for the treatment of epilepsy (1). Unfortunately, A₁Rs are widely expressed throughout the mammalian body, and systemic A₁R activation is associated with major—largely cardiovascular—side effects (3, 4). This is a major challenge for therapy development. If there was a therapy to limit the therapeutic activity of adenosine to an epileptogenic brain area, seizure suppression without side effects might become feasible. Several strategies have been developed to limit the therapeutic augmentation of A₁R signaling to the onset zones of seizure generation: adenosine kinase inhibitors have been developed to impair metabolic clearance of *endogenous* adenosine; however, the risk for ma-

nor systemic side effects precluded further drug development efforts (5). Further, a ketogenic diet was shown to suppress electrographic seizures in mice based on augmentation of A₁R signaling in the brain (6), and local adenosine-releasing brain implants were shown to effectively prevent seizures in a variety of rodent models of induced and spontaneous recurrent chronic seizures (7). However, with the exception of certain adenosine kinase inhibitors, to date there is no small-molecule drug available that could potentiate the endogenous antiepileptic functions of adenosine at the sites of seizure activity in an event-specific manner without widespread side effects.

In the study by Chen and colleagues, this challenge was addressed in an elegant series of molecular, biochemical, pharmacologic, and physiological experiments. The authors reasoned that while the A₁R is ubiquitously expressed, there might be an A₁R-dependent signal transduction mechanism that is specific to neurons in the brain. As a G-protein coupled receptor (2, 3), the A₁R couples either directly or indirectly to a group of proteins called regulators of G-protein signaling (RGS) whose function is the termination of G-protein signaling (8). The authors' reasoning was that a drug that inhibits RGS function might enhance and prolong the anticonvulsant activity of endogenous adenosine; if there was a brain-specific coupling mechanism, this strategy might be useful to augment endogenous adenosine signaling in a site- and event-specific manner. Using immunoprecipitation methods, Chen and colleagues first demonstrated an agonist-regulated interaction of the A₁R and neurabin, an adaptor protein that is



specific for neural tissues. Neurabin was found to be essential to mediate the attenuation of A₁R-mediated responses both in vitro and in vivo. Of importance, neurabin knock-out mice displayed enhanced sedation following A₁R stimulation, indicating that neurabin functionally couples to A₁Rs. Next, Chen and colleagues demonstrated the functional involvement of RGS4, which belongs to the R4 subfamily of RGS proteins that are highly expressed in brain and heart, in the neurabin-mediated attenuation of A₁R signaling. CCG-4986, a small molecule inhibitor of RGS4, as well as antisense oligonucleotides or small interfering RNAs abrogated the neurabin-dependent attenuation of A₁R signaling in cultured cells. Additional functional studies in cultured cells revealed that the A₁R, neurabin, and RGS4 form a complex following agonist stimulation to attenuate A₁R signaling. In this complex, neurabin serves as a scaffold to recruit RGS4 to the plasma membrane under conditions in which the A₁R was activated. These findings suggest that the neurabin/RGS4 complex might be a therapeutic target for epilepsy. Chen et al. reasoned that a block of neurabin/RGS4 signaling would potentiate the neuroprotective and antiseizure effects of endogenous adenosine. To test this hypothesis the authors used a mouse model of acute seizures and neuronal cell death, induced by systemic exposure to the excitotoxin kainic acid (KA). First, Chen and colleagues demonstrated attenuation of KA-induced acute seizures and reduction of neuronal cell death in neurabin knock-out mice. In addition, neurabin knock-out mice were less likely to develop spontaneous seizures 3 months after KA-injection; however, this result is not surprising because the attenuated acute response to KA in the mutant animals would limit subsequent epileptogenesis. Next, the authors demonstrated that the small molecule RGS4 inhibitor CCG-4986 attenuated acute KA-induced seizures and neuronal cell death to a similar degree as was observed in the neurabin knock-out mice. Protection was lost when KA-injection was paired with systemic administration of the A₁R antagonist DPCPX, whereas CCG-4986 had no effects in neurabin knock-out mice. Together these data suggest that the neurabin-RGS4 complex is engaged after agonist activation of the A₁R and negatively regulates A₁R activity in vivo. Because of tissue and cell-type specificity of neurabin and RGS4 expression, this process appears to be specific for neuronal signal transduction.

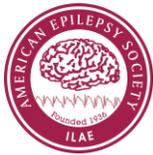
These findings are exciting for several reasons: A small molecule inhibitor was shown to promote the neuroprotective actions of endogenous adenosine. Since this process appears to be specific for neural signal transmission, the therapeutic modulation of A₁Rs appears to be feasible without the widespread side effects associated with conventional A₁R agonists. Of importance, RGS4 knock-out mice do not develop any major physiological impairment under baseline conditions; therefore systemic RGS4 inhibition appears to be relatively safe under normal circumstances. However, a word of caution is needed regarding the elimination of a physiological mechanism to protect the brain from the effects of excessive adenosine signaling, which for example has been implicated in one of the potential mechanisms underlying sudden unexpected death in epilepsy (9). Therapeutic targeting of RGS4 might therefore be limited

to pathologic conditions in which the potentiation of endogenous adenosine might provide acute therapeutic benefit (e.g., in stroke prevention). Whereas Chen and colleagues demonstrated potent neuroprotective effects of the RGS4 inhibitor CCG-4986 and attenuation of chemically induced seizures, it remains to be demonstrated whether RGS4 inhibitors can prevent or reduce spontaneous seizures in models of chronic epilepsy. Since adenosine deficiency is a pathologic hallmark of many chronic epilepsies (10), it appears unlikely that under conditions of reduced A₁R activation, RGS4 inhibition would provide sufficient signal augmentation to suppress spontaneous seizures in chronic epilepsy. Further, the expression of A₁Rs appears to be reduced in chronic epilepsy in humans (1). Thus, it remains to be demonstrated whether functional A₁R-neurabin-RGS4 coupling is maintained in chronic epilepsy. Despite those caveats, the findings from Chen and colleagues are a first step to harness the protective effects of adenosine pharmacologically in a tissue-specific manner. Perhaps the combination of an RGS4 inhibitor with an adenosine elevating agent might provide a therapeutic option to treat spontaneous seizures in chronic epilepsy under conditions in which endogenous adenosine signaling might be compromised.

by Detlev Boison, PhD

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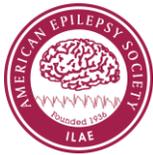
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