

ADENOSINE KINASE ELEVATION INCREASES ACTIVITY IN THE KAINIC ACID MODEL OF EPILEPSY

Overexpression of Adenosine Kinase in Epileptic Hippocampus Contributes to Epileptogenesis

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J Neurosci 2004;24:692–701

Endogenous adenosine in the brain is thought to prevent the development and spread of seizures via a tonic anticonvulsant effect. Brain levels of adenosine are regulated primarily by the activity of adenosine kinase. To establish a link between adenosine kinase expression and seizure activity, we analyzed the expression of adenosine kinase in the brain of control mice and in a kainic acid-induced mouse model of mesial temporal lobe epilepsy. Immunohistochemical analysis of brain sections of control mice revealed intense staining for adenosine kinase, mainly in astrocytes, which were more or less evenly distributed throughout the brain, as well as in some neurons, particularly in olfactory bulb, striatum, and brainstem. In contrast, hippocampi lesioned by a unilateral kainic acid injection displayed profound astrogliosis and therefore a significant increase in adenosine kinase immunoreactivity accompanied by a corresponding increase of enzyme activity, which paralleled chronic recurrent seizure activity in this brain region. Accordingly, seizures and interictal spikes were suppressed by the injection of a low dose of the adenosine kinase inhibitor 5-iodotubercidin. We conclude that overexpression of adenosine kinase in discrete parts of the epileptic hippocampus may contribute to the development and progression of seizure activity.

which are general CNS stimulants. Under control conditions, hippocampi stain for ADK diffusely in the CA fields and dentate molecular layer, as it does in cortex. More intense staining is seen in the stratum pyramidale and granule cell layer as well as the hilus, although even there, the staining is associated with astroglial cells.

However, after kainic acid (KA) injection in the ipsilateral hippocampus, ADK immunoreactivity changes are seen in the hilus as well as the stratum lucidum, oriens, and radiatum of this area as soon as 2 hours after the injection, such that these areas become devoid of ADK staining. Contralateral hippocampal staining is unaffected until days later. On day 1, after KA injection, intense staining occurs in the granule cells of the dentate gyrus, and ADK expression appears homogeneous in the affected hippocampus. One week after KA delivery, granular cell staining has disappeared, and the remaining hippocampal formation becomes infiltrated with ADK-positive cells, paralleling ongoing astrogliosis. This staining is most intense in the hilus of the dentate gyrus and the stratum lacunosum–moleculare; contralaterally, a diffuse increase is found in ADK-labeled cells. With the progression of gliosis, ADK immunoreactivity increases in a manner such that by the last time point studied (4 weeks), ADK staining is localized within processes as well as perinuclear zones in positively stained cells, which are now mainly evident in the dentate, stratum lacunosum, and stratum oriens (although CA3 remains unchanged).

In corroboration of these findings, biochemical assays of ADK activity showed both ipsilateral and contralateral hippocampi had significantly higher ADK activity than control hippocampi and, further, that ipsilateral preparations showed significantly higher enzymatic activity than contralateral ones. The neurophysiology that parallels these histochemical and biochemical changes shows that status epilepticus is triggered by the injection of KA, followed by a latent period of 2 weeks, which then gives way to the generation of spontaneous seizures in subsequent weeks.

This study is rather straightforward, and the data are quite consistent. The experimental setup is technically rather involved, and several lines of evidence have been pursued. However, the data obtained, which are largely histochemical, seem rather qualitative, and experimental group sizes have been limited to three to four animals per group to study time points

COMMENTARY

This article examines the potential role of adenosine and its metabolic enzyme adenosine kinase (ADK) in the expression of seizure activity. Adenosine is generally thought to have an inhibitory action on the CNS via adenosine A₁ and A_{2A} receptors and to be relieved by antagonists, such as caffeine,

ranging from 2 hours to 4 weeks. Although the figures displaying histochemical data appear to support the results described, it is uncertain what the variability was among samples, if any (e.g., was the background staining high in some preparations, making determinations difficult), in short, no measures are aimed at describing group data. Further, the biochemical data that are presented here use groups of four to five animals. Although it is typical to use to higher numbers of test subjects, perhaps the data are rather invariable.

The ADK antagonist 5-iodotubercidin clearly suppresses seizure activation in KA-lesioned animals, and subsequently, DPCTX, the adenosine A₁ antagonist, restores them. Quantifying seizure activity, in this instance in which seizure activity is completely abolished, is a bit superfluous, and the data, as presented, on seizure occurrence are misleading as presented. The authors refer to number of seizures per hour; for example, in KA-lesioned mice, an average of 32 seizures per hour was seen, each lasting an average of about 48 seconds. Thus about one seizure occurred every 2 minutes, each lasting almost 1 minute. Clinically, the rapidity of these events does not appear to allow for recovery, as we generally understand it, and thus would be considered status epilepticus. It would have been helpful to have the authors' method for quantifying seizures (i.e., where does one seizure end and the next one begin) clearly specified, perhaps graphically. Further, how were the EEG data analyzed:

was it done manually or by means of software? How long were EEG recordings for this analysis (the longer the period recorded, the more reliable the data)? The only figure that describes the neurophysiology displays a total of 360 seconds of EEG data obtained from a single experiment under each of three pharmacologic conditions (i.e., before and after 5-tubercidin and after the addition of DPCTX—120 seconds of EEG data for each). It is not clear from the data presented how measurements were determined.

How can it be determined whether the EEG data are truly representative of this group? Do all the pauses separate seizures? Given the stated average, it is possible, but the meaning of the pauses should be clarified. Perhaps, spike frequency, along with interspike interval, might have provided more useful measures of the data. The authors are to be complimented on the wide range of data collected. However, it is important to be mindful that what is being reported is quantified in a manner that (a) is useful to future studies that replicate and extend the present findings; (b) presents data that may be generalized (by using an adequate number of subjects to accomplish this end); (c) is the best way to illustrate the point at hand; (d) defines the terms that are used, conforming to accepted parlance; (e) is representative of the data; and (f) fully specifies the methods.

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