

ATP-DEPENDENT POTASSIUM CHANNELS: A CONVERGING TARGET FOR ENDOGENOUS ANTICONVULSANT FACTORS

Zinc Inhibits Glutamate Release via Activation of Presynaptic K Channels and Reduces Ischaemic Damage in Rat Hippocampus

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PURPOSE: Zinc is concentrated in certain CNS excitatory tracts, especially in hippocampal mossy fibers, where it has been suggested to modulate synaptic transmission and plasticity.

METHODS: By using rat mossy fiber synaptosomes depolarized by 4-aminopyridine, we show here that low zinc concentrations restore the membrane potential and reduce glutamate release. Both effects arose from activation of ATP-sensitive potassium channels (K_{ATP}), because they were mimicked by the K_{ATP} -opener diazoxide and antagonized by the K_{ATP} -blocker tolbutamide. By using recombinant channels expressed in COS-7 cells, we confirmed that micromolar zinc did activate K_{ATP} of the type found in hippocampus. We tested the hypothesis that this action of

zinc could be beneficial during an ischemic challenge by using organotypic hippocampal slice cultures.

RESULTS: When zinc was applied at micromolar concentrations during a brief anoxic-hypoglycemic episode, it significantly attenuated the ensuing neuronal death, whereas chelation of endogenous zinc markedly aggravated cell damage. Protective effect of zinc was mediated through K_{ATP} , as was shown by using the opener diazoxide and the blocker tolbutamide.

CONCLUSIONS: Thus, by activating presynaptic K_{ATP} channels, zinc protects neurons from hyperexcitation, excessive transmitter release, and excitotoxicity, and may thus act as an endogenous neuroprotector in conditions such as epilepsy or stroke.

COMMENTARY

The dentate gyrus is often described as a gateway in the propagation of seizure activity. Glutamatergic excitation from granule cells of the dentate gyrus propagates through the axons (mossy fibers) onto pyramidal neurons of the CA3 area of the hippocampus. Thus, elucidating factors that regulate the excitability of mossy fibers is important for understanding the mechanisms of epileptogenesis. Furthermore, identifying endogenous inhibitors of glutamatergic transmission in the synaptic connection between a dentate granule cell and a CA3 pyramidal neuron may translate into novel approaches for epilepsy therapy. Similar to that of other classic neurotransmitters, glutamatergic transmission is regulated by a diverse group of biologically active substances (i.e., neuromodulators). Neuromodulators are colocalized and coreleased with classic neurotransmitters and either facilitate or mitigate effects of the latter, both presynaptically (change in neurotransmitter release) and postsynaptically (change in functional state of the receptor).

One striking property of mossy fibers is the abundant presence of zinc. Zinc is localized in synaptic vesicles and is co-released with glutamate in response to an excitatory stimulus, thus meeting the definition of a neuromodulator. Numerous

studies have examined the role of zinc in the physiology of the hippocampus. Current views on the role of zinc in regulating hippocampal excitability are controversial. On the one hand, zinc has been implicated in potentiating neurotoxic effects of glutamate (1) and in compromising GABAergic inhibition (2). On the other hand, zinc has been shown to protect neurons against excitotoxic injury, attenuate excitability of mossy fibers, and inhibit *N*-methyl-D-aspartate (NMDA) receptors (3,4). A possible explanation for this discrepancy lies in different concentrations of zinc used. Studies that have used endogenous zinc or exogenous zinc in physiologically relevant concentrations report inhibitory and neuroprotective effects, whereas zinc in high concentrations seems to facilitate excitatory transmission. However, because the release of zinc is activity dependent, it is likely that the massive release that apparently occurs during seizures affords concentrations high enough to positively modulate glutamatergic transmission.

Another remarkable property of mossy fibers is high expression of ATP-sensitive potassium channels (K_{ATP}). K_{ATP} are potent regulators of neuronal excitability. Closing and opening of K_{ATP} depends on the intracellular concentration of ATP: when the intracellular ATP/adenosine diphosphate ratio increases, K_{ATP} close, and vice versa. Closing of K_{ATP} leads to

membrane depolarization and, thus, increases the excitability, whereas opening of K_{ATP} hyperpolarizes the membrane and is inhibitory. Hence, activation (i.e., opening) of K_{ATP} in mossy fibers is potentially protective against excitotoxicity, as it would impede glutamatergic transmission on the presynaptic level.

Mossy fibers are not the only site where zinc and K_{ATP} coexist. Their colocalization has also been found in pancreatic β cells, where zinc is coreleased with insulin, opens K_{ATP} , hyperpolarizes β -cell membrane, and inhibits further insulin release. The interaction between zinc and K_{ATP} in the pancreas prompted Bancila et al. to investigate whether similar interaction exists in mossy fibers and whether it modulates glutamatergic transmission in the dentate granule–CA3 pyramidal cell synapse.

The authors performed several lines of experiments. First, by using mossy fiber synaptosomes (isolated presynaptic terminals), they examined whether zinc regulates membrane excitability and whether this regulation is K_{ATP} dependent. The authors showed that zinc, although lacking any effect on resting membrane potential, blocked membrane depolarization induced by a nonselective, voltage-gated K^+ channel blocker, 4-aminopyridine. The effect of zinc was reversed by a K_{ATP} blocker, tolbutamide. Furthermore, zinc exhibited similar effects in a cell line transfected with K_{ATP} , thus directly proving that K_{ATP} was a target for its hyperpolarizing action.

An obvious consequence of mossy fiber hyperpolarization is inhibition of glutamate release. Zinc inhibited and tolbutamide increased 4-aminopyridine–induced glutamate release from mossy fiber synaptosomes. Furthermore, similar to the experiments with membrane potential, neither zinc nor synthetic K_{ATP} modulators affected basal release of glutamate.

The finding that zinc exerted its effects only during depolarization, but not under basal conditions, emphasized that opening of K_{ATP} may have little or no effect in the normally functioning hippocampal circuit, yet it becomes important during excessive excitation. Notwithstanding potential limitations of using K_{ATP} modulators as antiepileptic agents, such selectivity is useful. It is conceivable that K_{ATP} openers would be highly effective during pathologic excitation, without affecting normal neuronal functioning and related processes (e.g., learning and memory).

Inhibitory effects of zinc on glutamate release suggest that zinc, acting through K_{ATP} , may protect hippocampal neurons, particularly CA3 pyramidal cells, from excitotoxic injury. The authors found that neuronal hippocampal injury induced by hypoxia and hypoglycemia was dramatically attenuated after exogenous application of zinc, again via activation of K_{ATP} . More important, endogenous zinc was neuroprotective, because its chelation exacerbated hypoxia-induced neuronal cell death. Thus, it is conceivable that zinc-mediated K_{ATP} opening might be neuroprotective and anticonvulsant in other scenarios in-

volving excitotoxicity, such as limbic seizures. Furthermore, in temporal lobe epilepsy, which is characterized by recurrent excitatory connections among dentate granule cells (5), zinc might counteract this recurrent excitation on the presynaptic level, in addition to previously shown inhibition of NMDA receptors (4).

As Bancila and colleagues found, zinc acted as a K_{ATP} opener in concentrations that are believed to be relevant to the normal extracellular environment. In higher concentrations, which are induced by seizure activity, zinc could have neurotoxic effects if, for example, it acted in a manner similar to calcium at the NMDA receptor. Thus, it is conceivable that zinc potentiates glutamate excitotoxicity locally in epileptic foci, while inhibiting glutamatergic transmission and counteracting the propagation of seizure activity at distant synapses where its concentrations are lower (6).

The study by Bancila and colleagues emphasizes the importance of K_{ATP} in regulating neuronal excitability in excitotoxic conditions, including epilepsy. K_{ATP} , along with G protein-activated K^+ channels, belongs to the family of inward rectifier potassium channels, which set the membrane potential and transmitter release. Inward rectifying potassium channels are targets for antiepileptic effects of neuropeptides (e.g., galanin), some neurotransmitters (e.g., serotonin) (7), and as it occurs, for zinc. It is questionable, however, whether K_{ATP} openers soon can be translated into epilepsy therapy. K_{ATP} modulators have multiple effects, because peripheral (i.e., heart, pancreas, and vascular system) and brain K_{ATP} are similar (7). Nevertheless, it is conceivable that the ketogenic diet could act, in part, through activating K_{ATP} (8) and that a zinc-enriched diet might have anticonvulsant properties, as a result of the same mechanism (9).

by Andrey Mazarati, MD, PhD

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