

BRAIN WATER AND ION FLUXES: A HARD-TO-DIE HYPOTHESIS TO EXPLAIN SEIZURES

Increased Seizure Duration and Slowed Potassium Kinetics in Mice Lacking Aquaporin-4 Water Channels. Binder DK, Yao X, Zador Z, Sick TJ, Verkman AS, Manley GT. *Glia* 2006;53:631–636. The glial water channel aquaporin-4 (AQP4) has been hypothesized to modulate water and potassium fluxes associated with neuronal activity. In this study, we examined the seizure phenotype of AQP4 *-/-* mice using *in vivo* electrical stimulation and electroencephalographic (EEG) recording. AQP4 *-/-* mice were found to have dramatically prolonged stimulation-evoked seizures after hippocampal stimulation compared to wild-type controls (33 ± 2 s vs. 13 ± 2 s). In addition, AQP4 *-/-* mice were found to have a higher seizure threshold (167 ± 17 A vs. 114 ± 10 A). To assess a potential effect of AQP4 on potassium kinetics, we used *in vivo* recording with potassium-sensitive microelectrodes after direct cortical stimulation. Although there was no significant difference in baseline or peak $[K^+]_o$, the rise time to peak $[K^+]_o$ ($t_{1/2}$, 2.3 ± 0.5 s) as well as the recovery to baseline $[K^+]_o$ ($t_{1/2}$, 15.6 ± 1.5 s) were slowed in AQP4 *-/-* mice compared to WT mice ($t_{1/2}$, 0.5 ± 0.1 and 6.6 ± 0.7 s, respectively). These results implicate AQP4 in the expression and termination of seizure activity and support the hypothesis that AQP4 is coupled to potassium homeostasis *in vivo*.

COMMENTARY

Neuronal action potentials result from rapid influx of sodium or calcium ions and are terminated by a slower potassium efflux from the cell. These events, if not followed by a homeostatic response, lead to extracellular sodium and calcium depletion and potassium accumulation. In the CNS, neuronal ion pumps and transporters cooperate with glial ion channels in extracellular potassium clearance and refurbishment of $[Na^+]_o$ and $[Ca^{2+}]_o$ (1). Epileptic seizures are accompanied by abnormal synchronicity and hyperexcitability, which leads to increased inward sodium/calcium and outward potassium fluxes. Under these conditions, potassium ions reach a plateau in excess of the normal, low millimolar levels under resting or interictal conditions (2). This abnormal potassium accumulation can be explained by: 1) increased release from hyperactive neurons; 2) reduced glial capacity in potassium clearance; and 3) changes in extracellular space favoring potassium accumulation (2,3). The latter is controlled by a number of cellular mechanisms, including blood–brain barrier endothelial cells, production and absorption of CSF, and glial swelling in response to changes in osmolarity.

Binder et al. have extensively published data obtained from mice lacking the aquaporin water channel, AQP4, which is involved in the regulation of extracellular space and in osmotic brain homeostasis (4). The data show that animals lacking AQP4 have a higher seizure threshold, regardless of the models used. Both chemical (pentylentetrazol) and electrical stimu-

lation were less effective in inducing epileptiform activity in AQP4-null mice. The other main finding was that in AQP4-null mice the duration of seizures was dramatically reduced. The authors invoke a mechanism involving potassium homeostasis, supported by the fact that $[K^+]_o$ transients lasted longer in AQP-null mice. This finding suggests that rebound excitation may be enhanced by elevated $[K^+]_o$.

Both direct and indirect evidence show that management of extracellular potassium is deficient in epileptic brain (5) or under pathophysiological conditions associated with an increased propensity toward seizures, such as traumatic brain injury (6). The main cause underlying loss of potassium homeostasis seems to be loss of a specific subset of inwardly rectifying potassium (K_{IR}) channels that are also associated with cell cycle (7,8). Although Binder et al. did not find any correlation between either increased seizure duration or decreased seizure threshold and expression of K_{IR} 4.1, they did not evaluate other K_{IR} channels expressed in glial cells, so whether a correlation exists with these channels remains unknown. In addition, it is possible that these channels, while still present in glia, did not work properly because of failed positioning into the membrane or other factors. Further studies will be needed to clarify if a specific channel family is involved in the loss of potassium homeostasis in these mice.

Binder et al. show that water channels are relevant for the control of neuronal excitation, supporting the view that brain homeostasis plays an important role in controlling neuronal firing. These data as well as findings by others (2,3) support the possibility of alternative approaches to epilepsy therapy, such as drugs specifically involved in the reestablishment of brain homeostasis (e.g., diuretics) (9). The link between resistance to an initial seizure and decreased water channel levels may be relevant to the management of elevated risk of acute seizures. Aggressive monitoring and management of intracranial pressure

and brain water content after acute trauma or stroke is common practice. Thus, this work may provide a molecular correlate of an effective medical treatment and further increase our appreciation of how electrolytes and water balance are crucial aspects of CNS function and failure.

Unlike sodium ions, potassium ions are vasoactive and changes in $[K^+]_o$ have a powerful effect on the coupling of cerebral blood flow to neuronal activity (10). The combined effects of altered extracellular space and decreased potassium buffering thus may have many consequences. In addition, K_{IR} channel gating is affected by extracellular potassium. A moderate increase in potassium concentration, in the range of 1–15 mM, enhances potassium efflux through K_{IR} at physiologically relevant potentials. In some cells, including vascular smooth muscle, a moderate increase in extracellular potassium, paradoxically, leads to hyperpolarization and relaxation (11). Further increases in $[K]_o$ (to the plateau values seen during seizures), in contrast, cause vasoconstriction, depriving the brain of the nutrients needed during intense neuronal activity. The combined effects of altered potassium homeostasis and cerebral blood flow may be a crucial aspect of loss of aquaporin signaling.

In conclusion, the results presented support mechanisms of epileptogenesis that encompass nonneuronal cells and concentrate on the regulation of synaptic activity and neuronal synchronization by changes in extracellular ions. The latter impact not only the electrical properties of neurons but also the regulation of cerebral blood flow.

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References

1. Janigro D, McKhann GM. Electrophysiology of the CNS. In: Winn HR, ed. *Youman's Neurosurgery*. Philadelphia, PA: Saunders Co. 2003. pp. 153–173.
2. Dietzel I, Heinemann U, Lux HD. Relations between slow extracellular potential changes, glial potassium buffering, and electrolyte and cellular volume changes during neuronal hyperactivity in cat brain. *Glia* 1989;2:25–44.
3. Lux HD, Heinemann U, Dietzel I. Ionic changes and alterations in the size of the extracellular space during epileptic activity. *Adv Neurol* 1986;44:619–639.
4. Binder DK, Papadopoulos MC, Haggie PM, Verkman AS. In vivo measurement of brain extracellular space diffusion by cortical surface photobleaching. *J Neurosci* 2004;24:8049–8056.
5. Bordey A, Sontheimer H. Properties of human glial cells associated with epileptic seizure foci. *Epilepsy Res* 1998;32:286–303.
6. D'Ambrosio R, Maris DO, Grady MS, Winn HR, Janigro D. Impaired K homeostasis and altered electrophysiological properties of post-traumatic hippocampal glia. *J Neurosci* 1999;19:8152–8162.
7. MacFarlane SN, Sontheimer H. Changes in ion channel expression accompany cell cycle progression of spinal cord astrocytes. *Glia* 2000;30:39–48.
8. Cucullo L, Dini G, Hallene KL, et al. Very low intensity alternating current decreases cell proliferation. *Glia* 2005;51:65–72.
9. Schwartzkroin PA, Baraban SC, Hochman DW. Osmolarity, ionic flux, and changes in brain excitability. *Epilepsy Res* 1998;32:275–285.
10. Nguyen TS, Winn HR, Janigro D. ATP-sensitive potassium channels may participate in the coupling of neuronal activity and cerebrovascular tone. *Am J Physiol Heart Circ Physiol* 2000;278:H878–H885.
11. Kubo Y. Two aspects of the inward rectification mechanism. Effects of cytoplasmic blockers and extracellular K^+ on the inward rectifier K^+ channel. *Jpn Heart J* 1996;37:631–641.