Regulation of Brain Water: Is There a Role for Aquaporins in Epilepsy?

Aquaporin-4 Is Increased in the Sclerotic Hippocampus in Human Temporal Lobe Epilepsy

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The hippocampus of patients with mesial temporal lobe epilepsy is often hardened and shrunken, a condition known as sclerosis. MRI reveals an increase in the T_2-weighted signal, whereas diffusion-weighted imaging shows a higher apparent diffusion coefficient in sclerotic hippocampi, indicating increased water content. As water transport appears to be coupled to K^+ clearance and neuronal excitability, the molecular basis of the perturbed water homeostasis in the sclerotic hippocampus was explored. The expression of aquaporin-4 (AQP-4), the predominant water channel in the brain, was studied with quantitative real-time polymerase chain reaction analysis, light-microscopic immunohistochemistry, and high-resolution immunogold labeling. A significant increase in AQP-4 was observed in sclerotic, but not in non-sclerotic, hippocampi obtained from patients with medically intractable temporal lobe epilepsy. This increase was positively correlated with an increase in the astrocyte marker glial fibrillary acidic protein. AQP-4 was localized to the plasma membranes of astrocytes including the perivascular endfeet. Gene expression associated with increased AQP-4 was evaluated by high-throughput gene-expression analysis with Affymetrix GeneChip U133A, and related gene networks were investigated with Ingenuity Pathways Analysis. AQP-4 expression was associated with a decrease in expression of the dystrophin gene, a protein implicated in the anchoring of AQP-4 in perivascular endfeet. The decreased expression of dystrophin may indicate a loss of polarity in the distribution of AQP-4 in astrocytes. We conclude that the perturbed expression of AQP-4 and dystrophin may be one factor underlying the loss of ion and water homeostasis in the sclerotic hippocampus and hypothesize that the reported changes may contribute to the epileptogenic properties of the sclerotic tissue.

COMMENTARY

A growing body of evidence indicates that some cells have specialized channels in their plasma membranes that permit water to be transported though the lipid bilayer much more efficiently than by simple diffusion. The critical role of these bidirectional water transporters in biology and medicine was highlighted recently when Peter Agre was awarded the Nobel Prize for the discovery of the channels, which he has called "aquaporins" (1). At least 11 *aquaporin* (*AQP*) genes are present in mammals. These genes encode 30-kDa membrane proteins that are thought to assemble as homotetramers to form the water channels. By far the most abundant aquaporin in brain is AQP4, although AQP1 is present in the choroid plexus. Studies in AQP4-knockout mice have demonstrated a key role of this aquaporin in brain edema (2). In addition, AQP4 appears to be crucial for brain water and ion homeostasis during rapid neural activity (3).

Astrocytic endfeet that contact capillaries and pia abundantly express AQP4, which is present only at low levels elsewhere in the astrocyte. Interestingly, the C-terminus of AQP4 has a PDZ-binding domain, which may allow it to be anchored to cytoskeletal proteins. AQP4 is thought to associate with the syntrophins—scaffolding adaptor proteins that contain multiple protein-interaction domains (including a PDZ domain) and are present in the dystrophin protein complex. Dystrophin, a protein mutated in Duchenne muscular dystrophy, is a key component of these complexes in the muscle sarcolemmal membrane. Microvascular glial cells also have dystrophin-like complexes, which are localized to the glial–vascular interface. Studies with mice bearing the mutation for Duchenne muscular dystrophy have provided strong evidence that AQP4 is localized to astrocytic endfeet by the dystrophin complex (4).

What is the functional role of AQP4 in astrocytes? During high neuronal activity, water is transported away from the neuropil, which leads to shrinkage of the extracellular space. In the cortex, neuronal activity is associated with flux of water toward the subarachnoidal space, which serves as a water sink. This flow of water occurs too rapidly to depend on slow diffusion through lipid bilayers, thus implicating aquaporins. These activity-dependent shifts in water are thought to occur through AQP4-mediated uptake of water by nonendfeet

membranes around active synapses coupled to water efflux through the AQP4 pool in the endfeet (3).

Hippocampal sclerosis is the most common pathologic finding in mesial temporal lobe epilepsy. Hippocampal sclerosis involves neuronal loss in the CA1 and CA3 regions and in the dentate hilus, with relative preservation of dentate granule cells and a small zone of pyramidal cells in CA2, which is accompanied by dense gliosis as well as shrinkage and hardening of the tissue. The microvasculature proliferates within the sclerotic tissue, and astrocytes are thought to have abnormal interactions with the associated capillaries. The present study by Lee and colleagues, using human tissue, provides evidence that AQP4 is increased in sclerotic hippocampus, along with decreased expression of dystrophin and dystrophin-associated proteins. The authors speculate that the reduction in the dystrophin complex proteins leads to mislocalization of AQP4 in astrocytes, resulting in a loss of ion and water homeostasis. The disruption in the ability to handle metabolically produced and activity-dependent water fluxes is further hypothesized to account for the increases in water detected in brain-imaging studies of the sclerotic hippocampus and potentially contributes to epileptogenesis.

For this and many other studies showing increases in glial protein markers in epileptic tissue, an important question is whether greater expression of any such protein simply reflects astrocytic proliferation and is not specifically relevant to the pathologic process. In this study, another astrocytic marker, glial fibrillary acid protein, was increased to a similar extent as AQP4. Therefore, the increase in AQP4 expression in the present study may not be the critical factor in the postulated disrupted water balance in the sclerotic tissue. Rather, it could be that the mislocalization of AQP4, resulting from the observed altered expression in dystrophin-related proteins, is the more relevant factor.

It is interesting to speculate whether the alteration in water balance could play a pathologic role and enhance seizure susceptibility. Indeed, α-syntrophin–null mice, which have mislocated AQP4, exhibit hyperthermia-induced seizures of greater severity than do wild-type animals (5). A key factor in the greater seizure susceptibility is likely to be the impaired ability of astrocytes in the genetically altered mice to adequately clear K^+ from active neuropil. By using ion-selective microelectrodes in hippocampal brain slices, Amiry-Moghaddam and co-workers (5) demonstrated that K^+ clearance is prolonged in α-syntrophin–null mice. During ordinary neural activity, and especially during seizures, K^+ is released into the extracellular space, where it is cleared by astrocytes through several mechanisms, some of which lead to osmotic swelling. The process cannot continue if water elimination from the astrocyte is impaired, leading to suboptimal K^+ handling. Because excessive extracellular K^+ enhances seizure susceptibility, a ready explanation of how defective AQP4 function in the genetically modified animals could promote seizure susceptibility exists. Interestingly, dystrophin-deficient mice do not show clear evidence of enhanced seizure susceptibility (6). Because dystrophin also is localized to neuronal synapses, perhaps alterations in synaptic function complicate the situation in this model.

This new research by Lee et al. highlights the importance of another line of work that has demonstrated an association between the size of the extracellular space and seizure susceptibility. For more than 50 years, it has been realized that a rapid increase in the water content of the brain can lead to seizures and that dehydration provides protection against seizures (7,8). In neocortical and hippocampal slices, dilute medium enhances seizures or can induce them, by causing cell swelling and restriction of the extracellular space, even when active chemical synapses have been blocked (9). This effect leads to an increase in the concentration of extracellular K^+, which, as noted, enhances seizure susceptibility. Seizures themselves cause cell swelling and lead to shrinkage of the size of the extracellular space (10), providing a mechanism whereby seizures could promote further seizure activity. In contrast, treatment with impermeant solutes, such as mannitol, that cause cell shrinkage leads to expansion of the extracellular space, which can block electrographic seizure activity independent of the function of chemical synapses (9,11). Any alteration in the functional activity of AQP4 that compromises ion and water homeostasis after seizures, such as mistargeting due to diminished expression of dystrophin components, could lead to enhanced seizure susceptibility and contribute to epileptogenesis. Following this line of reasoning, strategies that reverse the defect in water handling could perhaps lead to new therapeutic approaches for intractable epilepsy. Extensive evidence suggests that the loop diuretic furosemide has antiseizure activity in the in vitro and animal seizure models (11) and possibly also in human epilepsy (12). Furosemide protects against activity-induced shrinkage of the extracellular space, and this effect may account for the ability of furosemide to inhibit seizures, although the precise mechanism is obscure. Perhaps in the future, it will be possible to evaluate potential therapeutic strategies that specifically target aquaporins.

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References

1. Agre P, King LS, Yasui M, Guggino WB,Ottersen OP, Fujiyoshi Y, Engel A, Nielsen S. Aquaporin water channels: from atomic structure to clinical medicine. J Physiol 2002;542:3–16.
2. Manley GT, Binder DK, Papadopoulos MC, Verkman AS. New insights into water transport and edema in the central nervous system from phenotype analysis of aquaporin-4 null mice. Neuroscience 2004;129:981–989.

3. Amiry-Moghaddam M, Ottersen OP. The molecular basis of water transport in the brain. Nat Rev Neurosci 2003;4:991–1001.
4. Neely JD, Amiry-Moghaddam M, Ottersen OP, Froehner SC, Agre P, Adams ME. Syntrophin-dependent expression and localization of Aquaporin-4 water channel protein. Proc Natl Acad Sci U S A 2001;98:14108–14113.
5. Amiry-Moghaddam M, Williamson A, Palomba M, Eid T, de Lanerolle NC, Nagelhus EA, Adams ME, Froehner SC, Agre P, Ottersen OP. Delayed K+ clearance associated with aquaporin-4 mislocalization: phenotypic defects in brains of alpha-syntrophin-null mice. Proc Natl Acad Sci U S A 2003;100:13615–13620.
6. De Sarro G, Ibbadu GF, Marra R, Rotiroti D, Loiacono A, Donato Di Paola E, Russo E. Seizure susceptibility to various convulsant stimuli in dystrophin-deficient mdx mice. Neurosci Res 2004;50:37–44.
7. Rowntree LG. The effects on mammals of the administration of excessive quantities of water. J Pharmacol Exp Ther 1926;29:139–159.
8. Andrew RD. Seizure and acute osmotic change: clinical and neurophysiological aspects. J Neurol Sci 1991;101:7–18.
9. Roper SN, Obenaus A, Dudek FE. Osmolality and nonsynaptic epileptiform bursts in rat CA1 and dentate gyrus. Ann Neurol 1992;31:81–85.
10. Dietzel I, Heinemann U, Hofmeier G, Lux HD. Transient changes in the size of extracellular space in the sensorimotor cortex of cats in relation to stimulus-induced changes in potassium concentration. Exp Brain Res 1980;40:432–439.
11. Hochman DW, Baraban SC, Owens JW, Schwartzkroin PA. Dissociation of synchronization and excitability in furosemide blockade of epileptiform activity. Science 1995;270:99–102.
12. Hesdorffer DC, Stables JP, Hauser WA, Annegers JF, Cascino G. Are certain diuretics also anticonvulsants? Ann Neurol 2001;50:458–462.