

## Response to “When can AQP4 assist transporter-mediated K<sup>+</sup> uptake?”

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We thank Hertz et al. for their comments and suggestions in this issue regarding alternative mechanisms that might be involved in the apparent coupling of K<sup>+</sup> and water in brain extracellular space (ECS). The mechanism examined in our model (Jin et al., 2013) is based on simple physical chemistry: uptake of ECS K<sup>+</sup> after release from neurons produces an osmotic driving force for water uptake by astrocytes, which reduces ECS volume and hence contributes to maintaining the electrochemical driving force for continued K<sup>+</sup> uptake. Reduced astrocyte water permeability in AQP4 deficiency slows the change in ECS volume, resulting in slowed K<sup>+</sup> uptake. To account quantitatively for experimental measurements in AQP4-null mice, it was necessary to include in the model diffusion-limited solute transport in astrocyte cytoplasm, which amplifies the sensitivity of K<sup>+</sup> uptake to astrocyte water permeability in which buildup of K<sup>+</sup> in astrocyte cytoplasm at the ECS interface reduces the driving force for K<sup>+</sup> uptake. Although additional mechanisms are likely involved in regulating ion and water movement between neurons, astrocytes, and the ECS, simple electro-osmotic coupling, without the need for AQP4-dependent K<sup>+</sup> conductance or NKCC1-facilitated water transport, was sufficient to account for the experimental data.

With regard to NKCC1-facilitated water transport, Hamann et al. (2005) reported that water permeability in pigmented ciliary epithelial cells from the eye was partially inhibited by the NKCC1 inhibitor bumetanide, and that the bumetanide-sensitive component of water transport was osmotic-gradient dependent. In a study to be reported separately (unpublished data), we reexamined NKCC1-dependent water transport using NKCC1-transfected cells, bumetanide, and astrocyte cultures from wild-type and AQP4-null mice. We were unable to detect NKCC1-facilitated water transport, even in AQP4-null astrocyte cultures where water permeability is low.

Hertz et al. (2013) suggest a possible role of pressure-driven, para-arterial convective solute transport in brain ECS, based on a report by Iliff et al. (2012), on K<sup>+</sup>/water coupling in the ECS. It is unclear to us how such long-range convection would impact short-term K<sup>+</sup>/water coupling. In any case, as discussed (Papadopoulos and Verkman, 2013), both the Iliff et al. (2012) model

for AQP4-dependent clearance of excess water from the brain and our original model (Papadopoulos et al., 2004) suffer from the fact that flow of solute-free water through AQP4 would be opposed by the osmotic gradients created by transmembrane movement of water without solute.

With regard to the role of the Na<sup>+</sup>/K<sup>+</sup> pump and NKCC1 in K<sup>+</sup> uptake by astrocytes, our model included Na<sup>+</sup>/K<sup>+</sup> pump-facilitated K<sup>+</sup> uptake explicitly, in which, based on published experimental data, pump activity was taken as a saturable function of the astrocyte-to-ECS K<sup>+</sup> gradient. Because our model focused on K<sup>+</sup> and water transport without explicit inclusion of Na<sup>+</sup> and Cl<sup>-</sup> ions, NKCC1 effects were modeled by considering different fractions of electrogenic versus electroneutral astrocyte K<sup>+</sup> transport. Our model did not explicitly include neuronal uptake mechanisms to minimize the number of uncertain parameters. Basal neuronal Na<sup>+</sup>/K<sup>+</sup> pump function was effectively included in an implicit manner in the astrocyte pump term; however, the temporal and absolute contributions of the neuronal Na<sup>+</sup>/K<sup>+</sup> pump to ECS K<sup>+</sup> clearance are not clear. We note that studies involving ouabain addition to brain slices or retinal preparations can be difficult to interpret because of the many secondary effects of metabolic depletion.

We hope that the ideas put forth in our model will stimulate further discussion and research in the challenging subject of K<sup>+</sup> and water homeostasis in brain ECS.

Edward N. Pugh Jr. served as editor.

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