When can AQP4 assist transporter-mediated K^+ uptake?

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We agree with Jin et al. (2013) that astrocytes accumulate extracellular K^+. They discuss how differences in (a) resting extracellular space (ECS) volume, (b) diffusion-limited water/K^+ transport, and (c) ECS contraction during K^+ reuptake may differently affect astrocytic K^+ uptake in wild-type animals and in mice with aquaporin-4 (*Aqp4*) knockout. The first of these factors is well studied, and it is logical that a certain extracellular K^+ concentration ($[K^+]_e$) increase in a larger volume takes longer to clear, prolonging neuroexcitation. But what causes the increase?

Recently, Iliff et al. (2012) proposed that (a) cerebrospinal fluid enters the brain parenchyma along para-arterial routes; (b) interstitial fluid (ISF) from the ECS with its waste products is cleared from the brain along para-venous routes; and (c) convective (bulk) flow-mediated ISF flow between these influx and clearance routes is facilitated by astrocytic AQP4–dependent water fluxes. They showed that *Aqp4* gene deletion slowed bulk flow–dependent solute clearance by ∼70% and suggested that during inhibition of bulk flow–dependent clearance, ECS dilation could be a compensatory mechanism to facilitate diffusional clearance of extracellular solutes, particularly of those with larger molecular weights, which is dependent on the ECS dimensions (Syková and Nicholson, 2008). The increased ECS might be created by the smaller effect of Aqp4 deletion on the arterial side than on the venous side of the system, indicated by lower arterial-side density of immunohistochemically determined AQP4 expression in the adjoining astrocytic endfeet (Iliff et al., 2012). Water permeability is not zero in astrocytes from Aqp4-deficient mice (Solenov et al., 2004), and the larger hydrostatic gradient on the arterial side may provide sufficient arterial water exit with less AQP4 dependence.

According to the second proposal by Jin et al. (2013), diffusion of K^+ and non-K^+ solutes in astrocyte cytoplasm should establish an osmotic driving force for transport of H_2O and K^+ into the cells, leading to significant uptake of K^+ in astrocytes. Such a mechanism is not consistent with the demonstration that cellular K^+ uptake from brain ECS in the adult mammalian brain cortex except at very highly elevated $[K^+]_e$ is almost entirely Na^+,K^+-ATPase dependent, indicated by its virtually complete inhibition by ouabain alkaloids (Xiong and Stringer, 2000; D'Ambrosio et al., 2002; MacAulay and Zeuthen, 2012), reasonably specific inhibitors of the Na^+,K^+-ATPase. Computer simulations have similarly shown that K^+ channel activity at rest and during low frequency firing does not contribute to astrocytic K^+ uptake, because the Nernst potassium equilibrium potential, EK, normally is more negative than the membrane potential (Somjen et al., 2008; Soe et al., 2009). However, at highly elevated $[K^+]_e$, channel activity aided transporter-mediated K^+ clearance to some degree (an astrocytic effect), an observation confirmed in a comparison between wild-type and Kir4.1$^{-/-}$ mice (Chever et al., 2010). D'Ambrosio et al. (2002) also showed that the only major effect of K^+ channel blockade normally is an increase in the post-stimulatory undershoot in $[K^+]_e$. A similar effect was reported by Chever et al. (2010) in Kir4.1$^{-/-}$ mice.

Na^+,K^+-ATPase expression is pronounced in both neurons and astrocytes (Peng et al., 1997; Li et al., 2013). After most normally occurring physiological neuronal activities, $[K^+]_e$ increases by ≤5 mM from its normal level of 3–5 mM, and this increase is handled by the Na^+,K^+-ATPase alone, both in the brain in vivo (MacAulay and Zeuthen, 2012) and in cultured astrocytes (Xu et al., 2013). Its action involves no direct association between transport of ions (combined Na^+ efflux and K^+ influx in a 3:2 ratio [Thomas, 1972]) and H_2O. It will therefore not create an osmotic driving force into astrocytes. A second mechanism, which operates at higher $[K^+]_e$, additionally enrolls NKCC1, which in the adult central nervous system is restricted to astrocytes (Deisz et al., 2011), and transports Na^+, K^+, 2 Cl^-, and water together (Epstein and Silva, 1985; Hamann et al., 2005, 2010). It is stimulated by vasopressin, and *Aqp4* knockout has no effect in cultured mouse astrocytes on vasopressin-stimulated, NKCC1-mediated increase in swelling, confirming that NKCC1-mediated uptake of H_2O occurs via the cotransporter itself and is AQP independent (Peng et al., 2012). In contrast, hypotonicity-induced swelling depended on AQP, confirming an AQP dependence found by Soe et al. (2009). These two forms for swelling are accordingly mechanistically different, as also shown

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by Cai et al. (2011). NKCC1 operation is Na^+,K^+-ATPase dependent, because it requires ion gradients established by Na^+,K^+-ATPase activity (Pedersen et al., 2006). A third mechanism that imports H_2O into brain cells is the operation of a Na^+/bicarbonate cotransporter, which also depends on ion gradients established by the Na^+,K^+-ATPase (Østby et al., 2009). This transporter serves as a pH regulator, is not directly activated by K^+, and promotes no K^+ uptake.

The concept that a Na^+,K^+-ATPase–mediated K^+ uptake occurs in astrocytes of the adult mammalian brain cortex (Hertz, 1965), which is supported by both Jin et al. (2013) and us (Xu et al., 2013), is gaining credibility (Walz, 2000; Somjen et al., 2008; MacAulay and Zeuthen, 2012; Wang et al., 2012a,b). However, K^+ exiting from excited neurons eventually must be returned to neurons. Bay and Butt (2012) showed that a Kir4.1-mediated release of K^+ from astrocytes allowed subsequent neuronal accumulation, but they provided no explanation as to why neurons could accumulate K^+ after its release from astrocytes but not immediately after neuronal release. The reason for this seems to be a difference between the Na^+,K^+-ATPase expressed in the two cell types. Not only is the maximum activity (V_{max}) higher in astrocytes, but the affinity of the extracellular K^+-stimulated site (K_D) is such that only the astrocytic enzyme is activated by increases in extracellular K^+ concentration above its resting level (Grisar et al., 1983; Hajek et al., 1996). Similar increases in extracellular K^+ concentration also stimulate glycogenolysis (Hof et al., 1988), an astrocyte-specific event in the brain (Ibrahim, 1975). A K^+-induced stimulation of glycogenolysis is also found in cultured astrocytes after, but not before, differentiating treatment with dibutyryl cyclic AMP (Hertz and Code, 1993). Active K^+ uptake in astrocytes requires glycogenolysis (DiNuzzo et al., 2012; Xu et al., 2013), because glycogenolytically derived energy is needed for fueling of signaling, allowing entry of Na^+ to activate the Na^+-sensitive intracellular site of the Na^+,K^+-ATPase in these nonexcitable cells (Xu et al., 2013). Once extracellular K^+ is no longer increased, the astrocytic Na^+,K^+-ATPase is unable to function, and astrocytically accumulated K^+ is released through Kir.1.4 channels perhaps in a gradual and spatially expanded manner, allowing the neuronal Na^+,K^+-ATPase to accumulate K^+. Exit of K^+ during the Kir4.1-mediated release might occur together with Cl^- and water, and reduction of KCl release in Aqp4-deficient hippocampal brain slices might therefore possibly explain the accentuation of shrinkage of the ECS in the mouse hippocampus (Haj-Yasein et al., 2012) during stimulation.

The higher increases in $[K^+]_e$ are generally limited to seizures, anoxia, and spreading depression (Somjen, 1979; Syková, 1992), where NKCC1 activity leads to massive intra-astrocytic uptake of H_2O ("cytosolic brain edema"). Two of the three studies providing the experimental basis for the computations in the Jin paper, Binder et al. (2006) and Padmawar et al. (2005), used such intense stimulation, whereas the third experimental study, Strohschein et al. (2011), did not, creating smaller increases in $[K^+]_e$. This study, performed in brain slices, found no changes between wild-type and $Aqp4^{-/-}$ animals at $[K^+]_e$ above 4 mM; a small decrease in the K^+ clearance rate in these mice below 4 mM might be explainable by a reported increase in gap junction coupling, as channel-mediated exit of astrocytically accumulated K^+ might counteract normalization of $[K^+]_e$. The two studies that used much more intense stimulation, Binder et al. (2006) and Padmawar et al. (2005), found a reduction in K^+ uptake in $Aqp4^{-/-}$ mice. This probably reflects the ability of channel-mediated K^+ transport to assist transporter-mediated K^+ clearance (Somjen et al., 2008; Chever et al., 2010), specifically at these high K^+ concentrations, and cooperativity between Kir4.1 and AQP, as reported by Padmawar et al. (2005) and Soe et al. (2009).

In conclusion, except at highly elevated $[K^+]_e$, effects of Aqp4 deletion on K^+ dynamics seem to be coincidental rather than caused by dependence of astrocytic K^+ uptake on AQP4 activity. This is because AQP4 does not interact with the K^+ transporters, the Na^+,K^+-ATPase, and NKCC1, which have the dominant effect on cellular, including astrocytic, K^+ uptake. Only at highly elevated $[K^+]_e$, where K^+ channel function can assist K^+ uptake by the transporters, is AQP4 able to enhance the channel-mediated activity.

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