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letters to the editor

The following is the abstract of the article discussed in the subsequent letter:

Sarfaraz, Darya, and Cosmo L. Fraser Effects of arginine vasodepression on cell volume regulation in brain astrocyte in culture. *Am J Physiol Endocrinol Metab* 276: E596–E601, 1999.—Astrocytes initially swell when exposed to hypotonic medium but rapidly return to normal volume by the process of regulatory volume decrease (RVD). The role that arginine vasopressin (AVP) plays in hypotonically mediated RVD in astrocytes is unknown. This study was therefore designed to determine whether AVP might play a role in astrocyte RVD. With the use of 3-*O*-[³H]methyl-D-glucose to determine water space, AVP treatment resulted in significantly increased 3-*O*-methyl-D-glucose water space within 30 s of hypotonic exposure ($P = 0.0001$) and remained significantly elevated above baseline (1.75 $\mu\text{l}/\text{mg}$ protein) at 5 min ($P < 0.021$). In contrast, in untreated cells, complete RVD was achieved by 5 min. At 30 s, cell volume with AVP treatment was 37% greater than in cells that received no treatment (2.9 vs. 2.26 $\mu\text{l}/\text{mg}$ protein, respectively; $P < 0.006$). The rate of cell volume increase (dV/dt) over 30 s was highly significant (0.038 vs. 0.019 $\mu\text{l}\cdot\text{mg}$ protein⁻¹·s⁻¹ in the AVP-treated vs. untreated group; $P = 0.0004$ by regression analysis). Additionally, the rate of cell volume decrease over the next 4.5 min was also significantly greater with vasopressin treatment ($-dV/dt = 0.0027$ vs. 0.0013 $\mu\text{l}\cdot\text{mg}$ protein⁻¹·s⁻¹; $P = 0.0306$). The effect of AVP was concentration dependent with $EC_{50} = 3.5$ nM. To determine whether AVP action was receptor mediated, we performed RVD studies in the presence of the V_1 -receptor antagonists benzamil and ethylisopropyl amiloride and the V_2 -receptor agonist 1-desamino-8-D-arginine vasopressin (DDAVP). Both V_1 -receptor antagonists significantly inhibited AVP-mediated volume increase by 40–47% ($P < 0.005$) whereas DDAVP had no stimulatory effects above control. Taken together, these data suggest that AVP treatment of brain astrocytes in culture appears to increase 3-*O*-methyl-D-glucose water space during RVD through V_1 -receptor-mediated mechanisms. The significance of these findings is presently unclear.

Effects of Arginine Vasopressin on Water Space in Astrocytes and in Whole Brain

To the editor: The role of arginine vasopressin (AVP) in regulation of astrocytic water content and volume by promoting transmembrane transport of water during dissimilarity between intra- and extracellular tonicity was investigated by Sarfaraz and Fraser (15). To achieve this objective, the authors exposed primary cultures of rat astrocytes to a medium containing reduced NaCl concentration (between 75 and 100 mM) and obtained an osmolality of 150–195 mosmol/kgH₂O. The massive reduction of extracellular tonicity that was used is a convenient tool, but it does not occur during either physiological or pathophysiological conditions. Moreover, AVP also facilitates “vasogenic brain edema” (4). This letter describes the effect of AVP during a hydro-

osmotic challenge that occurs in the brain in vivo and presents a unifying hypothesis for the role of AVP in regulation of water content in astrocytes and in whole brain.

An increase of extracellular K⁺ concentration ($[\text{K}^+]_e$) in the brain occurs physiologically as a response to neuronal activity (although the rise is at most a few mM) during seizures (where $[\text{K}^+]_e$ may become as high as 12 mM) and during brain ischemia or other insults where $[\text{K}^+]_e$ may increase to >60 mM and return to its normal level after restoration of physiological conditions (18, 20). This situation can be mimicked in vitro by increasing the K⁺ concentration of the medium. During incubation in medium containing 60 mM K⁺, mouse astrocyte cultures prepared like those used by Sarfaraz and Fraser take up K⁺ and Cl⁻ and show a moderate increase in water content (2). The increase in K⁺ and Cl⁻ may either be channel mediated (by a voltage-dependent opening of Cl⁻ channels combined with a permanently large K⁺ conductance) or occur actively by stimulation of the Na⁺,K⁺,Cl⁻ cotransporter (21), which is energetically driven by the Na⁺ gradient and operates together with the Na⁺,K⁺-ATPase, exchanging accumulated Na⁺ with K⁺ (19); both of these ion transporters are expressed in astrocytes, and both are stimulated by elevated $[\text{K}^+]_e$ (5, 21).

In astrocytes, but not in neurons, the K⁺-induced increase in water space is greatly enhanced by exposure to 10⁻¹²–10⁻¹⁰ M AVP (2); the increase amounts to almost 2 $\mu\text{l}/\text{mg}$ protein, or 50% of the nonstimulated water content. AVP has no similar effect on cultured cerebellar granule cell neurons, and it does not stimulate K⁺ uptake in astrocytes (2). Assuming that astrocytes account for 25–30% of cortical volume and the extracellular space half as much, an increase in astrocytic water space of 50% without concomitant movements of ions means that the concentrations of all extracellular ions double. Provided $[\text{K}^+]_e$ was normal, i.e., ~3.0 mM, before the onset of AVP's action, it would increase to 6.0 mM, a change that has a substantial effect on neuronal excitability (18, 20). Subsequent return to normal may be assisted by the exit of K⁺ across the blood-brain barrier, because an increase in brain $[\text{K}^+]_e$ triggers K⁺ removal through endothelial cells mediated by an abluminally located Na⁺,K⁺-ATPase (16).

The luminal surface of endothelial cells is the site of a K⁺,Na⁺,Cl⁻ cotransporter, and evidence is accumulating that it also expresses Na⁺,K⁺-ATPase activity (10, 11; M. Spatz, personal observation). In cultured endothelial cells, both of these ion transporters are stimulated by endothelin-1 (ET-1), and ET-1 release is enhanced by AVP (7, 8, 17). AVP-endothelin-induced

stimulation of uptake of blood-borne K^+ , Na^+ , and Cl^- in endothelial cells may, together with AVP-endothelin-mediated opening of abluminal inwardly rectifying quinine-inhibited Ca^{2+} -dependent K^+ channels (9, 13) and that of Na^+ and Cl^- channels, lead to transendothelial ion uptake in brain, which for osmotic reasons will be followed by accumulation of water. Such a process would explain the ability of AVP antagonists to reduce vasogenic AVP receptor-mediated brain edema (12, 14). During physiological brain activity, this mechanism may supply K^+ to normalize $[K^+]_e$ after astrocytic removal of K^+ and Cl^- without (or before) concomitant water uptake. During pathological conditions, cotransporter activity (1) and opening of ion channels lead to transmitter-mediated vasogenic brain edema and astrocytic swelling triggered by the excessive increase in $[K^+]_e$ and by AVP.

The effects of AVP on astrocytes and endothelial cells, both of which increase $[K^+]_e$ (albeit by different mechanisms), affect neuronal excitability (18, 20), and may thus contribute to AVP's effect on awareness and learning; this is in keeping with the observation that memory establishment is impaired by inhibitors of the cotransporter (3, 6) that supplies the driving force for water uptake in astrocytes and mediates ion accumulation from blood into endothelial cells. The analogous observation by Sarfaraz and Fraser (15) and Chen et al. (2) demonstrating AVP-induced changes in astrocytic water space during hydroosmotic challenge leaves little doubt that astrocytes play an important role in the regulation of water transport at the cellular level of the brain. However, the proposed interactions between endothelial cells and astrocytes require experimental confirmation and elucidation of the mechanisms involved.

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