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Nutrient- and neurotransmitter-transporters as molecular water pumps

T. Zeuthen

Department of Medical Physiology, University of Copenhagen, Copenhagen, Denmark

It is generally accepted that cotransporters act as passive water channels but an active mode of transport has also been suggested. Transmembrane osmotic gradients induce passive water transport in cotransporters and in a cell the magnitude of these fluxes may be significant: both the number of cotransporters and the unit water permeability are high. For the Na⁺-glutamate cotransporter (EAAT1) the unit water permeability is one tenth of that of the water channel AQP1. In addition, it is gated by the presence of substrate and by the membrane potential (MacAulay et al., 2001; MacAulay et al., 2002). Similar properties have been observed for other cotransporters of the symport type, e.g. the K⁺-Cl⁻ (KCC4), the Na⁺-K⁺-Cl⁻ (NKCC1), the H⁺-lactate (MCT1), and the Na⁺-glucose cotransporter (SGLT1), for a review see (Zeuthen & MacAulay, 2002b).

During cotransport another component of water transport emerges in parallel and independently of the passive water transport. By this mechanism, water can move uphill against the osmotic gradient since the energy contained in the substrate gradients is transferred to the transport of water. In short, cotransporters act as molecular water pumps. We have investigated active water transport in intact tissues by ionselective microelectrodes and fluorescence methods as well as in *Xenopus* oocytes, in which relevant cotransporters were over-expressed. In general, the stoichiometry between the cotransport of water and the non-aqueous substrates is fixed for a given transporter irrespective of transport conditions. Coupling ratios of 150 to 500 water molecules per charge translocated by the protein has been determined for different cotransporters, which mean that the tonicity of the transportate may be compatible to that of the surrounding tissues. Cotransport of water has been demonstrated in symports, such as those mentioned above, but not in the antiports for Na⁺/H⁺ and Cl⁻/HCO₃⁻. Given physiological values of intra- and extracellular diffusion coefficients, effects of unstirred layers can be ruled out (Zeuthen et al., 2002). For a recent review, see (Zeuthen & MacAulay, 2002a).

The concept of molecular water pumps is relevant for a number of well-established physiological phenomena, which cannot be explained by simple osmosis. In the small intestine, water is transported uphill from the lumen into the blood; during digestive processes, the lumen can attain hyperosmolarities of more than 100 mosm l⁻¹ relative to plasma (Reid, 1901). Glandular secretion also proceeds against an osmotic gradient; secretion of saliva, for example, can proceed against hydrostatic pressures of more than 2 meters of water (Ludwig, 1861). In brain, the synaptic cleft becomes hyperosmolar during neural activity (Dietzel et al., 1989); it is the role of neuroglia, in particular the astrocytes, to control the size and ion-concentrations of the extracellular space in the neuropil. Astrocytes are polarized with EAAT localized at the end facing the neuropil while the end abutting the blood circulation is rich in aquaporins, AQP4 (Nielsen et al.,

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Colonic crypt function in relation to fluid absorptionJ. Thiagarajah¹ and R. Naftalin²¹*Medicine and Physiology, UCSF, San Francisco, CA, USA and*²*Physiology, King's College London, London, UK*

The primary function of rat, mouse and human descending colon is the dehydration of faeces and the recovery of fluid and electrolytes from the intestinal digesta. In humans the colon receives around 1.5 L of fluid a day with electrolytes approximately at the same concentration as plasma. Small intestinal contents arrive in the colon at a water : solid ratio of 20:1 and leave having been dehydrated to a ratio of 2: 1. As in other epithelia water transport is secondary to active solute transport via membrane ion channels and transporters. The majority of fluid absorption in the descending colon occurs through the surface epithelium against a low hydraulic resistance. However as the luminal contents become more dehydrated it requires a significant force to further dehydrate faeces. Faeces have a similar structure to clay and dehydration on this scale requires a large compressive force of around 4 atm. The generation of a sufficiently hypertonic absorbate could provide the osmotic force required to transport water against a large hydraulic resistance and evidence is accumulating that this process is localized in colonic crypts. Colonic crypts are surrounded by a layer of myofibroblast cells and extracellular matrix components termed the pericryptal sheath. This sheath provides the physical barrier required to create a 'central' hypertonic compartment allowing fluid absorption via crypt epithelial cells and generating a suction force within the crypt lumen.

Initial evidence for the process of fluid absorption via crypts was provided by *in vitro* studies of colonic mucosa showing concentration polarization of fluorescently labeled dextrans within colonic crypt lumens¹. Additional *in vivo* experiments using agarose gel cylinders inserted into the colon showed that the descending colon, but not the caecum, is able absorb against large hydraulic resistances via colonic crypts². To characterize further the process of fluid convection into crypts, photobleaching studies of fluorescently labeled dextrans were performed in iso-

lated rat descending colonic mucosa and showed that recovery of fluorescence within the crypt lumen was the same for widely different molecular weight dextrans and abolished by inhibition of epithelial Na⁺ transport. These studies provide evidence that fluid flow in colonic crypts is convective³.

The generation of a sufficiently strong suction pressure at crypt openings requires a highly hypertonic compartment, postulated to be localized in the pericryptal space surrounding crypts. Confocal microscopy performed *in vivo* in mice using a low affinity ratiometric Na⁺ sensitive dye confirmed the presence of a pericryptal hypertonic compartment with a local Na⁺ concentration of 200–400 mM, which would be sufficient to provide the necessary osmotic pressure for convective flow into crypts. Inhibition of Na⁺ transport significantly reduced pericryptal Na⁺ concentration as did blockage of crypt opening using paraffin oil suggesting that pericryptal hypertonicity is dependent on salt transport from the crypt lumen.

Colonic fluid transport can be altered by in a number of states resulting in changes in colonic crypt function. High doses of ionizing radiation result in a reduction in fluid absorption. Examination of colonic mucosa post-irradiation showed that this reduction in fluid absorption was associated with increased leakiness of colonic crypt cells and the loss of cell adhesion molecules. Additionally it was shown that the pericryptal sheath was significantly disrupted after radiation and these changes were preceded by the release of apoptotic enzymes and signaling molecules. The loss of the pericryptal sheath and crypt epithelial adhesion molecules coincided with increased permeability of FITC dextrans out of crypts. In contrast to irradiation, low dietary Na⁺ results in increased fluid absorption through increases in plasma aldosterone and angiotensin II. Investigation of the effects of low Na⁺ diet in rat descending colon revealed a significant trophic effect on myofibroblast cells and extracellular components of the pericryptal sheath in contrast to rat proximal colon. These changes suggest that increased pericryptal barrier function may be contribute to increased fluid absorption after low Na⁺ diet.

Several *in vivo* and *in vitro* studies of colonic crypt function both in health and disease now provide evidence that crypts play an important role in colonic fluid absorption. Further studies are required to measure important determinants such as crypt luminal pressure gradients and the role of active secretion in states of disease on crypt and pericryptal function.

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REGULATION OF AQUAPORIN 2 FUNCTION

D. Marples

School of Biomedical Sciences, University of Leeds, Leeds, W. Yorkshire, UK

Body fluid balance is controlled predominantly by hormonal regulation of renal collecting duct function. The water permeability of the collecting duct is controlled by vasopressin, which causes the shuttling of aquaporin 2 (AQP2) water channels from intracellular vesicles to the apical plasma membrane of the cells. This shuttling is mediated by cAMP and activation of protein kinase A: phosphorylation of AQP2 seems to be a key step leading to its exocytic insertion into the plasma membrane. When vasopressin levels fall, AQP2 is retrieved endocytically.

This acute shuttling of AQP2 is modulated by changes in AQP2 expression: vasopressin infusion, or chronic dehydration, increase AQP2 expression, while water loading decreases it. This modulation can be partly explained by vasopressin effects mediated by cAMP, but it is now clear that other factors are also involved. A number of other hormones and local factors are known to modulate antidiuresis, although in many cases the mechanisms behind this remain unclear. We have been particularly interested in possible roles of prostaglandins, angiotensin, and bradykinin. Prostaglandin E2 and angiotensin II both appear to have some ability to increase cAMP and hence mimic the effects of vasopressin, while bradykinin antagonises the effects of vasopressin. We are currently investigating the signalling cascades underlying these effects. Pathological disorders of water balance have been shown to be associated with changes in both AQP2 expression and shuttling. In particular, many acquired forms of nephrogenic diabetes insipidus are associated with a decrease in AQP2 levels, which may be profound, while some, but not others, also show impaired trafficking of AQP2. In seeking treatments for such disorders, it is important to understand both why the disorder has arisen, and possible ways to bypass it. We hope that in the long term we can find stimuli that will alter both expression and shuttling of AQP2 independent of vasopressin, and that this will lead to new treatments for water balance disorders, and potentially for other problems such as hypertension.

This work was supported by the National Kidney Research Fund and the Medical Research Council

Where applicable, the experiments described here conform with Physiological Society ethical requirements.