

## WATER PUMPS

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A question that has intrigued physiologists for over a century is how water is transported across epithelial cells in the absence of external driving forces. Appealing explanations were put forward 35- 40 years ago: Peter Curran proposed a 3-compartment model; and Jared Diamond proposed a local osmosis model, later refined as the standing gradient hypothesis. In both cases, it was suggested that active sodium transport epithelium generated osmotic gradients within the tissue that caused water to flow. The local osmotic compartment was not defined in the Curran model, but Diamond proposed that the local osmotic gradient, or standing gradient, was generated in the lateral intercellular spaces by sodium pumps of the lateral membranes of the epithelium. While many investigators have attempted to test these models over the years, it has been difficult to obtain definitive answers, and so interest in the question has waned.

With Peter Agre's discovery of water channels, the aquaporins, there has been resurgence in interest in their role in epithelial water transport, especially in the kidney. In the case of the intestine however, the problem of how 8 liters of fluid are absorbed each day has remained an enigma because of the lack of significant expression of aquaporin genes in enterocytes. Fluid absorption in the intestine occurs in the absence of external osmotic and hydrostatic driving forces, and furthermore the rate of absorption is greatly enhanced during active glucose absorption ( $\text{Na}^+$ /glucose cotransport) and, to a lesser extent, active amino acids absorption. This link between sugar and fluid absorption is the basis for Oral Rehydration Therapy, which is so effective in combating secretory diarrhea.

In the course of our studies on the function of recombinant cotransporters we have found that they are multifunctional proteins. For example, the  $\text{Na}^+$ /glucose cotransporter (SGLT1) functions as a  $\text{Na}^+$ /glucose cotransporter, a  $\text{Na}^+$  uniporter, a channel for water and small non-electrolytes, and a water pump. The focus of this presentation is on the role of cotransporters in water transport. Our basic experimental approach has been to express SGLT1 in *Xenopus laevis* oocytes, and to measure the water transport properties of both control and SGLT1 expressing oocytes. Oocytes offer three advantages for these studies: 1). They are large cells, 1 mm in diameter; 2). Native oocytes have very low water permeabilities; and 3). Oocytes efficiently express cloned membrane proteins such as SGLT1. Thus is relatively simple to measure simultaneously SGLT1  $\text{Na}^+$ /glucose and water transport with great sensitivity and time resolution. Water transport is measured using an optical technique, and  $\text{Na}^+$ /glucose cotransport is measured using electrophysiological methods.  $\text{Na}^+$ /glucose cotransport is tightly coupled and is accurately recorded as a glucose stimulated inward  $\text{Na}^+$  current. The cotransporter is

activated, or inactivated, by the rapid addition or withdrawal of sugar, the rapid addition of phlorizin, or rapid changes in the membrane potential. In our system we can accurately record both water transport and  $\text{Na}^+$ /glucose cotransport in a time scale ranging from 1 second to tens of minutes.

There is no controversy about our conclusion that SGLT1 behaves as a water channel. The osmotic water permeability of oocytes increases with the level of expression of SGLT1, and the addition of phlorizin reduces the water permeability down to that observed for control oocytes. The osmotic water permeability of SGLT1 is independent of: 1) the size and direction of the osmotic gradient; 2) the presence or absence of sodium; 3) the presence or absence of glucose; and 4) is relatively independent of temperature ( $E_a$  5 kcal/mole). Since urea, ethylene glycol and formamide, but not mannitol, also permeate through SGLT1, we suggest that the channel has a radius of less than  $4\text{\AA}$ . This channel resides in the C-terminal domain of the protein. We have also provided evidence that other cotransporters behave as water channels. However, the absolute water permeability per cotransporter molecule is only 2-5% of that for AQP1.

There is also no controversy about our observation that water transport through SGLT1 is dramatically increased when cotransport is activated. However, there is some controversy about our

hypothesis that there is cotransport of water. Our evidence for Na<sup>+</sup>/glucose/water cotransport is the following. The glucose activated increase in water transport is immediate, i.e. occurs within 1 second when Na<sup>+</sup>/glucose cotransport is turned on, and immediately decreases when cotransport is turned off. The initial rate of water transport is stoichiometrically linked to the rate of Na<sup>+</sup>/glucose cotransport, i.e. for hSGLT1 264 water molecules for each glucose and 2 Na<sup>+</sup> ions. This coupling is independent of the expression level of SGLT1, the sugar and sodium concentrations, voltage and temperature. We conclude that this **initial** water flow is cotransport of water and NOT osmotic water transport because: 1). coupling is independent of the rate of Na<sup>+</sup>/glucose cotransport; 2) the coupling of water to Na<sup>+</sup>/sugar transport is independent of the osmotic gradient. Coupled water transport can actually occur **AGAINST** an osmotic gradient; 3) the coupling ratio varies from cotransporter to transporter, from 50 for a plant H<sup>+</sup>/amino acid cotransporter to 425 for the rabbit Na<sup>+</sup>/glucose cotransporter; 4). coupling occurs with H<sup>+</sup>/glucose cotransport by SGLT1 and H<sup>+</sup>/amino acid transport by the plant H<sup>+</sup>/amino acid cotransporter AAP5. Unlike Na<sup>+</sup> cotransport, the proton that enters the oocyte along with the substrate is expected to contribute little to any increase in the osmotic pressure of the oocyte due to buffering; 5). stimulation of ion transport into oocytes through ion channels does not produce an immediate water flow, and only induces a flow after a significant delay (> 40 seconds); and 6) the cotransport of water is accompanied by the cotransport of urea. Finally, the cotransport of water and urea along with Na<sup>+</sup> and glucose is readily explained by the large conformational changes in SGLT1 that underlie the turnover of the protein during the transport cycle.

A simple consequence of the fact that the SGLT1 cotransported fluid is hypertonic is that the oocyte will become hypertonic with time and that this in turn will lead to an increase in fluid transport into the cell. In the steady state the fluid transported should be close to isotonic. We actually observe that after the initial linear phase of water transport upon the addition of glucose there is a slow increase in the rate to a steady state value where the fluid transported is isotonic. At a modest level of SGLT1 expression the steady state flow is comprised of equal parts water cotransport, osmosis through SGLT1, and osmosis through the plasma membrane. Increasing the water permeability of the oocyte 10-fold by coexpressing AQP1 along with SGLT1 decreased the delay in achieving isotonic water transport, but even in such conditions cotransport accounts for one third of the total flow.

In summary, we have provided a wealth of evidence that cotransporters function as low conductance water channels, and in addition, serve as molecular water pumps when substrates are transported. Furthermore, we propose a model for water pumping that stems directly from our hypothesis to explain Na<sup>+</sup>/substrate cotransport. The physiological role for water transport through cotransporters depends on the level of cotransporter expression in the cell and the level of expression of other water transport proteins. In the case of the small intestine there are about 250,000 copies of SGLT1 in each enterocyte and there is little evidence for the presence of aquaporins in either the brush border or basolateral membranes of the epithelium. Thus we expect SGLT1 to play a major role in water transport across the brush border membrane of enterocytes. In the human intestine we estimate about half of the 8 liters absorbed each day occurs by cotransport and the other half occurs by osmosis through cotransporters. This would account for the intimate link between sugar, salt and water transport, and provides a rationale for ORT.

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