Comment to the Editor

The Mechanism of Water Transport in Na^+-Coupled Glucose Transporters Expressed in *Xenopus* Oocytes

It is well established that cotransporters transport water, but how they do it is debated. Two mechanisms have been suggested: cotransport of water along with the nonaqueous substrates, and osmosis where the cotransporters simply act as water channels. In a recent article, Charron et al. investigated this question for the Na^+-coupled glucose transporter expressed in *Xenopus laevis* oocytes. They focused upon the posttransport period, i.e., when external sugar was removed after a period of inwardly directed Na^+ and sugar transport, and argued, on partly empirical grounds, that the data could be explained exclusively by osmosis. In this Comment to the Editor, we have reinterpreted these data by a numerical model of the oocyte which reflects the physical transport processes taking place: cotransport and/or osmosis across the membrane and diffusion and mass balance of the nonaqueous substrates in the cytoplasm. We find that the experiments and analysis as performed in Charron et al. are inadequate with respect to distinguishing between the cotransport hypothesis and the osmotic hypothesis for water transport in cotransporters.

BACKGROUND

It is now generally accepted that cotransporters of the symport type transport water (1,2). Cotransporters such as the KCC, NKCC1, and a variety of Na^+-coupled cotransporters have capacities for water transport, which, per molecule, range between that of AQP0 and AQP1 (3–6). The nature of the transport mechanism, however, has been the subject of an intense debate, which has focused mainly on the water transport properties of the Na^+-coupled glucose transporter (SGLT1) expressed in *Xenopus* oocytes. Two principally different transport mechanisms have been proposed. We have presented the cotransport hypothesis in which a large fraction of the transported water moves along with Na^+ and glucose by a molecular mechanism within the cotransporter itself. Accordingly, this water flux is energized by the Na^+ flux and can proceed independent of the transmembrane osmotic gradient (6–10). In opposition to this, Charron and co-workers (11–13) have argued that the SGLT1 acts entirely as a water channel and that all water transport is osmotic. In this osmotic hypothesis, the driving force for the water transport is proposed to arise as an unstirred layer effect: the diffusion of Na^+ and sugar inside the oocyte is assumed to be so slow that significant concentrations build up at the inside of the membrane. This, in turn, leads to water transport by osmosis. It can be estimated that the intracellular diffusion coefficients need to be about three orders of magnitude lower than the free solution values for sufficient concentrations to build up (10). It will be of significant physiological relevance to decide between the two hypotheses. In the case of cotransport, water transport can proceed uphill, against the water chemical potential, energized by the (downhill) flux of Na^+. This would present a direct solution to several physiological problems, for example, how water can move uphill, from lumen into plasma, across the small intestine (14) and other epithelial cell layers (15).

In a recent article, Charron and co-workers used the human isoform hSGLT and focused upon the volume changes that took place immediately after a period of Na^+-coupled sugar transport in combination with an osmotic challenge (11). They employed the nonmetabolizable glucose-analog methyl-α-D-glucopyranoside (αMDG). The underlying assumptions were that only the osmotic hypothesis would explain an increase in intracellular osmolarity and osmotic water transport during the sugar stimulation, and that ''this contrasts with the prediction of the water cotransport hypothesis, which purports that 0% of the water transport would be passive during the first minute of transport'' (11). Thus, the osmotic hypothesis would predict a significant poststimulation swelling, whereas the cotransport hypothesis would not. In addition, the authors argued that the differences between the two hypotheses would be more pronounced for oocytes in which the passive water permeability had been increased by coexpression of AQP1, since the effects of the changes in intracellular osmolarities would be amplified. To analyze their data, the authors used two different numerical models. One was empirical and employed up to six arbitrary constants. Such models, however, do not reflect physical mechanisms. The other model described only central symmetrical diffusion in a sphere but did not incorporate the possibility of cotransport of water.

We would question these assumptions and argue that the numerical models used in Charron et al. (11) are inadequate. First, the assumption that the osmotic hypothesis predicts significantly larger changes in intracellular osmolarity during sugar transport than the cotransport hypothesis is untenable. This is particularly relevant for the experiments where sugar transport is combined with an increase in external osmolarity since water is removed by osmosis. Second, it appears that neither of the two numerical models used in Charron et al. (11) incorporates the correct equations to describe cotransport

Submitted August 16, 2006, and accepted for publication January 26, 2007.
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0006-3495/07/08/1413/04 $2.00

doi: 10.1529/biophysj.106.095380

of water. In fact, the principle of mass balance seems to be violated in the test between the osmotic and the cotransport hypotheses shown (Fig. 4 in Charron et al. (11)). Accordingly, we would like to reinterpret the experiments presented in Charron et al. (11) by a numerical model we have developed to describe transport of water in the *Xenopus* oocyte (10). This model is based on the physical mechanism involved and designed to distinguish clearly between osmosis and cotransport of water.

NUMERICAL MODEL AND CHOICE OF PARAMETERS

In our numerical model, SGLT1 was present in the plasma membrane where it mediated the coupled influx of Na^+, sugar, and possibly water. Application of sugar to the external surface of the oocyte under voltage clamp conditions initiated an inward clamp current, I_C, which was carried by two Na^+ ions and followed by one molecule of αMDG. To describe the cotransport hypothesis, this was accompanied obligatorily by a number of water molecules given by a coupling ratio (CR). Accordingly, the cotransported fluxes of Na^+, sugar, and water were, respectively, as follows:

$$J^+_{Na} = I_C F^{-1} \quad (1)$$

$$J_{\alpha MDG} = 0.5\, I_C F^{-1} \quad (2)$$

$$J_{H2O,CO} = 0.5\, CR\, V_w I_C F^{-}, \quad (3)$$

where F is Faraday's constant and V_w is the molar volume of water (18 $cm^3\ mol^{-1}$). To comply with the analysis in Charron et al. (11), we used a CR of 250 for the hSGLT1. When the osmotic hypothesis was tested, CR was set to zero. For both hypotheses, water also crosses the membrane by osmosis ($J_{H2O,OS}$), determined by the transmembrane osmotic gradient ($osm_i - osm_o$) and the passive osmotic water permeability L_p:

$$J_{H2O,OS} = L_p\, A\, V_w (osm_i - osm_o), \quad (4)$$

where A is the true surface area of the oocyte, ~0.4 cm^2. The external osmolarity (osm_o) was given by the experiment, but the osmolarity at the inside of the membrane (osm_i) depended on how fast the oocyte filled up with substrates and how readily they diffused in the cytoplasm. This is a function of the free fraction of the oocyte volume (V_F), in these calculations taken as 50% of the total volume (10,11), and of the intracellular diffusion coefficient D_i. In accordance with Charron et al. (11), we used a D_i of 0.15×10^{-5} $cm^2\ s^{-1}$ for both the Na^+ and αMDG, which, incidentally, agrees with our previous estimates (9). This value is about one-fifth of the diffusion coefficient for glucose in free solution. To describe the time course of substrate transport through the cytoplasm, the model oocyte was divided into 100 shells of equal thickness (division into 1000 shells gave the same results). Transport between shells was described by Fick's equation. Calculations were each performed for 0.01 s (for further details, see Zeuthen et al. (10)).

For both the cotransport hypothesis and the osmotic hypothesis, the L_p of the SGLT1-expressing oocytes consists of roughly equal contributions from the native oocyte membrane and from the SGLT1s (3). Coexpression of AQP1 can be used to increase the L_p of the membrane further by about one order of magnitude (8,11). In the analysis here, we used L_ps similar to those presented in Charron et al. (11). The *Xenopus* oocyte was assumed to be spherical, with an initial diameter of 1.2 mm. It is noted that clamp currents in the microelectrodes do not give rise to any significant changes in intracellular osmolarity as was ascertained both theoretically and experimentally in Zeuthen et al. (9).

SIMULATIONS

The question raised in Charron et al. (11) was which volume changes can be expected after a period of sugar transport? In Fig. 1 we simulated these volume changes for oocytes with relatively low passive water permeability (expression of hSGLT1 alone, Fig. 1, *A* and *B*) and for oocytes with high passive water permeability (coexpression of AQP1 and hSGLT1, Fig. 1, *C* and *D*). As outlined above, we used data and parameters from Charron et al. (11). In the cases where sugar transport was combined with an increase in external osmolarity (Fig. 1, *B* and *D*), the simulations showed clearly that the rates of posttransport swellings predicted by the cotransport hypothesis and the osmotic hypothesis were similar. This is due to two factors. First, the intracellular hyperosmolarities predicted by the two hypotheses are quite close at the time of substrate removal. This can be calculated from the integrated fluxes (Eqs. 1 and 2) and the final oocyte volumes. For the oocytes expressing only hSGLT1 (Fig. 1 *B*) the cotransport hypothesis predicted an intracellular hyperosmolarity of 1.5 mOsm l^{-1}. The osmotic hypothesis predicted a slightly higher hyperosmolarity of 2 mOsm l^{-1}, since the oocyte, on this model, has shrunk relatively more during the osmotic challenge, which results in an up-concentration of the intracellular contents. Second, the noninstantaneous wash-out of sugar from the external solution caused a gradual decline of the clamp current. This residual current contributed an additional swelling in the case of the cotransport scenario. For the oocytes expressing both hSGLT1 and AQP1 (Fig. 1 *D*), it was calculated that the cotransport hypothesis predicted an increase of 1.4 mOsm l^{-1} and the osmotic hypothesis an increase of 1.5 mOsm l^{-1} during the stimulation with sugar and hyperosmolarity. Here the currents were terminated abruptly by application of phlorizin, in analogy to the experiments in Charron et al. (11). This would avoid any ambiguities in regard to noninstantaneous removal of external sugar.

The results of these simulations conflict with Charron et al. (11), who assumed that only the osmotic hypothesis would give rise to significant increases in intracellular osmolarity and swellings after a period of sugar transport combined with an osmotic challenge. Our simulations underscore that the

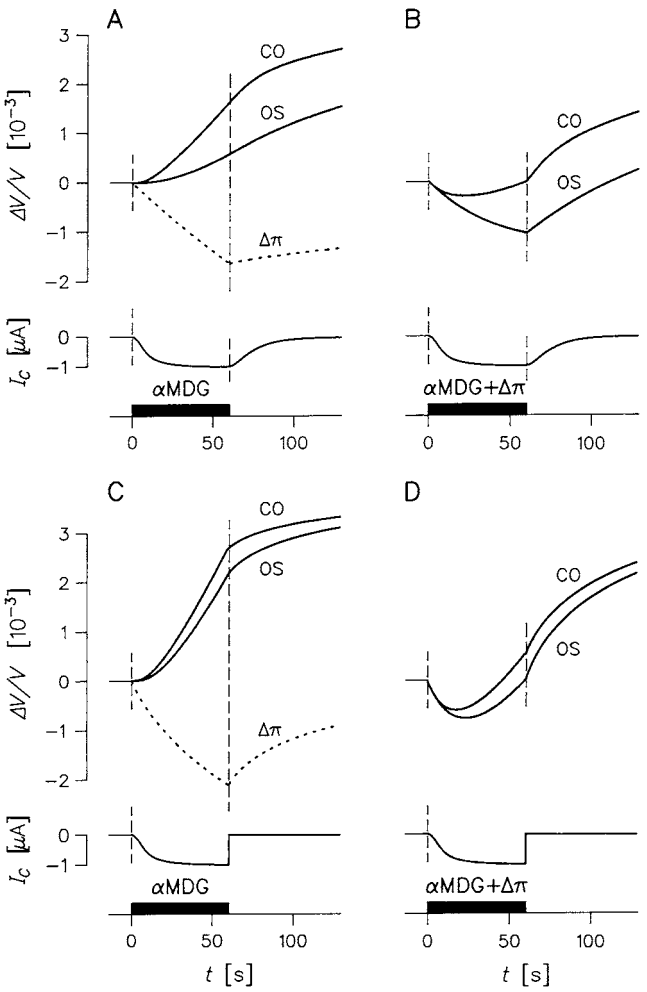

FIGURE 1 Simulation of sugar-induced water transport in hSGLT1-expressing oocytes. The transport parameters are from Charron et al. (11). Oocytes were voltage clamped, and the nonmetabolizable sugar αMDG applied at $t = 0$. (*A* and *B*) The results for oocytes expressing only hSGLT1, $L_p = 6.5 \times 10^{-4}$ cm s^{-1}. (*A*) The volume changes predicted by the cotransport model (CO) and the osmotic model (OS) in response to sugar addition only (*black bar*: αMDG). The clamp current (I_C) increased with a time constant of 10 s to a saturating level of 1 μA and decreased to zero with a similar time constant at sugar removal. For comparison, oocyte shrinkage induced by an increase in the external osmolarity of 5 mOsm is shown as a broken line ($\Delta\pi$); the initial rate of shrinkage defines the L_p. (*B*) The effects of adding sugar and increasing the external osmolarity (αMDG + $\Delta\pi$). (*C* and *D*) The effects of high values of L_p, 49×10^{-4} cm s^{-1}, which mimic the experiments obtained by coexpression of AQP1. The sugar-induced current was induced as in *A* but was terminated abruptly after 60 s by the addition of phlorizin. The osmotic challenge $\Delta\pi$ was 2 mOsm. Otherwise, parameters are as in *A* and *B*. In all simulations the oocyte diameter was taken as 1.2 mm, and the osmotically accessible volume fraction V_F as 0.5. The osmotic challenge was assumed to be fully effective throughout the 60-s period.

cotransport hypothesis also predicts significant changes in the intracellular osmolarity and rates of swelling. This is not surprising as the cotransport of 250 water molecules, 2 Na^+ ions, and 1 αMDG represent a hypertonic solution and, importantly, the imposed osmotic gradient removes water from the oocyte. In fact, a comparison between our simulations (Fig. 1) and the measurements presented in Figs. 1 and 4 in Charron et al. (11) shows that the cotransport hypothesis predicts the measurements better than the osmotic hypothesis. In general, the volumes predicted by the cotransport hypothesis are always higher and change faster than those predicted by the osmotic hypothesis (10).

If the L_p was increased by coexpression of AQP1, the difference between the predictions of the cotransport hypothesis and the osmotic hypothesis became smaller, and not larger as argued in Charron et al. (11). This follows from the fact that the relative importance of the cotransport component of water transport is diminished when the L_p and the capacity for passive water transport are increased.

Finally, it should be noted that after the removal of sugar, the volumes predicted by the cotransport and the osmotic hypotheses must approach the same value asymptotically. This follows from the principle of mass balance. During exposure to sugar, the amounts of Na^+ and αMDG transported into the oocyte are the same in either hypothesis. Accordingly, the number of osmotic active particles trapped inside the oocyte will be the same when sugar is removed. It follows that the final isosmotic steady-state volumes predicted by the osmotic and the cotransport hypotheses must be identical. The final steady state will be reached faster in the case of high water permeability (see Fig. 1).

A comparison between the cotransport and osmotic components of water transport in the SGLT1, the ion channel gramicidin, and the glucose monoport GLUT2 obtained by high resolution experiments has recently been presented (16).

CONCLUSION

Our analysis contrasts with that of Charron et al. (11) on four accounts. First, we find that at the termination of sugar transport combined with an osmotic challenge, the cotransport hypothesis and the osmotic hypothesis predict roughly the same rate of swelling. Second, the cotransport hypothesis gives the best fit to the recorded volume changes. Third, any increase in the passive water permeability by coexpression of AQP1 will minimize rather than maximize the differences between the changes predicted by the two hypotheses. In fact, lower L_ps (and higher clamp currents) will facilitate the distinction between cotransport and osmosis. See, for example, Loo et al. (6) where we used oocytes with L_ps of $\sim 2 \times 10^{-4}$ cm s^{-1} ($\sim 1/3$ of those employed in Charron et al. (11)) and currents in the range 1320–2980 nA. Finally, the problems with the numerical analysis in Charron et al. (11) are perhaps best illustrated by Fig. 4 in Charron et al. (11). Here the posttransport volumes calculated on the basis of the osmotic hypothesis are much larger than those calculated on the cotransport hypothesis, and the final steady states are different. This conflicts with the principle of mass balance as described above.

We do agree with the data and interpretations in Charron et al. (11) on one account: The D_i determined for Na^+ of 0.3×10^{-5} cm^2 s^{-1} and for αMDG of 0.15×10^{-5} cm^2 s^{-1}

are too large for significant, conventional unstirred layer concentrations to build up during transport. In other words, the hyperosmolarities that will build up at the inside of the membrane during transport cannot explain the experimentally observed influxes of water.

Comments on the manuscript by Drs. D. D. F. Loo, E. M. Wright, and R. Søgaard are gratefully acknowledged.

This study was supported by the Nordic Centre of Excellence program in Molecular Medicine and The Danish Research Council.

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