Water transport by the renal Na\(^{+}\)-dicarboxylate cotransporter

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A MAJOR FUNCTION OF the kidney is the reabsorption of salt and water from the glomerular filtrate. The mechanism of water transport is not well understood, although it is generally accepted that water transport is secondary to active solute transport (23). Of the 180 liters of plasma filtered by the glomerular apparatus every day, >80% is reabsorbed in the proximal tubule. Because aquaporins have been shown to be present both in the apical and basolateral membranes of proximal tubular cells, transcellular water transport is thought to be mediated through these water channels. However, in a recent study on aquaporin-1 knockout mice (21), the transepithelial water permeability of the proximal tubule was reduced 78%, whereas the fluid reabsorption was reduced only 50%. One interpretation of this result is that other membrane proteins are involved in fluid reabsorption. It has been proposed that ion-coupled solute transporters can mediate the transport of water, and there is evidence for the involvement of the intestinal Na\(^{+}\)-glucose cotransporter (SGLT1) in transport of water (10, 11, 14, 31). We speculate that renal cotransport proteins may contribute to water reabsorption in the proximal tubule.

The goal of this study was to investigate whether, in general, cotransport proteins could play a role in renal water reabsorption. Specifically, we tested the renal Na\(^{+}\)-dicarboxylate cotransporter (NaDC-1) for water transport. NaDC-1 belongs to a family of Na\(^{+}\)-dependent anion transporters, which includes the Na\(^{+}\)-dependent dicarboxylate transporters (1, 3, 16, 17) and the renal Na\(^{+}\)-sulfate cotransporter (13). NaDC-1 is primarily found in the apical membrane of the kidney proximal tubules where it reabsorbs tricarboxylic acid cycle intermediates (24). Defects in this transporter may play a role in the development of idiopathic hypocitraturia (see Ref. 18). NaDC-1 is electrogenic, with a stoichiometry of 3 Na\(^{+}\) transported/divalent carboxylate ion (6, 19). We measured substrate transport using the two-electrode voltage clamp and water transport as the changes in cell volume by an optical method on the cloned NaDC-1 expressed in Xenopus laevis oocytes. Our results indicate that NaDC-1 mediates both passive and solute-coupled water transport, and under physiological conditions, the cotransporter could contribute to fluid reabsorption across the proximal tubule.

MATERIALS AND METHODS

X. laevis oocytes were defolliculated, injected with 50 ng rabbit NaDC-1 cRNA (19), and maintained in Barth’s medium supplemented with 50 mg/ml gentamycin at 18°C for 3–8 days before use (11, 20). During the experiments, the oocyte was normally perfused with a NaCl control solution containing (in mM) 90 NaCl, 20 mannitol, 2 KCl, 1 MgCl\(_2\), 1 CaCl\(_2\), and 10 HEPES (pH 7.5, 212 mosM). In some experiments, 90 mM NaCl was exchanged with 90 mM LiCl or 90 mM choline chloride. Solutions containing substrates were made by isosmotic replacement of mannitol in the control solution with either 10 mM sodium citrate or 10 mM sodium succinate. In experiments studying lithium inhibition of water transport, the test solution contained 80 mM NaCl, 10 mM LiCl, and 10 mM citrate; the osmolality was the same as the control solution (212 mosM). Hyperosmotic solutions were prepared...
by addition of 20 mM mannitol to the appropriate solutions. The osmolarity of the solutions was measured using a vapor pressure osmometer (Wescor, Logan, UT).

The experimental protocol allowed simultaneous measurements of substrate transport and the oocyte volume. The experiments were performed under continuous superfusion of the chamber (functional volume 15 μl), and the half time for solution exchange was 1 s. Electrogenic substrate transport by NaDC-1 was studied using the two-electrode voltage-clamp method (8, 19). Because 3 Na⁺ are transported with each divalent carboxylate ion, the activity of NaDC-1 can be monitored as the inward current evoked by the substrate. The number of positive charges (Q) transported into the oocyte was obtained from the integral of the substrate-induced inward current.

Two general protocols were used to measure water fluxes. The osmotic water flux was estimated from the rate of change of cell volume when an osmotic gradient (20 mosM) was imposed by removal of mannitol from the superfusing solution. These experiments were performed in the absence of substrates (citrate and succinate) in the external solution. To measure water flow occurring during Na⁺-succinate (or citrate) cotransport, the oocyte membrane potential was held at -110 mV and the external solution was abruptly exchanged with a test solution in which mannitol in the control solution was isosmotically replaced by a transported substrate (succinate or citrate). Water fluxes across the oocyte membrane were calculated from the changes in cell volume. The oocyte cross-sectional area was measured every 0.5 s from bright-field images by a charged coupled device camera, and the images were digitized (11, 14, 31). The relative volume of the oocytes (V/V₀) is related to the relative cross-sectional area (A/A₀) by the following equation: V/V₀ = (A/A₀)², where V is the volume, V₀ is the initial volume, A is the cross-sectional area, and A₀ is the initial cross-sectional area. Volume flow (Jᵥ) was obtained from the following relation: Jᵥ = V/dt(V/V₀), where (d/dt)(V/V₀) is the relative volume change with time. Osmotic water permeability (Lᵥ) of the oocyte is related to the volume flow by the following equation: Lᵥ = ΣSᵥVᵥΔπ, where Sᵥ is the oocyte surface area (0.4 cm², Ref. 11), Vᵥ is the partial molar volume of water (18 cm³/mol), and Δπ is the osmotic difference. All experiments were repeated on at least three oocytes from different donor frogs. Statistics are given as means ± SE, and n is the number of experiments. When values were obtained from linear regression of the data, their error was obtained as the SE of the fits.

RESULTS

Osmotic water transport. Oocytes injected with NaDC-1 cRNA showed an increase in osmotic water permeability compared with control noninjected oocytes. This is illustrated in Fig. 1A where the volume changes in response to an osmotic challenge of 20 mosM (created by removal of mannitol from the superfusing control solution) to an oocyte injected with NaDC-1 cRNA and a control oocyte from the same batch are shown. Upon imposing the osmotic gradient in the absence of substrate, the NaDC-1 cRNA injected oocyte swelled at the rate of 53 pl/s. This rate was three times greater than that of the control oocyte (18 pl/s). When the osmotic gradient was removed, the swelling in both oocytes stopped. The osmotic water permeability Lᵥ of the oocyte injected with NaDC-1 cRNA was 3.6 × 10⁻⁴ cm/s, compared with 1.3 × 10⁻⁴ cm/s for the control oocyte. Figure 1B shows that, on average, oocytes injected with NaDC-1 cRNA had an Lᵥ of 2.8 ± 0.1 × 10⁻⁴ cm/s (n = 20 oocytes), which was twofold higher than the Lᵥ of control oocytes (1.2 ± 0.1 × 10⁻⁴ cm/s, n = 6 oocytes).

The increase in Lᵥ of oocytes injected with NaDC-1 cRNA was proportional to the number of transporters in the oocyte plasma membrane. In a series of experiments, the expression levels of NaDC-1 and osmotic water transport (expressed as Lᵥ) were measured in the same oocyte (Fig. 2). Expression level of NaDC-1 was...
determined as the total number transporters in the oocyte plasma membrane. This was estimated by measuring the maximal inward current ($I_{max}$) induced by a saturating concentration (10 mM) of citrate in the NaCl solution at $-110 \text{ mV}$ membrane potential. The number of transporters in the oocyte plasma membrane was obtained by dividing maximal transport rate ($I_{max}$) by the turnover rate of NaDC-1, which was assumed to be 50/s. The volume flow experiments were performed as described in Fig. 1A, with the external solution containing Na$^+$ (●), Li$^+$ (○), or choline (▼). The background $L_p$, which was determined in control oocytes ($1.2 \pm 0.1 \times 10^{-4} \text{ cm/s}$), has been subtracted. All data points were used to determine the slope of the regression line: $1.5 \pm 0.1 \times 10^{-14} \text{ cm}^2\text{s}^{-1}\text{protein}^{-1}$. The $L_p$ obtained in choline ($2.2 \pm 0.1 \times 10^{-14} \text{ cm}^2\text{s}^{-1}\text{protein}^{-1}$) was higher than those obtained in Na$^+$ ($1.4 \pm 0.2 \times 10^{-14} \text{ cm}^2\text{s}^{-1}\text{protein}^{-1}$) or Li$^+$ ($1.4 \pm 0.2 \times 10^{-14} \text{ cm}^2\text{s}^{-1}\text{protein}^{-1}$).

Solute-coupled water transport. We tested whether NaDC-1 exhibited solute-coupled water transport. The experiments were performed in the absence of an osmotic gradient, so the only driving force for water transport would be derived from the NaDC-1-dependent solute flux. In these experiments, transporter activity, which was measured as the inward current induced by citrate, and the oocyte volume were monitored simultaneously. Figure 3 shows the results of such an experiment. Initially, the oocyte was perfused with the control solution, and the membrane potential was held at $-110 \text{ mV}$. The solution was abruptly...
exchanged with a test solution, in which mannitol in the control solution was isosmotically replaced by 10 mM citrate. The isosmotic addition of citrate evoked an inward current, reaching a maximum of 1,280 nA (Fig. 3B). Concurrent with the increase in citrate-induced current, there was a linear increase in the volume of the oocyte (Fig. 3A, jagged trace) at a rate of 40 pl/s. The initial volume change was directly correlated with the inward charge (Q) obtained as the integral of the citrate-induced inward current. The smooth curve of Fig. 3A was drawn with each inward-positive charge accompanied by the transport of 165 water molecules (see below and Fig. 4). The curve, which represents the water flow coupled to Na·citrate cotransport, superimposed on the curve describing the volume change of the oocyte. The ratio between water and substrates (3Na, 1 dicarboxylate, 165 H2O) resulted in the transport of fluid which was hyperosmotic, and one would anticipate a small transient osmotic influx of water after removal of the substrate. This was observed when oocytes were exposed to citrate for periods longer than 30 s (see Fig. 3).

The volume flow under isosmotic conditions was directly correlated with the rate of Na·dicarboxylate cotransport. This is illustrated in Fig. 4. The data were obtained from a series of experiments where the volume flow and substrate transport rate were measured simultaneously. The solid circles in Fig. 4 represent the volume flow that resulted from the transport of citrate in the presence of Na+ solution. The slope as determined from a linear regression through the data was 32 ± 1 × 10⁻³ pl·s⁻¹·nA⁻¹ (24 trials on 10 oocytes). Citrate and succinate are both substrates of NaDC-1; however, a higher maximal transport rate is observed with citrate (19), indicating that the turnover rate of NaDC-1 is higher when citrate is transported. We also examined volume flow when NaDC-1 was transporting succinate (Fig. 4, ▽). The transport rate of NaDC-1 in succinate was ~60% of that in citrate (present study; Ref. 19), but the coupling ratio between water and succinate cotransport was slightly higher, 42 ± 5 × 10⁻³ pl·s⁻¹·nA⁻¹ (9 trials on 4 oocytes).

In the presence of Na⁺, Li⁺ inhibits substrate transport by NaDC-1 (6). Presumably, Na⁺ and Li⁺ are competing for the same binding site, and Li⁺ is a poor activator of substrate transport (19, 27). The presence of 10 mM Li⁺ (together with 80 mM Na⁺ and 10 mM citrate) reduced both the substrate-evoked inward current and the water flux to 33 ± 2 and 31 ± 3%, respectively (8 trials on 4 oocytes) (data not shown). The relationship between water flow and NaDC-1 transport rate is shown in Fig. 4 (○). The coupling ratio between water and current in the presence of 10 mM Li⁺ was 34 ± 3 × 10⁻³ pl·s⁻¹·nA⁻¹ (8 trials on 4 oocytes).

The data from the experiments with citrate, succinate, and Li⁺ could be described by the same linear function (Fig. 4). The slope of the regression line, 33 ± 2 × 10⁻³ pl·s⁻¹·nA⁻¹, corresponds to the transport of 176 ± 12 H₂O per inward positive charge, or 176 H₂O:3 Na⁺:1 dicarboxylate (since the stoichiometry of NaDC-1 is 3 Na⁺ per divalent dicarboxylate, Refs. 6 and 19).

Uphill water transport. NaDC-1 was capable of transporting water uphill, against the osmotic driving force (Fig. 5). In Fig. 5A, bottom trace, an oocyte expressing NaDC-1 was subjected to an osmotic challenge of +20 mosM, obtained by addition of 20 mM mannitol to the control solution. The oocyte volume immediately started to decrease at a rate ($J_{\text{passive}}$) predicted by the osmotic water permeability ($L_{\text{p}}$). When the same osmotic gradient was applied but under the condition that NaDC-1 was transporting citrate (addition of 20 mM of mannitol to the isosmotic citrate solution), then the rate of shrinkage of the oocyte and the volume flow ($J_{\text{V}_{\text{citrate}}}$) was decreased (Fig. 5A, top trace). The difference between the two volume flows (NaDC-1-coupled volume flow) is represented by the jagged line in Fig. 5B and corresponds to the water transport that was coupled to the influx of Na⁺ and citrate by NaDC-1. The smooth curve of Fig. 5B was obtained from the integral of the citrate-induced inward current with a coupling ratio of 245 water molecules/inward charge. The smooth curve superimposed on the volume curve, indicating that NaDC-1 transported water together with Na⁺ and citrate against the osmotic gradient. On average, the coupling ratio was 182 H₂O:3 Na⁺:1 dicarboxylate.

**DISCUSSION**

The increase in $L_{\text{p}}$ of oocytes expressing NaDC-1 and the linear relationship between expression level and...
A

![Graph showing volume changes and inward current](image)

**ΔV** (nl) vs. time (s)

- **Δπ = +20**
- **Δπ = 0**

**Citrate**

**Mannitol**

**0 20 40 60 80 100 time (s)**

- **NaDC-1**
- **Lp**

The Lp is due to a NaDC-1-specific water permeability. This increase is not simply due to an increase in protein density in the plasma membrane because specific inhibitors block passive transport through other cotransporters, for instance, phlorizin blocks the SGLT1 Lp and SKF blocks the GAT1 (Na\(^{+}\)-Cl\(^{-}\)-GABA transporter) Lp (10). The rate of water transport through the passive pathway is determined by the direction of the imposed osmotic gradient (Figs. 1A and 5A). The passive water permeability per protein can be obtained from the turnover rate of the cotransporter. In general, Na\(^{+}\)-coupled cotransporters have turnover rates in the range of 10–100 s\(^{-1}\) (12). Assuming a turnover rate for NaDC-1 of 50 per s would give an Lp of 1.5 × 10\(^{-14}\), cm·s\(^{-1}\)-protein\(^{-1}\). This is comparable to the Lp per protein found for the Na\(^{+}\)-glucose cotransporter SGLT1 (1.4 × 10\(^{-14}\), cm·s\(^{-1}\)-protein\(^{-1}\)) (10, 28). The Lp per protein found for cotransporters is ~100 times lower than that of the aquaporins (15). The activation energy for passive water transport by SGLT1 is 5 kcal/mol and is comparable to those of the aquaporins (10). Therefore, Na\(^{+}\)-dependent cotransporters may be described as being low-conductance water channels (10, 11, 28). In addition to a similar Lp, the passive water permeability was the same in Na\(^{+}\), Li\(^{+}\), and choline for both NaDC-1 and SGLT1, independent of the cation. These similarities suggest that SGLT1 and NaDC-1 share a common mechanism for passive water transport.

Solute-coupled water transport by NaDC-1 is summarized by the following observations: 1) secondary active transport of Na\(^{+}\) and citrate was accompanied by an increase in oocyte volume (Fig. 3); 2) the time courses of volume change and citrate-induced current (inward charge) were superimposable (Fig. 3); 3) there was a strict stoichiometric relationship between Na\(^{+}\), citrate (or succinate), and water, and the coupling ratio was 3 Na\(^{+}\), 1 dicarboxylate, and 176 water molecules per transport cycle (Figs. 3 and 4); 4) Li\(^{+}\) inhibited both water flow and NaDC-1 transport to the same extent; with no change in the coupling ratios (Fig. 4); and 5) the solute-coupled water flow occurred in the absence of, and even against, an osmotic gradient (Figs. 3 and 5). These findings suggest that there is a direct coupling between water flow and secondary active transport of the substrates via NaDC-1. Coupling may be related to conformational changes of the protein during the transport cycle (11, 14).

Solute-coupled water transport mediated by NaDC-1 shares a number of properties with that observed in SGLT1 (11, 14, 31): 1) independence from osmotic gradients, 2) strict stoichiometric relationship between water and substrates, and 3) both components of water transport (osmotic and solute coupled) are present under substrate transporting conditions. Water transport has also been observed in cotransport proteins from native tissue, such as the choroid plexus K\(^{+}\)-Cl\(^{-}\) and the retinal pigment epithelium H\(^{+}\)-lactate cotransporters (29, 30), and in the cloned human brain Na\(^{+}\)-Cl\(^{-}\)-GABA, rat thyroid Na\(^{+}\)-iodide, and the plant H\(^{+}\)-amino acid cotransporters expressed in oocytes (11). This is consistent, in view of the hypothesis that cotransporters from different families share common kinetic mechanisms (26), and suggests that there may be an essential role for water in the cotransport process.

The following observations support the hypothesis that solute-coupled water flow is not due to a local osmotic mechanism: 1) transport through ion channels,
such as connexin 50, nystatin, and gramicidin, does not result in initial solute-coupled water flow (14, 25, 31); 2) the osmotic effects have also been ruled out by theoretical considerations (11); and 3) if solute-coupled water transport were driven by local osmotic gradients, then the ratio of water molecules to solutes would be the same for all transporters. In fact, this ratio varies from transporter to transporter. In human SGLT1, 210 water molecules are coupled to 3 solutes (2 Na\(^+\) and 1 glucose) (14), whereas in rabbit SGLT1, the ratio was 360 H\(_2\)O:2 Na\(^+\):1 glucose (31). In both the K\(^+\)-Cl\(^–\) (29) and in the H\(^+\)-lactate cotransporter (30), the water-to-solute ratio was 500:2. The ratio for NaDC-1 was 175 water coupled to 4 solutes: 3 Na\(^+\) and 1 dicarboxylate. In preliminary experiments, the plant H\(^+\)-amino acid cotransporter had a ratio of 50:2 (1 H\(^+\) and 1 amino acid) (unpublished data).

Does NaDC-1 play a role in renal proximal tubular water transport? We note that citrate stimulates fluid absorption in rabbit isolated proximal tubule (2). In the case of human, the plasma concentration of all tricarboxylic acid cycle intermediates is ~0.25 mM (7), and on the basis of a glomerular filtration rate of 180 liters/day and a reabsorption efficiency of 90% (5), 40 mmol are reabsorbed in the proximal tubule per day. One millimole of tricarboxylic acid cycle intermediate is coupled to 175 mmol water, equivalent to 3.15 ml. Thus NaDC-1 could mediate the reabsorption of ~100 ml of solute-coupled water per day. The transport of 176 water molecules with 3 Na\(^+\) and 1 dicarboxylate is equivalent to a coupling ratio of 46 water molecules/solute molecule. Because isotonicity requires 275 water molecules/solute molecule (4), this results in a fluid that is hypertonic. The hypertonic fluid transported across the apical membrane by NaDC-1 per se would slowly create an osmotic gradient, which could then provide a driving force for water movement across the apical membrane. Most of the water is probably transported by aquaporin-1. With the fact that the overall water reabsorption is isotonic taken into account, a total of 800 ml of water could be reabsorbed as a result of the reabsorption of dicarboxylates by NaDC-1. Furthermore, because water transport appears to be a common property of cotransporters, the coupling of water to the absorption of all organic acids (which includes tricarboxylic acid cycle intermediates, short-chain fatty acids, and ketone bodies; in total, 2–3 mM) could account for ~6% of the total daily fluid reabsorption in the kidney proximal tubule.

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