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Correlation Between Transepithelial Na^+ Transport and Transepithelial Water Movement Across Isolated Frog Skin (*Rana esculenta*)

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Abstract. In the present work the coupling under short-circuited conditions between the net Na^+-influx across isolated frog skin and the transepithelial transport of water was examined i.e., the short-circuit current (I_{sc}) and the transepithelial water movement (TEWM) were measured simultaneously. It has been shown repeatedly that the I_{sc} across isolated frog skin is equal to the net transepithelial Na^+ transport. Furthermore the coupling between transepithelial uptake of NaCl under open-circuit conditions and TEWM was also measured.

The addition of antidiuretic hormone (AVT) to skins incubated under short-circuited conditions resulted in an increase in the I_{sc} and TEWM. Under control conditions I_{sc} was 9.14 ± 2.43 and in the presence of AVT 45.9 ± 7.3 neq cm^{-2} min^{-1} ($n = 9$) and TEWM changed from 12.45 ± 4.46 to 132.8 ± 15.8 nL cm^{-2} min^{-1}. The addition of the Na^+ channel blocking agent amiloride resulted in a reduction both in I_{sc} and TEWM, and a linear correlation between I_{sc} and TEWM was found. The correlation corresponds to that 160 ± 15 ($n = 7$) molecules of water follow each Na^+ across the skin. In another series of experiments it was found that there was a linear correlation between I_{sc} and the increase in apical osmolarity needed to stop the TEWM.

The data presented indicate that the observed coupling between the net transepithelial Na^+ transport and TEWM is caused by local osmosis.

Key words: Sodium — Water — Transport — Coupling — Frog skin — Osmosis

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Introduction

The isolated amphibian skin and bladder have been used extensively as models for studying the effect of ADH on the hydro-osmotic water flow. It is well established that the addition of antidiuretic hormone results in the insertion of water channels in the apical membrane, for ref. *see* e.g., (de Sousa & Grosso, 1981; Harris & Handler, 1988; Ecelbarger et al., 1995). The net water movement across these tissues under nonosmotic conditions (conditions where the apical and the basolateral side of the tissue are bathed in solutions of equal composition), has only been investigated in a few cases, but it has been shown that the uptake of water under nonosmotic conditions depends on the metabolism (House, 1964; Lau et al., 1979).

It is still an enigma how the transport of water across transporting epithelia under nonosmotic conditions is coupled to the transport of ions (Tripathi & Boulpaep, 1989; Whittembury & Reuss, 1992; Ussing, Lind & Larsen, 1996), although several models have been put forward and tested. These models suggest that electro-osmosis (House, 1964; Hill, 1975; Boulpaep et al., 1993), different forms of local osmosis (Diamond & Bossert, 1967, Barry & Hope, 1969; Sackin & Boulpaep, 1975; Spring, 1983; Reuss, 1985; Tripathi & Boulpaep, 1989), friction (Ussing & Eskesen, 1989), and ''osmotic engines'' (Zeuthen & Stein, 1994) are responsible for the coupling between the transport of ions and water.

The isolated frog skin contains three different types of cells engaged in the transepithelial transport of ions, namely the principal cells, the mitochondria-rich cells (MR-cells), and the glandular cells. The principal cells, which make up the major part of the epithelium, are responsible for the active transepithelial Na^+ uptake, whereas the MR-cells (which are scattered within in the epithelium) form the passive (potential-activated) pathway for Cl^-, for ref *see* e.g., Larsen (1991). The acinus

of the exocrine glands of frog skin is located in the dermis, and the glandular duct crosses the epithelial layer. Activation of the glands results in the secretion of a near isotonic solution (Bjerregaard & Nielsen, 1987).

In an attempt to characterize the coupling between ion flux and water flow via the different pathways in frog skin, the correlation between the Na^+ efflux via the paracellular pathway in the skin glands and transepithelial water movement was investigated (Nielsen, 1990*a*,*b*). The experiments showed that there was a linear correlation between the net Na^+ efflux and the transepithelial net outflow of water, and that 220 molecules of water follow each Na^+ via this pathway. In another investigation (Nielsen, 1995) it was found that in the presence of the antidiuretic hormone arginine-vasotocin (AVT) there was a significant correlation between the net influx of Cl^- via the MR-cells and water, and it was found that 70 molecules of water follow each Cl^- across the skin.

In the present work it was investigated whether under nonosmotic conditions there was a correlation between the net Na^+-influx via the principal cells and transepithelial transport of water. I found that in the presence of AVT there was a linear correlation between the net transepithelial transport of Na^+ and the net uptake of water. When Na^+ passes the skin via the principal cells about 160 water molecules follow each Na^+. The experiments presented indicate that the observed coupling is caused by local osmosis.

Materials and Methods

Skins from male and female frogs (*Rana esculenta*) were used. The frogs were kept at room temperature and had free access to food (meal worms) and water.

The Ringer's solution had the following composition (in mM): Na^+ 115.0, K^+ 2.5, Mg^{2+} 1.0, Cl^- 118.0 CO_3^{2-} 2.5, PO_4^{3-} 1.0; pH = 7.8. The osmolarity of the solutions was determined by measuring the freezing-point depression.

Flux Measurements

During flux measurements, one skin half was used for measurement of influx and the other for efflux. $^{22}Na^+$ and $^{36}Cl^-$ were added to the solution bathing one side of the skins. After a equilibration period of 20 min, an aliquot (1 ml) was withdrawn from the other side and replaced with fresh solution. The last procedure was repeated at intervals of 60 min throughout the experiment. In the experiments the fluxes of $^{22}Na^+$ and $^{36}Cl^-$ were measured simultaneously, the Cl^- was separated from Na^+ by precipitating Cl^- with $AgNO_3$. After centrifugation in a table top centrifuge, the $^{22}Na^+$ activity of the supernatant was assayed by liquid scintillation. The Cl^- precipitate was dissolved in 1 ml 0.5 M NaSCN. After centrifugation, the $^{36}Cl^-$ activity in the supernatant was counted by liquid scintillation.

Water Flow

The transepithelial current or potential and water flow were measured simultaneously. The flow of water was measured as described by Johnsen and Nielsen (1982). In short, the principle is that the skin is pressed against a stainless steel net with a pressure of 2 cm H_2O. One of the chamber halves (area 6 cm^2) is closed except for an outlet consisting of a capillary tube. The solution is allowed to flow into the capillary tube, whereby the light transmission of the tube changes markedly. The transmission is recorded by a detector consisting of a light-emitting diode and a photosensitive transistor. The signal from the detector controls a motor-driven syringe which appropriately adjusts the outside volume in order to keep the position of the meniscus constant. The signal to the motor (a stepper motor) drives a counter and the counts accumulated are recorded every minute or every third minute. The motor drives also a precision potentiometer which is used as potential divider, allowing the position of the syringe to be recorded continuously on a pen recorder. As current electrodes Ag/AgCl electrodes were used, the electrodes were mounted outside the chamber, and the current was passed through salt bridges consisting of a 3% agarose gel (Agarose Type VIII) made in the same solution as that present in the chamber. Agarose type VIII (which has a very low sulfate content) was used to abolish the water movement across the current bridges caused by electro-osmosis (Nielsen, 1995).

Materials

AVT and Agarose Type VIII were obtained from Sigma and $^{22}Na^+$ and $^{36}Cl^-$ from Amersham.

Definitions

Current, as usual a positive current, is defined as an inward-directed Na^+ flux (apical to basolateral), and an inward-directed water flow is in the present paper considered positive.

Results

Coupling Between Transepithelial Transport of NaCl and Water Under Open Circuited Conditions

In the experiments presented below the skins were mounted in an Ussing chamber and bathed in isotonic Ringer's solution. Addition of AVT (48 nM) to the skins resulted in an increase in the transepithelial potential and in the transepithelial water movement (TEWM) (Fig. 1). To get an estimate of the osmolarity of the solution transported, the TEWM was measured simultaneously with the transepithelial flux of $^{22}Na^+$ and $^{36}Cl^-$. The transepithelial influx was measured on one skin half and the transepithelial efflux on the other skin half. Under open circuit conditions (in the absence of AVT) the transepithelial potential was 30 mV, the net transport of Na^+ 5.14 neq cm^{-2} min^{-1}, of Cl^- 4.00 neg cm^{-2} min^{-1}, and of water 14.5 nL cm^{-2} min^{-1}, corresponding to (disregarding the osmotic coefficient) a calculated osmolarity of 0.629 Osmol (Table). After the addition of AVT the transepithelial potential was 52.8 mV, the net transport of Na^+ 11.5 neq cm^{-2} min^{-1}, of Cl^{-1} 9.69 neq cm^{-2} min^{-1}, and of water 52.1 nL cm^{-2} min^{-1}, corresponding to a calculated osmolarity of 0.406 Osmol. The calcu-

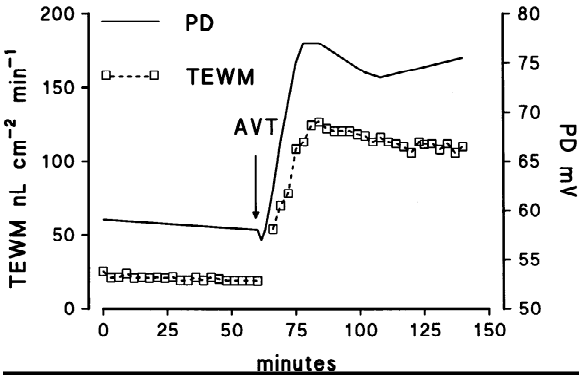

Fig. 1. Effect of the antidiuretic hormone arginine vasotocin (AVT) (48 nM, basolateral) on transepithelial potential (PD) and the transepithelial movement of water (TEWM).

lated osmolarity of the Ringer's solution used was 0.240 Osmol. Thus the solution transported is hypertonic.

During the experiment the transport of NaCl from the apical to the basolateral bathing solution resulted in a total change between the bathing solutions of about 0.5 mOsmol. To see whether this small change in the transepithelial osmolarity should have an effect on TEWM, the effect of transepithelial osmolarity on TEWM was investigated. The experiments were conducted on skins where the net transepithelial transport of NaCl was abolished by the addition of the Na^+ channel-blocking agent amiloride. The transepithelial osmotic water flow was measured both in the absence and in the presence of AVT. The osmolarity of the basolateral solution was kept constant, and the osmolarity of the apical solution was changed either by replacing a small amount of the Ringer's solution by water or by a Ringer's solution containing 0.5 M mannitol. The resulting osmolarity was measured by freezing point depression. The relation between apical osmolarity and TEWM could both in the presence and the absence of AVT be described by a linear relationship (Fig. 2). From the slope of the regression lines it was found that a change in the transepithelial osmolarity of 1 mOsmol in the absence and in the presence of AVT resulted in a change in the TEWM of 1.04 ± 0.17 or 7.95 ± 1.02 ($n = 4$) nL cm^{-2} min^{-1}, respectively, corresponding to a *Lp* value of 7.1×10^{-7} or 54.2×10^{-7} cm sec^{-1} Atm^{-1}, respectively. Thus the changes in the osmolarity of the bathing solutions caused by the transport NaCl were too small to account for the observed movement of water. Therefore the transepithelial movement of water must be caused by another mechanism.

Coupling Between Transepithelial Sodium Transport and Transepithelial Water Movement Under Short-Circuited Conditions

The addition of AVT (48 nM) to the isolated frog skin incubated under short-circuited conditions resulted in an increase in the short-circuit current (I_{sc}) and the transepithelial water movement (TEWM) (Fig. 3). It has been shown repeatedly that the only major transepithelial ion transport, which takes place under these conditions, is a transepithelial transport of Na^+, and that the I_{sc} measured is equal to the transepithelial transport of Na^+ (Ussing & Zerahn, 1951). Under control conditions I_{sc} was 9.14 ± 2.43, and in the presence of AVT 45.9 ± 7.3 neq cm^{-2} min^{-1}, and TEWM changed from 12.45 ± 4.46 to 132.8 ± 15.8 nL cm^{-2} min^{-1} ($n = 9$). Under short-circuited conditions Na^+ is transported across the skin, whereas Cl^- is delivered to, or removed from the bathing solutions by means of the current electrodes. The NaCl solution transported from the apical to the basolateral bathing solution is hypertonic, under control conditions it is 1.5 Osmol and in the presence of AVT 0.7 Osmol. The transport of this hypertonic NaCl from the apical to the basolateral bathing solution results during the period (50 min) presented in Fig. 3 in a change in the osmolarity between the bathing solutions of about 1 mOsmol. A change in the osmolarity of 1 mOsmol between the bathing solutions would in the presence of AVT cause a TEWM of 8 nL cm^{-2} min^{-1}. Thus the change in osmolarity of the bathing solutions, which happens during short circuiting, can not account for the observed movement of water.

The correlation between I_{sc} and TEWM was measured on skins where I_{sc} and TEWM were activated by addition of AVT. When I_{sc} and TEWM had reached a steady state, I_{sc} was reduced in a stepwise manner by addition of amiloride (Fig. 4). The addition of amiloride resulted in a reduction both in I_{sc} and TEWM. The results of 3 such experiments are presented in Fig. 5, and it is seen that in each experiment there is a linear correlation between I_{sc} and TEWM. The slope of the regression lines (Fig. 5) was 2.86 ± 0.26 nL neq^{-1} ($n = 6$), which corresponds to the fact that 160 ± 15 molecules of water follow each Na^+ across the skin. This indicates that there is a coupling (direct or indirect) between transepithelial Na^+ and water flow.

Electro- or Local Osmosis

If the observed coupling between ion and water flow is due to electro-osmosis or friction one would expect that a change I_{sc} should result in an immediate change in TEWM (Nielsen, 1995). Experiments of the type presented in Fig. 6 were carried out in order to measure the degree of coupling between I_{sc} and TEWM. The transport was activated by addition of AVT and after I_{sc}, and TEWM had reached a steady state, I_{sc} was inhibited by addition of amiloride (50 μM), and the change in I_{sc} and TEWM was measured. The observed decrease in I_{sc} and TEWM was fitted to a single exponential decay function, the half-time for the decrease in I_{sc} and TEWM was 0.59

Table. Effect of AVT (48 nM) on transepithelial potential (PD), Na^+ and Cl^- influx and efflux, and water uptake

Water flow nL cm^{-2} min^{-1}	PD mV	Na influx	Na efflux	Na netflux	Cl influx	Cl efflux	Cl-netflux
				neq cm^{-2} min^{-1}			
Control							
14.5 ± 2.2	30.0 ± 0.9	5.6 ± 2.0	0.46 ± 0.09	5.1 ± 2.1	7.0 ± 2.9	3.0 ± 1.2	4.0 ± 1.7
AVT							
52.1 ± 7.6	52.8 ± 5.7	12.6 ± 2.5	1.08 ± 0.20	11.5 ± 2.6	12.5 ± 4.2	2.8 ± 1.4	9.7 ± 2.9

The values are mean + SE (n = 5)

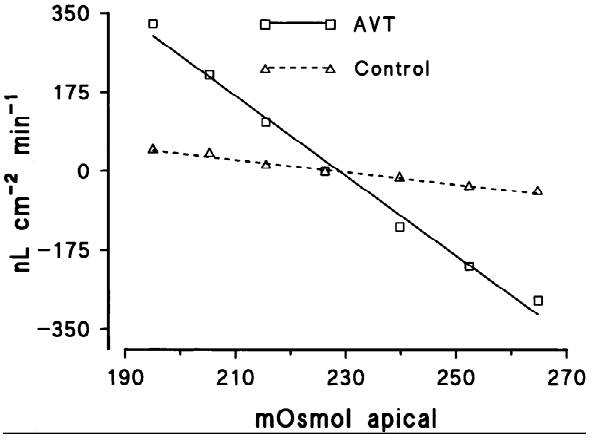

Fig. 2. Effect of changes in the apical osmolarity on the transepithelial movement of water (TEWM) across isolated frog skin in the absence and the presence of AVT (48 nM). The transepithelial Na^+ transport was blocked by the addition of the Na^+ channel blocking agent amiloride (50 μM, apical). The basolateral osmolarity (228 mOsmol) was kept constant during the experiment.

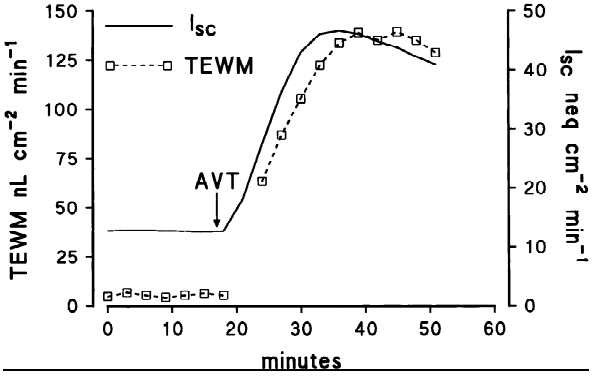

Fig. 3. Effect of AVT (48 nM, basolateral) on short circuit current (I_{sc}) and transepithelial movement of water (TEWM).

± 0.03 min and 1.69 ± 0.04 min, respectively, (n = 4). Thus, the decrease in TEWM is about 3 times slower than the decrease in I_{sc}, which indicates that the coupling between the movement of Na^+ and water is not direct (for further discussion *see* Discussion).

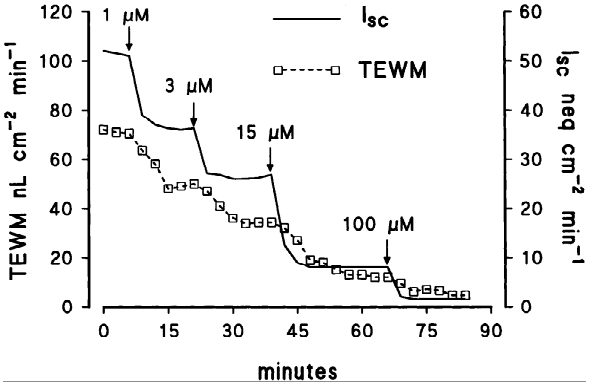

Fig. 4. Effect of amiloride (1, 3, 15 and 100 μM, apical) on the transepithelial movement of Na^+ (I_{sc}) and water (TEWM) across a skin incubated in the presence of AVT (48 nM, basolateral).

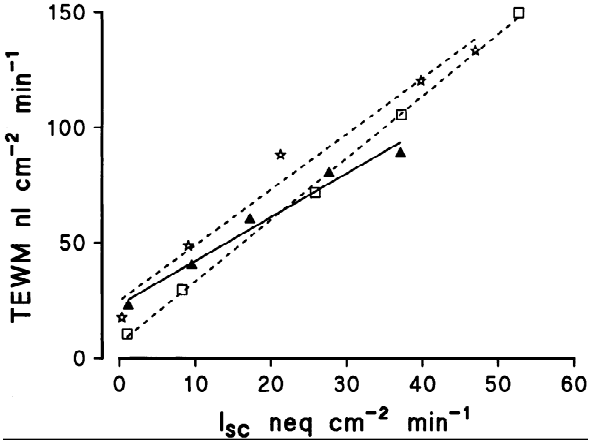

Fig. 5. Correlation between short-circuit current (I_{sc}) and transepithelial water movement (TEWM). Each experiment was carried out as outlined in Fig. 4. Open symbols are connected by broken lines and closed symbols by full lines.

Local Osmosis

If the TEWM is driven by local osmosis, then the transepithelial transport of Na^+ should lead to the formation of a local-osmotic gradient. The size of this gradient was estimated by increasing the osmolarity of the apical bath-

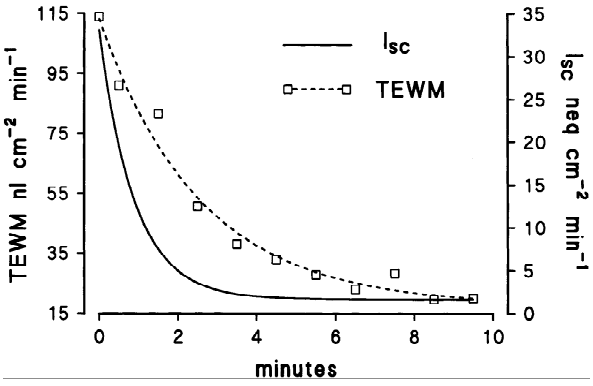

Fig. 6. Effect of amiloride (50 μM, apical) on short-circuit current (I_{sc}) and transepithelial water movement (TEWM). The experiment was carried out on an isolated frog skin where I_{sc} and TEWM were activated by the addition of AVT (48 nM, basolateral). At time zero amiloride was added to the apical solution. The decrease in I_{sc} and TEWM was fitted to a first-order decay function.

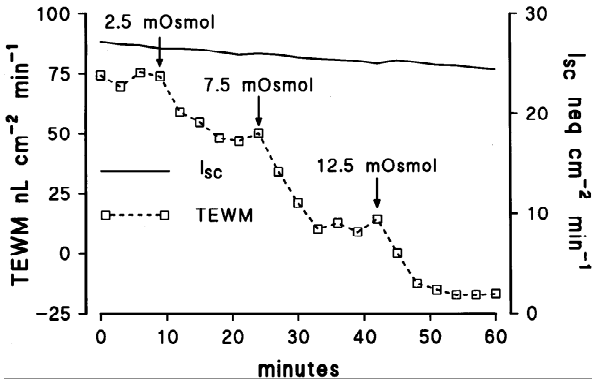

Fig. 7. Effect of an increase in the apical osmolarity (addition of mannitol) on the TEWM and I_{sc} in a frog skin incubated in the presence of AVT.

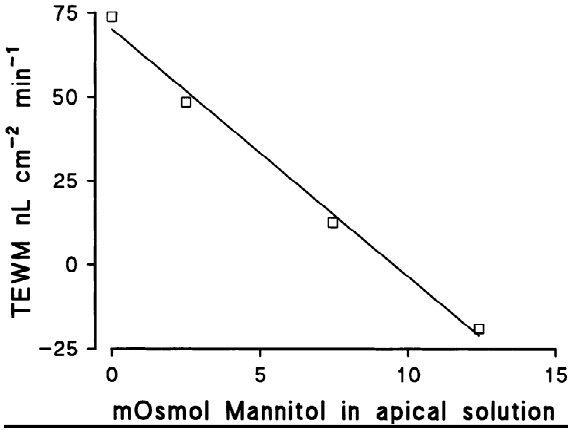

Fig. 8. Correlation between increase in apical osmolarity and TEWM. The osmolarity of the basolateral solution was kept constant during the experiment.

ing solution until the TEWM was abolished. The osmotic gradient was measured as outlined in Fig. 7. After I_{sc} and TEWM had reached a steady state, TEWM was reduced in steps by replacing small amounts of the Ringer's solution bathing the apical side of the skin with a Ringer's solution containing 0.5 M mannitol. From the relation between TEWM and the increase in osmolarity the change in osmolarity, needed to abolish TEWM, was found, (Fig. 8) i.e., the strength of transport was measured (Weinstein, Stephenson & Spring, 1981). The relation between I_{sc} and the strength of transport is presented in Fig. 9 ($n = 7$). The correlation between I_{sc} and the strength of transport, could be described by a linear correlation ($r^2 = 0.95$). The strength of transport was also determined under open-circuit conditions and it was found that the strength of transport was 15.5 ± 3.0 mOsmol when the transepithelial uptake of NaCl was 17.6 ± 4.3 nMole cm^{-2} min^{-1}. It appears to be the first time that this relationship has been measured in frog skin.

Discussion

From the data presented it is seen that in the presence of AVT both under open- and short-circuited conditions there is a transport of a hypertonic NaCl solution from the apical to the basolateral bathing solution. The change in osmolarity of the solutions caused by this transport of NaCl cannot account for the observed TEWM. The model presented in Fig. 10 is used to explain the observed coupling between transepithelial ion transport and TEWM. Under open-circuit conditions Cl^- passes the skin via specific Cl^- channels in the apical and the basolateral membrane of the mitochondria-rich cells, whereas under short-circuited conditions the current electrodes take care of the necessary adjustment of the Cl^- content of the bathing solutions. Both under open- and short-circuited conditions Na^+ mainly passes the apical membrane of the skin via Na^+-specific channels in the principal cells and the basolateral membrane via the Na,K-pump (for references *see* Larsen, 1988). The addition of AVT to the isolated frog skin under nontransporting conditions (in the presence of amiloride) resulted in about 8 times increase in the hydro-osmotic TEWM (Fig. 2). Addition of AVT results in the insertion of water channels in the apical membrane of the cells (for ref. *see* e.g., Harris & Handler, 1988; Ecelbarger et al., 1995). This indicates that in the presence of AVT most of the TEWM passes the apical membrane via a cellular pathway. The TEWM is not driven by a direct coupling in the apical membrane between Cl^- and water (Nielsen, 1995) or between Na^+ and water (Fig. 6). Since water passes the apical membrane via water channels the

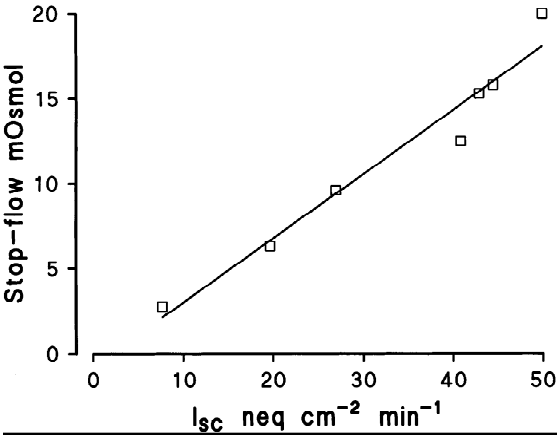

Fig. 9. Data from 7 experiments in which the increase in the apical osmolarity, needed to stop the TEWM (the strength of transport), is plotted as a function of the transepithelial Na^+ transport (I_{sc}).

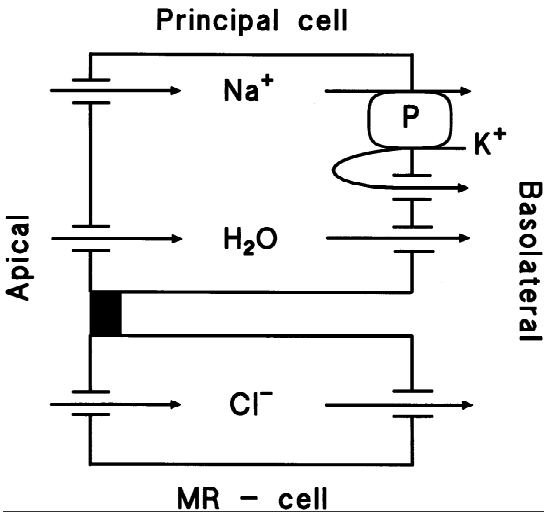

Fig. 10. Model used to explain the transepithelial transport of NaCl. For further explanation, *see text.*

movement of water across this membrane must be driven by an osmotic force. The basolateral membrane contains the Na,K-pump which is the primary active mechanism responsible (directly or indirectly) for the force driving the TEWM. The operation of the NaK-pump generates a recycling of K^+ across the basolateral membrane (Fig. 10). Thus, both the Na,K-pump and the K^+-channel are possible sites for an interaction between ion and water movement. Electroosmosis of the Schmid type (Schmid, 1950) might happen in the K^+-channel, and the Na,K-pump might act as an ''osmotic engine'' (Zeuthen and Stein, 1994). Under steady-state conditions each pump cycle removes 3 Na^+ from the cell. These ions are replaced by 3 Na^+ from the apical solution. Simultaneously with the net transepithelial movement of one Na^+ 160 water molecules follow (Fig. 5). Thus one pump cycle result in a TEWM of 480 molecules of water.

Zeuthen and Stein (1994) have shown that one cycle of the KCl-cotransporter (acting as an osmotic engine) results in a transmembrane transport of 500 water molecules. Electro-osmosis of the Schmid type has been shown to take place in the gramicidin channel, where it results in the transport of 6 molecules of water for each ion passing the channel (Finkelstein & Andersen, 1981).

If the TEWM is driven by one of these mechanisms, then under steady-state conditions the transepithelial transport of Na^+ would result in a net movement of water across the basolateral membrane, and consequently the osmolarity of the cell would increase. Because of this change in osmolarity of the cell, water would move into the cell. A TEWM driven by such a mechanism would require that the passive water permeability of the basolateral membrane is much lower than the water permeability of the apical membrane.

In frog skin it has been found that, under control conditions the ratio between the osmotic water permeability of the basolateral and the apical membrane is higher than 20, and in the presence of ADH, it is about 7 (MacRobbie & Ussing, 1961). The same has been found to be the case in toad bladder (Di Bona, Civan & Leaf, 1969). In the collecting duct it has been shown that the ADH-regulated water channel Aquaporin 2 is present in the apical membrane, whereas the water channel Aquaporin 3 is present predominately in the basolateral domain (for ref. *see* Ecelbarger et al., 1995). Aquaporin has also been cloned from the frog bladder (Abrami et al., 1994). Water channels have also been shown to be present in the basolateral membrane of the frog bladder (Van Der Goot, Corman & Ripoche, 1991). Thus available data imply that the basolateral membrane has a higher osmotic water permeability than the apical membrane. In a membrane with an osmotic engine and a high osmotic permeability a high degree of water circulation would take place across the membrane, but the net transport of water would be low. It is therefore suggested that the TEWM is not driven by an osmotic engine.

Local Osmosis Under Open Circuit Conditions

Local osmosis has repeatedly been suggested as the mechanism responsible for the coupling between transepithelial ion and water transport. However TEWM driven by local osmosis would always result in the transport of a solution which is more or less hypertonic (needless to say if it was isotonic there would be no osmotic gradient). Under open-circuit conditions in the presence of AVT there is a transepithelial transport of a hypertonic (0.4 Osmol) NaCl solution (Table). Since the solution, which passes the membrane, is hypertonic, then the solution left behind (at the apical side) will become hypo-

tonic. Thus, the presence of unstirred layers will maintain an osmotic gradient produced by an active mechanism within the membrane. This is opposite to the situation where the active transport of solute is abolished and the water is driven across the membrane by an applied osmotic gradient. The solution, which passes the membrane in this situation, is hypotonic, and consequently it results in an interepithelial solute polarization. Therefore an *Lp* value estimated from an applied osmotic gradient must be less than the true *Lp* value of the tissue (Weinstein et al., 1981; Barry & Diamond, 1984).

Under open-circuit conditions the rate of transepithelial transport of NaCl was about 10 nMole cm^{-2} min^{-1} and the TEWM 50 nL cm^{-2} min^{-1} (Table). A TEWM of that size can be driven by an applied osmotic gradient of about 6 mOsmol (Fig. 2). From the arguments presented below it appears that a transport of 10 nMole cm^{-2} min^{-1} NaCl produces a strength of transport of 8.8 mOsmol.

Weinstein et al. (1981) have, in their mathematical description of the coupling between transepithelial solute and volume flow, shown, that the osmotic gradient which can be produced by a transepithelial transport of NaCl can be described by an equation similar to Fick's first law:

$$J_{NaCl} = \Delta C \cdot D \cdot A/l \quad (1)$$

In Eq. 1 J_{NaCl} is the metabolically driven transport of NaCl, ΔC (the strength of transport) is the increase in bath concentration that nullifies transepithelial volume flow, D is the diffusion coefficient for NaCl ($D = 1.39 \cdot 10^{-5}$ cm^2 sec^{-1}), (A) is the area and l is the length of the unstirred layer. Under open-circuit conditions it was found that a transport of 17.6 nMole cm^{-2} min^{-1} of NaCl gave a strength of transport of 15.5 mOsmol. Based on these data one can calculate the ratio A/l and by the use of this value one can estimate that a transport of 10 nMole cm^{-2} min^{-1} of NaCl results in a ΔC of 8.8 mOsmol. Thus, the data presented indicate that the observed coupling between the transport of NaCl and water can be explained by the presence of a local osmotic gradient.

Coupling Between I_{sc} and TEWM

Under short-circuited conditions and in the presence of AVT there is a linear relationship between I_{sc} and TEWM (Fig. 5), and an I_{sc} of about 50 neq cm^{-2} min^{-1} results in a TEWM of 170 nL cm^{-2} min^{-1}, a TEWM which can be driven by an applied osmotic gradient of 21 mOsmol (Fig. 2).

If the observed TEWM under short-circuited conditions is driven by local osmosis, then it means that a transepithelial current of Na^+ *per se* results in the formation of an osmotic gradient somewhere in the tissue. Under short-circuited conditions the system can, as a first approximation, be described as a membrane with an electrogenic sodium pump and a water channel with an unstirred layer. The electrodes which measure the transmembrane potential are placed at the interface between the membrane and the bathing solutions. Under short-circuited conditions the transmembrane potential is kept at zero mV, and only Na^+ passes the membrane. The current electrodes (Ag/AgCl) take care of the necessary adjustment of the Cl^- content of the bathing solutions. The flow of a Na^+ current across the unstirred layer results in a potential drop across this layer. The *IV* drop across the unstirred layer (ΔV) was calculated from Eq. 2 (Ohms law for an electrolyte solution).

$$\Delta V = \alpha \cdot I_{sc} \cdot l \cdot A^{-1} \quad (2)$$

In Eq. 2 α is the specific resistance of the solution present in the extracellular space. The equivalent conductivity of the solution in the extracellular space is assumed to be equal to the conductivity of 115 mM NaCl solution:

$$\Lambda_{solution} = \Lambda_{Na} + \Lambda_{Cl} \quad (3)$$

However, under steady-state conditions only Na^+ flows across the extracellular space. Therefore, the conductivity of the solution in the extracellular space is equal to the equivalent conductivity for Na^+ (50.1 Ω^{-1} cm^2 Val^{-1}) times the Na^+ concentration (0.000115 M cm^{-3}), which gives a specific Na^+ resistance of 173.6 ohm cm^{-1}. l is equal to length of the unstirred layer, and A is the area. Neither l nor A is known but the ratio between l and A can be calculated from the strength of transport found under open-circuit conditions using Eq. 1. To drive a Na^+ current (I_{sc}) of 50 neq cm^{-2} min (80 μA cm^{-2}) across the unstirred layer requires, according to Eq. 2, a voltage of 5.1 mV. A voltage which is delivered from the voltage clamp apparatus. When the system is in a steady state there is no net movement of Cl^- across the unstirred layer and in this situation the electric force on Cl^-

$$\text{electrical force} = (-z \cdot F \cdot dE/dx) \quad (4)$$

has to balance the diffusion force on Cl^-

$$[-d\mu/dx = R \cdot T \cdot ln(C^x_{Cl}/C^{x+dx}_{Cl})] \quad (5)$$

By setting dE/dx equal to the potential drop across the extracellular space ΔV and C^x_{Cl} equal to the Cl^- concentration in the Ringer's solution (118 mM) and C^{x+dx}_{Cl} equal to the concentration at the apical end of the extracellular space, one gets by setting Eq. 4 = Eq. 5 the following equation (Eq. 6) where z, R, F and T have their usual meaning

$$C^{x+dx}_{Cl} = C^x_{Cl} \cdot \exp(z \cdot F \cdot \Delta V)/(R \cdot T) \quad (6)$$

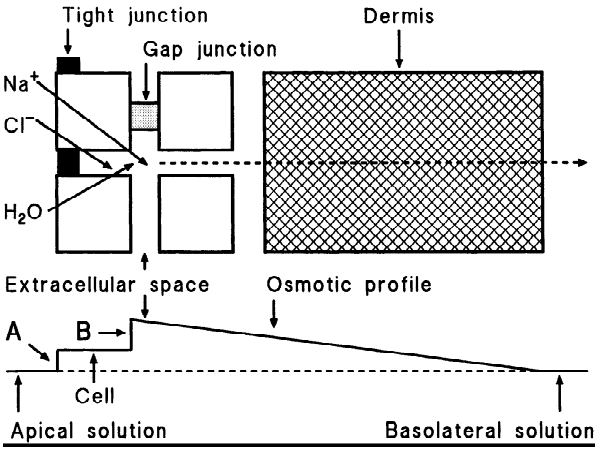

Fig. 11. The double-membrane model used to explain the coupling between transepithelial ion and water transport. The osmotic profile drawn is hypothetical. (*A*) osmotic gradient across the apical membrane of the epithelial cells, (*B*) osmotic gradient across the basolateral membrane of the epithelial cells. The principal cells are connected by gap junctions and form a functional syncytium. For further explanation, see text.

From Eq. 6 one can calculate that the Cl^- concentration at the apical end of the extracellular is 144.2 mM. Since electroneutrality has to be maintained the Na^+ concentration has to be same. This means that the calculated osmotic gradient across the unstirred layer (the skin) $2 \cdot (144.2–118)$ is 52.4 mOsmol). The data mentioned above (Fig. 7) show that a gradient of 21 mOsmol was sufficient to explain the observed TEWM. Thus, the observed coupling between transepithelial transport of Na^+ (I_{sc}) and TEWM can be explained by the presence of a local osmotic gradient. Furthermore, the calculation shows that because of the presence of an unstirred layer short circuiting of an epithelium will always result in the formation of an osmotic gradient across the tissue.

The data presented above indicate that active transepithelial transport of Na^+ both under open- and short-circuited conditions leads to the formation of a local osmotic gradient. Green and Giebisch (1989) and Timbs and Spring (1996) have shown the presence of a local osmotic gradient of 12 mOsmol in the proximal tubule of the rat kidney and of 27.5 mOsmol in MDCK cells respectively. In the presence of a local osmotic gradient the coupling between active solute transport and TEWM can be described by the double-membrane model (Curran, 1960). According to this model (Fig. 11) NaCl is transported from the apical side into the extracellular space of the epithelium, due to the presence of an unstirred layer (or other hindrances for diffusion) this transport of NaCl leads to a relatively high solute concentration in the extracellular space. The resulting osmotic gradient will cause water to move from the epithelial cells via specific water channels (reflection coefficient about 1) into the extracellular space. This movement of water would increase the osmolarity of the epithelial cells and water would move into the cells via specific water channels in the apical membrane. The opening of the extracellular space towards the apical solution is closed by tight junctions. The opening of the extracellular space towards the basolateral solutions happens via large pores (reflection coefficient about 0), and consequently no osmotic movement of water would take place from the basolateral solution into the extracellular space.

TEWM driven by an osmotic gradient ($\Delta\pi$) is equal to

$$TEWM = Lp \cdot \Delta\pi \tag{7}$$

where *Lp* is the hydraulic conductivity. In the presence of AVT the *Lp* of frog skin was 54.2×10^{-7} cm sec^{-1} Atm^{-1} (Fig. 2). The *Lp* values of tight epithelia such as frog skin are about a 100 times lower than *Lp* values for leaky epithelia such as the proximal tubule (Tripathi & Boulpaep, 1989). This means that a much smaller osmotic gradient than observed in the present experiments is able to drive a substantial TEMW in epithelia with high *Lp* values. Thus, the experiments presented support the notion (Spring, 1983) that a near isotonic transport across leaky epithelia can be driven by small local osmotic gradients (for further references *see* Tripathi & Boulpaep, 1989).

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