Physiol. Res. 43: 211–218, 1994

MINIREVIEW

Epithelial Sodium Transport: Basic Autoregulatory Mechanisms*

K. TURNHEIM

Institute of Pharmacology, Vienna University, Vienna, Austria

Received March 22, 1994

Accepted April 14, 1994

Summary

The epithelial cell is equipped with autoregulatory mechanisms that coordinate the rates of apical Na^+ entry and basolateral Na^+ extrusion, so that intracellular Na^+ activity is maintained relatively constant when the rate of active Na^+ transport changes. The increase of basolateral Na^+ extrusion *via* the ouabain-inhibitable Na^+,K^+-ATPase during Na^+ transport stimulation appears to be a result of both an increase in the number of operative Na^+,K^+-ATPase units in the basolateral cell membrane and in the Na^+ turnover per Na^+,K^+-ATPase unit. Further, it is possible that the number of epithelial cells, which are involved in active Na^+ transport, changes when the rate of Na^+ transport is altered. Not only apical Na^+ entry and basolateral Na^+ extrusion are coupled, the basolateral membrane K^+ conductance also changes in parallel with the rate of basolateral Na^+ extrusion ("pump-leak parallelism"). These regulatory mechanisms serve to prevent inordinate changes in intracellular ion composition, transmembrane electrical potential difference, and cell volume. The cellular events taking place during stimulation of active transport resemble the changes during osmotic cell swelling. Hence, it is possible that cell volume changes are responsible for the coordination of apical and basolateral membrane properties.

Key words

Epithelial sodium transport – Colon epithelium – Sodium-potassium ATPase – Epithelial ion channels – Aldosterone

Cell Model for Epithelial Na^+ absorption

Epithelial Na^+ transport is essential for the homeostasis of the effective circulating volume and the size of the extracellular space of the body. Since the end of the 19th century it is known that salts are necessary for fluid absorption. Heidenhain in Breslau showed in fundamental experiments that intestinal fluid absorption is not driven by hydrostatic or osmotic gradients across the epithelium, rather the driving force for fluid absorption resides in the epithelium itself. Curran in the 1950ies removed any reasonable doubt that there is no primary active transport of water, but that absorption of water is coupled to the transport of Na^+ (see Diamond 1978, Turnheim 1984). Na^+ transport is important not only for water absorption. Transport of sugars, aminoacids, nucleosides, chloride, phosphate, and H^+ ions is also coupled to that of Na^+. Hence Na^+ absorption plays a central role for many epithelial transport processes (Schultz 1986, Powell 1987).

* *Presented in part at the 12th Meeting of the European Intestinal Transport Group, Český Krumlov, September 1993*

Transepithelial Na^+ transport is a two-step process: Na^+ entry across the apical or luminal cell membrane followed by Na^+ extrusion across the basolateral cell membrane. Apical Na^+ entry occurs "downhill" or in the direction of a favourable electrochemical gradient for Na^+, since the electrical potential of the cell interior is negative and the Na^+ activity in the cells, $(Na^+)_c$, is low compared to the outside of the cell. Basolateral Na^+ extrusion, on the other hand, is directed "uphill" and therefore requires an active transport mechanism. This active transport mechanism, the "Na^+-pump", is the ouabain-inhibitable ATPase that exchanges Na^+ for K^+. The K^+ taken up into the cell when the pump turns over is recirculated to the blood-side of the epithelium *via* K^+-selective channels in the basolateral membrane. The combination of the Na^+-pump plus the K^+ leak (pump-leak system) in the basolateral membrane accounts for both the characteristic intracellular ion composition (low Na^+ and high K^+ activities) and the electrical cell membrane potential difference (Fig. 1).

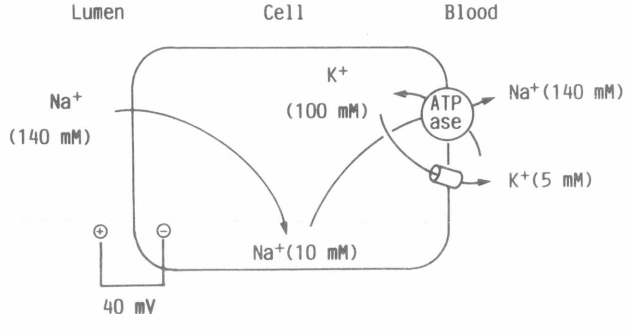

Fig. 1
Cell model for active Na^+ transport across epithelia.

The basic features of this cell model were first proposed 35 years ago for frog skin (Koefoed-Johnsen and Ussing 1958) and are still accepted today for Na^+-absorbing epithelia in general. However, this model represents a static view of cell function, all transport parameters are assumed to be in the steady-state. In the present review some of the cellular events during transport changes will be addressed, we will deal with the dynamic properties of the epithelium during changes in transport.

Intracellular Na^+ and apical membrane potential difference during changes in Na^+ transport rate

Interestingly, variations in the rate of apical Na^+ entry appear to be primarily a consequence of changes in apical membrane Na^+ permeability, P^m_{Na}, whereas the driving force for Na^+ entry remains comparatively constant. Both the steady-state intracellular Na^+ activity, $(Na^+)_c$, and the electrical potential difference across the apical membrane, V^{mc}, vary only within narrow limits when the rate of transcellular Na^+ transport is altered (Schultz and Hudson 1991, Turnheim 1991).

An example of these adaptive mechanisms are the cellular changes during stimulation of Na^+-transport in rabbit colon epithelium. This is a so-called tight or moderately tight epithelium with channel-mediated apical Na^+ entry that is blocked by the diuretic amiloride[1]. Channel-mediated Na^+ transport is stimulated by aldosterone, i.e. the rate of Na^+ transport is positively related to plasma aldosterone levels. Hence, the rate of Na^+ transport can be altered by dietary Na^+ loading or Na^+ restriction for several days, the latter causing secondary hyperaldosteronism. Stimulation of Na^+ transport in Na^+-deprived (high-aldosterone) animals is a result of a threefold increase in the maximal transport rate, whereas the half-saturation constant of Na^+ transport is unchanged (Fig. 2). Apical membrane Na^+ permeability, P^m_{Na}, is markedly increased during transport stimulation (Table 1). In fact, there is a linear relation between P^m_{Na} and I_{Na} (Fig. 2). These findings suggest that the number of conducting apical Na^+ entry sites is increased. Indeed, using noise analysis or the patch-clamp technique, evidence was obtained in other amiloride- and aldosterone-sensitive tissues, the toad urinary bladder or A6 cells (a cultured renal cell line from *Xenopus*), that the increase in P^m_{Na} is a result of an equal increase in the density of apical Na^+ channels, whereas the single channel current does not change (Palmer *et al.* 1982, Kemendy *et al.* 1992).

Using Na^+-selective microelectrodes or the current-voltage relations of the apical Na^+-entry pathway, steady-state $(Na^+)_c$ was shown to be more or less constant in rabbit descending colon despite wide variations in transcellular Na^+ transport (Table 1). The fact that $(Na^+)_c$ is practically unchanged when transcellular Na^+ transport varies indicates that basolateral Na^+ extrusion increases in parallel with apical Na^+ entry.

---

[1] The primary structure of the amiloride-sensitive epithelial Na^+ channel was recently identified by expression cloning in *Xenopus laevis* oocytes. This channel appears to consist of three homologous subunits (Canessa *et al.* 1994).

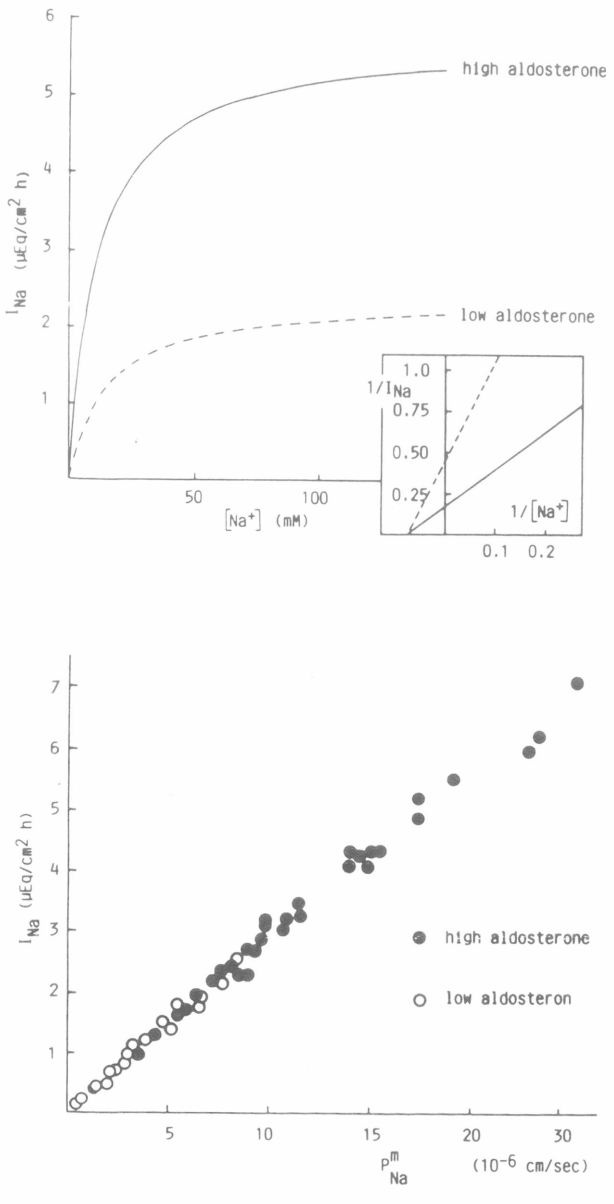

Fig. 2
Active Na^+ transport, I_{Na}, as a function of the Na^+ concentration in the bathing solution in colon epithelia from rabbits with high or low aldosterone levels (inset: double-reciprocal plot) and relation between I_{Na} and apical membrane Na^+ permeability, P^m_{Na} (data from Turnheim *et al.* 1986).

Not only apical Na^+ entry and basolateral Na^+ extrusion are coordinated, it was also shown for many epithelia that basolateral K^+ conductance increases during Na^+ transport stimulation (Schultz and Hudson 1991, Graf *et al.* 1993). In fact, there seems to exist a linear relation between basolateral membrane conductance and the rate of active Na^+ transport (Frömter and Gebler 1977, Thomas *et al.* 1983). In physiological terms, the increase in basolateral membrane K^+ conductance during Na^+ transport stimulation serves several purposes:

1) Increased transcellular Na^+ transport is accompanied by increased cellular K^+ uptake *via* the basolateral Na^+-K^+ exchange pump (see Fig. 1), hence cell K^+ may rise. This rise in cell K^+ is prevented by the parallel increase in basolateral K^+ conductance ("pump-leak parallelism").
2) Because of electrical coupling of the basolateral and apical membrane potential differences, an increase in basolateral K^+ conductance will hyperpolarize the electrical potential difference across the apical membrane and thereby increase or restore the driving force for conductive apical Na^+ entry.
3) An increase in basolateral K^+ conductance may be involved in the regulation of cell volume in response to cell swelling (Schultz and Hudson 1991).

Table 1
Parameters of active Na^+ transport across rabbit descending colon under conditions of dietary Na^+ loading (low aldosterone levels) or dietary Na^+ restriction (high aldosterone levels) (data from Turnheim *et al.* 1986, 1987)

	Low aldosterone	High aldosterone
I_{Na} (μEq/cm^{2}h)	0.8±0.1	3.2±0.3
P^m_{Na} (10^{-6} cm/s)	2.8±0.3	11.3±1.0
$(Na^+)_c$ (mM)	8.3–12.0	8.9–14.0
V^{mc} (mV)	−44±4	−39±4
r^m/r^s	2.0±0.4	1.8±0.3

I_{Na}: rate of active Na^+ absorption
P^m_{Na}: apical membrane Na^+ permeability
$(Na^+)_c$: intracellular Na^+ activity
V^{mc}: apical membrane electrical potential difference
r^m/r^s: ratio of the electrical resistance of the apical membrane to that of the basolateral membrane. The fact that r^m/r^s is not significantly changed during the marked increase in conductive apical Na^+ entry (as reflected by the rise in P^m_{Na}) indicates that basolateral membrane K^+ conductance is increased by a similar amount

The cellular events that prevent excessive changes in intracellular ion composition and hence cell volume during changes in transcellular Na^+ transport have been termed "homocellular regulation" (Schultz 1981) or "membrane cross-talk" (Diamond 1982). However, the precise mechanisms that coordinate

apical and basolateral transport properties are not entirely clear[2].

The close coordination between apical Na^+ entry and basolateral Na^+ extrusion can not only be demonstrated during chronic stimulation of Na^+ transport but also during acute changes. In *Necturus* small intestine apical Na^+ entry is increased by addition of sugars such as galactose, because there is a Na^+-sugar cotransport system in the luminal cell membrane. After addition of galactose V^{mc} depolarizes rapidly because of rheogenic apical Na^+ entry *via* the Na^+-sugar cotransporter. But then V^{mc} repolarizes despite the continued presence of galactose (Table 2). This repolarization is attributed to an increase in the K^+ conductance of the basolateral membrane, as repolarization is blocked by Ba^{2+} (Hudson and Schultz 1984). $(Na^+)_c$, measured continuously with Na^+-selective microelectrodes, increases transiently following addition of sugar, but after 10 min $(Na^+)_c$ does not differ significantly from the value before addition of galactose. It should be noted that $(Na^+)_c$ decreases although transcellular Na^+ transport continues to rise (Table 2).

Table 2
Effect of galactose on electrical parameters and intracellular Na^+ activity of *Necturus* small intestine (data from Hudson and Schultz 1984)

	Before addition of galactose	After addition of galactose	
		1–2 min	10 min
I_{sc} (μEq/cm^{2}h)	0.5±0.1	0.6±0.1	1.7±0.1
V^{mc} (mV)	−27±3	−5±5	−19±5
$(Na^+)_c$ (mM)	12±1	21±3	14±3
r^m/r^s *	0.8±0.2	0.2±0.1	1.0±0.2

I_{sc}: short-circuit current, measure of active Na^+ transport; other symbols as in Table 1
** The initial fall of r^m/r^s reflects the decrease in apical membrane electrical resistance because of rheogenic apical Na^+ entry. The subsequent rise of r^m/r^s is a result of an increase in basolateral membrane K^+ conductance*

The initial increase in $(Na^+)_c$ is consistent with an increase in the rate of apical Na^+ entry coupled to the entry of galactose. The subsequent decline in $(Na^+)_c$ may be a result of an increase in basolateral Na^+-pump activity and/or an increase in cell water content. In any case, the important fact is that in the steady-state of Na^+ transport stimulation, when Na^+ transport is approximately 3 times higher than without the sugar, $(Na^+)_c$ is not significantly increased. Since under these conditions basolateral Na^+-pump activity must also be increased threefold, the question arises, what signals the increase in pump rate, when steady-state $(Na^+)_c$ does not change, because pump activity is traditionally thought to be a function of $(Na^+)_c$.

Regulation of the basolateral Na^+-pump

As we have seen, $(Na^+)_c$, at least in the steady-state, remains relatively constant when Na^+ transport varies. Although relatively invariant intracellular Na^+ levels may be advantageous for cell homeostasis, as the internal ionic milieu is not markedly changed, the question is open how the basolateral Na^+-pump is activated. One possibility how basolateral Na^+ extrusion can increase despite unchanged $(Na^+)_c$ is an increase in the number of functional Na^+,K^+-ATPase units in the basolateral membrane. Indeed, when the rate of Na^+ absorption is stimulated threefold in rabbit descending colon by chronic dietary Na^+ restriction, the number of transporting Na^+-pump units in the basolateral membrane, measured by specific ouabain binding, was increased by 50 % in comparison to tissues transporting Na^+ at a low rate (Table 3).

Now, are we able to explain the mechanism that is responsible for the increase in basolateral Na^+ extrusion during stimulation of Na^+ transport when $(Na^+)_c$ remains relatively constant ? When dealing with the transport properties of the Na^+-pump one has to remember its positive cooperativity, i.e. a small increase in $(Na^+)_c$ may cause a much larger increase in pump rate, because 3 Na^+ ions are pumped per cycle. However, given the kinetic properties of the pump (Turnheim *et al.* 1983), a 50 % increase in the number of Na^+-pump units cannot explain the observed threefold increase in Na^+-pump rate, even when $(Na^+)_c$ is assumed to increase by 2 mM. Hence, it appears that in addition to an increase in the abundance of Na^+-pumps the turnover of the Na^+-pump is increased, i.e. the number of Na^+ ions pumped per min and per pump unit is increased during chronic stimulation of Na^+ transport. In fact, a twofold increase in Na^+-pump turnover can be calculated for the colon epithelium, when Na^+ transport is stimulated by aldosterone (Table 3).

[2] Procedures that inhibit basolateral Na^+ extrusion result in a decrease in apical membrane Na^+ permeability and consequently in the rate of apical Na^+ entry. In fact, the number of open apical Na^+ channels is markedly decreased when the Na^+-pump is inhibited by ouabain (Silver *et al.* 1993). This phenomenon was termed "feedback inhbition", its intracellular mediators may be Na^+ itself, Ca^{2+} or Ca^{2+}-dependent mechanisms, and pH (Turnheim 1991).

Table 3
Properties of the epithelial Na^+,K^+-ATPase of rabbit descending colon under conditions of low Na^+ transport rates (low aldosterone levels) or high Na^+ transport rates (high aldosterone levels) (data from Roden and Turnheim 1988)

	Low aldosterone	High aldosterone
N (pmol/mg)	1.0±0.1	1.5±0.2
K_D (μM)	0.7±0.1	0.7±0.1
Na^+ turnover	800	1 600

N: number of Na^+,K^+-ATPase units per mg tissue, measured using specific ouabain binding
K_D: apparent dissociation constant of saturable ouabain binding
Na^+ turnover: Na^+ ions pumped per Na^+,K^+-ATPase unit per min

Interestingly, the turnover of the Na^+-pump is much higher in isolated basolateral membrane vesicles (4 000–5 000 Na^+ ions per site and min) than in intact epithelia (800–1 600 per site and min), and it is even higher in purified enzyme (30 000 per site and min, Roden and Turnheim 1988). Hence, the turnover of the pump in intact epithelia is far below its maximal capacity, factors within the intact cell or the cell membrane appear to down-regulate pump turnover. It therefore seems more appropriate to speak of desinhibition of the pump rather than of stimulation under conditions of increased apical Na^+ entry.

The factors regulating pump turnover are unclear at present. The electrical potential difference across the basolateral membrane, V^{cs}, is unlikely to control pump turnover, because at least in the range of physiological potential differences the pump functions as a constant-current source, i.e. independent of the electrical potential difference. In addition, V^{cs} is not markedly changed at different rates of Na^+ transport (Lewis and Wills 1979). It is conceivable that the expression of another isoform of the Na^+,K^+-ATPase is responsible for the increase in pump turnover during chronic stimulation of Na^+ transport. But in epithelia only the α_1-isoform of the Na^+,K^+-ATPase was detected, irrespective whether the tissues transported Na^+ at high or low rates (Welling *et al.* 1990, Wiener *et al.* 1993).

The number of Na^+,K^+-ATPase units is not only changed when Na^+ transport is chronically stimulated by aldosterone. Rather it seems that the abundance of Na^+-pumps is generally a function of Na^+ transport rate. In A6 cells suppression of Na^+ transport by either amiloride or by Na^+ removal caused a large decrease in ouabain binding, at the same time Na^+,K^+-ATPase activity was also decreased (Lyoussi & Crabbé 1992). This decrease in the number of ATPase units was reversible upon reactivation of Na^+ transport. These observations indicate that apical Na^+ entry is a key regulator of the Na^+-pump number.

Table 4
Parameters of rabbit colon epithelium at high and low rates of Na^+ transport (high or low aldosterone levels) (data from Turnheim *et al.* 1986)

	Low aldosterone	High aldosterone
I_{Na} ($\mu Eq/cm^2h$)	0.8±0.1	3.2±0.3
$(Na^+)_c$ (mM)	8.3±1.2	8.9±0.6
Na^+ content of the Na^+ absorbing cells ($\mu mol/cm^2$)	0.11±0.02	0.32±0.03
volume of the Na^+ absorbing cells ($\mu l/cm^2$)	9	23
intracellular space of the epithelium ($\mu l/cm^2$)	21.1±0.9	26.9±1.8

Is the number of transporting epithelial cells variable ?

Up to now we have considered only the function of the individual Na^+ transporting cell. Ever since the advent of cellular biology we have been so focused on the single cell that we may have lost sight of the fact that cells of higher organisms function in concert, in connected multitude. I now want to discuss findings that raise the possibility that the number of transporting epithelial cells changes with varying Na^+ transport rates. Suggestive evidence that different cells of an absorptive epithelium may be in different functional states was provided by a comparison of intracellular Na^+ concentration and Na^+ content under conditions of high or low rates of Na^+ absorption in rabbit colon. An increase in transcellular Na^+ transport was associated with no or only a small increase in the steady-state Na^+ concentration, $(Na^+)_c$, as mentioned previously, whereas the intracellular Na^+ content of the Na^+-absorbing cells (i.e. the amiloride-sensitive cells) was markedly increased (Table 4). This

relative constancy of intracellular Na^+ concentration in combination with an increase in intracellular Na^+ content can be interpreted by an increase in the volume of individual Na^+ transporting cells (cell swelling) or an increase in the fraction of epithelial cells that take part in Na^+ transport (recruitment of new cells or previously inactive cells).

Using the values for $(Na^+)_c$ and the Na^+ content of the Na^+-absorbing cells, we can estimate the apparent volume of the cells involved in active Na^+ transport. This volume is almost tripled in epithelia with high Na^+ transport rates (Table 4).

Thus, stimulation of active Na^+ transport is associated with a marked increase in the volume of the epithelial cells that take part in Na^+ transport.

When comparing the apparent volume of the Na^+-transporting cells with the entire intracellular space of the epithelium, it seems that approximately 40 % of the intracellular space is involved in Na^+ transport in tissues with low Na^+ transport, but in epithelia with high Na^+ transport more than 80 % of the intracellular space participates in Na^+ transport (Table 4). Hence, the fraction of the intracellular space that takes part in Na^+ transport increases markedly during transport stimulation. This finding is at least consistent with the notion that there is recruitment of previously resting cells in addition to cell swelling. In other words, at a given time, different cells of an absorbing epithelium may be in different functional states, and the variation of the overall rate of transepithelial transport may be determined by the fraction of epithelial cells that is involved in transport (Fig. 3).

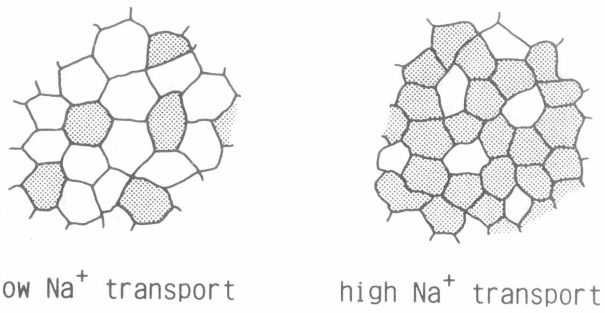

Fig. 3
Schematic illustration of the hypothesis that overall active Na^+ transport across an epithelium is determined by the number of cells that contribute to Na^+ transport. Active cells are stippled, quiescent cells are empty.

Physiologically, spreading the Na^+ transport load over a varying number of cells according to the macroscopic transport rate of the epithelium clearly has the advantage that the Na^+ flux across the individual cell varies only within a relatively narrow range, and hence the intracellular ionic composition is less affected as when greatly varying Na^+ fluxes would have to be accommodated by the individual cell. According to this concept the coupled cell ensemble of the epithelium and not solely the single cell is subject to regulation of Na^+ transport rate.

A somewhat related finding was recently reported for the distal intestine of the hen, the coprodeum. Under conditions of long-term dietary Na^+ restriction there is an increase in epithelial surface area which is primarily a result of an increase in the cell number and a smaller increase in the number of microvilli per cell (Mayhew *et al.* 1992).

Conclusions: role of cell volume

There are numerous adaptive changes that take place in an epithelium during variations in the rate of active or transcellular transport. Whereas the intracellular Na^+ activity and the electrical potential differences across the cell membranes remain comparatively constant, apical membrane Na^+ permeability increases in parallel with the rate of active Na^+ transport. Aside from other mechanisms, apical membrane Na^+ permeability may be altered by exocytotic expansion and endocytotic retrieval of the apical membrane area or by insertion of channel proteins into the apical membrane without increasing the apical membrane area (Lewis and Demoura 1984). These events appear to be mediated in part by cell swelling. It was shown repeatedly that changes in cell volume lead to parallel changes in transepithelial Na^+ transport (see Turnheim 1991). On the other hand, stimulation of Na^+ transport is known to cause an increase in cell volume (Czaky and Esposito 1969, Armstrong *et al.* 1970, Hudson and Schultz 1988, MacLeod and Hamilton 1991).

Further, the increase in basolateral K^+ conductance observed when epithelial cells swell osmotically resembles the increase in basolateral K^+ conductance in response to stimulation of Na^+ transport. Thus, it is possible that a common mechanism is responsible for volume regulation and for the parallelism between Na^+ transport rate and basolateral K^+ permeability (Schultz and Hudson 1991, Graf *et al.* 1993).

Swelling of the epithelial cells may also account for the increased activity of the Na^+-pump, as exposure to hypotonic solutions increases Na^+-pump rate markedly, although $(Na^+)_c$ is not changed. It was proposed that the number of Na^+-pump units in the basolateral membrane of epithelia increases during osmotic cell swelling (see Siebens 1985). In short, it is possible that cell swelling is the common regulating factor that ties the three major variables during changes in transepithelial transport together, the rate of apical Na^+ entry, the activity of the Na^+-pump, and the conductance of the basolateral K^+ channels.

Acknowledgement Supported by the Fonds zur Förderung der wissenschaftlichen Forschung (Austrian Research Council), projects P3688 and P6487.

References

ARMSTRONG W.McD., MUSSELMAN D.L., REITZUNG H.C.: Potassium and water content of isolated bullfrog small intestinal epithelium. *Am. J. Physiol.* **219:** 1023–1026, 1970.

CANESSA C.M., SCHILD L., BUELL G., THORNS B., GAUTSCHI I., HORISBERGER J.-D., ROSSIER, B.C.: Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits. *Nature* **367:** 463–467, 1994.

CSAKY T.Z., ESPOSITO G.: Osmotic swelling of intestinal epithelial cells during active sugar transport. *Am. J. Physiol.* **217:** 753–755, 1969.

DIAMOND J.M.: Solute-linked water transport in epithelia. In: *Membrane Transport Processes*. J.F. HOFFMAN (ed), Raven Press, New York, 1978, pp. 257–276.

DIAMOND J.M.: Transcellular cross-talk between epithelial cell membranes. *Nature* **300:** 683–685, 1982.

FRÖMTER E., GEBLER B.: Electrical properties of amphibian urinary bladder epithelium. III. The cell membrane resistances and the effect of amiloride. *Pflügers Arch.* **371:** 99–108, 1977.

GRAF J., GUGGINO W.B., TURNHEIM K.: Volume regulation in transporting epithelia. In: *Advances in Comparative and Environmental Physiology*, Vol. 14: *Interaction of Cell Volume and Cell Function.* F. LANG, D. HÄUSSINGER (eds), Springer-Verlag, Berlin, 1993, pp. 67–117.

HUDSON, R.L., SCHULTZ, S.G.: Effects of sodium-coupled sugar transport on intracellular sodium activities and sodium-pump activity in Necturus small intestine. *Science* **224:** 1237–1239, 1984.

HUDSON R.L., SCHULTZ S.G.: Sodium-glycine uptake by Ehrlich ascites tumor cells results in an increase in cell volume and plasma membrane channel activities. *Proc. Natl. Acad. Sci. USA* **85:** 279–283, 1988.

KEMENDY A.E., KLEYMAN T.R., EATON D.C.: Aldosterone alters the open probability of amiloride-blockable sodium channels in A6 epithelia. *Am. J. Physiol.* **263:** C825–C837, 1992.

KOEFOED-JOHNSEN U., USSING, H.: The nature of the frog skin potential. *Acta Physiol. Scand.* **42:** 298–308, 1958.

LEWIS S.A., DEMOURA L.C.: Apical membrane area of rabbit urinary bladder increases by fusion of intracellular vesicles: an electrophysiological study. *J. Membr. Biol.* **82:** 123–136, 1984.

LEWIS S.A., WILLS N.K.: Intracellular ion activities and their relationship to membrane properties of tight epithelia. *Feder. Proc.* **38:** 2739–2742, 1979.

LYOUSSI B., CRABBÉ J.: Influence of apical Na^+ entry on Na^+-K^+-ATPase in amphibian distal nephron cells in culture. *J. Physiol. (London)* **456:** 655–665, 1992.

MACLEOD R.J., HAMILTON J.R.: Volume regulation initiated by Na^+-nutrient cotransport in isolated mammalian villus enterocytes. *Am. J. Physiol.* **260:** G26–G33, 1991.

MAYHEW T.M., ELBROND V.S., DANTZER V., SKADHAUGE E.: Quantitative analysis of factors contributing to expansion of microvillus surface area in the coprodeum of hens transferred to a low NaCl diet. *J. Anat.* **181:** 73–77, 1992.

PALMER L.G., LI J.H.-Y., LINDEMANN B., EDELMAN I.S.: Aldosterone control of the density of sodium channels in the toad urinary bladder. *J. Membr. Biol.* **64:** 91–102, 1982.

POWELL D.W.: Intestinal water and electrolyte transport. In: *Physiology of the Gastrointestinal Tract* (2nd ed), L.R. JOHNSON (ed.), Raven Press, New York, 1987, pp. 1267–1305.

RODEN M., TURNHEIM K.: Sodium pump quantity and turnover in rabbit descending colon at different rates of sodium absorption. *Pflügers Arch.* **413:** 181–189, 1988.

SCHULTZ S.G.: Homocellular regulatory mechanisms in sodium-transporting epithelia: avoidance of extinction by "flush-through". *Am. J. Physiol.* **241:** F579–F590, 1981.

SCHULTZ S.G.: Cellular models of epithelial ion transport. In: *Physiology of Membrane Disorders* (2nd ed), T.F. ANDREOLI, J.F. HOFFMAN, D.D. FANESTIL, S.G. SCHULTZ (eds), Plenum Press, New York, 1986, pp. 519–534.

SCHULTZ S.G., HUDSON R.J.: Biology of sodium-absorbing epithelial cells: dawning of a new era. In: *Handbook of Physiology*, Section 6: *The Gastrointestinal System,* Vol. IV: *Intestinal Absorption and Secretion,* M. FIELD, R.A. FRIZZELL (eds), American Physiological Society, Bethesda, 1991, pp. 45–81.

SIEBENS A.W.: Cellular volume control. In: *The Kidney: Physiology and Pathophysiology.* D.W. SELDIN, G. GIEBISCH (eds), Raven Press, New York, 1985, pp. 91–115.

SILVER R.B., FRINDT G., WINDHAGER E.E., PALMER L.G.: Feedback regulation of Na channels in rat CCT. I. Effect of inhibition of Na pump. *Am. J. Physiol.* **264:** F 557–F564, 1993.

THOMAS S.R., SUZUKI Y., THOMPSON S.M., SCHULTZ S.G.: Electrophysiology of Necturus urinary bladder. I. "Instantaneous" current-voltage relations in the presence of varying mucosal sodium concentrations. *J. Membr. Biol.* **73:** 157–175, 1983.

TURNHEIM K.: Intestinal permeation of water. In: *Handbook of Experimental Pharmacology,* Vol. 70/I: *Pharmacology of Intestinal Permeation.* T.Z. CSÁKY (ed.), Springer, Berlin, 1984, pp. 383–463.

TURNHEIM K.: Intrinsic regulation of apical sodium entry in epithelia. *Physiol. Rev.* **71:** 429–445, 1991.

TURNHEIM K., THOMPSON S.M., SCHULTZ S.G.: Relation between intracellular sodium and active sodium transport in rabbit colon: current-voltage relations of the apical sodium entry mechanism in the presence of varying luminal sodium concentrations. *J. Membr. Biol.* **76:** 299–309, 1983.

TURNHEIM K., PLASS H., GRASL M., KRIVANEK P., WIENER H.: Sodium absorption and potassium secretion in rabbit colon during sodium deficiency. *Am. J. Physiol.* **250:** F235–F245, 1986.

TURNHEIM K., HUDSON R.L., SCHULTZ S.G.: Cell Na^+ activities and transcellular Na^+ absorption by descending colon from normal and Na^+-deprived rabbits. *Pflügers Arch.* **410:** 279–283, 1987

WELLING P.A., CAPLAN M., GIEBISCH G.: Aldosterone-dependent Na,K-ATPase expression is isoform specific. *J. Gen. Physiol.* **96:** 69a, 1990.

WIENER H., NIELSEN J.M., KLAERKE D.A., JØRGENSEN P.L.: Aldosterone and thyroid hormone modulation of α_1-, β_1-mRNA, and Na,K-pump sites in rabbit distal colon epithelium. Evidence for a novel mechanism of escape from the effect of hyperaldosteronemia. *J. Membr. Biol.* **133:** 203–211, 1993.

Reprint Requests
Dr. Klaus Turnheim, Institute of Pharmacology, Vienna University, Währingerstrasse 13a, A-1090 Vienna, Austria.