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Sodium Absorption, Volume Control and Potassium Channels: In Tribute to a Great Biologist

S.G. Schultz, W.P. Dubinsky
Department of Integrative Biology and Pharmacology, University of Texas Medical School, P.O. Box 20708, Houston, TX 77225, USA

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Abstract. It is well established, for all Na-absorbing epithelia, that an increase in the rate of transcellular Na^+ absorption is accompanied by an increase in the conductance of the basolateral membrane to K^+. For the case of small intestinal epithelial cells from the salamander *Necturus maculosus,* where the rate of transcellular Na^+ absorption can be increased manyfold by the addition of sugars or amino acids to the luminal bathing solution, it appears that this parallelism between Na-K pump rate and basolateral membrane K^+ conductance is closely related to volume regulation by the enterocyte. Recent studies have disclosed the presence of stretch-activated K^+ channels, in a highly enriched basolateral membrane fraction isolated from these epithelial cells, whose activity is increased by an increase in vesicle volume and inhibited by a decrease in vesicle volume or ATP. The activity of this channel also appears to be regulated by the degree of organization of the cortical actin cytoskeleton; activity is increased by depolymerization of the actin cytoskeleton and decreased by repolymerization of that structure. We postulate that the inhibitory effect of ATP is related to its role in promoting the polymerization of G-actin to form F-actin. We propose that enterocyte swelling that results from the intracellular accumulation of sugars or amino acids in osmotically active forms brings about disorganization of the cortical actin cytoskeleton and activates these channels and is, at least in part, responsible for the "pump-leak parallelism" in this amphibian.

Introduction

The modern era of epithelial physiology was ushered in by three papers emanating from the laboratories of the great Danish biologist, Hans Ussing. In the first (Ussing, 1949), it was demonstrated that the isolated frog skin is capable of bringing about the net transfer of Na^+ from the outer bathing solution to the inner bathing solution against both a concentration difference and an electrical potential difference indicating that this is an "active transport process". In the second, Ussing and Zerahn (1951) demonstrated that the electrical current necessary to abolish the spontaneous electrical potential difference across — in other words, to "short circuit"— isolated frog skin bathed by identical solutions, can be attributed entirely to the flow of Na^+ from the outer to the inner solution, thereby solving the problem posed by DuBois Reymond in 1848 (DuBois Reymond, 1848). On a personal note, in the early 1960's the late Peter Curran and I were supervising a group of first-year Harvard Medical School students in a laboratory exercise in which they were to compare the short-circuit current across isolated frog skin with the net Na^+ flux determined using isotopes. Professor Ussing was visiting Arthur K. Solomon's Biophysical Laboratories (of which Pete and I were members) and we prevailed upon Hans to lead the discussion after the laboratory exercise. To our students' delight, he opened, in his kind manner and in his ever gentle voice, by complimenting them on the fact that the agreement they had observed was much closer than he and Zerahn had found. And, it was! Indeed, I have wondered if I had been the reviewer of that manuscript whether I might not have insisted on more experiments; but, Ussing had a way of seeing the forest for the trees.

In the third paper, Koefoed-Johnsen and Ussing (1958) observed that the outer-facing membrane of isolated frog skin, bathed by a sulfate-Ringer solution, behaved as if it was solely permeable to Na^+, whereas the inner-facing membrane behaved as if it was solely permeable to K^+. These observations led to the now classic "double" or "series membrane" (KJU) model illustrated in Fig.1. The elegance of this model, and certainly one of the reasons for its almost

Correspondence to: S.G. Schultz; email: stanley.g.schultz@uth.tmc.edu

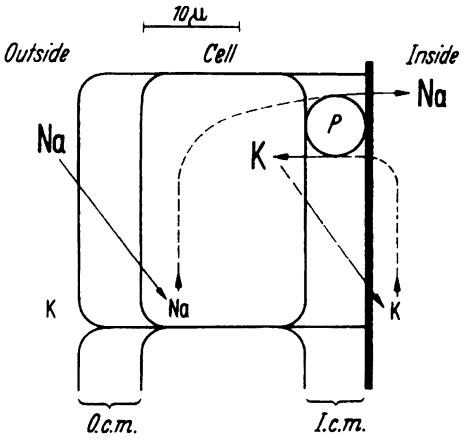

Fig. 1. The series of double membrane model proposed by Koefoed-Johnsen and Ussing for Na^+ transport by isolated frog skin (reproduced with permission from Koefoed-Johnsen & Ussing, 1958).

immediate acceptance by the scientific community, is that it simply segregated transport processes that had already been identified in symmetrical cells such as erythrocytes, nerve and muscle to either the apical (outer) or basolateral (inner) membranes, thereby creating the asymmetry needed for vectorial flow. Thus, by localizing Na-leaks to the apical membrane and everything else (i.e., K-leaks and Na-K pumps) to the basolateral membrane one could simultaneously account for transcellular Na^+ transport as well as the low intracellular Na^+ activity and high intracellular K^+ activity characteristic of virtually all animal cells. As pointed out previously by this author, the beauty of this model was in part derived from the fact that it was biologically parsimonious; it introduced no new demons. It was typical of the simple, aesthetic view of nature that characterized all of Ussing's thinking.

In the years that have followed its introduction, the KJU model for isolated frog skin has been successfully extended to describe a wide number of Na-absorbing epithelial cells with two basic modifications.

The first is that we know that there are four different mechanisms that may mediate Na^+ entry across the apical membrane into cells. These are (i) the originally described Na^+ channel, which is inhibited by very low, micromolar, concentrations of amiloride; (ii) rheogenic cotransporters that couple the entry of Na^+ to the entry of a wide variety of organic solutes such as sugars or amino acids; (iii) electroneutral cotransporters that couple the entry of Na^+ to the entry of Cl^- (e.g., NaCl or $KNaCl_2$) and are inhibited by a number of diuretics such as furosemide; and (iv) countertransporters that couple Na^+ entry to H^+ extrusions from the cell and are inhibited by high concentrations of amiloride (*c.f.*, Schultz & Lapointe, 2000).

Second, it is now clear that the two membranes that are arrayed in series do not operate independently but instead exhibit cross-talk designed to protect the cellular *milieu interior* (Schultz, 1981; Diamond, 1982). Thus, in "tight" epithelia such as frog skin or toad urinary bladder, inhibition of the basolateral membrane Na-K pump resulting in an increase in cell Na^+ activity brings about an inhibition of apical membrane Na^+ channels; this subject has been recently reviewed elsewhere (Schultz & Lapointe, 2000). Further, changes in the rate of Na^+ entry across the apical membrane, which result in changes in the rate of basolateral membrane pump activity and Na^+ absorption, are accompanied by parallel changes in the K^+ conductance of the basolateral membrane. This parallelism has been demonstrated in every Na-absorbing epithelium studied to date. Further, where examined, the parallelism appears to be very precise. Thus, in *Necturus* urinary bladder (Frömter & Gebler, 1977; Thomas et al., 1983), frog renal proximal tubule (Lang, Messner & Rehwald, 1986) and *Necturus* small intestine (Hudson & Schultz, 1984) increases in the rate of Na-K pump activity are paralleled *one-for-one* by increases in basolateral membrane K^+ conductance.

In the remainder of this paper, we will first review the evidence for the "pump-leak" parallelism, then examine the physiological utility of this ubiquitous phenomenon and, finally, briefly discuss recent findings suggesting the underlying mechanism in the light of our understanding of epithelial cell volume control. We will draw mainly upon data obtained in our laboratories on in vitro *Necturus* small intestine where the rate of Na^+ absorption can be rapidly and markedly increased by the addition of sugars or amino acids to the solution bathing the apical surface of the tissue. Comparisons with findings on other epithelia will be made when appropriate.

Evidence for the "Pump-Leak" Parallelism

The first direct evidence for an increase in basolateral membrane K conductance in response to an increase in the rate of Na entry across the apical membrane came from electrophysiological studies such as that illustrated in Fig. 2. Shown is the electrical potential difference across the apical membrane (ψ^{mc}) with respect to the mucosal solution and the ratio of the relative resistance of the apical membrane (r^m) to that of the basolateral membrane (r^s) [i.e., (r^m/r^s)] before and after the addition of alanine (or sugars) to the fluid perfusing the mucosal surface of the tissue. In the absence of the alanine, ψ^{mc} was approximately −40 mV cell interior negative with respect to the mucosal solution and (r^m/r^s) was close to unity. Immediately after the addition of 5 mM alanine to the mucosal solution, there was a rapid depolarization of

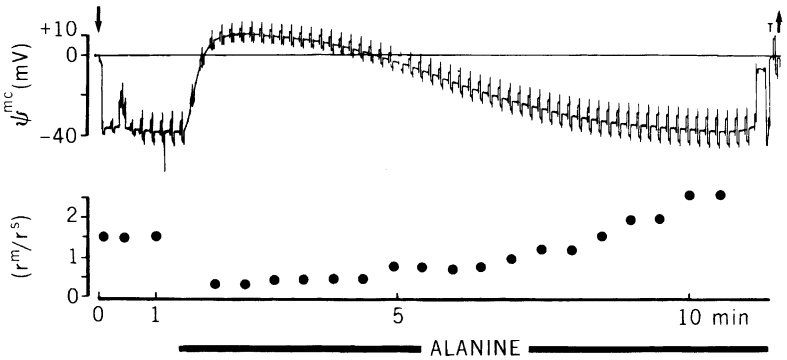

Fig. 2. The effect of alanine on the electrical potential difference across the apical membrane (ψ^{mc}) and the resistance ratio (r^m/r^s) of *Necturus* enterocytes (reproduced with permission from Gunter-Smith, Grasset & Schultz 1982).

ψ^{mc}, which often reversed polarity, accompanied by a marked, rapid decline in (r^m/r^s). The depolarization of ψ^{mc} is the result of the activation of rheogenic Na-amino acid cotransporters that were previously quiescent leading to a, transient, near doubling of the intracellular Na activity (Hudson & Schultz, 1984). The initial decline in (r^m/r^s) is due primarily to a decline in r^m due to the fact that these cotransporters are conductive. But, following these dramatic, rapid events, there is a slower repolarization of ψ^{mc} to near control values and an increase in (r^m/r^s) which often reaches values greater than control in the continued presence of the amino acid. As demonstrated by Grasset, Gunter-Smith & Schultz, (1983), these slow events are the result of an increase in basolateral membrane K^+ conductance. Those investigators demonstrated that a fourfold increase in Na-K pump activity, brought on by the addition of sugars or amino acids to the mucosal solution and, hence, a fourfold increase in the rate at which K^+ is pumped into the cell across the basolateral membrane, is accompanied by a decline in cell K^+ activity, but no change in the calculated electrochemical potential difference for K^+, i.e., the thermodynamic driving force for K^+ diffusion, across that barrier. Inasmuch as the rate of K^+ diffusion out of the cell across the basolateral membrane must have increased fourfold to maintain a steady-state, this must mean that the conductance of the basolateral membrane to K^+ must also have increased fourfold. The decline in cell K^+ activity is almost certainly due to cell swelling. Similar findings have been reported by Lang et al. (1986) for frog renal proximal tubule.

Physiological Utility of the "Pump-Leak" Parallelism

In all epithelia, the "pump-leak" parallelism serves to prevent inordinate swings in intracellular K^+ content when Na-K pump activity changes. Thus, if pump activity is increased, in the absence of a parallel increase in K^+ conductance, one might expect an increase in cell K^+ content, which must be accompanied by an increase in the content of some anion, presumably Cl^-, an increase in cell water content and cell swelling. This problem is compounded in small intestinal and renal proximal tubule epithelial cells when the increase in transcellular Na^+ absorption is due to an increase in Na-entry coupled to the entry of sugars or amino acids inasmuch as the latter are accumulated within the cells in osmotically active forms and thus obligate additional water accumulation. The increase in basolateral membrane K^+ conductance, perhaps in response to cell swelling (*see below*) almost certainly serves to limit volume increases.

A second value of the "pump-leak" parallelism is that, in leaky epithelia, the hyperpolarization of the basolateral membrane due to the increase in the K^+ conductance of that barrier also results in the hyperpolarization of the apical membrane. This increases the electrical driving force for the entry of all rheogenic Na-coupled carrier-mediated processes. The importance of this is shown in Fig. 3, which illustrates the rate of Na-coupled sugar entry across the apical membrane of *Necturus* small intestinal epithelial cells given in units of current, i.e., I^m_{NaS}, as a function of the electrical potential difference, ψ^{mc}. Clearly, the rate of coupled entry is markedly dependent upon ψ^{mc} and, indeed, is far more dependent upon the electrical driving force across the apical membrane than the chemical driving force. This finding has been confirmed for the case of the Na-coupled glucose carrier, SGLTI, expressed in *Xenopus* oocytes (Parent et al., 1992) and is consistent with the notion that the active site of the "naked" carrier protein is negatively charged and that one of the functions of Na^+ is to mask that charge.

The Link between the "Pump-Leak" Parallelism and Volume Control

Two sets of observations suggest that volume control by epithelial cells and the "pump-leak" parallelism may share a common mechanism. The first is that swelling of epithelial cells in response to exposure to hypotonic solutions results in a increase in the basolateral membrane K^+, and often Cl^-, conductance.

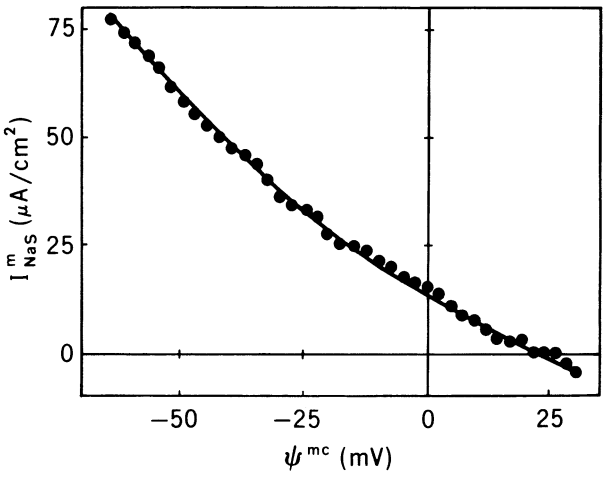

Fig. 3. An example of the effect of the electrical potential difference across the apical membrane (ψ^{mc}) on the rate of Na^+-coupled sugar entry across the barrier measured as the current $I^{m}_{SNa^+}$ (reproduced with permission from Lapointe, Hudson & Schultz, 1986).

This permits the exit of KCl accompanied by water, which limits and may even reverse the volume increase (Schultz, 1989). The second is the finding that in the course of transepithelial absorption, organic solutes are accumulated within epithelial cells in osmotically active forms and thus are accompanied by water. This results in cell swelling under isotonic conditions. In 1986, Lau, Hudson and Schultz demonstrated that the increase in basolateral membrane K^+ conductance that is associated with Na-coupled sugar absorption could be prevented by preventing or reversing cell swelling by exposure to a hypertonic solution, thereby linking the swelling induced by hypotonic conditions with that invoked by solute accumulation under isotonic conditions.

Studies on K^+ Channels in Basolateral Membrane Vesicles

In an effort to gain further insight into the mechanisms that regulate the K^+ channels in basolateral membranes of *Necturus* small intestinal epithelial cells, we have succeeded in isolating a membrane fraction of these cells that is highly enriched in Na–K ATPase, a universally accepted marker for those membranes, and minimally contaminated by enzyme markers for other plasma or intracellular membranes. Reconstitution of these membranes into artificial, phospholipid planar bilayers revealed a K^+ channel that is slowly inhibited by millimolar concentrations of MgATP in the solution facing the inner surface of the membrane (Fig. 4) (Mayorga–Wark et al., 1995). Further, inasmuch as these membranes spontaneously form vesicles, we were able to examine the effects of membrane stretch resulting from swelling or shrinking of vesicles exposed to anisotonic solutions, on K^+ channel activity. As shown in Fig. 5, exposure of the vesicles to a solution that is hypotonic to the intravesicular solution resulted in an increase in channel activity, as measured by $^{86}Rb^+$ uptake using the Garty technique (Garty, Rudy & Karlish, 1983), whereas exposure to a hypertonic solution could abolish channel activity (Dubinsky, Mayorga-Wark & Schultz, 1999). Depolymerization of the actin cytoskeleton using either cytochalasin D or a nonpharmacologic approach abolished these responses to anisotonic solutions (Dubinsky et al., 1999).

An increase in K^+ channel activity could also be elicited by exposing the vesicles to an isotonic solution containing glucose or alanine. Under these conditions, the sugar or amino acid enters the vesicle accompanied by the isotonic equivalent of water and also results in cell swelling. The increase in channel activity following isotonic swelling was indistinguishable from that following exposure to a hypotonic solution. Finally, increases in vesicle volume under isotonic or hypotonic conditions reversed the inhibitory effect of intravesicular MgATP on K^+ permeability (Dubinsky, Mayorga-Wark & Schultz, 2000), and inhibition of single-channel activity by ATP (*see* Fig. 4) could be rapidly reversed by cytochalasin B (unpublished observations); in short, vesicle swelling appears to mimic the action of cytochalasin, which depolymerizes F-actin, on the inhibitory effect of ATP on channel activity.

Stretch-activated K^+ channels have been identified in the basolateral membranes of *Necturus* proximal renal tubule cells (Sackin, 1989) and *Necturus* gallbladder (Vanoye & Reuss, 1999). They have also been identified by a number of investigators in the basolateral membranes of frog proximal tubule (Kawahara, 1990; Hunter, 1990) where direct evidence has been presented for their involvement in the increase in the K^+ conductance of that barrier in response to isotonic swelling accompanying Na-coupled solute absorption (Cermerikic & Sackin, 1993). Further, recently Schütt and Sackin (1997) have demonstrated stretch-activated channels in vesicles devoid of soluble cytoplasmic components.

Summary and Conclusions

Studies on the ATP-inhibitable K^+ channel identified in a basolateral membrane fraction isolated from *Necturus* small intestinal epithelial cells strongly suggest that this is a stretch-activated channel and that the effect of ATP is mediated by the well-established role of this nucleotide in promoting the polymerization of G-actin to form F-actin (*c.f.* Korn, Carlier & Pantoloni, 1987). A model summarizing our view of the relations among membrane stretch, the organiza-

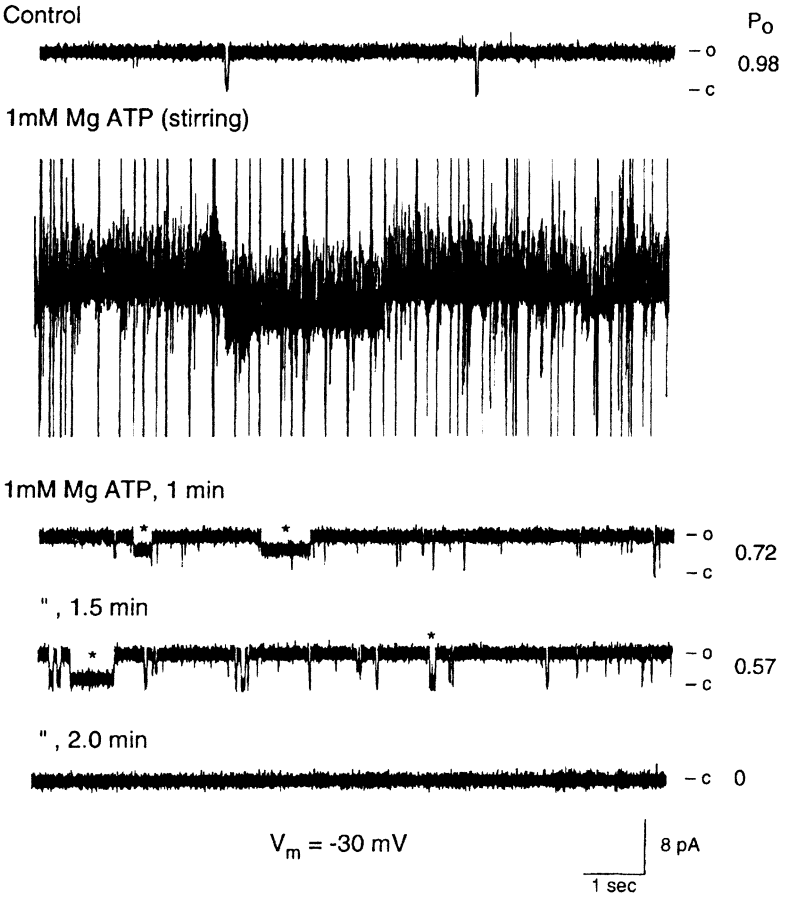

Fig. 4. Effect of ATP added to the inner solution on activity of a basolateral membrane K^+ channel reconstituted in a planar phospholipid bilayer. "o" denotes the open state and "c" the closed state. P_o is the open-probability. The noise in the second panel is due to stirring. (Reproduced with permission from Mayorga-Wark et al., 1995).

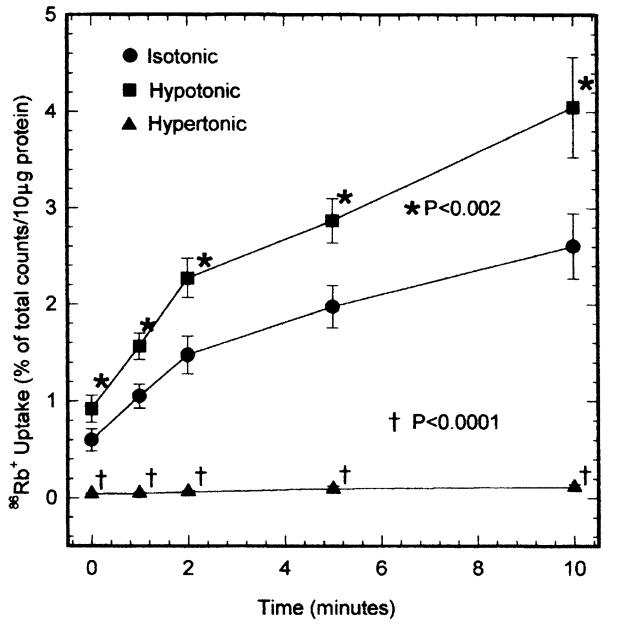

Fig. 5. Effect of exposure of basolateral membrane vesicles to hypotonic or hypertonic solutions on K^+ channel activity (measured as $^{86}Rb^+$ uptake) (reproduced with permission from Dubinsky et al., 1999).

tion of the cortical (submembranous) actin cytoskeleton, ATP and K^+ channel activity is illustrated in Fig. 6. We propose that membrane-stretch, secondary to cell swelling, brings about a disorganization of the cortical actin cytoskeleton, as has been observed by Cornet et al. (1987, 1993, 1994), and results in activation of the K^+ channel, whereas ATP and/or a reduction in membrane stretch has the reverse effect.

It should be emphasized that our findings do not exclude a possible role of other K^+ channel or cytoplasmic factors in regulating basolateral membrane conductance in response to changes in transcellular Na^+ transport, but are certainly consistent with the notion that alterations in cell volume and membrane tension, which accompany an increase in intracellular osmolytes, may be sufficient—at least for the case of *Necturus* small intestinal Na^+ absorbing cells.

Finally, it should be noted that the first hint of the "pump–leak" parallelism came from the undisputed father of epithelial biology himself, Hans Ussing, who, in a classic paper with Enid MacRobbie, published in 1961, reported that inhibition of Na^+ transport with a digitalis glycoside was accompanied by a decrease in the K^+ permeability of the inner membrane and suggested "...that the active ion transport and the passive fluxes are not entirely

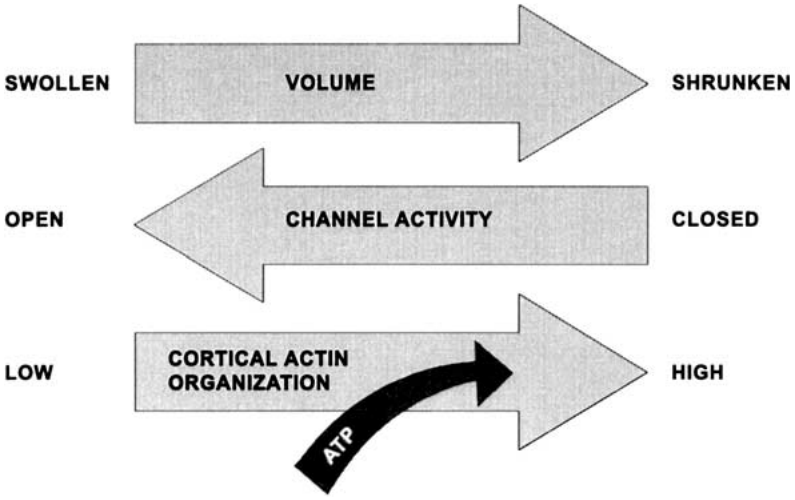

Fig. 6. Hypothetical model relating basolateral membrane K^+ channel activity to membrane stretch and cortical cytoskeletal organization. The inhibitory effect of ATP on channel activity is ascribed to its role in promoting the polymerization of G-actin to F-actin.

independent." It is hoped that, in the not too distant future, the cellular mechanisms responsible for this prescient observation will be fully resolved.

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