



Letters to the Editor

A. W. Cuthbert;, Sandy I. Helman and Bonnie Blazer-Yost

Am J Physiol Cell Physiol 273:1437-1439, 1997.

You might find this additional information useful...

This article cites 11 articles, 8 of which you can access free at:

<http://ajpcell.physiology.org/cgi/content/full/273/4/C1437#BIBL>

Medline items on this article's topics can be found at <http://highwire.stanford.edu/lists/artbytopic.dtl> on the following topics:

Biophysics .. Channel Protein

Physiology .. Homeostasis

Chemistry .. Ligand Binding

Updated information and services including high-resolution figures, can be found at:

<http://ajpcell.physiology.org/cgi/content/full/273/4/C1437>

Additional material and information about *AJP - Cell Physiology* can be found at:

<http://www.the-aps.org/publications/ajpcell>

This information is current as of December 21, 2006 .



letters to the editor

The following is the abstract of the article discussed in the subsequent letter:

Mitchell, Claire H., Jin Jun Zhang, Liwei Wang, and Tim J. C. Jacob. Volume-sensitive chloride current in pigmented ciliary epithelial cells: role of phospholipases. *Am. J. Physiol.* 272 (*Cell Physiol.* 41): C212–C222, 1997.—The whole cell recording technique was used to examine an outwardly rectifying chloride current activated by hypotonic shock in bovine pigmented ciliary epithelial (PCE) cells. Removal of internal and external Ca^{2+} did not affect the activation of these currents, but they were abolished by the phospholipase C inhibitor neomycin. The current was blocked by 5-nitro-2-(3-phenylpropylamino)benzoic acid, 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid, and 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) in a voltage-dependent manner, but tamoxifen, dideoxyforskolin, and quinidine did not affect it. This blocking profile differs from that of the volume-sensitive chloride channel in neighboring nonpigmented ciliary epithelial cells (Wu, J., J. J. Zhang, H. Koppel, and T. J. C. Jacob. *J. Physiol. Lond.* 491: 743–755, 1996), and this difference implies that the volume responses of the two cell types are mediated by different chloride channels (Jacob, T. J. C., and J. J. Zhang. *J. Physiol. Lond.* In press). Intracellular administration of guanosine 5'-O-(3-thiotriphosphate) ($\text{GTP}\gamma\text{S}$) to PCE cells induced a transient, time-independent, outwardly rectifying chloride current that closely resembled the current activated by hypotonic shock. DIDS produced a voltage-dependent block of the $\text{GTP}\gamma\text{S}$ -activated current similar to the block of the hypotonically activated current. Intracellular neomycin completely prevented activation of this current as did incubation of the cells in calphostin C, an inhibitor of protein kinase C (PKC). Removal of Ca^{2+} did not affect activation of the current by $\text{GTP}\gamma\text{S}$ but extended the duration of the response. Inhibition of phospholipase A_2 (PLA_2) with *p*-bromophenacyl bromide prevented the activation of the hypotonically induced current and also inhibited the current once activated by hypotonic solution. The findings imply that the hypotonic response in PCE cells is mediated by both phospholipase C (PLC) and PLA_2 . Both phospholipases generate arachidonic acid, and, in addition, the PLC pathway regulates the PLA_2 pathway via a PKC-dependent phosphorylation of PLA_2 .

The role of Cl^- channels in volume regulation in bovine pigmented epithelial cells

To the Editor: We would like to comment on a recently published paper by Mitchell et al. (6) on volume-sensitive Cl^- currents in bovine pigmented ciliary epithelial cells (BPCE). We believe there are some serious omissions of published findings that conflict with or question a number of statements made by the above authors. In the introduction, the authors state that very little is known about the response to osmotic swelling by pigmented ciliary epithelial cells. However, our paper (4) showed that acutely isolated BPCE cells fail to volume regulate following osmotic cell swelling. These findings contradict those of the authors; however, they are not cited in their paper. Our measurements were made on freshly isolated cells, whereas

those of the authors were cultured for up to 36 h. We believe that our model system more closely reflects the native transport properties of these cells. There are some striking differences between freshly isolated and cultured ciliary epithelial cells. For example, freshly isolated pigmented ciliary epithelial cells do not possess a $\text{Cl}^-/\text{HCO}_3^-$ exchanger (3), whereas pigmented epithelial cells in culture possess a robust exchange mechanism (7). It was not established by Mitchell et al. that these cells contain a swelling-activated Cl^- channel in fresh tissue, and thus the possibility is raised that this channel is not expressed in acutely isolated cells.

That a Cl^- channel is activated by cell swelling does not prove that it is involved in volume regulation (1), without the support of appropriate experiments that were not performed in the study of Mitchell et al. In fact, it has been shown that, in human breast cancer cells, the swelling-activated Cl^- conductance plays no role in volume regulation (2). Miley et al. reported in a previous abstract (5) that BPCE cells perform a regulatory volume decrease (RVD) in 2–4 min and that 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), and tamoxifen had no effect on RVD. However, Fig. 3 of their paper clearly shows that both NPPB and DIDS significantly reduce the swelling-activated Cl^- currents at membrane potentials in the physiological range. These contradictory findings suggest that additional experiments are necessary before we can assign a functional role for the swelling-induced Cl^- current in cultured BPCE.

Finally, in their DISCUSSION, Mitchell et al. (6) state, "Because depolarization is generated by the efflux of negative ions, this suggests that Cl^- , and not K^+ , is the predominant ion involved in RVD in these cells." Because conductive cation efflux must accompany conductive anion efflux for a cell shrinkage to occur following osmotic swelling, the above statement requires clarification. As pointed out by Altenberg et al. (2), three conditions are required for a Cl^- current to contribute to RVD: 1) a significant increase in Cl^- permeability must occur on swelling, 2) a net driving force favoring Cl^- efflux should accompany cell swelling, and 3) a parallel conductive pathway providing for a sizable loss of K^+ need also occur. Clearly, if Cl^- were the predominant conductance following cell swelling, without sizable K^+ efflux, RVD would be extremely slow or absent. Moreover, an RVD that presumably occurs in as little as 2 min (which is extremely rapid when compared with most cells that undergo RVD) must have comparable K^+ and Cl^- effluxes.

Because it has been suggested that the pigmented and nonpigmented cell acts in concert to secrete aqueous humor, the role each cell type plays in ion and water transport is fundamentally important. However, drawing physiological conclusions from isolated cells in

culture should be done with extreme caution, unless the mechanisms under study are also present in the native tissue. Recently, the hypothesis was put forth that the function of the pigmented epithelial cell was to take up ions and water and to transmit both via gap junctions into the adjacent nonpigmented epithelial cell. Once within the nonpigmented epithelial cell, ions and water are then transported by efflux pathways residing in the basolateral membrane of the nonpigmented epithelial cell into the aqueous chamber. In our paper (4), we presented data that supported the above hypothesis. What was striking was the obvious presence of a robust regulatory volume increase (RVI) in pigmented epithelial cells and the complete lack of RVD. On the other hand, the nonpigmented epithelial cells showed no RVI response, yet were very capable of undergoing a K^+ -dependent RVD. It will become clear with time whether the above hypothesis is correct, but only after appropriate experiments are conducted on preparations that are representative of the intact ciliary epithelium.

REFERENCES

1. Adorante, J. S., and P. M. Cala. Mechanisms of regulatory volume decrease in nonpigmented human ciliary epithelial cells. *Am. J. Physiol.* 268 (*Cell Physiol.* 37): C721–C731, 1995.
2. Altenburg, G. A., J. W. Dietmer, D. C. Glass, and L. Reuss. P-glycoprotein-associated Cl^- currents are activated by cell swelling but do not contribute to cell volume regulation. *Cancer Res.* 54: 618–622, 1994.
3. Butler, G. A. D., M. Chen, Z. Stegman, and J. M. Wolosin. Na^+ - Cl^- and HCO_3^- -dependent base uptake in ciliary body pigmented epithelium. *Exp. Eye Res.* 59: 343–359, 1994.
4. Edelman, J. L., G. Sachs, and J. S. Adorante. Ion transport asymmetry and functional coupling in bovine pigmented and nonpigmented ciliary epithelial cells. *Am. J. Physiol.* 266 (*Cell Physiol.* 35): C1210–C1221, 1994.
5. Miley, H. E., V. E. Walker, C. E. Pollard, and T. J. C. Jacob. Regulatory volume decrease in ciliary epithelial cells (Abstract). *Invest. Ophthalmol. Vis. Sci.* 36: S586, 1995.
6. Mitchell, C. H., J. J. Zhang, L. Wang, and T. J. C. Jacob. Volume-sensitive chloride current in pigmented ciliary epithelial cells: role of phospholipases. *Am. J. Physiol.* 272 (*Cell Physiol.* 41): C212–C222, 1997.
7. Weiderholt, M., H. Helbig, and C. Korbmacher. Ion transport across the ciliary epithelium: lessons from cultured cells and proposed role of the carbonic anhydrase. In: *Carbonic Anhydrase*, edited by F. Botre, G. Gross, and B. T. Storey. New York: Cambridge, 1991, p. 232–244.

Joseph S. Adorante
 Jeffrey L. Edelman
 Department of Biological Sciences
 Allergan, Inc.
 Irvine, CA 92715

REPLY

To the Editor: We thank Drs. Adorante and Edelman for creating this opportunity to address their criticisms and reconcile what they perceive to be a possible conflict between our work and theirs. “We are as men more sinned against than sinning” (to misquote Shakespeare, *King Lear* act 3, scene 2, line 57). In referring to our paper on the volume-sensitive Cl^-

current (5), Adorante and Edelman make the point that they were unable to find volume regulation in BPCE cells following osmotic cell swelling and that we do not mention this. In our hands these same cells do volume regulate, a fact that we have reported (4). Adorante and Edelman complain that we did not refer to their work. Our choice of references reflected the emphasis of our paper and its concern with the broader issues of second messenger control of volume regulation. However, since they have now drawn attention to this matter, we can pursue the possible reason(s) for the differences in our respective findings.

In their study they used acutely isolated BCPE cells and found no volume regulation following osmotic cell swelling as determined by Coulter counter. We, on the other hand, allowed our freshly dissociated cells to attach to glass for 12–36 h and, using optical image analysis, found RVD under similar osmotic conditions. Our cells are not cultured (in the sense that they have not undergone cell division while in culture), and therefore the comparison that Adorante and Edelman make with cultured cells is inappropriate. They should also be under no illusion that their system of trypsinization followed by Percoll gradient centrifugation is physiological. It has been shown that trypsinization causes damage to membrane proteins and leads to membrane leakiness (3). Thus there are two major differences between their work and ours: 1) the use of detached vs. attached cells and 2) the detection method.

To address the first difference, the cells that Adorante and Edelman studied were detached, whereas ours were attached. In this regard Han et al. (2) have made some interesting observations. They found that they could activate virtually no volume-sensitive Cl^- current in detached cells (human breast cancer cells) compared with the same cells when attached to glass. Therefore, the availability of swelling-activated Cl^- current depends, to some extent and through some unknown mechanism, on attachment to a substrate. Regarding the second difference, the use of image analysis allows us to select only those healthy cells and to follow the process of volume change from beginning to end in one cell. We can discard those cells that “bleb” following osmotic shock. Blebbing is an injury marker and indicates that the cells may be dying. The formation of blebs may affect the determination of cell volume by Coulter counter. Radically different time constants for RVD are determined by the two methods, and direct measurement by image analysis gives a value of 2–4 min (4) compared with the Coulter counter measurement in which the volume did not recover to original levels within 40 min of recording (Fig. 4B in Ref. 1). This difference needs to be addressed by those who chose to use the Coulter counter method.

Edelman and Adorante also draw attention to the differences in the pharmacology of RVD and the volume-activated Cl^- current. NPPB and DIDS, while blocking the volume-activated Cl^- current (5), did not significantly affect the RVD (4). First, both DIDS and NPPB give a voltage-dependent block (Fig. 3 in Ref. 5), and, at

the potentials the cells would be resting at in the volume studies, the block would be at its weakest; and, second, the currents are measured by an "invasive" technique of whole cell patch clamping. This can, as we explain in the DISCUSSION of Ref. 5, have the effect of artificially prolonging the volume-activated Cl^- current beyond its normal activation during RVD. When exposed to hypotonic solution, the cell can never equilibrate osmotically with the bathing solution because of the "infinite" (in comparison with the cell) supply of ions in the patch pipette.

Finally, the use of the term "predominant" (see DISCUSSION in Ref. 5), when referring to Cl^- efflux, does not preclude the involvement of K^+ . One partner in a relationship may be predominant, but that does not condemn the other partner to extinction. We mention the efflux of both ions in the introduction.

"No, I will be the pattern of all patience; I will say nothing more" (Shakespeare, *King Lear* act 3, scene 2, line 37).

REFERENCES

1. Edelman, J., G. Sachs, and J. S. Adorante. Ion transport asymmetry and functional coupling in bovine pigmented and nonpigmented ciliary epithelial cells. *Am. J. Physiol.* 266 (*Cell Physiol.* 35): C1210–C1220, 1994.
2. Han, E. S., C. G. Vanoye, G. A. Altenberg, and L. Reuss. P-glycoprotein-associated chloride currents revealed by specific block by an anti-P-glycoprotein antibody. *Am. J. Physiol.* 270 (*Cell Physiol.* 39): C1370–C1378, 1996.
3. Lamb, J. F., and P. Ogden. Transient leakiness of HeLa cells to Na and K during "rounding up" with trypsin, EDTA and pronase. *J. Physiol. (Lond.)* 358: 70P, 1985.
4. Miley, H. E., V. E. Walker, C. E. Pollard, and T. J. C. Jacob. Regulatory volume decrease in ciliary epithelial cells. *Invest Ophthalmol. Vis. Sci.* 36: S586, 1995.
5. Mitchell, C. H., J. J. Zhang, L. Wang, and T. J. C. Jacob. Volume-sensitive chloride current in pigmented ciliary epithelial cells: role of phospholipases. *Am. J. Physiol.* 272 (*Cell Physiol.* 41): C212–C222, 1997.

T. J. C. Jacob
C. H. Mitchell

*School of Molecular and Medical Biosciences
University of Wales, Cardiff CF1 3US, UK*

The following is the abstract of the article discussed in the subsequent letter:

Blazer-Yost, Bonnie L., and Sandy I. Helman. The amiloride-sensitive epithelial Na^+ channel: binding sites and channel densities. *Am. J. Physiol.* 272 (*Cell Physiol.* 41): C761–C769, 1997.—The amiloride-sensitive Na^+ channel found in many transporting epithelia plays a key role in regulating salt and water homeostasis. Both biochemical and biophysical approaches have been used to identify, characterize, and quantitate this important channel. Among biophysical methods, there is agreement as to the single-channel conductance and gating kinetics of the highly selective Na^+ channel found in native epithelia. Amiloride and its analogs inhibit transport through the channel by binding to high-affinity ligand-binding sites. This characteristic of high-

affinity binding has been used biochemically to quantitate channel densities and to isolate presumptive channel proteins. Although the goals of biophysical and biochemical experiments are the same in elucidating mechanisms underlying regulation of Na^+ transport, our review highlights a major quantitative discrepancy between methods in estimation of channel densities involved in transport. Because the density of binding sites measured biochemically is three to four orders of magnitude in excess of channel densities measured biophysically, it is unlikely that high-affinity ligand binding can be used physiologically to quantitate channel densities and characterize the channel proteins.

Binding sites for amiloride in intact epithelia

To the Editor: I agree with Blazer-Yost and Helman (3) that selective, high-affinity ligands for epithelial Na^+ channels (ENaCs) are still needed, but urgency is less than it was 25 years ago when to isolate, sequence, and clone the channel was the goal. In their review they found only a single instance in which ENaC binding data corresponded with the biophysical measurements, describing our study on the chicken coprodeum as unique (5). However, they are mistaken in dismissing other studies, as there are serious defects in their arguments, where they appear to have confused single-channel currents (i) at high Na^+ concentrations ($[\text{Na}^+]$) with those at low $[\text{Na}^+]$.

The classical papers by Lindemann and Van Driessche (7, 8), using noise analysis in frog skin, established the characteristics of ENaCs, now confirmed by single-channel recording. Their Fig. 2 (8) shows the linear relation between i and $[\text{Na}^+]$, which gives a value for i of 2×10^{-3} pA at 1 mM $[\text{Na}^+]$. Binding data with amiloride combined with biophysical measurements, carried out at 1 mM $[\text{Na}^+]$ quoted in the review (Table 1 of Ref. 3) gives values for i of $0.3\text{--}0.8 \times 10^{-3}$ pA, hardly the four orders of magnitude difference claimed by the reviewers. The channel density, N , increases as i falls with lowered $[\text{Na}^+]$ (1, 7, 8). Much later, in 1991, Els and Helman (6) reported similar data, showing that $[\text{Na}^+]$ could be reduced without affecting short-circuit current (I_{sc}), due to an increase in N balanced by a reduction in i , but failed to reduce $[\text{Na}^+]$ below the $[\text{Na}^+]$ that reduces I_{sc} to one-half its original value. From noise analysis (7), the number of channels in frog skin epithelium was $1/\mu\text{m}^2$, rising maximally to $50/\mu\text{m}^2$ at zero $[\text{Na}^+]$. In many studies, binding was measured at low $[\text{Na}^+]$ to increase the affinity of the ligand by reducing Na^+ /ligand competition. It is surprising that the reviewers failed to calculate the data from two papers describing benzamil binding in frog skin (1, 2), as they have done for the coprodeum. At near-zero $[\text{Na}^+]$, the binding site density was $130/\mu\text{m}^2$, i.e., less than threefold of the value from noise, but no specific binding was seen at 111 mM $[\text{Na}^+]$. The I_{sc} at 1 mM $[\text{Na}^+]$ was $6.25 \mu\text{A}/\text{cm}^2$ (Fig. 1 of Ref. 2), giving a value for i of 0.5×10^{-3} pA, within fourfold, not four-orders-fold, of the value from noise. Although no specific binding was detectable at high $[\text{Na}^+]$, I_{sc} increased to $28 \mu\text{A}/\text{cm}^2$. This current is supportable by a channel density of $1.5/\mu\text{m}^2$ with an i of 0.2 pA, dimensions

favored by the reviewers but undetectable by binding. Thus the appearance of detectable binding is only found when N is increased by lowering $[Na^+]$. Ligand binding is unlikely ever to give data as definitive as with noise but still may provide useful insights in intact epithelia. As the reviewers point out, we showed, in 1981 (4), that once tissues are disrupted multitudinous binding sites are revealed, with binding properties similar to channels. We termed these acceptor, but not receptor, sites.

REFERENCES

1. Aceves, J., and A. W. Cuthbert. Uptake of [3H]benzamil at different sodium concentrations. Inferences regarding the regulation of sodium permeability. *J. Physiol. (Lond.)* 295: 491–504, 1979.
2. Aceves, J., A. W. Cuthbert, and J. M. Edwardson. Estimation of the density of sodium entry sites in frog skin epithelium from the uptake of [3H]benzamil. *J. Physiol. (Lond.)* 295: 477–490, 1979.
3. Blazer-Yost, B. L., and S. I. Helman. The amiloride-sensitive epithelial Na^+ channel: binding sites and channel densities. *Am. J. Physiol.* 272 (*Cell Physiol.* 41): C761–C769, 1997.
4. Cuthbert, A. W., and J. M. Edwardson. Benzamil binding to kidney cell membranes. *Biochem. Pharmacol.* 30: 1175–1183, 1981.
5. Cuthbert, A. W., J. M. Edwardson, N. Bindselev, and E. Skadhauge. Identification of potential components of the transport mechanism for Na^+ in the hen colon and coprodaeum. *Pflügers Arch.* 392: 347–351, 1982.
6. Els, W. J., and S. I. Helman. Activation of epithelial Na channels by hormonal and autoregulatory mechanisms of action. *J. Gen. Physiol.* 98: 1197–1220, 1991.
7. Lindemann, B., and W. Van Driessche. Sodium-specific membrane channels of frog skin are pores: current fluctuations reveal high turnover. *Science* 195: 292–294, 1977.
8. Van Driessche, W., and B. Lindemann. Concentration dependence of currents through single sodium-selective pores in frog skin. *Nature* 282: 519–520, 1979.

A. W. Cuthbert
Department of Pharmacology
University of Cambridge
Cambridge CB2 1QJ, UK

REPLY

To the Editor: Reduction of apical solution Na^+ concentration ($[Na^+]$) does indeed increase open channel density (N_o) (4, 7, 9) due, in part, to increase of open probability (P_o) (4, 7), which should enhance detection of channels by ligand binding. For comparative purposes between papers cited by Cuthbert (1, 2) and our review (3), it must be recognized that experiments by Van Driessche and Lindemann (9) were done with K^+ -depolarized tissues in which single-channel current (i_{Na}) is reduced substantially (8). Furthermore, no biophysical measurements of i_{Na} exist below 5 mM Na^+ in nondepolarized tissues (4) or below 10.9 mM (6 mM activity) in K^+ -depolarized tissues (9). Linear extrapolation of i_{Na} to 1 mM is at best a rough approximation as $[Na^+]$ approaches its reversal potential difference. Assuming linearity for nondepolarized tissues, 100-fold decreases of $[Na^+]$ lead to 100-fold decreases of i_{Na} at high fractional transcellular resistances that exist at

very low rates of Na^+ entry into the cells. Compared with i_{Na} of 0.6 pA at 111 mM Na^+ (Fig. 1 of Ref. 3), the expected calculated i_{Na} at 1.1 mM $[Na^+]$ is 6×10^{-3} pA.

In one of two papers referred to by Cuthbert and in our review, short-circuit current (I_{sc} ; combined benzamil-sensitive and -insensitive currents) at 1.1 mM Na^+ was reported to be ~ 0.1 of the mean value at 110 mM Na^+ , or 1.87 $\mu A/cm^2$ with specific binding density of 130 sites/ μm^2 (2). Neglecting insensitive currents, maximum i_{Na} is 0.143×10^{-3} pA, which underestimates by 42 times or more the expected value. Alternatively, assuming specific ligand binding yields true open-channel density, I_{sc} is at least 78 $\mu A/cm^2$, far greater than I_{sc} measured at this binding density. Thus we agree that the enormous discrepancy between biochemical and biophysical estimates of channel densities is less at 1.1 mM but not at 111 mM Na^+ . Discrepancies of one to two orders of magnitude in near-maximally autoregulated tissues are an improvement but are no more acceptable.

We believe that we are not confused or mistaken on the primary issues of our review. It must be noted that 1) Lindemann and Van Driessche (6) estimated by extrapolation an upper limit of $N_o = 50/\mu m^2$ at 0 mM Na^+ for K^+ -depolarized tissues (exposed additionally to relatively high concentrations of amiloride required for noise analysis that may also autoregulate N_o). The upper extrapolated limit of N_o for nondepolarized tissues calculated with data from Aceves et al. (2) is $\sim 3.4/\mu m^2$. These upper limits are considerably less than any value of ligand binding at any I_{sc} (3); 2) Van Driessche and Lindemann (9) did not measure or calculate i_{Na} at $[Na^+] < 10.9$ mM, recognizing, no doubt, the uncertainty in doing so; 3) no matter how derived, i_{Na} (and, apparently, upper limits of N_o) of K^+ -depolarized tissues cannot be equated with i_{Na} of nondepolarized tissues as done by Cuthbert; 4) the I_{sc} of 6.25 $\mu A/cm^2$ at 1.1 mM Na^+ cited by Cuthbert is taken from a single experiment (1) and is not typical of a lower mean value (near 1.87 $\mu A/cm^2$) reported by his laboratory (2); and 5) Els and Helman (4) did in fact observe a decrease of I_{sc} at 5 mM Na^+ (nonpaired tissues) accompanied by increases of channel density and P_o . Their goal was not a study of discrepancies in estimation of channel densities.

The important problem remains to know how channel density is regulated at low $[Na^+]$ in terms not only of density but also P_o (4, 7). Changes of P_o further complicate interpretation of ligand binding data and $[Na^+]$ dependence of the macroscopic blocker equilibrium constant (5).

REFERENCES

1. Aceves, J., and A. W. Cuthbert. Uptake of [3H]benzamil at different sodium concentrations. Inferences regarding the regulation of sodium permeability. *J. Physiol. (Lond.)* 295: 491–504, 1979.
2. Aceves, J., A. W. Cuthbert, and J. M. Edwardson. Estimation of the density of sodium entry sites in frog skin epithelium from



- the uptake of [^3H]benzamil. *J. Physiol. (Lond.)* 295: 477–490, 1979.
3. **Blazer-Yost, B. L., and S. I. Helman.** The amiloride-sensitive epithelial Na^+ channel: binding sites and channel densities. *Am. J. Physiol.* 272 (*Cell Physiol.* 41): C761–C769, 1997.
 4. **Els, W. J., and S. I. Helman.** Activation of epithelial Na channels by hormonal and autoregulatory mechanisms of action. *J. Gen. Physiol.* 98: 1197–1220, 1991.
 5. **Helman, S. I., and L. M. Baxendale.** Blocker-related changes of channel density. Analysis of a three-state model for apical Na channels of frog skin. *J. Gen. Physiol.* 95: 647–678, 1990.
 6. **Lindemann, B., and W. Van Driessche.** Sodium-specific membrane channels of frog skin are pores: current fluctuations reveal high turnover. *Science* 195: 292–294, 1977.
 7. **Ling, B. N., and D. C. Eaton.** Effects of luminal Na^+ on single Na^+ channels in A6 cells, a regulatory role for protein kinase C. *Am. J. Physiol.* 256 (*Renal Fluid Electrolyte Physiol.* 25): F1094–F1103, 1989.
 8. **Tang, J., F. J. Abramcheck, W. Van Driessche, and S. I. Helman.** Electrophysiology and noise analysis of K^+ -depolarized epithelia of frog skin. *Am. J. Physiol.* 249 (*Cell Physiol.* 18): C421–C429, 1985.
 9. **Van Driessche, W., and B. Lindemann.** Concentration dependence of currents through single sodium-selective pores in frog skin. *Nature* 282: 519–520, 1979.

Sandy I. Helman
*Department of Molecular and Integrative
Physiology
University of Illinois
at Urbana-Champaign
Urbana, IL 61801*

Bonnie Blazer-Yost
*Department of Biology
Indiana University-Purdue University at
Indianapolis
Indianapolis, IN 46202*

