

Interactions of Sodium Transport, Cell Volume, and Calcium in Frog Urinary Bladder

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ABSTRACT The volume of individual cells in intact frog urinary bladders was determined by quantitative microscopy and changes in volume were used to monitor the movement of solute across the basolateral membrane. When exposed to a serosal hyposmotic solution, the cells swell as expected for an osmometer, but then regulate their volume back to near control in a process that involves the loss of KCl. We show here that volume regulation is abolished by Ba^{++} , which suggests that KCl movements are mediated by conductive channels for both ions. Volume regulation is also inhibited by removing Ca^{++} from the serosal perfusate, which suggests that the channels are activated by this cation. Previously, amiloride was observed to inhibit volume regulation: in this study, amiloride-inhibited, hyposmotically swollen cells lost volume when the Ca^{++} ionophore A23187 was added to Ca^{++} -replete media. We attempted to effect volume changes under isosmotic conditions by suddenly inhibiting Na^+ entry across the apical membrane with amiloride, or Na^+ exit across the basolateral membrane with ouabain. Neither of these Na^+ transport inhibitors produced the expected results. Amiloride, instead of causing a decrease in cell volume, had no effect, and ouabain, instead of causing cell swelling, caused cell shrinkage. However, increasing cell Ca^{++} with A23187, in both the absence and presence of amiloride, caused cells to lose volume, and Ca^{++} -free Ringer's solution (serosal perfusate only) caused ouabain-blocked cells to swell. Finally, again under isosmotic conditions, removal of Na^+ from the serosal perfusate caused a loss of volume from cells exposed to amiloride. These results strongly suggest that intracellular Ca^{++} mediates cell volume regulation by exerting a negative control on apical membrane Na^+ permeability and a positive control on basolateral membrane K^+ permeability. They also are compatible with the existence of a basolateral Na^+/Ca^{++} exchanger.

INTRODUCTION

The current view of transepithelial Na^+ transport by tight, or high-resistance, epithelia (e.g., frog skin, urinary bladder, distal tubule) originated with the model proposed by Koefoed-Johnson and Ussing (1958). A modern version of this

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model, graphically represented in Fig. 1, holds that Na^+ enters the cell by electrodiffusion across the apical membrane and is subsequently actively extruded across the basolateral membrane by the Na/K pump. K^+ that is pumped into the cell is recycled across the basolateral membrane by electrodiffusion since the electromotive force in this, as in other, tight epithelia is primarily determined by K^+ . However, the membrane is not a perfect K^+ electrode, and, in fact, a Cl^- conductance has been demonstrated in several tissues (Lewis et al., 1978; Wills et al., 1979; Thompson et al., 1982; Schultz et al., 1984; Demarest and Finn, 1987). Also incorporated into the model are features by which coordination of transport activities at the two membranes is achieved (see reviews by Taylor and

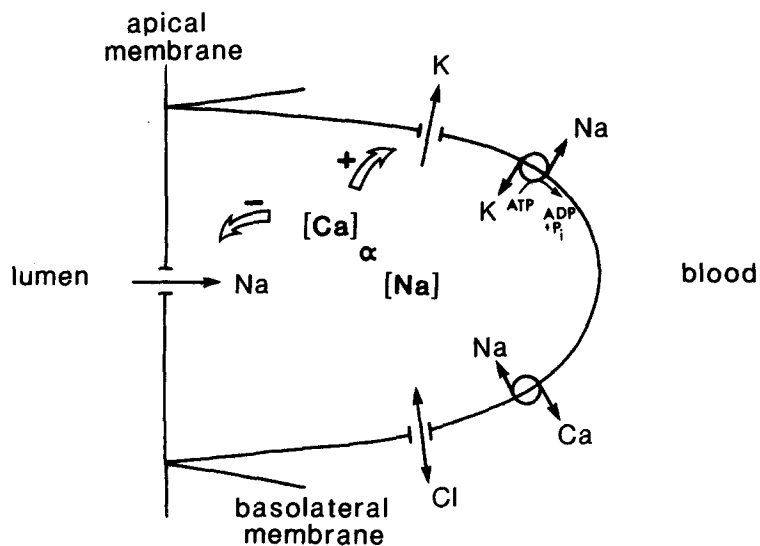


FIGURE 1. Modified Koefoed-Johnson-Ussing model for Na^+ transport across frog urinary bladder epithelium. Central to the model is the coupling of Ca^{++} and Na^+ by the Na/Ca exchanger, which causes cellular Ca^{++} activity to vary as a result of changes in cellular Na^+ activity.

Windhager, 1979; Schultz, 1981; Diamond, 1982; Chase, 1985; Davis and Finn, 1985a). Such coordination is necessary because the transporting cell must maintain a constant solute content and volume while engaged in transepithelial transport activity, an activity that involves a net flow of solute through its interior. As has been suggested by others (see Windhager and Taylor, 1983; Chase, 1985), a central role in the intermembrane coordination has been assigned to Ca^{++} . The evidence for a negative feedback relationship between intracellular Na^+ and apical Na^+ conductance and for the role of Ca^{++} in the feedback loop is compelling and has been recently reviewed by Chase (1985; cf. Lewis and Wills, 1983). The evidence for a role of Ca^{++} in the regulation of basolateral membrane permeability, on the other hand, is somewhat less compelling. Finally, there is some evidence that regulation of cell Ca^{++} activity is at least partly due to an Na/Ca exchange at the basolateral membrane (Grinstein and Elij, 1978; Chase and Al-Awqati, 1981; Friedman et al., 1981; Lorenzen et al., 1984; Palmer, 1985).

Many animal cells regulate their volume after osmotic swelling. The cells reach osmotic equilibrium after exposure to a hyposmotic medium and effect the volume loss with a dissipation of solute gradients (see Macknight and Leaf, 1977). We have previously shown (Davis and Finn, 1981, 1982*b*) that in frog urinary bladder, cell volume regulation is driven by the loss of KCl across the basolateral membrane. In this article, we present evidence that intracellular Ca^{++} levels control basolateral membrane solute movements both in the presence and absence of transport inhibitors.

MATERIALS AND METHODS

Solutions

The Ringer's solution had the following composition (mM): 109 NaCl, 2.5 KCl, 2.4 NaHCO_3 , 0.9 CaCl_2 . It was gassed with room air and had a pH of ~ 8.4 . In Ca^{++} -free solutions, no chelator was added. Hyposmotic Ringer's solution had one-third of the NaCl removed, which resulted in a difference in osmolality of 64 mosmol from control. In Na^+ -free solutions, all Na^+ was replaced with *N*-methyl-*D*-glucamine (NMDG; Aldrich Chemical Co., Milwaukee, WI). NMDG- HCO_3 was produced by bubbling a solution of the free base with CO_2 to a constant pH. Amiloride was a gift of Merck, Sharp & Dohme (West Point, PA) and ouabain was purchased from Sigma Chemical Co. (St. Louis, MO). Both agents were dissolved directly in Ringer's solution at a final concentration of 10^{-4} M. The Ca^{++} ionophore A23187, from Calbiochem-Behring Corp. (La Jolla, CA), was first dissolved in dimethylsulfoxide and then added to a Ringer's solution to a final concentration of 10^{-6} M.

Methods

Urinary bladders were removed from doubly pithed frogs (*Rana catesbeiana*), and a portion underlying the abdominal mesenteries from which the serosa was easily detached was separated from the rest of the tissue. After dissection of adherent smooth muscle bundles, the tissue was mounted in a miniature Ussing chamber designed for light microscopy (Spring and Hope, 1978). The method of determining cell volume has been described previously (Davis and Finn, 1981, 1982*a*, 1985*b*). Briefly, the tissue is viewed at high magnification with a Zeiss Universal microscope equipped with differential interference contrast (Nomarski) optics. The image is monitored with a video camera and the resulting video information is stored on video tape. An individual granular cell, chosen primarily for the clarity of its borders, is optically sectioned repeatedly before and after a change in solution. The sectioning process, a "volume scan," is under computer control and takes ~ 2 s. The video images of the resulting optical sections of the cell are later retrieved and the cross-sectional area of the cell in each of the optical sections is measured by computer-aided planimetry. The cell volume is finally calculated from these areas and the known distance between sections ($1 \mu\text{m}$).

In each experiment, the tissue was equilibrated in the chamber with Ringer's solution perfusing both sides for 30–40 min. Three control volume scans were then executed at 0.5-min intervals, the mucosal and/or serosal perfusate was switched to the experimental solution, and volume scans were executed thereafter at intervals appropriate to the experiment. After completion of the experiment, the data were analyzed in a "single-blind fashion": a single person analyzed the images from the entire experiment in a single session and in such a fashion that the results were unknown to him until the analyses were complete. The cell volume data are presented either as percent control, with the initial control volume taken as 100%, or as percent peak volume, where the maximum volume

determined after osmotically induced swelling is taken as 100%. The solid lines shown in the figures were fitted by eye. We have previously determined the mean volume of frog urinary bladder cells to be $\sim 2,100 \mu\text{m}^3$ (Davis and Finn, 1982*b*). The cell volume data are presented as the means \pm SE of three to six cells vs. time. Where appropriate, the mean rate of cell shrinkage or swelling was determined from the individual rates determined by regression analysis over a period specified in the text.

RESULTS

After osmotic swelling caused by exposure to a hyposmotic solution, the cells of frog urinary bladder epithelium spontaneously undergo a decrease in volume to levels close to control through a loss of fluid. Previous results (Davis and Finn, 1982*b*) support the notion that this loss of volume is due to an efflux of KCl across the basolateral membrane, and that it is dependent upon a functional transepithelial Na^+ transport system; i.e., that volume regulation is blocked by amiloride (mucosal exposure). In the first section of the Results, the nature and the control of this cell volume-regulatory system are explored further. In the second and third sections, the cell volume control mechanisms that function under isosmotic conditions are examined.

Anisosmotic Volume Control

Cell volume regulation (after cell swelling) was found to depend on the presence of Ca^{++} in the serosal medium. Fig. 2 depicts the results of experiments in which bladders were equilibrated in Ca^{++} -free isosmotic Ringer's solution serosal perfusate (with no chelator) and then exposed to Ca^{++} -free hyposmotic Ringer's solution. In three isosmotic control experiments, Ca^{++} removal alone had no effect on cell volume. As shown in the figure, the exposure of cells to an osmotic gradient resulted in a volume increase of 32%. This degree of swelling was the same as that determined previously for cells exposed to a similar gradient in the presence of Ca^{++} (Davis and Finn, 1982*b*). Unlike the previous result, however, the cells did not undergo volume regulation after osmotic equilibration; that is, Ca^{++} removal inhibited the process. In experiments in which Ca^{++} was not returned to the hyposmotic Ringer's solution, cell volume was unchanged from peak volume for up to 20 min, as shown in an example in the inset to Fig. 2. In the main experiment shown in Fig. 2, Ca^{++} was restored to the hyposmotic solution after a 5-min exposure, after which the volume of the cells spontaneously declined to near control levels at an initial rate of $3.8 \pm 0.7\%$ original volume/min. The presence of Ca^{++} in the medium is therefore necessary for cell volume regulation to occur. Since amiloride inhibits cell volume regulation in Ca^{++} -replete media (Davis and Finn, 1982*b*), we conclude that medium Ca^{++} is acting as a source for intracellular Ca^{++} rather than exerting control from the external aspect of the cell.

Since both Ca^{++} removal from the serosal perfusate and amiloride addition to the mucosal perfusate (Davis and Finn, 1982*b*) inhibit cell volume regulation in frog urinary bladder, we investigated the possibility that Ca^{++} acts as an intermediary in the inhibition by amiloride. Should the exposure of the apical membrane to amiloride cause a decrease in intracellular Ca^{++} , then inducing an increase in intracellular levels of the ion would be expected to restore volume

regulation. This maneuver was achieved through the serosal application of the ionophore A23187. To control for other effects of the ionophore (e.g., release of internal Ca^{++} stores), we added the drug to the serosal perfusate of tissues previously exposed first to Ca^{++} -free and then to Ca^{++} -free hyposmotic Ringer's solution. In three such experiments, the addition of the ionophore did not affect cell volume (the volume 10 min after addition was $100 \pm 1.8\%$ of the peak volume recorded after hyposmotic exposure). Thus, in the absence of Ca^{++} in the serosal perfusate, A23187 has no effect on the volume of osmotically swollen cells. Fig. 3 illustrates the action of the ionophore on cells exposed to both

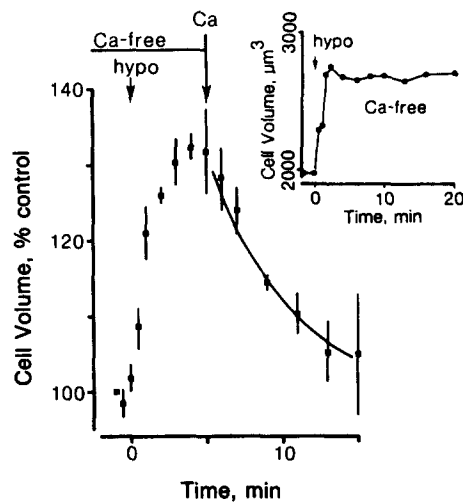


FIGURE 2. Effects of a Ca^{++} -free hyposmotic exposure on the volume of frog urinary bladder epithelial cells. Three tissues were pretreated for 15 min in a nominally Ca^{++} -free, isosmotic, serosal perfusate. At the first arrow, the osmolality of the serosal perfusate was reduced by removing one-third of the NaCl (-64 mosmol). At the second arrow, the hyposmotic serosal perfusate was switched to one containing normal amounts of Ca^{++} . The results are expressed as a percent of control with the initial control point taken as 100%, and they are presented as the means \pm SE. (Inset) Results of an individual experiment in which Ca^{++} was removed from, but not returned to, the serosal perfusate.

amiloride and hyposmotic solution in Ca^{++} -replete media. The tissues were first equilibrated with amiloride in the mucosal perfusate for 15 min (which, as demonstrated below, does not affect cell volume), and then with hyposmotic Ringer's solution (Ca^{++} -replete). As observed previously (Davis and Finn, 1982b), the cells swelled but did not undergo cell volume regulation. A23187 was added to the hyposmotic serosal perfusate after a 5-min period, and, as the figure shows, the cells then lost volume at an initial rate of $2.6 \pm 0.6\%/min$.

To determine whether loss of cell solute during volume regulation depends on an increase in membrane conductance, we tested the effect of Ba^{++} on this process. In four experiments, we exposed the basolateral surfaces of frog urinary bladders to hyposmotic Ringer's solution containing 1 mM Ba^{++} and followed

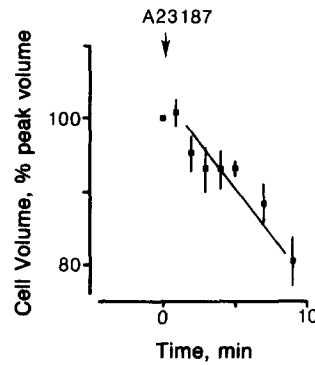


FIGURE 3. Effects of the Ca^{++} ionophore A23187 ($1 \mu\text{M}$) on cell volume in tissues exposed to amiloride and a Ca^{++} -replete, hyposmotic serosal perfusate. Three tissues were pretreated for 15 min in an amiloride-containing (0.1 mM) mucosal perfusate and then for 5 min in a hyposmotic serosal perfusate. Ionophore ($1 \mu\text{M}$) was then added to the serosal perfusate. The results are expressed as a percent of the peak volume achieved after hyposmotic exposure and begin at the time of ionophore addition; the cells were therefore swollen by $\sim 32\%$ at the beginning of the experiment.

the time course of the change in cell volume for 10 min (Fig. 4). Under these conditions, volume regulation was abolished: the cell volume 10 min after exposure (4–5 min after osmotic equilibration) was $129.8 \pm 4.9\%$ of the isosmotic cell volume, a value not different from that normally seen at the peak volume achieved after a hyposmotic exposure (e.g., see Fig. 2 and Davis and Finn, 1982*b*). This result strongly suggests that the KCl -driven volume loss is mediated by conductive pathways for K^{+} .

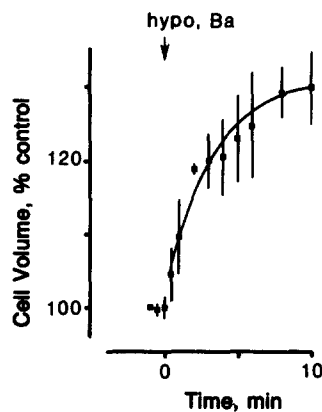


FIGURE 4. Effects of Ba^{++} on cell volume regulation in tissues exposed to a hyposmotic serosal perfusate. Four tissues, pre-equilibrated in Ringer's, were exposed to a hyposmotic perfusate containing Ba^{++} (1 mM) and cell volume was monitored for 10 min.

Isosmotic Volume Control

In this section, we address factors that control cell volume under isosmotic rather than hyposmotic conditions. As a first approach to this problem, inhibitors of transepithelial Na^+ transport were used to study the response of the cell to a sudden cessation of either Na^+ entry across the apical membrane (inhibition by amiloride) or Na^+ exit across the basolateral membrane (inhibition by ouabain). As described below (see Discussion), one would expect the cells to lose volume after amiloride addition and to swell after ouabain. As shown here, however, neither of these expected results occurred.

Amiloride, added to the mucosal perfusate, had no effect on cell volume under isosmotic conditions. In six cells, cell volume was unchanged after amiloride

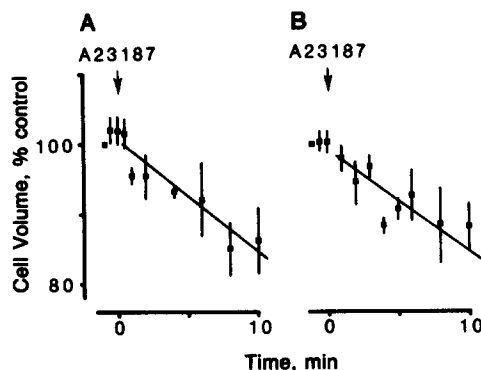


FIGURE 5. Effects of Ca^{++} ionophore on cell volume under isosmotic conditions. In both experiments depicted, A23187 (10^{-6} M) was added to the serosal, isosmotic perfusate at the time indicated by the arrow. In the control experiment (A, four tissues), the mucosal perfusate was Ringer's alone, whereas in the other (B, three tissues), the mucosal perfusate contained amiloride (10^{-4} M) for 15 min before the addition of the ionophore.

exposure over the duration of the experiment: 10 min after amiloride, cell volume was $99.4 \pm 2.3\%$ of control (the result of an individual experiment was presented previously; Davis and Finn, 1985a). This result is consistent with the notion that blockade of apical membrane Na^+ entry causes a decrease in basolateral membrane KCl permeability. To test whether increasing intracellular Ca^{++} in amiloride-blocked cells would cause a loss of volume under isosmotic conditions, Ca^{++} ionophore was added to the serosal perfusate of three tissues that had been pretreated with amiloride for 15 min. As shown in Fig. 5B, the addition of A23187 resulted in a volume loss, at an initial rate determined between 0 and 5 min of $2.0 \pm 0.1\%/min$. A similar loss of volume was observed, however, if ionophore was added to the serosal perfusate in the absence of amiloride. In this experiment (Fig. 5A), the initial rate of volume loss as determined between 0 and 6 min was $2.0 \pm 0.4\%/min$; cell volume was monitored for 20 min and an essentially linear loss of volume was observed over the full period (cell volume at 20 min was $76.1 \pm 5.7\%$ of control).

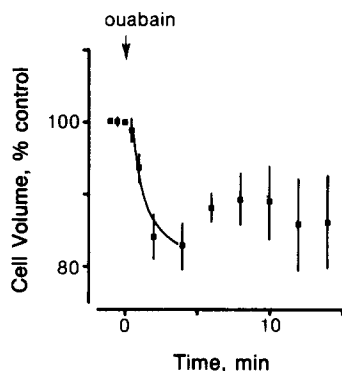


FIGURE 6. Effects of ouabain on cell volume. Five tissues were equilibrated under isosmotic conditions and then ouabain (0.1 mM) was added to the serosal perfusate.

Ouabain, applied to the serosal perfusate, caused a rapid loss of cell volume (Fig. 6). (In an earlier article [Davis and Finn, 1981], in which we were concerned with its effects on cell volume regulation, ouabain was stated to have variable effects on cell volume under isosmotic conditions. In an individual experiment, shown graphically, ouabain had no discernible effect under isosmotic control conditions because of a large variability. Collective examination of those results showed them to be consistent with the results in the current study: 20 min after ouabain, cell volume was $85.1 \pm 3.1\%$ of control in five cells from three tissues.) In five cells, the initial rate of loss, determined between 0.5 and 2 min after ouabain, was $9.8 \pm 2.8\%/min$. This highly significant rate is approximately one-half that of $18.6\%/min$ determined for cells undergoing volume regulation (Davis and Finn, 1985a). A minimum volume of $\sim 83\%$ of control was determined at 4 min; the points between 12 and 16 min after ouabain, inclusive, are only marginally different ($p < 0.1$) from control, which indicates that the volume of the cells may tend to increase secondarily. The initial loss of volume from cells exposed to ouabain indicates that K^+ leaves the cell across the basolateral membrane at a higher rate than Na^+ enters across the apical membrane. One possible explanation of these results is that intracellular Ca^{++} increases after ouabain because of its effect of increasing intracellular Na^+ . Consequently, we

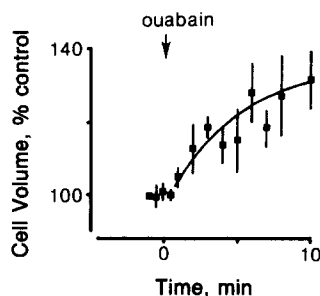


FIGURE 7. Effects of ouabain on cell volume under Ca^{++} -free conditions. Four tissues were pretreated with a Ca^{++} -free serosal perfusate for 15 min and then ouabain was added.

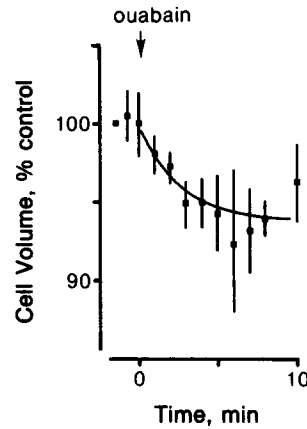


FIGURE 8. Effects of ouabain on cell volume under the conditions of amiloride pre-exposure, under Ca^{++} -free conditions. Four tissues were pretreated in amiloride-containing mucosal, and Ca^{++} -free serosal, perfusates for 15 min and then ouabain was added to the serosal perfusate.

pretreated tissues with Ca^{++} -free serosal perfusate for 15 min before ouabain addition. Under these conditions, ouabain caused cell swelling rather than shrinkage, as shown in Fig. 7. The initial rate of increase, determined between 0.5 and 3 min, was $7.4 \pm 1.6\%/min$ and steady state volume was 32% above control. Thus, only in the absence of Ca^{++} does ouabain have its expected effect of causing cell swelling.

We next tested the effect of ouabain in a situation where both apical Na^+ entry (amiloride) and basolateral Ca^{++} entry (Ca^{++} -free serosal perfusate) were diminished. As shown in Fig. 8, ouabain exposure under these conditions not only resulted in an inhibition of cell swelling (cf. Fig. 7), but may have caused a small loss of volume. The initial rate of decrease determined between 0 and 3 min was

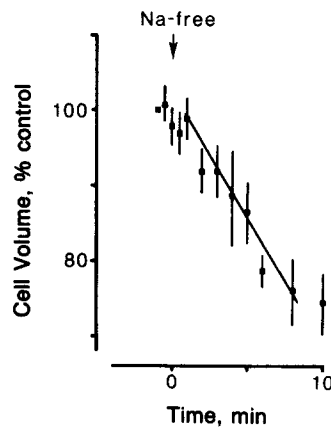


FIGURE 9. Effects of Na^+ removal on cell volume of cells exposed to amiloride. Three tissues were pretreated with amiloride-containing mucosal perfusate for 15 min and then all the Na^+ in the serosal perfusate was replaced with NMDG.

$1.6 \pm 0.9\%/min$, and the final volume was 6% below control. These results are therefore consistent with the notion that ouabain-induced cell swelling under Ca^{++} -free conditions is due to a higher rate of apical membrane Na^+ entry than basolateral membrane K^+ exit.

Basolateral Membrane Na/Ca Exchange

The results presented above are in agreement with the model shown in Fig. 1, which holds that the activity of intracellular Ca^{++} varies with that of intracellular Na^+ . In the final experiment, we investigated a second tenet of the model, which links the intracellular activities of Na^+ and Ca^{++} through an Na/Ca exchanger located in the basolateral membrane. Tissues were pretreated with amiloride to block apical membrane Na^+ movements and thereby simplify analysis of the experimental results. After 15 min of amiloride exposure, all the Na^+ in the serosal perfusate was replaced with NMDG. As shown in Fig. 9, this treatment resulted in a loss of volume. The initial rate of decrease, determined between 1 and 6 min, was $3.5 \pm 0.9\%/min$ ($n = 3$), and the final steady state volume was 74% of control (the cell volume at 20 min was not different from that determined at 10 min).

DISCUSSION

In the following discussion, we consider the possible mechanisms of solute movement (as inferred from changes in cell volume) across the basolateral membrane, the presumed changes in intracellular Ca^{++} activity and the effects of those changes, and the control of cell volume.

Basolateral Membrane Solute Transport and Intracellular Ca^{++}

We have used changes in cell volume to indicate net movements of solute across, primarily, the basolateral membrane of frog urinary bladder epithelial cells. As we have previously shown (Davis and Finn, 1982*b*), this solute is KCl, and it is likely that these K^+ movements are mediated by conductive channels. There are two lines of evidence supporting this notion. First, blockade of apical membrane Na^+ entry with amiloride is associated with decreases in basolateral membrane conductance (Davis and Finn, 1982*a*) and KCl permeability (Davis and Finn, 1982*b*). (In one of these references [Davis and Finn, 1982*b*], we showed that the urinary bladder of the frog has bioelectric properties similar to that of the toad. Both have positive-going stairstep potential profiles in the mucosal-to-serosal direction, and a transepithelial potential and short circuit that decline to 0 mV upon exposure to amiloride. The ratio of resistances is also unchanged for several minutes after amiloride, which shows that basolateral membrane resistance initially increases with the same time course as that of the apical membrane.) Second, Ba^{++} , which blocks K^+ conductance in a wide variety of tissues, including epithelia (Kirk et al., 1980; Latorre and Miller, 1983; Wills, 1985; Demarest and Finn, 1987), was found in the present study to block volume regulation. In keeping with this notion, Lewis et al. (1985) have recently identified apparent volume-sensitive changes in basolateral membrane electromotive force and conductance in toad urinary bladder.

The results of the current study are also consistent with the ionic pathway being sensitive to intracellular Ca^{++} , since maneuvers that can be expected to decrease or increase cell Ca^{++} caused corresponding changes in basolateral membrane permeability, as judged by changes in the rate of transmembrane water and solute movement. The simple maneuver of removing Ca^{++} from the serosal perfusate (with no added chelator) caused a decrease in the rate of solute movement across the basolateral membrane in two different experimental situations: cell volume regulation after hyposmotic swelling was blocked (Fig. 2), and cell swelling instead of shrinkage was observed after ouabain (compare Figs. 6 and 7). Observations similar to the former result have been made by others studying cell volume regulation (Grinstein et al., 1982; Cala, 1983). Experimentally increasing intracellular Ca^{++} , on the other hand, resulted in increased basolateral membrane solute movements in four different situations. In two of these, amiloride was present in the mucosal perfusate and, because it blocks Na^+ movements across the membrane, any change in cell volume in this circumstance can be ascribed to solute movements across the basolateral membrane alone. A loss of volume was observed after treatment by Ca^{++} ionophore (with and without amiloride) in cells under isosmotic conditions (Fig. 5), and in cells swollen by serosal exposure to hyposmotic Ringer's solution in which amiloride blocked volume regulation (Fig. 3). In the final case, the return of Ca^{++} to a hyposmotic medium restored the ability of the cells to undergo volume regulation (Fig. 2). Our data are therefore consistent with the idea that increases in intracellular Ca^{++} cause a loss of intracellular solute, whereas decreases inhibit this loss. As noted above, this solute must be predominantly or solely KCl.

The results of other experiments, in which we neither removed Ca^{++} nor added ionophore, are also consistent with the notion that intracellular Ca^{++} varies in concert with Na^+ . In addition to the dependence of the change in cell volume upon Ca^{++} availability after ouabain addition (Figs. 6 and 7), amiloride had no effect on cell volume. Hence, the observed changes in basolateral membrane permeability correlate with the changes in Na^+ activity that have been repeatedly shown to occur in epithelia after exposure to ouabain and amiloride (e.g., see Harvey and Kernan, 1984). The activities of Na^+ and Ca^{++} therefore appear to be linked. There is a growing body of evidence in epithelia, in general, supporting this linkage (see Chase, 1985), and, as noted above, direct evidence for Na/Ca exchange in epithelial basolateral membranes has been indicated in studies by Grinstein and Erlj (1978), Lorenzen et al. (1984), and Chase and Al-Awqati (1981). Our observation that cell shrinkage in frog urinary bladder follows Na^+ removal from the serosal perfusate (Fig. 9) is consistent with such a mechanism. In this experimental situation, the presence of amiloride in the mucosal perfusate assured that any gain or loss of cell volume resulted from the net movement of solute (KCl) across the basolateral membrane alone. Because removal of Na^+ would be expected to provide an increased outward driving force for Na^+ via the Na/Ca exchanger, cell Ca^{++} would be expected to rise as a consequence of its net inward movement on the exchanger. Consequently, the loss of cell volume observed upon the substitution of NMDG for Na^+ probably resulted from this increase in cell Ca^{++} as mediated by Ca^{++} -activated pathways for KCl. We

therefore conclude that the changes in intracellular Ca^{++} that presumably occurred in this study were caused, at least in part, by the activity of an Na/Ca exchanger. Recent observations by Palmer (1985) on the modulation of apical Na^+ pathways by manipulation of Ca^{++} concentrations support this contention.

Control of Cell Volume

As we have previously shown (Davis and Finn, 1981, 1982*b*) and further investigated in the current study, frog urinary bladder cells, like many others (see Macknight and Leaf, 1977), undergo a volume-regulatory process after osmotically induced swelling. Amiloride in the mucosal perfusate and Ba^{++} -containing and Ca^{++} -free serosal solutions block this process, which suggests that the volume loss is due to solute movement through conductive K^+ channels. If, under isosmotic control conditions, the basolateral membrane has a finite, but limiting, Cl^- conductance such that K^+ is maintained above electrochemical equilibrium, then during volume regulation this anion pathway must be activated to allow net movement of KCl. Such a change in Cl^- conductance has been demonstrated in human lymphocytes and hamster ovary cells (Grinstein et al., 1983; Sarkadi et al., 1984). The activation of a Cl^- conductance does not preclude increases in K^+ conductance as well, and in fact, such increases have been demonstrated in lymphocytes undergoing volume regulation (Grinstein et al., 1982). Thus, it is likely that cell volume regulation in frog urinary bladder is associated with increases in the conductance of the basolateral membrane to both Cl^- and K^+ , a conclusion that is supported by a recent study of Lewis et al. (1985). It should be stressed, however, that a complete analysis of the problem of cell volume regulation in this epithelium must await the determination of the ionic driving forces that exist during the net solute flux that drives the regulatory process. Because all existing electrophysiological data have been collected under isosmotic conditions, we cannot be absolutely certain whether solute movements are due to changes in permeability or in driving forces.

One important conclusion to be drawn from this study is that the epithelial cells also regulate basolateral membrane permeability under isosmotic conditions, presumably to maintain volume or K^+ homeostasis during changes in transepithelial Na^+ transport. In the steady state, the cells maintain a constant volume by balancing the flows of solute across the apical and basolateral membranes. Direct evidence for this cellular function is provided by the results of the experiments in which amiloride was used to block apical membrane Na^+ entry, and in which ouabain was used to block the Na/K pump. In the case of apical membrane Na^+ entry blockade, and in the absence of a compensatory response, one would expect the cell to lose volume, first, because of a loss of cell Na^+ owing to continued Na/K pump activity, and second, because of a net K^+ loss that would result from an eventual decrease in pump activity (e.g., see Cox and Helman, 1983). In most high-resistance epithelia, amiloride leads to a hyperpolarization of the basolateral membrane potential, and hence to a decrease in the driving force for K^+ . In contrast, in frog and toad urinary bladder, the driving force for the passive K^+ loss is increased after amiloride because the basolateral membrane potential is depolarized rather than hyperpolarized (see Davis and

Finn, 1982a). Irrespective of the direction of the change in the basolateral membrane potential, however, a driving force for K^+ exit generally exists across the basolateral membrane of all high-resistance epithelia after amiloride because E_K is significantly higher than the post-amiloride steady state potential. Thus, to the extent that Na^+ entry is blocked after amiloride addition, Cl^- would be obligated to leave the cell with K^+ to achieve electroneutrality, and the cell would be expected to suffer a net loss of KCl and water. As we showed, however, in frog urinary bladder, where the forces favor an elevated rather than a diminished KCl loss, amiloride had no effect on cell volume. Therefore, the cells of the epithelium regulate their volume after amiloride exposure by reducing the permeability of the basolateral membrane such that the expected net solute movement is blocked.

In the case of blockade of the Na/K pump by ouabain and in the absence of a compensatory response, one would expect the cell to swell. In the steady state, with the Na/K pump operating with a 3 Na/2 K ratio (Nielsen, 1979; Kirk et al., 1980), 3 equivalents of Na^+ must enter the transporting cells via the apical conductive entry pathway for every 2 equivalents of K^+ that exit via conductive pathways across the basolateral membrane (see Fig. 1). The difference in charge, 1 equivalent, is carried by the Na/K pump, as postulated by several workers (Nielsen, 1979; Kirk et al., 1980; Davis and Finn, 1982a). Thus, more solute enters the cell across the apical membrane through conductive pathways than leaves through conductive pathways across the basolateral membrane. Consequently, upon Na/K pump inhibition, the cell should swell, owing to the accumulation of NaCl and osmotically obligated water. Sufficient Cl^- would enter in this situation to compensate electrically for the charge transfer that is normally associated with the pump. The expected result with ouabain inhibition, however, was not realized; rather, the cells rapidly lost volume when ouabain was added (Fig. 6). Therefore, basolateral membrane permeability is increased, secondarily, after ouabain exposure, such that the cell suffers a net loss of solute and volume.

In each of the situations above, the steady state was upset by selectively blocking a single pathway for solute movement, and in each case it would be possible to explain the observed change in cell volume by postulating modifications in basolateral membrane permeability only. When cell Na^+ activity was decreased by exposure to amiloride, cell volume was unchanged because of a decreased basolateral membrane permeability, and when cell Na^+ activity was increased by exposure to ouabain, the cells shrank because of an increased permeability. The results expected in the absence of changes in basolateral membrane permeability were realized only when intracellular Ca^{++} activity was experimentally increased or decreased, respectively. As discussed above, these latter results can be explained by Na/Ca exchange-mediated changes in cell Ca^{++} activity. To account for other results, however, it is also necessary to postulate changes in apical membrane permeability. One situation stands out in this regard: as shown in Fig. 5A, the addition of A23187 under isosmotic conditions caused a loss of volume that proceeded at the same rate as the volume loss observed in tissues that were pretreated with amiloride (Fig. 5B). One obvious conclusion from this experiment is that inundation of cells with Ca^{++} by the serosal addition of A23187 is

as effective in blocking apical Na^+ permeability as amiloride added to the mucosal perfusate. These data consequently support the notion of intracellular Ca^{++} acting to effect a negative feedback loop on apical membrane Na^+ entry (see Chase, 1985).

In conclusion, we have shown, using simple experimental manipulations to change its intracellular activity, that Ca^{++} exerts powerful control over the maintenance of the cell volume of frog urinary bladder under both isosmotic and hyposmotic conditions, probably by exerting positive control over the permeability of the basolateral membrane and negative control over that of the apical membrane.

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