Pflügers Arch (1989) 414:393–400

Pflügers Archiv
European Journal of Physiology

Mechanism of active K^+ secretion by flounder urinary bladder

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Abstract. We investigated the mechanism of active K^+ transport by the urinary bladder of the winter flounder by measuring transepithelial properties in Ussing Chambers and by determining the cellular electrical potential profile using conventional microelectrodes. In the absence of transmural electrochemical potential gradients isolated bladders can exhibit a serosa-to-mucosa short circuit which is due entirely to net K^+ secretion. The properties of transcellular K^+ movement can be adequately described by a model which provides for active K^+ uptake across the basolateral membrane via an electrogenic Na/K ATPase and K^+ exit from the cell across the apical membrane down an electrochemical potential gradient via K^+ channels which are blocked by mucosal barium. The conductance of the apical membranes of the transporting cells appears to be due almost solely to K^+ while that of the basolateral membrane may be due largely to Cl^-.

Key words: Potassium secretion – Urinary bladder – Active transport

Introduction

Renfro [15, 16] studied ion and water transport by the urinary bladder of the winter flounder using isolated sacs and a perfused bladder preparation. These studies showed that the epithelium actively absorbed Na^+ and Cl^- and suggested that the salt absorption process was electrically silent. The isolated bladder exhibited a moderately high electrical resistance ($1{,}000\,\Omega \cdot cm^2$) and a small (4–5 mV) transepithelial potential difference (P.D.) oriented mucosal-side positive. Ouabain abolished both NaCl absorption and the transepithelial P.D. but the mechanistic relation between salt transport and the electrical potential was not established.

We report here studies of ion transport by isolated portions of flounder bladder mounted as flat sheets so that the transepithelial ion fluxes and electrical properties could be more conveniently determined. In addition we measured the intracellular electrical potential profile during ion replacement. The results are consistent with a model for cellular transport for the bladder in which a mucosa-positive transepithelial P.D. is due solely to active K^+ secretion which is linked to a NaCl absorption via a basolateral Na/K ATPase. The bladder exhibits the unique property of generating a short circuit current which is due entirely to net K^+ secretion so that the tissue may provide a valuable model for the study of this process. These experiments were carried out during various months in the summers from 1979 to 1984 at the Mount Desert Island Biological Laboratory, Salsbury Cove, Maine, and some of the results have been reported in preliminary form [1–3].

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Materials and methods

Transmural studies. Urinary bladders were removed from flounder which had been maintained for several days to 1 week in flowing sea water. The bladders were opened longitudinally and pinned out, mucosal side up, on a soft plastic sheet. A portion of the tissue was then gently compressed between two concentric plastic rings which were designed to "snap" together and thus secure the edges of the tissue. Depending on the size of the flounder, from one to four 1.25 cm^2 pieces could be obtained in this way. The tissue, secured by the plastic rings, was mounted in an Ussing chamber and bathed on both sides by solutions containing (mMol/l): Na^+ 147.5, Cl^- 147.5, K^+ 2.5, Ca^+ 1.5, Mg^{2+} 1.0, HEPES 15.0, glucose 5.0. Both sides were vigorously stirred with air, the pH being approximately 7.5. All experiments were conducted at room temperature which averaged about 20° C.

The chamber was equipped with four electrodes; two for monitoring the P.D. and two for passing current across the tissue. An electronic voltage clamp maintained the transmural P.D. at 0mV and the tissue conductance (G_T) was determined from the change in transepithelial current produced by a brief (500 ms), 10 mV change in clamping potential. Preliminary experiments revealed that the conductance of the bladder could increase spontaneously, sometimes by as much as a factor of five. It appeared that these changes were the result of smooth muscle contractions. Increases in G_T often followed a pulse of current (conductance determination) and were elicited promptly by the addition of 10^{-6} M carbamyl choline to the serosal bathing solution. Spontaneous and carbamyl choline induced increases in G_T were abolished by the addition of 10^{-5} M verapamil to the serosal bathing solution. Preliminary experiments suggested that this concentration had no effect on net NaCl absorption or short circuit current (I_{sc}), thus, in all experiments reported below verapamil was present in the serosal bath.

Transmural fluxes of ^{22}Na or ^{36}Cl were measured simultaneously; mucosa to serosa or serosa to mucosa, on paired

Table 1. Transmural fluxes and electrical parameters under control conditions

	J_{Na+} (μEq/cm² · h)	J_{Cl-} (μEq/cm² · h)	I_{sc} (μA/cm²)	G_T (mS/cm²)
M to S	3.91 ± 0.60	4.51 ± 0.65	6.76 ± 0.51	0.69 ± 0.22
S to M	0.47 ± 0.08	1.22 ± 0.19	6.05 ± 0.31	0.53 ± 0.09
net or avg.	3.44	3.29	6.41	0.61

Transmural fluxes of Na^+ and Cl^- in the presence of symmetrical NaCl Ringer's. Values for J_{Na+} and J_{Cl-} are mean ± SE for 11 pairs of bladders. The value for each bladder was the average of at least three successive, 30 min flux periods. Since I_{sc} and G_T did not vary greatly between the m to s and s to m groups the values were averaged for economy. Positive current is in the s to m direction

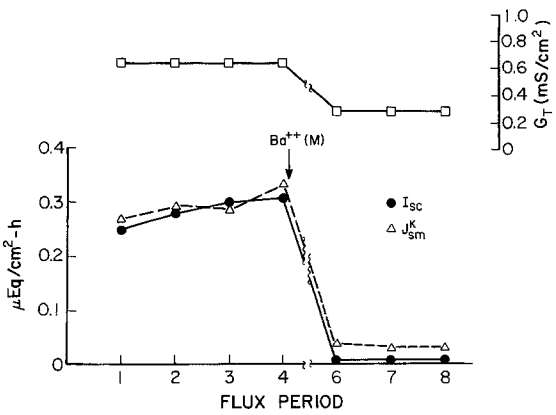

Fig. 1. Plot of values of I_{sc}, J^K_{sm} and G_T versus flux period from a representative experiment. Values for I_{sc} and G_T represent the average for each 30 min flux period. Barium (2 mMol/l, mucosal) was added immediately following the fourth flux period and markedly reduced I_{sc}, J^K_{sm}, and G_T

tissues. In all experiments transmural "control fluxes" were measured for at least three half-hour periods prior to any experimental maneuver, and then for at least four additional half-hour periods following the addition of a blocker or inhibitor. The period immediately following the addition was excluded from the data analysis due to the possibility of non-steady tracer flow. Although we did not routinely control for time dependent effects, preliminary experiments indicated that I_{sc} and G_T were stable at room temperature for 6 h. Fluxes of ^{22}Na and ^{36}Cl were separated by counting each sample twice, once in a liquid scintillation spectrometer and then in a gamma spectrometer as previously described [4]. Transmural ^{42}K fluxes were measured in an identical fashion except that the counts were corrected for decay.

Measurement of intracellular potential. Bladder segments were mounted horizontally (mucosal surface up) in a chamber with an aperture of 0.13 cm² and impaled from above with conventional glass microelectrodes. The procedures employed for measurements of apical (V_a), basolateral (V_b) and transepithelial (V_t) potentials and the fractional apical membrane resistance (f^R_a) have been described in detail [8, 9]. As for the transepithelial studies, verapamil was included in the serosal solution to block spontaneous tissue movement.

Results

I_{sc} and NaCl absorption

Values for simultaneously measured unidirectional fluxes of Na^+ and Cl^- under control conditions are grouped together in Table 1. Qualitatively the results from these experiments on flat sheets of bladder are consistent with those obtained by Renfro [15, 16] in the perfused bladder preparation; namely equimolar net absorption of Na^+ and Cl^- and a small but non-zero I_{sc} consistent with positive charge flow from serosa to mucosa. The average tissue resistance measured across the bladders mounted as flat sheets, about 1,600 Ω · cm², was approximately 50% greater than that reported by Renfro. Likewise the serosa to mucosa Na^+ flux was about 50% less suggesting that these differences were the result of the difference in the effective surface area of the perfused and flat sheet preparations. Another difference which cannot be attributed to area was the ratio of the simultaneously measured serosa to mucosa Na^+ and Cl^- fluxes (J^{Cl}_{sm}/J^{Na}_{sm}) which was about 1.3 in Renfro's experiments and averaged about 2.6 in the present study.

Demarest [5] and Demarest and Machen [6] obtained similar results from in vitro studies of the urinary bladder of the Starry Flounder. Bladders from sea water adapted animals absorbed Na^+ and Cl^- at equal rates and exhibited a relatively high resistance (1,748 Ω · cm²). The average value of V_t for 76 animals was 2.8 mV, mucosa negative, but it was reported that about 10% of these exhibited mucosa-positive potentials.

Renfro [15] reported that serosal ouabain inhibited both V_t and NaCl absorption in a perfused bladder preparation. We confirmed this result showing that serosal ouabain abolished both I_{sc} and NaCl absorption in the flat sheet preparation used in this study [1]. In this previously published study we found that there was no obligatory relation between I_{sc} and NaCl absorption. In about 30–50% of bladders it was not possible to detect an I_{sc}, but bladders with no detectable I_{sc} nevertheless absorbed NaCl.

I_{sc} is due to active K^+ secretion

Preliminary experiments showed that in bladders which generated a I_{sc} addition of barium (1–5 mM) to the mucosal bathing solution abolished this current, raising the possibility that I_{sc} was due to K^+ secretion. Figure 1 shows the results of a representative experiment in which I_{sc} and the serosa to mucosa K^+ flux (J^K_{sm}) were measured before and after the addition of 2 mM barium to the mucosal bath. The mucosa to serosa K^+ flow averaged 0.01 ± 0.005 μEq/cm² · h for the eight flux periods and was not affected by mucosal barium, so that the serosa to mucosa K^+ flux was, for practical purposes, equal to the net K^+ flux. As shown in the figure net K^+ secretion was identical to the I_{sc} and both were abolished by mucosal barium. Barium inhibition of I_{sc} was accompanied by a 60% reduction in the total tissue conductance, G_T. Although barium abolished K^+ secretion we found in two experiments no inhibition of NaCl absorption (data not shown).

The basolateral ATPase is electrogenic

The observation that active K^+ secretion by flounder urinary bladder produced an I_{sc} which was blocked by mucosal

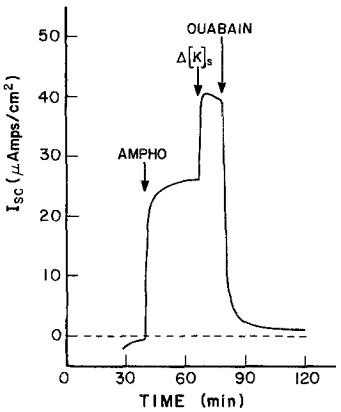

Fig. 2. Record of I_{sc} versus time showing the effect of mucosal addition of 10 μMol/l amphotericin-B (Ampho). Tissue was bathed by Na-benzene sulfonate Ringer's on both sides and initial I_{sc} was near zero. Addition of amphotericin-B to the mucosal bath induced a current which was positive in the mucosal-to-serosal direction. The current was enhanced by raising serosal K^+ concentration by 3 mMol/l ($\Delta[K]_s$) and was abolished by the addition of ouabain (10^{-4} Mol/l) to the serosal bath

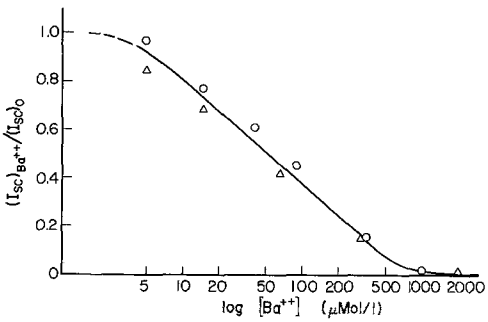

Fig. 3. Dose response relation for the inhibition of I_{sc} (K^+ secretion) by mucosal barium. Data from experiments on two bladders is shown. Note that the inhibition is half maximal at about 50 μMol/l

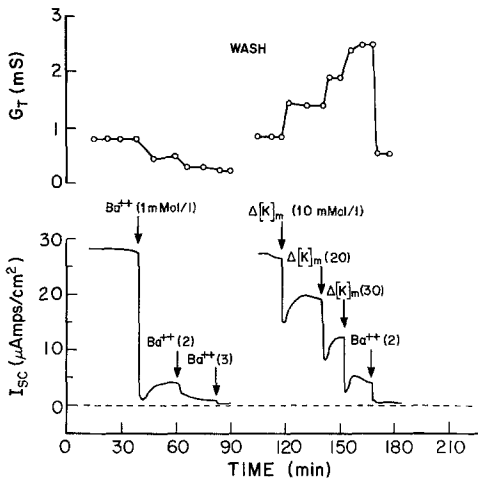

Fig. 4. Time course of a representative experiment utilizing a single bladder showing the effect of mucosal barium and mucosal K^+ on I_{sc} and G_T. The addition of Ba^{2+} to the mucosal bath rapidly reduced I_{sc}. The inhibition was reversed by washing and then the K^+ concentration in the mucosal bath was increased in a stepwise fashion from 2.5 mM to 30 mMol/l. Each increment in mucosal K^+ was associated with a decline in I_{sc} and an increase in G_T. Note that the addition of 3 mMol/l mucosal barium in the presence of 30 mMol/l K^+ reduced G_T to a value below that measured in the presence of 2.5 mMol/l mucosal K^+

barium or serosal ouabain suggested a model for transcellular K^+ movement: active basolateral uptake via a Na/K ATPase and subsequent K^+ exit via K^+ channels in the apical membrane. Renfro et al. [17] used [^{3}H] ouabain autoradiography to show that the Na/K ATPase was localized in the basolateral membranes of the bladder cells. Kirk et al. [11] showed that it was possible to measure directly the current generated by electrogenic Na/K exchange at the basolateral membrane of the turtle colon in tissues which were treated with the polyene antibiotic, amphotericin-B, to eliminate the apical membrane as a barrier to monovalent cation flow. We conducted similar experiments using portions of flounder bladder which were bathed on both sides by Ringer's solutions identical to those described above except that all of the NaCl was replaced by sodium-benzene sulfonate, in order to reduce cell swelling due to salt entry into the cells via the polyene channels. The results of a representative experiment are shown in Fig. 2. In the presence of symmetric Na-benzene sulfonate Ringers I_{sc} was near zero, probably due in part to the requirement for Cl^- to facilitate Na^+ entry into the cell [16, 19]. Abolishing Na^+ entry could reduce or eliminate the turnover of the Na/K ATPase.

The addition of amphotericin-B to the mucosal bath (10 μMol/l) resulted in the rapid development of an I_{sc} consistent with net positive charge flow from mucosa to serosa, i.e. a current opposite to that due to K^+ secretion. This current was promptly increased by the addition of 3 mM K^+ to the serosal bath. The direction of the change in I_{sc} induced by serosal K^+ addition was opposite to that expected due to a K^+ diffusional emf, but was consistent with the notion that added K^+ stimulated electrogenic Na/K exchange. The I_{sc} was abolished by the addition of ouabain to the serosal bath. It was also of interest to note that in the presence of ouabain the introduction of a 10:1 transmural K^+ gradient across polyene-treated tissue did not lead to the development of transcellular K^+ currents as previously observed in the turtle colon [11, 12] under similar conditions (data not shown). The lack of such effects suggested that the basolateral K^+ conductance was small. Similarly, the response of the "pump current" to serosal K^+ addition was unaffected by the addition of 2 mM serosal barium (not shown).

Evidence for apical K^+ channels

Ussing chamber results. The inhibitory effect of mucosal barium on active K^+ secretion was consistent with the notion that K^+ exit from the cell was due to apical K^+ channels which were blocked by barium. In two experiments a dose-response for inhibition of I_{sc} by mucosal barium was determined as shown in Fig. 3. Inhibition of I_K was half-maximal at about 50 μMol/l and was readily reversed by washing. The first half of Fig. 4 illustrates the reversibility of the barium inhibition. Following the addition of 3 mMol/l $BaCl_2$ three changes of the mucosal bath restored the current to its control value. Note also that G_T was reversibly decreased by the application of mucosal barium.

As an additional test of the conductive properties of the apical membrane we investigated the effects of raising the

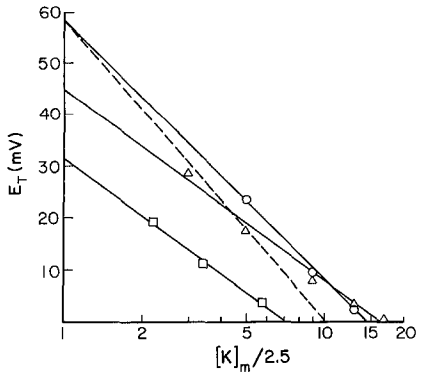

Fig. 5. Plot of the calculated values of the apparent emfs for K^+ secretion (E_T) versus the mucosal K^+ concentration normalized to 2.5 mMol/l K^+

mucosal K^+ concentration. In most experiments the K^+ concentration was progressively raised by adding small volumes of KCl to the mucosal bath. In a few experiments, however, K^+ addition was accomplished via equimolar replacement of Na^+ and this procedure produced identical results. The second half of Fig. 4 shows a representative experiment in which mucosal K^+ concentration was progressively raised from 2.5 to 32.5 mMol/l. Each addition resulted in a prompt decrease in I_{sc} and concomitant increase in G_T. Although G_T was increased more than 2.5-fold when the mucosal potassium concentration was 32.5 mMol/l, the addition of barium (3 mMol/l final concentration, mucosal) reduced G_T to a value near that measured previously in the presence of barium with 2.5 mM K^+, suggesting that the majority of the increase in G_T was due to an increase in the transcellular conductance.

The results presented in Fig. 4 can be placed in a more quantitative framework if the cellular pathway for K^+ secretion is represented by a simple equivalent circuit consisting of a battery in series with a conductance. The use of this simple model was justified on the basis of the results of microelectrode impalements described below which showed that the fractional resistance of the apical membrane remained near unity even if mucosal K^+ was raised 10-fold. The data presented in Fig. 4 indicated that raising mucosal K^+ not only changed the transepithelial driving force for K^+ transport but also the conductance of the cellular path. At any value of mucosal K^+ concentration the total, transepithelial emf for K^+ flow is the sum of the "intrinsic" emf due to active cellular transport and the applied emf due to the increased mucosal K^+ concentration. The total apparent emf for K^+ transport associated with each mucosal K^+ concentration can be calculated from the barium-inhibitable I_{sc} and the barium-inhibitable conductance, i.e.

$$E_T = \Delta I_{sc}/\Delta G_T , \quad (1)$$

where E_T is the transepithelial emf, ΔI_{sc} is the barium-blockable I_{sc} and ΔG_T is the barium-sensitive portion of the total tissue conductance. Figure 5 shows values for E_T from several such experiments on different tissues plotted versus the log of $[K^+]_m/2.5$. In all cases E_T varied linearly with the log of the mucosal potassium concentration with slopes which were similar to the Nernst slope of 58 mV/decade (dotted line) as expected if raising mucosal K^+ reduced the total driving force for K^+ secretion. The intercepts, however, which represent the value of the apparent driving force for K^+ secretion in the absence of a transmural K^+ gradient ($[K^+]_m$ = 2.5 mMol/l) varied from tissue to tissue.

Microelectrode results

Figure 6 shows the results of a representative impalement of a urinary bladder cell. After the apical membrane potential (V_a) stabilized at about 60 mV, cell interior negative, the mucosal K^+ concentration was raised from 2.5 mMol/l to 9 mMol/l and V_a depolarized by about 14 mV. A subsequent increase in mucosal K^+ to 30 mMol/l further depolarized V_a. As shown in the figure these K^+-induced changes in V_a were reversible and repeatable. Corresponding changes in V_t paralleled those in V_a, and were consistent with a direct contribution of changes in cellular emf to V_t. The results of impalements are summarized in Table 2. The values in the table illustrate two effects of raising mucosal K^+ concentrations, depolarization of V_a and a decrease in the fractional resistance of the apical membrane. Furthermore in the presence of mucosal Ba^{2+} (1 mMol/l) changes in mucosal K^+ concentration had relatively little effect on V_a. These observations are consistent with the presumption derived from macroscopic measurements, namely that K^+ channels make an important contribution to the conductance of the apical membrane. The response of V_a to a change in mucosal K^+ varied from tissue to tissue. Figure 8 shows the values for the slope of V_a on $[K^+]_m$ measured under open-circuit conditions plotted vs. the corresponding value of I_{sc} for each tissue. In tissues which exhibited low rates of K^+ secretion changes in $[K^+]_m$ had little effect on V_a or V_t and control V_a values averaged 40 mV. In tissues which secreted K^+ at higher rates, V_a hyperpolarized and both V_a and V_t became increasingly K^+-dependent.

Mechanism of basolateral K^+ exit

A consideration of models for transport by flounder urinary bladder (see Discussion) raised the issue of the mechanisms by which potassium and chloride might exit the cell on the basolateral side. Several lines of evidence suggested that the K^+ conductance of this membrane was low relative to that of the apical membrane and that the short-circuit current might be carried across the basolateral membrane by another ion, namely chloride. Values of V_b recorded during elevation of serosal solution $[K^+]$ were consistent with this notion. At the serosal $[K^+]_s$ of 2.5 mMol/l V_b averaged 38.1 ± 2.3 mV and was 35.8 ± 1.5 mV when $[K^+]_s$ was raised to 30 mM (n = 13). Barium added to the serosal bath (5 mM) had no detectable effect on I_{sc} or the cellular electrical potential profile (not shown). If a major portion of cellular potassium exit occurred via a conductive pathway in the basolateral membrane one might expect at least a transient increase in the K^+ secretory current due to serosal barium. In addition, when amphotericin was added to the mucosal bath in the presence of a mucosal-to-serosal K^+ gradient it was not possible to induce a transmural K^+ current (not shown). Finally, in non-amphotericin-treated tissues abruptly raising the mucosal K^+ concentration from 2.5 to 15 or 20 mMol/l gave rise to a rapid, transient reversal of I_{sc} or "spike" which rapidly returned to the normal orientation. This behavior is exactly what is expected if K^+ does not exit readily across the basolateral membrane. Apical membrane currents induced by perturbing mucosal K^+ must be carried across the basolateral membrane by some other ion, for

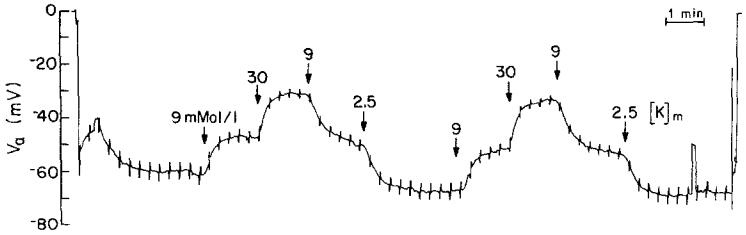

Fig. 6. Representative impalement of flounder bladder cells showing V_a as a function of time. The pulses represent changes in V_a due to a bipolar, constant current pulse of 8 μA/cm^2 at 10 s intervals. At the *left* of the trace the microelectrode punctured the cell. After V_a stabilized at about 60 mV, inside negative, the K^+ concentration in the mucosal bath was changed in a step-wise fashion. Note that increasing mucosal K^+ depolarized V_a and decreased the resistance of the apical membrane. Both effects were reversed by restoring the original mucosal K^+ concentration. The total tissue resistance was 450 Ω · cm^2

Table 2. Intracellular electrical potentials: effects of mucosal [K^+], barium and amphotericin-B

$[K^+]_m$ (mM)	V_T (mV)	V_a (mV)	f_a^R
2.5	−10.6 ± 1.25	−61.0 ± 3.3	0.91 ± 0.02
9.0	−3.4 ± 0.8	−49.2 ± 3.0	0.84 ± 0.03
30.0	+9.8 ± 1.3	−32.0 ± 2.5	0.81 ± 0.03
2.5 (Ba^{2+})	−0.2 ± 0.1	−39.2 ± 3.5	[a]
9.0 (Ba^{2+})	+1.1 ± 0.1	−37.7 ± 3.3	[a]
30 (Ba^{2+})	+3.0 ± 0.1	−34.6 ± 3.5	[a]
2.5 (Ampho)	+24.0 ± 2.0	−5.0 ± 1.0	0.59 ± 0.08

All values are x ± SEM. V_T, V_a and f_a^R were determined using four bladders. For each impalement values are obtained at three K concentrations first in the absence and then in the presence of 1 mMol/l mucosal barium. Values of V_T, V_a and f_a^R in the presence of 10 μMol/l amphotericin-B (Ampho) were determined in a separate experiment utilizing nine bladders in which control values did not differ from those shown. [a] f_a^R was not distinguishable from unity in the presence of mucosal barium regardless of the mucosal K^+ concentration

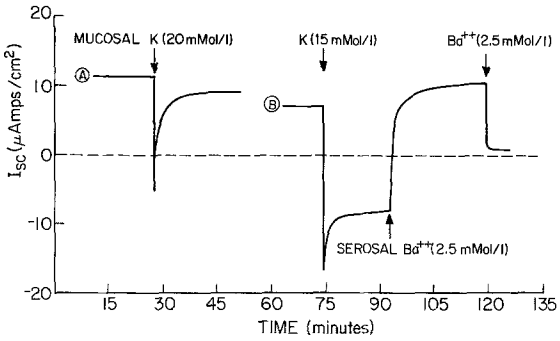

Fig. 8. Representative traces showing examples of the two types of response of I_{sc} to sudden increases in mucosal K^+. *Type A* (spike response) was more common but occassionally the response shown in *B* was obtained. Here the imposition of a transmural K^+ gradient led to the development of a reversed I_{sc} (positive m to s). Subsequent addition of Ba^{2+} (2.5 mMol/l) to the serosal bath caused the current to reverse again to the secretory direction, and the application of mucosal Ba^{2+} abolished the current

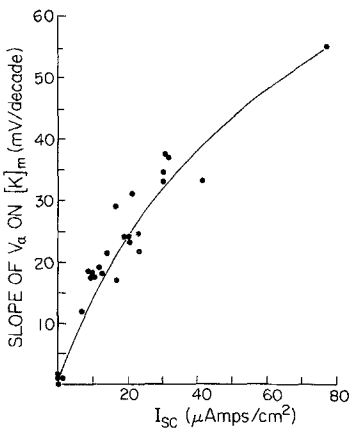

Fig. 7. Relation between the slope of the relation between V_a and the log of $[K^+]_m$ for 26 bladders. Note that as I_{sc} (K^+ secretion) increased the slope approached a value of approximately 60 mV/decade

instance chloride. These currents would be transient due to the accumulation of chloride ions in the cells and consequent alterations in ionic emfs. An exception to this behavior was the occasional observation of a "reversed" K^+ current induced by raising mucosal K^+. Examples of two types of responses to raising mucosal K^+ concentration are illustrated in Fig. 9. Most frequently the response of I_{sc} to increases in mucosal K^+ was similar to that shown in trace A, a spike of reversed I_{sc} which returned rapidly to its original serosal orientation but at a reduced magnitude. In about 20% of the bladder tested, however, the response was that shown in trace B; an initial spike and subsequent decline to a new steady state in which the direction of current flow was from mucosal to serosal. This steady-state reversed I_{sc} was abolished by mucosal barium (not shown) indicating that the current was due to net K^+ flow from mucosa to serosa through the cells. The addition of barium to the serosal bath also had a dramatic effect on the reversed current causing it to reverse a second time and assume a new steady state in which the direction of I_{sc} was again consistent with net K^+ secretion, despite the imposed mucosal to serosal K^+ emf of about 40 mV. This current was abolished by mucosal barium. In other experiments it was shown that the K^+ secretory current which was induced by serosal barium in the presence of a mucosal to serosal K^+ gradient was also abolished by ouabain, consistent with the notion that the current was due to active K^+ secretion.

The basolateral membrane is Cl^--selective

We attempted to test for the presence of a basolateral Cl^- conductance, by exploiting the fact that amphotericin-B, when added to one side of a biological membrane, forms

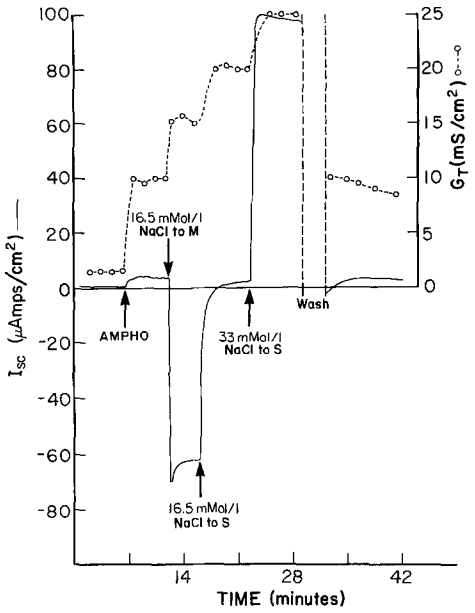

Fig. 9. Representative trace showing transcellular Cl^- currents induced in amphotericin-treated bladders by imposing a transmural Cl^- gradient. Note that the direction of the current was determined by the orientation of the applied anion gradient. Positive values represent current flow from the mucosal to serosal side

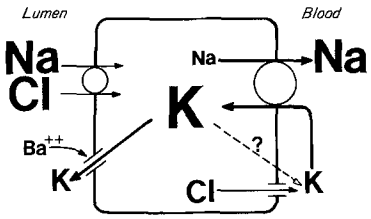

Fig. 10. Schematic model for the cellular transport processes which are thought to co-exist in cells of the flounder bladder

pores which are cation selective but which also possess a significant Cl^- conductance [13, 14]. Bladders were treated with serosal ouabain (0.1 mMol/l) and were bathed on both sides by Ringer's which was identical to that described above except that all but 5.0 mM of the Cl^- was replaced by gluconate. Amphotericin-B (10 μMol/l) was added to the mucosal bathing solution and transmural Cl^- gradients were imposed by adding a small volume of concentrated NaCl (2 Mol/l) to either bathing solution.

Figure 10 shows the results of an experiment in which the polyene was added to the mucosal bath in the absence of any transmural chloride gradient. The transmural conductance increased more than 6-fold (from 1.5 to 10 mS/cm^2) but the effect on the current was slight as expected in the absence of driving forces for transmural ion flow. The addition of 16.5 mMol/l NaCl to the mucosal bath resulted in the prompt development of a current, positive in the serosal to mucosal direction, i.e. consistent with net Cl^- flow from mucosa and serosa. This current could only have been due to net Cl^- flow from mucosa to serosa because net Na^+ flow would have produced the opposite result. The direction of the current was determined by the direction of the transmural chloride gradient. Addition of 16.5 mMol/l NaCl to the serosal bath to abolish the Cl^- gradient reduced the current to zero although the conductance was further increased. Finally a further increase in the serosal Cl^- concentration to 54.5 mMol/l created a serosal to mucosal chloride gradient and resulted in a positive current from mucosa to serosa as expected for serosal to mucosal net Cl^- flow. Identical results were obtained when the tissue was bathed symmetrically in K-gluconate Ringer's and the Cl^- added as KCl (not shown).

The currents induced by amphotericin-B in the presence of transmural chloride gradients were consistent with the notion that the flounder bladder contains an anion selective conductance in the basolateral membrane. In the discussion we show that the expected anion conductance of the one-sided polyene pore is sufficient to account for the measured transcellular Cl^- currents.

Discussion

The observations reported here can all be reconciled at least qualitatively with the model shown in Fig. 10. Flounder bladder epithelial cells are envisioned as consisting of an apical membrane which contains an electrically silent NaCl entry mechanism in parallel with a conductive K^+ channel which is blocked by barium. The basolateral membrane is the locus of an electrogenic Na/K ATPase and a conductive Cl^- channel. The series arrangement of a basolateral Na/K ATPase and apical K^+ channels is necessary to account for the observation that active K^+ secretion is quantitatively equal to the I_{sc} and is blocked by serosal ouabain and mucosal barium. The reversible inhibition of secretion by mucosal barium and the concomitant reduction in transcellular conductance strongly suggest that apical K^+ exit occurs via K^+ channels in the apical membrane. Measurements with polyene-treated tissues in the absence of ion gradients are consistent with the presumption that the driving force for K^+ secretion resides in a basolateral Na/K ATPase, which also mediates active Na^+ exit from the cell. A basolateral Cl^- conductance could in principle provide a primary route for net Cl^- exit from the cell.

Apical membrane

The rapid, reversible response of I_{sc} and V_a to the addition of either potassium or barium to the mucosal bath suggested that K^+ channels serve as the route of K^+ exit from the cell. Changes in total cellular conductance induced by raising mucosal K^+ are compatible with a model in which the apical K^+ conductance is highly dependent on the concentration of mucosal K^+. Additional evidence for the presence of apical K^+ channels was provided by Van Driessche et al. [20] who measured spontaneous and barium-induced fluctuations in K^+ currents.

Although these studies did not directly address the mechanism of NaCl entry, the observations that NaCl absorption is under some circumstances independent of I_{sc} and G_T, coupled with the apparent high Cl^- conductance of the basolateral membrane are consistent with the notion that the apical NaCl entry step is electrically silent. Renfro [15] and Stokes [19] reported that mucosal ion substitution studies revealed an interdependence of Na^+ and Cl^- entry compatible with obligatory co-transport of the two ions into the cell. In addition, Stokes [19] has obtained evidence that this co-transport is specifically blocked by hydrochlorothiazide.

Basolateral membrane

The use of the pore-forming antibiotic, amphotericin-B, to functionally eliminate the apical membrane of flounder urinary bladder enabled us to obtain evidence for at least two transport elements in the basolateral membrane, an electrogenic Na/K ATPase and a Cl^- conductance. The Na/K ATPase was identified by measuring an apparent pump current. Although Na^+ and K^+ fluxes were not measured as in similar studies on turtle colon [11], the activation of ouabain sensitive current by serosal K^+ is indicative of coupled electrogenic exchange. It seems likely that this transport element is responsible for the ouabain sensitivity of NaCl absorption and K^+ secretion.

In the presence of mucosal amphotericin and serosal ouabain the bladder exhibited a transmural conductance which was chloride selective despite the fact that the antibiotic is known to form cation selective pores when added to one side of biological and artificial membranes [13, 14]. More recent studies [10] confirmed and extended this result showing that gradients of bromide, nitrate iodide and thiocyanate all produced transmural currents in amphotericin-treated bladders regardless of the accompanying cation (Na^+, K^+, choline). If the amphotericin-treated apical membranes may be regarded as cation selective under the conditions of these experiments then the observed anion selectivity for transmural currents must reflect the properties of the basolateral membrane. To support this interpretation we must inquire as to whether the amphotericin pores would be expected to confer upon the apical membrane sufficient anion conductance to support the observed currents. Although it is tempting to speculate that some amount of the mucosally applied polyene was internalized, thus forming a small number of two-sided pores (which are anion selective), studies on the effects of the polyenes on the lipid bilayers suggest that this is unlikely [14]. The anion conductance conferred on the apical membrane by the polyene can be accounted for on the basis of the known selectivity of the polyenes. The measurements of Marty and Finkelstein [14] are consistent with a value for P_{Cl^-}/P_{Na^+} of about 0.1 for the one-sided pore. The minimum apical conductance necessary to account for the observed chloride currents can be estimated from the steady state value of I_{Cl^-} and the applied Cl^- emf. Using the values from Fig. 10, an applied E_{Cl^-} of about 30 mV yields an I_{Cl^-} of 60 μA so that the total, transmural Cl^- conductance was about 2 mS. Due to the series arrangement of the apical and basolateral membranes the apical membrane must have had at least this much chloride conductance. To estimate the total polyene-induced apical conductance we used the value of the cellular component of the small-signal conductance (G_T). From Fig. 10 we take the amphotericin-induced increase in G_T (the cellular conductance) to be 15 mS. Again this serves as a minimum estimate of the total polyene-induced conductance. Thus for transmural anion currents to traverse the polyene pores, the fractional anion conductance must be of the order of 2 mS/15 mS = 0.13, a value comparable to that derived for nystatin by Marty and Finkelstein from measurement of NaCl dilution potentials. It would appear, therefore, that the anion conductance of the cation selective polyene pores would be sufficient to account for the measured Cl^- currents, but the results do not reveal which membrane, apical or basolateral, was the rate-limiting barrier for anion currents. Although we do not have a direct measure of the ion selectivity of the amphotericin-treated apical membrane under these conditions the generation of pump currents (Fig. 2) and the high values of G_T induced by amphotericin are consistent with a large apical cation conductance.

The results obtained from polyene-treated bladders strongly suggest that in this condition the basolateral membrane is highly anion selective. Despite this we observed occasionally, as detailed above, electrical behavior which was consistent with the presence of a K^+ conductance in the basolateral membrane. In this small percentage of bladders it was possible to induce reverse (absorptive) net K^+ flows which were blocked by serosal as well as mucosal barium. In polyene-treated bladders, however, we were never able to detect a significant basolateral K^+ conductance. These observations may be due to variability in the properties of the isolated bladders. We experienced great variability, for instance, in the rate of K^+ secretion. It is also possible that the polyene-treated cells were altered in some way which favored the observation of the basolateral chloride conductance.

Quantitative considerations

The model presented accounts qualitatively for our observations on K^+ transport. It provides mechanisms for active K^+ entry into the cells (via the Na/K ATPase) and for conductive exit of K^+ and Cl^-. A coupled apical entry mechanism provides a means for electrically silent apical NaCl entry and these two ions can leave the cell via the Na/K ATPase and the basolateral Cl^- conductance respectively. This model presents some problems for quantitative analysis, however. If we assume provisionally that the mechanisms for NaCl absorption and K^+ secretion reside in the same cell and that the Na/K ATPase has a fixed stoichiometry of 3 Na^+:2 K^+ then it is clear from the values for net Na^+ absorption and I_{sc} in Table 1 that only a fraction of the K^+ which would be expected to enter the cell via a 3 Na^+:2 K^+ ATPase is actively secreted. Thus the maintenance of a steady state requires another mechanism for basolateral K^+ exit, or a basolateral Na^+:K^+ pump of another (non 3/2) stoichiometry.

In the absence of a significant K^+ conductance an electrically silent co- or counter-transport mechanism must be considered. For instance, the addition of an electrically silent KCl co-transport system such as that proposed by Reuss [18] for gallbladder would resolve this discrepancy. The presence of such a mechanism would permit mass balance to be maintained at variable rates of K^+ secretion. Continuity of electrical current would be maintained at the basolateral membrane by conductive Cl^- exit. I_{Cl^-} would have to be equal to the sum of the pump current (due to 3 Na^+:2 K^+ exchange) plus the apical current due to conductive K^+ exit.

The flounder bladder as a model for active K^+ secretion

The flounder bladder actively absorbs NaCl and actively secretes K^+, but because the absorptive process is electrically silent, the bladder exhibits the unique property of generating a short-circuit current which is due entirely to net K^+ secretion. At the apical membrane this K^+ flow represents an outward K^+ current through a population of barium-blockable K^+ channels. The accessibility of these K^+ channels from the mucosal bath and the ease with which the K^+ current can be monitored should make the flounder bladder

a useful model for the study of the factors which regulate apical K^+ conductance in epithelial cells.

The cellular model which accounts for active K^+ secretion by the flounder urinary bladder (Fig. 10) is remarkably similar to that proposed by Frizzell et al. [7] and Halm et al. [8, 9] to describe the cellular basis for active K^+ secretion by the intestine of the same animal, with one important exception. Frizzell et al. [7] proposed that the short-circuit current generated by isolated flounder intestine is carried at the apical membrane by outward K^+ flow via barium-sensitive channels. In contrast to the bladder, however, in the intestine only a fraction of this outward K^+ current appears as net secretion because much of the secreted K^+ is "recycled" by a $Na^+:K^+:2\ Cl^-$ co-transport element which is responsible for furosemide-sensitive NaCl absorption by intestine. Stokes [19] showed that NaCl absorption by Flounder bladder was independent of mucosal K^+ and only modestly sensitive to furosemide. Thus both flounder intestine and bladder appear to possess apical K^+ channels but the role of each tissue in net K^+ secretion is modified by the nature of the NaCl absorptive process.

Acknowledgements. The authors are grateful to David Andrew for his help with the early phases of this work, and to John Stokes for the loan of some equipment used for a portion of these studies. This research was supported by grants DK 29786 (DCD) and DK 31091 (RAF) from the National Institutes of Diabetes and Digestive and Kidney Diseases. David Dawson was supported by a Career Development Award from the same institute during a portion of this work.

References

1. Dawson DC, Andrew D (1979) Differential inhibition of NaCl absorption and short-circuit current in the urinary bladder of the winter flounder, *Pseudopleuronectes americanus*. Bull Mt Desert Isl Biol Lab 19:46–49
2. Dawson DC, Andrew D (1980) Active potassium secretion by flounder urinary bladder: role of a basolateral Na-K pump and apical potassium channel. Bull Mt Desert Isl Biol Lab 20:89–92
3. Dawson DC, Andrew D (1983) Basolateral potassium conductance in flounder urinary bladder. Bull Mt Desert Isl Biol Lab 23:26–27
4. Dawson DC, Cooke AR (1978) Parallel pathways for ion transport across rat gastric mucosa: effect of ethanol. Am J Physiol 235(1):E7–E15
5. Demarest JR (1984) Ion and water transport by the flounder urinary bladder: salinity dependence. Am J Physiol 246:F395–F401
6. Demarest JR, Machen T (1984) Passive and active ion transport by the urinary bladder of a euryhaline flounder. Am J Physiol 246:F402–F408
7. Frizzell RA, Halm DR, Musch MW, Steward CP, Field M (1984) K transport by flounder intestinal mucosa. Am J Physiol 246:F946–F951
8. Halm DR, Krasny EJ Jr, Frizzell RA (1985) Electrophysiology of flounder intestinal mucosa. I. Conductance properties of the cellular and paracellular pathways. J Gen Physiol 85:843–864
9. Halm DR, Krasny EJ Jr, Frizzell RA (1985) Electrophysiology of flounder intestinal mucosa. II. Relation of the electrical potential profile to coupled NaCl absorption. J Gen Physiol 85:856–883
10. Keller J, Ernst SA, Dawson DC (1984) Anion currents across basolateral membrane of urinary bladder of winter flounder *Pseudopleuronectes americanus*. Bull Mt Des Isl Biol Lab 24:94–95
11. Kirk KL, Halm DR, Dawson DC (1980) Active sodium transport by turtle colon via an electrogenic Na-K exchange pump. Nature 287:237–239
12. Kirk KL, Dawson DC (1983) Basolateral potassium channel in turtle colon: Evidence for single-file ion flow. J Gen Physiol 82:297–313
13. Kleinberg ME, Finkelstein A (1984) Single-length and double-length channels formed by Nystatin in lipid bilayer membranes. J Membr Biol 80:257–269
14. Marty A, Finkelstein A (1975) Pores formed in lipid bilayer membranes by Nystatin: differences in its one-sided and two-sided action. J Gen Physiol 65:515–526
15. Renfro JL (1975) Water and ion transport by the urinary bladder of the teleost *Pseudopleuronectes americanus*. Am J Physiol 228:52–61
16. Renfro JL (1978) Interdependence of active Na^+ and Cl^- transport by the isolated urinary bladder of the teleost, *Pseudopleuronectes americanus*. J Exp Zool 199:383–390
17. Renfro JL, Miller DS, Karnaky KJ Jr, Kinter WB (1976) Na-K-ATPase localization in teleost urinary bladder by (^3H) ouabain autoradiography. Am J Physiol 231:1735–1743
18. Reuss L (1983) Basolateral K:Cl co-transport in a NaCl absorbing epithelium. Nature (Lond) 305:723–726
19. Stokes JB (1984) Sodium chloride absorption by the urinary bladder of the winter flounder. J Clin Invest 74:7–16
20. Van Driessche W, Chang D, Dawson DC (1985) Fluctuation analysis of apical K currents in the urinary bladder of the winter flounder (*Pseudopleuronectes americanus*). Bull Mt Des Isl Biol Lab 25:1–9

Received November 16, 1988/Received after revision January 6/ Accepted March 6, 1989