Mechanisms of K^+ Transport in Isolated Turtle Urinary Bladder

INDUCTION OF ACTIVE K^+ SECRETION IN A K^+-ABSORBING EPITHELIUM

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ABSTRACT Transepithelial K^+ movement was studied in vitro in the short-circuited turtle bladder by increasing luminal K^+ permeability and by inhibiting the basolateral Na/K pump. Luminal addition of amphotericin B caused net K^+ secretion (180±52 nmol/h) compared with net K^+ absorption (42±6 nmol/h) in control bladders. Serosal ouabain and luminal amiloride abolished K^+ secretion in amphotericin-treated bladders; ouabain restored net absorption (45±16 nmol/h). The direction and rate of net K^+ transport are controlled by the relative K^+ permeabilities and the Na/K pump sites at the two cell membranes of the epithelium.

INTRODUCTION

Several epithelia are capable of K^+ absorption as well as K^+ secretion. Thus, the distal nephron (1, 2) or the colon (3–6) either absorb or secrete K^+ depending on the conditions. Net K^+ transport may occur between cells in response to an electrochemical gradient or across cells as a result of active K^+ transport at one of the cell membranes. Transcellular K^+ absorption requires active translocation of K^+ across the luminal cell membrane by means of a pump or a cotransport system. Active secretion of K^+ has been attributed either to a secretory pump at the luminal cell membrane or to basolateral K^+ uptake in series with a passive step at the luminal membrane.

In the present study we further examine the mechanisms of active transcellular K^+ transport. We induce active K^+ secretion in the turtle urinary bladder, an epithelium that normally absorbs K^+. The study demonstrates that steady state K^+ secretion can be brought about by increasing the K^+ permeability of the luminal membrane (with amphotericin B) and that secretion depends on active K^+ uptake by the Na/K pump at the basolateral membrane.

METHODS

Urinary bladders were removed from fresh-water turtles, *Pseudemys scripta* (Lemberger Co., Germantown, WI). Each bladder was divided into two portions and the hemibladders were mounted in Lucite chambers. The area of exposed tissue was 8 cm^2 and all results are expressed for this membrane area. The mucosal (M)[1] and serosal (S) bathing fluids were identical Ringer's solutions bubbled with CO_2-free air. The Ringer's solutions contained (in millimoles per liter): 118.4 Na, 3.5 K, 1.8 Ca, 0.5 Mg, 122.5 Cl, 2.0 HPO_4.

Tissues were maintained in the short-circuit state except for brief periods when the spontaneous potential difference was measured. The bridges used to deliver current and to measure potential difference were filled with 3 M $NaNO_3$-3% agar. Unidirectional potassium fluxes were determined using ^{42}K as previously described (7).

Gramicidin was obtained from Sigma Chemical Co., St. Louis, MO, and was ~87% gramicidin A. Gramicidin was dissolved in dimethyl sulfoxide (DMSO) and added to the mucosal bathing solution to give final concentrations of 10 nM to 50 μM. Amphotericin B (dissolved in DMSO) was added to the mucosal solution to achieve a concentration of 5 μg/ml (5.4 μM). Amiloride (0.1 mM) was added to the mucosal solution and ouabain (0.5 mM) was added to the serosal solution. These concentrations are sufficient to block sodium absorption by the turtle bladder under control conditions (8). Steady-state K^+ fluxes were measured during four 30-min periods beginning 3 h after amphotericin and 2 h after amiloride or ouabain.

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[1] *Abbreviations used in this paper:* M, mucosal; S, serosal.

Data were analyzed by t test or analysis of variance, as appropriate. Results are reported as mean±SE.

RESULTS

The K^+ permeability of the luminal membrane of the turtle bladder was increased by the addition of either gramicidin or amphotericin to the luminal solution. With gramicidin the maximal increases in the unidirectional S → M K^+ flux were obtained at concentrations of 100 nM. The mean increase, however, was only 11±2 nmol/h (all doses ≥100 nM; n = 17) and, therefore, too small to induce net K^+ secretion. With amphotericin it was possible to induce substantial increases in the S → M flux at 5 μg/ml, one-third of the concentration used previously to induce a defect in the passive H^+ permeability (9). At this concentration the transport activities of the bladders were transiently altered but then remained stable for many hours.

After luminal addition of amphotericin, the short-circuit current fell for ~30 s, then rose to reach a peak value between 2 and 8 min. The current then declined somewhat below the control level and reached stable values after 90 min. The rate of appearance of ^{42}K in the mucosal fluid increased ~60-fold during the first 30 min of exposure to amphotericin. This initial outflow of ^{42}K probably represents loss of cellular K^+. The rate of ^{42}K appearance then decreased until a steady S → M K^+ flux was reached ~3 h after exposure to amphotericin.

Fig. 1 shows the unidirectional K^+ fluxes under steady-state conditions (open bars). Net K^+ fluxes are indicated by shaded bars. Under control conditions, net transport occurred in the absorptive direction. After amphotericin the S → M flux was greatly increased and the direction of net transport was reversed. Net K^+ secretion was 180±52 nmol/h. As shown in the third set of bars, inhibition of Na^+ absorption by serosal ouabain abolished net secretion and restored net K^+ absorption, this reversal being caused by a large reduction in S → M K^+ flux.

Inhibition of Na transport by amiloride is not necessarily complete in the presence of amphotericin, since the amphotericin channels have a finite Na conductance that is unaffected by amiloride. In these studies, amiloride caused ~95% inhibition of Na absorption as judged from the short-circuit current. There was no net K^+ movement in bladders exposed to amiloride and amphotericin, probably as a result of a fortuitous balance between active secretion (inhibited from the amphotericin level) and active absorption (as seen in control bladders or in the presence of amphotericin and ouabain).

DISCUSSION

The pathways of K^+ movement in control and amphotericin-treated turtle bladder are shown in Fig. 2. K^+ is taken up at both the luminal and basolateral membranes by Na-K dependent pumps with similar characteristics (7, 10). The rate of uptake at the luminal membrane is, however, only ~1% of that at the basolateral membrane. All of the potassium taken up

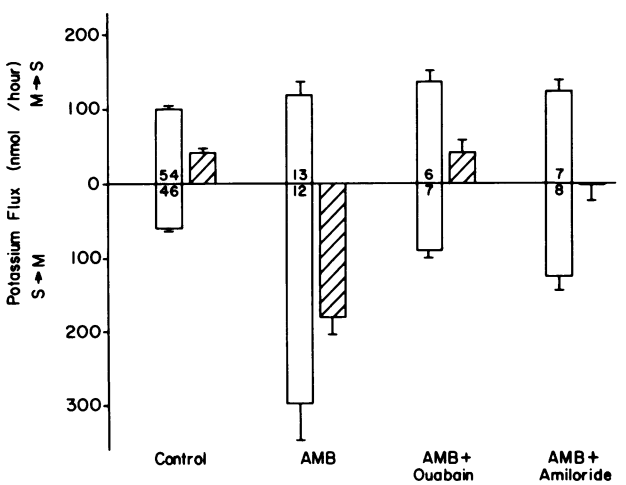

FIGURE 1 Unidirectional and net potassium fluxes in control hemibladders and after treatment with amphotericin B (AMB), AMB + serosal ouabain, or AMB + mucosal amiloride. Control data are from a previous study (7). Number of experiments is indicated within the bars. Net flux (shaded bars) was calculated as the difference between mean unidirectional fluxes (open bars).

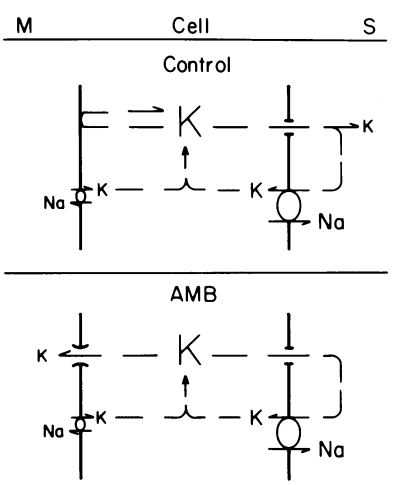

FIGURE 2 Direction of K^+ transport as a function of K^+ pumps and permeabilities. Na^+-dependent K^+ uptake occurs at both membranes. The larger pump at the basolateral membrane is to indicate the greater rate of transport at this membrane. In the control, only the basolateral membrane has a significant K^+ permeability. After amphotericin B (AMB) both membranes are K^+ permeable.

by the epithelium exits the cells across the basolateral membranes because the luminal membrane has such a low permeability to K^+ under control conditions. Thus, although the rate of K^+ uptake from the serosal solution is much higher than that from the mucosal solution, the serosal K^+ recycles across the basolateral membrane and K^+ from the mucosal solution is transported across the epithelium. After amphotericin, K^+ that enters the cells can exit across either the luminal or basolateral membrane. Since the rate of uptake from the serosal solution is higher than from the mucosal solution, there is net secretion of K^+. When the rate of basolateral K^+ uptake is inhibited, the rate of K^+ secretion is reduced or abolished.

The importance of the luminal K^+ permeability as a determinant for the direction of net K^+ transport in turtle bladder is in accord with studies in other epithelia. Thus, amphotericin and related polyene antibiotics induce K^+ secretion in epithelia that do not normally transport K^+, such as toad bladder (11) and frog skin (12). Several investigators (3, 13–15) have reported that the luminal membranes of some segments of the intestinal tract and the distal nephron have a high K^+ permeability. These epithelia normally secrete K^+ but are capable of active K^+ absorption under the appropriate conditions (1–6).

The apparent stoichiometry of sodium absorption to potassium secretion in the present study ranged from 4 to 75 (median 13). These values are far in excess of the value of 3:2 expected for (Na + K)-ATPase, probably due to significant K^+ recycling across the basolateral membrane. Support for this interpretation comes from studies in frog skin (12) and turtle colon (16) treated with polyene antibiotics. In those studies it was possible to bring the ratio close to 3:2 by blocking the K channels of the basolateral cell membrane with barium (12, 16).

In summary, our studies in the turtle bladder indicate that the direction of K^+ transport is controlled by (*a*) the rate of sodium absorption that determines the rate of K^+ uptake at the basolateral membrane, (*b*) the rate of luminal K^+ uptake, (*c*) the luminal K^+ permeability that controls K^+ movement from cell to lumen, and (*d*) the basolateral K^+ permeability that controls K^+ movement from cell to blood. Such an arrangement of pumps and permeabilities may be responsible for secretion and absorption of K in a variety of epithelia.

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