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Apical Membrane K Conductance in the Toad Urinary Bladder

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Summary. The conductance of the apical membrane of the toad urinary bladder was studied under voltage-clamp conditions at hyperpolarizing potentials (mucosa negative to serosa). The serosal medium contained high KCl concentrations to reduce the voltage and electrical resistance across the basal-lateral membrane, and the mucosal solution was Na free, or contained amiloride, to eliminate the conductance of the apical Na channels. As the mucosal potential (V_m) was made more negative the slope conductance of the epithelium increased, reaching a maximum at $V_m = -100$ mV. This rectifying conductance activated with a time constant of 2 msec when V_m was changed abruptly from 0 to -100 mV, and remained elevated for at least 10 min, although some decrease of current was observed. Returning V_m to $+100$ mV deactivated the conductance within 1 msec. Ion substitution experiments showed that the rectified current was carried mostly by cations moving from cell to mucosa. Measurement of K flux showed that the current could be accounted for by net movement of K across the apical membrane, implying a voltage-dependent conductance to K (G_K). Mucosal addition of the K channel blockers TEA and Cs had no effect on G_K, while 29 mM Ba diminished it slightly. Mucosal Mg (29 mM) also reduced G_K, while Ca (29 mM) stimulated it. G_K was blocked by lowering the mucosal pH with an apparent pK_I of 4.5. Quinidine (0.5 mM in the serosal bath) reduced G_K by 80%. G_K was stimulated by ADH (20 mU/ml), 8-Br-cAMP (1 mM), carbachol (100 μM), aldosterone (5×10^{-7} M for 18 hr), intracellular Li and extracellular CO_2.

Key Words toad urinary bladder · K channels · ADH · carbachol · Li · quinidine

Introduction

The toad urinary bladder has been used extensively as a model for the mammalian distal nephron, particularly the cortical collecting tubule (CCT) (Macknight et al., 1980). Na transport by the toad bladder and the CCT appear to involve similar mechanisms. K transport, on the other hand, is quite different. The CCT actively secretes K (Grantham et al., 1970) through a transcellular pathway. One element of this pathway is a Ba-sensitive apical K permeability (O'Neil & Sansom, 1984; Stokes, 1984) whereas in the bladder the apical K permeability is thought to be quite low (Robinson & Macknight, 1976).

We reported earlier that the conductance of the apical membrane of the toad bladder in the absence of mucosal Na increases as the mucosal potential becomes negative (Palmer et al., 1980). In this paper I demonstrate that this voltage-dependent pathway is a K conductance which is completely inactive under the short-circuited conditions commonly used to study this tissue. The conductance is opened by large transepithelial potentials of the polarity maintained in open-circuited conditions. It may be activated when transepithelial Na transport rates are high.

Materials and Methods

Toads (*Bufo marinus*, female, Dominican origin) were obtained from National Reagents (Bridgeport, Connecticut). They were kept in tanks with access to fresh water prior to use. Urinary bladders were excised from double-pithed toads and mounted in Lucite® chambers. Mounting and electrical connections were as described previously (Palmer, 1982).

Solutions

Control serosal solutions contained (in mM): 85 KCl, 50 sucrose, 1 $CaCl_2$, 0.5 $MgCl_2$, 5 glucose, and 3.5 K phosphate, buffered to pH 7.5. Control mucosal solutions contained 115 KCl, 1 $CaCl_2$, 0.5 $MgCl_2$, and 3.5 K phosphate, buffered to pH 6.0.

Data Acquisition

Current-voltage relationships were obtained under voltage-clamp conditions by application of voltage ramps as described previously (Palmer, 1984*a*). Currents were digitized and recorded with either a digital oscilloscope (Nicolet) or a laboratory computer (PDP 11-23). The apical K rectifying current (I_r),

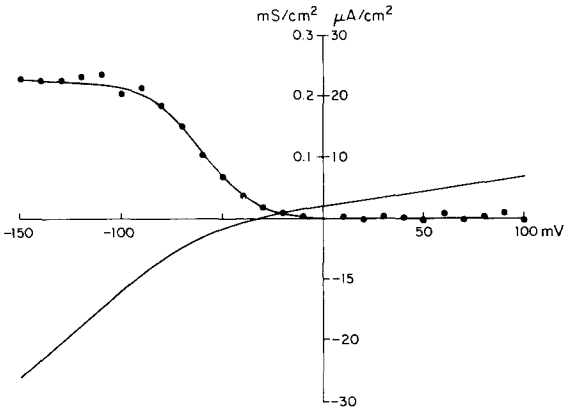

Fig. 1. Voltage dependence of current and conductance in the absence of mucosal Na. The *I-V* relationship was obtained using a rapid voltage ramp. The slope conductance was then computed at different transepithelial voltages (●). The solid line is a best fit of the conductance data to Eq. (1), with $V_0 = -61$ mV and $q = 0.18$

which was found to equal the K current across the membrane (I_K) was computed from *I-V* relationships by taking the current at a large negative transepithelial voltage (V_T), usually −100 mV, and subtracting the current which was obtained at the same voltage by extrapolating the linear part of the *I-V* relationship, measured between ±20 mV. To measure the time course of activation of the conductance, V_T was charged rapidly from zero, where G_K is inactive, to −100 mV, where G_K is active. The resulting current transients were corrected for linear resistive and capacitative components by applying an identical pulse of opposite polarity and adding the two current transients electronically. To measure the time course of the decay of G_K, V_T was held at +100 mV, changed to −100 mV for 20 msec and returned to +100 mV. Linear components were corrected for by applying an identical pulse to +50 mV, multiplying the resulting current transient by 4 and subtracting this from the first current transient.

To measure the serosal-to-mucosal flux of K, the mucosal side of the tissue was washed extensively with K-free solution containing (in mM): 75 Na_2SO_4, 1 $CaSO_4$, 3.5 mM Na phosphate, buffered to pH 7.5, and 10 μM amiloride to block the apical Na conductance. The mucosal bath was replaced with 4 ml of this solution and stirred by aeration. The mucosal fluid was sampled at 5- to 10-min intervals and analyzed for K with an atomic absorption spectrophotometer (Perkin-Elmer Model 360). The K concentration was computed from a calibration curve using KCl standards in the presence of excess Na and was corrected for K contamination by reading blank samples, obtained in the same way as the experimental samples, but with parafilm in the chamber instead of a bladder.

Reagents

Antidiuretic hormone (ADH) was added as aqueous Pitressin® (Parke Davis, Detroit) to the serosal bath at a final concentration of 20 mU/ml. Carbachol was dissolved in water at 100 mM and added to the serosal solution to a final concentration of 100 μM. 8-Bromo cAMP was dissolved in the serosal bathing solution at 10 mM and added to the serosal solution at a final concentration of 1 mM. Quinidine was dissolved at 0.5 M in methanol and added to the serosal solution at a final concentration of 0.5 mM. Methanol alone had no effects on electrical properties at this concentration. Aldosterone was dissolved in methanol at a concentration of 5×10^{-4} M and added to the serosal solution at a final concentration of 5×10^{-7} M. Control hemibladders received methanol only. Mucosal CO_2 tension was increased by bubbling the solution with 6 or 9% CO_2 and adding the appropriate concentration of $KHCO_3$ to maintain pH 6.0.

Table 1. Effect of mucosal Cl replacement on voltage-dependent currents[a]

Mucosal solution	G_L (msec/cm²)	I_r (μA/cm²)
KCl 115 mM	0.10 ± 0.02	5.7 ± 1.3
KCl 25 mM + Sucrose 85 mM	0.08 ± 0.02	5.7 ± 1.3
Ratio	0.82 ± 0.03	1.03 ± 0.05
KCl 115 mM	0.14 ± 0.03	8.6 ± 1.6
K Gluconate 115 mM	0.14 ± 0.03	9.0 ± 1.6
Ratio	1.04 ± 0.04	1.04 ± 0.04

[a] G_L was the slope conductance at $V_T = 0$. I_r is the voltage-dependent currents at $V_T = -100$ mV. Data are given as mean ± SEM for eight experiments (sucrose replacement) or seven experiments (gluconate replacement).

Results

The basic phenomenon under investigation is illustrated in Fig. 1. The current-voltage relationship for a representative bladder in which the Na transport system was not functioning due to the Na-free mucosal solution shows that the non-Na conductance increases sharply as the voltage is increased in the negative direction. Similar results were reported previously (Palmer et al., 1980). The plot of slope conductance *vs.* V_T was corrected for the linear conductance observed at $V_T = 0$. The curve could be described by the empirical equation:

$$G = G_{max}/(1 + \exp(q(V - V_0)) \quad (1)$$

where $G_{max} = 0.23$ mS/cm², the maximal conductance, $V_0 = -61$ mV, the voltage at which the conductance is half-maximal, and $q = 0.18$, indicating the steepness of the voltage dependence. Although the magnitude of G_{max} varied considerably from bladder to bladder, and was dependent on hormonal status (*see below*), values of V_0 and q were fairly consistent. In a series of ten experiments analyzed in this way values of V_0 averaged −59 ± 5 mV (mean ± standard deviation) and q averaged 0.17 ± 0.02.

As pointed out by Palmer et al. (1980) the volt-

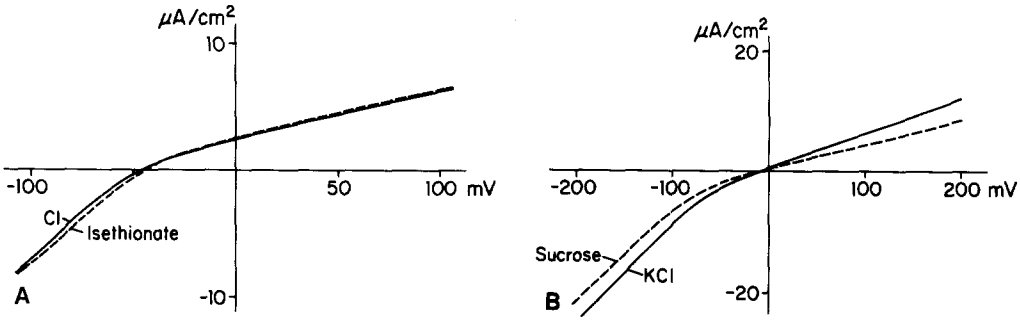

Fig. 2. Effect of mucosal Cl substitution on the voltage-dependent conductance. In *A*, *I-V* relationships were obtained with mucosal KCl in the mucosal medium, and then again after replacement of KCl with K-isethionate. In *B*, *I-V* relationships were obtained with mucosal KCl (115 mM) and then again after reducing mucosal KCl to 25 mM, adding sucrose (180 mM) to maintain constant osmolarity

age-dependent conductance is likely to reside in the apical membrane, as the apical resistance should be high relative to that of the basal-lateral membrane under these conditions (high serosal K, zero mucosal Na). In addition, the activation curve shifts toward less negative voltages when the series basal-lateral membrane potential is reduced with high K.

To test if the rectifying current (I_r) is carried by outward movement of cations across the apical membrane, or inward movement of anions, mucosal Cl was replaced with the presumably impermeant anions isethionate or gluconate. There was no detectable effect on I_r (Fig. 2, Table 1). In another series of experiments, mucosal KCl was reduced to 25 mM with sucrose added to maintain constant osmolarity. While the currents at negative V_T were slightly smaller, the reduction could be accounted for by a decrease in the linear conductive component, measured as the slope conductance at $V_T = 0$. Thus, I_r was apparently unaffected (Table 1). Both of these observations are consistent with the idea that I_r reflects an outward movement of intracellular cations across the apical membrane. Replacement of mucosal K with N-methyl-D-glucamine (NMDG), choline or Na in the presence of amiloride also had no effect on I_r (*data not shown*). This is to be expected, as at large mucosal negative potentials mucosal cations would in any case carry very little current across the apical membrane.

Since the most abundant cellular cation is K, the hypothesis that I_r is a K current was tested. The appearance of K in the mucosal medium was measured directly during periods when V_T was maintained at −120 mV. Before these experiments could be interpreted, however, it was necessary to establish whether I_r was maintained in the steady state. Figure 3 shows responses to changes in V_T to −120 mV for several minutes. With KCl in the mucosal medium, I_r was large initially, declined over the next 10 sec, then increased again. The secondary

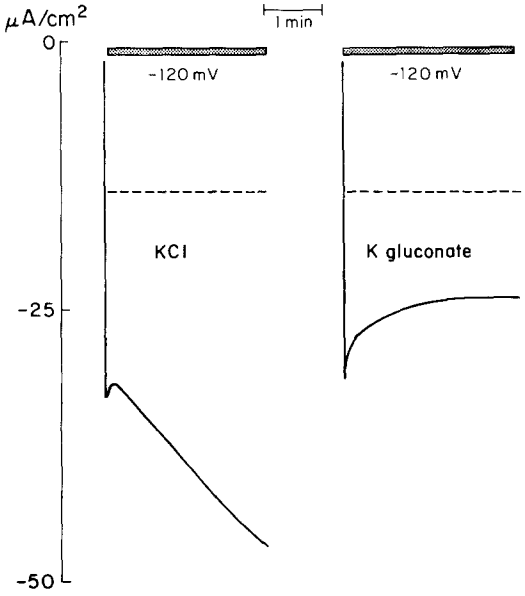

Fig. 3. Steady-state currents with hyperpolarizing voltages. The clamping voltage was changed abruptly from zero to either +120 or −120 mV in the presence of mucosal KCl or K gluconate. The solid lines show the response to a change in −120 mV. The dotted lines show the response to +120 mV. These currents, which are positive, have been inverted in the figure for comparison with larger currents obtained at negative voltages

increase in current was dependent on mucosal Cl, as it was eliminated by replacement of Cl with gluconate (Fig. 3) or SO_4^{2-} (*not shown*). In the presence of impermeant mucosal anions, I_r declined with time but remained measurable up to 10 min after the voltage change. The decline in I_r may be due to depletion of permeant ions from the cytoplasm. Furthermore, like the instantaneous current, the steady-state current was stimulated by ADH and inhibited by quinidine (*see below*) confirming the assumption that the instantaneous and steady-state currents are mediated by the same pathway.

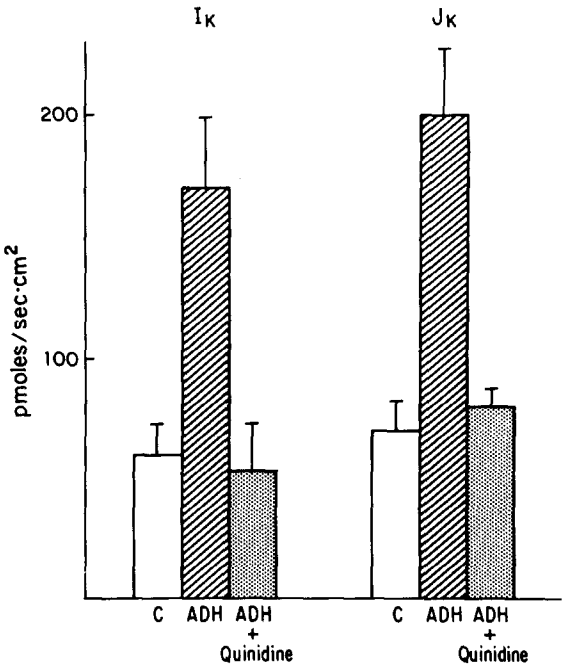

Fig. 4. Comparison of current and K fluxes at hyperpolarizing voltages. Bladders were equilibrated with K-free mucosal solutions. Average currents and average K fluxes were determined over 10-min intervals in which the voltage was maintained at −100 mV. ADH (20 mU/ml) was then added to the serosal medium, and the currents and fluxes determined from 20 to 40 min later. Quinidine (0.5 mM) was then added to the serosal medium and the parameters remeasured after 5 to 10 min. Data represent means ± SEM of five experiments

As can be seen in Fig. 4, the steady-state current at $V_T = -100$ mV could be accounted for by the unidirectional movement of K (J_K) into the mucosal medium, which in the absence of mucosal K equals the net K flux. Presumably, both the current and the flux measurements include contributions from the linear pathway (tentatively identified with the paracellular shunt) as well as the voltage-dependent pathway. Furthermore, the increase in the voltage-dependent current elicited by ADH was accompanied by a quantitatively similar increase in J_K. Finally, inhibition of I_r by quinidine was associated with a parallel decrease in J_K. Quinidine did not change the slope conductance or the K flux at $V_T = 0$, while ADH slightly increased both the conductance and the flux at $V_T = 0$. We conclude from these experiments that within the accuracy of the measurements that $I_r = I_K$. That is, the rectifying or voltage-dependent current is carried across the apical membrane by an outward movement of K.

Time Course of Activation

The data in Fig. 3 indicate that I_K activates rapidly in response to changes in V_T. To see if the activation was instantaneous or if, like other voltage-dependent channels, it was a time-dependent event, the time course of the development of I_K was examined. Since activation occurs at least as fast as the capacitance spike, this was corrected for by adding the current responses to changes in V_T of the opposite polarity. All currents associated with linear components of the epithelial impedance will cancel with this method, but I_K, which is activated during the pulse to negative but not to positive V_T, will remain.

A typical time course of I_K is shown in Fig. 5*A* to consist of a very rapid component followed by a slower component that was approximately exponential. The time constant for the exponential phase was 2 msec and was not markedly voltage dependent between $V_T = -50$ and -150 mV.

To investigate the rate at which I_K turns off at positive voltages, V_T was held at +100 mV and switched briefly to −100 mV and then back to +100 mV. Correction for linear components was made using the current responses for switches to +50 mV, a voltage at which I_K is not activated. At +100 mV, the driving force for I_K should be inward—from mucosa to cell. However, no inward transient was seen when V_T was returned to +100 mV (Fig. 5*B*). Thus it appears that the voltage-dependent conductance inactivates extremely rapidly, at least at large positive V_T where an inward K current would be expected.

Table 2. Effect of divalent cations on I_K[a]

Ion	I_K (μA/cm²)	Fraction of control
NMDG	5.9 ± 0.4	1
Mg	2.9 ± 0.3	0.49 ± 0.05
Ca	7.9 ± 1.0	1.37 ± 0.18
Sr	7.3 ± 0.6	1.08 ± 0.05
Ba	5.0 ± 0.7	0.86 ± 0.11

[a] I_K was measured at $V_T = -150$ mV, with mucosal solutions containing NaCl (29 mM) plus NMDG (86 mM), and with replacement of NMDG Cl by 29 mM $MgCl_2$, $CaCl_2$, $SrCl_2$ and $BaCl_2$. This concentration of divalent cation was chosen to preserve ionic strength. All solutions contained 10^{-5} M amiloride. Data represent means ± SEM for five experiments.

Inhibition of I_K by K Channel Blockers

Many types of K channels in different cell types are blocked by the impermeant cations Cs^+, TEA^+ and Ba^{2+}. Neither Cs^+ nor TEA^+ had any effect on I_K even at concentrations of up to 900 mM in the mucosal bath. Ba^{2+} had only a small blocking effect, reducing I_K by about 14% when added to the mucosal solution at a concentration of 29 mM. Surprisingly, Mg^{2+} was more potent than Ba^{2+} in reducing I_K (Ta-

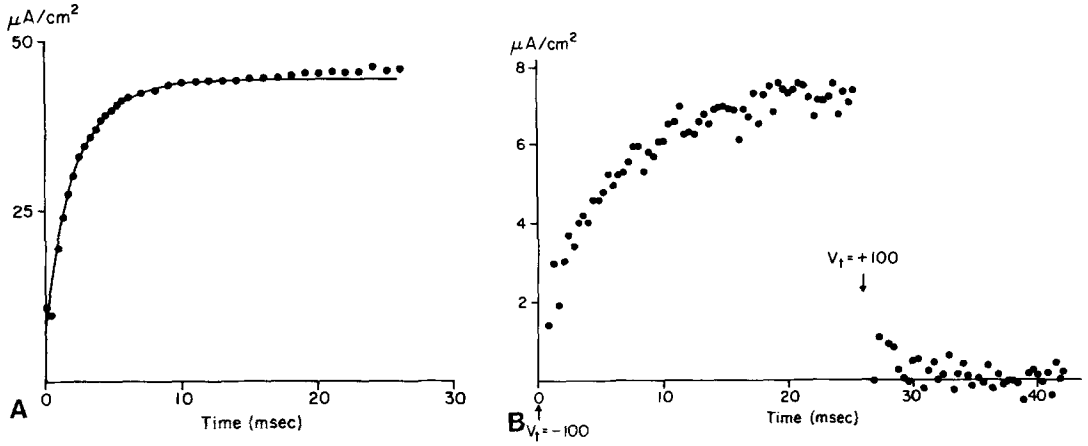

Fig. 5. Time course of the development of I_K. In *A*, the voltage clamp was switched from 0 to −100 mV and from 0 to +100 mV. The currents obtained at +100 mV were added to those obtained at −100 mV to cancel linear components of the tissue conductance. The solid line represents an exponential curve with a time constant of 2.2 msec. In *B*, the clamping voltage was switched from +100 to either −100 or +50 mV and then back to +100 mV. The changes in current observed at +50 mV were multiplied by 4 and subtracted from those at −100 mV to cancel linear components of the tissue conductance. The remaining current is plotted as a function of time

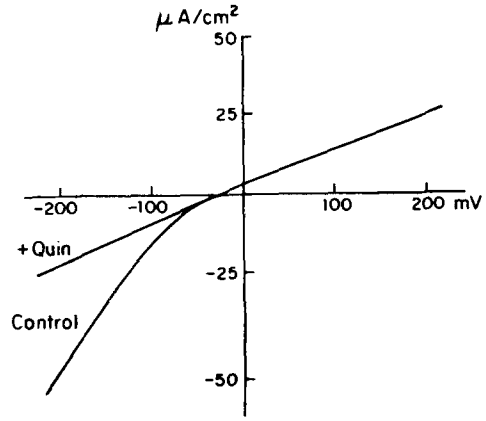

Fig. 6. Effect of quinidine on I_K. *I-V* relationships were obtained before and 5 to 10 min after, addition of quinidine (0.5 mM) to the serosal solution

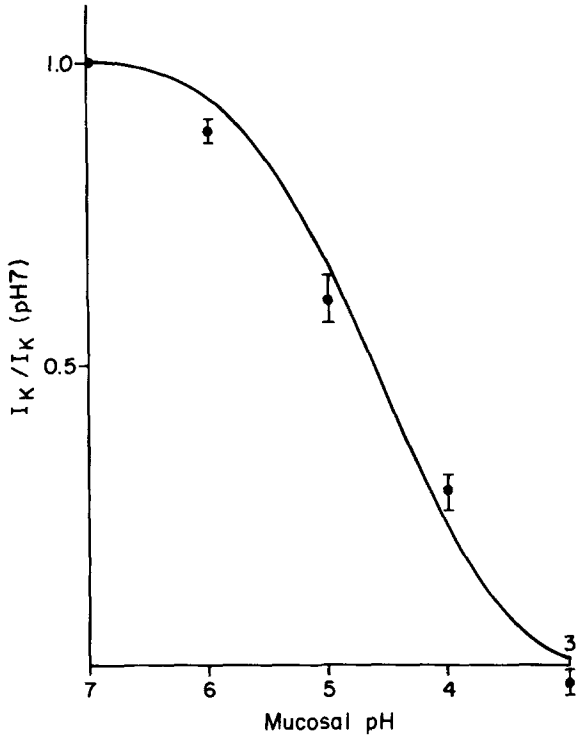

Fig. 7. Effect of mucosal pH on I_K. *I-V* relationships were obtained at different mucosal pH in the range of 6.0 to 3.0. The voltage-dependent current was computed at −160 mV. Data are plotted as the values at low pH normalized to those at pH 6.0, and represent means ± SEM for five experiments. The solid line represents the titration curve for a site with $pK_a = 4.5$

ble 2). Both Ca^{2+} and Sr^{2+}, on the other hand, stimulated the voltage-dependent conductance.

Quinidine reduced I_K by about 80% when added to the serosal bath at a concentration of 0.5 mM (Fig. 6). A similar effect was seen when the drug was added to the mucosal bath. This effect of quinidine was complete within 10 min after addition to the medium, and was irreversible, at least within 30 min after washout. Since quinidine works from either side, it is not clear whether its effect is on the apical membrane conductance or the series basal-lateral conductance. However, in the presence of mucosal Na, the reduction in the amiloride-sensitive conductance by quinidine is relatively small (*unpublished observations*), suggesting that the basal-lateral conductance is not the major site of quinidine inhibition.

I_K decreased as mucosal pH was decreased (Fig. 7). The results of this experiment are consistent with the titration by protons of a single inhibitory site at the outer surface of the membrane with an apparent pK_a of 4.5.

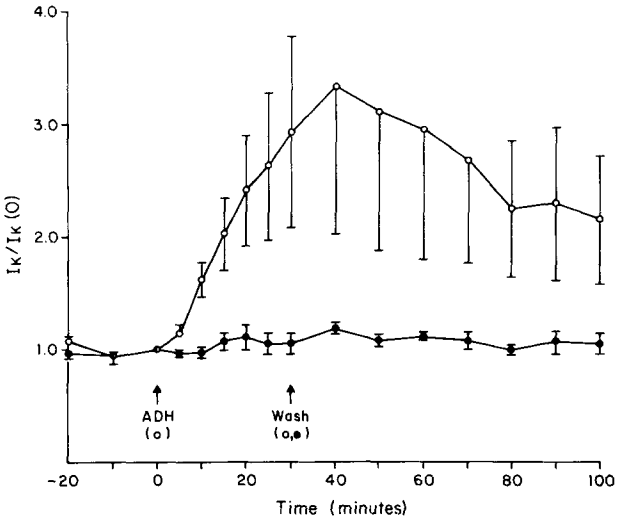

Fig. 8. Effect of ADH on I_K paired hemibladders. Bladders were maintained under short-circuit conditions except for determination of voltage-dependent currents. Currents were measured at −100 mV. At time zero, ADH (20 mU/ml) was added to the serosal side of one hemibladder. After 30 min, the serosal media of both hemibladders was replaced with hormone-free solution. Data represent means ± SEM for five experiments

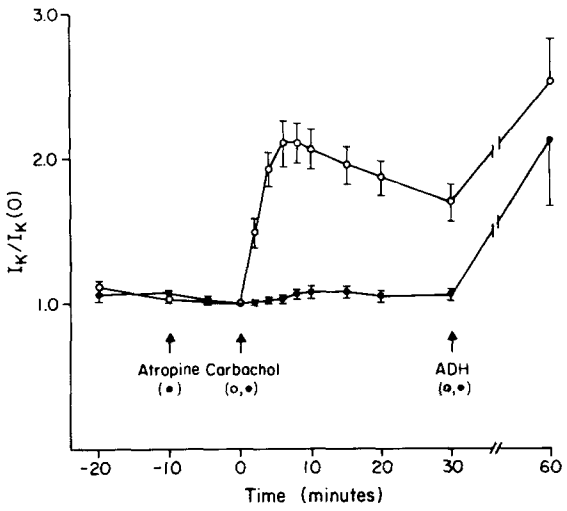

Fig. 9. Effects of carbachol on I_K. Paired hemibladders were maintained under short-circuited conditions except for determination of voltage-dependent currents. Currents were measured at −100 mV. Atropine (10^{-5} M) was added to the serosal side of one hemibladder, and diluent to the other hemibladders. After 10 min, carbachol (10^{-4} M) was added to the serosal side of both hemibladders. Data were normalized to values obtained at $t = 0$ just before addition of carbachol. Data represent means ± SEM for four experiments

Table 3. Agents which stimulate I_K[a]

Agent		Fractional increase in I_K	n
8-Bromo-cAMP	1 mM (serosal)	2.8 ± 0.7	9
Li (mucosal)	25 mM (mucosal)	2.0 ± 0.2	6
CO_2 (mucosal)	6–9% (mucosal)	1.4 ± 0.1	7
Carbachol	100 μM (serosal)	2.6 ± 0.3	17
ADH	20 mU/ml (serosal)	3.5 ± 0.5	19
Aldosterone	5×10^{-7} M (serosal)	1.9 ± 0.1	8

[a] I_K was measured at $V_T = -100$ to -160 mV. Data represent mean ± SEM. n is the number of experiments. In all cases except aldosterone, each hemibladder served as its own control. In the case of aldosterone, paired hemibladders served as controls.

Regulation of I_K

As was reported previously, ADH stimulates the voltage-dependent conductance at concentrations used to enhance Na and water transport in the toad bladder (Li et al., 1982). The time course of the effect of ADH addition and removal from the serosal medium is shown in Fig. 8. Hemibladders were maintained in the short-circuited state except during periodic determinations of I_K. Paired hemibladders which did not receive the hormone served as controls. The most remarkable aspect of the time course is the very slow return to control levels after washout of the hormone. I_K was still elevated 1 hr after removal of ADH. Using an identical experimental procedure, Palmer and Lorenzen (1983) found that ADH-dependent water flow returned to control levels within 15 min after washout. ADH-dependent apical membrane capacitance, on the other hand, returned to normal with a much slower time course. This suggests the possibility that K channels are retrieved from the apical membrane along with additional membrane area that is inserted during ADH stimulation.

Like other effects of ADH, the increase in I_K appears to involve the generation of cAMP, as exogenous nucleotide (8-bromo cAMP, 1 mM to the serosal solution) mimics this effect of the hormone (Table 3).

Carbachol, an acetylcholine agonist, is another agent which strongly stimulates I_K, as is illustrated in Fig. 9. Paired hemibladders were equilibrated under short-circuit conditions while checking I_K periodically. Atropine, a muscarinic antagonist, was added to the serosal side of one hemibladder, and 10 min later carbachol was added to both hemibladders. In the tissues not exposed to atropine, I_K increased rapidly, doubling after about 6 min and remaining elevated for 30 min. There was very little effect on the atropine-treated hemibladders. When ADH was added at the end of the experiment, both

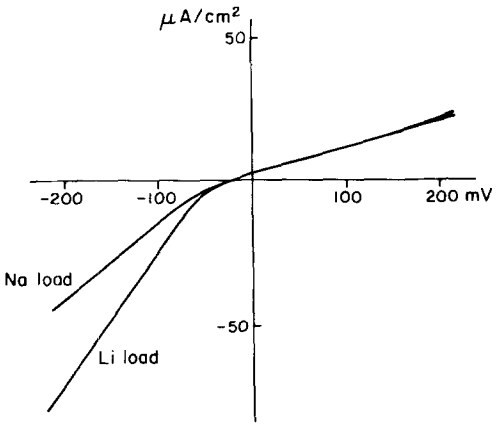

Fig. 10. Effect of Li on I_K. *I-V* curves were obtained after exposure of the mucosal side to 115 mM LiCl for 3 min, followed by removal of the Li and replacement with solution containing 115 mM KCl + 10^{-5} M amiloride. The maneuver was repeated, with NaCl instead of LiCl in the loading solution

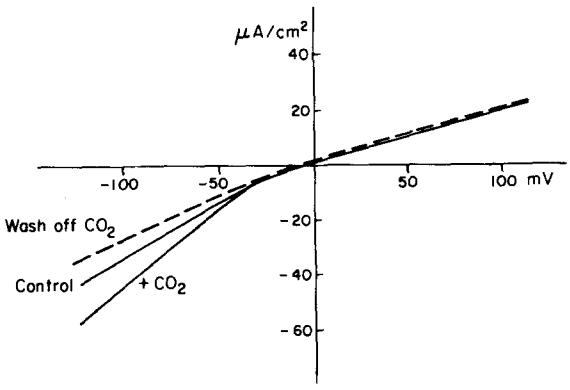

Fig. 11. Effect of mucosal CO_2 on I_K. *I-V* curves were obtained under control conditions, and 5 min after changing the mucosal solution for one which was bubbled with 9% CO_2. $KHCO_3$ was added to maintain constant pH. A third *I-V* relationship was determined after returning control solution to the mucosal bath

hemibladders responded with a normal increase in I_K. These experiments indicate that carbachol stimulates I_K rapidly, that the response is muscarinic in nature, and that it is additive with the response to ADH.

A third maneuver which stimulates I_K is the introduction of Li into the cell. In the experiment shown in Fig. 10, *I-V* relationships were measured after exposure of the mucosal surface to either NaCl or to LiCl, followed by removal by washing with KCl in the presence of 10^{-5} M amiloride. I_K was significantly larger after the Li treatment. The *I-V* relation after Na loading was similar to that of the untreated hemibladders. Exposure to LiCl in the continuous presence of amiloride had no effect on I_K. This implies that Li must enter the cell through amiloride-sensitive Na channels (Palmer, 1982) to have an effect.

Finally, addition of CO_2 to the mucosal bath at constant extracellular pH also stimulates I_K (Fig. 11, Table 3). This effect may be the result of acidification of the cytoplasm, which is expected to occur during acute increases in P_{CO_2} (Boron & DeWeer, 1976). Washout of the CO_2 sometimes produced an overshoot effect, with I_K falling below the pre-CO_2 levels (Fig. 11).

Aldosterone also appears to regulate the K conductance. Paired hemibladders were incubated for 18 hr with and without aldosterone (5×10^{-7} M) in the serosal medium. I_K was increased by 90% in the steroid-treated tissues relative to controls. In the same experiments apical Na permeability, measured by fitting the amiloride-sensitive *I-V* relationship with the constant field-equation (Palmer et al., 1980), was increased sevenfold in the aldosterone-treated tissues relative to controls.

Discussion

Comparison with Other Epithelia

Several tight epithelia with amiloride-sensitive Na transport systems like that of the toad bladder also appear to have K channels in their apical membranes. These include frog skin (Zeiske & Van Driessche, 1979), rabbit colon (Wills et al., 1982) and rabbit cortical collecting tubule (O'Neil & Sansom, 1984; Stokes, 1984). In contrast to the K conductance in the toad bladder described here, those in the frog skin and rabbit colon are active under short-circuited conditions. That in the collecting tubule has been studied under open-circuit conditions, but can be observed in the presence of amiloride, when the transepithelial voltage is very small (O'Neil & Sansom, 1984; Stokes, 1984). Thus none of the K conductances in these systems seems to show the voltage-dependence of that of the bladder. Another difference is in the sensitivity to Ba, which blocks K channels in the frog skin, rabbit colon and CCT in millimolar concentrations. In the toad bladder the voltage-dependent conductance is only partially blocked with 29 mM Ba.

Comparison with Other Conductances Induced by Hyperpolarization

It has been shown previously that hyperpolarization of the toad bladder to voltages of 120 mV or more

increases the total tissue conductance (Finn & Rogenes, 1980). In those studies, the voltage-dependent conductance was apparently paracellular, as serosal-to-mucosal fluxes of Na and sucrose, which are presumed to be paracellular, increased concomitantly. This phenomenon differs from the conductance increase reported here in that higher transepithelial voltages are required to open the paracellular shunt, and much longer times are required for this conductance to be expressed. It is interesting, however, that in nondepolarized bladders, where the apical conductance increase begins at about −100 mV (Palmer et al., 1980) the rectifying K current would be activated at about the same voltage as the paracellular path.

In amphibian skin, a Cl-specific conductance is activated upon hyperpolarization of the tissue (Hviid-Larsen & Kristensen, 1978). This conductance is distinguishable from the voltage-dependent K conductance in that it requires several minutes to develop and is abolished by replacement of mucosal Cl with an impermeant anion such as gluconate. It is likely that the slow increase in conductance seen at $V_T = -120$ mV in the presence of mucosal Cl but not gluconate in Fig. 3 involves a similar mechanism to that reported by Hviid-Larsen and Kristensen for amphibian skin.

Thus at least three different conductive transport systems, distinguishable by their kinetics and their ion specificities, are activated by hyperpolarization of the toad urinary bladder epithelium. Studying the bladder under short-circuited conditions maintains all of these conductances in the closed state and greatly simplifies the transport characteristics of this tissue.

Comparison with Other Voltage-Dependent K Channels

The voltage-dependence of the apical K conductance in the toad bladder is qualitatively similar to that of other channels such as the delayed rectifier of squid axon (Hodgkin & Huxley, 1952) or the Ca-activated K channel (Latorre & Miller, 1983; Moczydlowski & Latorre, 1983) in that the conductance is activated by making the cell interior more positive (less negative) with respect to the outside of the cell (the mucosal medium in the case of the bladder). The steepness of the voltage dependence is also comparable. The K conductance described here can be empirically described to be related to the voltage according to Eq. (1). The opening and closing process can be considered as a simple two-state equilibrium in which the apparent equilibrium constant is an exponential function of voltage: $K_{eq} = \exp(zFV/RT)$ where z is approximately −4.4. This compares with analogous values of about −4 for the squid axon K channels (Hodgkin & Huxley, 1952) and −2 to 3 for Ca-activated K channels (Moczydlowski & Latorre, 1983). The K channel of the bladder is unusual, however, in that the cell potential must actually be positive to the outside to activate the conductance. In the presence of high serosal K, it has been assumed that the cell potential approximates that of the serosal medium (Palmer, 1984*b*). If this is the case, then the apical membrane potential must be 50 or 60 mV, cell positive, to achieve 50% activation.

The kinetics of the K channel in toad bladder are at least superficially similar to those of the squid axon channel. Both conductances increase with a time constant of a few milliseconds. We could not measure the rate of closing of the K conductance. In the squid axon this process also requires a few milliseconds (Hodgkin & Huxley, 1952).

Regulation

Two hormones that increase Na channel activity in the apical membrane of the toad bladder—ADH (Li et al., 1982) and aldosterone (Palmer et al., 1982)—also stimulate the voltage-dependent apical K conductance. It appears that this effect of ADH, like others in this tissue (Orloff & Handler, 1962), is mediated by intracellular cAMP, as addition of the 8-bromo form of the nucleotide to the bath mimicked the action of the hormone (Table 3). The effect of aldosterone was unexpected as it was previously reported that this hormone did not increase the amiloride-insensitive conductance of the toad bladder (Palmer et al., 1982). In that study, however, the exposure to aldosterone was for up to 6 hr, whereas the results reported here were obtained after 18 hr of incubation. Thus the increase in K conductance may be a late effect of the mineralocorticoid (Truscello et al., 1983; Geering et al., 1984). It is of interest in this regard that Sansom and O'Neil (1985) found that injection of rabbits with DOCA, another potent mineralocorticoid, increased apical K conductance with a delay of one day.

Other agents have opposite effects on Na and K conductances. These include carbachol, which inhibits Na transport (Sahib et al., 1978) and CO_2, which reduces apical Na permeability (Palmer, 1985). Both of these agents stimulate K conductance (Table 3). The intracellular messengers mediating the carbachol response are unknown. In other cells, muscarinic cholinergic agonists stimulate phosphoinositol turnover, leading presumably to an increase in intracellular free Ca and to stimulation

of protein kinase C (Berridge & Irvine, 1984). In preliminary experiments, no effects of the addition of Ca ionophores (A23187 or ionomycin) or phorbol esters, thought to stimulate protein kinase C, on K conductance were observed.

Intracellular Li is also involved in the PI turnover pathway, by blocking the dephosphorylation of 1-phosphoinositol into inositol (Berridge & Irvine, 1984). There is no evidence to suggest that this mechanism could account for the effects of Li observed here. In fact, the possibility that Li is transported by this pathway more effectively than K cannot be ruled out.

The stimulation of K conductance by CO_2 presumably results from acidification of the cytoplasm (Boron & DeWeer, 1976). Obviously, we do not know if this is a direct effect of lowered cell pH.

Physiological Significance

The physiological role of the apical K conductance could be twofold. Obviously, it could mediate K secretion by the bladder. Cell K is accumulated above its electrochemical equilibrium in toad bladder cells, as in other cell types (Delong & Civan, 1978). Hyperpolarization of the tissue would both increase the driving force for K efflux across the apical membrane, as well as increasing the K conductance. It is not clear, however, if K is secreted to any appreciable extent by the bladder.

A second role for the K conductance would be to depolarize the apical membrane under conditions of high rates of active Na transport. Thus, the electrogenic outward movement of K would balance the electrogenic inward movement of Na, the net result being effectively a Na-for-K exchange. This mechanism could be useful when the membrane potential tends to slow Na reabsorption.

A caveat to this hypothesis is that the selectivity of this pathway for K over Na is not known, since all the measurements made here were under conditions of very low cell Na. The possibility that Na is also conducted does not, however, rule out the hypothesis that the opening of the pathway could serve to depolarize the apical membrane under conditions of high rates of Na transport and cell potentials which are positive to the mucosal solution. Under these circumstances Na ions would be relatively close to electrochemical equilibrium, whereas the driving force for K would be large and in the secretory direction. Thus even a nonspecific cation conductance would tend to reduce the cell-positive potential.

A second caveat is that the K conductance described here is not necessarily in the same cell type as are the apical Na channels. A direct, electrically mediated exchange of Na for K is only applicable to the case where the two conductances are in the same cells, or in electrically coupled cells. However, a less direct coupling of Na and K fluxes across the epithelium, rather than across the apical membrane, could still take place even if the two pathways were in different cells.

Finally, the question arises of whether these channels are ever open under physiological conditions. According to Fig. 1, the apical membrane potential (V_a) must be at least -30 mV to obtain a measurable K current, and about -60 mV to activate the conductance by 50%. These are probably overestimates since they assume that the cell potential is zero under short-circuited conditions, and that the resistance of the basal-lateral membrane is negligible. A negative cell potential or finite basal-lateral resistance would imply that V_a must be less negative than V_T in Fig. 1, and that therefore smaller negative values of V_a are required to activate the K conductance. In the *Necturus* urinary bladder under open-circuit conditions, Higgins et al. (1977) found that V_a was correlated with the spontaneous transepithelial potential such that V_a became negative (i.e. cell positive to mucosa) when V_T was more negative than -80 mV. With very large V_T (-130 to -150 mV) V_a approached -40 to -60 mV. Thus V_a may, at least under extreme conditions, be sufficiently negative to open the K conductance.

A similar conclusion can be reached by considering that in the toad bladder under non-K-depolarized conditions, I_K is half-maximal when V_T is about -110 mV (Palmer et al., 1980). Certainly some bladders can achieve potentials this large *in vitro* (Higgins et al., 1975; Erlij, 1976; L.G. Palmer, *unpublished data*). On the other hand, it is not clear if the transepithelial potential is ever as large as 110 mV *in vivo*. Toad urine generally contains a relatively low Na concentration of 1 to 30 mM (Leaf et al., 1958). Thus rates of Na transport, and hence the transepithelial potential, will be low. The K conductance reported here may be dormant under most *in vivo* conditions, except when the urine Na is particularly high.

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