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Voltage-Dependent Interaction of Barium and Cesium with the Potassium Conductance of the Cortical Collecting Duct Apical Cell Membrane

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Summary. The influence of Ba^{++} and Cs^+ on the K^+ conductive properties of the rabbit isolated perfused cortical collecting tubule were assessed using electrophysiological methods. As before, elevation of K^+ from 5 to 25 or 50 mM ($choline^+$ for K^+ substitution) in the luminal perfusate, caused a marked hyperpolarization (lumen negative) of the transepithelial voltage, V_{te}, and increase in the transepithelial conductance, G_{te} – indicative of a high luminal (apical) K^+ conductance – whereas a similar elevation of K^+ in the bath caused only minor changes in V_{te} and G_{te}. In the presence of 5 mM K^+ in perfusate and bath, addition of 5 mM Ba^{++} to the perfusate caused G_{te} to decrease from 16.1 to 7.4 $mScm^{-2}$ (10 mM Cs^+ had qualitatively similar effects) and greatly diminished the response of V_{te} and G_{te} to K^+ elevation in the lumen, reflecting a decrease in the apical membrane K^+ conductance. In contrast, a similar addition of 5 mM Ba^{++} to the bath caused only a modest reduction in G_{te} of 0.4 $mScm^{-2}$, consistent with a relatively low K^+ conductance of the basolateral membrane and tight junction. In other studies the effects of luminal addition of Ba^{++} and amiloride were found to be relatively independent, with the magnitude of the Ba^{++}-sensitive G_{te} (8.7 $mScm^{-2}$) being several-fold greater than the amiloride-sensitive G_{te}(1.4 $mScm^{-1}$), indicative of a dominant K^+ conductance at that border which is spatially distinct from the Na^+ conductance. Furthermore, from the input current-voltage relation of the tubule, the effects of Ba^{++} (0.1–5 mM) and Cs^+(10 mM) at the luminal border on tissue conductance were found to be highly voltage-dependent – the effects on conductance being diminished with lumen-negative voltages and more pronounced, approaching a maximum, with lumen-positive voltages. It is concluded that the apical (luminal) cell membrane contains a dominant K^+ conductive pathway that is blocked by luminal addition of Ba^{++} and Cs^+. The voltage-dependent nature of the block is consistent with a K^+ conductive pathway which has a binding site for Ba^{++} and Cs^+, and presumably K^+, located deep within the channel.

Key Words renal epithelium · cortical collecting duct · barium · cesium · potassium transport · potassium channel · potassium conductance

Introduction

The rabbit cortical collecting duct is known to avidly secrete K^+ into the tubular lumen (Grantham, Burg & Orloff, 1970; Stoner, Burg & Orloff, 1974; O'Neil & Helman, 1977; Iino & Imai, 1978; Schwartz & Burg, 1978; Stokes, 1981). This is thought to occur by a two-step process: K^+ is taken up into the cell across the basolateral membrane via the Na^+:K^+ exchange pump (*see* Koeppen, Biagi & Giebisch, 1983; O'Neil, 1981; Stokes, 1981) and is then secreted from the cell into the lumen, supposedly by diffusing down its electrochemical gradient through a recently identified K^+ conductive pathway in the apical (luminal) cell membrane (O'Neil & Boulpaep, 1979, 1982; O'Neil, 1982*a*, *c*; Koeppen et al., 1983). While this apical cell membrane K^+ conductance likely plays a central role in the regulation of K^+ secretion, little is known about the properties of this or other K^+ conductive pathways in this tissue. However, the properties of K^+ channels in several other tissues, most notably, excitable tissues, have been particularly well characterized. These properties have in part been elucidated by studying the blocking actions of various monovalent and divalent cations such as Cs^+ and Ba^{++} (e.g., Sperelakis, Schneider & Harris, 1967; Hagiwara, Miyazaki & Rosenthal, 1976; Standen & Stanfield, 1978; Coronada & Miller, 1979; Armstrong & Taylor, 1980; Eaton & Brodwick, 1980). Recent studies of several epithelial tissues have demonstrated that Ba^{++} and Cs^+ may also block K^+ conductive pathways in epithelial cells (e.g., Nielsen, 1979; Wills, Eaton, Lewis & Ifshin, 1979; Nagel & Hirschmann, 1980; Biagi, Sohtell & Giebisch, 1981; Gogelein & Van Dreissche, 1981; Greger, 1981; Planelles, Teulon & Anagnostopoulos, 1981; Koeppen et al., 1983) although little is known of the nature of this interaction. These similar effects of Ba^{++} and Cs^+ among tissues likely indicate that the properties of K^+ channels from various cell types may have certain common characteristics.

The purpose of the present study was to investigate further the properties of the K^+ conductive pathways in the rabbit isolated cortical collecting duct, particularly of the apical cell membrane, and to evaluate the nature of the interaction of Ba^{++} and Cs^+ with this membrane pathway. It was found that Ba^{++} and Cs^+ block the K^+ conductive pathway of the apical cell membrane, which, as before, was shown to be the major K^+ conductive pathway in this tissue. Furthermore, the blocking actions were highly voltage depenent, implicating a binding site for Ba^{++} and Cs^+ deep within the membrane channel. Preliminary results of these studies have been reported (O'Neil, 1982*a, b*).

Materials and Methods

Isolation and Perfusion of Tubules

New Zealand white female rabbits were maintained on standard purina rabbit chow (0.4% Na^+, 1.4% K^+) and tap water *ad libitum*. The rabbits were killed by cervical dislocation, the right kidneys removed, and segments of cortical collecting duct dissected free as previously described (O'Neil & Boulpaep, 1979). A segment of tubule was connected to a perfusion apparatus and studied *in vitro*, as before (O'Neil & Boulpaep, 1979), as modified from the methods of Burg and co-workers (Burg Grantham, Abramow & Orloff, 1966). In brief, a glass perfusion pipette was inserted into one end of the tubule lumen for perfusion while the other end of the tubule was held in a glass collecting pipette. The tubules were perfused with a media containing (in mM): 45, NaCl; 40, Na gluconate; 55, choline chloride; 5, KCl; 10, PIPES (or Tris Cl); 1, $CaCl_2$; and 1, $Mg\ Cl_2$; pH 7.4. The tubules were continuously bathed at 37 °C in a media either identical to the perfusate or in a solution containing (in mM): 65, NaCl; 13, Na gluconate; 25, $NaHCO_3$; 1.2, NaH_2PO_4; 1, Na acetate; 45, choline chloride; 5, KCl; 1, $CaCl_2$; 1, $MgCl_2$ and gassed with 95% O_2–5% CO_2 to maintan the pH at 7.4. Both bathing solutions also contained 5 mM glucose. The properties of the tissue did not differ significantly between the two bathing solutions. The effects of various cations on the tissue were evaluated by adding these ions to either the perfusate or bath. Potassium, Cs^+ and Ba^{++} were added as the Cl salts in exchange for choline Cl such that the chloride concentration was maintained constant. With the perfusion apparatus described, both the perfusate and bath could be exchanged rapidly with a test solution within 15 to 20 sec (O'Neil & Boulpaep, 1982).

Electrical Measurements

The transepithelial potential difference, V_{te}, was measured as before (O'Neil & Boulpaep, 1979) through the perfusion pipette using an agar salt bridge (0.9% NaCl) and Ag–Ag Cl wire connected to a high impedance electromoter (10^{11} Ω, WPI KS-700 dual electrometer) whose output was recorded on one channel of an MFE 1400 recorder. The bath was grounded via a second agar bridge so that all voltages reflected the voltage of the lumen relative to the bath. A voltage reference source was used to null all voltage asymmetries before the tubule was connected. In those studies in which the K^+ concentration was elevated unilateraly, in either the perfusate or bath, a voltage asymmetry existed because of the asymmetric diffusion potentials at the solution-bridge interfaces. Correction for this asymmetry potential was made by estimating the change in the potential at the bridge interface, relative to a flowing 3-M KCl electrode, upon elevation of K^+.

The transepithelial conductance, G_{te}, of the tissue was estimated as before using cable analysis (Helman, Grantham & Burg, 1971; O'Neil & Helman, 1977). Briefly, constant current pulses up to ±200 nA and 500 msec duration were injected into one end of the tubule lumen via the perfusion pipette using a bridge arrangement and the resulting voltage deflections at both ends of the tubule recorded. At the perfusion end the voltage deflections were recorded via the voltage-sensing bridge and electrometer (making appropriate corrections for changes in the pipette resistance due to changes in the volume resistivity of the various perfusates) while the voltage changes at the opposite end of the tubule were measured via an agar bridge inserted into the collection pipette which was connected via a Ag–Ag Cl wire to a second channel of the KS-700 electrometer and recorded on a second channel of the MFE recorder. All voltage deflections were measured within 25 to 100 msec after the onset of the current pulse. The response or rise time of the recorder and pipette system in the absence of a tubule was approximately 10 msec. In those studies in which Ba^{++} and Cs^+ were added to the tubular lumen, the effects on conductance were found to be highly voltage dependent particularly upon injection of negative currents, i.e., positive current flow from bath to lumen (lumen-negative voltage deflections). The decrease in conductance associated with the addition of these cations to the lumen was maximal and relatively voltage independent upon passage of positive current pulses (lumen-positive voltage). Hence, since it is assumed in the cable analysis that the transepithelial conductance is constant along the length of the tubule, the estimates of G_{te} in the presence of Ba^{++} and Cs^+ were obtained from passage of positive current pulses only.

Statistical Analysis

The data are summarized as mean values ± SEM. Differences between means were evaluated by either a paired or unpaired t test as appropriate. Relations between parameters were evaluated with linear regression analysis.

Results

Luminal Membrane Properties

The addition of either Ba^{++} or Cs^+ to the luminal perfusate caused a marked but reversible reduction in the transepithelial conductance, G_{te}, of the cortical collecting tubule as shown in Fig. 1. This was often accompanied by a transient hyperpolarization of the transepithelial voltage, V_{te}, which relaxed over 1 to 2 min to a new steady-state value close to the original control value. This transient hyperpolarization of V_{te} was highly variable but appeared to be related to active Na^+ transport since addition of 50 μM amiloride (a Na-channel blocker) to the luminal perfusate, greatly reduced the hyperpolarization (*see* Discussion). Therefore, to simplify the analysis of the effect of Ba^{++} and

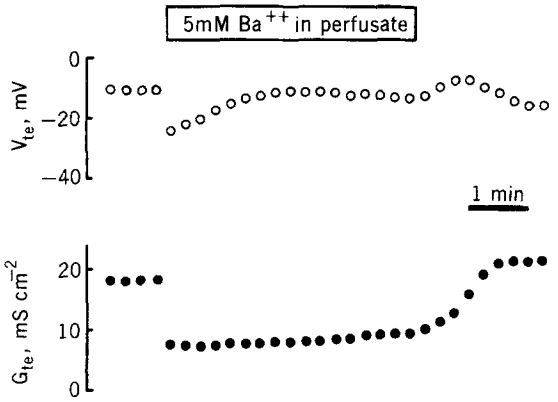

Fig. 1. Time-course of the influence of addition of 5 mM Ba^{++} to the luminal perfusate on V_{te} and G_{te}

Table 1. Summary of the steady-state effects of luminal addition of Ba^{++} and Cs^{+} on V_{te} and G_{te}*

A. Influence of luminal addition of Ba^{++} ($N=34$)

	$-Ba^{++}$	$+5$ mM Ba^{++}	Difference
V_{te}, mV	-1.0 ± 2.1	-3.0 ± 2.0	-2.0 ± 0.6[a]
G_{te}, $mScm^{-2}$	16.1 ± 1.6	7.4 ± 0.7	-8.7 ± 1.4[a]

B. Influence of luminal addition of Cs^{+} ($N=4$)

	$-Cs^{+}$	$+10$ mM Cs^{+}	Difference
V_{te}, mV	2.4 ± 3.2	2.4 ± 2.6	0.0 ± 0.6
G_{te}, $mScm^{-2}$	17.2 ± 3.4	11.2 ± 2.8	-6.0 ± 2.2[a]

* Includes all tubules perfused both in the absence and presence of amiloride (50 μM).

[a] $P<0.05$.

Cs^{+} on the K^{+} conductive properties, most studies were done in the presence of 50 μM amiloride in the perfusate (*see* below).

The steady-state changes in V_{te} and G_{te} upon the addition of either 5 mM Ba^{++} or 10 mM Cs^{+} to the luminal fluid are summarized in Table 1 for all tubules. (The initial low values of V_{te} are due to the presence of amiloride in most experiments.) Only minimal changes in the steady-state value of V_{te} were observed. However, in every case the steady-state value of G_{te} was decreased. Upon addition of 5 mM Ba^{++} to the luminal fluid, G_{te} decreased from 16.1 to 7.4 $mScm^{-2}$. Likewise upon the addition of 10 mM Cs^{+}, G_{te} decreased from 17.2 to 11.2 $mScm^{-2}$. In general, 5 mM Ba^{++} appeared to be more effective in reducing G_{te} than 10 mM Cs^{+}.

While the addition of Ba^{++} or Cs^{+} to the luminal fluid consistently caused a decrease in the G_{te}, the magnitude of the response was highly variable. As shown in Fig. 2, the variations in the magnitude of the Ba^{++}-sensitive G_{te} were directly correlated with the control G_{te} before the addition of Ba^{++}. Hence, part of the wide variations in G_{te} from tubule to tubule (O'Neil & Boulpaep, 1982) may reflect a variable Ba^{++}-sensitive conductance of the apical cell membrane.

It has been shown previously that the apical cell membrane of the cortical collecting duct possesses a major K^{+} conductance, and, as a result, elevation of K^{+} in the luminal fluid causes a hyperpolarization of the V_{te} (lumen negative potential) and an increase in G_{te} (O'Neil & Boulpaep, 1979, 1982; Koeppen et al., 1983). This response to K^{+} was therefore used to evaluate the effect of Ba^{++} on the apical cell membrane K^{+} conductance. The results of elevating K^{+} in the luminal perfusate (choline-K^{+} substitution) from 5 to either 25 or 50 mM are summarized in Fig. 3. The change in the V_{te} upon elevation of K^{+} in the luminal fluid averaged -11.6 mV before the addition of Ba^{++} and only -6.9 mV after the addition of 5 mM Ba^{++} to the perfusate. Likewise the increase in the G_{te} upon elevation of K^{+} was decreased from 13.3 $mScm^{-2}$ before the addition of Ba^{++} to only 2.2 $mScm^{-2}$ after the addition of Ba^{++} to the perfusate. Clearly, luminal addition of Ba^{++} causes a pronounced reduction in the apparent K^{+} conductance of the luminal border.

Basolateral Cell Membrane and Tight Junction Properties

In contrast to the effect of K^{+} and Ba^{++} at the luminal border, these two cations had only minimal effects at the peritubular border. As shown in Table 2, elevation of K^{+} from 5 to 50 mM in the peritubular bath caused only a small depolarization of the V_{te} of 2.6 mV and a modest increase in the G_{te} of 0.9 $mScm^{-2}$. Likewise, the addition of 5 mM Ba^{++} to the bath was accompanied by a modest depolarization of V_{te} of 0.9 mV and a decrease in G_{te} of 0.4 $mScm^{-2}$. These results are most consistent with the notion that the basolateral cell border and tight junction have a *relatively* low K^{+} permeability.

Interaction between Ba^{++} and Amiloride

In many of the present studies, 50 μM amiloride was added to the luminal perfusate to abolished

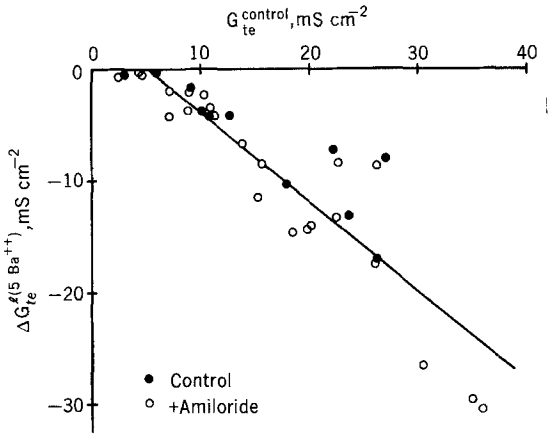

Fig. 2. Relation between the initial control transepithelial conductance, $G_{te}^{control}$, and the luminal Ba^{++}-sensitive conductance, $\Delta G_{te}^{l(5\ Ba^{++})}$. Tubules were perfused either in the absence (control) or presence (+amiloride) of 50 μM amiloride in the perfusate. The equation of the linear regression of $\Delta G_{te}^{l(5\ Ba^{++})}$ on $G_{te}^{control}$ is:

$\Delta G_{te}^{l(5\ Ba^{++})} = -(0.81 \pm 0.07)\ G_{te}^{control} + (4.3 \pm 1.3)$

$N = 34$, $r = 0.902$

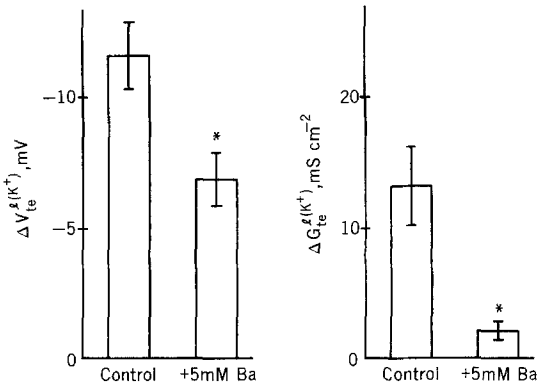

Fig. 3. Influence of luminal addition of Ba^{++} (5 mM) on the response to luminal elevation of K^{+} from 5 to either 25 or 50 mM. In all cases, the response of V_{te}, $\Delta V_{te}^{l(K^+)}$, and G_{te}, $\Delta G_{te}^{l(K^+)}$, to elevation of K^{+} before the addition of Ba^{++} (control) is reduced after the addition of Ba^{++} (+5 mM Ba^{++}). $N = 13$

Table 2. Summary of the effects of bathing medium K^{+} and Ba^{++}

A. Influence of K^{+} elevation in bath ($N = 5$)

	Control (5 mM K^{+})	+50 mM K^{+}	Difference
V_{te}, mV	−11.2±8.6	−8.6±9.1	2.6±0.6[a]
G_{te}, mScm^{-2}	13.7±3.2	14.6±3.6	0.9±0.4

B. Influence of Ba^{++} addition to bath ($N = 7$)

	Control	+5 mM Ba^{++}	Difference
V_{te}, mV	−10.5±7.0	−9.6±7.0	0.9±0.6
G_{te}, mScm^{-2}	17.1±3.3	16.7±3.2	−0.4±0.1[a]

[a] $P < 0.05$.

Table 3. Influence of amiloride on the steady-state response to Ba^{++}

A. Influence of luminal addition of Ba^{++} (5 mM) in the absence of amiloride[a]

	−Amiloride		
	−Ba^{++} (Control)	+Ba^{++}	Difference
V_{te}, mV	−10.6±5.9	−12.4±5.1	−1.8±1.4
G_{te}, mScm^{-2}	16.2±2.8	9.1±1.5	−7.1±2.0[b]

B. Influence of luminal addition of Ba^{++} (5 mM) in the presence of 50 μM Amiloride[a]

	+50 μM amiloride		
	−Ba^{++}	+Ba^{++}	Difference
V_{te}, mV	4.6±1.3	3.2±0.9	−1.4±0.7
G_{te}, mScm^{-2}	15.4±2.8	7.9±1.5	−7.5±1.9[b]

[a] Paired studies, $N = 10$ [b] $P < 0.01$.

active Na^{+} transport. It was of interest to determine whether amiloride and Ba^{++} may interact in this system by assessing the effect of amiloride on the tissue's response to Ba^{++} and vice versa. The results are summarized in Table 3. The steady-state changes in V_{te} and G_{te} upon the addition of 5 mM Ba^{++} to the perfusate were similar in the presence or absence of amiloride. Most importantly, the decrease in G_{te} averaged 7.1 mScm^{-2} in the absence of amiloride and 7.5 mScm^{-2} in the presence of amiloride, indicating little or no effect of amiloride on the tissue response to Ba^{++} (*see* Discussion).

To investigate further a possible interaction between Ba^{++} and amiloride at the luminal border, the response of the tissue to addition of 50 μM amiloride to the perfusate was compared in the presence and absence of 5 mM Ba^{++} in the perfusate (Table 4). The changes in V_{te} and G_{te} upon addi-

Table 4. Effects of luminal Ba^{++} on the steady-state response to amiloride

A. Influence of luminal addition of amiloride (50 μM) in the absence of Ba^{++} [a]

	$-Ba^{++}$		
	−Amiloride (control)	+Amiloride	Difference
V_{te}, mV	−17.9±8.8	3.8±1.5	21.7±8.6[b]
G_{te}, $mScm^{-2}$	14.6±3.9	13.2±3.7	−1.4±0.3[b]

B. Influence of luminal addition of amiloride (50 μM) in the presence of Ba^{++} [a]

	+5 mM Ba^{++}		
	−Amiloride	+Amiloride	Difference
V_{te}, mV	−17.6±7.7	3.7±1.2	21.3±7.5[b]
G_{te}, $mScm^{-2}$	8.0±1.8	6.4±1.8	−1.5±0.4[b]

[a] Paired studies, $N=6$ [b] $P<0.05$.

tion of amiloride averaged 21.7 mV and 1.4 $mScm^{-2}$, respectively, in the absence of Ba^{++} and remained unchanged after the addition of 5 mM Ba^{++} to the perfusate. As pointed out in the Discussion, while the effects of Ba^{++} on the tissue's response to amiloride appear to be minimal this is not to say that Ba^{++} does not influence Na^{+} transport in this tissue.

Voltage-Dependent Effects of Ba^{++} and Cs^{+}

The effects of Ba^{++} and Cs^{+} on the G_{te} were observed to be highly voltage dependent, particularly upon passage of negative current pulses where V_{te} became more lumen negative. As a consequence, estimates of the G_{te} upon injection of negative current pulses were not valid (*see* Materials and Methods). Hence, in order to evaluate the voltage-dependent effects of Ba^{++} and Cs^{+}, the input characteristics of the tubule were studied. This was done by evaluating the relation between the current injected into the perfusion end of the tubule, I_o, and the resulting voltage recorded at that point, V_o. Representative examples showing the effects of luminal addition of Ba^{++} and Cs^{+} on the input current-voltage relation ($I_o - V_o$) of the tubule are shown in Fig. 4*a* and *b*. In the absence of Ba^{++} or Cs^{+} the control $I_o - V_o$ relation was only slightly curvilinear; however, in the presence of either Ba^{++} or Cs^{+} the $I_o - V_o$ relation was highly curvilinear, particularly in the lumen-negative voltage region. It should be noted that in previous studies it was demonstrated that sharp nonlinearities in the $I_o - V_o$ plot existed, particularly at voltages near −120 mV, lumen negative, and to a lesser extent at voltages of near 45 mV, lumen positive. The present $I_o - V_o$ plots differ from the previous studies in that the voltage range did not extend to −120 mV, lumen negative, and when "bends" occurred near 45 mV, lumen positive, the $I_o - V_o$ relation was fitted with a smooth curve.

The voltage-dependent effects of Ba^{++} and Cs^{+} on the conductance of the tissue were particularly apparent from the relation between the input slope conductance, G_o (obtained from the slope of the $I_o - V_o$ relation), and V_o. As can be seen from the data of Fig. 5, the control G_o was slightly voltage dependent. In the presence of 0.1 mM Ba^{++} in the luminal fluid, the G_o was highly voltage dependent, being similar to the control value at −50 mV and approaching a minimum G_o at 50–75 mV, lumen positive. In the presence of 5 mM Ba^{++} in the luminal perfusate the G_o was still voltage dependent in the lumen-negative region, but approach a constant maximum value for all lumen-positive voltages. Likewise, in the presence of 10 mM Cs^{+} in the luminal perfusate, the G_o was particularly voltage dependent between −50 and +50 mV.

It is apparent from both Figs. 4 and 5, that in the presence of 10 mM Cs^{+} or 5 mM Ba^{++} in the perfusate, the effects of these cations on the conductance were relatively voltage independent for the more positive voltages. Hence, the use of cable equations to estimate the G_{te} in this study were only valid upon passage of large positive current pulses as was done througout the study (*see* Materials and Methods).

Time-Dependent Voltage Polarizations

When a constant current pulse was injected into the tubule lumen, the voltage response was often ohmic and approached a new steady-state voltage within a few msec. In some tubules, particularly those with a high G_{te}, the voltage response to constant current injection was initially abrupt, supposedly reflecting the ohmic properties of the tissue, but then slowly continued to polarize with continued current injection into the lumen as shown in Fig. 6*A*. This time-dependent polarization did not appear to be related to changes in the conductance of the tissue as the ohmic abrupt step-changes at

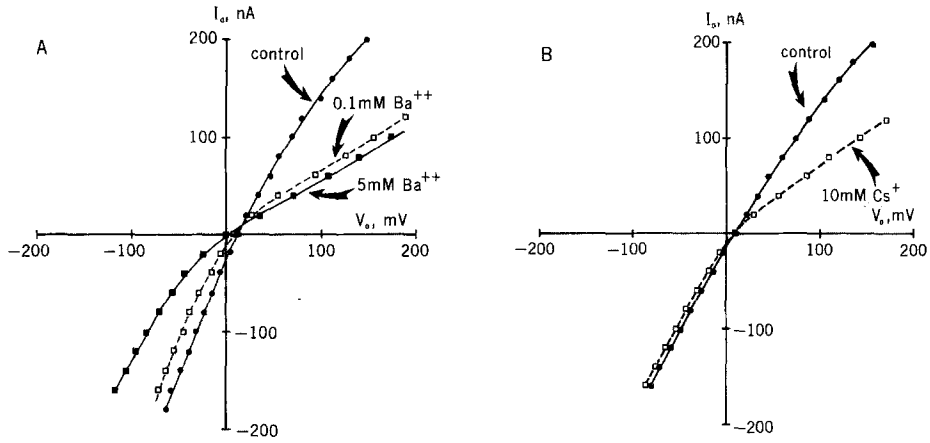

Fig. 4. Relation between the input current, I_o, and input voltage, V_o. (*A*): Influence of luminal addition of Ba^{++}. (*B*): Influence of luminal addition of Cs^{+}

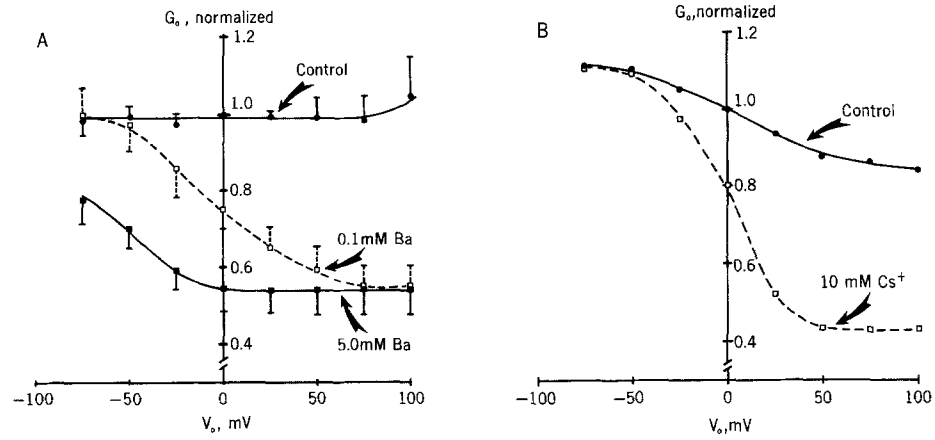

Fig. 5. Relation between the input slope conductance, G_o, and the input voltage, V_o. The G_o was obtained from the slope of the $I_o - V_o$ relation, $G_o = \Delta I_o / \Delta V_o$. (*A*): Influence of luminal addition of Ba^{++} ($N=7$). (*B*): Influence of luminal addition of Cs^{+} in one tubule

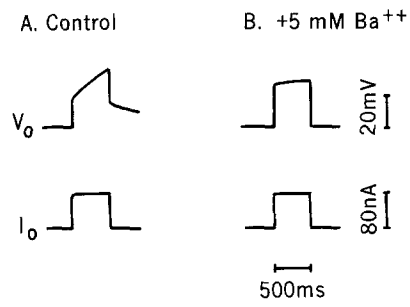

Fig. 6. Influence of luminal Ba^{++} on the current-induced voltage polarizations. (*A*): Example of the voltage polarization to a 500-msec current pulse in the absence of Ba^{++}. (*B*): Voltage polarization to a 500-msec current pulse in the presence of 5 mM Ba^{++} in the perfusate in the same tubule as in *A*

the beginning and end of the current pulse were similar as has been noted previously (Helman et al., 1971; Koeppen et al., 1983). The addition of Ba^{++} to the luminal fluid appeared to markedly reduce this polarization phenomenon (Fig. 6*B*), in agreement with the report by Koeppen et al. (1983), indicating that it is likely related to K^{+} movements across the apical cell border. The origin of the polarization would be most consistent with the view that upon passage of current, K^{+} movements bring about changes in the K^{+} concentration gradient across the K^{+} permeable apical cell border, thereby altering the K^{+} diffusion potential across that border and, in turn, the apical cell membrane and transepithelial potential difference. The involvement of the basolateral membrane in this phenomenon remains to be assessed for the cortical collecting duct.

Discussion

The present study provides additional evidence that the apical (luminal) cell border of the cortical collecting duct (tubule) possess a high K^{+} conductance. Previous studies demonstrated that a significant K^{+} permeability of the luminal border could be attributed to a high K^{+} conductance of the apical cell membrane and not of the tight junction (O'Neil & Boulpaep, 1979, 1982), a view recently confirmed using microelectrode techniques (O'Neil, 1982*c*; Koeppen et al., 1983). This apical

cell membrane K^+ conductance was readily blocked by the luminal addition of either Ba^{++}, as shown also by Koeppen et al. (1983), or Cs^+. A similar action of Ba^{++} and Cs^+ has been reported for K^+ conductive pathways from a variety of tissues, including both epithelial tissues (e.g., Nielsen, 1979; Wills et al., 1979; Nagel & Hirschmann, 1980; Gogelein & Van Dreissche, 1981; Greger, 1981; Planelles et al., 1981; Koeppen et al., 1983) and nonepithelial tissues alike (e.g., Hagiwara et al., 1976; Sperelakis et al., 1977; Standen & Stanfield, 1978; Coronada & Miller, 1979; Armstrong & Taylor, 1980; Eaton & Brodwick, 1980). Thus, the effects of Ba^{++} and Cs^+ would appear to be selective for K^+ conductive pathways, regardless of the tissue.

Nature of the Interaction of Ba^{++} and Cs^+ with the K^+ Conductance of the Apical Cell Membrane

An important feature of the blocking action of Ba^{++} and Cs^+ is that their effect on the tissue conductance was highly voltage dependent. This was particularly evident in the presence of either 0.1 mM Ba^{++} or 10 mM Cs^+ where a very steep voltage-dependent block was observed (*see* Fig. 5). With a lumen-negative voltage of -50 mV, essentially no blockage was observed, while a maximum block was observed with lumen-positive voltage of 50 to 75 mV. Where such a steep voltage-dependent block of K^+ channels has been observed in excitable tissues (*see* Eaton & Brodwick, 1980), this phenomenon has been explained as a binding site for Ba^{++} and Cs^+ which is located deep within the membrane channel so that changes in the membrane voltage influence the probability of finding Ba^{++} and Cs^+ at the blocking site. Furthermore, in preliminary studies it has been shown that with increasing K^+ concentration in the luminal fluid, the effects of Cs^+ on G_{te} are greatly diminished, indicating competition between Cs^+ and K^+ for a common site (R.G. O'Neil, *unpublished*). A similar competition has been reported between Cs^+ and K^+, as well as between Ba^{++} and K^+, in other tissues (Zieske & Van Driessche, 1979; Eaton & Brodwick, 1980). Hence the nature of the interaction of Ba^{++} and Cs^+ with the K^+ conductive pathway in the apical cell border of the cortical collecting duct appears to be very similar in some respects to that of K^+ channels in excitable tissues.

The above properties of the apical cell membrane K^+ conductive pathway cannot be reconciled readily with a K^+ pathway in which ions move via free diffusion through an aqueous pore. Rather, as discussed in detail for some K^+ channels in excitable tissues (Adelman & French, 1978; Hille & Schwarz, 1978; Begenisich & De Weer, 1980; Labarca & Miller, 1981) the properties would be most consistent with a single- or multisite channel in which ions move across the membrane by "jumping" from site to site, i.e. single-file diffusion, and where at some point through the channel, the movements of Ba^{++} and Cs^+ are restricted, supposedly via steric hindrance. This model could account for the steep voltage dependence of the Ba^{++} and Cs^+ block of the channel as well as the apparant competition for K^+ and Cs^+ (and supposedly between K^+ and Ba^{++}) for a common site. Clearly, extensive testing will be required to investigate the validity of this model for K^+ movements in the cortical collecting duct.

Cell Membrane Sidedness of Ba^{++} and Cs^+ Inhibition

It has been shown recently in squid axon that K^+ channels are exquisitely sensitive to internal Ba^{++} (Eaton & Brodwich, 1980). Hence a small leakage flux of Ba^{++} into a cell could block K^+ channels by binding to a site accessible from the inside of the cell only. From consideration of the voltage dependence of the Ba^{++} block in the cortical collecting duct, Ba^{++} would appear to exert its blocking action by binding to a site accessible from the luminal side of the cell only. With increasing lumen-positive voltages, the block by Ba^{++} increases as would be anticipated if Ba^{++} were to move from the lumen toward the cell interior, down its electrochemical gradient. In contrast, if the site of Ba^{++} blockage of the K^+ channel was accessible from the interior of the cell, a lumen-positive voltage would favor Ba^{++} movement from the blocking site into the cell interior so that the Ba^{++}-block would decrease. Since the opposite dependence on voltage was observed for both Ba^{++} and Cs^+, it is most probable that the blocking site is accessed from the lumen side of the apical cell membrane.

Relation Between Apical Membrane Na^+ and K^+ Conductances

The apical cell border of the cortical collecting duct possesses both a Na^+ and K^+ conductance. Since there would appear to be little direct interaction between these two ions, this may indicate that Na^+ and K^+ traverse this membrane via spatially separate paths. This is supported both by the results of earlier studies in which amiloride was shown

to block selectively the Na^{+} conductance without influencing the apparent K^{+} conductance (O'Neil & Boulpaep, 1979) and by the results of the present study in that amiloride did not appear to influence the effects of Ba^{++} on the G_{te} – recognizing, of course, that the effects of amiloride on G_{te} are relatively small compared to those of Ba^{++}. Likewise it may superficially appear that Ba^{++} did not influence the apical cell membrane Na^{+} transport in the present study since Ba^{++} addition to the luminal fluid did not influence the amiloride-sensitive G_{te}. This result may be somewhat misleading, however, since it can be shown from circuit analysis of an epithelium that blocking a major ion pathway in parallel with the Na^{+} conductive pathway at the apical cell border should result in an increased amiloride-sensitive G_{te}. Since this was not observed after Ba^{++} addition, it is possible that Ba^{++} either directly or indirectly caused a modest reduction in the apical cell membrane Na^{+} conductance. Regardless, the primary effects of Ba^{++} and Cs^{+} at the apical cell border would appear to be on the K^{+} conductance, while that of amiloride would appear to be on the Na^{+} conductance, in accord with the results of other studies (O'Neil & Boulpaep, 1979; O'Neil, 1982*c*; Koeppen et al., 1983). These results are supportive of the view that the Na^{+} and K^{+} channels of the cortical collecting duct apical cell membrane are spatially distinct pathways.

While the major action of luminal Ba^{++} and Cs^{+} in the cortical collecting duct would appear to be on the apical cell membrane K^{+} conductance, other effects, such as changes in the tight junction conductance, cannot be unequivocally ruled out. In this regard, Reuss, Cheung and Grady (1981) have reported that Ba^{++} may cause a small decrease (approx. 10%) in the tight junction conductance of the gallbladder. Since in the present study addition of Ba^{++} to the bathing medium had little influence on G_{te}, a direct action of luminal addition of Ba^{++} on the tight junction conductance seems unlikely. However, indirect effects of Ba^{++} (and Cs^{+}) are possible on this or other conductive pathways. This remains to be evaluated in separate studies.

Significance of a High Apical Cell Membrane K^{+} Conductance

The K^{+} conductance of the basolateral cell membrane and tight junction would appear to be relatively low in the cortical collecting duct since the changes in both the V_{te} and G_{te} were relatively small upon elevation of K^{+} in the bath or upon addition of Ba^{++} to the bathing media. This is consistent with the results of previous studies (O'Neil & Helman, 1977; O'Neil & Boulpaep, 1982). Others, however, have noted that elevation of K^{+} in the bath can bring about a significant depolarization of the V_{te} and basolateral membrane potential difference (Grantham et al., 1970; Koeppen et al., 1983). The reason for the differences among studies is not known, but these differences serve to point out that the basolateral cell membrane K^{+} conductance may be variable like other ionic conductive pathways in this tissue (O'Neil & Boulpaep, 1982). Nonetheless, the dominant K^{+} conductance in this tissue would appear to be at the apical cell border.

The physiological importance of a low K^{+} conductance of the basolateral cell membrane relative to that of the apical cell membrane, may poise the cortical collecting tubule for K^{+} secretion. There is considerable evidence that K^{+} uptake into the cell is active and occurs via the Na^{+}:K^{+} exchange pump at the basolateral cell membrane (*see* O'Neil, 1981). Since a major K^{+} conductance resides at the apical cell border, K^{+} movement from the cell into the tubular lumen would be favored, particularly in the presence of a lumen-negative V_{te}, bringing about net K^{+} secretion. Furthermore, simply regulating this apical cell membrane K^{+} conductance could bring about regulation of K^{+} secretion. Indeed, it has been shown in preliminary studies that reducing the luminal pH not only reduces the rate of K^{+} secretion into the tubular lumen (Boudry, Stoner & Burg, 1976) but also reduces the apical cell membrane K^{+} conductance (O'Neil, 1982*b*). Thus, it is likely that regulation of this K^{+} conductance serves as a major site for regulation of K^{+} secretion.

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