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Basolateral Membrane Potassium Conductance of A6 Cells

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Summary. To study the properties of the basolateral membrane conductance of an amphibian epithelial cell line, we have adapted the technique of apical membrane selective permeabilization (Wills, N.K., Lewis, S.A., Eaton, D.C. 1979*b*, *J. Membrane Biol.* **45**:81–108). Monolayers of A6 cells cultured on permeable supports were exposed to amphotericin B. The apical membrane was effectively permeabilized, while the high electrical resistance of the tight junctions and the ionic selectivity of the basolateral membrane were preserved. Thus the transepithelial current-voltage relation reflected mostly the properties of the basolateral membrane. Under "basal" conditions, the basolateral membrane conductance was inward rectifying, highly sensitive to barium but not to quinidine. After the induction of cell swelling either by adding chloride to the apical solution or by lowering the osmolarity of the basolateral solution, a large outward-rectifying K^+ conductance was observed, and addition of barium or quinidine to the basolateral side inhibited, respectively, 82.4 ± 1.9% and 90.9 ± 1.0% of the transepithelial current at 0 mV. Barium block was voltage dependent; the half-inhibition constant (K_i) varied from 1499 ± 97 μM at 0 mV to 5.7 ± 0.5 μM at −120 mV.

Cell swelling induces a large quinidine-sensitive K^+ conductance, changing the inward-rectifying basolateral membrane conductance observed under "basal" conditions into a conductance with outward-rectifying properties.

Key Words A6 cells · amphotericin B · basolateral membrane · K^+ conductance · cell volume · barium · quinidine

Introduction

In Na^+-reabsorbing epithelia, according to the model of Ussing (Koefoed-Johnsen & Ussing, 1958), the basolateral potassium conductance is the recirculating pathway for K^+ accumulated into the cell by the basolateral Na^+,K^+-ATPase. Indeed, the basolateral membrane conductance of a number of different epithelial cells has been shown to be largely potassium selective (Reuss et al., 1984). This K^+ conductance is involved in the regulation of the cell volume (Dawson, 1987; Eveloff & Warnock, 1987).

In the basolateral membrane of Na^+-reabsorbing epithelia, two types of K^+ conductances have been described: a "basal" or "resting" conductance and a volume-activated conductance observed only when the cells are swollen (Germann et al., 1986*b*; Dawson & Richards, 1990); similar results have been reported in the chloride-secreting tracheal epithelium (Butt, Clapp & Frizzell, 1990). Both conductances are blocked by barium, but only the volume-activated K^+ conductance is sensitive to quinidine.

In tight epithelia, patch-clamp technique has allowed the description of a number of basolateral K^+ channels with different voltage dependence (Lewis & Hanrahan, 1985; Richards & Dawson, 1986; Taniguchi, Yoshitomi & Imai, 1989). The relative importance of these channels in the macroscopic conductances is not completely understood (Dawson, 1987).

The study of the voltage dependence of the basolateral membrane K^+ conductance is complicated by the presence of the "in series" apical membrane high resistance. Depending on the techniques or the preparations, different types of voltage dependence have been observed. Direct observation of the current-voltage (*I-V*) relation of the basolateral membrane conductance can be obtained when intracellular potential measurements are possible (Wills, Lewis & Eaton, 1979*b*; Thompson, Suzuki & Schultz, 1982; Thomas et al., 1983; Schoen & Erlij, 1985; Horisberger & Giebisch, 1988); in these studies, inward-rectifying or linear conductances have been found. Alternatively, the basolateral membrane conductance can be studied when the apical membrane resistance is lowered enough by the use of ionophores or detergents (Lewis et al., 1977). This method has been used in some natural tight epithelia such as the rabbit urinary bladder (Lewis & Wills, 1982), the rabbit colon (Wills et al., 1979*a*,*b*), the turtle colon (Germann et al., 1986*b*;

Table 1. Composition of the perfusing solutions (in mM)

	Na	K	Ca	Mg	Gluconate	Cl	SO_4	HCO_3	H_2PO_4	MOPS	Sucrose
Apical solutions:											
No Cl^-: (A1)	1	94	—	1	94	—	1	—	—	10	—
25 mM Cl^-: (A2)	1	94	1.8	1	73.6	25	—	—	—	10	—
Basolateral solutions:											
Control: (B1)	100.8	3	1.8	1	58.6	25	—	25	0.8	—	—
High K^+: (B2)	100.8	12	1.8	1	67.6	25	—	25	0.8	—	—
Sucrose: (B3)	37.2	3	1.8	1	—	20	—	25	0.8	—	119.2
Low osM: (B4)	37.2	3	1.8	1	—	20	—	25	0.8	—	—

Dawson, 1987), the toad urinary bladder (Reuss, Gatzy & Finn, 1978; Lewis et al., 1985*b*) or the frog skin (Garty, 1984). Investigators using the selective permeabilization of the apical membrane have reported the presence of an outward-rectifying K^+ conductance in the basolateral membrane (Lewis & Wills, 1982).

Our study was designed to determine the voltage dependence, the physiological and pharmacological characteristics of the basolateral membrane K^+ conductance of a tight epithelium in culture, using known K^+ channel blockers. For this purpose, we have adapted the apical membrane permeabilization technique to the A6 cell line deriving from the *Xenopus laevis* kidney. The A6 cells exhibit many properties of the naturally occurring Na^+-reabsorbing tight epithelia (Handler et al., 1979). They have amiloride-sensitive Na^+ channels in their apical membrane (Hamilton & Eaton, 1986) and basolateral Na^+,K^+-ATPase (Verrey et al., 1987). Little is known concerning their basolateral K^+ conductance, except that it contains a barium-sensitive K^+ conductance (Granitzer et al., 1991).

Materials and Methods

Cell Culture

A6 cells were obtained from the American Tissue Type Collection and cloned by limiting dilution (clone A6–2F3: Verrey et al., 1987). Cells at passages 88–98 were grown in a humidified atmosphere of 5% CO_2 in air at 28° C in an amphibian medium (Handler et al., 1979) supplemented with 5% fetal calf serum (PAA, Linz, Austria), 100 U/ml penicillin G and 130 μg/ml streptomycin.

The Transwell® permeable supports of 4.7 cm^2 (Costar, Cambridge, MA) were prepared as follows: the nitrocellulose filters were coated with rat tail collagen (Paccolat et al., 1987). After a 30-min polymerization with ammonia vapor, the supports were allowed to dry for 3 hr. The collagen was then fixed and sterilized with 2.5% glutaraldehyde and washed extensively with water and culture medium. Then the cells were seeded on these permeable supports at a density of 2×10^6 cells/cm^2.

One day after seeding, the culture medium on both sides of the supports was changed and 10^{-7} M dexamethasone (Sigma, St. Louis, MO) was added according to the protocol of Preston, Muller and Handler (1988).

The cells were used for electrical measurements between 7 and 29 days of culture on permeable supports.

Solutions and Drugs

The compositions of the solutions used to perfuse the apical and the basolateral sides of the epithelia are given in Table 1. Variations in Cl^- concentration of the apical media were used to induce or not cell swelling after the permeabilization of the apical membrane (Germann, Ernst & Dawson, 1986*a*). In the basolateral medium, a constant Cl^- concentration of 25 mM was used to prevent excessive cell swelling and to reduce Cl currents. To study the cell volume changes induced by another method, two other basolateral solutions (B3, B4) of different osmolarities were used. In B3, 58.6 mM of Na gluconate were replaced by 119.2 mM sucrose to keep the osmolarity similar to B1 (205 ± 1 mosM), and in B4, sucrose was omitted yielding an osmolarity of 83 ± 1 mosM. The basolateral bicarbonate-containing solutions were gassed with 95% O_2 and 5% CO_2 (pH 7.4). The pH of the [3-[N-morpholino]propanesulfonic acid] (MOPS)-buffered apical solutions was adjusted to 7.0 with NaOH. All the experiments were performed with solutions at a temperature of 28°C.

From a stock solution of 5 mg/ml, amphotericin B (Fungizone®, Squibb: amphotericin 50 mg/Na-cholate/41.2 mg/$NaHPO_4$ 2.52 mg) was added to the apical solution to final concentrations of 2.7 or 10.8 μM. All solutions containing amphotericin B were protected from light. Barium was added as $BaCl_2$ to the basolateral solution while keeping Na^+ and Cl^- concentrations constant by adjustment of the NaCl and Na-gluconate concentrations. When used, quinidine (Sigma) as quinidine-chloride was added to the basolateral solution.

Electrical Measurements

The electrical measurements were performed in a modified Ussing chamber allowing the continuous perfusion of both the apical and basolateral sides at a flow rate of 10 ml/min. The volume of the chamber was 3 ml for the apical part of the chamber and 5 ml for the basolateral part. Solutions could be exchanged without interruption of the electrical measurements.

Transepithelial potential was measured through Hg-HgCl half-cells connected to the apical and basolateral sides of the epithelium through 1 M KCl-3% agar bridges in polythene tubing

and referenced to the basolateral side. By convention, positive current was flowing from the apical to basolateral side. Current was injected through 2-mm internal diameter polythene tubing filled with 1 M KCl-3% agar.

The electrodes were connected to a current-voltage clamp apparatus (Physiologic Instrument VCC 600, San Diego, CA). Transepithelial current (I_t) and transepithelial voltage (V_t) were continuously recorded on a chart-paper recorder (Graphtec WR 7500, Tokyo, Japan). The series solution resistance was measured with a test-permeable support at the start of the experiment and compensated for by the voltage-clamp apparatus.

The Transwell® filter supporting the cell monolayer was placed in the measurement chamber, and the V_t was first measured in the presence of the culture medium on the apical side and the control basolateral solution (B1, Table 1). We limited our experiments to monolayers producing a V_t larger than 30 mV (apical negative). Then the apical solution (A1 or A2, Table 1) was perfused, and V_t was clamped at −60 mV (a voltage value that approximates the physiological basolateral voltage (Granitzer et al., 1990)). The short-circuit current (I_{sc}) was measured every minute by clamping the V_t to zero for 3 sec.

The transepithelial (chord) conductance (G_t) was calculated from the difference between the transepithelial current (ΔI_t) at −60 and at 0 mV:

$$G_t = \Delta I_t / \Delta V_t. \quad (1)$$

Current-voltage (I-V) curves were obtained by generating square pulse voltage changes and monitoring the corresponding currents. Starting from −60 mV the holding potential was set for 3-sec periods to 0, −20, and −40 mV and then to −80, −100, and −120 mV with a return to −60 mV between each step.

Known inhibitors of the K^+ channels were studied by adding increasing concentrations of the blockers to the basolateral solution. I-V curves were obtained 5 min after solution change. The log concentration-effect relationships of barium and quinidine were analyzed by fitting the results to a single site model:.

$$\text{Inhibition} = \text{Inh}_{max} \cdot C/(K_i + C) \quad (2)$$

where Inh_{max} is the maximal inhibition, C is the inhibitor concentration, and K_i is the half-inhibition constant. The best fit was obtained by a least-square fit method using a simplex algorithm (Nelder & Mead, 1965).

The data are presented as mean values ± SEM. Differences between means were analyzed using the Student's t test for paired experiments unless otherwise specified.

Results

Apical Membrane Permeabilization

The result of a typical permeabilization of the apical membrane of the A6 cells is shown in Fig. 1*A* and *C*. The addition of amphotericin B to the chloride-free apical solution (A1, Table 1) induced an increase of the I_{sc} from 3 to 11 $\mu A/cm^2$. The transepithelial conductance (G_t) followed a similar increase (from 0.06 to 0.20 mS/cm^2). Both variables stabilized simultaneously after about 4 min (mean 4.3 ± 0.1 min in 21 experiments). According to the equivalent circuit analysis (Wills et al., 1979*b*), a selective increase of the apical membrane conductance (G_a) would result in proportional changes of G_t and I_{sc} corresponding to the following equation:

$$G_t = (1/\text{EMF}_{bl}) \cdot I_{sc} + G_s \quad (3)$$

where EMF_{bl} is the electromotive force of the basolateral membrane and G_s is the conductance of the paracellular "shunt." Equation (3) indicates that the relation of G_t *versus* I_{sc} is linear if EMF_{bl} and G_s are not modified.

Indeed, during exposure to amphotericin B as shown on Fig. 1*C*, G_t increased linearly with I_{sc}. A regression analysis of the G_t *versus* I_{sc} plot yields an EMF_{bl} (slope^{-1}) of −63.1 mV and a shunt conductance (G_s) (corresponding to the zero G_t intercept) of 0.012 mS/cm^2. In all experiments (n = 21), the G_t *versus* I_{sc} plot was linear (correlation coefficient >0.9995). The mean values of the I_{sc} and conductances before and after amphotericin B are given in Table 2.

The same experiments were repeated in the presence of 25 mM Cl^- in the apical medium (A2, Table 1). A parallel increase of the I_{sc} and G_t was also observed when amphotericin B was added to the apical solution (Fig. 1*B*). However, both increases were much larger than the values observed in the absence of Cl^- and occurred with a slower time course (104 min) before stabilization (mean 106 ± 12 min (SD) in 33 experiments). As in several experiments in the presence of 25 mM Cl^-, 10.8 μM amphotericin induced a loss of the paracellular resistance. Therefore, we modified the permeabilization protocol, as shown in Fig. 1*B*, by decreasing the amphotericin B concentration to 2.7 μM when I_{sc} had reached 20 $\mu A/cm^2$ (after a time range of 10 to 45 min); this solution containing 2.7 μM amphotericin B was perfused throughout the experiment. Using this protocol, no significant difference was observed between the first I_{sc} and G_t values after the initial stabilization, and 90 min later (I_{sc}: 162.3 ± 30.8 $\mu A/cm^2$ *versus* 164.4 ± 30.9 $\mu A/cm^2$; G_t: 2.61 ± 0.50 mS/cm^2 *versus* 2.59 ± 0.51 mS/cm^2; n = 6, NS). Figure 1*D* shows that the I_{sc} *versus* G_t relation also fit with Eq. (3), confirming that neither the paracellular conductance (G_s) nor the basolateral electromotive force (EMF_{bl}) were modified. The mean values of the I_{sc} and conductances are given in Table 2. It should be noticed that the determination of G_s by circuit analysis depends on the assumption of the absence of paracellular current at 0 mV. Our measurements were performed with a 100 to 1 mM basolateral to apical Na^+ gradient and a 94 to 3 mM apical to basolateral K^+ gradient. Our values of G_s

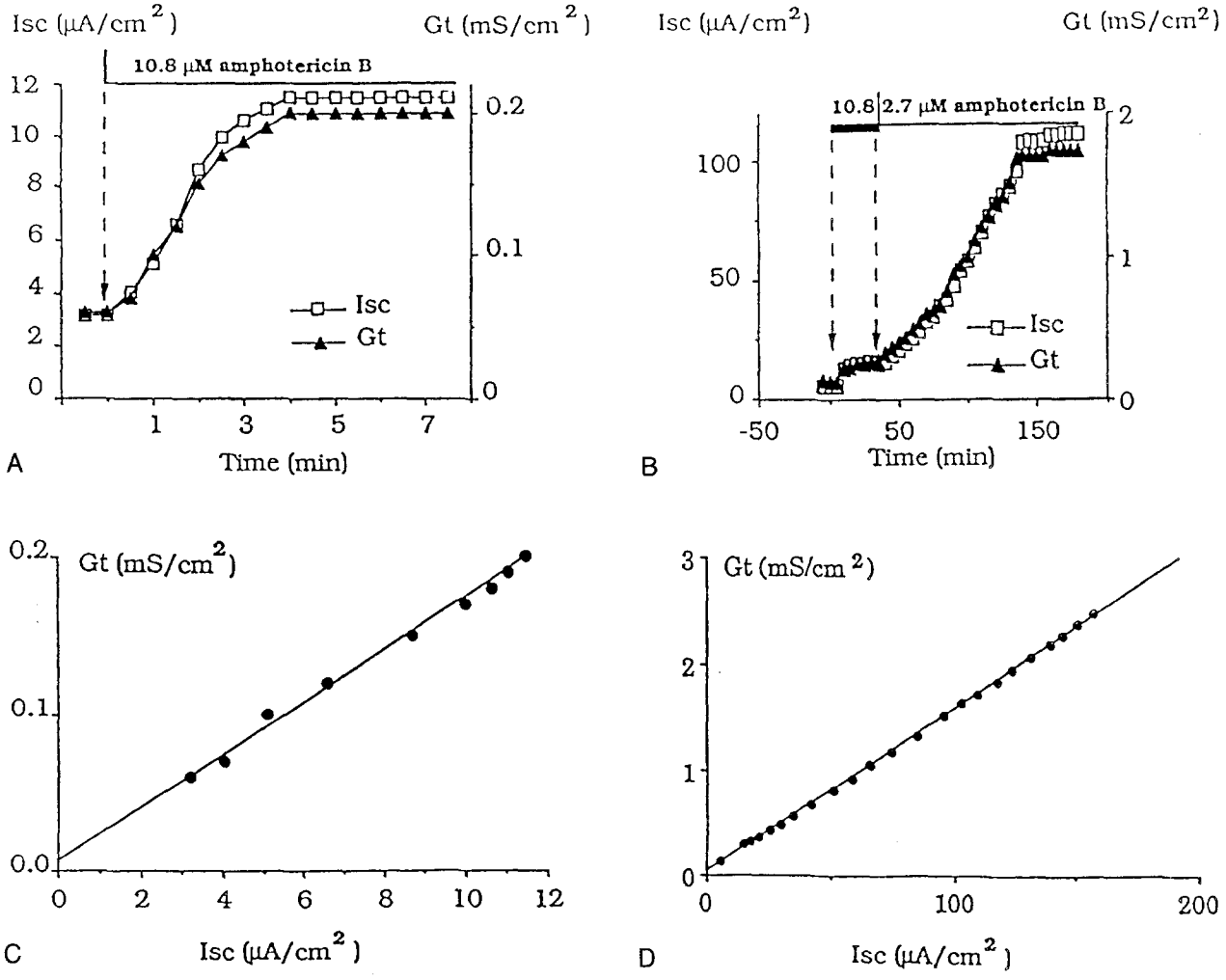

Fig. 1. (*A* and *B*) Time course of amphotericin B effects on the short-circuit current (I_{sc}) and on the transepithelial conductance (G_t). (*A*) In the absence of Cl^- in the apical solution (solution A1, Table 1), amphotericin B was added to the apical solution as indicated. (*B*) In the presence of 25 mM Cl^- in the apical solution (solution A2, Table 1), amphotericin B was added to the apical solution first at 10.8 μM, and, after the I_{sc} had reached 20 μA · cm^{-2}, the concentration of amphotericin B was reduced to 2.7 μM. (*C* and *D*) Plots of the transepithelial conductance (G_t) *versus* the short-circuit current (I_{sc}). Each point represents a simultaneous G_t and I_{sc} measurement during the progressive permeabilization of the apical membrane of the experiments shown in *A* and *B*, respectively. As described in the text, the linearity of the plot indicates the selective effect of amphotericin B on the apical membrane.

Table 2. Effect of amphotericin B on the short-circuit current and the transepithelial conductance in the absence or presence of 25 mM chloride in the apical solution

	Control		Amphotericin B			
	I_{sc} (μA/cm²)	G_t (mS/cm²)	I_{sc} (μA/cm²)	G_t (mS/cm²)	E_{bl} (mV)	G_s (mS/cm²)
No Cl (n = 21)	3.08 ± 0.21	0.07 ± 0.01	11.46 ± 1.14	0.21 ± 0.01	−63.6 ± 1.6	0.025 ± 0.004
25 mM Cl (n = 33)	5.31 ± 0.34	0.12 ± 0.01	156 ± 9	2.46 ± 0.14	−64.6 ± 0.6	0.039 ± 0.05

might have been overestimated if the paracellular current due to the Na^+ gradient was larger than the paracellular current due to the K^+ gradient and conversely. Considering the extremely low values of G_s, an overestimation appears improbable; on the other hand, G_s must be smaller than the total G_t before apical membrane permeabilization (*see* Table 2).

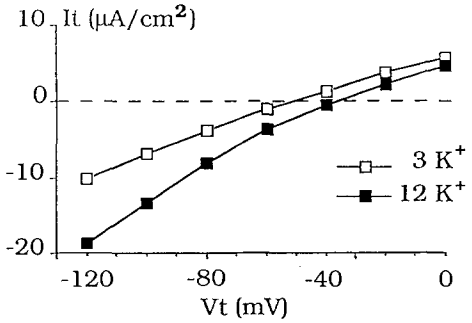

Fig. 2. Transepithelial current-voltage relation under resting conditions, in the presence of 3 mM K^+ (solution B1, Table 1) or 12 mM K^+ (solution B2, Table 1). This *I-V* curve was obtained after permeabilization of the apical membrane by amphotericin B, with a chloride-free apical solution (solution A1, Table 1). In both cases the transepithelial conductance was rectifying towards the apical side.

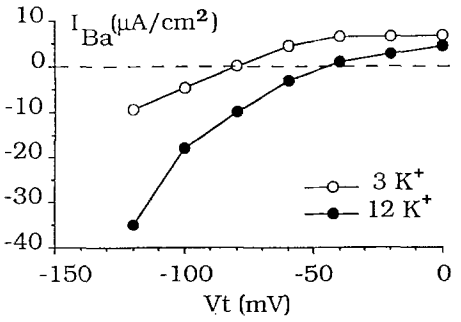

Fig. 3. Current-voltage relation of the Ba^{2+}-sensitive current (I_{Ba}) under "resting" conditions. I_{Ba} is the difference between the current measured in the presence or in the absence of the highest tested Ba^{2+} concentration (5 mM). These measurements were obtained after permeabilization of the apical membrane by amphotericin B, with a chloride-free apical solution (solution A1, Table 1), with a 3 or 12 mM K^+ concentration in the basolateral solution. The reversal potentials of I_{Ba} were −80 and −41 mV, with 3 and 12 mM K^+, respectively (mean values in the text). The *I-V* plot shows that the Ba^{2+}-sensitive conductance of the basolateral membrane is inward rectifying.

The effects of amphotericin B were slowly reversible (a complete reversibility of the amphotericin B effects was obtained when the apical solution without amphotericin B was perfused for about 70 min (n = 2)); therefore, all the experiments were done in the continuous presence of amphotericin B in the apical solution.

Basolateral Conductance Under "Resting" Conditions

After a complete permeabilization of the apical membrane, in the absence of chloride in the apical solution, the transepithelial equilibrium potential was −53.8 ± 1.6 mV (n = 21). The transepithelial *I-V* curves showed an inward-rectifying aspect. The calculated conductances were of 0.034 ± 0.005 mS/cm² between −20 and −40 mV *versus* 0.11 ± 0.01 mS/cm² between −100 and −120 mV, n = 21, $P <$ 0.01 (Fig. 2). The transepithelial *I-V* curves were even more inward rectifying when the K^+ concentration of the basolateral solution was increased to 12 mM K^+ (solution B2, Table 1). Under these conditions, transepithelial conductances of 0.054 ± 0.006 mS/cm² between −20 and −40 mV and of 0.197 ± 0.037 mS/cm² between −100 and −120 mV (n = 7, $P < 0.02$) could be calculated (*see* example in Fig. 2). The equilibrium V_t was −46.1 ± 1.8 mV, a value to compare with a Nernst potential of −52.7 mV for a perfectly K^+-selective membrane.

Barium is a well-known blocker of several types of K^+ channels (Latorre & Miller, 1983), in particular the K^+ channels present in the basolateral membrane of epithelial cells (Germann et al., 1986*a*,*b*; Horisberger & Giebisch, 1988). When this blocker was added to the basolateral solution there was a large dose-dependent reduction of the inward current with a K_i of 1.69 ± 0.28 μM Ba^{2+} (n = 5) and 1.31 + 0.22 μM (n = 6) at −120 mV for K^+ concentrations of 3 and 12 mM, respectively. The effect on outward currents (at low V_t) was small and did not allow for a precise estimation of a K_i. The barium-sensitive current (I_{Ba}: difference of I_t with or without 5 mM Ba^{2+}) was −5.85 ± 0.78 or −45.39 ± 5.86 μA/cm² at −120 mV and was inward rectifying (*see* example in Fig. 3) with a mean equilibrium potential of −78.4 ± 3.4 or −46.1 ± 1.7 mV (n = 7), with 3 and 12 mM K^+, respectively. Quinidine did not induce any detectable reduction of inward current and reduced the outward current by 3.6 ± 0.4 μA/cm² (n = 8) at 0 mV. This effect was very small compared to the inhibition by quinidine of the I_{sc} observed after cell swelling (*see below*).

Chloride-Activated K Conductance

When the apical membrane permeabilization was performed in the presence of 25 mM Cl^-, more then 10 times larger I_{sc} and G_t were observed (Table 2). Thus, under these conditions, the basolateral membrane conductance was largely dominated by another type of conductance apparently activated by the presence of Cl^- in the apical solution. The *I-V* curves of 33 experiments allowed the calculation of an I_{sc} of 156 ± 13 μA/cm² and an equilibrium potential of −69.9 ± 0.9 mV. All the transepithelial *I-V* curves had an outward-rectifying aspect (*see*, for example, the "Control" curve of the experiment

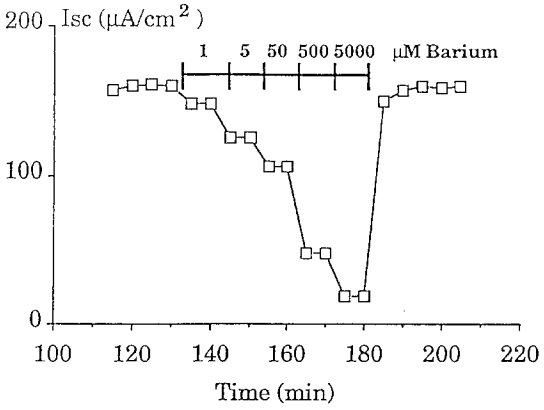

Fig. 4. Time course of Ba^{2+} effect on the short-circuit current (I_{sc}). After a full permeabilization of the apical membrane in the presence of 25 mM Cl , Ba^{2+} was added to the basolateral solution. The abscissa indicates the time starting at the first addition of amphotericin B.

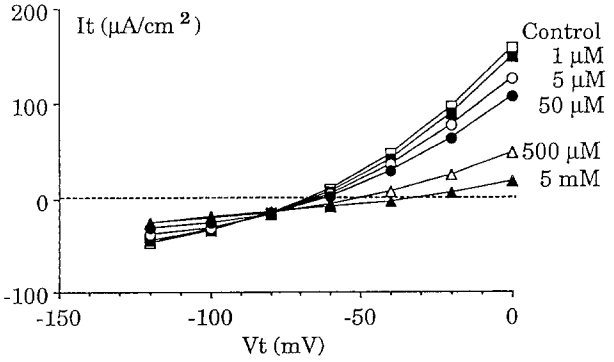

Fig. 5. Effects of Ba^{2+} on the transepithelial current-voltage (I_t/V_t) relation under cell swelling conditions. After apical membrane permeabilization in the presence of 25 mM Cl , increasing Ba^{2+} concentrations were added to the basolateral solution, and transepithelial *I-V* curves were recorded after 5-min equilibration for every tested concentration.

shown in Fig. 5) with conductances of 3.15 ± 0.11 mS/cm² calculated between 0 and −20 mV *versus* 0.85 ± 0.03 mS/cm² between −100 and −120 mV, $n = 33$, $P < 0.001$.

Effect of Barium

When Ba^{2+} was added to the basolateral solution, I_{sc} was instantaneously reduced (Fig. 4). The effect of Ba^{2+} was dose dependent and completely reversible within 5 min. In 25 experiments, 5 mM Ba^{2+} inhibited 82.4 ± 1.9% of the I_{sc}. The equilibrium potential was decreased from −69.1 ± 1.4 to −32.9 ± 1.3 mV ($n = 25$, $P < 0.001$). A family of *I-V* curves were obtained with increasing concentrations of Ba^{2+} (1–5000 μM) in 17 experiments (*see* example in Fig. 5).

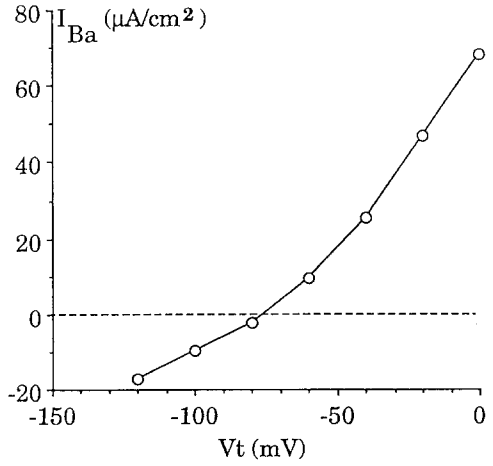

Fig. 6. Current-voltage relation of the Ba^{2+}-sensitive current (I_{Ba}) under cell swelling conditions (with 25 mM Cl in the apical solution (A1, Table 1)). I_{Ba} is the difference between the current measured in the presence or in the absence of the highest tested Ba^{2+} concentration (5 mM).

The Ba^{2+}-sensitive current (I_{Ba}) defined as the difference between I_t with or without 5 mM Ba^{2+} was 131.5 ± 12.8 μA/cm² ($n = 25$) at 0 mV. The I_{Ba} *I-V* curve was outward rectifying (*see* example in Fig. 6) with a mean equilibrium potential of −76.8 ± 0.8 mV ($n = 25$).

Voltage and Time Dependence of Ba^{2+} Block

Log concentration inhibition curves at holding potentials of 0 and −120 mV are shown in Fig. 7. Membrane hyperpolarization induced a left shift of the inhibition curve. K_i was 1499 ± 97 μM Ba^{2+} at 0 mV and 5.7 ± 0.5 μM at −120 mV ($n = 17$, $P < 0.001$). The voltage dependence of the apparent K_i was estimated by computing the regression line of log K_i *versus* V_t. The slope of this line indicated an e-fold change per 24.6 ± 1.7 mV ($n = 17$).

When the holding potential was stepped from −60 mV to a more negative potential, a stable current value was attained immediately, within the time resolution of our measurement system (100 msec). In contrast, as illustrated in Fig. 8*A*, when the holding potential was stepped from −60 to 0 mV there was first a fast change of current (within 100 msec) and then a slower increase. Although the initial fast phase was above the time resolution of our measurement system, the slow phase could be fitted to a single exponential with a time constant of 0.92 ± 0.09 sec^{-1} ($n = 5$) (at 500 μM Ba^{2+}). This time-dependent part of the block represented 55% of the total block due to 500 μM Ba^{2+} at 0 mV (Fig. 8*B*). As

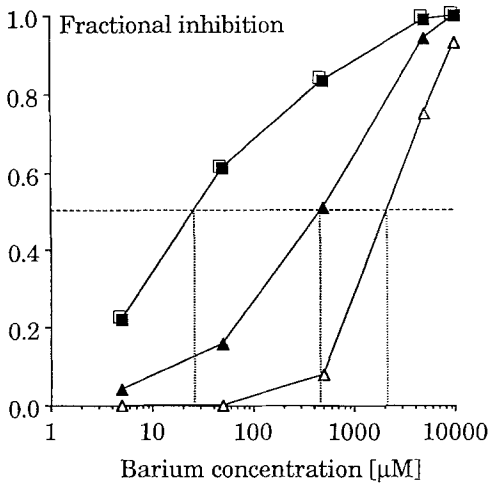

Fig. 7. Relation of the fractional inhibition of the transepithelial current to the Ba^{2+} concentration in a typical experiment. The currents were measured at 0 mV (triangles) and at −120 mV (squares), and 100 msec (filled symbols) and 3 sec (open symbols) after the voltage change. Fractional inhibition was obtained by dividing the Ba^{2+}-induced decrease of the current at each Ba^{2+} concentration by the decrease of current in the presence of the maximal Ba^{2+} concentration (values at 100 msec). At −120 mV, the inhibition curves were similar after 100 msec or 3 sec (filled and open squares).

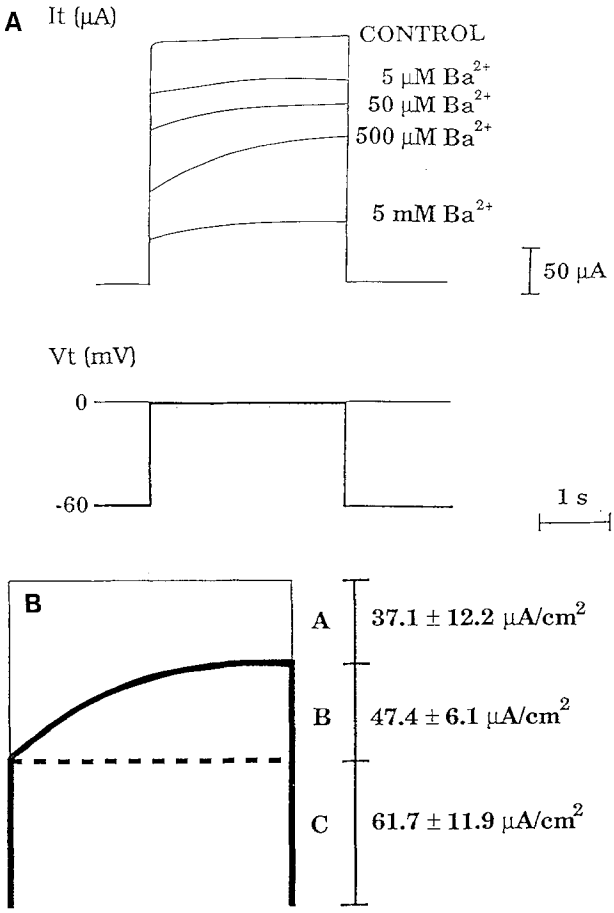

Fig. 8. Time dependence of Ba^{2+} block. (*A*) Original recordings of the transepithelial current when the holding potential was changed for 3 sec from −60 to 0 mV. The measurements were repeated in the absence (*CONTROL*) and in the presence of increasing Ba^{2+} concentrations. In the absence of Ba^{2+}, the current reached a steady state within the time resolution of the measurement system. In the presence of Ba^{2+}, after a first rapid change, there was a slow increase of the current, most obvious at 500 μM. (*B*) Schematic representation indicating that the transepithelial current measured at 0 mV (I_{sc}) can be divided into three parts: (i) an I_{sc} part permanently blocked by 500 μM Ba^{2+} at 0 mV, (ii) an I_{sc} part for which Ba^{2+} block was relieved with a slow time constant of 0.92 sec, and (iii) an I_{sc} part resistant to 500 μM Ba^{2+}. The values are the means of five experiments.

shown in Fig. 7, when the current was sampled 100 msec after the holding potential change to 0 mV, a different inhibition curve was observed with a K_i of 256 ± 27 μM (n = 17).

Effect of Quinidine

Quinidine is a potent blocker of a number of K^+ channels: the Ca^{2+}-dependent K^+ channels (Chang & Dawson, 1988), the K^+ channels induced by cell swelling (Germann et al., 1986*a*,*b*) and other K^+ channels of the basolateral membrane of the tight epithelia (Van Driessche & Hillyard, 1985). We have tested the effect of increasing quinidine concentrations (0.1 to 1000 μM) on I_t (*see* example in Fig. 9). The addition of 1 mM quinidine caused a marked inhibition of I_{sc}: 90.93 ± 1.04% (n = 11). The quinidine-sensitive component of I_t (I_{quin}) defined as the difference between I_t with or without 1 mM quinidine was 153.07 ± 11.91 μA/cm² at 0 mV. A $K_{1/2}$ of 11.4 ± 2.4 μM was obtained for quinidine at 0 mV. Within a 0.1- to 3-sec range, no time dependence of quinidine block was found (*data not shown*). Quinidine effect was only slowly and partly reversible. The current-voltage behavior of I_{quin} (*see* example in Fig. 10) showed an outward rectification with an equilibrium potential of −77.64 ± 0.97 mV (n = 11), a value very close to the K^+ equilibrium potential. In Fig. 10, we compared the I_{quin} *I-V* curve with the theoretical prediction of Goldmann-Hodgkin-Katz (GHK) equation (Schultz, 1980). The fit was reasonably good, and no systematic deviations were obvious.

Germann et al. (1986*a*,*b*) had shown that quinidine inhibited the K^+ conductance induced under cell swelling conditions (in the presence of high Cl^- concentration). As the large quinidine-sensitive conductance was present with 25 mM Cl^- but not in the absence of apical Cl^-, we have repeated our experiments decreasing the Cl^- concentrations on

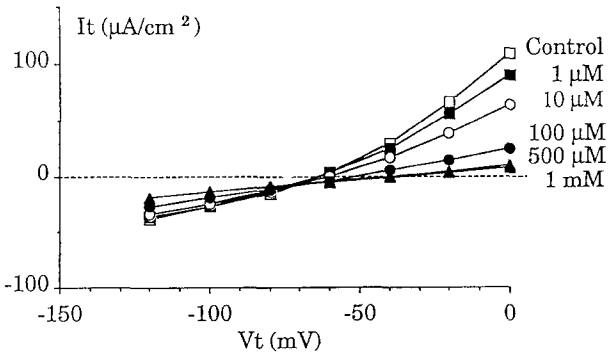

Fig. 9. Effects of quinidine on the transepithelial current-voltage (I_t/V_t) relation under cell swelling conditions (25 mM Cl in the apical solution). Increasing concentrations of quinidine were added to the basolateral side.

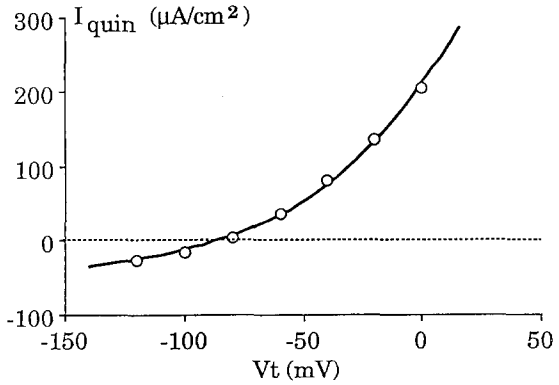

Fig. 10. Current-voltage relation of the quinidine-sensitive current (I_{quin}) under cell swelling conditions (25 mM Cl^- in the apical solution). The solid line is the best fit corresponding to the GHK model with a $[K_o]$ of 3 mM, a $[K_i]$ of 68.4 mM and a P of 40.5 × 10^{-6} cm/sec, for this experiment, assuming a perfectly K^+-selective quinidine-sensitive conductance.

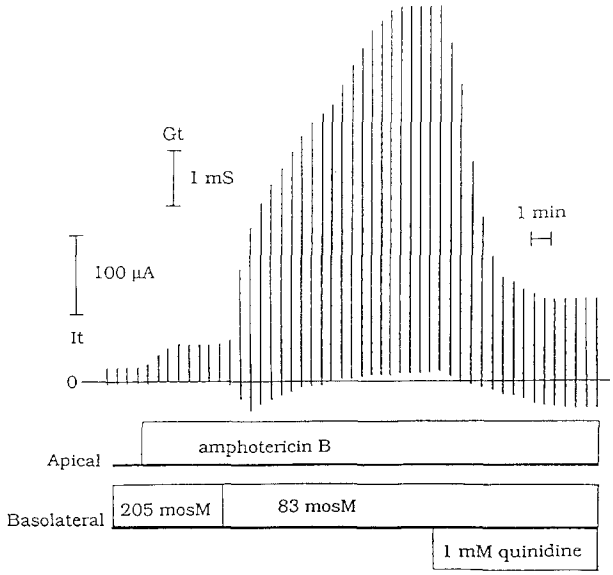

Fig. 11. Original recording of the transepithelial current (I_t) at 0 mV (I_{sc}, top of the bars) and at −60 mV (bottom of the bars). The length corresponds to the transepithelial conductance (G_t). This experiment was performed in the absence of Cl^- in the apical solution. Addition of 10.8 μM amphotericin B induced a first increase of I_t and G_t. After stabilization, cell swelling obtained by lowering the basolateral solution osmolarity induced large increases of I_t and G_t that could be blocked by the addition of 1 mM quinidine. The values of I_t and G_t were recorded on a 4.7-cm^2 area of epithelium.

both sides of the epithelium from 25 to 5 mM Cl^-. This did not reduce the quinidine-sensitive part of the transepithelial current (94.63 ± 0.98%, n = 5, NS, unpaired t test, when compared with the experiments with 25 mM Cl^-). Thus, the stimulating effect of Cl^- in the apical solution on the basolateral conductance appeared between the concentrations of 0 to 5 mM Cl^-.

When the concentration of Cl^- was increased from 0 to 25 mM (A1 to A2) after apical membrane permeabilization, a slow increase of G_t and I_{sc} was observed (from 10.3 ± 2.2 to 140.6 ± 25.3 μA/cm^2 and 0.18 ± 0.03 to 2.31 ± 0.42 mS/cm^2). This effect was very similar to the changes observed when the apical membrane was permeabilized in the presence of 25 mM Cl^-. The aspect of the transepithelial conductance changed from inward to outward rectifying, and a large part of the conductance became sensitive to quinidine.

Osmotically Induced Changes of the Basolateral K Conductance

In a separate set of experiments, we have tested the effects of cell swelling by lowering the osmolarity of the basolateral solution from 205 to 83 mosM (solution B4, Table 1). Such a change immediately induced a parallel increase of the I_{sc} and G_t (Table 3, Fig. 11). This process was similar to the one observed when the apical Cl^- concentration was increased.

The I-V curves obtained under these conditions also had an outward-rectifying aspect with an equilibrium potential of −71.8 ± 1.3 mV (n = 5). Quinidine was found to be an excellent inhibitor of this osmotically activated K^+ conductance with a maximal I_{sc} inhibition at 1 mM quinidine of 68.9 ± 2.3 μA/cm^2 (corresponding to 76 ± 1% of total I_{sc} (n = 5)), and a decrease of the transepithelial conductance from 1.45 ± 0.03 to 0.46 ± 0.02 mS/cm^2 (n = 5, $P < 0.001$).

Table 3. Effect of basolateral osmolarity on the short-circuit current and the transepithelial conductance ($n = 5$)

Control		Amphotericin B			
I_{sc} ($\mu A/cm^2$)	G_t (mS/cm^2)	I_{sc} ($\mu A/cm^2$)	G_t (mS/cm^2)	I_{sc} OSM ($\mu A/cm^2$)	G_t OSM (mS/cm^2)
3.57 ± 0.06	0.061 ± 0.003	9.31 ± 0.40	0.16 ± 0.01	87.45 ± 2.32	1.45 ± 0.03

Discussion

Selective Permeabilization of the Apical Membrane

According to the equivalent electrical circuit (Wills et al., 1979*b*), V_t reflects exclusively the electrical properties of the basolateral membrane if two conditions are fulfilled: the tight junctions conductance must be small relative to the transcellular conductance and the apical membrane resistance should become negligible in comparison with the basolateral resistance. In our A6 cells model, the first point was well demonstrated by the good linearity of the G_t *versus* I_{sc} plot during the onset of the amphotericin B effect. After full permeabilization, G_s estimated from the G_t *versus* I_{sc} plot was only 11 and 1.6% of the total transepithelial conductance, in the absence or presence of Cl, respectively (*see* Table 2) and thus contributed little to the whole transepithelial current.

Concerning the second point, amphotericin B induced a very large increase of G_t, more than 20-fold, when the cells were swollen either in the presence of 25 mM Cl^- in the apical solution or when the cells were exposed to a basolateral hyposmotic solution (Tables 2 and 3). In the absence of apical Cl^-, the increase of G_t was smaller, because G_t was limited by the low basolateral membrane conductance, when the cells were not swollen. Considering that the apical membrane is the main resistive barrier in a tight epithelium, in series with the lower resistance of the basolateral membrane, the apical conductance must have increased by an even larger factor. After addition of amphotericin B, either osmotic changes or the addition of chloride induced similar large increases of the transepithelial conductance, and this activated conductance was sensitive to the basolateral addition of quinidine. This indicates that under these conditions, the transepithelial resistance is dominated by the basolateral membrane resistance. It is therefore probable that after amphotericin B treatment, the apical membrane resistance becomes negligibly small as compared with the basolateral resistance. This fact has been demonstrated by direct measurements of the voltage divider ratio in the rabbit urinary bladder (Lewis et al., 1977). As the aqueous channels formed by amphotericin B are poorly selective for monovalent ions (De Kruijff et al., 1974), an equilibration of K^+, Na^+ and Cl^- between the apical and the intracellular content is likely to occur.

In addition, in order to study the physiological properties of the basolateral membrane, this membrane must not be permeabilized by the ionophore. The effects of K^+ channel blockers demonstrated that the basolateral membrane conductance was still highly selective for K^+ after permeabilization. When the cells were swollen, from the K^+ and Na^+ concentrations of the apical and basolateral solutions and the transepithelial equilibrium potential, we calculated a P_K/P_{Na} ratio of 32, despite the fact that the transepithelial conductance includes the poorly selective paracellular conductance. This indicates that under our experimental conditions the basolateral membrane was still highly K^+ selective and not permeabilized by amphotericin B. Thus, the observed transepithelial conductance was mainly due to basolateral membrane native channels activated or not by cell swelling. As there was only 1 mM Na^+ in the apical solution, the contribution of the Na^+,K^+-ATPase to the transepithelial current was small, as shown by the basolateral addition of ouabain (200 μM) which did not modify detectably this transepithelial current (*data not shown*).

In addition, the values of basolateral membrane conductance, from 0.21 to 2.46 mS/cm^2 in the absence or presence of apical Cl^- are in the same range as those obtained by intracellular electrodes (0.5 mS/cm^2, Thomas & Mintz, 1987; 0.29 mS/cm^2, Granitzer et al., 1991).

Under our experimental conditions (−60 mV holding potential), when the apical membrane was permeabilized, the cell preparations were highly stable for extended periods of time (>90 min). Moreover the effects of amphotericin B were fully reversible, as measured by the I_{sc} and the G_t values. Thus, the apical membrane exposure to amphotericin B resulted in a stable preparation, where transepithelial current and voltage measurements re-

flected directly the properties of the basolateral membrane under "resting" or stimulated conditions.

Amphotericin B pores are permeable to Cl^- ions as well as to Na^+ and K^+ ions (De Kruijff et al., 1974). It has been shown that amphotericin B treated epithelial cells placed in Cl^- containing solutions undergo extensive swelling (Germann et al., 1986*a*; Butt, Clapp & Frizzell, 1990). The similar appearance of a quinidine-sensitive K^+ conductance observed in A6 cells with permeabilized apical membrane when chloride was added to the apical medium or when the osmolarity of the basolateral solution was lowered, strongly support the hypothesis that both responses are secondary to conductance activation by cell swelling, although we have not actually measured the cell volume.

Basolateral "Resting" K Conductance

The transepithelial equilibrium potential was close to the basolateral membrane potential measured with intracellular microelectrodes in A6 cells (Thomas & Mintz, 1987; Granitzer et al., 1991) or in other tight epithelia (Wills et al., 1979*b*; Schoen & Erlij, 1985; Horisberger & Giebisch, 1988). Both the basolateral membrane reversal potential and the effect of barium indicated that the largest part of the conductance was selective for K^+, and that this conductance was inward rectifying, even in the presence of a large outwardly directed K^+ gradient.

Patch-clamp studies have identified K^+ channels with inward-rectifying kinetics in the basolateral membrane of renal proximal tubule (Kawahara, Hunter & Giebisch, 1987; Sackin & Palmer, 1987). Similar results have been reported for K^+ channels reconstituted from the basolateral membrane of enterocytes (Costantin et al., 1989). If similar channels are present in tight epithelia, they could mediate the inward-rectifying macroscopic conductance that we have observed.

Although no effect of quinidine could be detected on the inward K^+ currents under these conditions, a small but significant inhibition of the outward current was measured. The amplitude of this quinidine-sensitive current amounted only to about 2% of the quinidine-sensitive current measured when the cells were swollen. This small outward conductance might result from a little and partial activation of volume-sensitive K^+ channels, even in the absence of apical chloride.

Volume-Activated K Conductance

After the induction of cell swelling, either by Cl^- or by osmotic changes, there was a large increase of the basolateral membrane conductance due to a quinidine-sensitive K^+ conductance. The current-voltage relationship of the quinidine-sensitive conductance was outward rectifying, making the whole transepithelial *I-V* curve of the permeabilized epithelium outward rectifying. This quinidine-sensitive *I-V* curve conformed closely with the GHK constant-field flux equation over a 120 mV-range (Fig. 10). Thus, the observed rectification could be attributed to the asymmetrical K^+ concentrations.

Mechanism of Block by Barium

Similar to several investigators who have studied the effects of extracellular Ba^{2+} on K^+ conductances (Standen & Stanfield, 1978; Armstrong, Swenson & Taylor, 1982; De Wolf & Van Driessche, 1986; Hanrahan et al., 1986; Zeiske, Van Driessche & Ziegler, 1986; Merot et al., 1989; Turnheim et al., 1989), we have found that the blocking potency of Ba^{2+}, after activation of the volume-sensitive conductance, was clearly voltage dependent. A more than 250-fold increase of the K_i was observed between holding potentials of 0 and -120 mV. Some characteristics of Ba^{2+} block observed in this study deserve some comments. It must be noted that, for Ca^{2+}-activated K^+ channels, Ba^{2+} is a much more potent inhibitor when applied to the intracellular side of the membrane (Eaton & Brodwick, 1980; Neyton & Miller, 1988). In epithelial cells, a K_i around 100 nM for intracellular Ba^{2+} block has been measured (Guggino et al., 1987). Thus, even a small amount of Ba^{2+} entering the cells could have a significant blocking effect by binding to an intracellular site. Although we cannot exclude this possibility, a direct effect of Ba^{2+} on an extracellular site of the A6 cell's basolateral membrane appears more probable. First, the observed Ba^{2+} block was rapidly reversible either by membrane depolarization or by removal of Ba^{2+} from the basolateral solution. Second, the direction of the voltage dependence was opposite to that described for internal Ba^{2+} block (Armstrong et al., 1982; Neyton & Miller, 1988). Third, the K_i of the Ba^{2+} block (at 0 mV) was in the same range as that observed in patch-clamp studies on the apical membrane of epithelial cells (Merot et al., 1989), using right side-out excised patches, a situation where the direct effect of Ba^{2+} ions on the extracellular part of the channel is most probable. In addition, a number of patch-clamp studies on basolateral membrane have demonstrated K^+ channel block by external Ba^{2+} in the same range of concentration (Richards & Dawson, 1986; Kawahara et al., 1987; Loo & Kaunitz, 1989; Taniguchi et al., 1989). A similar voltage dependence has been described for the Ba^{2+}

block of the K^+ current across the apical membrane of frog skin and has been interpreted as resulting from the location of the Ba^{2+} binding site within the transmembrane electrical field at a relative distance δ of 0.72 (de Wolf & Van Driessche, 1986). Applying the same analysis to our data (an *e*-fold change of K_i per 24 mV) yields a δ of 0.54.

The value of 0.92 sec^{-1} for the time constant of the Ba^{2+} block relief is very similar to that obtained by Hanrahan et al., (1986) for K^+ channels of the basolateral membrane of locust midgut. It is also in the same range as that estimated for the inward-rectifier K^+ channel of frog skeletal muscle (Standen & Stanfield, 1978); however, the Ba^{2+}-sensitive current was not inwardly rectifying in A6 cells. Very different values for the dissociation rate constant have been reported in other tissues, much slower for the delayed-rectifier K^+ current of the squid axon (Armstrong et al., 1982) or faster for the apical membrane of the frog skin (Van Driessche & Zeiske, 1980).

Voltage Dependence

Different types of voltage dependence have been described for the basolateral membrane conductance of epithelial cells. Basolateral membrane outward-rectifying *I-V* curves conforming to the GHK model have also been observed in another tight epithelium using a similar technique (Wills et al., 1979*a*). In several other experiments the basolateral membrane *I-V* relationship was linear (Thompson et al., 1982; Schultz et al., 1984; Schoen & Erlij, 1985) or inward rectifying (Garty, 1984; Nagel, 1985; Horisberger & Giebisch, 1988). The basolateral K^+ conductance of leaky epithelia was also found to be inward rectifying (Lang, Messner & Rehwald, 1986). In view of the opposite rectifying properties of the "resting" and volume-activated K^+ conductances that we have observed, it is possible that differences in previously published data resulted from experimental conditions inducing various degrees of cell swelling.

The basolateral K^+ conductance in swollen cells is not only larger than in "resting" cells but differs in sensitivity to inhibitors and in voltage dependence. Thus, it is probable that cell swelling activates a population of K^+ channels specifically involved in cell volume regulation.

In conclusion, the technique of apical membrane permeabilization by an ionophore antibiotic can be applied to A6 cells grown on permeable supports. In this stable preparation, a high paracellular resistance is maintained, and investigations of the basolateral membrane electrical properties can be carried out by transepithelial measurements. We have demonstrated that the basolateral membrane of A6 cells is highly selective for K^+. Under "basal" or "resting" conditions the K^+ conductance is inward rectifying, highly sensitive to barium but not to quinidine. After maneuvers inducing cell swelling a much larger quinidine-sensitive K^+ conductance is induced. This quinidine-sensitive conductance is outward rectifying in the presence of physiological asymmetrical K^+ concentrations.

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