

Differentiation of Two Distinct K Conductances in the Basolateral Membrane of Turtle Colon

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ABSTRACT The K conductance of the basolateral membrane of turtle colon was measured in amphotericin-treated cell layers under a variety of ionic conditions. Changing the composition of the bathing solutions changed not only the magnitude but also the physical properties of the basolateral K conductance. The results are consistent with the notion that altered ionic environments can lead to changes in the relative abundance of two different populations of K channels in the basolateral membrane, which can be differentiated on the basis of pharmacological specificity, ion selectivity, and tracer kinetics. In the following article (Germann, W. J., S. A. Ernst, and D. C. Dawson, 1986, *Journal of General Physiology*, 88:253–274), we present evidence consistent with the hypothesis that one of these conductances was due to the same channels that give rise to the normal resting basolateral K conductance of the transporting cells, while the other was associated with experimental maneuvers that led to extreme swelling of the epithelial cells.

INTRODUCTION

In their seminal model for active Na absorption by frog skin, Koefoed-Johnsen and Ussing (1958) proposed that a dominant feature of the basolateral membrane of the epithelial cell was a K conductance, and subsequent investigations of a variety of tight, Na-absorbing epithelia confirmed the existence of a substantial basolateral conductance to K (Lewis et al., 1978; Wills et al., 1979; Koeppe et al., 1983). More recently, attention has focused on the possibility that alterations in the rate of active Na absorption may involve obligatory changes in the magnitude of the basolateral K conductance (Smith and Frizzell, 1984). Schultz (1981) has proposed that such changes in the basolateral K conductance would allow changes in the rate of Na absorption without major changes in the cellular ionic composition.

We studied changes in the basolateral K conductance of amphotericin-treated

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turtle colon that were induced by changing the ionic composition of the bathing solutions. We found several maneuvers that produced changes not only in the magnitude but also in the physical properties of the basolateral K conductance. In this article, we present evidence that two basolateral K conductances can be distinguished on the basis of pharmacological specificity, ion selectivity, and tracer kinetics. These results derive almost exclusively from experiments in which one conductance was induced by bathing the tissues in solutions containing the organic anion benzene sulfonate. In the following article (Germann et al., 1986), we present evidence that is consistent with the notion that one of these conductances is due to the same channels that comprise the normal resting conductance of the epithelial cells, whereas the other conductance, which was specifically blocked by quinidine or lidocaine, was apparent only under conditions of extreme osmotic stress, which led to pronounced swelling of the epithelial cells.

MATERIALS AND METHODS

Colons were removed from turtles (*Pseudemys scripta*) and stripped of musculature, as described previously (Dawson, 1977a). Isolated pieces of tissue were mounted in Ussing chambers ($A = 5.2 \text{ cm}^2$) and the mucosal and serosal bathing solutions were vigorously stirred and aerated with room air. The pH of all solutions was ~ 8.3 at room temperature. All experiments were conducted under short-circuit conditions, i.e., the electrical potential difference across the tissue was held at zero by means of a voltage clamp. Unless otherwise indicated, the basolateral Na/K-ATPase was inhibited with 10^{-4} M ouabain in the serosal bath (Kirk et al., 1980), and $5 \times 10^{-5} \text{ M}$ amiloride was present in the mucosal bath to block apical Na channels (Thompson and Dawson, 1978).

Measurement of Basolateral K Conductance

The basolateral K conductance was measured using the method of Kirk and Dawson (1983), in which a K gradient was imposed across the tissue and the pore-forming antibiotic amphotericin B was added to the mucosal bath to functionally eliminate the apical membrane as a barrier to cation flow. Kirk and Dawson (1983) showed that in this condition, properties of the basolateral K conductance can be assessed directly by measuring transmural K currents. The present experiments differed from those of Kirk and Dawson in that K gradients were imposed by replacing the mucosal bath with an isosmotic Ringer's solution in which all of the Na had been replaced by K rather than by nonisosmotic addition of K salts to the mucosal or serosal bath. In a typical experiment, tissues were first incubated in standard amphibian NaCl Ringer's solution (112 mM NaCl, 2.5 mM KHCO_3 , 1 mM CaCl_2). Next, the mucosal bath was changed to an Na-free, high-K Ringer's solution (54.75 mM K_2SO_4 , 2.5 mM KHCO_3 , 1 mM CaCl_2 , 54.75 mM sucrose). Transmural K gradients in all experiments were 112:2.5 mM, mucosa to serosa, unless indicated otherwise. The presence of an Na-free mucosal solution reduced the short-circuit current (I_{sc}) to $\sim 10\%$ of the initial value. After the establishment of the K gradient, ouabain was added to the serosal bath. After the current had declined to a steady value near zero, amiloride was added to the mucosal bath to block apical Na channels, and this was followed by addition of amphotericin. In early experiments, ouabain and amiloride were always added simply to ensure that neither the electrogenic pump current (Halm and Dawson, 1983) nor apical Na channels (Kirk and Dawson, 1985) contributed to the measured currents. Subsequent experiments have shown, however, that identical results are obtained in the absence of these inhibitors, presumably because an Na-free mucosal

bath effectively eliminates Na/K-ATPase activity, and the amiloride-sensitive apical conductance is trivial compared to that induced by amphotericin. Amphotericin (9 μ M, mucosal bath) caused a rapid increase in the I_{sc} , in the direction consistent with the passive flow of K down the chemical potential gradient from mucosa to serosa. The I_{sc} induced by amphotericin in the presence of a transepithelial K electromotive force (emf) was used as a measure of the basolateral K conductance.

In experiments involving benzene sulfonate (BS), as the anion in the serosal bath, tissues were initially bathed in symmetrical NaBS Ringer's solutions (112 mM NaBS, 2.5 mM KHCO_3 , 1 mM CaCl_2). Ouabain (10^{-4} M) was then added to the serosal bath. After the current had declined to near zero, the mucosal bath was then switched to an Na-free, high-K Ringer's (see above), and the experiment proceeded as previously described. Preliminary experiments showed that results were unaffected if ouabain was added after the mucosal solution change.

Tracer Flow Measurements

In some experiments, transmural fluxes of ^{42}K were measured using a sample-and-replace paradigm, as described previously (Dawson, 1977a). Unidirectional K movement was expressed in terms of rate coefficients, λ_{ij} , defined according to

$$\lambda_{ij} = J_{ij}/C_i,$$

where J_{ij} is the flux of K from i to j and C_i is the K concentration on side i . In order to correct for paracellular tracer flow, amphotericin-induced fluxes, $J_{ij}^{\text{ampho}} - J_{ij}^{\text{control}}$, were used in the calculation of rate coefficients. In preliminary experiments, we found that amphotericin treatment produced as much as a doubling of the transmural flux of [^3H]mannitol, a marker for paracellular permeability. ^{42}K fluxes, however, were increased by 10-fold, so that the contribution of the paracellular path to the amphotericin-induced ion flow was negligible and the type of correction used had no effect on the results. In addition, comparing amphotericin-induced increases or blocker-induced decreases in fluxes or currents gave the same values. In one set of experiments, ^{42}K and ^{86}Rb flows were measured simultaneously. In this case, the samples were counted twice: once immediately after sampling and again after at least 2 wk to allow virtually complete decay of ^{42}K . Net counts owing to ^{42}K and ^{86}Rb were obtained by subtraction with appropriate corrections for the decay of both isotopes.

Mucosal Uptakes of Tracer K

In a number of experiments, unidirectional influxes of tracer K across the amphotericin-treated apical membrane were determined by measuring the initial rate of K uptake from the mucosal bath, as described by Kirk and Dawson (1983).

Ion Selectivity

In experiments comparing the effects of serosal Cl and BS, the mucosal bath contained Na-free K_2SO_4 Ringer's (54.75 mM K_2SO_4 , 2.5 mM KHCO_3 , 1 mM CaCl_2 , 54.75 mM sucrose), and the serosal bath contained a low-K solution of composition 110 mM NaX, 2.5 mM NaHCO_3 , 2 mM KX, 1 mM CaCl_2 , where X = Cl or BS. We determined the cation selectivity of the basolateral K conductance by measuring currents owing to K and Rb in the presence of identical gradients of these cations. In these experiments, mucosal bathing solutions were used in which K was replaced with equimolar amounts of Rb (56 mM Rb_2SO_4 , 1 mM CaCl_2 , 56 mM sucrose). The serosal bath contained solutions that were identical to those described above except that 2.5 mM K was replaced with Rb. For experiments involving serosal anion substitutions other than Cl or BS, the serosal bath

contained the Ringer's solution described above, where X = nitrate, isethionate, aspartate, or benzoate. The mucosal bath contained Na-free K_2SO_4 Ringer's as described above.

Inhibitors

Quinidine (Sigma Chemical Co., St. Louis, MO) was added to a final concentration of 0.2 mM in the mucosal bath. The effects of quinidine were identical if the drug was added to the serosal bath, although the onset was slower, presumably because of the unstirred connective tissue layer on the serosal side of the tissue. The effects of quinine (Sigma Chemical Co.), a stereoisomer of quinidine, were identical to those of quinidine. Carbachol (Sigma Chemical Co.) was always added to the serosal bath (final concentration, 10 μ M). The drug was found to be completely ineffective when added to the mucosal bath. Ba was always added as $BaCl_2$ to a final concentration of 5 mM in the serosal bath.

RESULTS

Identification of Two Types of K Conductance

Fig. 1 shows representative examples of currents induced by mucosal amphotericin in the presence of mucosal-to-serosal (M-to-S) K gradients (112:2.5 mM). To facilitate comparison, the currents are plotted here as a percent of the maximum value. The mucosal solutions both contained Na-free K_2SO_4 Ringer's and Na/K pump activity was inhibited by adding ouabain (0.1 mM) to the serosal bath. Before the addition of amphotericin, the I_{sc} values were near zero despite the substantial K emf across the tissues because the apical membrane was relatively impermeable to K in the absence of the polyene. In addition, the paracellular path is characterized by a low ion selectivity and relatively high resistance (Dawson, 1977a; Halm and Dawson, 1984). The addition of amphotericin to the mucosal bath induced currents that were consistent with K flow from mucosa to serosa down a chemical potential gradient, as previously reported (Kirk and Dawson, 1983). Tracer flux data, presented below, confirmed that both currents were, in fact, due to net K flow.

The two tissues represented in Fig. 1 differed only in that one had been preincubated with regular (NaCl) Ringer's (dashed line), whereas the other was preincubated in NaBS Ringer's (solid line) (see Materials and Methods). The two tissues behaved similarly in that transmural K currents were induced in both by mucosal amphotericin, but differed markedly with respect to the action of quinidine, an inhibitor of K conductance (Lew and Ferreira, 1978). In the presence of Cl-free NaBS Ringer's solution in the serosal bath, the K current, which is abolished by serosal Ba (Kirk and Dawson, 1983), was rapidly inhibited by the addition of 0.2 mM quinidine. In contrast, when the serosal bathing solution contained a standard NaCl Ringer's solution, the K current was either unaffected or, at most, slightly reduced by quinidine. Doubling or tripling the dose of the drug did not produce further inhibition. However, the quinidine-insensitive current was abolished by the addition of 5 mM Ba to the serosal bath.

Table I summarizes the effects of a variety of inhibitors on the basolateral K currents measured under these two conditions. Ba abolished both currents. Quinine, a stereo-isomer of quinidine, blocked the quinidine-sensitive but not the quinidine-insensitive current. Lidocaine, a local anesthetic, blocked the

quinidine-sensitive but not the quinidine-insensitive I_K . Carbachol, a muscarinic agonist that blocks the quinidine-insensitive I_K (Venglarik and Dawson, 1986), did not inhibit the quinidine-sensitive I_K . Of the remaining agents, papaverine inhibited both currents, whereas neither tetraethylammonium (TEA) nor 4-aminopyridine inhibited either current.

Ion Selectivity

The marked difference in pharmacological specificity exhibited by the basolateral K conductance measured under different ionic conditions raised the possibility that the changes in bathing solution composition could have induced a qualitative change in the nature of the basolateral K conductance, such as that which would

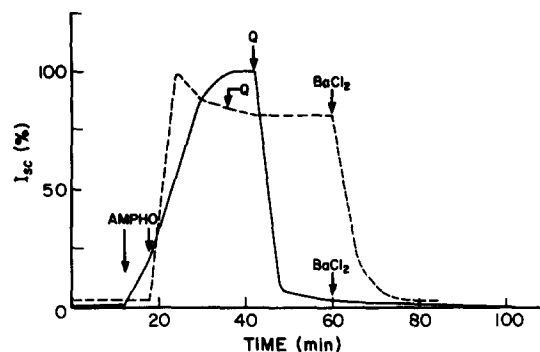


FIGURE 1. Short-circuit current (I_{sc}), as a percent of the maximum, plotted as a function of time for the two conditions described in the text. All tissues were treated with ouabain (0.1 mM, serosal) and amiloride (0.05 mM, mucosal). 9 μ M amphotericin was added to the mucosal bath. Peak values for the two currents (μ A/cm²) were 286.5 for the quinidine-sensitive current (solid line), and 61.5 for the quinidine-insensitive (dashed line). Quinidine and BaCl₂ were added in small volumes of concentrated aqueous solution at the points indicated by the arrows. BaCl₂ was added to the serosal bath to a final concentration of 5 mM. The effects of quinidine (or the lack of it) did not depend on the side to which the drug was added, so for convenience the drug was usually added to the mucosal bath to a final concentration of 0.2 mM.

be brought about by the substitution of one set of K channels for another. To test this hypothesis, we measured the cation selectivity of the basolateral membrane under the two conditions. Table II compares currents induced by mucosal amphotericin in the presence of M-to-S gradients of either K or Rb. Gradients of Cs were also examined but gave rise to no significant currents under any condition.

Two important results emerge from Table II. First, the quinidine-sensitive K currents were two to three times larger than the quinidine-insensitive currents. Second, the basolateral membrane differed markedly in its selectivity for K over Rb under the two conditions. The ratio I_K/I_{Rb} for the quinidine-sensitive conductance was $\sim 6.7:1$, whereas that for the quinidine-insensitive conductance was

TABLE I
Effects of Inhibitors on Basolateral K Currents

Inhibitor	Percent of I_{Kc} inhibited	
	Quinidine-sensitive conductance (serosal BS)	Quinidine-insensitive conductance (serosal Cl)
Ba (5 mM, S)	95-100	95-100
Quinidine (0.2 mM, M or S)	95-100	0
Quinine (0.2 mM, M or S)	95-100	0
Lidocaine (0.2 mM, M)	95-100	0
Carbachol (10 μ M, S)	0	95-100
TEA (20 mM, S)	0-10	0-10
Papavarine (0.3 mM, M)	95-100	95-100
4-Aminopyridine (20 mM, S)	0-10	0-10

Transmural K currents were generated as shown in Fig. 1 in the presence of either serosal Cl or BS. Inhibitors were added to either bathing solution to the final concentration indicated in the parentheses. Values represent the range of inhibition for at least three experiments in which the inhibitors were classified as effective or ineffective. For ineffective inhibitors (0-10% inhibition for both types of conductances), the concentration represents the maximum dose tested. For effective inhibitors (95-100% inhibition of both conductances), the routinely used dose is indicated.

~1.6:1. The dramatic difference in basolateral membrane selectivity is underlined by the fact that although the quinidine-sensitive K currents were more than two times higher than the quinidine-insensitive currents, the quinidine-sensitive Rb currents were only half of the quinidine-insensitive Rb currents. The table

TABLE II
K and Rb Currents in the Presence of Serosal Cl or BS

	Serosal BS (n = 6)		Serosal Cl (n = 5)	
	ΔI_K	ΔI_{Rb}	ΔI_K	ΔI_{Rb}
	$\mu A/cm^2$		$\mu A/cm^2$	
Amphotericin-induced	120.3 \pm 20.2	17.8 \pm 2.8	52.8 \pm 10.7	33.8 \pm 4.8
Quinidine-sensitive	104.9 \pm 20.5	15.7 \pm 2.7	3.3 \pm 0.9	2.0 \pm 1.1
Ba-sensitive	110.4 \pm 21.1	17.6 \pm 6.6	48.6 \pm 19.2	30.8 \pm 4.7
Amphotericin-induced	$\Delta I_K/\Delta I_{Rb} = 6.76$		$\Delta I_K/\Delta I_{Rb} = 1.56$	
Quinidine-sensitive	$\Delta I_K/\Delta I_{Rb} = 6.68$		—	—
Ba-sensitive	$\Delta I_K/\Delta I_{Rb} = 6.27$		$\Delta I_K/\Delta I_{Rb} = 1.58$	

Values of I_{Kc} are means \pm SEM from paired experiments in the presence of M-to-S gradients (112:2.5 mM) of K or Rb. Changes in the current (ΔI_{Kc}) after the addition of mucosal amphotericin followed by the addition of quinidine (0.2 mM) and BaCl₂ (5 mM) to each tissue are shown. Values from several tissues are averaged for each condition. The quinidine-sensitive I_{Kc} is calculated as the I_{Kc} immediately before quinidine addition minus the steady state I_{Kc} after quinidine addition. The Ba-sensitive I_{Kc} is the quinidine-sensitive I_{Kc} plus any further changes in I_{Kc} brought about by the subsequent addition of Ba, since Ba inhibits the current completely in the absence of quinidine.

shows that comparisons based on polyene-induced increases in I_{sc} or inhibitor-induced reductions in I_{sc} gave similar results.

The ratio of the macroscopic currents measured in the presence of K and Rb, respectively, may not only be a measure of relative ionic permeability, but may also reflect differential effects of the two ions on channel properties and gating (Hille, 1984). To obtain an accurate measurement of the relative permeabilities of K and Rb under the two conditions, we simultaneously measured transmural M-to-S fluxes of K and Rb in the presence of an M-to-S K gradient (in the absence of abundant Rb) during quinidine-sensitive and quinidine-insensitive currents. Fig. 2 shows the results presented as a plot of the ratio of the rate coefficients (λ_K/λ_{Rb}) vs. time. Before the addition of amphotericin, the ratio was unity, as expected since transmural isotope flow is largely via a paracellular path. After addition of polyene, the flux ratios increased in both conditions, but more markedly in the presence of quinidine-sensitive currents. After cellular currents

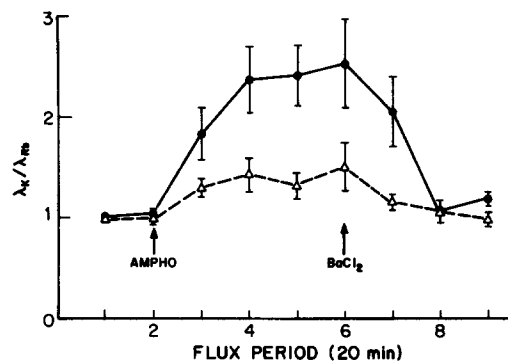


FIGURE 2. Plot of the ratio of the M-to-S rate coefficients for K and Rb (λ_K/λ_{Rb}) measured simultaneously in the presence of transmural K gradient (112:2.5 mM) under quinidine-sensitive (solid line) and quinidine-insensitive (dashed line) conditions. Quinidine-sensitive and quinidine-insensitive K currents were generated as described in the text using either BS- or Cl-containing Ringer's in the serosal bath.

were blocked with Ba, both ratios returned to unity. This result confirmed that the basolateral membrane selectivity differed appreciably under quinidine-sensitive and quinidine-insensitive conditions even in an identical ionic milieu.

Tracer K Fluxes

An advantage of the polyene-treated epithelial cell layer is the relative ease with which tracer flux determinations can be carried out under conditions in which these fluxes pertain to a single membrane. We measured the transmural flow of ^{42}K under conditions in which we could induce quinidine-sensitive and quinidine-insensitive K currents. As shown in Table IIIA, the results of tracer flux measurements confirmed that in both conditions, the amphotericin-induced I_{sc} was equal to the net flow of K. The results of tracer flow measurements are presented in Table IIIB in the form of the tracer rate coefficients (see Materials and Methods). A consideration of the tracer rate coefficient is informative because the ratio of the rate coefficients will directly reveal possible "anomalies"

TABLE IIIA
Transcellular K Fluxes in the Presence of Amphotericin and a K Gradient

Transmural K fluxes	J_{MS}^K	J_{SM}^K	J_{net}^K	I_{sc}
	$\mu\text{eq}/\text{cm}^2 \cdot \text{h}$			
Quinidine-sensitive conductance (serosal BS)	3.00 ± 0.46 ($n = 7$)	0.02 ± 0.04 ($n = 7$)	2.98 ± 0.46 ($n = 7$)	3.46 ± 0.41 ($n = 14$)
Quinidine-insensitive conductance (serosal CI)	1.19 ± 0.28 ($n = 9$)	0.01 ± 0.01 ($n = 9$)	1.18 ± 0.28 ($n = 9$)	1.18 ± 0.14 ($n = 18$)

Values are means \pm SEM for n tissues. For each tissue, the value of the flux or current was obtained from the mean of at least three 20-min flux periods in the presence of amphotericin. All values were corrected for paracellular flow by calculating the amphotericin-induced component of the flux or current, ΔJ or ΔI .

in the mechanism of tracer flow (Dawson, 1977*b*). If the tracer crosses the membrane by simple diffusion without interacting with any other species, the ratio of the rate coefficients should be unity.

A comparison of the rate coefficients for tracer K flow in the presence of quinidine-sensitive and quinidine-insensitive K currents provided additional evidence that the properties of the basolateral membrane differed markedly under these two conditions. The ratio of the rate coefficients for quinidine-sensitive cellular K flow was highly anomalous. The ratio suggests that in the presence of the 112:2.5 M, M-to-S K gradient, ^{42}K movement from M to S (with the gradient) was favored by a factor of nearly sixfold over tracer flow in the reverse direction. In contrast, the ratio of rate coefficients in the quinidine-insensitive condition was near unity in the presence of an identical gradient. The anomalous flux ratio observed in the quinidine-sensitive condition is consistent with the results of Kirk and Dawson (1983), who showed that coupling between the basolateral membrane flows of abundant and tracer K was of the sort expected for a single-file mechanism of K flow.

To demonstrate that the anomalous tracer flow observed under quinidine-sensitive conditions was dependent on the direction of the K gradient, additional experiments were conducted (Table IIIB) in which ^{42}K rate coefficients were determined in the presence of a serosal-to-mucosal (S-to-M) K gradient (KBS

TABLE IIIB
Transmural K Rate Coefficients in the Presence of K Gradients

	λ_{MS}	λ_{SM}	$(\lambda_{MS}/\lambda_{SM})$
	$\text{cm}/\text{h} \times 100$		
Quinidine-sensitive conductance	2.65 ± 0.41 ($n = 7$)	0.48 ± 0.10 ($n = 7$)	5.52
Quinidine-insensitive conductance	1.05 ± 0.25 ($n = 9$)	1.02 ± 0.14 ($n = 9$)	1.03
S-to-M K gradient:			
Quinidine-sensitive conductance	0.49 ± 0.35 ($n = 5$)	3.43 ± 0.50 ($n = 5$)	0.14

All gradients were 112:2.5 mM. Amphotericin-induced fluxes were used in all calculations to correct for paracellular tracer flow. The values in the first two lines were obtained from the flux data shown in Table IIIA.

Ringer's on the serosal side, Na₂SO₄ Ringer's on the mucosal side). As shown in the table, reversing the K gradient inverted the ratio of the rate coefficients under quinidine-sensitive conditions. Similar experiments under quinidine-insensitive conditions were not possible, because "reversed" currents (with KCl Ringer's in the serosal bath) had a substantial quinidine-sensitive component, presumably caused by cell swelling induced by the serosal KCl (see also Germann et al., 1986).

Serosal Anion Substitutions

To determine whether the development of a quinidine-sensitive conductance depended on the presence of BS or on the absence of Cl in the serosal bath, we conducted additional experiments in which serosal Cl was replaced with equimolar amounts (112 mM) of nitrate, aspartate, or isethionate (see Table IV). The mucosal solutions always contained Na-free K₂SO₄ Ringer's. In all cases, the

TABLE IV
Effect of Serosal Anion Substitutions on Amphotericin-induced K Currents

Serosal anion	Mucosal anion	ΔI_{Kc}		n
		Amphotericin-induced $\mu A/cm^2$	Quinidine-sensitive $\mu A/cm^2$	
Cl	SO ₄	47.4±4.8	1.5±0.8	7
NO ₃	SO ₄	33.5±15.2	3.8±3.8	3
Isethionate	SO ₄	38.2±6.5	3.0±0.8	3
Aspartate	SO ₄	65.9±2.1	1.4±1.4	3
Benzoate	SO ₄	138.9±10.9	99.0±16.1	3

Values of I_{Kc} are from experiments in the presence of M-to-S gradients of K (112:2.5 mM). Experiments in which serosal Cl was replaced with another anion were always done with a control containing serosal Cl. The amphotericin-induced and quinidine-sensitive I_{Kc} values were calculated as in Table II.

I_{sc} induced by amphotericin was unaffected by quinidine, which demonstrates that the elimination of serosal Cl was not sufficient to bring about the appearance of the quinidine-sensitive conductance. When serosal Cl was replaced by benzoate (Table IV), however, the amphotericin-induced current was almost completely abolished by quinidine.

Apical Membrane Properties in Amphotericin-treated Colon

In a previous study of basolateral K currents, Kirk and Dawson (1983) measured the influx of ⁴²K from the mucosal bath into the cells in the presence of the polyene and showed that the basolateral membrane was the site of action of Ba and the locus of the observed cation-cation interaction. Because the conditions of the present experiments differed substantially from those of Kirk and Dawson's experiments, we also measured mucosal ⁴²K uptake to determine to what degree (if any) the properties of transmural K flows reflected characteristics of the apical, rather than the basolateral, membrane.

Table V shows the unidirectional influx of ⁴²K from the mucosal solution into

the cell under quinidine-sensitive conditions. We examined this condition rather than the quinidine-insensitive condition because (a) we had not previously tested the action of quinidine on K influx, and (b) we wished to test the hypothesis that the properties of transmural K flows are determined solely by the basolateral membrane (i.e., that the basolateral membrane is the rate-limiting barrier to K flow). The quinidine-sensitive condition is the worst case, because the quinidine-sensitive conductance is larger than the quinidine-insensitive conductance. The values for ^{42}K influx show that although quinidine abolished the K current, the drug had no effect on the influx of K across the apical membrane. In addition, the table shows that the values for K influx were also unchanged when net K flow was abolished by eliminating the transmural K gradient. The observation that ^{42}K influx was independent of net K flow is consistent with the notion that positive coupling between ^{42}K and unlabeled K does not occur in the polyene channels. Kirk and Dawson (1983) showed that by comparing the unidirectional K influx with the transmural K current, it was possible to estimate the ratio of the conductances of the apical and basolateral membranes. Using values from Table V for simultaneously measured K influx and K current, we obtained a

TABLE V
K Influx Across Apical Membrane

	J^{K} $\mu\text{eq}/\text{cm}^2 \cdot \text{h}$	I_{sc} $\mu\text{eq}/\text{cm}^2 \cdot \text{h}$	n
Amphotericin	44.1±1.3	5.2±0.4	24
Quinidine	39.4±2.1	0.8±1.0	25
$[\text{K}]_{\text{M}} = [\text{K}]_{\text{S}}$	37.7±1.4	1.2±0.1	20

J^{K} is the K influx across the apical membrane. The transmural K gradient was M to S, 112:2.5 mM, except for the final entry where $[\text{K}]_{\text{M}} = [\text{K}]_{\text{S}} = 112$ mM. Each value is the mean ± SE for n tissues, since only one determination can be done per tissue.

ratio of apical K conductance to basolateral K conductance of ~30:1. These measurements confirm that the transmural K currents in amphotericin-treated colon are a direct measure of the properties of the basolateral membrane.

DISCUSSION

Two Types of Basolateral K Conductances

The results presented here are consistent with the notion that the basolateral membrane of turtle colon epithelial cells can exhibit at least two qualitatively different types of K conductance. These two conductance states were differentiated on the basis of three criteria: (a) pharmacological specificity, (b) ion selectivity, and (c) tracer kinetics. Any attempt to differentiate basolateral K conductance states on the basis of one of these criteria alone would doubtlessly be subject to a variety of ambiguities, some of which are discussed below. However, the fact that the K conductances could be distinguished with respect to three independent criteria lends strong support to the notion that there was a fundamental physical difference in the basolateral K conductance under the two conditions. It is also important to note that in these experiments we observed

an almost complete dichotomy in the state of the basolateral membrane under the two conditions, i.e., the K conductance was abolished by quinidine in one case and was totally insensitive to the drug in the other. If we adopt a simple model in which each conductance is the result of a separate population of ion channels, this complete dichotomy means that the experimental maneuver that leads to the induction of one conductance also leads to the suppression of the other. In the following article, we show that the conditions that led to the activation of the quinidine-sensitive conductance were also associated with extreme swelling of the epithelial cells. Furthermore, it became apparent that under some conditions, it was possible to activate the quinidine-sensitive conductance without suppressing the quinidine-insensitive conductance. Recent results obtained by Venglarik and Dawson (1986) strongly suggest that the suppression of the quinidine-insensitive conductance in some experiments was due to the release of endogenous acetylcholine by submucosal nerves in response to the organic anions or ouabain in the serosal bath.

Pharmacological Specificity

Ba ion added to the serosal bath blocked both quinidine-sensitive and quinidine-insensitive basolateral K currents. In view of the fact that Ba has been shown to block K conductances in a variety of cell types (Nagel, 1979; Eaton and Brodwick, 1980; Kirk and Dawson, 1983), this result might not be regarded as surprising. The result should not necessarily be taken as indicating any fundamental similarity in the two K conductances, however. Although it has been suggested that Ba ions can block K channels by entering the channel (Eaton and Brodwick, 1980), it is also known that Ca channels are quite permeable to Ba, so that in our experimental paradigm, an intracellular action of the divalent ion cannot be excluded (Hagiwara and Ohmori, 1982).

Quinidine and lidocaine, in contrast to Ba, provided useful pharmacological probes for distinguishing two conductance states of the basolateral membrane. The mode of action of these compounds on K channels is unknown. Quinidine (or quinine) has been shown to block Ca-induced K loss from human erythrocytes (the so-called "Gardos effect") (Gardos, 1956; Lew and Ferreira, 1978), and the compound blocks K movements in a variety of other systems, including guinea pig hepatocytes (Burgess et al., 1981), rat pancreatic B cells (Lebrun et al., 1983), and human lymphocytes (Grinstein et al., 1982). Since much of the data in the cited studies derives from tracer flow measurements, it is not clear whether quinidine blocks conductive flows in these cells. Quinidine is known to inhibit K currents in excitable cells (Hermann and Gorman, 1984), however, and quinidine and quinine block basolateral K conductance in frog skin (Ambramcheck, 1984). In addition, Richards and Dawson (1985*a, b*, 1986) recently reported that single K channels in isolated epithelial cells from turtle colon were blocked by quinidine and lidocaine. The data of Cala et al. (1985), however, suggest that the action of this compound may not be restricted to conductive K flows, since electroneutral K efflux from erythrocytes is inhibited as well.

Ion Selectivity

We chose to define the ion selectivity of the basolateral membrane using a paradigm that we assumed would approximate cellular function under normal

conditions, i.e., a net cation flux driven by an outwardly directed K or Rb gradient. In this condition, the selectivity of the basolateral membrane differed markedly under quinidine-sensitive and quinidine-insensitive conditions by two criteria. First, there was a marked difference in the ratios of the macroscopic currents carried by K and Rb under the two conditions. Second, tracer flux ratios for the two cations in the presence of K also differed under the two conditions. The discrepancy between these two definitions of "cation selectivity" may reflect the fact that the two cations differ not only with regard to their permeability but also with regard to their effect on parameters such as channel gating. Furthermore, we cannot even be certain that either macroscopic conductances can be attributed to a single population of channels. The difference in the overall selectivity of the basolateral membrane suggests, however, that under the two conditions, there was a substantial difference in the densities of certain types of ionic channels in the basolateral membrane.

Tracer Kinetics

The marked difference in the ratio of unidirectional ^{42}K rate coefficients under quinidine-sensitive and quinidine-insensitive conditions provides additional support for the contention that these two conditions represent different physical states of the basolateral membrane with regard to K transport. In addition, the tracer kinetics may provide some insight into the mechanism of ion translocation across the basolateral membrane. The anomalous, non-unity ratio of forward and reverse rate coefficients for quinidine-sensitive currents is consistent with the previous report by Kirk and Dawson (1983) of positive coupling between the flow of the abundant and tracer isotopes of K. This conclusion is strengthened by the observation that reversing the orientation of the K gradient inverted the ratio of rate coefficients. Taken together, these observations are consistent with the notion that K flow through the quinidine-sensitive conductance occurs predominantly via pathways characterized by strong ion-ion interactions, as would be expected, for instance, in a single-file pore (Hodgkin and Keynes, 1955).

The unity ratio for the tracer rate coefficients under quinidine-insensitive conditions provides a clear distinction between this and the quinidine-sensitive condition, but the mechanistic interpretation is less certain. The unity ratio could reflect an absence of ion-ion interaction under quinidine-insensitive conditions. Unfortunately, however, whereas it is easy to show that the rate coefficients are anomalous, it is more difficult to argue convincingly for the absence of interaction. The unity ratio is a ratio of the total cellular K fluxes and could include contributions from nonconducting paths that might gratuitously counteract the contribution of a single-file pore.

Biological Significance of the Two Conductances

A comparison of the present results with those of Kirk and Dawson (1983) strongly suggests that the earlier results pertain exclusively to the quinidine-sensitive conductance. The experiments of Kirk and Dawson were conducted on tissues bathed by BS Ringer's and treated with ouabain. In fact, Germann and

Dawson (1982) reported that K currents generated using the method of Kirk and Dawson were blocked by quinidine. The experiments presented here do not in themselves provide any insight into the roles that these conductances might play in the life of the epithelial cells. On the basis of observations detailed in the following article, however, we argue that the quinidine-insensitive basolateral K conductance is, in fact, the normal resting conductance of the epithelial cells. The quinidine-sensitive conductance, on the other hand, is due to a different population of channels that is not normally active but can be induced or activated under extreme conditions such as osmotic stress.

The advent of the patch-clamp technique has revealed a wide variety in the types of K channels that can account for the K conductance of biological cell membranes. In their review, Latorre and Miller (1983) list six different types of K channels. Although it is not possible to identify with certainty channels in other cell systems that could account for the properties reported here, it seems clear that K channels can differ markedly with regard to ion selectivity and inhibitor specificity. For instance, although most K channels that have been characterized by reversal potential measurements exhibit little selectivity for K over Rb (Latorre and Miller, 1983), it was recently reported (Gallacher et al., 1984) that a class of channels from the pancreatic acinar cell was characterized by a very high selectivity for K over Rb in conductance measurements. Richards and Dawson (1985*a, b*, 1986) have identified single channels in isolated colonic cells that are blocked by either quinidine or lidocaine and may be the molecular basis for the macroscopic quinidine-sensitive conductance in the colonic epithelium.

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