

Resting and Osmotically Induced Basolateral K Conductances in Turtle Colon

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ABSTRACT Two types of K conductance can be distinguished in the basolateral membranes of polyene-treated colonic epithelial cells (see Germann, W. J., M. E. Lowy, S. A. Ernst, and D. C. Dawson, 1986, *Journal of General Physiology*, 88:237–251). The significance of these two types of K conductance was investigated by measuring the properties of the basolateral membrane under conditions that we presumed would lead to marked swelling of the epithelial cells. We compared the basolateral conductance under these conditions of osmotic stress with those observed under other conditions where changes in cell volume would be expected to be less dramatic. In the presence of a permeant salt (KCl) or nonelectrolyte (urea), amphotericin-treated colonic cell layers exhibited a quinidine-sensitive conductance. Light microscopy revealed that these conditions were also associated with pronounced swelling of the epithelial cells. Incubation of tissues in solutions containing the organic anion benzene sulfonate led to the activation of the quinidine-sensitive g_K and was also associated with dramatic cell swelling. In contrast, tissues incubated with an impermeant salt (K-gluconate) or nonelectrolyte (sucrose) did not exhibit a quinidine-sensitive basolateral conductance in the presence of the polyene. Although such conditions were also associated with changes in cell volume, they did not lead to the extreme cell swelling detected under conditions that activated the quinidine-sensitive g_K . The quinidine-sensitive basolateral conductance that was activated under conditions of osmotic stress was also highly selective for K over Rb, in contrast to the behavior of normal Na transport by the tissue, which was supported equally well by K or Rb and was relatively insensitive to quinidine. The results are consistent with the notion that the basolateral K conductance measured in the amphotericin-treated epithelium bathed by mucosal K-gluconate solutions or in the presence of sucrose was due to the same channels that are responsible for the basolateral K conductance under conditions of normal transport. Conditions of extreme osmotic stress, however, which led to pronounced swelling of the epithelial cells, were associated with the activation of a

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new conductance, which was highly selective for K over Rb and was blocked by quinidine or lidocaine.

INTRODUCTION

In the preceding article (Germann et al., 1986), we showed that by changing the composition of the solutions bathing the isolated turtle colon, it was possible to promote the appearance of one of two distinct types of basolateral K conductance. When the major anion in the serosal bathing solution was Cl, the basolateral K conductance was blocked by muscarinic agonists, was relatively insensitive to quinidine or lidocaine, and was relatively nonselective for K over Rb. When the serosal anion was benzene sulfonate, the basolateral K conductance was not blocked by muscarinic agonists, but was blocked by quinidine or lidocaine and was highly selective for K over Rb. The two states of the basolateral membrane also differed with regard to their tracer kinetic behavior. The striking difference in the properties of the basolateral membrane under these two conditions suggested the possibility that the change in the bathing solution composition brought about a change in the relative abundance of two different populations of K channels.

Grinstein et al. (1982*b*) showed that the swelling of peripheral lymphocytes was associated with the activation of a K efflux that was blocked by quinidine and which appeared to occur via conductive pathways (Grinstein et al., 1982*a*). These observations raised the possibility that the conditions that led to the appearance of the quinidine-sensitive g_K might also be associated with cell swelling. Since we could not measure cell volume changes directly during the determination of basolateral K currents, we determined the properties of basolateral K conductance under conditions that we presumed would lead to dramatic cell swelling and then examined the tissues for evidence of cell swelling using light microscopy. We compared these results with those obtained under other conditions, which were not expected to produce profound change in cell volume and which were associated with a quinidine-insensitive basolateral K conductance. The results support the hypothesis that the basolateral membrane of polyene-treated colonic cells can exhibit two distinct K conductance states. One of these is due to the same channels that comprise the normal resting K conductance of the basolateral membrane, i.e., that which is blocked by muscarinic agonists but not by quinidine or lidocaine and is only moderately selective for K over Rb. This conductance may dominate the basolateral membrane under conditions of normal cell volume and also, perhaps, over a range of "physiological" volume changes. The quinidine-sensitive K conductance is due to a separate population of channels that appear to be activated under extreme conditions. One of these conditions is osmotic stress, which leads to profound increases in cell volume. This conductance is unaffected by muscarinic agonists, is blocked by quinidine and lidocaine, and is highly selective for K over Rb.

MATERIALS AND METHODS

Measurement of Basolateral K Conductance

K currents across the basolateral membranes were measured as the short-circuit current (I_{sc}) in the presence of a transmural K gradient and mucosal amphotericin B as described

in the previous article (Germann et al., 1986). Tissues were routinely treated with mucosal amiloride (0.05 mM) and serosal ouabain (0.1 mM) to block apical Na channels and the electrogenic pump current (Kirk et al., 1980; Kirk and Dawson, 1985), respectively, but subsequent experiments have shown that none of the results are contingent on the presence of these blockers.

Solutions

Tissues were always initially bathed symmetrically with a standard amphibian NaCl Ringer's solution (112 mM NaCl, 2.5 mM KHCO₃, 1 mM CaCl₂). In experiments comparing the effects of different mucosal anions, the mucosal bath was switched to an Na-free, high-K Ringer's with the composition 109.5 mM KX, 2.5 mM KHCO₃, 1 mM CaCl₂, where X = gluconate, Cl, nitrate, or thiocyanate. For experiments in which the mucosal anion was sulfate, the solution contained 54.75 mM K₂SO₄, 2.5 mM KHCO₃, 1 mM CaCl₂, and 54.75 mM sucrose.

For studies comparing the effects of nonelectrolytes, tissues were first incubated in standard amphibian NaCl Ringer's. K gradients were established using the mucosal (Na-free K₂SO₄ Ringer's) and serosal (NaCl Ringer's) solutions indicated above, which were diluted 1:1 with isotonic solutions of urea or sucrose.

Inhibitors

Quinidine (0.2 mM final concentration, added to the mucosal bath) and Ba (5 mM final concentration, added to the serosal bath) were used as inhibitors of amphotericin-induced K currents, as described in the preceding article (Germann et al., 1986). In studies involving normally transporting tissues, the effects of quinidine and carbachol (10 μM, serosal) were compared.

Morphological Studies

Once stable electrical responses to transmural K gradients were achieved after exposure of tissues to different mucosal anions or to nonelectrolytes, either in the absence or presence of amphotericin, tissues were fixed acutely for 5 min by the addition of a concentrated mixture of glutaraldehyde and formaldehyde to the mucosal and serosal baths to give final concentrations of 2 and 3%, respectively. Subsequently, the tissues were removed from the chambers, fixed for an additional hour in the same fixative, post-fixed with 1% OsO₄ for 60 min, and then dehydrated and embedded in Spurr resin. Sections (1 μM thick) were cut with glass knives using a Sorvall MT-2B ultramicrotome, stained with azure II, and photographed using a Leitz Orthoplan light microscope. To facilitate comparisons between the various experimental conditions, care was taken to orient tissue blocks so that sections were perpendicular to the long axis of the epithelial sheet and to treat all sections with an identical staining protocol. Sections were cut from two to four tissue blocks from each experimental condition. At least two separate experiments were examined and photographed. The micrographs presented in the Results are representative, and although there was some variation in detail between replicate tissue blocks, particularly between experiments, the general morphological responses were comparable for a given incubatory condition. All photomicrographs are presented at the same final magnification.

RESULTS

Mucosal Anion Substitutions

We used several strategies in an attempt to produce a degree of cell swelling that would be easily detectable at the light-microscopic level. In the first of these, we

exploited the fact that "one-sided" channels formed by amphotericin are not perfectly cation selective. Experiments on planar bilayers (Marty and Finkelstein, 1975; Kleinberg and Finkelstein, 1983), *Aplysia* neurons (Russell et al., 1977), and rabbit urinary bladder cells (Lewis et al., 1977) indicated that one-sided polyene pores exhibit a measurable permeability to Cl ions such that the ratio of the permeability to K to that of Cl was in the range of 10–20:1. Thus, cell layers bathed on the mucosal side by K salts of Cl or other permeant ions would be expected to gain salt from the mucosal bath and the consequent osmotic water flow would lead to cell swelling. In contrast, with an impermeant ion such as gluconate in the mucosal bath, the epithelial cells would not be expected to gain salt from the mucosal bath, and although polyene treatment might induce volume changes, profound swelling would not be anticipated. Studies of the one-sided polyene pore in planar bilayers showed that Cl, nitrate, and thiocyanate permeate the one-sided pore, whereas sulfate and glucose do not (Kleinberg, 1984; Kleinberg and Finkelstein, 1984). Table I shows values for basolateral K currents measured in the presence of identical K gradients, but also in the presence of permeant and impermeant mucosal anions. The serosal bathing solution was in all cases regular NaCl Ringer's ($[K] = 2.5$ mM). I_K was insensitive to quinidine when the mucosal anion was sulfate or gluconate. In contrast, when the mucosal anion was Cl, nitrate, or thiocyanate, I_K was highly sensitive to quinidine, i.e., quinidine-sensitive currents were observed only when the mucosal bath contained anions that could permeate amphotericin channels. The association of the quinidine-sensitive basolateral conductance with the presence of permeant salts in the mucosal bath was consistent with the notion that this conductance was induced by cell swelling.

Effect of Permeant and Impermeant Nonelectrolytes on the Basolateral Conductance

To impose the different osmotic conditions without altering the ionic composition of the bathing solutions, we exposed tissues to solutions containing either urea or sucrose. We assumed that the membranes of the epithelial cells might be expected to be more permeable to the former than the latter. In addition, tracer and osmotic flow measurements on planar bilayers (Holz and Finkelstein, 1970; Holz, 1979; Kleinberg and Finkelstein, 1984) and red blood cells (Cass and Dalmark, 1973) indicated that urea readily permeates polyene pores, whereas sucrose is impermeant. We compared the quinidine sensitivity of basolateral K currents in tissues exposed to these two nonelectrolytes with the expectation that cells exposed to urea would gain urea, and hence water, whereas those exposed to sucrose would, if anything, be expected to lose water because of salt exit from the cells. Tissues were bathed by solutions that were diluted 1:1 with an isosmotic solution of either sucrose or urea to maintain the total solute concentration at normal tonicity (320 mosmol).

Fig. 1 shows the results of a representative experiment in which two tissues were exposed to identical K gradients (56:1.25 mM, mucosa to serosa), the major anions in both cases being sulfate on the mucosal side and Cl on the serosal side. In the presence of amphotericin and urea, the K current was approximately five

TABLE I
Effect of Mucosal Anion Substitutions on Amphotericin-induced K Currents

Mucosal anion	Serosal anion	Amphotericin-induced I_{Kc} $\mu A/cm^2$	Quinidine-sensitive I_{Kc} $\mu A/cm^2$	I_{Kc}^Q/I_{Kc}^A
Sulfate	Cl	47.4±4.8	1.5±0.8	0.04±0.03 (n = 7)
Gluconate	Cl	52.6±9.1	2.3±1.5	0.05±0.02 (n = 5)
Cl	Cl	180.6±15.3	134.9±28.3	0.74±0.14 (n = 5)
Nitrate	Cl	210.0±24.8	202.9±28.3	0.96±0.03 (n = 4)
Thiocyanate	Cl	110.2±6.2	107.0±4.2	0.98±0.05 (n = 3)

Values of I_{Kc} are from experiments in the presence of mucosal-to-serosal gradients of K (112:2.5 mM). The amphotericin-induced I_{Kc} (I_{Kc}^A) was calculated as the steady state I_{Kc} after amphotericin addition minus the I_{Kc} immediately before amphotericin addition. The quinidine-sensitive I_{Kc} (I_{Kc}^Q) was calculated as the steady state I_{Kc} after amphotericin addition minus the steady state I_{Kc} after quinidine addition. The ratio of quinidine-sensitive I_{Kc} to amphotericin-induced I_{Kc} was calculated for each tissue and then averaged for all experiments done under a particular set of conditions.

times the magnitude of that observed in the presence of sucrose and was reduced ~70% by quinidine. In contrast, the tissue bathed by sucrose exhibited a smaller current, and this current was insensitive to quinidine, although it was blocked by Ba. Fig. 2 shows the results of a representative experiment in which both tissues were bathed by a urea-containing Ringer's solution as described above, but in one case the bathing solutions contained 50 mM sucrose as well. It can be seen that the excess sucrose prevented the induction of a quinidine-sensitive K current despite the presence of urea. These results are consistent with the premise that the induction of the quinidine-sensitive conductance by urea was due to an osmotic effect rather than some effect of urea unrelated to cell swelling.

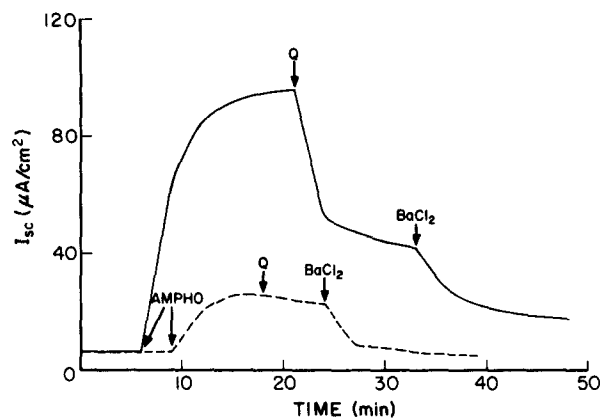


FIGURE 1. Representative paired experiment comparing the effect of bathing tissues (mucosal [M] and serosal [S]) with solutions that were diluted 1:1 with isotonic urea (solid line) or isotonic sucrose (dashed line). In the presence of an M-to-S K gradient (56.5:1.25 mM), the amphotericin-induced I_{Kc} was markedly reduced by quinidine (Q), whereas the current in the tissue exposed to sucrose was unaffected. Both currents were inhibited to a similar extent by BaCl₂.

Note that in the presence of urea, a substantial portion of the current remained after the addition of quinidine, in contrast to the results obtained in the presence of serosal benzene sulfonate (see Germann et al., 1986). Even doubling or tripling the dose of quinidine had no further effect (not shown). Serosal Ba inhibited a portion of the remaining current, but the current was not reduced to its pre-amphotericin value. The nature of the residual current is unknown, but it is consistent with Cl flow down a concentration gradient from serosa to mucosa. Further experimentation is necessary before firm conclusions can be drawn, however.

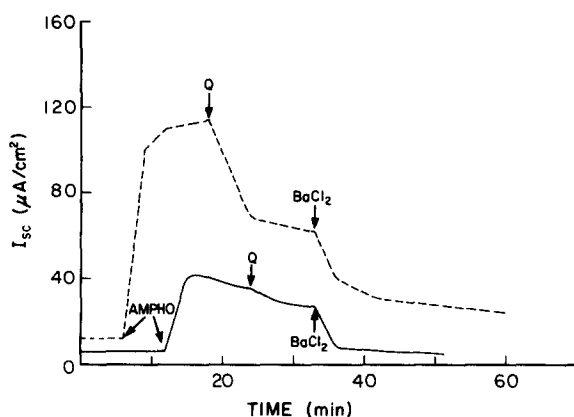


FIGURE 2. Representative paired experiment showing the effect of hyperosmotic sucrose on urea-induced quinidine-sensitive I_K . Both tissues were bathed as described under Fig. 1 by mucosal and serosal solutions diluted 1:1 with isotonic urea, but one received 50 mM additional sucrose (solid line). The presence of hyperosmotic sucrose prevented the development of an appreciable quinidine-sensitive I_K .

Effect of Hyperosmolarity on the Quinidine-sensitive Conductance Induced by Benzene Sulfonate

In the previous article (Germann et al., 1986), we showed that the quinidine-sensitive basolateral K conductance was induced by exposing the colonic cell layer to solutions in which the Cl in the serosal bath was replaced by the organic anion benzene sulfonate (BS), but we had no reason to suspect, a priori, that this maneuver would lead to cell swelling. To test for a possible role of cell swelling in the induction of quinidine-sensitive currents by serosal BS, we compared the magnitude of quinidine-sensitive K currents in tissues bathed by BS Ringer's (see the legend to Fig. 3) in the presence of solutions made hyperosmolar by the addition of sucrose. Fig. 3 shows a representative result in which the solutions contained 150 mM added sucrose. No current was induced in the hyperosmotic condition. This result is consistent with the view that incubation in BS Ringer's induced cell swelling. Note also, however, that in contrast to the observations presented above, the quinidine-insensitive current was also suppressed. This is consistent with observations detailed in the preceding article, which indicated

that BS solutions reduce or eliminate the quinidine-insensitive conductance, perhaps by causing the release of acetylcholine from submucosal nerves.

Induction of Quinidine-sensitive Basolateral Conductances

As an additional test of the hypothesis that the quinidine-sensitive I_K was induced by osmotic stress, we conducted experiments in which basolateral K conductance was measured in a single tissue before and after maneuvers that we presumed would produce cell swelling. Fig. 4 shows the results of a representative experiment. A quinidine-insensitive current was first generated as previously described

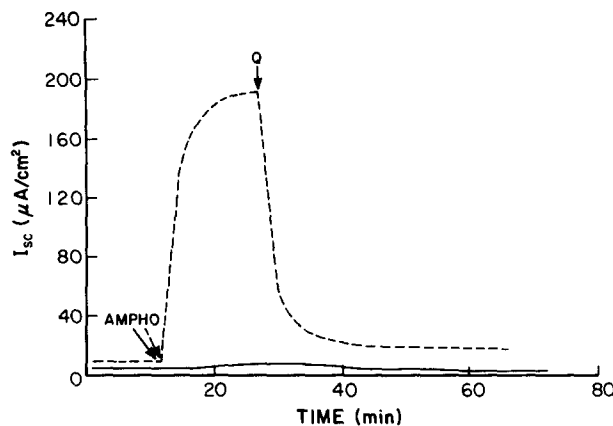


FIGURE 3. Inhibition of quinidine-sensitive currents in BS Ringer's by hyperosmotic sucrose. Paired tissues were bathed by mucosal K-gluconate Ringer's and serosal NaBS Ringer's (as described in the preceding article) to induce a quinidine-sensitive I_K , but one tissue received 150 mM additional sucrose (solid line). In the tissue bathed by hyperosmotic solutions, no current was induced by amphotericin, whereas a quinidine-sensitive I_K was present in the presence of isotonic Ringer's with serosal BS.

using a mucosal bath containing K-gluconate Ringer's. This current was then abolished by the addition of carbachol ($10 \mu\text{M}$) to the serosal bath. After the reduction of basolateral K conductance, the mucosal solution was changed to KCl Ringer's. The presence of the permeant mucosal salt led to the rapid development of a substantial current that was abolished by quinidine. Quinidine-sensitive currents induced by mucosal KCl were also blocked by lidocaine (data not shown). This result lends further support to the notion that these experimental maneuvers, which we presumed would cause cell swelling, activated a K conductance in the basolateral membrane that was distinct from that which was blocked by muscarinic agonists.

Cation Selectivity of Osmotically Induced K Currents

In the preceding article, we showed that a characteristic feature of the quinidine-sensitive basolateral K conductance was a high selectivity for K over Rb. Fig. 4

shows that quinidine-sensitive currents induced by the presence of permeant salts in the mucosal bath also exhibited this characteristic high selectivity for K over Rb. Fig. 4 compares the induction of a quinidine-sensitive current by KCl and RbCl. Although gradients of either ion produced carbachol-sensitive currents in the presence of mucosal sulfate or gluconate, only mucosal KCl led to the induction of a quinidine-sensitive I_{sc} . Similarly, in urea-containing solutions, a quinidine-sensitive current was induced in the presence of a K but not an Rb gradient (data not shown). These results are consistent with the idea that exposure

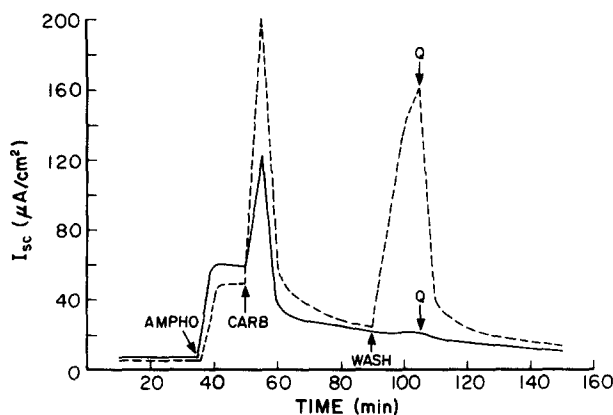


FIGURE 4. Induction of quinidine-sensitive K current after inhibition of the quinidine-insensitive K current with carbachol and comparison of gradients of K and Rb. One tissue (dashed line) was exposed to a K gradient (mucosal K-gluconate, serosal NaCl Ringer's, $[K] = 2.5$ mM) and the amphotericin-induced current was blocked by carbachol ($10 \mu\text{M}$, serosal). The addition of carbachol produced the characteristic biphasic response (Venglarik and Dawson, 1986), which consisted of a transient increase followed by inhibition of I_K . A virtually identical response was obtained in the presence of an Rb gradient (solid line, mucosal Rb_2SO_4 , serosal NaCl Ringer's, $[\text{Rb}] = 2.5$ mM). After inhibition of these currents, the mucosal bathing solutions were both washed and replaced with solutions of the Cl salts of the two cations ("wash"). Mucosal KCl led to the prompt development of a quinidine-sensitive K current (Q), whereas no current was induced by mucosal RbCl.

of the tissue to permeant mucosal salts or urea is associated with the activation of a conductance that is characterized by a high selectivity for K over Rb.

Morphological Evidence for Cell Swelling

Epithelial tissues were examined by light microscopy to determine whether experimental maneuvers that led to the activation of a quinidine-sensitive I_K also resulted in epithelial cells that appeared to be swollen in comparison with other tissues that did not exhibit a quinidine-sensitive I_K . Three comparisons were made: mucosal KCl vs. mucosal K-gluconate (serosal NaCl Ringer's), urea vs. sucrose as described above, and serosal BS vs. serosal NaCl (mucosal K-gluconate). Tissues were fixed in the Ussing chambers either before treatment with amphotericin or after quinidine-sensitive and -insensitive currents had reached a steady

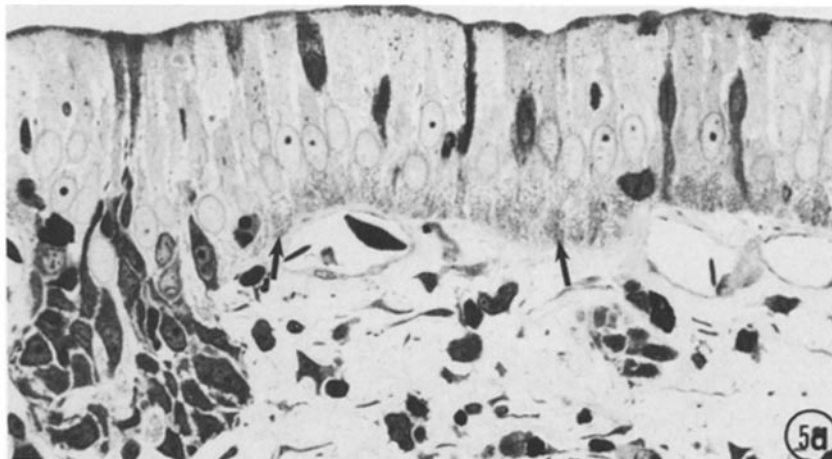
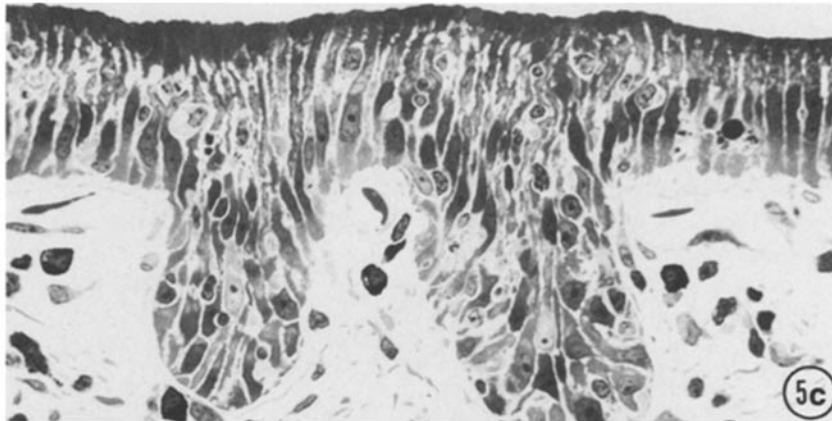
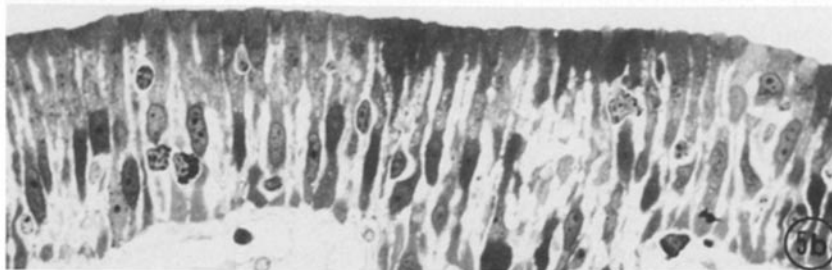
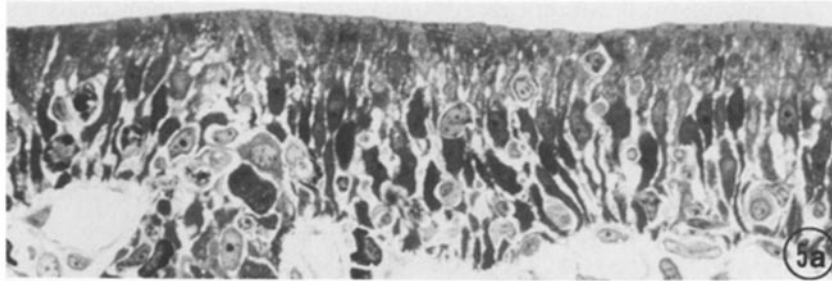
state, as described in Materials and Methods. Representative results are shown in Figs. 5–7.

The colonic epithelium is composed of a single layer of columnar cells that extends from the basement membrane to the free surface (Fig. 5). In addition, at the basal aspect of the epithelium, epithelial cells are seen extending periodically into the subjacent supportive connective tissue (lamina propria) as apparent solid “knots” of cells (Figs. 5–7). The cells may function in a generative capacity since mitotic figures are occasionally seen in these aggregates. These cells do not appear to reach the free surface of the colon.

In the absence of amphotericin, tissues exposed to a K gradient in the presence of mucosal gluconate (Fig. 5*a*) or Cl (Fig. 5*b*) consisted of thin, columnar cells with clearly discernible intercellular spaces extending from the basement membrane to just beneath the expanded (with mucous granules) subapical zone. The cytoplasm (as well as the nuclei) of the surface epithelial cells was intensely stained, obscuring to a large extent any intracellular detail.

Tissues fixed after the addition of amphotericin, with gluconate as the mucosal anion (Fig. 5*c*), were superficially similar in appearance to tissues fixed before the addition of the polyene with either gluconate (5*a*) or Cl (5*b*) as the mucosal anion, exhibiting clearly discernible intercellular spaces and dense, intensely stained cytoplasm. In contrast, when tissues were examined that had been fixed after the addition of amphotericin in the presence of mucosal Cl Ringer's, morphological indices of cell swelling were clearly apparent (Fig. 5*d*). These included the disappearance of most of the open intercellular spaces, the appearance of enlarged nuclei with faintly stained nucleoplasm, and a pronounced loss of cytoplasmic background staining intensity. Because of the last effect, cellular organelles such as mitochondria were clearly visible. The apparent “dilution” of cytoplasmic content, as judged by stain intensity, was even more marked than that illustrated in Fig. 5*d*; photometer compensation for the weak staining of the surface epithelial cells prolonged the photographic exposure. This also accounts for the darkly stained appearance of the subepithelial cellular knots (and connective tissue cells). These cells, which for the most part do not appear to be exposed directly to the mucosal bath, were moderately stained as in Fig. 5, *a–c*, but had their staining intensity enhanced photographically because of overexposure. The lack of clear signs of swelling in these cells implies that they are not coupled to the surface epithelial cells.

Although we did not undertake a detailed morphometric analysis of these light microscopic sections, we used the height of the cell layer as a rough measure of cell volume under each of these experimental conditions. Cell height was determined for two tissues (two blocks) for each condition using from two to four representative sections. The cell height in each section was defined as the mean of measurements at three points along the surface. The results (in microns) for the conditions represented in Fig. 5 were: K-gluconate/pre-amphotericin = 22.5 ± 4.5 (6); KCl/pre-amphotericin = 41.0 ± 2.6 (6); K-gluconate/post-amphotericin = 28.9 ± 0.7 (7); KCl/post-amphotericin = 52.8 ± 2.1 (5), where the number of sections examined is given in parentheses. Of central importance was the comparison between mucosal K-gluconate and KCl after amphotericin treatment,



since the basolateral K current could only be determined in polyene-treated cell layers. Cell height measurements indicated that tissues exposed to mucosal KCl exhibited an apparent cell volume that was ~ 1.8 times that of tissues exposed to mucosal K-gluconate. The cell height measurements also suggested that there may have been cell volume differences in tissues exposed to the different anions even in the absence of amphotericin, i.e., that for KCl/pre-amphotericin was ~ 1.8 times that for K-gluconate/pre-amphotericin. The electrophysiological consequences of this difference, if any, are unknown because we could not determine basolateral K conductance in the absence of the polyenes. The difference could indicate that a significant difference in cell volume between the two conditions existed before the addition of the polyene. Cell height measurements also suggested that the addition of amphotericin may have been associated with as much as a 30% increase in cell volume in tissues bathed by mucosal K-gluconate (Fig. 5, *a* and *c*), a condition that we know to be associated with a quinidine-insensitive basolateral K conductance. This observation could be taken to indicate that only the most extreme examples of cell swelling were associated with the activation of a quinidine-sensitive g_K . It must be emphasized, however, that cell height is but one measure of cell swelling. As judged by cell height, the conditions KCl/pre-amphotericin and KCl/post-amphotericin differed by $\sim 30\%$, whereas the same measurements indicate an 80% difference between the KCl-pre-amphotericin and K-gluconate/pre-amphotericin conditions. The appearance of the cells, however, suggests a more dramatic difference in cell volume in the former pair than in the latter.

FIGURE 5. (*opposite*) Micrographs illustrating morphological effects of mucosal anions on colonic epithelial cells after exposure to a K gradient in the absence or presence of mucosal amphotericin. Tissue sections were stained under identical conditions and photographed using a spot photometer positioned on the surface epithelium. The final magnification for all micrographs is 570. (*a*) Mucosal gluconate in the absence of amphotericin. The cytoplasm and nuclei of the thin columnar epithelial cells are densely stained and intercellular spaces are expansive. Mucous granules pack the apical cytoplasm. (*b*) Mucosal Cl in the absence of amphotericin. The morphological appearance is similar to that shown in *a*. (*c*) Mucosal gluconate in the presence of amphotericin. The appearance of the epithelium is similar to that shown in the previous figures. The extensions of the surface epithelium into the underlying connective tissue to form solid knots of cells are included in the micrograph. The cells lack exposure to a lumen or the free surface. These cells exhibit moderate staining intensity. (*d*) Mucosal Cl in the presence of amphotericin. Cell swelling is indicated by a marked reduction in cytoplasmic staining intensity and obliteration of most of the intercellular spaces (compare with preceding figures). Nuclei are enlarged and lightly stained, and mitochondrial aggregates (arrows) are now readily distinguishable in the basal cytoplasm. The epithelial aggregates at the base of the epithelium and the connective tissue cells had staining intensities similar to that shown in the preceding figures. They appear quite dark in this micrograph because of the longer exposure and development time required to compensate for the poor stain intensity of the surface epithelium. In this section, occasional darkly staining cells are present in the cell layer. These are found only occasionally and in small numbers. Their significance is unclear.

Fig. 6 shows representative sections from tissues fixed after the addition of amphotericin in the presence of sucrose (*a*) or urea (*b*). The figure illustrates the striking difference in the appearance of the epithelial cells under the two conditions. In the presence of sucrose, the cellular morphology was superficially similar to that observed with gluconate as the mucosal anion (Fig. 5*b*), namely,

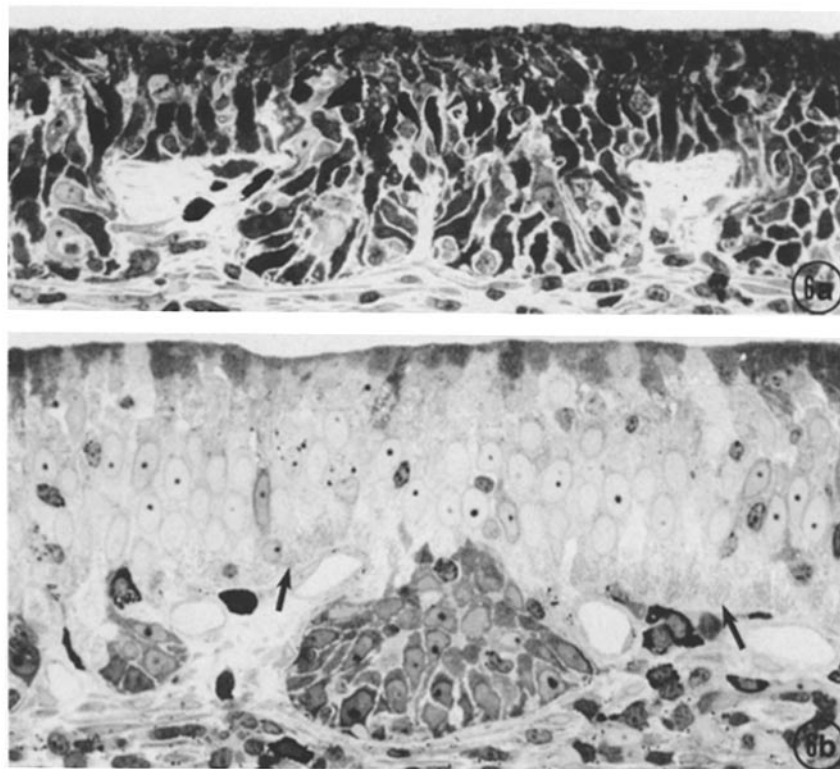


FIGURE 6. (*a*) Mucosal sucrose in the presence of amphotericin. The surface epithelial cells are stained intensely and intercellular spaces are open. The morphological appearance is similar to that shown in Fig. 5, *a-c*. (*b*) Mucosal urea in the presence of amphotericin. The indices of cell swelling described in Fig. 5*d* for exposure to mucosal Cl in the presence of amphotericin apply equally to that for urea with the exception that the reduction in stain intensity was not quite as dramatic. The arrows indicate basal aggregations of mitochondria.

readily discernible intercellular spaces and dense, intensely stained cytoplasm. In contrast, the tissue exposed to urea exhibited lightly stained cytoplasm, and intercellular spaces were barely detectable. Thus, two conditions, mucosal KCl and urea, that gave rise to quinidine-sensitive basolateral K currents were also associated with pronounced swelling of epithelial cells when compared with conditions (K-gluconate and sucrose) that did not produce a quinidine-sensitive I_K . In the Discussion, we consider the possible significance of this dramatic swelling in relation to the other apparent volume differences between experimental conditions.

The appearance of epithelial cells bathed by serosal BS Ringer's was of particular interest since this treatment produced quinidine-sensitive K currents (see above and Germann et al., 1986), but, in contrast to mucosal KCl and urea, there was no reason to suspect that this treatment would cause cell swelling. Fig. 7*a* is a representative section taken from a tissue fixed after exposure to

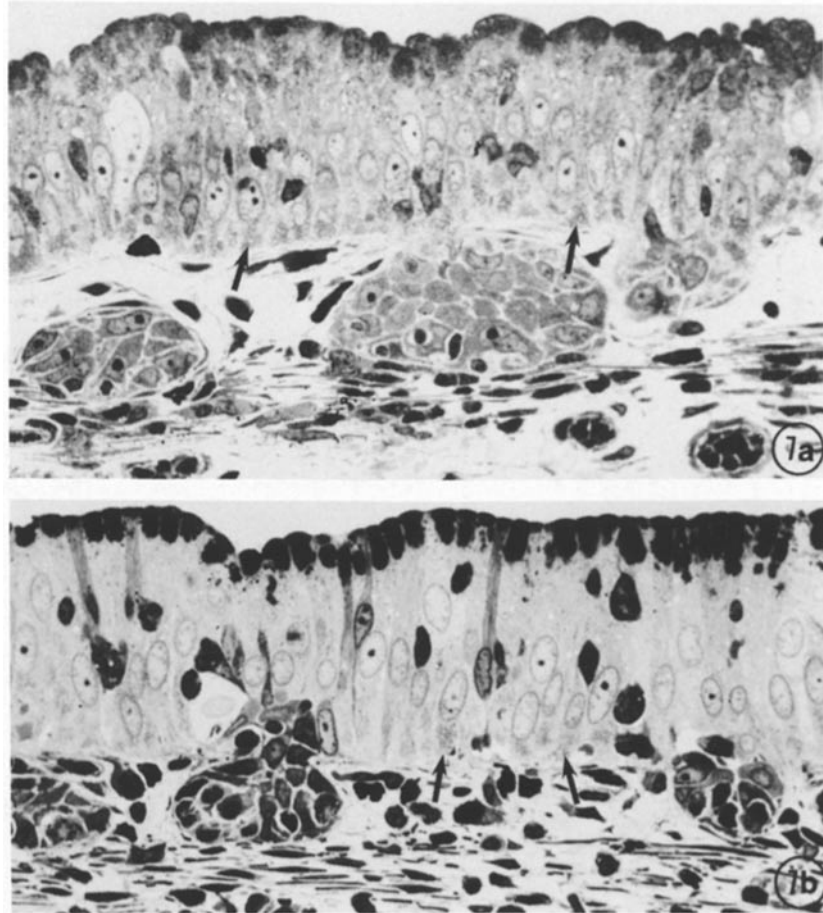


FIGURE 7. (a) Serosal BS in the presence of mucosal K-gluconate plus amphotericin. The surface epithelial cells are moderately swollen. The intercellular spaces are closed, the nuclei are enlarged and lightly stained, and the background cytoplasmic staining intensity is reduced, thereby revealing basal aggregations of mitochondria (arrows). (b) Serosal plus mucosal NaBS Ringer's in the absence of amphotericin. Cell swelling is pronounced. The arrows indicate mitochondrial aggregates.

amphotericin in the presence of serosal BS Ringer's. The mucosal bath was K-gluconate Ringer's. When Figs. 7*a* and 5*b* (mucosal K-gluconate, serosal NaCl, post-amphotericin) are compared, a striking difference is apparent, i.e., a loss of discernible intercellular space and a diminution of cytoplasmic background staining. The appearance of the tissues bathed by serosal BS was comparable to

that observed in the presence of mucosal KCl/post-amphotericin (Fig. 5*d*), although the extent of the apparent cytoplasmic dilutions was less marked. It is of interest that control tissues exposed to mucosal plus serosal NaBS Ringer's in the absence of amphotericin appeared even more swollen (Fig. 7*b*). This suggests that a partial restitution of cytosolic volume occurred when the mucosal solution was replaced with K-gluconate, followed by the addition of amphotericin (cf. Fig. 7*a*). It is not immediately obvious why incubation of tissues in BS Ringer's should cause such dramatic cell swelling. We do not have any data that allow us to compare directly the membrane permeability of BS and gluconate, for instance. It is striking, however, that even in this rather obscure instance, the correlation between extreme cell swelling and the induction of a quinidine-sensitive conductance was maintained.

Effect of Quinidine on Normally Transporting Tissues

The observation that the quinidine-insensitive conductance was blocked by carbachol, coupled with the observation that the same agonist inhibited active transport in normally transporting colons (Venglarik and Dawson, 1984, 1985, 1986; Germann et al., 1986), strongly suggested that the quinidine-insensitive conductance was the normal resting conductance of the basolateral membranes of the epithelial cells. To further explore possible roles for the quinidine-sensitive and quinidine-insensitive conductances in the normal function of the cell, we studied the effect of quinidine on normally transporting tissues bathed on both sides by NaCl Ringer's solutions. In this condition, the spontaneous I_{sc} is due to active Na transport from mucosa to serosa (Dawson, 1977). The active transport of Na by turtle colon is well described by a variant of the Koefoed-Johnson-Ussing model, in which the basolateral Na/K pump has a fixed stoichiometry of 3 Na:2 K (Kirk et al., 1980). In such a model, conductive basolateral K exit is necessary to sustain active Na absorption, and blocking this conductance should substantially reduce active Na absorption.

We have reported previously (Venglarik and Dawson, 1984, 1985, 1986; Germann et al., 1986) that serosal carbachol in a dose sufficient to abolish the quinidine-insensitive conductance also inhibited the I_{sc} of normally transporting tissues. In contrast, in three experiments, quinidine inhibited I_{sc} by only $14 \pm 2\%$, even though the dose (0.2 mM) was sufficient to bring about the complete inhibition of the quinidine-sensitive K conductance in amphotericin-treated tissues. These results suggest that under normal conditions, the dominant K conductance in the basolateral membrane is the quinidine-insensitive conductance.

Reversibility of Amphotericin Effects on Epithelial Cells

The method used in these studies to measure basolateral membrane K currents exploits the pore-forming properties of amphotericin to functionally eliminate the apical membrane as a barrier to cation flow. At this point, it is not certain what changes in the functional state of the cell might result from permeabilizing the apical membrane in this fashion. It is clear, for instance, that polyene-induced changes in apical salt permeability can influence basolateral K permeability. In

experiments such as those described above, there is always the possibility that treatment with amphotericin might have led to irreversible changes in cell function that have no bearing on the normal behavior of the cells. To address this issue, we assessed the reversibility of amphotericin treatment on normally transporting tissues. Fig. 8 shows the results of representative experiments, in which quinidine-sensitive and quinidine-insensitive currents were generated in the absence of amiloride and ouabain, but in the presence of mucosal KCl and K-gluconate Ringer's. Subsequently, the mucosal bath was washed several times

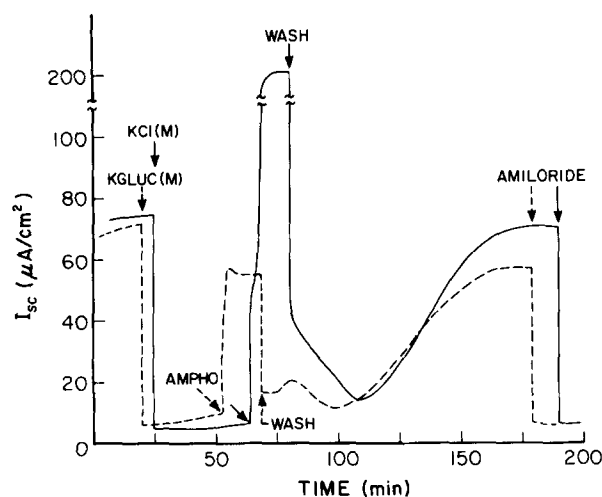


FIGURE 8. Reversibility of amphotericin effect. Plot of I_{sc} for paired tissues. Tissues were initially bathed in symmetrical NaCl Ringer's. Subsequently, the mucosal bath was changed to either K-gluconate (dashed line) or KCl (solid line) Ringer's with no ouabain added. At "ampho," $9 \mu\text{M}$ amphotericin was added to the mucosal bath. After a steady state K current was achieved, the mucosal solution of both tissues was washed ("wash") and replaced with regular NaCl Ringer's. After I_{sc} returned to its initial values, amiloride (5×10^{-5} M final concentration) was added to the mucosal bath.

and replaced with a polyene-free NaCl Ringer's solution. The short-circuit currents returned to their initial (pre-amphotericin) values and were inhibited by amiloride. Thus, as far as electrical parameters can tell us, epithelial cells were able to recover their normal functions even after undergoing the relatively drastic treatment of exposure to amphotericin. This suggests that amphotericin treatment does not irreversibly alter cell function.

DISCUSSION

Physiological Significance of the Two Types of K Conductances

In the preceding article, we distinguished two distinct K conductance states of the basolateral membrane of colonic epithelial cells on the basis of pharmacological specificity, ion selectivity, and tracer kinetics. The results presented in the

present article are consistent with the notion that these two types of K conductance could play different roles in the life of the cells.

Several lines of evidence suggest that the quinidine-insensitive conductance measured in amphotericin-treated cell layers is due to the same channels that give rise to the normal resting basolateral K conductance of the epithelial cells, whereas the quinidine-sensitive g_K represents a new conductance that is induced or activated only under rather extreme conditions such as profound cell swelling. Carbachol, a muscarinic agonist that completely blocked the quinidine-insensitive conductance, also profoundly inhibited active Na absorption (Venglarik and Dawson, 1986). This action is consistent with a blockade of the basolateral g_K , since K recycling is required to sustain active, rheogenic Na absorption (Venglarik and Dawson, 1986). We cannot, of course, rule out the possibility that carbachol might have other effects on the normal tissue. In contrast, quinidine, which abolishes the quinidine-sensitive conductance, had little or no effect on normal transport. It is important to recognize that if the apical membrane is the dominant resistive barrier in the normal tissue, then small changes in basolateral g_K caused by quinidine might not be detectable. It seems certain, however, that a profound reduction in the basolateral g_K of the sort produced by quinidine in the presence of mucosal KCl, for instance, would be detectable in this setting.

A second characteristic of the quinidine-insensitive basolateral K conductance was a relatively low selectivity for K over Rb. This relative interchangeability of Rb for K has also been borne out in additional studies on normally transporting tissues. If such tissues are washed repeatedly with K-free, Rb-containing Ringer's over a period of 4–8 h, normal transport is maintained (Dawson, D. C., unpublished observation), whereas in tissues washed with K-free Ringer's, active transport is abolished within 1 h. These results suggest that complete replacement of cellular K with Rb does not greatly affect normal transport, as would be expected if Rb could readily permeate K channels. In addition, tissues that are bathed by Rb Ringer's exhibit the characteristic inhibition of normal transport and basolateral Rb currents by carbachol (Venglarik and Dawson, 1986). (Previous studies have shown that the affinity of the basolateral Na/K-ATPase is, if anything, greater for Rb than for K [Halm and Dawson, 1983].) In contrast, the relatively high selectivity of the quinidine-sensitive conductance for K over Rb seems incompatible with the apparent interchangeability of K and Rb in supporting active Na absorption. It should be emphasized here that we do not contend that the magnitude of the quinidine-insensitive basolateral K conductance is comparable to that in normally transporting tissues, but only that the two are due to the same population of channels. The correspondence of the K/Rb selectivity and sensitivity to muscarinic agonists exhibited by the normal and polyene-treated tissue argues strongly for this conclusion.

We have used three rather different experimental maneuvers to activate the quinidine-sensitive basolateral K conductance: K salts of permeant anions, a permeant nonelectrolyte (urea), and the organic anion BS. It was striking to find that each of these maneuvers was also associated with a profound swelling of the epithelial cells. This observation naturally raised the question of a possible relation of this conductance to some sort of cellular volume-regulatory mecha-

nism. It has been shown for a variety of cell types, including red blood cells (Cala, 1983), human peripheral lymphocytes (Grinstein et al., 1982*b*), rat hepatocytes (Kristensen and Folke, 1984), and epithelial cells from frog skin (MacRobbie and Ussing, 1961), frog urinary bladder (Davis and Finn, 1982), and *Necturus* gallbladder (Larson and Spring, 1984), that cell swelling induces a rapid loss of KCl, which leads to cell shrinkage and a regulatory decrease in cell volume. These responses to cell volume increases have not been characterized electrophysiologically, but Grinstein et al. (1982*a*) obtained evidence that, in lymphocytes, the exit of K and Cl occurs via conductive pathways, and showed that this volume-induced K efflux is blocked by quinidine (Grinstein et al., 1982*b*). Lau et al. (1984) used microelectrode measurements to demonstrate that cell swelling increases a Ba-inhibitable K conductance in the basolateral membrane of *Necturus* small intestine.

It is not clear whether the present experiments make any contribution to our understanding of cellular volume regulation. We showed that experimental conditions that lead to the activation of a quinidine-sensitive g_K are associated with a profound swelling of the epithelial cells. In fact, it was only the dramatic nature of this swelling response that rendered the differences between the controls and the experimentals discernible. We cannot contend that these changes necessarily represent a "physiologically significant" volume change. In studies on lymphocytes, for instance, cells were swollen by 20–70% in order to evoke regulatory volume decreases (Grinstein et al., 1984). In the present studies, it is likely that cell swelling was significantly greater than 80%. Measurements of cell height also suggested that cell volume might vary under conditions where the basolateral K conductance would be expected to be quinidine insensitive, i.e., K-gluconate, before and after amphotericin (Fig. 5, *a* and *c*), so that our experiments do not exclude the possibility that the magnitude of the quinidine-insensitive conductance could be modulated by smaller, more physiological changes in cell volume. Finally, our experimental approach precluded any study of the time course of cell volume changes. Rather, we compared experimental conditions where, judging from electrical properties, the cells were in a steady state. Thus, our experiments provide no information as to possible "regulatory" volume changes that may have occurred as a result of an alteration in the composition of the bathing solution. We suspect, however, that the use of permeant mucosal salts or urea produced in amphotericin-treated cells what might be termed a "volume-clamp" condition caused by the overwhelming effect of the added solute on the cell water.

Despite some ambiguity as to the physiological significance of the response, it seems clear that maneuvers that uniformly produced profound swelling of the cells also led to the activation of a basolateral K conductance that was not present in the membrane of the cells under normal transport conditions. The significance of this activation and its relation to more subtle regulatory volume changes remains to be determined. It may be that the conductance evoked in this study, by rather extreme cell swelling, is in fact a part of a completely different cellular regulatory mechanism that is not directly related to cell swelling but was nevertheless activated under these extreme conditions.

Cellular Mechanisms for Modulation of Basolateral K Conductance

This study does not directly address the nature of the cellular mechanisms that underlie the dramatic changes in the nature of the basolateral K conductance. In view of the wide variety of K channel types that have been identified in patch-clamp and reconstitution studies (Latorre and Miller, 1983), the simplest hypothesis consistent with the results is that the basolateral membrane conductance can be dominated by one of two different populations of K channels under various conditions. In some experiments (see the preceding article), the membrane conductance appears to be due exclusively to one type of channel or another. This state of affairs would also be consistent with a mechanism for reversible interconversion of channel types. This type of mechanism seems unlikely, however, for several reasons. First, in experiments in which urea was used as a permeant solute to swell epithelial cells, quinidine did not inhibit I_K entirely, and the quinidine-insensitive (Ba-inhibitable) current was identical in the presence of urea or sucrose. This observation suggests not only that two channel types could coexist in the basolateral membrane, but also that activation of the quinidine-sensitive g_K could occur without any change in the magnitude of the quinidine-insensitive g_K . In addition, we have observed that a quinidine-sensitive K current can be induced during an ongoing quinidine-insensitive current simply by changing the mucosal bath from K-gluconate to KCl. The addition of quinidine abolishes only the extra current induced by mucosal KCl and brings the current down to the level of the previous quinidine-insensitive I_K (unpublished observations). Taken together, these observations support a model based on two separate populations of channels. It should be noted that the coexistence of the two presumed channel types discussed above is in contrast to observations on tissues in which the quinidine-sensitive conductance was induced by incubation in BS Ringer's (see the preceding article). In this instance, the quinidine-insensitive conductance was suppressed. A likely cause for this inhibition is the release of endogenous acetylcholine, which may be caused by the organic anion.

Additional support for the notion that the quinidine-sensitive K conductance is due to the activation of a distinct population of K channels was provided by recent studies of the basolateral membrane using the techniques of fluctuation analysis and single channel recording. Dawson et al. (1985) measured fluctuations in basolateral K currents induced by lidocaine in tissues treated with amphotericin and bathed by mucosal K-gluconate or KCl. They found that lidocaine, a reversible blocker of the K current activated by mucosal KCl, induced a Lorentzian component in the power density spectrum only when the mucosal bath contained KCl Ringer's. Furthermore, Richards and Dawson (1985a, b, 1986) used the patch-clamp technique to record single channel K currents in isolated turtle colon epithelial cells. When cells were bathed by KCl Ringer's, it was possible to identify a K channel with a conductance of ~ 17 pS that was blocked by lidocaine or quinidine. These observations provide strong support for the notion that the quinidine-sensitive conductance is due to a distinct population of K channels in colonic cells.

The observation that basolateral K currents induced by KCl or urea were blocked by quinidine raised the possibility that a Ca-activated K conductance was

involved in the response. The observations of Grinstein et al. (1982b) on the swelling-induced K loss in lymphocytes suggested a possible role for intracellular Ca as an activator of K efflux, but direct measurements of intracellular calcium using the fluorescent dye Quin 2 did not reveal any changes in intracellular Ca (Rink et al., 1983). Recently, however, Chase (1985) presented evidence that cell swelling in cells isolated from the toad bladder is associated with a rise in cell calcium. Chang and Dawson (1985) treated isolated colonic sheets in the turtle colon with mucosal digitonin to remove the apical membrane. Using this preparation, it was possible to demonstrate that acute increases in cell Ca activated a basolateral K conductance that was blocked by quinidine. This result raised the possibility that increases in cell Ca could activate the same K conductance that is turned on under conditions associated with profound cell swelling. Recent studies, however (Dawson, D. C., unpublished data), argue against this conclusion, since in digitonin-treated cells Ca can also activate Rb currents.

The Use of Mucosal Amphotericin to Study Basolateral Ion Channels

We (Kirk et al., 1980; Kirk and Dawson, 1983; Halm and Dawson, 1983), as well as others (Wills et al., 1979; Garty, 1984), have exploited the pore-forming properties of amphotericin by using this drug to functionally eliminate the apical membrane as a barrier to transcellular cation flow in isolated epithelia. In principle, this method provides a direct and technically straightforward approach to measuring the properties of the basolateral membranes of epithelial cells. Compared with microelectrode techniques, the use of amphotericin-treated tissues has several advantages, including the physical stability of the preparation, the ability to measure membrane current rather than only membrane potential, and the ability to measure tracer fluxes. There are several potential problems with this approach, however. The measurement of transcellular currents in polyene-treated epithelial cell layers is clearly a macroscopic, global measurement that represents the parallel sum of ionic currents across the basolateral membranes of many epithelial cells. Thus, the interpretation of the total current might be compromised by the presence of several different cell types in the cell layer, which would contribute differentially to the current. Our morphological studies (this article and unpublished observations) suggest that the turtle colon is a very homogeneous epithelium. Unlike the mammalian colon, the turtle colon lacks crypts and consists of a simple, flat layer of columnar cells. At the electron-microscopic level, the basic form of the surface epithelial cells seems to be invariant. Although this alone does not prove that the cells are identical, the turtle colon seems to be characterized by a much greater degree of homogeneity than the mammalian colon.

A second concern about these studies centers on polyene-induced changes in cell volume and possible irreversible alterations in cell function. It is clear that polyene treatment can induce major alterations in cellular cation content and cell volume. We exploited these changes in order to study the modulation of the basolateral K conductance. It might not be surprising, therefore, to find that there are other changes, in cell pH or Ca concentration, for instance, that contribute to the observed changes. We have shown, however, that the epithelial

cells have the ability to recover from even the most drastic of insults, since after amphotericin treatment these tissues can regain normal, amiloride-sensitive active Na absorption. Thus, although the polyene-induced changes in cell composition may exceed anything normally experienced by the cell, the perturbations do not exceed the capacity of the cell regulatory mechanisms to restore normal cell function. In the end, one makes a compromise in these experiments not unlike that which must be made in reconstitution studies. To obtain some degree of isolation of the basolateral membranes, as well as limited control over intracellular composition, we must tolerate some ambiguity as to the precise relation of the measured properties to those that would be obtained in the normal cell. Some of this ambiguity can be removed by careful "fingerprinting" of ion channels by means of blocker specificity and ion selectivity.

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