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Apical membrane potassium conductance in guinea pig gallbladder epithelial cells

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GUNTER-SMITH, PAMELA J. *Apical membrane potassium conductance in guinea pig gallbladder epithelial cells.* Am. J. Physiol. 255 (Cell Physiol. 24): C808-C815, 1988.—The fractional resistance of the apical membrane (f_{Ra}) of guinea pig gallbladder epithelial cells was observed to vary with changes in apical membrane potential (V_a). Depolarizing V_a from a base-line potential of −60 to −30 mV decreased f_{Ra} from 0.79±0.03 to 0.59±0.05. A comparable hyperpolarization had no effect on f_{Ra}. The potassium channel blocker tetraethylammonium (TEA) inhibited the changes in f_{Ra} induced by voltage when added to the mucosal but not when added to the serosal solution. Mucosal addition of Ba^{2+} and decreased pH also inhibited changes in f_{Ra}, whereas quinidine and 4-amino-pyridine did not. These results indicate that an increase in the K^+ conductance of the apical membrane is responsible for changes in f_{Ra} with membrane depolarization. The current-voltage relation of this TEA-sensitive pathway was determined from differences in transepithelial current in the presence and absence of maximally effective concentrations of TEA and analyzed with respect to the Goldman constant-field equation. Computer-generated, best-fit analysis to the data indicated that they cannot be easily reconciled with K^+ movement through a voltage-independent pathway or channel. Taken together, the results suggest that activation of a voltage-dependent K^+ conductance in the apical membrane is responsible for changes in f_{Ra} with V_a. This conductance also appears to be Ca^{2+}-sensitive as ionomycin caused a shift in the relation between V_a and f_{Ra}.

voltage; fractional resistance; apical membrane potential

WITH THE APPLICATION of electrophysiological and patch-clamp techniques to the study of ion transport by epithelial cells, evidence generated by numerous laboratories indicates that conductive ion movements across the individual cell membranes of these cells occur through ion channels similar to those extensively studied in excitable cell membranes (for recent reviews see Refs. 31 and 32). Although a major role for intracellular mediators such as Ca^{2+}, pH, and cyclic nucleotides has been given in the regulation of these channels in epithelia, more recently, the importance of membrane voltage in the regulation of ion channels in these nonexcitable tissues has become recognized. For example, voltage-dependent anion conductances have been reported in toad skin (18), rabbit proximal tubule (10), dogfish rectal gland (12), and MDCK cells (19); and K^+ conductances have been reported in choroid plexus (3), medullary thick ascending limb cells (13), cortical collecting tubule (5, 9, 17, 25), toad urinary bladder (24), MDCK cells (1), *Necturus* (7) and *Triturus* gallbladders (23), and cultured renal cells (20). These voltage-dependent K^+ channels are also regulated by intracellular mediators such as Ca^{2+}. Furthermore, several distinct subsets of channels with respect to single channel conductance, pharmacology, and mode of regulation often reside in the same membrane (8, 12, 13) making individual contributions to the overall permeability of the membrane for a given ion. Although in most cases the exact physiological functions of these various channels are not known, channels in epithelia are thought to be important in maintaining the normal membrane potential of the cell, the vectorial transport of ions across the epithelial cell layer, and the regulation of cell volume.

In this study, I report on the apical membrane K^+ conductance and its regulation in guinea pig gallbladder epithelial cells. Evidence will be presented indicating that this conductance is regulated by both voltage and Ca^{2+}. Additionally, I will present evidence that the population of channels comprising the apical membrane K^+ conductance are distinct from those comprising that of the basolateral membrane. [Sections of these results have appeared previously in abstract form (14).]

METHODS

Animals and solutions. Male Hartley guinea pigs (300–800 g) used in these experiments were screened for evidence of disease before use. They were housed in groups in stainless steel cages, maintained in rooms at 21°C, 50% rh, 12-h light-dark cycle (no twilight), and allowed access to commercial chow and tap water ad libitum. Gallbladders were removed after death of the animals by halothane inhalation followed by cervical dislocation. The bladders were stripped of their underlying musculature by blunt dissection and mounted in a chamber having a diameter of 0.13 cm^2. Both mucosal and serosal surfaces were continuously superfused by Ringer solution from reserviors by gravity feed. Standard Ringer solution contained (in mM) 130 Na^+, 108 Cl^-, 5 K^+, 1.0 Ca^{2+}, 1.0 Mg^{2+}, 35 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), and 10 glucose (pH 7.4) and gassed with 100% O_2. Bath pH was decreased by replacement of HEPES with Na_2HPO_4-NaH_2PO_4. Bath K^+ was increased by equimolar replacement of *N*-methyl-D-glucamine (NMDG). In these experiments, 50 mM NaCl was initially replaced by an equivalent amount of NMDGCl.

Tetraethylammonium chloride (TEA) and $BaCl_2$ were added by equimolar substitution of NaCl. All other drugs were added directly to normal Ringer except ionomycin, which was initially dissolved in dimethyl sulfoxide (DMSO). Unless indicated differently, changes from and

additions to the standard Ringer were made to the mucosal bath alone. Experiments were performed at 37°C.

Electrical measurements. The transepithelial potential with respect to the mucosal bath (V_t) was monitored by means of calomel cells in contact with the bathing solution via Ringer-agar bridges. Transepithelial current (I_t) was passed by means of an automatic voltage-clamp device (Physiologic Instruments, Houston, TX) and Ag-AgCl electrodes. Tissues were normally maintained under short-circuit conditions except for brief periods (300 ms) during which they were clamped to ±10 mV to measure transepithelial resistance (R_t). The intracellular potential across the apical membrane (V_a) was measured by 0.5 M KCl-filled microelectrodes having a resistance of 80–100 MΩ. The intracellular potential across the basolateral membrane V_b was calculated from $V_t = V_a + V_b$. Values of V_a and V_b are reported with respect to their respective bathing solutions. The fractional resistance of the apical membrane (f_{Ra}) was calculated from the ratio of the deflections produced in V_a and V_t by the bipolar voltage pulses.

Changes in V_a with V_t were determined from two protocols. In the first (*protocol 1*), V_t was stepped in 10-mV increments to increasingly more positive or negative potentials with a duration of 2 s until $V_t = \pm 100$ mV; ~40 s were required to complete the sequence. f_{Ra} was calculated from $\Delta V_a/\Delta V_t$ produced by 200 ms bipolar deflections of 10 mV superimposed on the clamping step. In the second protocol (*protocol 2*), V_t was stepped from 0 to a positive potential for 100 ms, back to 0 for 200 ms, then to a negative potential for 100 ms. The value of the clamp potential in either direction was increased by 10 mV until $V_t = \pm 100$ mV. f_{Ra} was calculated from $\Delta V_a/\Delta V_t$. All clamping sequences were under computer control (INDEC Systems, Sunnyvale, CA) as were data collection and analysis. Deflections in V_a and V_t during the clamping sequences were measured 25–50 ms after initiation of the pulse and averaged over a 20-ms interval. As will be shown later, any existing voltage transients were over by this time. For calculation of transmembrane current (I), outward current is considered positive.

Statistics. Significant differences between means were determined by the Student's t test for paired or unpaired observations. Unless indicated otherwise, n refers to the number of tissues studied.

RESULTS

Effect of high K^+. The effect of increasing mucosal bath K^+ (5–50 mM) on V_a and f_{Ra} is shown in Fig 1. In three such experiments (10 cells), V_a and f_{Ra} averaged 60.8 ± 2.3 mV and 0.82 ± 0.03, respectively. V_a depolarized ($\Delta V_a = 11.9 \pm 1.9$) when mucosal K^+ was increased as predicted for a K^+-selective membrane. In most cells, f_{Ra} also decreased ($\Delta f_{Ra} = 0.05 \pm 0.01$, $n = 7$), whereas in others ($n = 3$), there was no change.[1] TEA (15 mM), which has previously been shown to block K^+ channels in a variety of tissues (22, 31, 32), inhibited the high K^+ depolarization and concomitant change in f_{Ra} but had little effect on initial V_a or f_{Ra}.

Effect of voltage. Also shown in Fig. 1 is the effect of clamping the V_t from zero to more serosa-positive potentials on V_a. Not only does V_a depolarize but f_{Ra} decreases as well. Although TEA did not block the depolarization induced by clamping V_t, it did block the accompanying change in f_{Ra}. TEA was ineffective when added to the serosal solution and, further, had no effect on high-K^+ depolarizations of the basolateral membrane induced by elevating serosal bath K^+ (data not shown).

The effect of transepithelial voltage on membrane potential of one of these cells is shown in more detail in Fig. 2 in which *protocol 1* was used. Increasing V_t depolarized V_a linearly until V_a reached aprroximately −30 mV. Basolateral membrane potential (V_b) hyperpolarized in a similar fashion. The magnitude of the change in V_a is clearly larger than that in V_b. This is expected because f_{Ra} is ~0.8 in these cells, indicating that the apical membrane resistance accounts for 80% of the total cellular resistance with the basolateral membrane comprising only 20%. At V_a more depolarized than −30 mV, the relation between V_a, V_b, and V_t becomes distinctly curvilinear and a greater percent change in V_b is observed than in V_a. In the presence of TEA, however, the relation between V_a, V_b, and V_t remains linear throughout the range of voltages used in the study.

Figure 3 shows the relation between V_a (varied by clamping V_t) and f_{Ra} determined for the same cell in Fig. 2. As was observed for the relation between V_a and V_t, depolarizing V_a below −30 mV drastically changes the relation such that depolarization beyond this voltage causes a progressive decrease in f_{Ra}. The potential (−30 mV) at which changes in f_{Ra} were observed was surprisingly constant from tissue to tissue. In a few cases, however, changes in f_{Ra} occurred at higher (−50 mV) or lower (−20 mV) potentials. In contrast to depolarizing pulses, hyperpolarization of V_a generally had little effect on f_{Ra}. In experiments in which hyperpolarization was effective (3 of 14), f_{Ra} increased rather than decreased.

Despite the magnitude of the changes in V_a and f_{Ra} discussed above, clamping V_t had little effect on R_t (Fig. 4). Clamping V_t to ±100 mV changed R_t by 7.1 ± 1.9% ($n = 14$). Further, the addition of TEA altered R_t by only 6.0 ± 2.6% ($n = 6$).

The time course of changes in V_a on clamping V_t is shown in greater detail with the oscilloscope traces in Fig. 5 of a cell in which clamping *protocol 2* was used. Hyperpolarizing pulses resulted in equal increments in V_a. As shown above, depolarizing V_a beyond −30 mV, however, resulted in an obvious departure from linearity and an increase in the time required to reach a steady state (~25 ms). In the presence of TEA, hyperpolarizing and depolarizing pulses are symmetrical. The relations between V_a and f_{Ra}, and V_a and V_t obtained using this clamping protocol (data not shown) are similar to that obtained using *protocol 1* (Figs. 2 and 3) in that changes in f_{Ra} were observed at V_a more depolarized than −30 mV. However, the absolute value of f_{Ra} obtained for a particular value of V_a differed from that obtained from

[1] Because changes in f_{Ra} were small, it is reasonable to suspect that sometimes changes were within the noise of the measuring system, perhaps accounting for the observation that in some cells no change was observed. It is not clear why the magnitude of changes in f_{Ra} with K_m elevation is so small. However, considering the magnitude of depolarization (~11 mV), it may reflect a small partial conductance of the membrane to K^+ under basal conditions. A similar conclusion was reached by Cremaschi et al. (4) who in addition demonstrated Na^+ and Cl^- conductances in the apical membrane of this tissue.

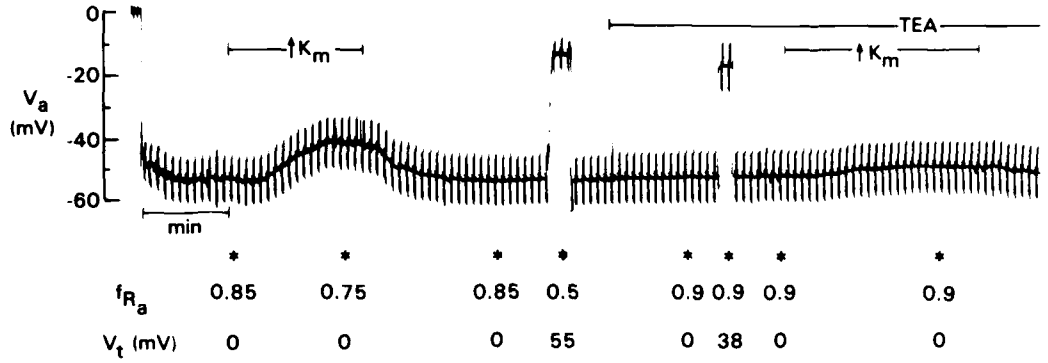

FIG. 1. Effect of increasing mucosal K^+ (K_m) and V_t on V_a and f_{Ra} in presence and absence of TEA (15 mM). K_m was increased from 5 to 50 mM. See text for definitions.

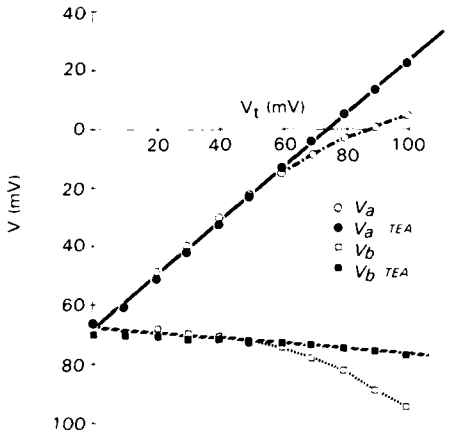

FIG. 2. Effect of clamping V_t on V_a and V_b in the absence and presence of TEA. Representative data obtained from a single impalement using *protocol 1*. See text for definitions.

protocol 1. This is expected because *protocol 2* calculates f_{Ra} from large changes in V_a (±100 mV) over regions in which the relation is not linear. Thus f_{Ra} calculated using *protocol 1* (i.e., 10 mV deflections superimposed on the

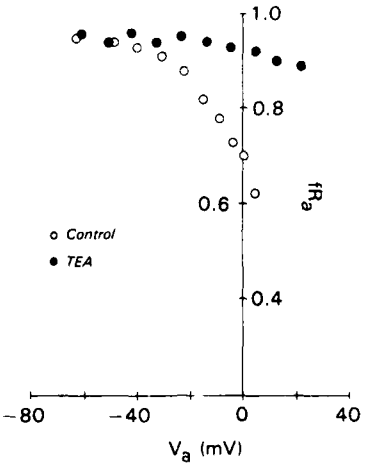

FIG. 3. Relation between V_a and f_{Ra} in absence and presence of TEA. Data from same impalement used in Fig. 2. See text for definitions.

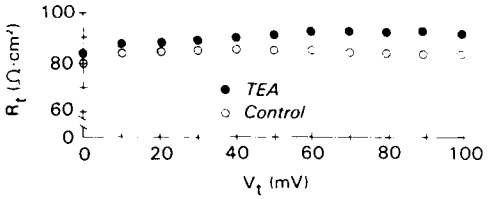

FIG. 4. Effect of clamping V_t on R_t in absence and presence of TEA. R_t was calculated as described in METHODS. Data are from same experiment as in Figs. 2 and 3. See text for definitions.

clamping step) more accurately reflects changes in relative membrane resistances with changes in membrane voltage.

Effect of K^+ channel blockers. Because changes in f_{Ra} with V_a were inhibited by the K^+ channel blocker, TEA, the ability of several other K^+ channel blockers to inhibit these changes was assessed to gain more information concerning the type of pathway responsible for the decline in f_{Ra} with voltage. Those tested included Ba^{2+}, reduced pH, 4-amino-pyridine (4-AP), and quinidine. Of these, Ba^{2+} (5 mM) and reduced pH (<5) affected the relation between V_a and f_{Ra} (Fig. 6). In both cases, the relation was shifted such that substantially greater depolarization was required to induce changes in f_{Ra}, consistent with voltage dependence of the inhibition. Unlike TEA, Ba^{2+} and pH also decreased basal V_a and increased f_{Ra} indicative of a decrease in basal K^+ conductance of the membrane (data not shown).

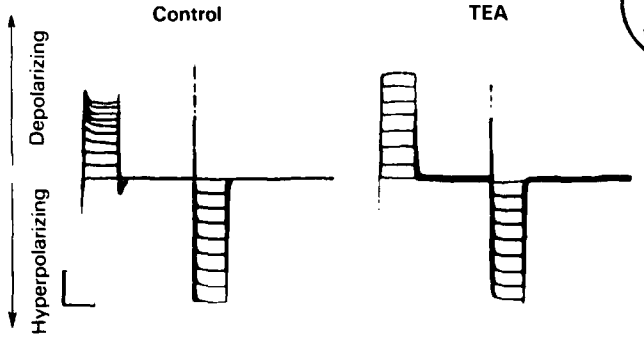

FIG. 5. Oscilloscope tracings of changes in V_a with V_t using clamping *protocol 2* before (*left*) and after (*right*) TEA. Results for both depolarizing and hyperpolarizing pulses are shown for a single impalement. Scale indicates 20 mV and 100 ms. See text for definitions.

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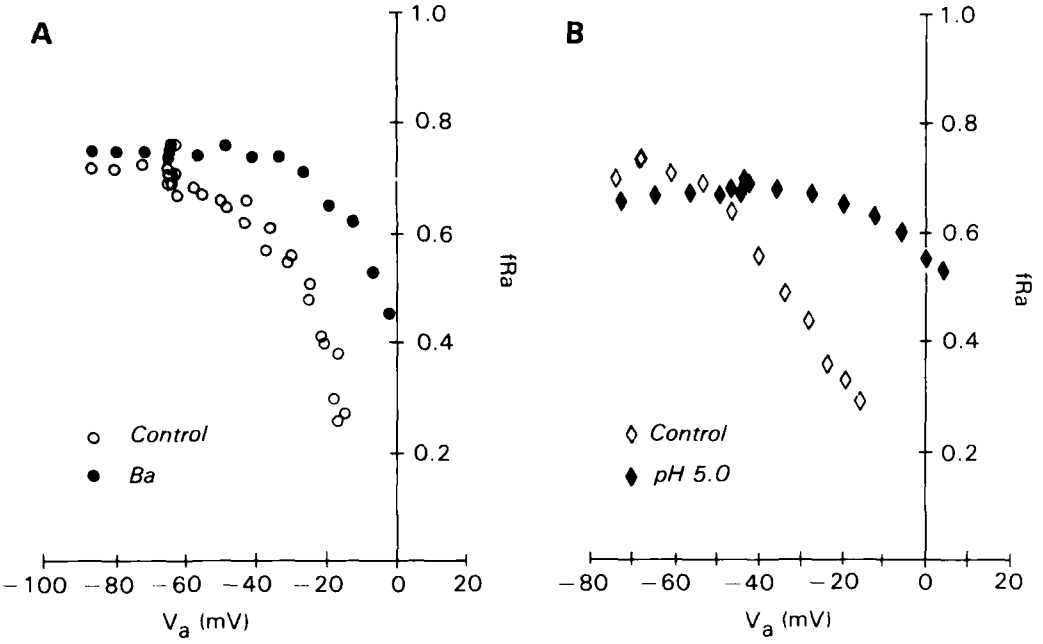

FIG. 6. Effect of Ba^{2+} (*A*) and pH (*B*) on V_a and f_{Ra} relation. *A* and *B*: representative experiments from different tissues (n = 4 and 3, for Ba^{2+} and pH, respectively). Control and inhibitor data, however, are from a single cell. See text for definitions.

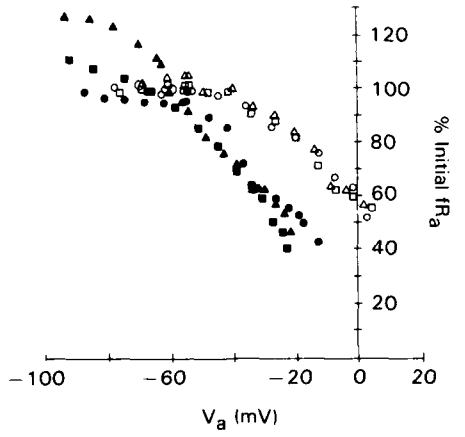

FIG. 7. Effect of ionomycin on the relation between V_a and f_{Ra}. Data are from a single tissue for three cells impaled before and 3 cells impaled after the addition of ionomycin. See text for definitions.

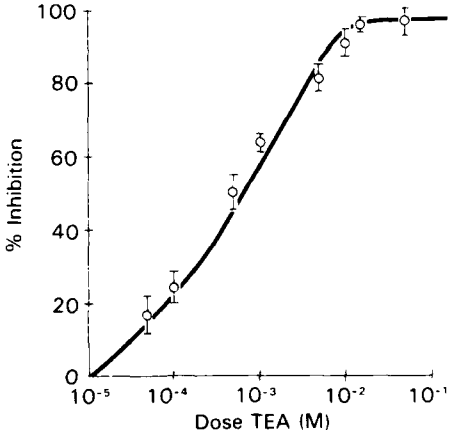

FIG. 8. Dose-response relation for TEA inhibition of changes in f_{Ra} with voltage. %Inhibition of f_{Ra} change for each dose was determined for V_a = −20 mV. Each point represents mean for at least 4 tissues. See text for definitions.

Effect of Ca^{2+} ionophore. The presence of 10^{-6} M ionomycin in the mucosal bath significantly altered the relation between V_a and f_{Ra}. As shown for the experiment in Fig. 7, changes in f_{Ra} occurred at more hyperpolarized potentials in the presence of the ionophore. In three experiments, depolarizing V_a to −30 mV decreased f_{Ra} to 83 ± 5%, whereas in the presence of ionophore depolarization to the same potential decreased f_{Ra} to 69 ± 2% of its initial value. Changes in f_{Ra} with V_a in the presence of ionomycin were also inhibited by mucosal TEA (data not shown).

Current-voltage relation of TEA-sensitive pathway. Of the K^+-channel blockers evaluated in this study, the most effective blocker of voltage induced changes in f_{Ra} was TEA. Assuming that TEA completely blocks the conductive pathway responsible for the changes in f_{Ra} without affecting other pathways, maximally effective concentrations of TEA can be used to evaluate the current-voltage (*I-V*) relation of this pathway as done previously by Thompson et al. (30) for the amiloride-sensitive pathway in rabbit colon provided that current through this pathway is not negligible with respect to paracellular current flow. The dose-response relation shown in Fig. 8 demonstrates that at concentrations exceeding 10 mM, TEA is virtually 100% effective in preventing changes in f_{Ra} over the range of voltages studied. Furthermore, as shown in Fig. 9 (V_a vs. I_t), I_t was substantially different in the presence and absence of 15 mM TEA. Thus subtraction of the two curves in Fig. 9 gives the current

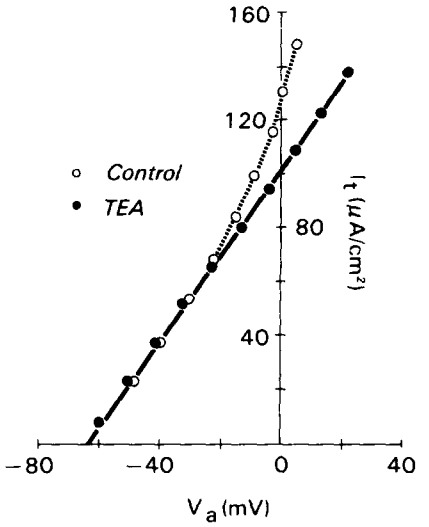

FIG. 9. V_a vs. current required to clamp V_a (I_t) before and after TEA. Data are from same impalement as that in Figs. 2–4 (*protocol 1*). Identical results were obtained from *protocol 2*. See text for definitions.

through the TEA-sensitive pathway. The resulting I-V relation is shown in Fig. 10 where I_{TEA} is the current through this pathway at a given V_a. This relation closely approximates that of the TEA-sensitive current across the apical membrane assuming that driving forces for TEA-insensitive pathways at this membrane are not affected by the inhibitor.

Because this current is blocked by Ba^{2+} and low-mucosal pH in addition to TEA, the most likely ionic species carrying this current is K^+. Therefore, the data shown in Fig. 10 were fit to the Goldman constant-field equation for voltage-independent K^+ movement across the apical membrane

$$I_K = V_a P_K F^2/RT \frac{[K_m - K_c \exp(V_a F/RT)]}{1 - \exp(V_a F/RT)}$$

where I_K is the K^+ current across the membrane, P_K is

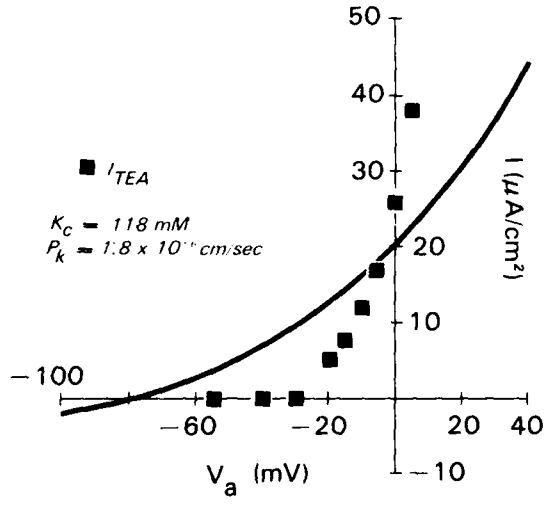

FIG. 10. I-V relation of TEA-sensitive pathway calculated from subtraction of two curves shown in Fig. 9. Curve is computer-generated best fit of data to Goldman constant-field equation giving values for P_K and K_c shown. See text for definitions.

the K^+ permeability, K_c and K_m are the intracellular and mucosal bath K^+ activities, respectively, and RT and F have their usual meanings. Initial values of K_c were selected from measurements made in this tissue by Cremaschi et al. (4). Because P_K has not been measured, initial values of P_K were based on that determined by Reuss et al. (28) for *Necturus* gallbladder.[2] Clearly the computer-generated, best-fit curve shown in Fig. 10 does not adequately describe the relation between I_{TEA} and V_a. Similar results were obtained from the analysis of the I-V relations of five additional cells from three tissues regardless of the clamping protocol used. The implications of these data with respect to this curve will be discussed below.

DISCUSSION

Interpretation of data. The results of this study indicate that a voltage-dependent K^+ conductance resides in the apical membrane of guinea gallbladder epithelial cells. This is supported by the observations that *1*) depolarization of the apical membrane is accompanied by a decrease in the fractional resistance of the apical membrane, *2*) the change in fractional resistance can be inhibited by classical K^+-channel blockers Ba^{2+}, TEA, and pH, *3*) TEA has no effect when applied from the basolateral side, and *4*) the I-V relation of the TEA-sensitive pathway cannot be fit by the Goldman constant-field equation.

There are several issues central to this interpretation that must be addressed. First, does the fractional resistance reflect changes in cellular membrane resistance rather than paracellular resistance? As discussed by Boulpaep and Sackin (2), changes in fractional resistance may reflect differences in the relative values of junctional and lateral intercellular space resistances rather than cellular resistances. Although this issue was not directly addressed in this study, several lines of evidence support a cellular origin of changes in fractional resistance. Clamping the transepithelial potential to positive or negative values had little effect on transepithelial resistance. In a leaky tissue such as this, the transepithelial resistance mainly reflects the resistance of the paracellular pathway. In addition, although TEA blocked changes in f_{Ra} observed with clamping, it had little effect on transepithelial resistance. Finally, changes in f_{Ra} were blocked by classical inhibitors of membrane K^+ conductances, TEA and Ba^{2+}. Thus the changes in f_{Ra} observed in this study most likely have a cellular rather than a paracellular origin. A similar conclusion was reached for changes in f_{Ra} with voltage in *Necturus* gallbladder by Garcia-Diaz et al. (7). However, because in *Necturus* gallbladder voltage clamping changed transepithelial resistance by 50%, these investigators used La^{3+} to block

[2] In the analysis of Na conductance in rabbit colon (30), the I-V relation was analyzed for conformity to the Goldman constant-field equation using values of permeability and intracellular ion activity from fit of the equation to I when $V_a = E_t$ (the reversal potential or zero current) and $V_t = 0$. This approach was not used in the present study as V_a at which the TEA-sensitive current reversed was not clear. The data suggest that this conductance is essentially zero around the negative potentials at which I_K is expected to reverse in these cells (~−75 mV) and thus current is negligible at hyperpolarized potentials and within the noise of the measurement.

changes in transepithelial resistance with clamping, yet still observed changes in fractional resistance.

Second, does the relation between f_{Ra} and V_a reflect a voltage-dependent conductance change or Goldman rectification through a voltage-independent pathway? To answer this question, the *I-V* relation of the TEA-sensitive pathway was analyzed for fit to the Goldman constant-field equation for voltage-independent K^+ movement across the apical membrane. *I-V* relations were determined from differences in transepithelial current in the presence and absence of the inhibitor, TEA. As discussed by Lapointe et al. (21), this approach is generally not applicable to the study of ion transport across "leaky" epithelia. The preponderance of transepithelial current flow through paracellular rather than cellular pathways can preclude the determination of transcellular current flow. However, fortuitously, this does not appear the case for the TEA-sensitive pathway in guinea pig gallbladder. Although it was difficult to reliably determine ΔI_t at hyperpolarized potentials, the reduction of I_t by TEA was substantial within the range of voltages at which the TEA-sensitive conductance appears activated (< -30 mV). At very depolarized values of V_a, in some cases, TEA reduced I_t by ~80 $\mu A/cm^2$. Thus, although there may be some uncertainty at hyperpolarized regions of the *I-V* curve, this is not the case for that portion of the curve of particular interest.

Central to the analysis of these *I-V* relations are the criteria that *1*) TEA affects only a transcellular pathway, *2*) the *I-V* relation is instantaneous with respect to changes in ionic composition, and *3*) that other conductive pathways are not affected by the inhibitor. All of these criteria were met in the study. As noted earlier, TEA predominantly affects the transcellular pathway. Neither voltage nor TEA had much effect on transepithelial resistance with changes in transepithelial resistance being < 10%. The error introduced in the analysis by this change is small. In addition, TEA has previously been shown to block membrane K^+ conductances in other epithelia with little effect on paracellular transport (7, 11).

The *I-V* relations were obtained under conditions that appeared to be instantaneous with respect to changes in intracellular composition. One of the clamping protocols used was essentially identical to that employed by Thompson et al. (30) in which the sequence is bipolar (in *protocol 2*, the potential was rapidly clamped to alternately a positive or negative voltage from 0). Such a sequence would not be expected to cause shifts in intracellular composition. Although the other protocol employed was unipolar (in *protocol 1*, the clamping voltage became progressively positive or negative without returning to 0) and more prone to produce changes in intracellular composition, the results obtained were essentially identical, perhaps due to the rapidity with which the clamping sequence was accomplished. The *I-V* relation, however, was not instantaneous with respect to changes in membrane voltage. Depolarizing the apical membrane beyond −30 mV produced a progressive increase in the time required for a steady-state voltage to be reached (~25 ms). Similar changes were observed previously for *Necturus* gallbladder under current clamp by Reuss and Finn (27) and voltage-clamp conditions by Garcia-Diaz et al. (7). Reuss and Finn (27) suggested that these changes were related to polarization effects at the membrane (changes in ionic composition in extracellular compartments). Such an explanation would bring into question the appropriateness of the data analysis presented here. However, as discussed by Garcia-Diaz et al. (7) and Garcia-Diaz and Essig (6) for *Necturus* gallbladder and more recently for *Necturus* small intestine by Lapointe et al. (21), the voltage transients observed here are more likely capacitative (charging of linear RC elements). The apparent increase in the time constant results from the voltage-dependent decline of the apical membrane resistance and a subsequent decrease in the ratio of apical to basolateral membrane time constants. This does not adversely affect the interpretation of the data provided that values of V_a and f_{Ra} are taken after the transients, as was the case.

It is also unlikely that the addition of TEA affects conductive pathways other than that of the apical membrane K^+ conductance. TEA only blocked the conductance associated with depolarization. The inhibitor had no effect on basal membrane potential and little effect on f_{Ra}. Thus driving forces for other ion flows should be unchanged and the *I-V* relation of the transcellular TEA-sensitive pathway approximate that of the apical membrane without requiring corrections employed by others (30).

Thus because the *I-V* relation of the TEA-sensitive pathway met the necessary criteria described above, analysis of this relation with respect to the Goldman constant-field equation is appropriate. Using reasonable values for apical membrane potassium permeability and intracellular potassium activity , the TEA-sensitive current could not be fit by this equation. This was particularly evident for the dramatic increases in current observed at V_a corresponding to values at which f_{Ra} was noted to decrease. Thus the changes in current and f_{Ra} observed with voltage are consistent with activation of a voltage-dependent K^+ conductance. Garcia-Diaz et al. (7) reached a similar conclusion for *Necturus* gallbladder, however, these investigators did not analyze the *I-V* relations of this pathway.

Nature of voltage-dependent K^+ conductance and its relation to those in other epithelia. To gain additional information concerning the nature of the pathway responsible for the voltage dependence of the apical membrane conductance, two series of experiments were performed. First, the efficacy of several K^+-channel blockers that have been shown to block different types of K^+ channels (22) was assessed. In addition to TEA, both Ba^{2+} and decreased pH (5.0) blocked changes in fractional resistance with voltage. Quinidine and 4-AP had no effect on the voltage-dependent conductance. Ba^{2+} and pH also had effects on basal potential and fractional resistance consistent with blocking basal K^+ conductance, whereas TEA, quinidine, and 4-AP did not. Thus TEA appears to specifically block the voltage-dependent K^+ conductance with little effect on the K^+ conductance comprising the basal K^+ conductance of the membrane, whereas Ba^{2+} and pH have effects on both. This raises the possibility that at least two different types or states

of K^+ conductance reside in the apical membrane.

In a second series of experiments, the effect of the Ca^{2+} ionophore, ionomycin, was determined. A shift in the voltage dependence was observed such that changes in fractional resistance occurred at more hyperpolarized potentials. Thus the voltage-dependent conductance also appears to be calcium sensitive.

This conductance was localized to the apical membrane. There was no effect of TEA when applied from the serosal side. In a previous study (15), serosal Ba^{2+} and quinidine were observed to inhibit basolateral membrane K^+ conductance as was also observed for turtle colon (8, 29). These pharmacological differences suggest that the individual conductances contributing to the K^+ conductance of the serosal membrane are distinct from those of the apical membrane.

Given the pharmacology of the voltage-dependent conductance reported in the present study, the membrane voltage at which the conductance appears activated (−30 mV) and its calcium sensitivity the data suggest that activation of channels generally referred to as "maxi-K^+" channels underlie the observed changes in fractional resistance with membrane voltage. Although this awaits confirmation, these channels have been observed in the cell membranes of several other epithelia (3, 19, 22) including the apical membrane of Triturus gallbladder (23). It is distinctly different from that described recently by Palmer (24) for the apical membrane of toad urinary bladder that is blocked by Ba^{2+}, pH and quinidine, but not TEA, and activated at V_a exceeding 50 mV.

The physiological significance of a voltage-dependent K^+ conductance in the apical membrane of guinea pig gallbladder epithelial cells is presently unclear. The lack of an effect of TEA on basal V_a and f_{Ra}, or membrane hyperpolarization on f_{Ra} suggest that in contrast to *Necturus* gallbladder (7), this conductance does not generally contribute to the basal K^+ conductance of these cells. The voltage sensitivity of the conductance indicated by this study, however, suggests that it would become significant at membrane potentials less than −30mV. Activation of such a conductance would partially repolarize the membrane, reestablishing the membrane potential and the driving force for various ion movements. Although it is unlikely that the apical membrane would be depolarized to this potential under physiological conditions, in the presence of increased intracellular Ca^{2+} the conductance would be activated at more hyperpolarized potentials. Guinea pig gallbladder secretes fluid and electrolytes in the presence of secretagogues such as prostaglandin (16). HCO_3^- secretion has been observed to be accompanied by K^+ secretion. Although the electrophysiological response of this tissue to secretagogues has not been studied, the notion that the voltage-dependent conductance is involved in this secretory response is attractive. Along these lines, in preliminary experiments, TEA was observed to increase the short-circuit current elicited by prostaglandin in guinea pig gallbladder (unpublished data). Basolateral membrane Ca^{2+}-activated, voltage-dependent K^+ channels have been observed to be involved in the secretory response of several secretory epithelia including lacrimal, pancreatic, and salivary glands (see Ref. 26). Determination of the role of similar channels in the apical membrane of epithelial cells, however, has been more elusive (5).

In summary, the present study reveals a voltage-dependent K^+ conductance in the apical membrane of guinea pig gallbladder epithelial cells. In addition, the conductance appears to be activated by Ca^{2+}. Future studies on the mode of regulation of this conductance by voltage and Ca^{2+} with respect to its physiological role should yield valuable information concerning the regulation of transport processes in epithelial cells.

NOTE ADDED IN PROOF

Stoddard and Reuss (29a) have very recently reevaluated apical membrane potential and fractional resistance in *Necturus* gallbladder under current clamp conditions. They also conclude that the results (which are qualitatively similar to those reported here) reflect a voltage-sensitive K conductance in the apical membrane.

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