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Electrogenic Bicarbonate Secretion in the Turtle Bladder: Apical Membrane Conductance Characteristics

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Summary. We have recently shown that stimulation of electrogenic HCO_3^- secretion is accompanied by a simultaneous increase in short-circuit current (I_{sc}, equivalent to HCO_3^- secretion rate under these conditions), apical membrane capacitance (C_a, proportional to membrane area), and apical membrane conductance (G_a, proportional to membrane ionic permeability). The current experiments were undertaken to explore the ionic basis for the increase in G_a and the possibility that the rate of electrogenic HCO_3^- secretion is regulated by changes in G_a. Membrane electrical parameters were measured using impedance-analysis techniques before and after stimulation of electrogenic HCO_3^- secretion with cAMP in three solutions which contained different chloride concentrations. In another series of experiments, the effects of an anion channel blocker, anthracene-9-carboxylic acid (9-AA), were measured after stimulation of electrogenic HCO_3^- secretion with cAMP. The major conclusions are: (*i*) a measurable apical Cl^- conductance exists in control hemibladders; (*ii*) the transport-associated increase in G_a includes a Cl^--conductive component; (*iii*) G_a also appears to reflect a HCO_3^- conductance; (*iv*) the relative magnitudes of the apical membrane conductances to Cl^- and HCO_3^- are similar; (*v*) 9-AA reduces G_a and I_{sc} in cAMP-stimulated hemibladders; and (*vi*) alterations in I_{sc} appear to be mediated by changes in G_a.

Key Words bicarbonate transport · turtle bladder · equivalent-circuit analysis · impedance analysis · membrane conductance · anion transport

Introduction

The urinary bladder of the freshwater turtle contains discrete mechanisms for the active transport of acid and base. Electrogenic bicarbonate secretion is known to be selectively stimulated by cAMP. Electron micrographic studies have shown that cAMP results in morphological alterations in the apical membrane of a single cell type, the β-type carbonic anhydrase-rich cell and, therefore, HCO_3^- transport is thought to be mediated by the β cells (Stetson, 1988). The currently accepted model for electrogenic HCO_3^- secretion incorporates a Cl^-/HCO_3^- exchange mechanism in parallel with an anion conductive pathway in the apical membrane of the β cell (Stetson et al., 1985). The Cl^- that enters the cell in exchange for HCO_3^- is thought to cycle out of the cell via the conductive pathway, thereby generating current. A proton ATPase in the basolateral membrane reabsorbs acid, which ultimately provides the driving force for the process.

In Cl^--free solutions, stimulation of HCO_3^- secretion with cAMP also results in an increase in I_{sc}, which has been shown to be equivalent to the rate of HCO_3^- secretion (Satake et al., 1983). Under these conditions Cl^-/HCO_3^- exchange is not functional and HCO_3^- is thought to be secreted via the conductive pathway. Hence, the apical membrane is thought to be permeable to both Cl^- and HCO_3^-.

Recently, using impedance-analysis techniques, we have shown that stimulation of HCO_3^- secretion with isobutyl methylxanthine (IBMX, a phosphodiesterase inhibitor) and cAMP is accompanied by a concomitant increase in apical membrane capacitance (C_a, proportional to apical membrane area), and apical membrane conductance (G_a, proportional to ionic permeability) (Rich, Dixon & Clausen, 1990). Carbachol, another agent that selectively stimulates electrogenic HCO_3^- secretion, was shown to produce a transient increase in I_{sc} that is accompanied by a similar transient increase in C_a and G_a. These results are consistent with the current model for HCO_3^- secretion by the β cell and further suggest that electrogenic HCO_3^- secretion may be regulated by apical membrane remodeling processes that result in alterations in apical membrane conductive properties.

The regulation of salt and water transport in a

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variety of epithelia has been shown to be mediated by a cAMP-activated apical membrane chloride conductance (Landry et al., 1987). For example, the apical membrane in cultured cells from human airway epithelium contains a chloride channel which is activated in response to intracellular cAMP (Frizzell et al., 1986), and the large secretory response observed in the small intestine due to cholera toxin results from a cAMP-induced increase in apical chloride conductance. Finally, patch-clamp studies in rabbit intercalated cells grown in culture, which are functionally and morphologically similar to the carbonic anhydrase-rich cells in the turtle bladder, have confirmed that the apical membrane contains chloride channels (Light et al., 1988). Hence, it is conceivable that the cAMP-stimulated increase in G_a in the turtle bladder may also be due to an increase in Cl^- conductance.

The current model for electrogenic bicarbonate secretion, originally proposed by Stetson and Steinmetz (1985), proposes that the conductive pathway in the apical membrane of the β cell is chloride selective. However, no direct measurements of apical chloride conductive properties have been made to date. The current studies were undertaken to determine whether the increase in G_a, associated with stimulation of electrogenic HCO_3^- secretion, is due to an increase in Cl^- conductance and to examine further the relationship between G_a and the rate of electrogenic HCO_3^- secretion.

Materials and Methods

Dissection, Chamber, and Solutions

Freshwater turtles, *Pseudmys scripta elegans,* were double pithed and their urinary bladders were removed with a minimal amount of handling. Hemibladders were mounted in Ussing chambers specially designed to minimize edge damage (Lewis et al., 1977). The nominal exposed surface area of the chamber was 2.0 cm^2 and each half-chamber had a volume of 15 ml.

In all experiments the mucosal and serosal solutions were bubbled with 5% CO_2, 95% O_2 and gently stirred. Three different bathing solutions with varying amounts of chloride were used. The first solution, a modified Ringer's solution contained (in mM): 90 NaCl, 20 $NaHCO_3$, 1.0 NaH_2PO_4, 0.5 Na_2HPO_4, 3.5 KCl, 1.0 $MgCl_2$, and 1.0 $CaCl_2$. The second solution, a low-chloride Ringer's solution, contained Na-gluconate in place of NaCl, and was identical in other respects with the chloride-containing Ringer's solution. The third solution was chloride free and contained (in mM): 90 Na-gluconate, 20 $NaHCO_3$, 1.0 NaH_2PO_4, 0.5 Na_2HPO_4, 3.5 K-gluconate, 1.0 $MgSO_4$, and 1.0 Ca-gluconate. In all cases the pH was adjusted to 7.3. *These three solutions will be referred to as normal-, low-, and zero-chloride solutions, respectively.* In a separate series of experiments, the pH of the mucosal solution was buffered at 4.5 in order to enhance uptake of 9-AA, a weak acid. The composition of this solution was (in mM): 75 NaCl, 10 Na_3-citrate, 10 citric acid, 1.0 Na_2HPO_4, 3.5 KCl, 1.0 $MgCl_2$, and 1.0 $CaCl_2$. In all cases the serosal solution contained 2% bovine serum albumin (Fraction V, Sigma) and 5.0 mM D-glucose. Otherwise the mucosal and serosal bathing solutions were identical. A small amount of silicon oil (Antifoam A, Dow Corning, Midland, MI) was sprayed atop the serosal solution to control foam resulting from bubbling the albumin-containing solution.

Electrical Measurements

Transepithelial voltage (V_t) was measured differentially using a high-speed, high-impedance amplifier (Model 113, Princeton Applied Research, Princeton, NJ) connected to a pair of Ag/AgCl electrodes mounted close to both surfaces of the epithelium. A second pair of Ag/AgCl electrodes mounted at opposite ends of the chamber were used to pass transepithelial current. Agar bridges were used in place of Ag/AgCl electrodes in chloride-free solutions. The bridges used for voltage measurements were made of 3% agar and 2.0 M K-gluconate and were mounted adjacent to the epithelium, and the current-passing electrodes were made of 3% agar and 2.0 M KCl and were mounted at opposite ends of the chamber. Care was exercised in the construction of the bridges to minimize bridge resistance (*see below*). Constant current was generated using a calibrated 1 MΩ carbon series resistor connected to the mucosal current electrode; the serosal electrode was connected to ground.

During an experiment the bladder was held under open-circuit conditions and in the majority of experiments, short-circuit current (I_{sc}) was measured intermittently by passing a 500-msec pulse that depolarized transepithelial voltage (V_t) to zero. A positive current is defined as flowing from mucosa to serosa.

In one series of experiments I_{sc} was calculated as the ratio of V_t and the transepithelial resistance (determined by analysis of impedance, *see below*). We verified that calculated and measured estimates of I_{sc} were in good agreement; in 30 cases measured in three preparations, calculated estimates of I_{sc} differed from measured estimates by -0.07 ± 0.14 $\mu A/cm^2$ (mean ± SD).

Measurement of Bicarbonate Secretion Rate

In addition to electrogenic proton and HCO_3^- secretion, the turtle bladder actively reabsorbs sodium via an electrogenic process. Sodium reabsorption was inhibited in all experiments by the mucosal addition of 0.1 mM amiloride (Merck, Sharp and Dohme, Rahway, NJ). Application of amiloride resulted in a rapid reduction of V_t, which reversed in polarity from mucosal-side positive to mucosal-side negative. The remaining I_{sc} is a combination of proton and bicarbonate electrogenic secretion. Electrogenic HCO_3^- secretion was stimulated with the mucosal and serosal addition of 50 μM IBMX and the serosal addition of 1.0 mM 8-bromo cAMP, a membrane-permeable analog of cAMP. Henceforth, when we refer to stimulation with cAMP we are implying these experimental conditions. The change in I_{sc} (ΔI_{sc}) stimulated by cAMP has been shown to be equivalent to the rate of bicarbonate secretion, as measured by pH-stat techniques (Satake et al., 1983). Therefore the cAMP-stimulated increase in I_{sc} was used as a measure of electrogenic bicarbonate secretion.

Impedance Analysis

Transepithelial impedance was measured using the method of Clausen and Fernandez (1981). A complete description of the technique can be found in Clausen, Reinach and Marcus (1986) and its application in the turtle bladder may be found in Clausen and Dixon (1986). Briefly, a wide-band pseudorandom binary signal was generated digitally, converted to a constant current, and applied transepithelially. The resulting voltage response was amplified, passed through an antialiasing filter, digitized, and recorded by computer. The impedance was calculated by dividing the cross-spectral density of the voltage and the current by the power-spectral density of the applied current.

At the start of each experiment the current was recorded and characterized by measuring the voltage response to the current across a calibrated 1.5 kΩ carbon resistor. The impedance of the empty chamber and electrodes was verified to be totally resistive; the resulting phase angle deviations were less than 0.5 degrees over the frequency range used. This small deviation is expected since phase deviations arising from our major stray capacitance, the input capacitance of the amplifier (about 15 pF), are negligible due to the low resistance of the Ag/AgCl electrodes (ca. 1 kΩ) or the agar bridges (ca. 4 kΩ).

The data were represented as Bode plots which plot phase angle and log impedance magnitude *versus* frequency. Estimates of the different membrane electrical parameters were obtained by fitting the data by a morphologically based equivalent circuit model using a nonlinear least-squares curve-fitting algorithm. The model is described in detail in Clausen et al. (1986). Briefly, the apical membrane was represented as a parallel resistor-capacitor (RC) circuit and the basolateral membrane was represented as a distributed, parallel RC circuit accounting for the path resistance of the narrow lateral space. The junctional paracellular resistance was assumed to be infinite and a small series resistance was included to represent the resistance between the voltage electrodes and the epithelial surface. Note that this method has previously been shown to result in reliable estimates of apical and basolateral capacitance (C_a and C_b), apical and basolateral conductance (G_a and G_b), and lateral-space distributed resistance (R_p) in the turtle bladder during stimulation of proton or bicarbonate secretion (Clausen & Dixon, 1986; Rich et al., 1990).

Membrane capacitance may be used as an indirect measure of membrane area since the specific capacitance of nearly all biological membranes is nearly constant at approximately 1 μF/cm^2 (Cole, 1972). Membrane conductance is proportional to ionic permeability. The specific conductance of the membranes ($G_{a\text{-norm}}$ and $G_{b\text{-norm}}$) can be estimated by normalizing each membrane by its respective capacitance (i.e., $G_{a\text{-norm}} = G_a/C_a$ and $G_{b\text{-norm}} = G_b/C_b$). R_p provides an indirect measure of lateral-space geometry; R_p is directly proportional to lateral-space length and inversely proportional to lateral-space width. R_p is also proportional to the resistivity (ρ) of the solution filling the lateral spaces.

Statistics

Statistical analyses were performed using the MINITAB system (Minitab, State College, PA). Results presented in tables and text are reported as mean ± SEM, unless stated otherwise. Paired and unpaired *t*-tests were used to determine the statistical significance of observed changes in membrane parameters where $P < 0.05$ was accepted as significant. Differences, or changes in parameters, are indicated in tables as "Δ".

For each curve fit to the measured data the Hamilton R-factor (Hamilton, 1964) was calculated. The R-factor is a measure of the relative difference between the data and the model-predicted impedance and therefore is an objective measure of the relative quality of the fits. The average R-factor from 229 randomly selected impedance runs was 1.04 ± 0.21% (mean ± SD). Occasionally data were poorly fit by the equivalent circuit model resulting in a relatively large R-factor. Runs were discarded if the R-factor exceeded 1.5%, a value more than 2 SD greater than the mean R-factor. Of the 229 runs selected here only 4 runs were rejected based on this criterion.

Results

Time Course of cAMP-Stimulated Changes in Apical Membrane Electrical Parameters

Cyclic-AMP is known to stimulate electrogenic HCO_3^- secretion selectively, without affecting proton secretion, or Na^+ reabsorption (Satake et al., 1983). In an earlier study (Rich et al., 1990), we showed that stimulation of HCO_3^- transport by the serosal application of cAMP produces an increase in I_{sc}, which is accompanied by concomitant increases in C_a and G_a. We proposed that the increase in I_{sc} might be mediated by the insertion of apical membrane, reflected by the increase in C_a, possessing Cl^-/HCO_3^- exchangers and/or Cl^--selective channels. The Cl^-/HCO_3^- exchangers are thought to translocate Cl^- and HCO_3^- in an electroneutral fashion, and would therefore not be expected to alter the conductive properties of the apical membrane. However, the increase in G_a is consistent with the notion that the inserted membrane possesses Cl^--conductive channels. To investigate further the relationship between I_{sc}, C_a and G_a, we measured the detailed time course of the changes in these three parameters, following stimulation with cAMP.

These particular experiments were performed in hemibladders bathed with low-pH mucosal solution. Note that under these conditions, electrogenic proton secretion is inhibited, and I_{sc} reflects the absolute rate of electrogenic HCO_3^- secretion. Impedance data were acquired every 2 min following the application of cAMP, thereby providing good time resolution of the changes in membrane parameters. For these experiments *only*, I_{sc} was calculated (*see* Materials and Methods), in order to avoid possible alterations in the kinetics of the response resulting from frequent transient changes in transcellular current caused by short circuiting the epithelium.

The time course in the change in I_{sc}, C_a and G_a is shown in Fig. 1, which is a representative response measured in one hemibladder following application of cAMP. These results show a close temporal association between the three parameters, consistent

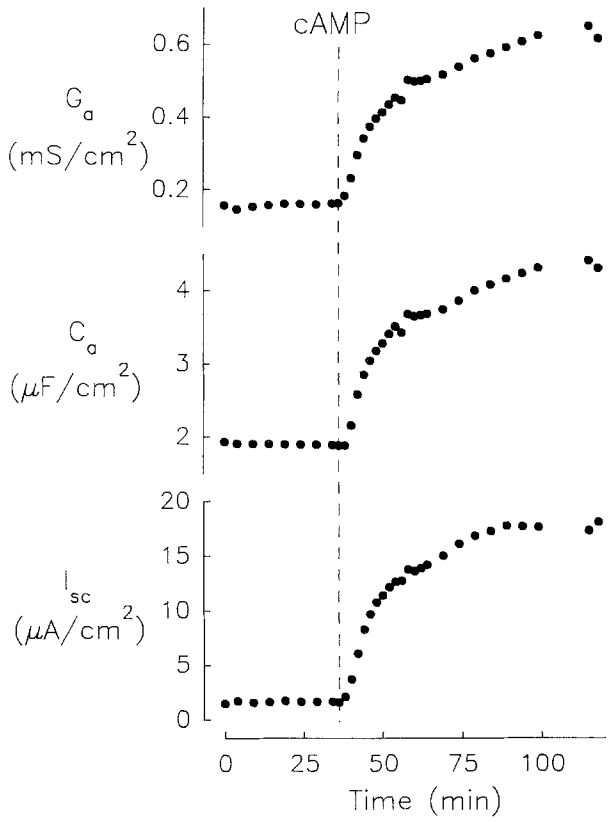

Fig. 1. G_a (upper panel), C_a (middle panel), and I_{sc} (lower panel) from a representative hemibladder plotted as a function of time. At the point marked "cAMP" 1 mM cAMP was added to the serosal solution and 50 μM IBMX was added to the mucosal and serosal solutions

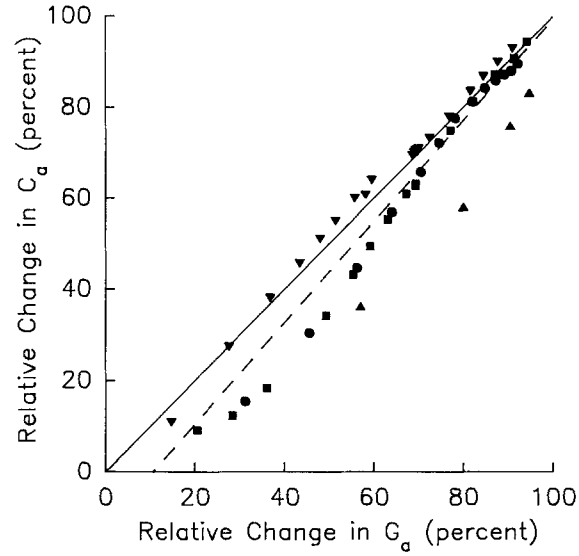

Fig. 2. Relative change in C_a (ordinate) plotted against the corresponding relative change in G_a (abscissa). Each symbol is an individual time point taken from the response following the addition of cAMP (*see* Fig. 1). The different symbols denote data acquired from four preparations. The solid line is the line of identity, and the broken line is the linear regression through the data

with our earlier hypothesis that the increase in I_{sc} is mediated by an increase in apical membrane conductance resulting from the insertion of channel-containing apical membrane.

Further support for this hypothesis is shown in Fig. 2 where the *relative* change in G_a is plotted *versus* the *relative* change in C_a for each time point from four separate experiments. The parameters were each normalized by subtracting the baseline (pre-cAMP) value, and subsequently dividing by the resulting maximum value, thereby producing coordinates that ranged from 0 to 100%. So as not to unduly weight the 0 and 100% coordinates, only values ranging between 5 and 95% were plotted. The different symbols in Fig. 2 denote results obtained from four different preparations. The solid diagonal line is the line of identity, and the dashed line is the linear regression through the data.

Figure 2 shows a strong near-linear correlation ($r = 0.96$) between C_a and G_a. This strongly supports the notion that the transport-associated rise in G_a results primarily from the addition of apical membrane area and that this added membrane possesses conductive channels. However, the slope of the regression line (1.11 ± 0.05) is statistically different from unity ($P = 0.02$), thereby indicating that G_a and C_a are not perfectly correlated. The abscissa intercept shows that a small fraction, approximately 11%, of the transport-associated increase in G_a is independent from the changes in C_a and may result from opening of channels already present in the apical membrane.

Apical Membrane Conductance and Capacitance in Reduced Cl^- Solution

A series of experiments were done in which the Cl^- concentration in the bathing solutions was lowered from 97.5 to 7.5 mM by replacement of 90 mM NaCl with equimolar Na-gluconate. Although reduced, the Cl^- concentration is still expected to be above the reported K_m's for the Cl^--HCO_3^- exchange processes. For example, the K_m for proton transport in the α cells, which is dependent on basolateral membrane Cl^--HCO_3^- exchange, is estimated at 0.13 mM serosal Cl^- (Fischer, Husted & Steinmetz, 1983). Therefore any changes resulting from the reduced Cl^- levels are not expected to be due to

Table 1. Apical membrane parameters in low chloride[a]

	$[Cl^-]$ mM	C_a $\mu F/cm^2$	G_a mS/cm^2	$G_{a\text{-norm}}$ $\mu S/\mu F$	I_{sc} $\mu A/cm^2$
Control	97.5	3.2 ± 0.4	0.26 ± 0.06	80 ± 13	−8.8 ± 4.0
Control	7.5	3.0 ± 0.4	0.19 ± 0.04	66 ± 8.3	−6.1 ± 2.8
Δ		−0.2 ± 0.06	−0.06 ± 0.03	−14 ± 6.1	2.7 ± 1.3
P		0.02	NS	0.05	NS
Control	97.5	3.0 ± 0.4	0.24 ± 0.05	86 ± 15	−7.7 ± 3.1
cAMP	97.5	3.9 ± 0.6	0.37 ± 0.07	100 ± 15	−2.7 ± 2.5
Δ		0.9 ± 0.2	0.13 ± 0.04	15 ± 15	−5.0 ± 1.1
P		0.005	0.01	NS	0.003
cAMP	7.5	3.6 ± 0.5	0.29 ± 0.05	86 ± 13	−3.8 ± 2.4
Δ		−0.30 ± 0.1	−0.08 ± 0.02	−15 ± 4	−1.0 ± 0.6
P		0.04	<0.0001	0.008	NS

[a] Data are mean ± SEM from eight hemibladders. "Control" indicates hemibladders prior to stimulation with cAMP, "Δ" indicates the difference between the preceding two states, and "*P*" is probability comparing the preceding two states (paired *t* test).

changes resulting from alterations in electroneutral Cl^--HCO_3^- exchange. If the membrane exhibits a Cl^--conductive pathway, then this protocol should reveal it without affecting I_{sc} due to alterations in Cl^-/HCO_3^- exchange.

The results of reducing extracellular Cl^- under control (unstimulated) conditions are shown in Table 1. Reducing Cl^- causes small decreases in G_a and $G_{a\text{-norm}}$, but only the decrease in $G_{a\text{-norm}}$ reached statistical significance (the decrease in G_a approaches significance, $P = 0.09$). Recall that $G_{a\text{-norm}}$ is the apical membrane conductance normalized to membrane capacitance and provides an estimate of the apical membrane specific conductance. These results show that under control conditions, the apical membrane exhibits a low, but measurable, Cl^- conductance.

Reducing the Cl^- concentration also resulted in a small decrease in C_a, that was found to be significant. The basis for this decrease is not clear. It is notable that restoration to normal Cl^- concentrations failed to reverse this change. Finally, reducing Cl^- had no significant effect on I_{sc}, consistent with the notion that the experimental maneuver did not significantly alter HCO_3^- or proton secretion. Note that negative I_{sc} indicates that the epithelium is secreting protons in excess of HCO_3^-.

Table 1 also shows the apical membrane electrical parameters after restoring the bath Cl^- concentration to 97.5 mM and stimulating electrogenic HCO_3^- secretion with cAMP. Cyclic-AMP produced a significant increase in C_a, G_a and I_{sc}, consistent with the notion that stimulation of HCO_3^- secretion is mediated by an increase in channel-containing apical membrane area. Although G_a increased, $G_{a\text{-norm}}$ did not increase significantly, suggesting that the added membrane has a specific conductance similar to the existing membrane. An estimate of the specific conductance of the added membrane can be calculated by normalizing the increase in G_a (ΔG_a) by the increase in C_a (ΔC_a). $\Delta G_a/\Delta C_a$ equals 202 ± 82 $\mu S/\mu F$, a value that was not statistically different from $G_{a\text{-norm}}$, as determined by a paired *t* test ($P = 0.2$). Finally, we should note that the changes observed in all apical membrane parameters, as well as the increase in I_{sc}, are statistically indistinguishable from values previously published (Rich et al., 1990), as determined by unpaired *t* tests.

In cAMP-stimulated hemibladders, reducing the Cl^- concentration in the bathing solutions to 7.5 mM resulted in significant decreases in G_a and $G_{a\text{-norm}}$, and this is also shown in Table 1. This suggests that the added apical membrane, in response to cAMP stimulation, is Cl^- conductive. We cannot, however, exclude other possibilities, such as a cAMP-induced activation of existing channels unrelated to the increase in membrane area. Finally, reducing the concentration of Cl^- produced a small but significant decrease in C_a, similar to that seen in the unstimulated case. Although the basis for this change is poorly understood, we do not feel that it is involved in the regulation of electrogenic HCO_3^- transport, since C_a decreased after Cl^- reduction in both control and stimulated hemibladders but no significant changes were observed in I_{sc}.

These data, taken as a whole, show that the apical membrane possesses a Cl^- conductance under control conditions. After stimulation of electrogenic HCO_3^- secretion with cAMP, we observe a significant increase in apical membrane Cl^- conduc-

tance, suggesting that cAMP may cause the addition of apical membrane possessing Cl^- channels. Finally, reduction of bath Cl^- to 7.5 mM does not result in statistically significant changes in the rate of electrogenic transport processes.

Apical Membrane Conductance and Capacitance in Zero-Chloride Solutions

A second series of experiments in Cl^--free solutions was performed to examine further the dependence of G_a on bath Cl^- concentration. Immediately following dissection, hemibladders were washed in Cl^--free solution, mounted in the chambers, and washed again with approximately 75 ml (5 chamber volumes) of Cl^--free solution. We felt that this procedure resulted in virtual elimination of all exogenous Cl^- at the start of each experiment. We should note, however, that throughout the experiments, other sources of Cl^- were unavoidable. These sources include 0.1 mM Cl^- arising from the mucosal application of amiloride-HCl, leakage of Cl^- from KCl-containing agar electrode bridges, and residual Cl^- from imperfect solution changes. Since both HCO_3^- and proton secretion may be affected by low Cl^- concentration in the bathing solution due to alterations in Cl^-/HCO_3^- exchange we felt that it was important to quantify the actual Cl^- concentration under "Cl^--free" conditions.

The amount of Cl^- arising from electrode leakage of KCl was estimated to raise bath Cl^- concentration by a maximum of 0.25 mM during the duration of an experimental maneuver, which rarely exceeded 30 min. This was computed by considering the diffusion distance over 30 min (ca. 0.2 cm), bridge geometry (tubing with internal diameter 0.114 cm), and bridge KCl concentration (2 M). The 0.25 mM increase is a worst-case estimate, where it is assumed that all KCl in a 0.2-cm length of tubing diffuses into the chamber.

Bathing solution changes were performed isovolumically in order to avoid hydrostatic gradients across the epithelium. The procedure involves flushing each half-chamber with between 7 and 8 chamber volumes of solution (105 to 120 ml), while aspirating the overflow. When changing from normal Cl^--containing solutions (97.5 mM) to Cl^--free solutions, each change in chamber volume should theoretically produce an e-fold reduction in the Cl^- concentration, leaving 0.03 to 0.09 mM residual Cl^-. We tested this experimentally by measuring the washout of FD&C green dye in chambers assembled with a plastic film in place of the epithelium. The dye levels were measured spectrophotometrically. After flushing dye-containing bath with 8 chamber volumes of dye-free solution, we measured 0.17% residual dye remaining. This indicates that 0.17 mM Cl^- would remain after the solution change. This is somewhat higher than what is expected theoretically, and may reflect the existence of poorly perfused unstirred layers. For example, we calculate that a 100-μm unstirred layer adjacent to the epithelium would retain enough Cl^- to account for a residual concentration after washout of 0.13 mM. *From these sources of Cl^-, we estimate that the Cl^- concentration in "Cl^--free solutions" is greater than 0.1 mM, but less than 1 mM.*

The apical membrane parameters in Cl^--free solutions are summarized in Table 2. It is notable that under these conditions, we still observed an appreciable negative short-circuit current, presumably due to a significant baseline rate of proton secretion. Upon subsequent washing of the bathing solutions with normal Cl^--containing solution, we observed a significant increase in G_a and $G_{a\text{-norm}}$ (41 and 52%, respectively), thereby indicating that even in unstimulated hemibladders the apical membrane possesses a substantial permeability to Cl^-. Washing with normal Cl^--containing solution also resulted in a small decrease in I_{sc}, consistent with an increase in proton secretion rate. Finally, no change was observed in C_a when going from Cl^--free to normal levels of Cl^-.

Apical membrane parameters following the return to Cl^--free bathing solution are also shown in Table 2. This maneuver did not result in a return of G_a or $G_{a\text{-norm}}$ to control values. This is not thought to be due to significant residual Cl^- levels following the solution change for the reasons discussed above. I_{sc} also failed to return to control values. It is noteworthy that the basolateral membrane parameters, in contrast to the apical membrane parameters and I_{sc}, returned to control values following restoration of normal Cl^- concentration (*see below*).

In chloride-free solution cAMP has been shown to stimulate electrogenic bicarbonate secretion and the increase in transport rate was also shown to be reflected by an increase in I_{sc} (Satake et al., 1983). Stimulation with cAMP resulted in an increase in I_{sc} that was accompanied by an increase in C_a and G_a (Table 2), and these increases were statistically indistinguishable from those observed in normal Cl^--containing solutions (*cf.*, Table 1, $P > 0.5$, 0.3 and 0.8 for C_a, G_a, and I_{sc}, respectively). No significant change occurred in $G_{a\text{-norm}}$, suggesting that the inserted membrane possesses conductive properties similar to that of the existing membrane. The specific conductance of the inserted membrane ($\Delta G_a/\Delta C_a$) is calculated to be 112 ± 25 μS/μF, a value which was not significantly different from the specific conductance of the apical membrane in Cl^--free solution ($P = 0.24$). These results suggest that the apical

Table 2. Apical membrane parameters in zero chloride[a]

	$[Cl^-]$ mM	C_a $\mu F/cm^2$	G_a mS/cm^2	$G_{a\text{-norm}}$ $\mu S/\mu F$	I_{sc} $\mu A/cm^2$
Control	0	3.1 ± 0.5	0.16 ± 0.02	52 ± 6.7	−9.3 ± 2.1
Control	97.5	3.0 ± 0.5	0.22 ± 0.03	78 ± 10	−13.2 ± 2.8
Δ		−1.3 ± 0.07	0.07 ± 0.02	27 ± 6.3	−3.9 ± 0.8
P		NS	0.02	0.008	0.003
Control	0	3.0 ± 0.5	0.21 ± 0.03	74 ± 7.9	−12.1 ± 2.8
cAMP	0	3.9 ± 0.8	0.30 ± 0.06	79 ± 6.4	−5.6 ± 2.4
Δ		0.9 ± 0.4	0.09 ± 0.03	4.6 ± 3.0	6.5 ± 3.6
P		0.05	0.03	NS	0.02
cAMP	97.5	3.8 ± 0.7	0.51 ± 0.09	146 ± 23	−8.5 ± 2.7
Δ		−0.06 ± 0.2	0.22 ± 0.05	67 ± 19	−2.9 ± 2.0
P		NS	0.005	0.02	NS

[a] Data are from eight hemibladders.

membrane is conductive to an anion other than Cl^-, and the cAMP-stimulated increase in apical membrane area is also not solely conductive to Cl^-.

Finally, the effects of restoring bathing solution Cl^- concentration to normal levels after cAMP stimulation is also seen in Table 2. This resulted in a 73% increase in G_a and an 85% increase in $G_{a\text{-norm}}$. The increases resulting from Cl^- addition were significantly greater than the increases observed in unstimulated hemibladders ($P = 0.002$ and 0.05, respectively). This demonstrates that the apical membrane possesses a higher Cl^- conductance after cAMP stimulation of electrogenic bicarbonate secretion. No significant change in I_{sc} or C_a was observed.

Effects of Anthracene-9-Carboxylic Acid on Apical Membrane Parameters

Stetson et al. (1985) report that the mucosal application of 9-AA inhibits electrogenic HCO_3^- secretion in turtle bladder. Since 9-AA is a known Cl^--channel blocker in other tissues, these authors suggest that its inhibitory action in turtle bladder is due to the reduction of apical membrane conductance to Cl^-.

Mucosal application of up to 1 mM 9-AA did not change I_{sc} or the other apical membrane parameters when the mucosal solution was buffered at pH 7.4. However, when mucosal solution pH was buffered at pH 4.5 in order to facilitate cellular entry of 9-AA (a weak acid), 9-AA produced a dose-dependent decrease in I_{sc}, G_a and $G_{a\text{-norm}}$. These results are summarized in Table 3.

The experimental protocol for these results was as follows. Hemibladders were first bathed in normal Cl^--containing solution with a mucosal pH of 7.4. Electrogenic HCO_3^- transport was then stimulated by the addition of serosal cAMP. Finally, the mucosa was washed with a citrate-buffered solution at pH 4.5. The first row in Table 3 shows control parameters measured under these conditions. I_{sc} is positive, consistent with net electrogenic HCO_3^- secretion; lowering mucosal pH effectively inhibits electrogenic proton secretion (*cf.*, Stetson et al., 1985). The subsequent mucosal addition of 0.1 and 0.5 mM 9-AA resulted in a dose-dependent decrease in both G_a and $G_{a\text{-norm}}$, suggesting that 9-AA blocks apical membrane channels. A similar dose-dependent decrease in I_{sc} was also observed, further suggesting that the blockage of apical membrane channels is associated with a reduction of electrogenic HCO_3^- transport. A small but significant increase in C_a was also observed at the higher concentration of 9-AA, when compared to control values.

These results are consistent with the notion that alterations in mucosal conductance mediate changes in I_{sc}. To investigate this further, we examined the relationship between I_{sc} and G_a. Figure 3 shows I_{sc} plotted as a function of G_a. Data points are parameters from stimulated hemibladders in normal Cl^--containing solution with 0, 0.1, or 0.5 mM 9-AA (from experiments summarized in Table 3). A linear-regression analysis (solid line) shows a significant slope of 8.0 ± 2.8 mV ($P < 0.02$, $r = 0.7$), but the current extrapolated at zero-conductance is not significantly different from zero (0.91 ± 0.75 $\mu A/cm^2$, $P > 0.25$).

Basolateral Membrane Parameters and Lateral-Space Resistance

The Cl^- dependence of the basolateral membrane is shown in Table 4, which are results obtained from the same hemibladders used in the Cl^--free experiments described above. Addition of Cl^- results in an

Table 3. Effects of 9-AA in cAMP-stimulated hemibladders[a]

	$[Cl^-]$ mM	C_a $\mu F/cm^2$	G_a mS/cm^2	$G_{a\text{-norm}}$ $\mu S/\mu F$	I_{sc} $\mu A/cm^2$
Control	97.5	3.4 ± 0.4	0.29 ± 0.04	85 ± 4	3.7 ± 0.3
0.1 9-AA	97.5	3.5 ± 0.5	0.25 ± 0.04	71 ± 4	2.9 ± 0.5
Δ		0.1 ± 0.05	−0.04 ± 0.006	−14 ± 2	−0.7 ± 0.3
P		NS	0.007	0.01	NS
0.5 9-AA	97.5	3.7 ± 0.5	0.22 ± 0.04	61 ± 7	2.3 ± 0.2
Δ		0.2 ± 0.06	−0.07 ± 0.02	−24 ± 4	−1.4 ± 0.2
P		0.02	0.03	0.007	0.004

[a] Data are from four hemibladders. In these experiments, "Control" indicates hemibladders previously stimulated with 50 μM IBMX and 1.0 mM 8-bromo-cAMP in 97.5 mM Cl^- solution. Specified concentrations of 9-AA are in mM.

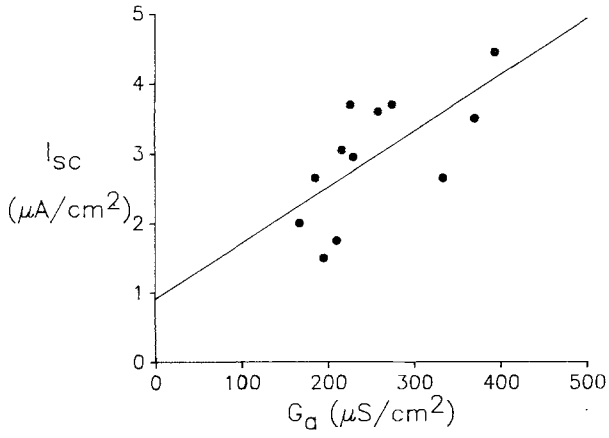

Fig. 3. Relationship between I_{sc} and G_a. Individual data points are taken from experiments summarized in Table 3. The slope of the regression line is 8.0 ± 2.8 mV ($P = 0.02$, $r = 0.7$). The zero-conductance intercept is 0.91 ± 0.75 $\mu A/cm^2$ and is not significantly different from zero ($P > 0.25$)

increase in both G_b and $G_{b\text{-norm}}$, and the subsequent return to Cl^--free solution is accompanied by a return to baseline values. In Cl^--free solutions, stimulation of electrogenic bicarbonate secretion with cAMP had no effect on G_b or $G_{b\text{-norm}}$. Finally, the subsequent return to normal Cl^--containing solutions resulted in a large increase in G_b and $G_{b\text{-norm}}$. These results suggest that the basolateral membrane also possesses Cl^--conductive channels and that the basolateral conductance is not affected by stimulation of transport with cAMP.

The existence of basolateral Cl^--permeable channels is also supported by the experiments with 9-AA described above. In those experiments, $G_{b\text{-norm}}$ decreased from 570 ± 81 $\mu S/\mu F$ ($n = 4$) in control hemibladders to 430 ± 74 $\mu S/\mu F$ ($P < 0.01$, paired *t* test) after the application of 0.1 mM mucosal 9-AA. A further reduction of $G_{b\text{-norm}}$ to 390 ± 67 $\mu S/\mu F$ ($P < 0.002$ compared to control) was observed after the application of 0.5 mM mucosal 9-AA.

The lateral-space resistance, R_p, decreased when bath Cl^- concentration was increased from zero to normal levels. R_p is interpreted in the distributed equivalent-circuit model as the resistance of the lateral spaces. This resistance is proportional to the length of the spaces and inversely proportional to the cross-sectional area of the spaces. Hence, if lateral-space geometry is unaffected by alterations in bathing solution Cl^- concentration, one should not observe a Cl^- dependent change in R_p. However, R_p is also directly proportional to the resistivity of the solution bathing the lateral spaces. Substitution of Cl^- salts with gluconate salts, which have lower diffusion constants, is expected to increase solution resistivity. Assuming that the solution filling the lateral spaces has the same resistivity (ρ) as the serosal bathing solution, then R_p/ρ provides an estimate of the lateral-space length-to-area ratio, a quantity independent of solution composition. Using a conductivity bridge, the measured ρ in normal Cl^- and Cl^--free solutions was found to be 105 and 181 Ωcm, respectively. As seen in Table 4, R_p/ρ was found to be independent of solution Cl^- composition in both unstimulated and stimulated hemibladders. These results suggest that the observed changes in R_p are attributable to changes in solution resistivity, rather than changes in lateral-space geometry. These results also indicate that the lateral spaces are adequately perfused with bulk serosal solution. Finally, the constancy of R_p/ρ provides further independent support of the adequacy of the distributed equivalent-circuit model.

In control hemibladders C_b was unaffected by changes in bath Cl^- concentration and also did not change upon cAMP stimulation of transport. However, C_b decreased when Cl^- concentration was increased from 0 to 97.5 mM in stimulated hemibladders (Table 4).

Table 4. Basolateral membrane parameters in zero chloride[a]

	$[Cl^-]$ mM	C_b $\mu F/cm^2$	G_b mS/cm^2	$G_{b\text{-norm}}$ $\mu S/\mu F$	R_p Ωcm^2	R_p/ρ cm
Control	0	7.5 ± 1.0	3.0 ± 0.4	427 ± 66	246 ± 43	1.4 ± 0.2
Control	97.5	7.8 ± 1.1	4.9 ± 0.9	678 ± 140	142 ± 27	1.4 ± 0.3
Δ		0.4 ± 0.5	1.9 ± 0.7	251 ± 72	−104 ± 21	−0.0 ± 0.1
P		NS	0.04	0.008	0.005	NS
Control	0	7.3 ± 1.1	3.6 ± 0.7	519 ± 110	247 ± 50	1.4 ± 0.3
cAMP	0	6.5 ± 0.8	3.5 ± 0.4	561 ± 72	206 ± 32	1.1 ± 0.2
Δ		−0.8 ± 0.7	−0.1 ± 0.4	43 ± 55	−41 ± 25	0.3 ± 0.1
P		NS	NS	NS	NS	NS
cAMP	97.5	5.5 ± 0.7	5.5 ± 0.4	1067 ± 100	112 ± 13	1.1 ± 0.1
Δ		−1.4 ± 0.2	2.0 ± 0.3	506 ± 74	−94 ± 21	0.0 ± 0.1
P		0.006	<0.001	0.02	0.007	NS

[a] Data are basolateral membrane parameters summarized from the same experiments as Table 2 (eight hemibladders).

Discussion

The currently proposed model for electrogenic bicarbonate secretion by the β cell incorporates a Cl^-/HCO_3^--exchange mechanism in parallel with an anion-conductive pathway in the apical membrane (Stetson et al., 1985). Bicarbonate is secreted in exchange for Cl^- in an electroneutral manner, and Cl^- is then thought to exit the cell via the conductive pathway. Hence, it has been proposed that changes in apical membrane Cl^- conductance may be involved in the regulation of electrogenic HCO_3^- secretion. We have shown previously that stimulation of electrogenic HCO_3^- secretion is accompanied by an increase in apical membrane capacitance and conductance (Rich et al., 1990). The results are consistent with the notion that changes in conductance may be involved in the regulation of transport rate.

The purpose of this study was (*i*) to quantify the apical membrane Cl^- conductance, (*ii*) to determine if the transport-associated increase in G_a is Cl^- conductive, and (*iii*) to determine whether changes in transport rate are dependent on changes in G_a.

Apical Membrane Conductance in Unstimulated Hemibladders

The results demonstrate that in control hemibladders G_a varies with extracellular Cl^- concentration, and therefore the apical membrane is Cl^- conductive. The turtle bladder epithelium is comprised of three cell types, and G_a represents the conductance characteristics of the entire epithelium. Therefore, from impedance measurements alone, we cannot determine membrane parameters for a specific cell type.

The granular cell is the predominant cell type in the epithelium and is thought to mediate Na^+ reabsorption. This transport process is blocked with the mucosal addition of amiloride. In general, since most Na^+-transporting tight epithelia do not possess a significant apical membrane Cl^- conductance, the granular cell is not thought to contribute to the change in G_a resulting from alterations in bath Cl^- concentration. The remainder of the epithelium is comprised of α- and β-type carbonic anhydrase-rich cells which are thought to mediate proton and HCO_3^- secretion, respectively. Patch-clamp studies in intercalated cells, which are morphologically and functionally similar to the carbonic anhydrase-rich cells, have demonstrated the presence of Cl^- channels in the apical membrane (Light et al., 1988). Hence, the apical Cl^- conductance measured under control conditions is thought to represent properties of the α and β cell types.

The Cl^- substitution experiments also revealed the presence of a Cl^--induced apical membrane conductance. This effect was observed in control hemibladders bathed in Cl^--free solution; increasing Cl^- concentration to normal levels resulted in an increase in G_a and $G_{a\text{-norm}}$, but the subsequent return to Cl^--free solution had no effect on either parameter (*see* Table 2). Note that the effects of the solution change on the basolateral membrane parameters were reversible; I_{sc}, R_p, G_b, and $G_{b\text{-norm}}$ returned to control values when the bathing solution Cl^- concentration was returned to 0 mM from 97.5 mM, indicating that the solution changes were complete (*see* Results). Since G_a and $G_{a\text{-norm}}$ remain elevated when bath Cl^- concentration was returned to 0 mM from 97.5 mM, the Cl^--induced increase in G_a is not specific for Cl^-.

The Cl^--induced conductance was observed

only in bladders which had been preincubated in Cl^--free solution. Changes in G_a and $G_{a\text{-norm}}$ were reversible when bath Cl^- concentration was lowered from normal (97.5 mM) to low (7.5 mM) levels, and returned (Table 1). The fundamental difference between the two protocols is the time of incubation in Cl^--free solution and the magnitude of the change in Cl^- concentration. Hence, the results suggest that Cl^- may activate an apical membrane conductive pathway in bladders that were previously Cl^- deprived, but the nature of the process, as well as the physiological relevance, is not understood at this time.

Apical Membrane Conductance in Stimulated Hemibladders

After stimulation of HCO_3^- secretion, G_a is proportional to the bathing solution Cl^- concentration, and a substantial apical membrane Cl^- conductance is present. Comparing the magnitude of the change in G_a (ΔG_a) in control and stimulated hemibladders resulting from alterations in bath Cl^- concentration should reveal whether the transport-associated increase in conductance is due to the insertion of Cl^--conductive channels. Decreasing Cl^- concentration in the bathing solution in control hemibladders resulted in no significant change in G_a, but in stimulated hemibladders G_a decreased by 23% (Table 1). However, the ΔG_a in the stimulated case was not statistically greater than the ΔG_a in control hemibladders. A larger change in bath Cl^- concentration (0 to 97.5 mM) resulted in a 41% increase in G_a in control hemibladders and a 73% increase in stimulated hemibladders, and in this case ΔG_a was significantly greater in the stimulated case ($P = 0.002$). Hence, these results show that after stimulation of electrogenic HCO_3^- secretion with cAMP, the apical membrane possesses a higher Cl^- conductance consistent with the notion that the increase in G_a is mediated via an increase in the number of Cl^--conductive channels.

Stimulation of Electrogenic Bicarbonate Secretion with cAMP

Stimulation of electrogenic HCO_3^- secretion results in a simultaneous increase in I_{sc}, C_a, and G_a in both normal and Cl^--free solutions. The observed increases in C_a and G_a were statistically indistinguishable in either solution (unpaired t test). This shows that the membrane-remodeling processes associated with stimulation of transport are not dependent on the extracellular Cl^- concentration. The increase in I_{sc} was also found to be independent of extracellular Cl^- concentration (unpaired t test). Recall that in normal Cl^--containing solutions I_{sc} is thought to be due to Cl^- exiting the cell via the conductive pathway, and in Cl^--free solutions, I_{sc} is thought to result from HCO_3^- passing through the conductive pathway. Hence, these results suggest that the conductive pathway is permeable to both Cl^- and HCO_3^-. Furthermore, since the magnitude of ΔI_{sc} does not vary with bathing-solution Cl^- concentration, the selectivity of the conductive pathway for either ion is expected to be similar.

The specific conductance of the inserted membrane can be calculated as $\Delta G_a/\Delta C_a$. In normal chloride-containing solution $\Delta G_a/\Delta C_a$ was 202 ± 82 μS/μF; in Cl^--free solution $\Delta G_a/\Delta C_a$ was 112 ± 25 μS/μF. The specific conductance of the inserted membrane in Cl^--free solution appeared lower than in Cl^--containing solution, but did not achieve statistical significance (unpaired t test, $P = 0.2$). These data show that the conductive channels inserted into the apical membrane in response to cAMP are permeable to an ion other than Cl^-, the most likely candidate being HCO_3^-. However, these results should not be interpreted as indicating that the inserted membrane is impermeable to Cl^-. Mean estimates of $\Delta G_a/\Delta C_a$ will invariably exhibit large coefficients of variation, since each $\Delta G_a/\Delta C_a$ value is a calculated quantity dependent on four estimated parameters determined from two impedance runs. Pooling the above data with the additional estimates of $\Delta G_a/\Delta C_a$ resulting from the earlier time-course experiments (*cf.*, Figs. 1 and 2) results in a mean value of 266 ± 79 μS/μF ($n = 12$) in normal Cl^--containing solution. This value is statistically larger than the value in Cl^--free solution ($P = 0.04$). Hence we conclude that the inserted membrane is Cl^- permeable, but that about 42% of the measured conductance can be attributed to another ion, presumably HCO_3^-. Finally, if the conductance in Cl^--free solutions is in fact a HCO_3^- conductance, then the data also suggest that the pathway is more permeable to HCO_3^- than to Cl^- since concentrations of HCO_3^- are substantially lower than normal concentrations of Cl^-.

The relative conductance to Cl^- as compared to the total membrane conductance, or the effective transference number (t_{Cl^-}), can be calculated from the measured G_a in normal and Cl^--free solutions. The apical conductance to Cl^- (G_{Cl^-}) may be calculated as G_a in normal Cl^- solution minus G_a in Cl^--free solution, and t_{Cl^-} is then G_{Cl^-}/G_a. For the six experiments in Table 2, t_{Cl^-} is 0.30 ± 0.06 in control hemibladders and 0.42 ± 0.05 in stimulated hemibladders. These values are statistically indistinguishable ($P = 0.1$, paired t test), suggesting that

the increased conductance following cAMP stimulation is not due to the insertion of Cl^--selective channels into the apical membrane, but rather, the channels also may be permeable to other anions. Furthermore, the magnitude of t_{Cl^-} in normal Cl^- solution suggests that G_a is at least as conductive to another anion as it is to Cl^-. We should note that single-channel studies have recently shown that Cl^- channels in a pancreatic cell line are also permeable to HCO_3^-, and the permeability ratio for HCO_3^- to Cl^- was approximately 0.5 (Tabcharani et al., 1989). Our data show that G_a is not selective for Cl^- and would be consistent with a channel that is conductive to both HCO_3^- and Cl^-.

Effects of 9-AA on G_a

9-AA is known to block the Cl^- conductance in muscle membrane (Palade & Barchi, 1977) and an apical Cl^- conductance in canine tracheal epithelium (Welsh, 1984). The apical Cl^- conductance in tracheal epithelium has also been shown to be stimulated by cAMP (Frizzel et al., 1986). In this study we have shown that the mucosal application of 9-AA to stimulated hemibladders results in a dose-dependent decrease in G_a and $G_{a\text{-norm}}$, providing further evidence for an anion-conductive pathway in the apical membrane of stimulated hemibladders.

Short-Circuit Current Dependence on G_a

If changes in I_{sc} are mediated by alterations in G_a, then any experimental maneuver which results in changes in G_a might be expected to also result in concomitant changes in I_{sc}. However, alterations in bath Cl^- concentration resulted in alterations in G_a and $G_{a\text{-norm}}$ that were not accompanied by alterations in I_{sc} (Table 1 and 2). Recall that I_{sc} under these conditions (mucosal pH of 7.3) is determined by the sum of proton and HCO_3^- secretion. Also recall that proton secretion has been shown to be sensitive to serosal Cl^- concentration with a reported K_m of 0.13 mM. In Cl^--containing solution the I_{sc} associated with HCO_3^- secretion is thought to result from Cl^- exciting the β cell via the conductive pathway and therefore is dependent upon the Cl^- electrochemical gradient as well as G_a. In Cl^--free solution the I_{sc} associated with electrogenic HCO_3^- secretion is thought to result from HCO_3^- passing across the conductive pathway, and therefore under these conditions I_{sc} will be determined by the electrochemical gradient for HCO_3^- as well as Cl^-. Hence, alterations in bath Cl^- concentration alter both electrogenic proton and HCO_3^- secretion and the relative effect on either transport process is difficult to determine.

The mucosal application of 9-AA to stimulated hemibladders resulted in a dose-dependent reduction in G_a, $G_{a\text{-norm}}$ and I_{sc} (*see* Table 3). In this case mucosal pH was lowered to enhance uptake of 9-AA; lowering mucosal pH also inhibits proton secretion and therefore simplifies the interpretation of I_{sc}. Hence, under these conditions I_{sc} is largely accounted for by electrogenic HCO_3^- secretion. The results suggest that the effects of 9-AA on HCO_3^- secretion may result from a reduction in G_a. Figure 3 shows that I_{sc} and G_a are linearly related with a slope of 8.0 ± 2.8 mV and a y intercept not significantly different from zero. The slope of the regression line represents the driving force for Cl^- exit and is a reasonable physiological value for Cl^- efflux across the apical membrane; for example, in frog cornea (a Cl^--secreting epithelium) the net driving force for apical membrane Cl^- efflux is 10 mV (Clausen et al., 1986). The apparent correlation between I_{sc} and G_a also suggests that changes in the rate of transport are mediated by changes in G_a, and not by changes in the driving force for Cl^- or HCO_3^-. Finally, at zero conductance, the extrapolated current is not statistically different from zero, suggesting that the apical membrane possesses little or no conductance that is unrelated to HCO_3^- secretion under these conditions.

Summary and Conclusions

According to the current model for electrogenic bicarbonate secretion, HCO_3^- is secreted in exchange for Cl^- which subsequently exits the cell via a conductive pathway in the apical membrane thereby resulting in a short-circuit current (Stetson & Steinmetz, 1985). In Cl^--free solutions, Cl^-/HCO_3^- exchange is blocked and HCO_3^- is thought to be secreted solely through the conductive pathway. The data presented here provide firm experimental support for this model. We have shown that the apical membrane possesses a measurable Cl^- conductance under control conditions, and this Cl^- conductance is increased following stimulation of transport with cAMP. In addition, the data show that G_a is not highly selective for Cl^- since an appreciable conductance and short-circuit current remains in Cl^--free solutions. Additionally, these data suggest that G_a is conductive to another anion, presumably HCO_3^-, and that both Cl^- and HCO_3^- have similar relative conductances. Finally, using the anion channel blocker 9-AA, it has been demonstrated that alterations in I_{sc} are mediated by changes in G_a.

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References

Clausen, C., Dixon, T.E. 1986. Membrane electrical parameters in turtle bladder measured using impedance analysis techniques. *J. Membrane Biol.* **92:**9–19

Clausen, C., Fernandez, J.M. 1981. A low-cost method for rapid transfer function measurements with direct application to biological impedance analysis. *Pfluegers Arch.* **390:**290–295

Clausen, C., Reinach, P.S., Marcus, D.C. 1986. Membrane transport parameters in frog corneal epithelium measured using impedance analysis techniques. *J. Membrane Biol.* **91:**213–225

Cole, K.S. 1972. Membranes, Ions, and Impulses. p. 12. University of California Press, Berkeley

Fischer, J.L., Husted, R.F., Steinmetz, P.R. 1983. Chloride dependence of the HCO_3 exit step in urinary acification by the turtle bladder. *Am. J. Physiol.* **245:**F564–F568

Frizzell, R.A., Halm, D.R., Rechkemmer, G., Showmaker, R.L. 1986. Chloride channel regulation in secretory epithelia. *Fed. Proc.* **45:**2727–2731

Hamilton, W.C. 1964. Statistics in Physical Science. Ronald Press, New York

Landry, D.W., Reitman, M., Cragoe, E.J., Jr., Al-Awqati, Q. 1987. Epithelial chloride channel: Development of inhibitory ligands. *J. Gen. Physiol.* **90:**779–798

Lewis, S.A., Eaton, D.C., Clausen, C., Diamond, J.M. 1977. Nystatin as a probe for investigating the electrical properties of a tight epithelium. *J. Gen. Physiol.* **70:**427–440

Light, D., Fejes-Toth, G., Naray-Fejes-Toth, A., McCann, F., Keller, T., Stanton, B. 1988. Voltage-dependent chloride channels in apical membrane of intercalated cells in culture. *Kidney Int.* **33:**422 (*Abstr.*)

Palade, P.T., Barchi, R.L. 1977. On the inhibition of muscle membrane chloride conductance by aromatic carboxylic acids. *J. Gen. Physiol.* **69:**879–896

Rich, A., Dixon, T.E., Clausen, C. 1990. Changes in membrane conductances and areas associated with bicarbonate secretion in turtle bladder. *J. Membrane Biol.* **113:**211–219

Satake, N., Durham, J.H., Ehrenspeck, G., Brodsky, W.A. 1983. Active electrogenic mechanisms for alkali and acid transport in turtle bladders. *Am. J. Physiol.* **244:**C259–C269

Stetson, D.L. 1988. Cyclic AMP-stimulated apical membrane amplification in turtle bladder β-cells: Activation of chloride channels? *Kidney Int.* **33:**427 (*Abstr.*)

Stetson, D.L., Beauwens, R., Palmisano, J., Mitchell, P.P., Steinmetz, P.R. 1985. A double-membrane model for urinary bicarbonate secretion. *Am. J. Physiol.* **249:**F546–F552

Stetson, D.L., Steinmetz, P.R. 1985. α and β types of carbonic anhydrase-rich cells in the turtle bladder. *Am. J. Physiol.* **249:**F553–F565

Tabcharani, J.A., Jensen, T.J., Riordan, J.R., Hanrahan, J.W. 1989. Bicarbonate permeability of the outwardly rectifying anion channel. *J. Membrane Biol.* **112:**109–122

Welsh, M.J. 1984. Anthracene-9-carboxylic acid inhibits an apical membrane chloride conductance in canine tracheal epithelium. *J. Membrane Biol.* **78:**61–71

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