J. Membrane Biol. 113, 211–219 (1990)

The Journal of
Membrane Biology

Changes in Membrane Conductances and Areas Associated with Bicarbonate Secretion in Turtle Bladder

Adam Rich, Troy E. Dixon†, and Chris Clausen
Department of Physiology and Biophysics, State University of New York, Stony Brook, New York 11794-8661, and †Department of Medicine, VA Medical Center, Northport, New York 11768

Summary. Transepithelial impedance-analysis studies were performed in turtle bladder epithelium in order to measure changes in the different epithelial membranes resulting from stimulation of electrogenic bicarbonate secretion. Changes in membrane conductance relate to changes in ionic permeability, whereas changes in membrane capacitance relate to changes in membrane area, since most biological membranes exhibit a specific capacitance of $\sim 1\ \mu F/cm^2$. The results of this investigation are summarized as follows: (i) cAMP and carbachol, agents which have been shown previously to stimulate electrogenic bicarbonate secretion, result in increases in apical-membrane conductance and capacitance; (ii) these changes occur concomitantly with the observed change in transport (measured using the short-circuit-current technique), thereby suggesting that bicarbonate secretion may be regulated in part by changes in the chloride conductance of the apical membrane; (iii) the increase in conductance does not reflect an increase in the membrane's specific conductance, thereby indicating that it results from the addition of membrane possessing similar ionic permeability as the existing apical membrane; (iv) the magnitude of the changes in capacitance indicate that a minor cell population (β-type carbonic-anhydrase-rich cells) increase their apical-membrane area by several-fold; (v) a lack of transport-associated changes in the basolateral-membrane parameters suggest that transport is not regulated by alterations in basolateral-membrane ionic conductance or area; (vi) a lack of colchicine sensitivity, coupled with the magnitude of the changes in apical-membrane capacitance, indicate that the membrane remodeling processes are different from those involved in the regulation of proton secretion in a different cell population (α-type carbonic-anhydrase-rich cells).

Key Words bicarbonate transport · proton transport · turtle bladder · equivalent-circuit analysis · impedance analysis · endocytosis · exocytosis · carbonic anhydrase

Introduction

Many of the details regarding acid and base transport by the kidney have been determined from studies performed in the urinary bladder of the freshwater turtle. This epithelium possesses transport processes for the secretion of protons and bicarbonate. Electrogenic proton secretion can be stimulated specifically with CO_2 (Gluck, Cannon & Al-Awqati, 1982). Bicarbonate secretion is composed of two components: one electroneutral and one electrogenic (Stetson et al., 1985). Electrogenic bicarbonate secretion is stimulated by cAMP, carbachol, and other agents, all of which do not apparently affect electrogenic proton secretion (Schneider et al., 1988). The different sensitivities to these agents led to the proposal that the two transport processes are functionally distinct.

Further evidence for this assertion arose from morphological studies of the epithelium. The predominant cell type observed has a distinct granular appearance (Rosen, 1970), but possesses low carbonic-anhydrase (CA) activity. These granular cells, which comprise ~80% of the cell number, have been found to mediate electrogenic sodium reabsorption in the epithelium (Schwartz, Bethencourt & Rosen, 1982). The remaining ~20% of the cells were found to be characteristically rich in CA and are currently thought to mediate the acid and base transport processes. High-resolution electronmicrographic studies of these cells resulted in a further subdivision into two morphologically distinct cell types (Stetson & Steinmetz, 1985). Alpha-type CA-rich cells (α cells), the major CA-rich cell type, possess apical microplicae and a dense population of rod-shaped intramembrane particles associated with the apical membrane. The α cells also exhibit morphological changes that are dependent on the CO_2 tension. The remaining CA-rich cell population, termed β-type CA-rich cells (β cells), do not exhibit apical microplicae, possess rod-shaped particles within the basolateral membrane, and exhibit a morphology that appears independent of CO_2 tension. Furthermore, stimulation of electrogenic bicarbonate secretion results in morphological alterations of the β-cells; no effects are observed in the

other cell types (Stetson, 1988). Hence, it has been proposed that the α cells mediate electrogenic proton secretion and that the β cells mediate electrogenic bicarbonate secretion.

Impedance-analysis techniques have been used to examine the association between electrogenic proton secretion and membrane electrical parameters in the turtle bladder (Clausen & Dixon, 1986; Dixon et al., 1986). These techniques allow the determination of the conductance and the capacitance of each of the membranes that comprise the epithelium. Membrane conductance is proportional to membrane ionic permeability, and capacitance is proportional to membrane area. Analysis of impedance, coupled with the simultaneous measurement of transport rate using the short-circuit-current technique, showed that apical-membrane endocytotic processes were requisite for the inhibition of proton transport (Dixon, Clausen & Coachman, 1988). This confirmed the notion, originally proposed by Gluck et al. (1982), that regulation of electrogenic proton secretion involves altering the number of apical-membrane proton pumps, via the insertion and removal of pump-containing vesicles.

The purpose of this study was to investigate the relationship between changes in electrogenic bicarbonate secretion and changes in the electrical characteristics of the different epithelial membranes. We wished to investigate whether changes in membrane ionic permeability properties and/or recently observed changes in membrane morphology (Stetson, 1988) are involved in the regulation of the rate of bicarbonate secretion. Membrane electrical parameters were measured by analyzing transepithelial impedance. Changes in the rates of electrogenic bicarbonate secretion were measured using the short-circuit-current technique.

Materials and Methods

Dissection, Chamber, and Solutions

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

In all experiments, the mucosa and serosa were bathed in a modified Ringer's solution with the following composition (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 $CaCl_2$. In addition, 2% bovine serum albumin (Fraction V, Sigma) and 5 mM D-glucose were added to the serosal solution. The mucosal and serosal solutions were continuously bubbled with 5% CO_2 and gently stirred with magnetic fleas. A small amount of silicon oil (Antifoam A, Dow Corning, Midland, MI) was sprayed on the serosal solution to control the foam resulting from bubbling the albumin-containing solution. Electrogenic bicarbonate secretion was stimulated with the cholinergic agonist carbachol, or the membrane-permeable cAMP analog 8-bromo-cAMP in the presence of the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX). The doses of these agents (obtained from Sigma, St. Louis, MO) are described in the text. In a separate series of experiments, colchicine (Sigma), in doses described in the text, was used to inhibit microtubular function.

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 the preparation. A second set of Ag/AgCl electrodes mounted at opposite ends of the chamber was used to pass transepithelial current. Constant current was generated using a calibrated 1-MΩ carbon series resistor connected to the mucosal electrode; the serosal electrode was connected to ground. The bladder was held under open-circuit conditions, and short-circuit current (I_{sc}) was measured intermittently by passing a 500-msec pulse which depolarized V_t to zero. A positive current is defined as one flowing from mucosa to serosa.

Measurement of the Rate of Bicarbonate Secretion

In addition to electrogenic proton and bicarbonate transport processes, the turtle bladder actively reabsorbs sodium via an electrogenic process. This process was inhibited in all experiments by the mucosal addition of 0.1 mM amiloride (Merck, Sharp & Dohme, Rahway, NJ). Prior to the application of amiloride, V_t is mucosal-side negative, thereby reflecting active sodium reabsorption. Application of amiloride rapidly reduces this potential which usually reverses in polarity, thereby unmasking electrogenic proton and bicarbonate secretion. Eight-bromo cAMP, IBMX and carbachol result in an increase in I_{sc}, which has been shown to be equivalent to bicarbonate secretion by pH-stat techniques. In addition, these agents are not thought to affect proton secretion (Satake et al., 1983). Hence this increase in I_{sc} was used as a measure of the resulting increase in 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 to the turtle bladder may be found in Clausen and Dixon (1986). Briefly, a small-amplitude, constant-current, wide-band signal was applied transepithelially. The resulting voltage response was filtered, digitized, and recorded by computer, and the impedance, as a function of frequency, was calculated using standard Fourier-analysis techniques. The different membrane electrical parameters were determined by fitting the data by a morphologically-based equivalent circuit model using a nonlinear least-squares curve fitting algorithm. 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 (Clausen & Dixon, 1986).

Membrane capacitance can be used as an indirect measurement of membrane area since the specific capacitance of nearly all biological membranes is remarkably constant at approximately 1 μF/cm^2 (Cole, 1972). In addition, measurement of membrane capacitance allows estimation of the specific conductance of the membranes by normalizing G_a and G_b to unit area (i.e., $G_{a\text{-norm}} = G_a/C_a$ and $G_{b\text{-norm}} = G_b/C_b$). Finally, the path resistance of the lateral space, R_p, provides an indirect measure of lateral space geometry; R_p is directly proportional to lateral-space length and indirectly proportional to lateral-space width. Changes in R_p are expected to also reflect changes in cell volume (e.g., an increase in cell volume might be expected to reduce the width of the lateral spaces, thereby increasing R_p).

Statistics

Except where specifically noted, results presented in tabular form and in text are reported as mean ± SEM. Paired and unpaired t tests were used to determine the statistical significance of observed changes in membrane parameters, where $P > 0.05$ was interpreted as indicating "not significant" (NS). Differences or changes in parameters are indicated in the tables and text as Δ.

Results

Impedance Data

Impedance-analysis techniques have been successfully used in the study of electrogenic proton transport in turtle-bladder epithelium (*cf.*, Clausen & Dixon, 1986; Dixon et al., 1986), but it is possible that stimulation of electrogenic bicarbonate secretion might result in data that could not be readily analyzed using these same techniques. This was not found to be the case. Figure 1 shows representative transepithelial impedance measured from a single hemibladder stimulated to secrete bicarbonate by the serosal and mucosal application of 50 μM IBMX (I_{sc} increased by 1.6 μA/cm^2). The symbols are the measured impedance, and the solid line is the best fit by the equivalent-circuit model used in the previous impedance studies of electrogenic proton transport.[1]

For each curve fit to the measured data, we calculated the Hamilton R-factor (Hamilton, 1964), which is an objective measure of the quality of the fits. The R-factor is the relative discrepancy between the data and the model-predicted impedance. In 206 randomly-selected impedance runs measured from 12 different hemibladders, the average R-factor was 0.9 ± 0.3% (mean ± SD); the R-factor calculated for the analysis shown in Fig. 1 was 1.0%. The low R-factors indicate that the equivalent-circuit model accurately represents the measured impedance of the tissue. Nevertheless, occasionally we observed data that could not be accurately fit by the model. These runs were discarded if they resulted in R-factors that exceeded 1.5%, a value two standard deviations above the overall mean R-factor. We should emphasize, however, that this occurred rarely; only seven out of the above-mentioned 206 runs exhibited R-factors greater than 1.5%.

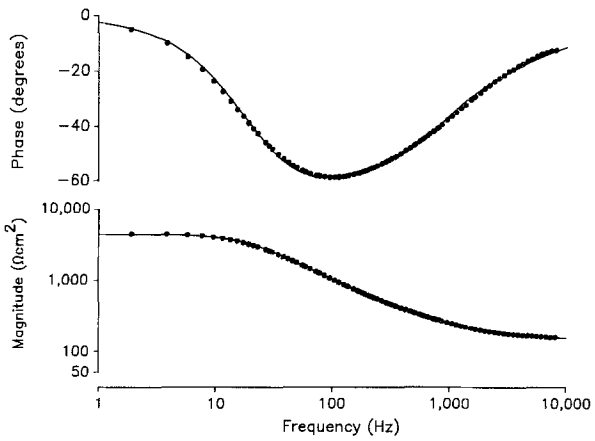

Fig. 1. A single representative impedance run from a hemibladder after addition of 50 μM IBMX to the serosal and mucosal bathing solutions. The upper panel shows phase angle and the lower panel shows impedance magnitude, both plotted as a function of frequency. The line drawn through the points shows the resulting fit by the equivalent-circuit model. The agreement between the model and the data is typical (*see* text)

Other statistical techniques were also used to insure that the equivalent-circuit electrical parameters were well determined by the measured impedance; these methods are described in Clausen et al. (1986).

Membrane Parameters under Control Conditions

Control values from Tables 1 and 2 were pooled (15 hemibladders) and compared with values previously published. G_a (0.22 ± 0.03 mS/cm^2), $G_{a\text{-norm}}$ (0.10 ± 0.01 mS/μF), C_b (6.1 ± 0.6 μF/cm^2), G_b (5.3 ± 0.04 mS/cm^2), and R_p (225 ± 24 Ωcm^2) were found to be statistically indistinguishable from previously reported values (Clausen & Dixon, 1986), as judged by unpaired t tests ($P > 0.05$). However, C_a

[1] Also evident in the data are effects resulting from a small series resistance (~100 Ωcm^2), which reduces the phase angle at high frequencies and causes a high-frequency asymptote in the impedance magnitude. This resistance is caused simply by the finite resistance of the unstirred layers between the voltage electrodes and the membrane surfaces.

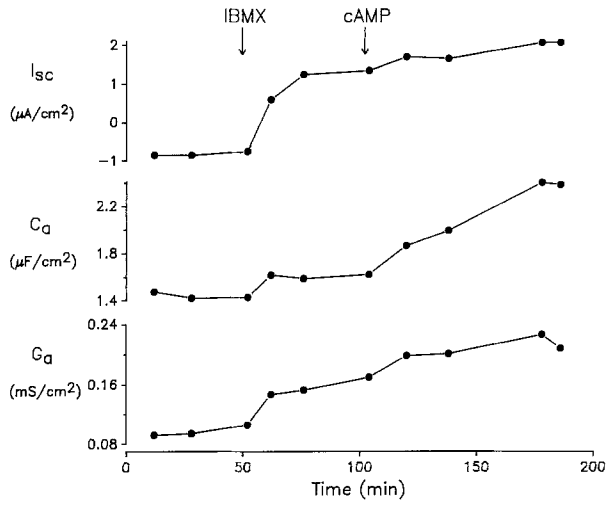

Fig. 2. I_{sc} (upper panel), C_a (middle panel), and G_a (lower panel) plotted as a function of time from a single representative hemibladder. At the point marked *IBMX*, 50 μM IBMX was added to both the mucosal and serosal solutions. At the point marked *cAMP*, 1 mM 8-bromo-cAMP was added to the serosal solution

(2.1 ± 0.2 μF/cm²) was lower ($P = 0.02$) than the earlier reported value (3.2 ± 0.4 μF/cm², $n = 17$), which may simply reflect a slightly higher degree of mounting stretch of the epithelium. $G_{b\text{-norm}}$ (0.94 ± 0.10 mS/μF) is somewhat larger ($P = 0.02$) than previously reported (0.64 ± 0.07 mS/μF, $n = 17$), but this is not surprising since the composition of the bathing solutions is different in the two studies (e.g., the earlier study was done using bicarbonate-free solutions).

Response to cAMP and IBMX

Cyclic AMP is a known stimulator of electrogenic bicarbonate secretion; a representative response to an increase in intracellular cAMP is shown in Fig. 2. Mucosal and serosal addition of 50 μM IBMX results in an increase in I_{sc} (upper panel). Subsequent addition of 1 mM 8-bromo-cAMP to the serosal solution results in an additional increase in I_{sc}. Both of these responses are consistent with an increase in bicarbonate secretion. Two items should be addressed regarding the change in I_{sc} shown in Fig. 2. First, recall that the increase in I_{sc} is interpreted as an increase in electrogenic bicarbonate secretion, but the absolute baseline rate of bicarbonate secretion is unknown. Second, we observed variability in the *relative* response to IBMX and 8-bromo-cAMP. In some cases, as is shown in Fig. 2, IBMX resulted in a larger increase in I_{sc} than the subsequent addition of 8-bromo-cAMP; in other cases, IBMX resulted in a relatively smaller increase in I_{sc} than observed after the subsequent addition of 8-bromo-cAMP. In all cases, however, both agents resulted in an increase in I_{sc}.

Concomitant with the observed increase in I_{sc}, we also observed an increase in C_a and G_a, and this is illustrated in the middle and lower panels, respectively, of Fig. 2. The increase in C_a and G_a follow similar time courses as the increase in I_{sc}. We interpret these data as indicating a transport-related increase in apical-membrane area and ionic conductance.

A series of experiments were performed using 1 mM 8-bromo-cAMP in the presence of 50 μM IBMX. Henceforth, when we refer to results of cAMP, we are referring to this experimental condition.

The steady-state response to cAMP in eight hemibladders is shown in Table 1. The increase in transport was accompanied by a 0.9 ± 0.2 μF/cm² increase in C_a, and a 0.13 ± 0.04 mS/cm² increase in G_a. However, the increase in G_a did not indicate an increase in the specific conductance (hence ionic permeability) of the apical membrane, since $G_{a\text{-norm}}$ failed to increase significantly. These results indicate that cAMP stimulation results in an increase in apical-membrane area caused by the addition of membrane possessing similar specific conductance. Note that the specific conductance of the added membrane can be estimated directly by normalizing the increase in G_a (ΔG_a) by the increase in C_a (ΔC_a) (*see* Dixon et al., 1986). $\Delta G_a/\Delta C_a$ equals 0.12 ± 0.04 mS/μF, a value statistically indistinguishable from the apical-membrane specific conductance.

The basolateral-membrane parameters, as well as R_p, did not change significantly after stimulation with cAMP, and this is also shown in Table 1. This indicates that cAMP-induced stimulation of transport results in changes in the electrical parameters that are predominantly limited to the apical membrane. In addition, the results also indicate that stimulation does not result in a significant change in lateral-space morphology, that might otherwise result from transport-associated changes in cell volume. Recall that changes in lateral-space geometry are reflected in changes in R_p.

Response to Carbachol

Carbachol has been reported to stimulate electrogenic bicarbonate secretion in the turtle bladder via a mechanism involving the phospho-inositol cascade (Schneider et al., 1988). This process is *not* thought to involve cAMP. The effects of serosal application of 10 μM carbachol in a representative

Table 1. Effect of cAMP on electrical parameters[a]

	I_{sc} (μA/cm^2)	C_a (μF/cm^2)	G_a (mS/cm^2)	$G_{a\text{-norm}}$ (mS/μF)	C_b (μF/cm^2)	G_b (mS/cm^2)	$G_{b\text{-norm}}$ (mS/μF)	R_p (Ωcm^2)
Control	−4.2 ± 3.1	2.0 ± 0.2	0.19 ± 0.03	0.10 ± 0.01	6.0 ± 0.9	5.2 ± 0.6	0.98 ± 0.16	229 ± 32
cAMP	−0.2 ± 2.4	2.8 ± 0.3	0.32 ± 0.05	0.11 ± 0.01	4.4 ± 0.5	4.2 ± 0.4	0.97 ± 0.05	220 ± 35
Δ	4.0 ± 1.0	0.9 ± 0.2	0.13 ± 0.04	0.01 ± 0.01	−1.6 ± 0.9	−1.0 ± 0.4	−0.02 ± 0.13	−9 ± 22
P	0.003	<0.0001	0.008	NS	NS	NS	NS	NS

[a] Data are from eight hemibladders. The first row shows control values. The second row shows parameter values after cAMP-induced stimulation of bicarbonate secretion. The third row shows the difference between control and cAMP states. The last row shows probabilities comparing the two states (paired *t* test).

Table 2. Effect of carbachol on electrical parameters[a]

	I_{sc} (μA/cm^2)	C_a (μF/cm^2)	G_a (mS/cm^2)	$G_{a\text{-norm}}$ (mS/μF)	C_b (μF/cm^2)	G_b (mS/cm^2)	$G_{b\text{-norm}}$ (mS/μF)	R_p (Ωcm^2)
Control	−8.3 ± 2.1	2.3 ± 0.2	0.26 ± 0.05	0.11 ± 0.02	6.3 ± 0.6	5.4 ± 0.6	0.90 ± 0.12	220 ± 39
Carb.	−3.1 ± 2.4	2.8 ± 0.3	0.37 ± 0.06	0.13 ± 0.01	6.6 ± 0.9	5.2 ± 0.6	0.84 ± 0.11	165 ± 44
Δ	5.2 ± 0.8	0.5 ± 0.1	0.11 ± 0.02	0.02 ± 0.01	0.3 ± 0.7	−0.2 ± 0.7	−0.06 ± 0.16	−55 ± 33
P	0.003	0.002	0.001	NS	NS	NS	NS	NS

[a] Data are from seven hemibladders. The first row shows control values. The second row shows parameter values after carbachol-induced stimulation of bicarbonate secretion. The third row shows the difference between control and carbachol states. The last row shows probabilities comparing the two states (paired *t* test).

experiment are shown in Fig. 3. Application of carbachol produces a rapid rise in I_{sc} (upper panel), with time of onset similar to that observed with cAMP stimulation. However, in contrast to cAMP, the effects of carbachol on I_{sc} are transient; within one hour, I_{sc} returns to control levels (paired *t* test). Concomitant with the rise in I_{sc}, one also observes a similar transient increase in C_a (middle panel) as well as G_a (lower panel), and both these responses follow a nearly identical time course as I_{sc}. It is interesting to note, however, that both C_a and G_a do not return completely to control levels. Seven hemibladders exhibited a control value for C_a of 2.3 ± 0.2 μF/cm^2, which, after carbachol, attained a steady-state value of 2.6 ± 0.2 μF/cm^2 ($P < 0.025$ by paired analysis). Similarly, G_a started at a control value of 0.26 ± 0.05 mS/cm^2 and attained a steady-state value of 0.35 ± 0.07 mS/cm^2 ($P < 0.02$).

Table 2 summarizes the reponses of the membrane electrical parameters measured at the *peak* of the transient increase in I_{sc} following the serosal application of 10 μM carbachol; the data are from seven hemibladders. Carbachol stimulation results in increases in C_a and G_a, but the rise in G_a does not appear to be caused by an increase in the apical membrane's ionic permeability since $G_{a\text{-norm}}$ showed no statistically significant increase. In addition, the basolateral-membrane parameters, as well as R_p, showed no transport-associated changes. It is interesting to note that the *peak* changes in I_{sc}, C_a, and G_a were statistically indistinguishable (unpaired analysis) with the *sustained* increases observed after stimulation of transport resulting from cAMP.

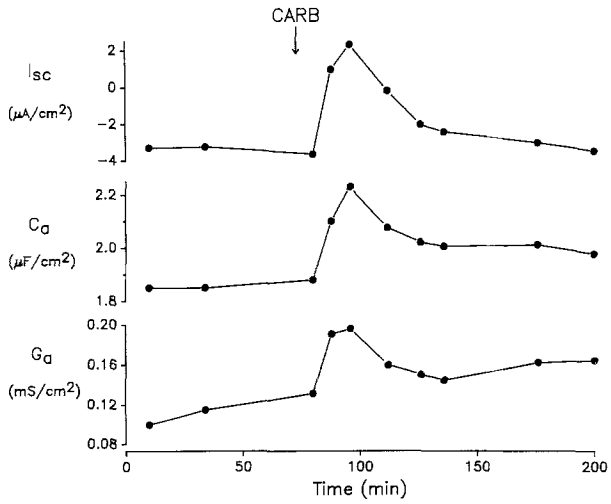

Fig. 3. I_{sc} (upper panel), C_a (middle panel), and G_a (lower panel) plotted as a function of time from a single representative hemibladder. At the point marked *CARB*, 10 μM carbachol was added to the serosal solution. Carbachol produces a transient increase in I_{sc} and the membrane parameters, which differs from the response to cAMP which produces a sustained increase

Table 3. Effect of colchicine pretreatment[a]

	I_{sc} (μA/cm^2)	C_a (μF/cm^2)	G_a (mS/cm^2)	$G_{a\text{-norm}}$ (mS/μF)	C_b (μF/cm^2)	G_b (mS/cm^2)	$G_{b\text{-norm}}$ (mS/μF)	R_p (Ωcm^2)
Colch.	-11.1 ± 2.7	2.0 ± 0.2	0.23 ± 0.03	0.11 ± 0.01	6.2 ± 1.7	4.3 ± 0.6	0.89 ± 0.20	164 ± 19
cAMP	-7.9 ± 2.8	2.6 ± 0.2	0.34 ± 0.04	0.14 ± 0.01	5.2 ± 0.7	4.2 ± 0.4	0.85 ± 0.08	128 ± 18
Δ	3.3 ± 1.3	0.6 ± 0.2	0.12 ± 0.02	0.02 ± 0.01	-1.0 ± 1.1	-0.1 ± 0.6	-0.04 ± 0.16	-36 ± 10
P	0.02	0.01	0.001	0.04	NS	NS	NS	0.01

[a] Data are from seven hemibladders. The first row shows values after incubation for 2 hr in 0.1 mM colchicine. The second row shows parameter values after cAMP-induced stimulation of bicarbonate secretion in the continued presence of colchicine. The third row shows the difference between these two states. The last row shows probabilities comparing the two states (paired t test).

Again, these data are consistent with the notion that stimulation of bicarbonate transport is associated with increases in apical-membrane area resulting from the addition of membrane possessing similar specific ionic conductance, and hence ionic permeability.

Effects of Colchicine

Colchicine, a microtubule destabilizer, has been shown previously to block exocytotic processes involved in the regulation of proton secretion in the α cells in the turtle bladder (Gluck et al., 1982). In order to investigate whether the observed changes in C_a noted above involved microtubular function, we performed a series of experiments in hemibladders pretreated with colchicine. The results obtained from seven hemibladders are shown in Table 3.

The hemibladders were incubated with 0.1 mM serosal colchicine for two hours. This treatment resulted in no statistically significant changes in the membrane parameters as compared to control hemibladders (unpaired analysis comparing Tables 1 and 3). Colchicine failed to prevent the cAMP-induced stimulation in I_{sc}, and the associated increase in C_a and G_a. Furthermore, the changes in the parameters were found to be statistically indistinguishable from the earlier experiments in hemibladders not exposed to colchicine (unpaired analysis comparing the changes shown in Table 3 with those in Table 1). The colchicine-treated hemibladders exhibited a small transport-associated decrease in R_p after a cAMP stimulation, and a small increase in $G_{a\text{-norm}}$, both of which were not observed in the earlier results. These data show that 0.1 mM colchicine is not effective in blocking the transport-associated change in the apical membrane parameters, thereby indicating that microtubular function is not requisite. The small decrease in R_p might reflect a slight increase (~20%) in the width of the lateral spaces, which is not unexpected since colchicine might disrupt the cytoskeleton.

The effects of other agents which alter the cytoskeleton were not investigated in this study. Note that transepithelial conductance is increased by cytochalasin B (Stetson & Steinmetz, 1983), as well as cytochalasin D (*unpublished observation*), presumably resulting from disruption of the integrity of the tight junctions. The impedance-analysis technique requires low paracellular conductance (*see* Clausen & Dixon, 1986).

Discussion

The purpose of this study was to investigate the relationship between changes in the rate of electrogenic bicarbonate secretion and the associated changes in the epithelial membrane electrical parameters. Transepithelial impedance measurements were analyzed to obtain estimates of membrane capacitance, which is proportional to membrane area, and membrane conductance, which is proportional to membrane ionic permeability. Changes in I_{sc} resulting from either cAMP or carbachol were used as an indirect measure of bicarbonate secretion. Recall that cAMP and carbachol are thought to stimulate transport via different regulatory pathways.

Membrane Electrical Parameters

The addition of cAMP results in an increase in I_{sc} reflecting an increased rate of bicarbonate secretion (Fig. 2), and this increase is temporally associated with an increase in C_a and G_a. These findings suggest that an increase in apical-membrane area may be requisite for an increase in transport, hence alteration of apical-membrane area may be the mechanism for cellular regulation of transport.

Further support for this notion arises from the results obtained using a different transport-stimulating agent, which causes a different response in I_{sc}. Serosal addition of carbachol causes a *transient* increase in I_{sc} (Fig. 3), and this response is also accompanied by transient changes in C_a and G_a. Fur-

thermore, these changes in the apical-membrane electrical properties closely follow the time course of the changes in I_{sc}.

It is interesting to note that the peak increases in I_{sc}, C_a and G_a following the application of carbachol are each statistically indistinguishable from the sustained increases observed after application of cAMP. Carbachol is thought to bind to a surface receptor which ultimately activates the phosphoinositol pathway, and cAMP is thought to exert its effects via the activation of protein kinase-A (Schneider et al., 1988). Hence, the two distinct signaling pathways appear to stimulate a common distal mechanism that regulates electrogenic alkali secretion. The data are consistent with the notion that this mechanism involves altering the number of transport elements in the apical membrane, via the insertion and/or removal of apical membrane.

The basolateral-membrane parameters did not change significantly after stimulation of bicarbonate secretion with cAMP or carbachol. This indicates that this transport process is not closely regulated by changes in basolateral ionic conductance. A plausible means for regulation of transport would be by altering the number of proton pumps thought to be located within the membrane (*see* below), via endo- and exocytotic processes. We found no evidence for this, since basolateral capacitance also did not change significantly.

Apical Membrane Ionic Conductance

The current working hypothesis for bicarbonate secretion incorporates an apical-membrane Cl^--HCO_3^- exchange process in parallel with a chloride-conductive leak pathway (Stetson et al., 1985). Bicarbonate is secreted in exchange for chloride, a process thought to be electrically silent, whereas chloride is recycled across the apical membrane via chloride-permeable channels. Active proton reabsorption across the basolateral membrane by a proton ATPase is thought to drive the process. The observed increase in G_a with increased transport is consistent with this proposed model of bicarbonate transport; alterations in the apical-membrane chloride conductance might be an important regulator of transport rate. Our results are consistent with the notion that the putative increase in chloride conductance results from addition of apical membrane possessing ionic channels. The estimated specific conductance of the added membrane is $\Delta G_a/\Delta C_a$ and is indistinguishable from $G_{a\text{-norm}}$, the existing membrane specific conductance. Note, however, that we cannot rule out the possibility that the increase in G_a involves the activation of existing apical-membrane channels, coupled with the simultaneous addition of membrane which lacks conductive channels. Addition or removal of membrane containing an electrically-silent Cl^--HCO_3^- exchange process would not be expected to alter the conductance of the membrane.

Effects of Colchicine

Stimulation of proton transport in the turtle bladder involves the exocytosis of proton-pump-containing vesicles with the apical membrane (Gluck et al., 1982). This process is inhibited by colchicine, thereby indicating that microtubular function is involved. In regards to electrogenic bicarbonate secretion, colchicine was not effective in blocking cAMP-induced increases in I_{sc}. These results indicate that electrogenic bicarbonate secretion is not dependent on normal microtubular function. If regulation of this process truly involves alterations in apical membrane area, then the membrane remodeling processes appear to be different from those involved in the regulation of proton secretion.

Cellular Heterogeneity

Recall that the turtle bladder epithelium may be divided into two major cell types, granular and CA-rich cells, which account for approximately 80 and 20% of the total cell number, respectively. Available micrographic evidence indicates that the β cells, which account for roughly 20% of the CA-rich cell type, mediate bicarbonate secretion (Stetson & Steinmetz, 1985). Hence a small portion of the epithelial cells, about 5% of the total, are thought to mediate bicarbonate secretion.

Stimulation of bicarbonate secretion with cAMP results in a 45 ± 7% increase in C_a, and the peak response observed after stimulation with carbachol results in a 23 ± 5% increase in C_a (recall that the absolute increases in C_a were not statistically different). Clausen and Dixon (1986) showed that impedance analysis results in estimates of C_a which reflect the capacitance of all the cells in the epithelium. It is therefore quite surprising that stimulation of bicarbonate secretion would result in such a large change in C_a, considering that the changes are thought to occur in a minor cell population comprising roughly 5% of the cell population.

This apparent paradox can be investigated by consideration of the hypothesis for electrogenic bicarbonate transport mentioned above. Bicarbonate is thought to be transported in an electroneutral fashion across the apical membrane, and the current measured under short-circuit conditions is thought to be carried via a conductive pathway permeable to chloride. Hence this current may be described by

$$I_{sc} = g_{Cl}(E_{Cl} - V_a)$$

where g_{Cl} is the apical-membrane chloride conductance, V_a is the apical-membrane potential under short-circuit conditions, and E_{Cl} is the chloride equilibrium potential. The difference $E_{Cl} - V_a$ can be thought of as the net electrochemical driving force for chloride exit under these conditions.

If we suppose for the moment that the transport-associated increase in C_a is completely unrelated to electrogenic bicarbonate secretion, and if we assume that 5% of the apical-membrane area is involved in the transport, then we can compute the effective driving force.[2] The estimated apical-membrane capacitance of the transporting cells (*see* Table 1) would be 5% of C_a and would equal 0.10 μF/cm^2 of epithelium. g_{Cl} could then be estimated as the product of this capacitance and $G_{a\text{-norm}}$ and would equal 10 μS/cm^2 of epithelium. The observed change in I_{sc} (4.0 μA/cm^2) would therefore require a net driving force for chloride exit of 400 mV. Hence, electrogenic bicarbonate secretion would require an apical-membrane potential estimated at close to −450 mV, a nonphysiological value. The only resolution to this problem is additional membrane area, or a significantly higher apical-membrane specific conductance.

The question of additional membrane area can be addresssed if we assume that the measured increase in C_a, added to the 5% nominal capacitance accounted for by the minor cell type, reflects the effective area of transporting membrane. A similar calculation can then be used to estimate the driving force. In this case, the apical capacitance of the transporting cells is estimated as 1.0 μF/cm^2 of epithelium (*see* Table 1). g_{Cl} would then equal the product of this capacitance and the specific conductance of the inserted membrane ($\Delta G_a/\Delta C_a$, *see* results above), and would equal 120 μS/cm^2 of epithelium. Therefore, the predicted net driving force for chloride exit would be 33 mV, a physiologically reasonable value (*cf.*, Clausen et al., 1986).

We cannot rule out the possibility that the transporting cells exhibit a substantially higher specific conductance than $G_{a\text{-norm}}$, but this conductance would have to be at least an order of magnitude greater. Since our results show no evidence for a dramatic increase in $G_{a\text{-norm}}$ with transport, we believe that a far more plausible conclusion is that electrogenic bicarbonate secretion is mediated by an effective membrane area far greater than that accounted for by 5% of the measured apical-membrane capacitance. If the secretion is mediated by the β cells, then this would indicate that stimulation of transport results in fourfold (carbachol-induced) to ninefold (cAMP-induced) increase in the apical-membrane area of these cells.

Substantial transport-related changes in apical-membrane area are observed in a number of epithelia. For example, quantitative microscopy has shown that stimulation of proton secretion in turtle bladder results in a twofold increase in apical-membrane area (Stetson & Steinmetz, 1983), and in gastric mucosa, histamine-induced stimulation of proton transport results in a 6- to 10-fold increase in area (Forte & Machen, 1986). Note also that in both of these preparations the area changes compared quantitatively to estimates of C_a derived from impedance-analysis studies (Clausen, Machen & Diamond, 1983; Diamond & Machen, 1983; Clausen & Dixon, 1986). The question remains whether the measured increase in C_a can be accounted for by changes in the β cells, or whether cAMP and/or carbachol result in morphological changes in other cell types as well.

Morphological studies which would permit quantitative comparison with our electrical measurements are currently unavailable. Although Stetson and Steinmetz (1985) did not observe numerous intracellular vesicles in the apical regions of the β cells, Stetson (1988) reported that stimulation of transport with cAMP resulted in the formation of a network of tubular invaginations which extended 5 μm into the cytoplasm from the apical membrane. This finding is similar to that observed in gastric mucosa, which possesses tubulovesicles that fuse with the apical membrane upon stimulation of acid secretion (Forte & Machen, 1986). Stetson (1988) also reported that in turtle bladder the observed changes were found only in the β cells; cAMP stimulation produced no observable changes in the other cell types. Hence these morphological studies suggest that the transport-associated increase in capacitance results from an increase in apical-membrane area due to the formation of the tubular invaginations in the β cells. In contrast, the transport-associated changes in the α cells results from the endo- and exocytosis of discrete vesicles (Dixon et al., 1986).

Note also that Durham and Nagel (1986) provided physiological evidence that stimulation with cAMP does not affect the granular cells. In their microelectrode study, cAMP had no effect on the resistance ratio measured in these cells. Hence, their results also indicate that cAMP-induced stimulation of bicarbonate secretion is specific to CA-rich cells.

[2] We also assume that the apical membrane is essentially chloride permselective and that the membrane exhibits a linear current-voltage relationship.

Conclusions

Stetson and Steinmetz (1985) and Stetson et al. (1985) suggest that the possibility that proton and bicarbonate transport in turtle bladder are both mediated by similar cells that differ in the polarity of the specific transport elements; namely, the proton-secreting α cells possess an apical-membrane proton pump, and a Cl^--HCO_3^--exchange process and chloride conductance located in the basolateral membrane. The bicarbonate-secreting β cells have, in essence, reversed polarity, with the proton pump located in the basolateral membrane and bicarbonate and chloride membrane transport processes located in the apical membrane. Our results, which are consistent with this proposal, provide additional information regarding the regulatory processes involved in electrogenic proton and bicarbonate transport.

In both cases, the limiting membrane conductance appears to reside in the apical membrane, which possesses a conductance that is several-fold lower than that of the basolateral membrane and which appears to be the site of regulation of the transport processes. Regulation of electrogenic proton transport involves alterations in the number of proton pumps, whereas regulation of bicarbonate secretion appears to involve alterations in chloride conductance (and possibly the number of Cl^--HCO_3^- exchangers). Some notable differences exist between these two membrane remodeling processes. In proton secretion, the magnitude of the transport-associated changes in C_a can be accounted for by a twofold change in area, involving endo- and exocytosis and discrete membrane vesicles. In bicarbonate secretion, the magnitude of the changes in C_a appear to result from significantly larger alterations in membrane area involving a network of tubular structures. In addition, the α and β cells appear to possess different pharmacology regarding the membrane remodeling processes: α cells are colchicine sensitive; β cells apparently are not.

This work was supported in part by a Veterans Administration Merit Review Grant, a National Institutes of Health (NIH) Biomedical Research Support Grant, and NIH grant AM-28074.

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., Machen, T.E., Diamond, J.M. 1983. Use of the AC impedance analysis to study membrane changes related to acid secretion in amphibian gastric mucosa. *Biophys. J.* **41:**167–178

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

Diamond, J.M., Machen, T.E. 1983. Impedance analysis in epithelia and the problem of gastric acid secretion. *J. Membrane Biol.* **72:**17–41

Dixon, T.E., Clausen, C., Coachman, D. 1988. Constitutive and transport-related endocytotic pathways in turtle bladder epithelium. *J. Membrane Biol.* **102:**49–58

Dixon, T.E., Clausen, C., Coachman, D., Lane, B. 1986. Proton transport and membrane shuttling in turtle bladder epithelium. *J. Membrane Biol.* **94:**233–243

Durham, J.H., Nagel, W. 1986. Evidence for separate cellular origins of sodium and acid-base transport in the turtle bladder. *Am. J. Physiol.* **250:**C609-C616

Forte, J.T., Machen, T.E. 1986. Ion transport by gastric mucosa. *In*: Physiology of Membrane Disorders. T.E. Andreoli, J.F. Hoffman, D.D. Fanestil, and S.G. Schultz, editors. pp. 535–558. Plenum, New York

Gluck, S., Cannon, C., Al-Awqati, Q. 1982. Exocytosis regulates urinary acidification by rapid insertion of H^+ pumps into the luminal membrane. *Proc. Natl. Acad. Sci USA* **79:**4327–4331

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

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

Rosen, S. 1970. The turtle bladder. I. Morphological studies under varying conditions of fixation. *Exp. Mol. Pathol.* **12:**286–296

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

Schneider, E.S., Durham, J.H., Matons, C., Brodsky, W.A. 1988. Alkali secretion in the turtle bladder: Up-regulation by the phospho-inositol cascade and inhibition by diphenylaminecarboxylate (DPC). *In*: Membrane Biophysics III: Biological Transport. M.A. Dinno and W.M. Armstrong, editors. pp. 81–92. Alan R. Liss, New York

Schwartz, J., Bethencourt, D., Rosen, S. 1982. Specialized function of carbonic-anhydrase-rich and granular cells in turtle bladder. *Am. J. Physiol.* **242:**F627–F633

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

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. 1983. Role of membrane fusion in CO_2 stimulation of proton secretion by turtle bladder. *Am. J. Physiol.* **245:**C113–C120

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

Received 3 May 1989; revised 14 September 1989