Effects of Changes in Mucosal Solution Cl^- or K^+ Concentration on Cell Water Volume of *Necturus* Gallbladder Epithelium

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ABSTRACT An electrophysiologic technique was used to measure changes in cell water volume in response to isosmotic luminal solution ion replacement. Intracellular Cl^- activity (aCl_i^-) was measured and net flux determined from the changes in volume and activity. Reduction of luminal solution [Cl^-] from 98 to 10 mM (Cl^- replaced with cyclamate) resulted in a large fall in aCl_i^- with no significant change in cell water volume. Elevation of luminal solution [K^+] from 2.5 to 83.5 mM (K^+ replaced Na^+) caused a small increase in aCl_i^- with no change in cell water volume. Exposure of the *Necturus* gallbladder epithelium to agents that increase intracellular cAMP levels (forskolin and/or theophylline) induces an apical membrane electrodiffusive Cl^- permeability accompanied by a fall in aCl_i^- and cell shrinkage. In stimulated tissues, reduction of luminal solution [Cl^-] resulted in a large fall in aCl_i^- and rapid cell shrinkage, whereas elevation of luminal solution [K^+] caused a large, rapid cell swelling with no significant change in aCl_i^-. The changes in cell water volume of stimulated tissues elicited by lowering luminal solution [Cl^-] or by elevating luminal solution [K^+] were reduced by 60 and 70%, respectively, by addition of tetraethylammonium (TEA^+) to the luminal bathing solution. From these results, we conclude that: (*a*) In control tissues, the fall in aCl_i^- upon reducing luminal solution [Cl^-], without concomitant cell shrinkage, indicates that the Cl^- entry mechanism is electroneutral (Cl^-/HCO_3^-) exchange. (*b*) Also in control tissues, the small increase in aCl_i^- upon elevating luminal solution [K^+] is consistent with the recent demonstration of a basolateral Cl^- conductance. (*c*) The cell shrinkage elicited by elevation of intracellular cAMP levels results from conductive loss of Cl^- (and probably K^+). (*d*) Elevation of cAMP inhibits apical membrane Cl^-/HCO_3^--exchange activity by 70%. (*e*) The cell shrinkage in response to the reduction of mucosal solution [Cl^-] in stimulated tissues results from net K^+ and Cl^- efflux via parallel electrodiffusive pathways. (*f*) A major fraction of the K^+ flux is via a TEA^+-sensitive apical membrane K^+ channel.

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INTRODUCTION

The mechanisms of salt and water transport across *Necturus* gallbladder epithelium have been studied by measurements of transepithelial fluid absorption, membrane electrical properties, intracellular ion activities, and changes in cell volume. In particular, the pharmacologic sensitivity and ionic dependence of many of these parameters have been used to establish the apical and basolateral cell membrane transport mechanisms responsible for salt and water absorption. There is general agreement that apical entry of Na^+ and Cl^- is electroneutral; however, at least three different mechanisms (NaCl cotransport, $NaKCl_2$ cotransport, and parallel Na^+/H^+ and Cl^-/HCO_3^- exchanges) have been proposed to be operative, singularly or in combination (Reuss, 1989).

The original proposal that Na^+ and Cl^- entry are directly coupled was based upon measurements of changes in Na^+ and Cl^- intracellular activities (aNa_i^+ and aCl_i^-, respectively) at steady state, after replacement of the luminal bathing solution counter-ion (Reuss and Grady, 1979; García-Díaz and Armstrong, 1980). Recent evidence supporting NaCl and/or $NaKCl_2$ cotransport mechanisms was derived primarily from measurements of changes in cell volume in response to ion substitutions and/or pharmacologic agents (Ericson and Spring, 1982; Spring and Ericson, 1982; Davis and Finn, 1985). Steady-state measurements of ion activities cannot discriminate between cotransport and parallel Na^+/H^+ and Cl^-/HCO_3^- exchange (Reuss and Stoddard, 1987). Further, the proposed cotransport mechanisms are not consistent with the results of studies in which luminal solution pH, aCl_i^-, pH_i, and aNa_i^+ were measured during luminal solution ion replacements (Baerentsen et al., 1983; Reuss, 1984; Weinman and Reuss, 1984). On the basis of measurements of transient changes in intracellular ion activities, it was concluded that Na^+/H^+ and Cl^-/HCO_3^- exchange could account quantitatively for salt entry across the apical membrane, estimating the latter from the rate of fluid absorption (Reuss, 1984).

Fluid absorption is reduced in several leaky epithelia by maneuvers that elevate intracellular cAMP levels. The primary effects of elevation of cAMP in *Necturus* gallbladder epithelium include activation of an apical membrane Cl^- conductance and inhibition of both apical membrane Na^+/H^+ and Cl^-/HCO_3^- exchangers, with the net effect of reducing NaCl and fluid absorption (Petersen and Reuss, 1983; Reuss and Petersen, 1985; Reuss, 1987). Physiologically relevant hormonal regulation of ion transport by VIP and secretin via cAMP has been suggested for mammalian gallbladder (Wood and Svanvik, 1983; O'Grady et al., 1989).

The present experiments were designed to evaluate the effects of a reduction of luminal solution $[Cl^-]$ or an elevation of luminal solution $[K^+]$ on cell water volume and aCl_i^- in *Necturus* gallbladder epithelial cells before and during elevation of intracellular cAMP levels. Under control conditions, the elevation of $[K^+]$ and the reduction of $[Cl^-]$ induced significant changes in aCl_i^- without measurable changes in cell water volume. Since net influx or efflux of salt would cause changes in cell volume, these results do not support NaCl or $NaKCl_2$ cotransport as the dominant apical membrane entry pathway. Elevation of intracellular cAMP induced an apical membrane Cl^- conductance, caused cell shrinkage, and inhibited Cl^-/HCO_3^- exchange by ~70%. Furthermore, under these conditions isosmotic ion substitutions in

the luminal bathing solution elicited large and rapid changes in cell water volume. We conclude that net fluxes through apical membrane Cl^- channels and TEA^+-sensitive K^+ channels underlie these volume changes.

Parts of this work have been published in abstract form (Cotton and Reuss, 1988, 1990).

METHODS

Preparations, Solutions, and Drugs

Mudpuppies (*Necturus maculosus*) were maintained in aquaria at 5–10°C and anesthetized with tricaine methanesulfonate. Gallbladders were removed, opened, rinsed free of bile, and mounted in a chamber, apical side up. The upper compartment of the chamber was open, had a volume of ~0.2 ml, and was exchanged at a rate of 20–30 ml/min. The lower compartment was closed, had a volume of ~0.8 ml, and was perfused at a rate of 10–15 ml/min.

The control bathing solution (NaCl Ringer's solution) contained (in mM): 90 NaCl, 10 $NaHCO_3$, 2.5 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, and 0.5 NaH_2PO_4, and was equilibrated with 1% CO_2/99% air. The pH was ~7.65 and the osmolality was ~200 mosmol/kg. During the TMA^+ loading procedure the NaCl in the mucosal solution was replaced by TMA_2SO_4 (osmolality was maintained by addition of sucrose). Low Cl^- solutions were prepared by isomolar replacement of Cl^- with cyclamate, and high K^+ solutions were prepared by isomolar replacement of Na^+ with K^+. Theophylline, forskolin, and tetraethylammonium chloride (TEACl) were purchased from Sigma Chemical Co. (St. Louis, MO). Theophylline was dissolved directly in Ringer's solution and used at a final concentration of 3 mM. TEA^+-containing solutions were prepared by replacing 9 mM NaCl with 9 mM TEACl. Forskolin was stored as a 25-mM stock solution in ethanol, and was diluted to a final concentration of 2.5 μM with Ringer's solution.

Microelectrode Fabrication and Calibration

Glass microelectrodes were used to measure voltages and intracellular $aTMA^+$ and aCl^- (Altenberg et al., 1990). Cell membrane voltages and intracellular ion activities were determined by either simultaneous impalements of two cells with two single-barrel microelectrodes or by impalement with a double-barrel microelectrode. The single-barrel microelectrodes were prepared from borosilicate glass with internal fiber (1 mm o.d., 0.5 mm i.d.; Friedrich and Dimmock, Inc., Milville, NJ). Conventional microelectrodes were filled with 3 M KCl and had resistances that ranged from 20 to 50 MΩ when immersed in Ringer's solution. Single-barrel TMA^+-sensitive microelectrodes were constructed from micropipettes baked overnight in an oven at 200°C. Hexamethyldisilazane (Sigma Chemical Co.) was introduced into the oven and the electrodes were baked for an additional 1–2 h. A short column of resin was introduced into the shaft and the tips were allowed to fill. The electrodes were back-filled with NaCl Ringer's solution and a chloridized silver wire was inserted and sealed in place with dental wax. The resistances ranged from 10 to 15 GΩ when immersed in NaCl Ringer's solution. Double-barrel microelectrodes were prepared from fused borosilicate glass (each barrel o.d. = 1.0 mm, i.d. = 0.43 mm; Hilgenberg, Malsfeld, Germany) or from nonfused borosilicate glass with internal fiber (EN-1, Omega Dot; reference barrel, o.d. = 1.0 mm, i.d. = 0.5 mm; resin barrel, o.d. = 1.0 mm, i.d. = 0.75 mm; Friedrich and Dimmock, Inc.) as previously described (Altenberg et al., 1990). The K^+/TMA^+ selective resin consisted of 5% potassium tetrakis (*p*-chlorophenylborate) in 3-nitro-*O*-xylene. The reference barrel was filled with 1 M Na formate/10 mM KCl solution and the ion-selective barrel was backfilled with NaCl Ringer's solution. The resistance of the conventional barrel usually exceeded 400 MΩ and the resistance of the resin barrel was usually 100–200 GΩ. Microelectrodes with high TMA^+ selectivity were

consistently obtained only after breaking the tips of the double-barrel electrodes by impaling the tissue. Useful electrodes had resistances of 80–150 MΩ (conventional barrel) and 5–10 GΩ (TMA^+-selective barrel). The Cl^--sensitive resin was Corning Cl^- exchanger 477913 (Corning Medical, Medfield, MA). The reference barrel was filled with a 1 M Na formate/10 mM KCl solution and the ion-selective barrel was backfilled with NaCl Ringer's solution. Useful electrodes had resistances of 50–150 MΩ for the reference barrel and 30–100 GΩ for the Cl^--selective barrel when immersed in NaCl Ringer's solution.

TMA^+-sensitive electrodes were calibrated with solutions that contained 120 mM KCl plus 0.1–20 mM TMACl. Slopes ranged from 54 to 62 mV/decade $\Delta[TMA^+]$ and the selectivity ratio for (TMA^+/K^+) was 10^2–10^3. The results of intracellular measurements were expressed as TMA^+ concentration, which assumes that the activity coefficients inside and outside the cell are the same. Cl^--sensitive microelectrodes were calibrated with HEPES (1 mM)-buffered NaCl solutions (10–100 mM) in which the osmolality was kept constant by addition of sucrose. Slopes were calculated taking into account differences in ionic strength and ranged from 53 to 59 mV/decade $\Delta[Cl^-]$. Intracellular Cl^- activity was calculated from the Nicolsky equation. The selectivity ratio for Cl^-/HCO_3^- was ~10 and the expected $[HCO_3^-]_i$ was ~5 mM; therefore, no correction was made for intracellular HCO_3^-.

Electrical Measurements

The transepithelial voltage (V_{ms}) was referred to the serosal fluid compartment. The lower compartment electrode was an Ag-AgCl pellet in series with a Ringer/agar bridge. The upper compartment electrode was a calomel half-cell in series with a flowing, saturated KCl macroelectrode constructed from a fiber-filled glass pipette (Ultrawick; World Precision Instruments, New Haven, CT) pulled to a tip diameter of ~1 mm, which was placed in the upper compartment next to the suction (outflow) pipette. At the superfusion rates used, the KCl leakage into the mucosal solution compartment did not elicit a measurable elevation in K^+ activity. Apical (V_{mc}) and basolateral (V_{cs}) membrane voltages were referred to the adjacent bathing solution. Ion-sensitive microelectrodes were connected to a high-input impedance electrometer (model FD-223; World Precision Instruments). The Ag-AgCl pellet in the lower compartment served as ground. Intracellular impalements were made with hydraulic micromanipulators (model MO-103; Narishige, Tokyo, Japan). The tissue was observed with a microscope (Leitz Diavert) equipped with Hoffman modulation-contrast optics at 300×.

Transepithelial constant current pulses (50–100 μA/cm², 2–3 s duration) were passed between an Ag-AgCl pellet in the lower compartment and an Ag-AgCl wire in the upper compartment. The resulting voltage deflections (measured at 800 ms from the onset of the pulse) were corrected for series solution resistances and used to calculate transepithelial resistance (R_t) and the apparent ratio of cell membrane resistances ($R_a/R_b = \Delta V_{mc}/\Delta V_{cs}$) (see Stoddard and Reuss, 1988*b*). Transepithelial voltage (V_{ms}), cell membrane voltages (V_{mc} and V_{cs}), and differential ion-sensitive electrode voltages ($V_{ion} - V_{cs}$) were low-pass filtered, digitized, sampled at 10 Hz with a data acquisition system (ASYST; Macmillan Software Co., New York, NY), and stored in a microcomputer for later analysis.

TMA^+ Loading

Since the native membrane TMA^+ permeability is undetectably small, it was necessary to transiently permeabilize the membrane to load the epithelial cells with TMA^+ (Reuss, 1985). The tissue was mounted in the chamber and allowed to equilibrate for 60–90 min during bilateral superfusion with NaCl Ringer's solution. The mucosal solution was then changed to TMA_2SO_4 Ringer's for ~2 min, and then nystatin (10 mg/ml in dimethylsulfoxide) was added at a final concentration of 7.5–30 μg/ml (40–160 U/ml). After 5–10 min the nystatin exposure was

terminated and the mucosal TMA_2SO_4 Ringer's superfusion was continued until the membrane voltages and apparent ratio of the cell membrane resistances recovered (usually 60–90 min), indicating "resealing" and restoration of the native membrane permselectivity. SO_4^{2-} was used to minimize the uptake of anions, since net uptake of salt and water during the loading procedure would result in cell swelling. Detailed validation of the reversibility of the exposure to nystatin and the time course for restoration of the membrane properties has been presented previously (Reuss, 1985; Cotton et al., 1989). Holz and Finkelstein (1970) showed that the effects of nystatin on water and ion permeability of lipid bilayers are linearly related. Therefore, the reversibility of the effect of nystatin on electrodiffusive ion permeation supports the notion that the water fluxes measured a long time after removal of the antibiotic are not mediated by nystatin remaining in the cell membranes.

Calculations

Intracellular TMA^+ concentration was calculated from $V_{TMA^+} - V_{cs}$ for each data point. Changes in cell water volume were calculated from the following equation:

$$\Delta V(t) = [V(t) - V(0)] = V(0)\left(\frac{[TMA]\,(0)}{[TMA]\,(t)} - 1\right) \quad (1)$$

where $\Delta V(t)$ = cell water volume change (nl/cm²), $V(t)$ = cell water volume at time t (nl/cm²), $V(0)$ = initial cell water volume (2,870 nl/cm²), $[TMA^+]$ (0) = intracellular $[TMA^+]$ at time 0 (mM), and $[TMA^+]$ (t) = intracellular $[TMA^+]$ at time t (mM).

The value for initial cell water volume was calculated for an epithelial sheet 35 μm tall with a cell water content of 82% (Cotton et al., 1989). The initial rate of change in cell water volume was calculated from the slope of the least-squares linear regression analysis of the linear portion of the curve (usually 5–15 s; 50–150 data points) after the cell water volume began to change.

RESULTS

Effects of Low Cl^- or High K^+ Luminal Bathing Solutions on Electrical Properties, Cell Water Volume, and aCl_i^- of Unstimulated Gallbladders

The effects of isomolar replacement of luminal $[Cl^-]$ (Cl^- replaced by cyclamate) on voltages, resistance ratio, aCl_i^-, and cell volume are illustrated in Figs. 1 *A* and 2 *A* and summarized in Table I. In agreement with previous results, changes in cell membrane voltages and resistance ratio are small, and R_t increased from 147 ± 13 to 158 ± 13, $P < 0.05$ (Petersen and Reuss, 1983; Reuss, 1987). The concomitant changes in cell water volume and aCl_i^- are also summarized in Table I. Cell water volume was not significantly altered during the 2-min exposure to low $[Cl^-]$ in spite of the rapid (−17.4 mM/min) fall in aCl_i^- from 19.4 to 10.5 mM. Similar results were obtained in tissues in which the luminal $[Cl^-]$ was lowered and TEA^+ (9 mM) was simultaneously added to the luminal solution (see Figs. 1 *B* and 2 *B* and Table I).

In contrast with the effects of reducing $[Cl^-]$, isomolar replacement of Na^+ with K^+ resulted in large depolarizations of V_{mc} and V_{cs}, a lumen-negative change in V_{ms}, and a fall in the resistance ratio, as depicted in Figs. 3 *A* and 4 *A* and summarized in Table II. These results agree with previous studies in which the apical cell membrane of this tissue was shown to be largely potassium permselective (Reuss and Finn, 1975; Van Os and Slegers, 1975). In tissues simultaneously exposed to elevated luminal $[K^+]$ and 9 mM TEA^+, the changes in membrane voltages were attenuated compared

with elevation of luminal [K^+] without TEA^+ (Figs. 3, *A* and *B*, 4, *A* and *B*, and Table II). The effects of elevated luminal solution [K^+] with or without TEA^+ on aCl_i^- and cell water volume are also summarized in Table II. Cell volumes did not change significantly. However, a small increase in aCl_i^- was observed during elevation of luminal solution [K^+], and this increase was abolished when TEA^+ was included in the bathing solution. Not shown, in the absence of other ion substitutions, addition of TEA^+ to the luminal solution (9 mM, partially replacing Na^+) caused only small changes in membrane voltages (V_{cs} depolarized by 2.0 ± 0.4 mV, n = 3) and had no effects on either aCl_i^- or cell water volume (see Discussion).

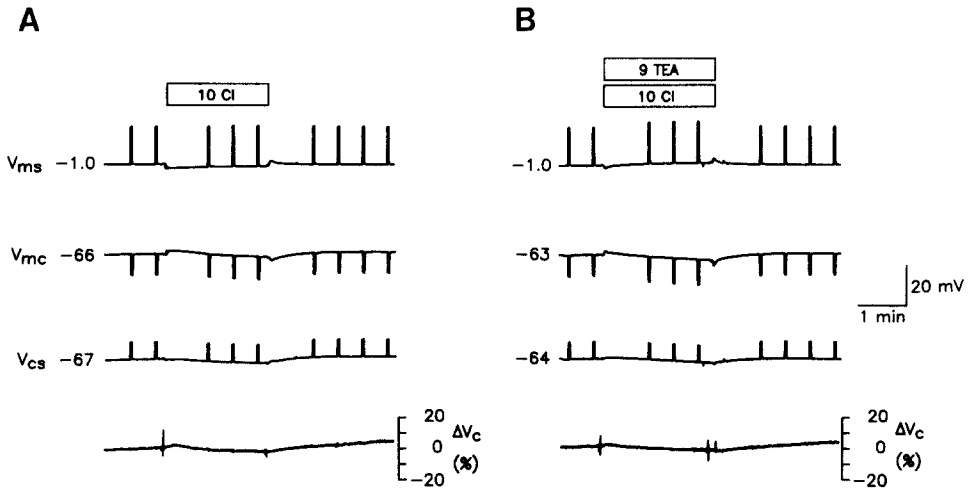

FIGURE 1. Effects of reducing luminal solution [Cl^-] on voltages, resistances, and cell water volume. V_{ms}, V_{mc}, and V_{cs} denote transepithelial, apical membrane, and basolateral membrane voltages, respectively. Upward deflections denote mucosa-positive (V_{ms}) or cell-positive (V_{mc}, V_{cs}) voltage changes. (*A*) Luminal solution [Cl^-] was reduced from 98 to 10 mM (Cl^- replaced with cyclamate) during the indicated interval. The voltage deflections result from transepithelial constant current pulses. The change in cell water volume (ΔV_c) was calculated as described in Methods. (*B*) Same experimental maneuver as in *A*, except 9 mM TEA^+ (TEA^+ replaced Na^+) was included in the low Cl^- bathing solution.

As seen in Figs. 1–4, the time courses and magnitudes of the changes in membrane voltages upon changes in external [Cl^-] and [K^+] vary considerably from tissue to tissue. This variability appears to be partly seasonal (Stoddard and Reuss, 1989*a*, *b*), but is poorly understood.

Effects of Theophylline and Forskolin on Electrophysiological Properties, Cell Water Volume, and aCl_i^-

Exposure of gallbladder epithelium to a luminal solution containing 3 mM theophylline and 2.5 μM forskolin, agents that elevate intracellular cAMP levels, induced rapid changes in membrane voltages and resistance ratios (Fig. 5, *A* and *B* and Table III). The voltage and resistance changes are in agreement with previous reports in

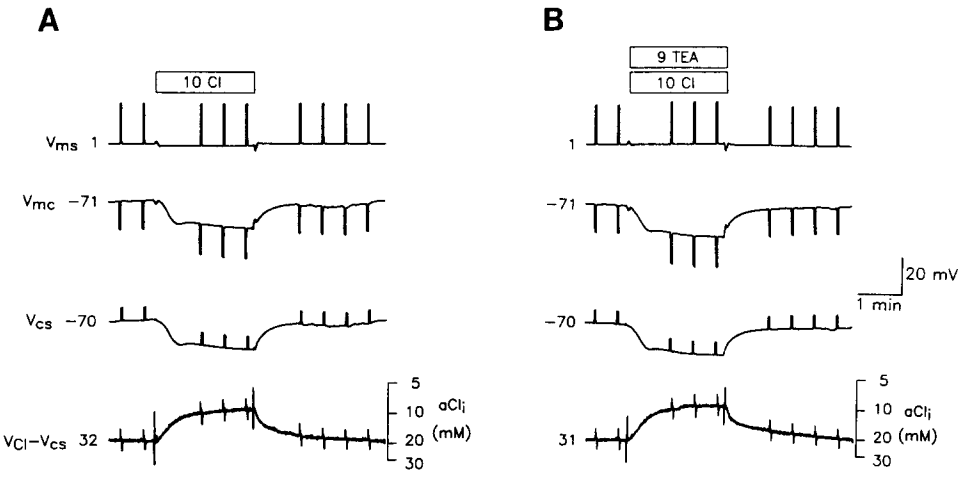

FIGURE 2. Effects of reducing luminal solution [Cl^-] on voltages, resistances, and aCl_i^-. (*A*) Luminal solution [Cl^-] was reduced from 98 to 10 mM (Cl^- replaced with cyclamate) during the indicated interval. $V_{Cl} - V_{cs}$ is the difference between the voltage outputs of the intracellular Cl^- and conventional microelectrode barrels. (*B*) Same experimental maneuver as in *A*, except 9 mM TEA^+ (TEA^+ replaced Na^+) was included in the low Cl^- bathing solution.

which the electrophysiological effects of cAMP were attributed to induction of an apical membrane Cl^- conductance (Petersen and Reuss, 1983). Cell shrinkage and a fall in aCl_i^- were also observed (Fig. 5, *A* and *B* and Table III). The fall in aCl_i^- from 20 to 15 mM was sustained, but it is not known if the cells remain shrunken at later times or whether cell volume is recovered.

TABLE I

Effects of Reducing Luminal Solution [Cl^-] without or with TEA^+ on Voltages, Resistances, aCl_i^-, and Cell Volume

Condition	V_{ms}	V_{mc}	R_a/R_b	aCl_i^-	$\Delta aCl_i^-/\Delta t$	Δ Cell water volume	Δ Volume/ Δt
	mV	*mV*		*mM*	*mM/min*	*%*	*%/min*
98 Cl	0.2 ± 0.6	−59 ± 2	6.26 ± 2.17	19.4 ± 1.3	—	—	—
10 Cl	−1.3 ± 0.7	−67 ± 3	7.02 ± 2.53	10.5 ± 1.2	−17.4 ± 2.4*	−2.3 ± 1.3	−2.4 ± 1.2
Difference	−1.4 ± 0.3*	−7 ± 2*	0.77 ± 0.58	−8.9 ± 0.2*	—	—	—
98 Cl	0.1 ± 0.6	−59 ± 3	4.99 ± 1.72	19.9 ± 2.1	—	—	—
10 Cl+TEA	0.0 ± 0.7	−66 ± 4	7.71 ± 2.04	10.2 ± 1.2	−17.9 ± 1.8*	−3.2 ± 1.7	−3.6 ± 1.8
Difference	−0.1 ± 0.4	−7 ± 3*	2.73 ± 0.56*	−9.7 ± 1.0*	—	—	—

Values are means ± SEM; $n = 9$ (voltages and resistance), $n = 4$ (aCl_i^-), and $n = 5$ (cell volume). V_{ms} and V_{mc} are transepithelial and apical membrane voltages, respectively. R_a/R_b is the apparent ratio of membrane resistances (apical/basolateral). Mucosal [Cl^-] was reduced from 98.1 to 9.8 mM for ~2 min by replacement with cyclamate. [TEA^+] was 9.0 mM. Values in 10 Cl^- or 10 Cl^- + TEA^+ were measured ~1.5 min after the ion substitution. $\Delta aCl_i^-/\Delta t$ and $\Delta volume/\Delta t$ represent the initial rates (first 10–15 s) of change in aCl_i^- or cell water volume, respectively. *Significant change, $P < 0.05$.

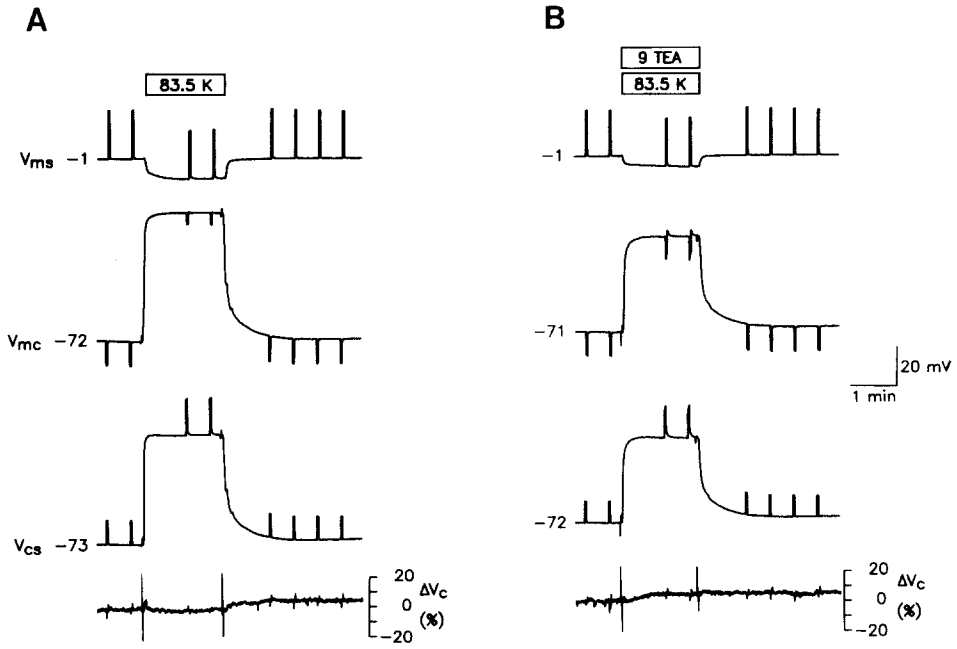

FIGURE 3. Effects of elevating luminal solution $[K^+]$ on voltages, resistances, and cell water volume. (*A*) Luminal solution $[K^+]$ was elevated from 2.5 to 83.5 mM (K^+ replaced Na^+) during the indicated interval. (*B*) Same experimental maneuver as in *A*, except 9 mM TEA^+ (TEA^+ replaced Na^+) was included in the high K^+ bathing solution. For additional details see Fig. 1.

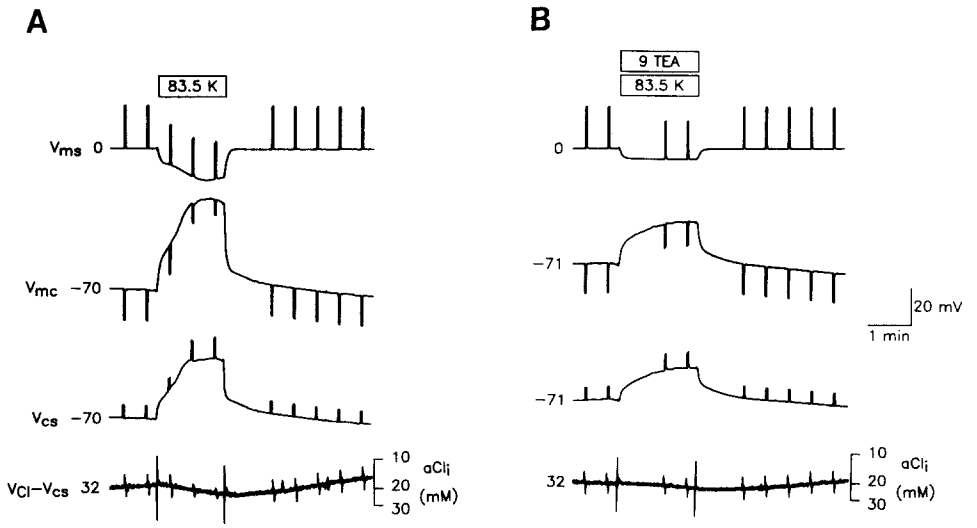

FIGURE 4. Effects of elevating luminal solution $[K^+]$ on voltages, resistances, and aCl_i^-. (*A*) Luminal solution $[K^+]$ was increased from 2.5 to 83.5 mM (K^+ replaced Na^+) during the indicated interval. (*B*) Same experimental maneuver as in *A*, except 9 mM TEA^+ (TEA^+ replaced Na^+) was included in the high K^+ bathing solution.

TABLE II

Effects of Elevating Luminal [K⁺] without or with TEA⁺ on Voltages, Resistances, aCl_i^-, and Cell Volume

Condition	V_{ms}	V_{mc}	R_a/R_b	aCl_i^-	$\Delta aCl_i^-/\Delta t$	Δ Cell water volume	Δ Volume/ Δt
	mV	*mV*		*mM*	*mM/min*	*%*	*%/min*
2.5 K	0.3 ± 0.5	−60 ± 3	5.62 ± 1.48	18.5 ± 1.0	—	—	—
83.5 K	−10.0 ± 1.3	−14 ± 3	2.25 ± 0.75	23.0 ± 1.0	4.3 ± 0.8*	−0.8 ± 1.4	−6.6 ± 5.4
Difference	−10.3 ± 1.3	46 ± 4*	−3.36 ± 1.38*	4.5 ± 0.8*	—	—	—
2.5 K	0.7 ± 0.6⁺	−61 ± 3	6.83 ± 1.44	18.9 ± 1.1	—	—	—
83.5 K+TEA	−4.9 ± 0.6	−34 ± 3	2.45 ± 0.39	20.7 ± 1.6	1.5 ± 0.6	1.1 ± 1.2	−0.6 ± 4.2
Difference	−5.5 ± 0.2	27 ± 4*	−4.39 ± 1.25*	1.8 ± 0.9	—	—	—

Values are mean ± SEM; n = 9 (voltages and resistance), n = 4 (aCl_i^-), and n = 5 (cell volume). Mucosal solution [K⁺] was increased from 2.5 to 83.5 mM for ~2 min (K⁺ replaced Na⁺). [TEA⁺] was 9.0 mM. Values in 83.5 K⁺ or 83.5 K⁺ + TEA⁺ were measured ~1.5 min after the ion substitution. *Significant change, $P < 0.05$. For further details, see Table I.

Effects of Low Cl^- or High K^+ Luminal Bathing Solutions on Electrical Properties, Cell Water Volume, and aCl_i^- of Stimulated Gallbladders

Tissues were initially exposed for 10 min to 3 mM mucosal theophylline and 2.5 μM forskolin in order to increase intracellular cAMP and activate the apical membrane Cl^- conductance. Throughout the remainder of the experimental protocol the apical

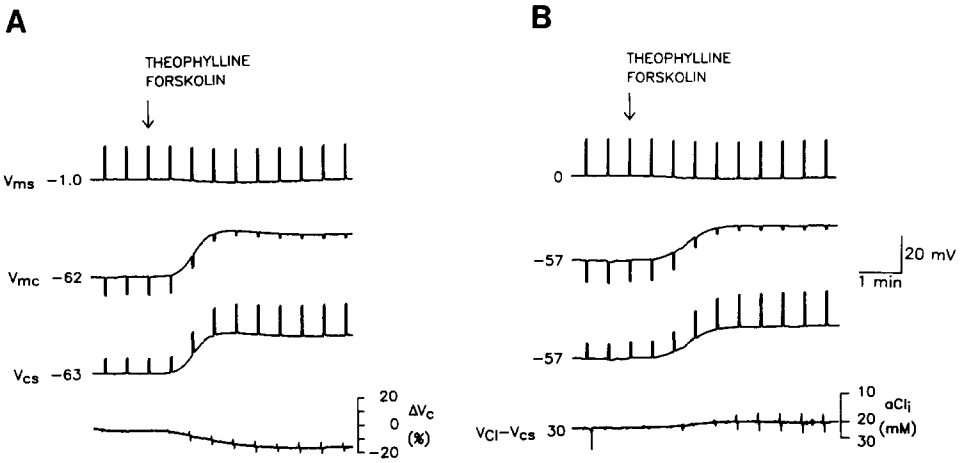

FIGURE 5. Effects of forskolin and theophylline on voltages, resistances, cell water volume, and aCl_i^-. At the indicated time the mucosal bathing solution was changed to one that contained forskolin (2.5 μM) and theophylline (3 mM). The serosal bathing solution was changed simultaneously to one that contained theophylline (3 mM). Due to the large dead volume of the serosal compartment, 3–5 min is required to fully exchange the serosal bathing solution. (*A*) In this tissue the change in cell water volume was recorded. (*B*) In another tissue the change in aCl_i^- was recorded.

TABLE III

Effects of Elevation of Intracellular cAMP on Voltages, Resistances, aCl_i^-, and Cell Water Volume

Condition	V_{ms}	V_{mc}	V_{cs}	R_a/R_b	R_t	aCl_i^-	Δ Cell water volume
	mV	*mV*	*mV*		*Ω · cm²*	*mM*	*%*
Control	−0.1 ± 0.4	−62 ± 3	−61 ± 3	7.50 ± 2.17	140 ± 16	20.3 ± 0.5	—
Stimulated	−2.6 ± 0.6	−42 ± 2	−44 ± 2	0.03 ± 0.01	141 ± 16	15.5 ± 1.2	—
Difference	−2.5 ± 0.3*	19 ± 2*	17 ± 2*	−7.48 ± 2.17*	1 ± 1	−4.8 ± 0.8*	−10.5 ± 1.7*

Values are mean ± SEM; n = 9 (voltages and resistances), n = 4 (aCl_i^-), and n = 5 (cell volume). aCl_i^- and Δ cell water volume were measured 3–5 min after onset of exposure to 3 mM theophylline (mucosal and serosal solutions) and 2.5 μM forskolin (mucosal solution) to elevate intracellular cAMP levels. *Significant change, $P < 0.05$.

Cl^- conductance was maintained by continued exposure to theophylline (3 mM, added to the serosal solution).

In contrast with the results obtained before stimulation, isomolar replacement of Cl^- with cyclamate induced a rapid, large, transient depolarization of V_{mc} and V_{cs} (Figs. 6*A* and 7*A*, Table IV). Furthermore, the resistance ratio increased and a lumen negative change in V_{ms} was observed. Nearly mirror-image changes were

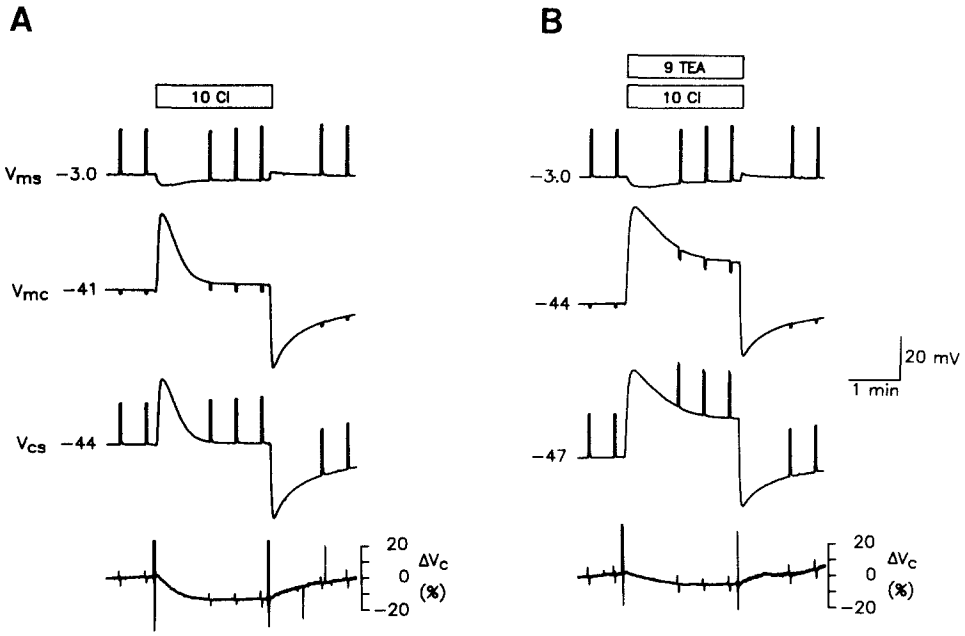

FIGURE 6. Effects of reducing luminal solution $[Cl^-]$ on voltages, resistances, and cell water volume during elevation of cAMP levels. (*A*) Luminal solution $[Cl^-]$ was reduced from 98 to 10 mM (replaced with cyclamate) during the indicated interval. (*B*) Same experimental maneuver as in *A*, except 9 mM TEA^+ (TEA^+ replaced Na^+) was included in the low Cl^- bathing solution. For additional details see Fig. 1.

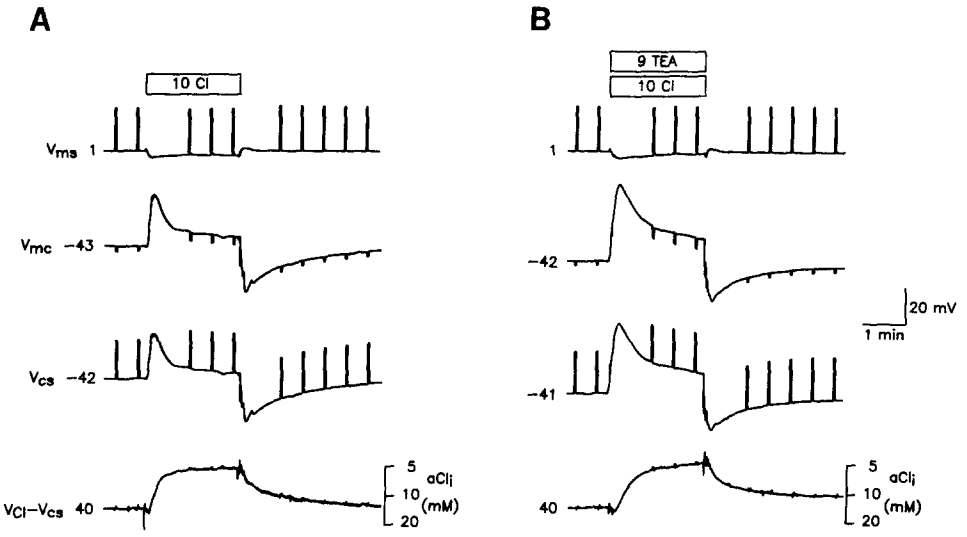

FIGURE 7. Effects of reducing luminal solution [Cl^-] on voltages, resistances, and aCl_i^- during exposure to theophylline. (*A*) Luminal solution [Cl^-] was reduced from 98 to 10 mM (replaced with cyclamate) during the indicated interval. (*B*) Same experimental maneuver as in *A*, except 9 mM TEA^+ (TEA^+ replaced Na^+) was included in the low Cl^- bathing solution.

observed upon return to control Ringer's solution. These responses have been previously attributed to the coexistence of the apical membrane Cl^- conductance induced by cAMP and the native K^+ conductances of the apical and basolateral cell membranes (Petersen and Reuss, 1983; see also Hodgkin and Horowicz, 1959). Concurrent with the voltage changes upon lowering [Cl^-], rapid reductions in cell water volume and aCl_i^- were observed (Figs. 6*A* and 7*A* and Table IV). The initial rate of cell shrinkage was 27.6 ± 5.4%/min and the cells were shrunken at steady state

TABLE IV

Effects of Reducing Luminal Solution [Cl^-] without or with TEA^+ on Voltages, Resistances, aCl_i^-, and Cell Volume in Theophylline-treated Tissues

Condition	V_{ms}	V_{mc}	R_a/R_b	aCl_i^-	$\Delta aCl_i^-/\Delta t$	Δ Cell water volume	Δ Volume/ Δt
	mV	*mV*		*mM*	*mM/min*	*%*	*%/min*
98 Cl	−1.0 ± 0.7	−43 ± 2	0.02 ± 0.01	14.9 ± 0.4	—	—	—
10 Cl	−4.8 ± 0.9	−35 ± 4	0.12 ± 0.02	7.1 ± 1.0	−30.8 ± 2.7*	−12.4 ± 1.0*	−27.6 ± 5.4*
Difference	−3.8 ± 0.4*	8 ± 3*	0.10 ± 0.02*	−7.8 ± 0.7*	—	—	—
98 Cl	−0.8 ± 0.6	−45 ± 2	0.01 ± 0.01	15.1 ± 0.6	—	—	—
10 Cl+TEA	−5.6 ± 1.1	−26 ± 6	0.19 ± 0.03	7.8 ± 1.3	−18.2 ± 1.6*	−8.3 ± 1.0*	−12.0 ± 1.2*
Difference	−4.8 ± 0.6*	19 ± 5*	0.18 ± 0.03*	−7.3 ± 0.8*	—	—	—

Values are mean ± SEM; n = 9 (voltages and resistances), n = 4 (aCl_i^-), and n = 5 (cell volume). Tissues were exposed initially to theophylline and forskolin and then to serosal theophylline (3 mM). For further details see Table I. [TEA^+] was 9.0 mM. *Significant change, $P < 0.05$.

(within 1 min) by 12.4 ± 1.0%. aCl_i^- fell from 15 ± 1 to 7 ± 1 mM with an initial rate of decline of 30.8 ± 2.7 mM/min. This rate is somewhat faster than previously reported, although the steady-state changes were similar (Reuss, 1987). Upon return to control Ringer's solution, both the cell water volume and the aCl_i^- returned to the values recorded before ion replacement.

The effects of simultaneous replacement of Cl^- and exposure to TEA^+ are shown in Figs. 6 *B* and 7 *B* and summarized in Table IV. The peak depolarization of V_{mc} was increased from 32 ± 2 to 42 ± 1 mV ($P < 0.001$) and the rate of repolarization was slowed dramatically with TEA^+. The extent of the repolarization during continued exposure to low [Cl^-] was also reduced by TEA^+ (i.e., at 1.5 min the cells remained depolarized). Furthermore, TEA^+ reduced the initial rate of decrease in cell water

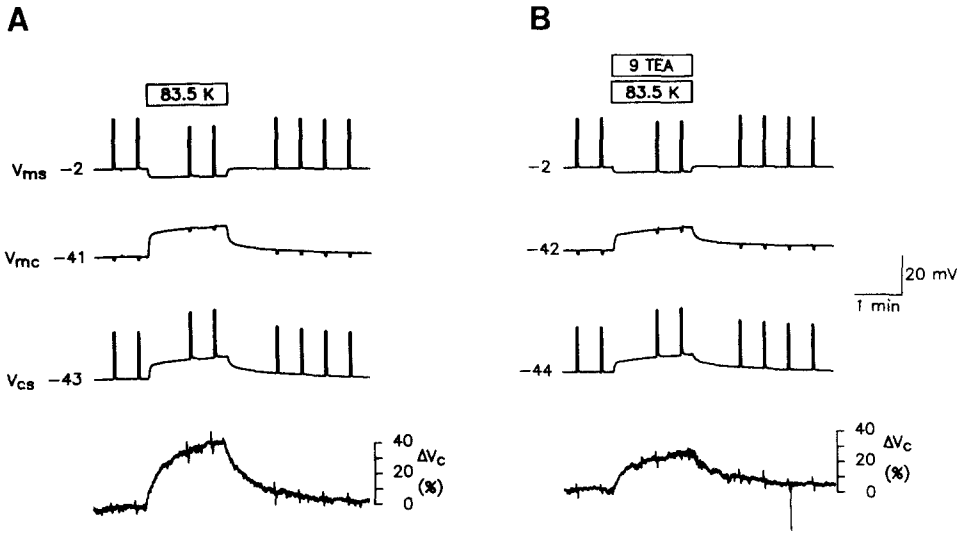

FIGURE 8. Effects of elevating luminal solution [K^+] on voltages, resistances, and cell water volume during elevation of cAMP levels. (*A*) Luminal solution [K^+] was elevated from 2.5 to 83.5 mM (K^+ replaced Na^+) during the indicated interval. (*B*) Same experimental maneuver as in *A*, except 9 mM TEA^+ (TEA^+ replaced Na^+) was included in the high K^+ bathing solution.

volume by ~60%. The steady-state shrinkage was also reduced (from 12.4 ± 1.0 to 8.3 ± 1.0%, $n = 5$, $P < 0.05$). The initial rate of fall in aCl_i^- upon reduction of luminal solution [Cl^-] was reduced by TEA^+ from 30.8 ± 2.7 to 18.2 ± 1.5 mM/min ($n = 4$, $P < 0.05$; see Table IV). The steady-state value of aCl_i^- during exposure to low luminal [Cl^-] was not altered by TEA^+ (Table IV).

The response to elevation of luminal solution [K^+] was also different in stimulated tissues (compare Figs. 3 and 4 with 8 and 9). All voltage changes were reduced in stimulated gallbladders compared with unstimulated tissues, as expected from the large apical membrane Cl^- conductance elicited by cAMP. Elevating luminal solution [K^+] in stimulated tissues caused cell swelling at an initial rate of 88.2 ± 26.4%/min (see Fig. 8 *A*). After 1.5 min, cells were swollen by 17.6 ± 3.7% (Table V). The period

TABLE V

Effects of Elevating Luminal [K⁺] without or with TEA⁺ on Voltages, Resistances, aCl_i^-, and Cell Volume in Theophylline-treated Tissues

Condition	V_{ms}	V_{mc}	R_a/R_b	aCl_i^-	$\Delta aCl_i^-/\Delta t$	Δ Cell water volume	Δ Volume/ Δt
	mV	*mV*		*mM*	*mM/min*	%	*%/min*
2.5 K	−0.8 ± 0.5	−46 ± 2	0.03 ± 0.01	15.4 ± 1.7	—	—	—
83.5 K	−6.3 ± 0.7	−35 ± 4	0.01 ± 0.00	20.9 ± 1.7	2.2 ± 0.4*	17.6 ± 3.7*	88.2 ± 26.4*
Difference	−5.6 ± 0.4*	11 ± 4*	−0.02 ± 0.01*	5.5 ± 1.5*	—	—	—
2.5 K	−0.8 ± 0.0	−45 ± 3	0.04 ± 0.02	16.7 ± 2.2	—	—	—
83.5 K+TEA	−4.4 ± 0.6	−40 ± 2	0.03 ± 0.02	17.7 ± 2.2	1.0 ± 0.4	9.1 ± 2.8*	33.6 ± 10.2*
Difference	−3.7 ± 0.3*	5 ± 1*	0.01 ± 0.01	1.0 ± 0.4	—	—	—

Values are mean ± SEM; $n = 9$ (voltages and resistances), $n = 4$ (aCl_i^-), and $n = 5$ (cell volume). The period of exposure to 83.5 K^+ or to 83.5 K^+ + TEA^+ was 1.5 min. The measurements were taken after ~1.0 min of exposure. For further details see Table I. *Significant change, $P < 0.05$.

of exposure to high K^+ bathing solution was limited to 1.5 min because prolonged exposure to high K^+ solution led to continued swelling (>25% in 3 min) from which the cells recovered slowly. If the period of exposure to high K^+ was limited to 1.5 min, membrane voltages and cell volume fully recovered within 5 min after returning to the control Ringer's solution.

In stimulated tissues, the changes in aCl_i^- upon elevation of luminal solution [K^+] were small and variable. The initial rate of change in aCl_i^- was only 2.2 ± 0.4 mM/min ($n = 4$, $P < 0.01$). At the end of the 1.5-min exposure to elevated [K^+], aCl_i^- had increased from 15.4 ± 1.7 to 20.9 ± 1.7 mM ($n = 4$, $P < 0.05$). An example of the

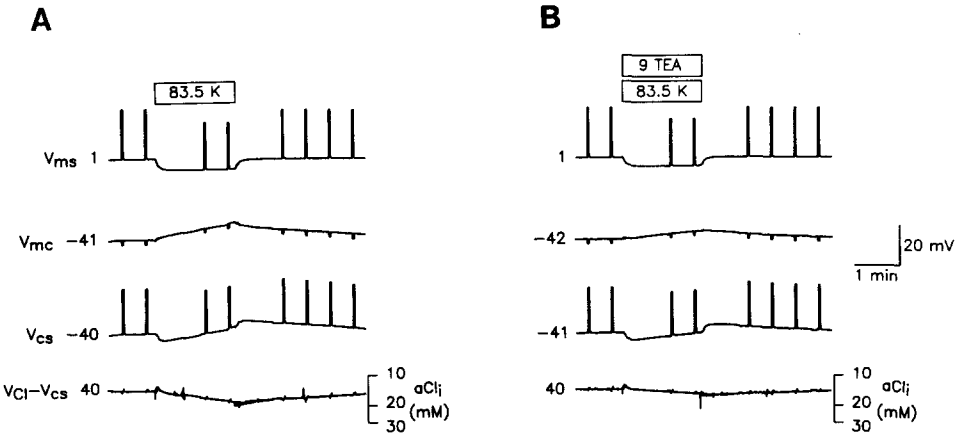

FIGURE 9. Effects of elevating luminal solution [K^+] on voltages, resistances, and aCl_i^- during exposure to theophylline. (*A*) Luminal solution [K^+] was increased from 2.5 to 83.5 mM (K^+ replaced Na^+) during the indicated interval. (*B*) Same experimental maneuver as in *A*, except 9 mM TEA^+ (TEA^+ replaced Na^+) was included in the high K^+ bathing solution.

effect of elevation of mucosal solution $[K^+]$ on aCl_i^- in a stimulated tissue is shown in Fig. 9 *A*. The results are summarized in Table V.

Also in stimulated tissues, elevating luminal solution $[K^+]$ concomitantly with addition of TEA^+ resulted in a small depolarization of V_{mc} and V_{cs} and a ~60% decrease in the initial rate of cell swelling, compared with the same ion substitution without TEA^+ (Fig. 8, *A* and *B*, and Table V). Cell swelling after 1.5 min of exposure to high K^+ was reduced by 40% by TEA^+. No change in aCl_i^- was detected when mucosal solution $[K^+]$ was elevated in the presence of TEA^+ (Fig. 9, *A* and *B*, and Table V). Exposure to TEA^+ in the absence of changes in luminal solution $[K^+]$ or $[Cl^-]$ had no effect on cell water volume in stimulated tissues (data not shown).

DISCUSSION

We have combined measurements of changes in aCl_i^- and cell water volume to examine apical membrane transport properties of *Necturus* gallbladder epithelium before and after elevation of intracellular cAMP levels. Although K^+ represents the major intracellular cation, we did not measure aK_i^+ because the control value of aK_i^+ is high (~90 mM) and therefore the small changes expected in these experiments would be difficult to measure. Three mechanisms have been proposed to account for NaCl entry across the apical cell membrane of *Necturus* gallbladder epithelium: namely, NaCl cotransport, $NaKCl_2$ cotransport, and parallel Na^+/H^+ and Cl^-/HCO_3^- exchanges. In the experiments reported here we reduced luminal solution $[Cl^-]$ and demonstrated that aCl_i^- falls without a concomitant reduction in cell water volume. Furthermore, elevation of mucosal solution $[K^+]$ caused a small increase in aCl_i^- with no change in cell water volume. As explained below, the observation of changes in aCl_i^- without concomitant changes in cell volume rules out cotransport (of NaCl or $NaKCl_2$) as the main mechanism for apical membrane NaCl entry. After elevation of intracellular cAMP levels, reducing luminal solution $[Cl^-]$ or elevating luminal solution $[K^+]$ caused rapid and large shrinkage or swelling of the cells, respectively. These volume changes result from net fluxes of K^+ and Cl^-, largely via parallel apical membrane conductances.

Isosmotic Luminal Ion Replacements in Unstimulated Tissues

A large number of electrophysiological experiments have provided evidence that apical membrane Cl^- entry is via Cl^-/HCO_3^- exchange in *Necturus* gallbladder epithelium (Reuss, 1984; Reuss and Costantin, 1984). Contrary evidence has been presented by Spring and co-workers (Spring and Hope, 1979; Ericson and Spring, 1982; Larson and Spring, 1983) and by Davis and Finn (1985) and is based primarily on changes in cell volume measured optically. Ericson and Spring (1982) concluded that salt entry is via NaCl cotransport, whereas Davis and Finn (1985) concluded that salt entry across the apical cell membrane is via both cotransport (NaCl or $NaKCl_2$) and parallel exchange (Na^+/H^+ and Cl^-/HCO_3^-).

If transapical membrane Cl^- influx is electroneutral because it is by cotransport with one or more cations (rather than by anion exchange), the change in cell volume during the reduction in luminal solution $[Cl^-]$ can be estimated from the fall in

aCl_i^- according to the following equation (Giraldez, 1984; Alvarez-Leefmans et al., 1988):

$$\frac{1}{v}\frac{dv}{dt} = \frac{\sum_j^n z_j(da_j^i/dt)}{\sum_j^n z_j a_j^i} \tag{2}$$

where v represents the initial cell water volume, dv/dt is the rate of change in cell water volume, j denotes the jth ion, z is the valence, a_j^i is the initial intracellular activity, and da_j^i/dt is the rate of change in intracellular activity.

If we assume that (*a*) transapical membrane Cl^- flux is via NaCl or $NaKCl_2$ cotransport, (*b*) Na^+ and K^+ represent the total extracellular and intracellular cations and together with the accompanying anions account for most of the osmolality of both compartments, and (*c*) the transported salt is accompanied in isosmotic proportions by water, then the sum of $aNa_i^+ + aK_i^+$ must be constant, and therefore, $daNa_i^+/dt + daK_i^+/dt = 0$ and Eq. 2 simplifies to:

$$\frac{1}{v}\frac{dv}{dt} = \frac{daCl_i^-/dt}{aNa_i^+ + aK_i^+ - aCl_i^-} \tag{3}$$

From the initial rate of fall of aCl_i^- (17.4 mM/min) upon lowering mucosal solution $[Cl^-]$ from 98 to 10 mM, and the values of aNa_i^+ (11 mM), aK_i^+ (95 mM), and aCl_i^- (20 mM) under control conditions, we calculate with Eq. 3 that dv/dt should be ~20%/min. However, there was no significant change in cell water volume in spite of the large fall in aCl_i^- (Table I). For the above calculation we assume that the salt flux is by cotransport and accompanied by water in isosmotic proportions. The latter is reasonable to assume in light of the high osmotic water permeability of both cell membranes, i.e., ~0.05 cm/s (Persson and Spring, 1982; Cotton et al., 1989). Clearly, the fall in aCl_i^- is electroneutral since the concomitant changes in membrane voltages are slow and small. The Cl^- loss is ultimately due to a change in the Cl^- flux across the apical membrane, which is via cotransport (e.g., NaCl, $NaKCl_2$) or exchange (e.g., Cl^-/HCO_3^-). If Cl^- is cotransported then the fall in aCl_i^- must be accompanied by a fall in aNa_i^+ or aK_i^+ and thus a decrease in cell water volume. However, if Cl^- enters the cells by anion exchange, then the fall in aCl_i^- will be accompanied by an increase in $aHCO_{3i}^-$ and no change in cell volume. Reuss (1987) found that when mucosal $[Cl^-]$ was reduced to 10 mM the calculated net efflux of Cl^- was equal to the calculated net influx of HCO_3^-. If the osmotic and reflection coefficients for Cl^- and HCO_3^- are similar, the steady-state osmotic difference will be preserved and cell water volume will not change. Since $aHCO_{3i}^-$ is partially buffered, the situation is somewhat more complex.

The dominant electrodiffusive permeability of the apical membrane of the unstimulated *Necturus* gallbladder epithelium is for K^+. Intracellular microelectrode (Stoddard and Reuss, 1988*b*) and patch-clamp studies (Segal and Reuss, 1990*a*, *b*) suggest the existence of at least two types of apical K^+ conductances, one of which is a maxi K^+ channel gated by membrane depolarization and by elevation of $[Ca^{2+}]_i$. This pathway can be almost totally blocked by TEA^+. As stated in the Results, TEA^+ has a very small depolarizing effect on the apical membrane under control conditions, but

its effect is sizeable in high K^+ medium (Figs. 3 and 4). This difference is attributable to activation of apical membrane maxi K^+ channels by the depolarization elicited by elevating external [K^+] (Segal and Reuss, 1990*a*).

Elevation of luminal solution [K^+] from 2.5 to 83.5 mM would be expected to favor net K^+ influx across the apical membrane, and should thereby promote cell swelling. However, no cell swelling was observed (Fig. 3 *A* and Table III). The most likely explanation for the lack of change in cell volume is the basolateral membrane depolarization. Calculations from the control value of aK_i^+ (Reuss and Stoddard, 1987), the changes in aK_o^+, and measured membrane voltages (Table II) indicate that the changes in K^+ electrodiffusive driving forces across the cell membranes are similar in magnitude. Therefore, net K^+ influx across the apical cell membrane (because of the change in E_K) may be balanced by net K^+ efflux across the basolateral membrane (because of the change in V_{cs}).

The small increase in aCl_i^- during the elevation of luminal solution [K^+] is expected based on the recent demonstration of a significant basolateral membrane Cl^- conductance in *Necturus* gallbladder when bathed in a HCO_3^-/CO_2 buffered solution (Stoddard and Reuss, 1988*a*, 1989*a*, *b*). We can estimate from Eq. 4 the change in net Cl^- flux because of the fall of V_{cs}:

$$I^{Cl} = \Delta V_{cs} \cdot G_b^{Cl} \tag{4}$$

where I^{Cl} = Cl^- current, ΔV_{cs} = change in V_{cs} induced by elevation of mucosal [K^+] (35.6 mV), and G_b^{Cl} = basolateral Cl^- conductance (0.5 mS/cm^2). This calculation yields I^{Cl} = 17.8 μA/cm^2. For a cell water volume of 2.87 μl/cm^2 of epithelium, $da Cl_i/dt$ is ~3 mM/min, remarkably close to the measured rate (4.3 mM/min; Table II). Consistent with the above interpretation is the observation that TEA^+ blocked the change in aCl_i^-, an effect that we attribute to the reduced depolarization of V_{cs} (Table II).

The results of our experiments with elevation of mucosal solution [K^+] can be compared with results obtained by other investigators, also in *Necturus* gallbladder. Giraldez (1984) found that aNa_i^+ fell and aK_i^+ and aCl_i^+ increased, and from these changes he calculated (from Eq. 2 above) that cell volume should increase. Using optical techniques, Hermansson and Spring (1986) found that increasing mucosal or serosal solution [K^+] to 25 mM had no effect on cell volume, whereas elevation of mucosal solution [K^+] to 88 mM caused cell shrinkage. Davis and Finn (1988), also using optical techniques, reported that elevation of mucosal solution [K^+] to 95 mM caused cell swelling. In spite of the differences in experimental design among these groups, it is difficult to reconcile the results. The period of exposure to high K^+ luminal solution in the experiments of Hermansson and Spring (1986) and Davis and Finn (1988) ranged from 15 to 25 min, whereas we limited the period of exposure to <2 min. This may account, at least in part, for the different results.

Stimulation with Forskolin and Theophylline

Agents that increase intracellular [cAMP] inhibit net salt and water absorption in a number of leaky epithelia. Electrophysiologic studies indicate that a major effect of agents that increase cAMP in *Necturus* gallbladder epithelium is the activation of an apical membrane Cl^- conductance (Petersen and Reuss, 1983). Patch-clamp studies

have revealed the existence of a Cl^- channel in dissociated gallbladder cells stimulated by cAMP (Segal and Reuss, 1989). In addition, elevation of [cAMP] in *Necturus* gallbladder epithelium inhibits both apical membrane Na^+/H^+ and Cl^-/HCO_3^- exchange by ~50% (Reuss and Petersen, 1985; Reuss, 1987).

Under control conditions, aCl_i^- is maintained above electrochemical equilibrium (Reuss and Weinman, 1979; Reuss and Costantin, 1984) by operation of the Cl^-/HCO_3^- exchanger of the apical membrane. Upon elevation of cAMP, apical membrane P_{Cl} increases and aCl_i^- falls to values near equilibrium across the apical membrane (Petersen and Reuss, 1983). If the fall in aCl_i^- occurs *pari passu* with net cation efflux, the expected rate of cell shrinkage (Eq. 3) is 3.6 ± 1.1%/min (n = 4). The observed rate was 5.4 ± 1.8%/min (n = 5), statistically not different from the expected one. At the steady state (3 min) we predict that the cells should shrink by 6.3 ± 1.0%, but we observed a 10.5 ± 1.7% decrease in cell water volume ($P < 0.05$). The reason for this difference is unknown. It is likely that the cell shrinkage upon increases in intracellular cAMP levels is due in large part to net effluxes of Cl^- and a cation, probably K^+, since it is the most abundant one and its electrodiffusive permeability is high across both cell membranes.

Isosmotic Luminal Ion Replacements in Stimulated Tissues

In tissues with high intracellular cAMP levels, lowering luminal solution [Cl^-] caused cell shrinkage, whereas increasing luminal solution [K^+] produced cell swelling. These results could be explained by fluxes via parallel conductive pathways (Cl^- and K^+ channels) or via KCl cotransport. When TEA^+ was added simultaneously with the change in [Cl^-] or [K^+], the volume changes were reduced by ~60–70% (Tables IV and V), suggesting that net fluxes of Cl^- and K^+ are via apical membrane channels. This conclusion is supported by the following observations: (*a*) TEA^+ enhanced the peak depolarization of V_{mc} elicited by lowering luminal solution [Cl^-] because of both greater relative P_{Cl} permeability and slower fall in aCl_i^-. (*b*) The rate of repolarization of V_{mc} during exposure to low Cl^- solution was slowed by TEA^+, because K^+ efflux (inhibited by TEA^+) becomes rate limiting, decreasing the rate of Cl^- efflux and thus the repolarization. (*c*) The increase in R_a/R_b in low Cl^- solution was enhanced by TEA^+, i.e., G_a^{Cl} decreases because $[Cl^-]_o$ is reduced and G_a^K decreases because TEA^+ blocks apical K^+ channels. The depolarization of V_{mc} would be expected to activate apical membrane voltage- and Ca^{2+}-gated maxi K^+ channels that are normally inactive at resting membrane voltages.

From Eq. 3, the estimated rate of cell shrinkage is 29.0 ± 2.5%/min, close to the observed value of 27.6 ± 5.4%/min. The agreement is good because cAMP stimulates the Cl^- conductance and inhibits Cl^-/HCO_3^- exchange. Conductive Cl^- efflux is coupled, via the changes in membrane voltage, to K^+ efflux. With low [Cl^-] plus TEA^+ we predict initial cell shrinkage at a rate of 19.7 ± 1.4%/min compared with the observed value of 12.0 ± 1.2%/min. The discrepancy is explained by the greater fractional contribution of Cl^-/HCO_3^- exchange to total Cl^- efflux: conductive Cl^- efflux is reduced by TEA^+, whereas Cl^-/HCO_3^- exchange is unchanged (see below for calculation of apical Cl^- fluxes via conductive and exchange pathways).

Elevation of luminal solution [K^+] in tissues with high cAMP levels led to a large, rapid increase in cell water volume. Because of the high P_{Cl}, raising [K^+] produced

only a small depolarization of V_{mc} and V_{cs}. Consequently, there was a large change in apical $\Delta\tilde{\mu}K^+/F$ favoring K^+ entry, and almost no change in basolateral $\Delta\tilde{\mu}K^+/F$. Net Cl^- influx is probably driven by the small K^+-induced depolarization. The apical membrane G^{Cl} under these conditions is likely to be high, since R_a/R_b is low (~ 0.01) and the relative apical membrane P_{Cl} is high (Petersen and Reuss, 1983).

We were unable to accurately measure the initial rate of increase in aCl_i^- upon elevation of mucosal solution $[K^+]$ because of the long time constant of the Cl^- electrodes. If cell swelling is due to isosmotic KCl influx, then intracellular Cl^- content would be expected to increase from ~ 60 to ~ 110 nmol/cm^2 epithelium. However, we measured an increase to only ~ 90 nmol/cm^2 after ~ 1.5 min. It is likely that a portion of the increase in intracellular Cl^- is "shunted" by exchange for HCO_3^- across the apical membrane.

cAMP Inhibition of Cl^-/HCO_3^- Exchange

The rates of Na^+/H^+ and Cl^-/HCO_3^- exchange are inhibited $\sim 50\%$ by maximal concentrations of cAMP (Reuss and Petersen, 1985; Reuss, 1987). If the cell shrinkage induced by the reduction in luminal solution $[Cl^-]$ is due to isosmotic loss of Cl^- plus a cation via conductive pathways, then we can calculate the contributions of electrodiffusion and Cl^-/HCO_3^- exchange to the fall in aCl_i^-. From Eq. 3 we calculate that the net flux of Cl^- through the conductive pathway was 1.1 nmol/cm^2·s (for a cell volume of 2.58 μl/cm^2). Furthermore, we can calculate total net flux (M_j) from Eq. 5:

$$M_j = \frac{1}{\gamma}\left(\frac{da_i^j}{dt}v + a_j^i\frac{dv}{dt}\right) \tag{5}$$

where γ is the activity coefficient. The calculated total net efflux was 1.3 nmol/cm^2·s. The difference, 0.2 nmol/cm^2·s, represents the rate of Cl^-/HCO_3^- exchange in stimulated tissues. Since the only pathway in unstimulated tissues is Cl^-/HCO_3^- exchange, we conclude that elevation of [cAMP] inhibits Cl^-/HCO_3^- exchange by $\sim 70\%$ (0.83 nmol/cm^2·s vs. 0.24 nmol/cm^2·s) in *Necturus* gallbladder epithelium.

Conclusions

We have used measurements of aCl_i^- and changes in cell water volume to study the effects of isosmotic mucosal solution ion substitutions in control and cAMP-stimulated gallbladders. In the absence of cAMP stimulation, we find that reduction of mucosal solution $[Cl^-]$ induces net Cl^- efflux with no change in cell volume, a result consistent with apical membrane Cl^-/HCO_3^- exchange but not with NaCl or $NaKCl_2$ cotransport. In contrast, in tissues with elevated intracellular cAMP levels reductions of mucosal solution $[Cl^-]$ produced cell shrinkage and elevations of mucosal solution $[K^+]$ induced cell swelling. Net movements of Cl^- and K^+ under these conditions are predominantly via cAMP-activated apical membrane Cl^- channels and TEA^+-sensitive apical membrane K^+ channels.

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