Cell Swelling Activates the K⁺ Conductance and Inhibits the Cl⁻ Conductance of the Basolateral Membrane of Cells from a Leaky Epithelium

Ruben J. Torres, Muthangi Subramanyam, Guillermo A. Altenberg, and Luis Reuss

From the Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77555-0641

Abstract: Necturus gallbladder epithelial cells bathed in 10 mM HCO₃⁻/1% CO₂ display sizable basolateral membrane conductances for Cl⁻ (G_{Clb}^b) and K⁺ (G_{Kb}^b). Lowering the osmolality of the apical bathing solution hyperpolarized both apical and basolateral membranes and increased the K⁺/Cl⁻ selectivity of the basolateral membrane. Hypersmotic solutions had the opposite effects. Intracellular free-calcium concentration ([Ca²⁺]ᵢ) increased transiently during hyposmotic swelling (peak at ~90 s, return to baseline within ~90 s), but chelation of cell Ca²⁺ did not prevent the membrane hyperpolarization elicited by the hypsomotic solution. Cable analysis experiments showed that the electrical resistance of the basolateral membrane decreased during hypsomotic swelling and increased during hypersomotic shrinkage, whereas the apical membrane resistance was unchanged in hypsomotic solution and decreased in hypersomotic solution. We assessed changes in cell volume in the epithelium by measuring changes in the intracellular concentration of an impermeant cation (tetramethylammonium), and in isolated polarized cells measuring changes in intracellular calcine fluorescence, and observed that these epithelial cells do not undergo measurable volume regulation over 10–12 min after osmotic swelling. Depolarization of the basolateral membrane voltage (V_{α}) produced a significant increase in the change in V_{α} elicited by lowering basolateral solution [Cl⁻], whereas hyperpolarization of V_{α} had the opposite effect. These results suggest that: (a) Hyposmotic swelling increases G_{Kb} and decreases G_{Clb}. These two effects appear to be linked, i.e., the increase in G_{Kb} induces membrane hyperpolarization, which in turn reduces G_{Clb} (b) Hypersomotic shrinkage has the opposite effects on G_{Kb} and G_{Clb}. (c) Cell swelling causes a transient increase in [Ca²⁺]ᵢ, but this response may not be necessary for the increase in G_{Kb} during cell swelling.

Key words: transport • channel • gallbladder • cross talk • calcium

Introduction:
The Necturus gallbladder (NGB) epithelium is a useful model system for functional studies of a family of epithelia that perform near-isosmotic fluid absorption. The mechanisms accounting for ion and water transport in NGB epithelium under control conditions have been characterized in detail and considerable information is also available on regulation of transport (for reviews, see Reuss, 1988; Reuss and Altenberg, 1995). An issue requiring further study is the regulation of basolateral membrane Cl⁻ and K⁺ channels, especially its relationship to the mechanism of cross talk between apical and basolateral membranes, in which cell volume changes could play a major role (Schultz and Hudson, 1991). For instance, we have proposed that elevation of cAMP causes a reduction in Cl⁻ efflux across the basolateral membrane. This is based on the effects of cAMP on fluid transport (Petersen and Reuss, 1983), intracellular ion contents, and cell volume (reviewed in Reuss and Altenberg, 1995; Reuss et al., 1991). Further, exposure to cAMP reduces basolateral membrane conductance (Copello et al., 1993). We suspected that this may include a decrease in basolateral membrane G_{Cl} (G_{Clb}) brought about by the cell shrinkage produced by cAMP. In other words, the hypothesis is that the changes in apical membrane conductances elicited by cAMP result in a loss of cell KCl, which in turn causes cell shrinkage, and that the shrinkage results in a decrease in basolateral membrane G_{Cl}. Accordingly, we assessed the effects of cell volume and membrane voltage on the Cl⁻ and K⁺ conductances of the basolateral membrane. Specifically, we investigated the rapid effects of cell swelling and shrinkage on these conductances, in order to test the hypothesis that cell volume changes are involved in cross talk between apical and basolateral membranes. Inasmuch as G_{Clb} is small relative to the basolateral K⁺ conductance (G_{Kb}), in addi-
tion to osmotic-shrinkage we carried out osmotic-swelling experiments. If cell swelling increases GbCl, then swelling experiments would yield relative changes in GbCl easier to measure than those elicited by cell shrinkage. In contrast with the apical membrane pathway (Copello et al., 1993; Heming et al., 1994), the GbCl appears to be cAMP independent (Copello et al., 1993) and is activated by HCO3−/CO2 (Stoddard and Reuss, 1988a). The results of these studies demonstrate that GbCl is increased by cell shrinkage and decreased by cell swelling, probably via changes in cell membrane voltage (depolarization during cell shrinkage, hyperpolarization during cell swelling). The basolateral K+ conductance (GbK) is apparently voltage insensitive (Stoddard and Reuss, 1988b), and possibly activated by elevations in internal Ca2+ (Bello-Reuss et al., 1981). Here, we demonstrate that the K+ conductance is stimulated by cell swelling and inhibited by cell shrinkage.

**METHODS**

**General**

Mudpuppies (Necturus maculosus) were purchased from Kon’s Scientific (Germantown, WI) or Nasco Biologicals (Ft. Atkinson, WI) and maintained at 5–10°C. Anesthesia was accomplished by immersion in a 1 g/liter tricaine methanesulfonate solution. The gallbladder was excised, opened, washed, and mounted in a modified Ussing chamber (Altenberg et al., 1990). When the gallbladders were mounted serosal side up, a patch of connective tissue was removed by dissection, to allow for microelectrode impalements through the basolateral membrane (Altenberg et al., 1990). Some experiments were carried out with polarized epithelial cells isolated from the intact tissue (“figure-eight cells”), using a method previously described (Torres et al., 1996). The control bathing solution (bPSS) contained, in mM: 90 NaCl, 10 NaHCO3, 2.5 KCl, 1.8 CaCl2, 1.0 MgCl2, and 0.5 NaH2PO4, gassed with 1% CO2/99% air, pH 7.66, at room temperature, with an average osmolality of 207 mosmol/kg. A low-NaCl but isomotic solution was prepared by replacing 33 mM NaCl with 70 mM sucrose. This solution was made hypsomotic (by ~17 or 34%) by removing sucrose, or hyperosmotic (also by ~17 or 34%) by adding a further 35 or 70 mM sucrose. Changes in bathing solution osmolality were made only in the apical bathing medium, inasmuch as reductions in basolateral solution [NaCl], with or without changes in osmolality, elicited long-lasting oscillations in membrane voltage and conductance. To assess cell membrane ionic selectivity, Cl− was replaced with gluconate, and Na+ was replaced with K+. These replacements were isomolar.

**Electrophysiological Techniques**

Transepithelial voltage (Vm, referenced to the basolateral bathing solution; Vma, referenced to the apical bathing solution) and cell membrane voltages (apical = Vma, basolateral = Vmb, referenced to the respective adjacent solutions) were measured as previously described (Altenberg et al., 1990). The ground electrode was an Ag-AgCl pellet separated from the apical bathing solution by a short Ringer-agar bridge. The basolateral bathing solution electrode was a flowing, saturated-KCl bridge in series with a calomel half-cell. Hence, liquid junction potentials upon changes in the basolateral solution were minimized. The transepithelial resistance, R, (ΔVm/I, where ΔVm is the change in Vm elicited by a constant current pulse of density I), and the apparent ratio of cell membrane resistances (Ra/Rb, where the subscripts a and b denote apical and basolateral membranes, respectively) were determined from the steady-state voltage deflections elicited by DC pulses of 50 μA/cm2 and 1 s duration, applied across the tissue through Ag-AgCl electrodes. The voltage deflections were corrected for series resistances. The absolute values of Ra and Rb were estimated from two-point cable analysis, as previously described (Petersen and Reuss, 1985; Copello et al., 1993). Intracellular-microelectrode studies were also carried out on isolated polarized cells attached with Cell-Tak® (Collaborative Medical Products, Bedford, MA) to a dialysis membrane mounted in the microelectrode chamber (Torres et al., 1996b).

Intracellular Cl− activity (aCl) and tetramethylammonium (TMA+) activity (aTMA) were measured with double-barrel ion-sensitive microelectrodes constructed and calibrated as previously described (Altenberg et al., 1990). Validation of impalements was as described before (Altenberg et al., 1990). To measure changes in cell water volume, epithelia were loaded with TMA+ using transient exposure to mystatin, and the intracellular activity of TMA+ was determined with double-barrel, TMA+-sensitive microelectrodes (Reuss, 1985; Altenberg et al., 1990).

**Fluorescence Techniques**

Changes in cell water volume were assessed in isolated polarized cells attached to a coverslip coated with Cell-Tak®, loaded with calcine-AM (3 μM, for 20 min) and then superfused with isomotic solution for 15 min before starting the experiment. Calcine is a good choice among fluorescent indicators to assess changes in cell water volume because it undergoes less photobleaching than other probes and is retained better in the cells (Altenberg et al., 1994; Alvarez-Leefmans et al., 1995; Crowe et al., 1995). The experiments were performed using a digital video confocal laser imaging system (Odyssey; Noran Instruments, Middleton, WI). Excitation light was 488 nm, and emitted light was measured at wavelengths longer than 515 nm. Pictures were obtained at 30-s intervals, and fluorescence of a 15–20 μm² area in the center of a cell was measured. The records were corrected for fluorescence decay independent of cell volume changes (primarily due to dye photobleaching), which was fit by a single exponential. The data are presented as Fo/Ft, where Fo = fluorescence in isomotic solution, at t = 0, and Ft = corrected fluorescence at time = t. The ratio Fo/Ft is proportional to cell volume and was ~75% of the ideal (“osmometric”) responses after exposure to hypo or hyperosmotic solutions.

Intracellular free [Ca2+] ([Ca2+]i) was estimated from the 340/380 nm Fura-2 fluorescence ratio (F340/380) in isolated polarized cells. Isolated polarized cells (Torres et al., 1996b) were loaded at room temperature with Fura-2,AM (5 μM for 1 h). The cells were then attached to a coverslip coated with Cell-Tak®, mounted in a chamber and superfused with isomotic solution for ~15 min before starting the experiment. Measurements were carried out essentially as described (Altenberg et al., 1994). Data were acquired at 1 Hz. At the end of each experiment, cells were exposed to 10–20 μM ionomycin to obtain saturating free [Ca2+]i, and then to 1–2 mM MnCl2 in the continuous presence of ionomycin, to quench the dye and thus correct for background fluorescence. Data are presented as F540/380 after background correction. In some experiments, cells were loaded with acetoxymethyl ester of 1,2-bis(2-amino-phenoxo)ethane-N,N,N′,N′′-tetraacetic acid (BAPTA,AM), Half-BAPTA,AM, or N,N,N′,N′′-tetrakis(2-pyridylmethyl) ethylendiamine (TPEN), a heavy-metal chelator (Kao, 1994). All of these chemicals were obtained from Molecular Probes (Eugene, OR).
Statistical Analysis

Results are given as means ± SEM. Statistical comparisons were done by t tests for paired or unpaired data, as appropriate. A value of \( P < 0.05 \) was considered significant.

RESULTS

Effects of Changes in Apical Bathing Solution Osmolality on Voltages and Resistances

The effects of lowering the apical solution osmolality (from 200 to 135 mosmol/kg) on the basic electrophysiological properties of the epithelium are illustrated in Fig. 1. In this experiment, a cell was impaled from the apical side with a conventional microelectrode. Voltages and resistances were recorded before, during, and after exposure to hyposmotic solution on the apical side. Reducing bathing solution osmolality caused a hyperpolarization of \( V_{cs} \) and an increase in \( R_a/R_b \). Both effects were reversible. Table I summarizes the results of experiments such as the one shown in Fig. 1. The hyperpolarization at 3 min was \( 8.6 \) mV, and the repolarization was complete after 4 min of perfusion with isosmotic solution.

The experiment shown in Fig. 2 illustrates the effects of exposure to a hyposmotic solution (the apical solution osmolality was increased from 200 to 270 mosmol/kg), and Table II summarizes the results with this experimental protocol. The main effects of hyposmotic solution were cell membrane depolarization and a decrease in \( R_a/R_b \), i.e., opposite changes to those elicited by hypsomotic solution. As in the experiments with hypsomotic solution, the effects were reversible. The effect of anisosmotic solutions on \( V_{ms} \) was small, but significant, apical side negative with hypsomotic solution and opposite in polarity with hypsomotic solution. These changes can be explained by paracellular pseudo-streaming potentials caused by water flow from apical to basolateral solution with hypsomotic solution and from basolateral to apical solution with hypsomotic solution. These voltage changes, because of their small magnitude, do not contribute significantly to the changes in cell membrane voltages (Reuss et al., 1992a, b). There were also significant changes in \( R_s \); a decrease with hypsomotic solution and an increase with hypsomotic solution. These changes are in the directions expected for widening and narrowing of lateral-intercellular spaces, respectively (Reuss et al., 1992a), but changes in junctional resistance cannot be ruled out.

Effects of Changes in Apical Bathing Solution Osmolality on Relative \( K^+ \) and \( Cl^- \) Conductances of the Basolateral Membrane

The changes in \( V_{cs} \) elicited by increasing basolateral \([K^+]\) from 2.5 to 25 mM (\( \Delta V_{cs}^K \)), or by lowering basolateral \([Cl^-]\) from 98.1 to 8.1 mM (\( \Delta V_{cs}^{Cl^-} \)), were measured in the same cells, during exposure to isosmotic apical

### Table I

|          | \( V_{ms} \) | \( V_{mc} \) | \( V_{cs} \) | \( R_a/R_b \) | \( R_s \) | \( \Delta V_{cs}^K \) | \( \Delta V_{cs}^{Cl^-} \) |
|----------|-------------|-------------|-------------|-------------|---------|-----------------|-----------------|
| Isosmotic| \(-0.2 \pm 0.2\) | \(-65 \pm 1\) | \(-65 \pm 2\) | \(4.9 \pm 0.4\) | \(245 \pm 30\) | \(28 \pm 4\) | \(5 \pm 1\) |
| Hyposmotic| \(-0.9 \pm 0.3^*\) | \(-73 \pm 2^*\) | \(-74 \pm 2^*\) | \(9.0 \pm 1.4^*\) | \(222 \pm 26^*\) | \(45 \pm 3^*\) | \(1 \pm 1^*\) |
| Recovery | \(-0.2 \pm 0.3\) | \(-67 \pm 2\) | \(-67 \pm 2\) | \(5.2 \pm 0.5\) | \(245 \pm 38\) | — | — |

For voltages and \( R_a/R_b \) values shown were obtained at 3 min of exposure to hypsomotic solution. Values during recovery (\( n = 9 \)) were taken at 4 min. \( \Delta V_{cs}^K \) and \( \Delta V_{cs}^{Cl^-} \) were measured at \(-20\) min (see text and Fig. 3). Values are means ± SEM (for the first five columns, \( n = 11 \) paired experiments; for the last two columns, \( n = 7 \)). *Significantly different from value in isosmotic solution (\( P < 0.05 \)).
bathing solution and at 20 min of exposure to hypoosmotic solution. Similar results were obtained between 5 and 20 min in hypoosmotic bathing medium (not shown). As illustrated in Fig. 3 and summarized in Table I, exposure to hypoosmotic solution increased $\Delta V_{cs}$ and decreased $\Delta V_{cs}^{Cl}$, relative to the respective values measured in isosmotic solution. Further, $\Delta V_{cs}^{Cl}$ in hypoosmotic solution was not significantly different from zero.

Similar experiments were carried out to assess the changes in basolateral membrane ionic selectivity after a 5–10 min exposure to hypoosmotic solution. The results are illustrated in Fig. 4 and summarized in Table II. In hypoosmotic medium, $\Delta V_{cs}^{K}$ was reduced significantly, while $\Delta V_{cs}^{Cl}$ was increased. Both changes are opposite to those elicited by exposure to hypoosmotic solution.

Because of the low paracellular electrical resistance of the NGB epithelium, the values of $\Delta V_{cs}^{K}$ and $\Delta V_{cs}^{Cl}$ are less than the corresponding changes in zero-current membrane voltages (the ionic substitutions produce changes in intraepithelial current flow). There are also concomitant paracellular diffusion potentials, but these are small and in both instances (high-K$^+$ and low-Cl$^-$) tend to compensate in part for the shunting effect of the paracellular pathway (see Reuss and Finn, 1975).

These results demonstrate that the relative $G_{Kb}^V$ increases and relative $G_{Clb}^V$ decreases during exposure to hypoosmotic solution. Conversely, the relative $G_{Kb}^V$ decreases, and the relative $G_{Clb}^V$ increases during exposure to a hyperosmotic solution. To estimate the changes in absolute values of $G_{Kb}^V$ and $G_{Clb}^V$ in both sets of experiments, we performed the experiments described in the next section.

**Effects of Exposure to Anisosmotic Solutions on Cell Membrane Resistances**

Fig. 5, A and B, depicts representative two-point cable analysis experiments under control conditions and during transient exposure to hypoosmotic and hypoosmotic solutions. Two cells were simultaneously impaled, and both transepithelial and intracellular current pulses were applied at intervals. Downward (hyperpolarizing) voltage spikes denote $\Delta V_{x}$, i.e., the voltage changes elicited by current injection into another (electrically coupled) cell. Exposure to hypoosmotic solution caused a decrease in $\Delta V_{x}$, which reached a stable value in ~4 min and returned to control levels within 4–5 min of restoring superfusion with isosmotic solution (Fig. 5 A). In contrast, exposure to hypoosmotic solution increased $\Delta V_{x}$. This effect was complete within 4 min and returned to control levels after a 4-min exposure to isosmotic solution (Fig. 5 B). Fig. 5 C depicts the $K_{r}$ fits of the Bessel function $K_{r}$ to the two-point cable analysis values obtained with hypoosmotic and hyperosmotic solutions, respectively. From these data, we calculated $R_{r}$ (resistance for current flow out of the cell, i.e., $R_{r}$ and $R_{p}$ in parallel); from $R_{r}$ and the concomitant values of $R_{f}$ and $R_{p}$, we calculated the resistances of the cell membranes and the paracellular pathway. The method used requires normalization of the data to the values observed under control conditions. The bases,
assumptions, and limitations of this method have been
discussed in detail elsewhere (Petersen and Reuss, 1985;
Copello et al., 1993).

The results of these calculations are summarized in
Table III. Exposure to anisosmotic solutions elicits
mostly changes in basolateral membrane resistance: \( R_b \)
decreases during superfusion with hyposmotic solution
and increases during superfusion with hyperosmotic solu-
tion. Whereas hyposmotic solution causes exclusively
a decrease in \( R_b \), hyperosmotic solution both increases
\( R_b \) and decreases \( R_a \). The latter effect could be due to
activation of apical membrane maxi-K\(^+\) channels by de-
polarization (Segal and Reuss, 1990).

The ionic-substitution experiments illustrated in Figs.
3 and 4 and summarized in Tables I and II demon-
strated an increase in relative \( G_{K_b} \) with hyposmotic solution
and a decrease in relative \( G_{K_b} \) with hyperosmotic solution.
These results, together with the resistance data, conclusively demonstrate that the changes in ba-
solateral membrane conductance are principally due to
changes in \( G_{K_b} \) in the same direction: increase with hy-
posmotic solution and decrease with hyperosmotic so-
lution. Rather unexpectedly, we found that the changes
in \( G_{K_b} \) and \( G_{Cl_b} \) were in opposite directions: when \( G_{K_b} \)
increased (hyposmotic solution), \( G_{Cl_b} \) decreased; when
\( G_{K_b} \) decreased (hyperosmotic solution), \( G_{Cl_b} \) increased.
We next investigated the mechanism of the changes in
\( G_{K_b} \) and \( G_{Cl_b} \).

**Cell Swelling Causes Elevation of \([Ca^{2+}]_i\), But the Latter Is
Not Necessary for the Increase in \( G_{K_b} \)**

Entry of \( Ca^{2+} \) has been shown to play a signaling role in



![Figure 3](image3.png)

**Figure 3.** Effects of apical surface superfusion with isosmotic
(A) or hyposmotic solution (B) on the ionic selectivity of the ba-
solateral membrane. Changes in \( V_{cs} \) were elicited by increasing ba-
solateral solution \([K^+]_o\) from 2.5 to 25 mM (\( \Delta V_{cs}^K \), top) or by lower-
ing basolateral solution \([Cl^-]_o\) from 98.1 to 8.1 mM (\( \Delta V_{cs}^{Cl^-} \), bot-
tom). All records were obtained in the same cell. During expo-
sure to hyposmotic solution, \( \Delta V_{cs}^K \) increased and \( \Delta V_{cs}^{Cl^-} \) decreased, compared to the respective values in isosmotic solution.

![Figure 4](image4.png)

**Figure 4.** Effects of apical surface superfusion with isosmotic
(A) or hyperosmotic solution (B) on \( \Delta V_{cs}^K \) (top) and \( \Delta V_{cs}^{Cl^-} \) (bottom). All records were obtained in the same cell. During exposure to hy-
perosmotic solution, \( \Delta V_{cs}^K \) decreased and \( \Delta V_{cs}^{Cl^-} \) increased.
cells preloaded with BAPTA, the elevation in \([\text{Ca}^{2+}]_i\), by exposure to hyposmotic solution was abolished. Experiments were also carried out with 50 \(\mu\)M Half-BAPTA,AM and 50 \(\mu\)M TPEN, a heavy-metal chelator (Kao, 1994). Neither compound binds \(\text{Ca}^{2+}\) in the nanomolar range. In both instances, the change in \([\text{Ca}^{2+}]_i\), after exposure to hyposmotic solution was similar to that observed under control conditions (not shown). Hence, the effect of BAPTA cannot be attributed to nonspecific toxic effects or to heavy-metal chelation. Because of the effect of pH on Fura-2 fluorescence (Reers et al., 1989), we also measured \(p\text{Hi}\) with BCECF, using the same protocol as for the \([\text{Ca}^{2+}]_i\), measurements. Hyposmotic solution did not cause measurable changes in \(p\text{Hi}\) (data not shown). These control experiments validate the conclusion that \([\text{Ca}^{2+}]_i\) does in fact rise transiently during exposure of NGB epithelial cells to hyposmotic solutions.

**Figure 5.** Two-point two-dimensional cable analysis under control conditions and during exposure to hyposmotic (−34%) and hyperosmotic (+34%) solutions. (A) Effect of exposure to hyposmotic solution. Cell-negative pulses of 40 nA, and 1-s duration were applied at 20-s intervals. The changes in \(V_\mu_\text{v}\) induced by the applied current were measured in another cell ∼260 μm away. \(\Delta V_\mu_\text{v}\) (downward spikes in the \(V_\mu_\text{v}\) record) decreased in amplitude during superfusion with hyposmotic solution, returning to control levels after superfusion with isosmotic solution. The interruption in the record denotes a 2-min interval. (B) Effect of exposure to hyperosmotic solution (inter-electrode distance, 415 μm). \(\Delta V_\mu_\text{v}\) increased during superfusion with hyperosmotic solution, returning to control levels in isosmotic solution. (C) Fits of Bessel function \(K_\mu_\text{v}\) to cable analysis data obtained during continuous impalements across the apical membrane before, during, and after exposure to either hyposmotic (open circles, \(n = 4\) tissues) or hyperosmotic solution (filled circles, \(n = 3\) tissues) on the apical side. Control values of \(\Delta V_\mu_\text{v}\) (in isosmotic solution) were superimposed on a curve \(\Delta V_\mu_\text{v} = A K_\mu_\text{v}(\mu_\text{v}/\lambda)\) characterized by previously measured average parameters in NGB epithelium under control conditions: \(A = 2.15\) mV and \(\lambda = 260\) μm (solid line). A normalized distance \(\mu_\text{v}\) was assigned to each point, and data obtained 180 s after exposure to apical hypomotic or hyperosmotic solution were plotted for the same value of \(\mu_\text{v}\). Best fits of the same Bessel function to these data (segmented line) yielded \(A = 2.17\) mV and \(\lambda = 200\) μm in hypomotic solution and \(A = 2.19\) mV and \(\lambda = 327\) μm in hyperosmotic solution. From the values of \(A\) and \(\lambda\), the values of \(R_\mu\) in hyper- and hypomotic solution were calculated as previously described (Petersen and Reuss, 1985; Copello et al., 1993).

### Table III

| Condition       | \(R_\mu/R_\text{b}\) | \(R_\text{f}\) | \(R_\text{t}\) | \(R_\text{z}\) | \(R_\text{s}\) |
|-----------------|-----------------|----------------|----------------|----------------|----------------|
| Isosmotic       | 4.9             | 245            | 927            | 5469           | 1116           | 254            |
| Hypomotic       | 9.0             | 222            | 546            | 5460           | 607            | 230            |
| Recovery        | 5.2             | 245            | 878            | 5444           | 1047           | 255            |
| Isosmotic       | 5.2             | 184            | 913            | 5669           | 1089           | 189            |
| Hypomotic       | 1.3             | 301            | 1498           | 3445           | 2650           | 317            |
| Recovery        | 4.9             | 173            | 950            | 6538           | 1108           | 178            |

First two columns were taken from Tables I (hypomotic) and II (hyperomotic), respectively. Other data are averages calculated as described in METHODS. See also Fig. 5.
To determine the role of \([\text{Ca}^{2+}]\) in the response of isolated polarized cells to hyposmotic swelling, we examined the effect of swelling (34% hyposmotic solution) on membrane voltage \((V_m)\) with or without pre-loading the cells with 50 \(\mu\)M BAPTA (see METHODS). Without BAPTA, \(V_m\) hyperpolarized by 4 ± 1 mV; with BAPTA, the hyperpolarization was 3 ± 1 mV (not significantly different; \(n = 4\) paired experiments).

### \(G_{Cl}^b\) Is Modulated by Membrane Voltage

The changes in \(G_{Cl}^b\) described above could result from: (a) direct effect of changes in cell volume, (b) changes in membrane voltage produced by the activation or inhibition of \(G_{Cl}^b\), or (c) other mechanisms. In other experiments, we have observed decreases in \(G_{Cl}^b\) associated with basolateral membrane hyperpolarization (Stoddard and Reuss, 1989; Altenberg et al., 1992; Altenberg et al., 1993). Cell swelling activates \(G_{Cl}^b\); this in turn would hyperpolarize the membrane (because \(E_K > V_m\)), and the hyperpolarization would cause a decrease in \(G_{Cl}^b\). The opposite effects, i.e., decrease of \(G_{Cl}^b\), depolarization, and activation of \(G_{Cl}^b\), would be elicited by cell shrinkage. To test the hypothesis that the effects of cell volume changes on \(G_{Cl}^b\) are mediated by changes in membrane voltage, we studied the effects of baseline \(V_C^b\) on \(\Delta V_{Cl}^b\). Depolarization of \(V_C^b\) was accomplished either by elevating apical solution \([K^+]\) from 2.5 to 25 mM (the change in \(V_C^b\) results from a loop-current change, see Reuss and Finn, 1975); hyperpolarization of \(V_C^b\) was accomplished by a transepithelial current clamp. The results are shown in Fig. 7. With depolarization of \(V_C^b\), \(\Delta V_{Cl}^b\) increased, and with hyperpolarization of \(V_C^b\), it decreased significantly. In the latter experiment, an extracellular electrode within 5 \(\mu\)m from the impaled cell was used as reference to avoid the effect of changes in voltage drops in the solutions (produced by the different conductivities of high-\(Cl^–\)and low-\(Cl^–\)solutions). We conclude that \(G_{Cl}^b\) is voltage dependent: decreased by hyperpolarization and increased by depolarization of \(V_C^b\).

In conclusion, these experiments show that after swelling of NGB epithelial cells \(G_{Cl}^b\) increases, causing basolateral membrane hyperpolarization, which may explain the parallel decrease in \(G_{Cl}^b\). In previous studies, we found no increase of apical membrane \(Cl^–\) conductance after cell swelling by exposure to hyposmotic solutions (Heming et al., 1994). This is consistent with the lack of change in \(R_P\) (see above). Hence, our studies do not support the notion that NGB epithelial cells undergo volume-regulatory decrease by loss of \(K^+\) and \(Cl^–\) via channels. However, cell volume measurements using an optical-sectioning technique have been reported to show virtually complete cell volume regulation within a few minutes of the onset of the osmotic perturbation (Persson and Spring, 1982; Larson and Spring, 1984; Furlong and Spring, 1990). Hence, it was of interest to test whether cell volume regulation takes place regardless of the decrease in \(G_{Cl}^b\). This was done in the next series of experiments.

### Effects of Exposure to Hyposmotic Solution on Cell Water Volume and Intracellular \(aCl_i\)

Cell water volume and \(aCl_i\) were measured before, during, and after exposure to hyposmotic solution (apical side alone). In Fig. 8 A we illustrate a cell water volume measurement in TMA\(^+\)-loaded cells. Exposure to hyposmotic bathing solution caused an increase in cell volume, evidenced by the decrease in \(G_{Cl}^b\). However, there was no measurable cell volume regulation, although during this time \(G_{Cl}^b\) is clearly activated (see Figs. 1 and 3 and Tables I and III). One could argue that the experimental method, namely the TMA\(^+\) loading by transient exposure to nystatin, caused a fall in \(G_{Cl}^b\) or otherwise changed the properties of the cells, abolishing the volume regulatory response. To test this possibility, we measured \(aCl_i\) before, during, and after exposure to hyposmotic solution, without TMA\(^+\) loading. The expectations are that hyposmotic solution will initially cause a dilution of intracellular \(Cl^–\); if there is
cell volume regulation by KCl efflux, then aCl would fall further during the regulatory response; if there is cell volume regulation by a different mechanism, without Cl− loss, then aCl would rise during the regulatory response. Fig. 8 B illustrates the result of this experiment. Exposure to hyposmotic apical solution produced a monotonic change in aCl, i.e., there was no indication of more than one process (water influx) changing aCl. Further, Fig. 8 C depicts the intracellular TMA⁺ and Cl− activities during the first 5 min of exposure to the hyposmotic solution (records from Fig. 8, A and B). The linear relationship indicates that the rates of change in both ion activities are the same, i.e., the time courses of aCl and aTMA⁺ mirror each other. In addition, the maximal fractional changes in the two activities did not differ significantly (see Fig. 8). Inasmuch as TMA⁺ is a cell water volume marker, i.e., effectively impermeant, this result indicates that during the 5-min period Cl− was also effectively impermeant, i.e., its cell content did not change. If cell volume regulation were very fast, one could envision a monotonic “subosmometric” change in aTMA⁺ after hyposmotic swelling (cell water volume tends to increase because of water influx and to decrease because of regulatory efflux of solute and water). However, the magnitude of the change in aTMA⁺ is that expected for a cell behaving as an ideal osmometer, and the parallel measurements of aCl do not show the higher rate of decrease predicted if Cl− were involved in the regulatory response, or the lower rate of decrease expected if the regulatory volume decrease did not involve Cl−.

The possibility of cell volume regulation after exposure to hyposmotic solution was further explored in a preparation of isolated NGB epithelial cells that retain structural and functional polarity (Torres et al., 1996a, b). Changes in cell water volume were estimated from the changes in intracellular fluorescence in cells loaded with calcein, as illustrated in Fig. 9 A, B, and C. Exposure of isolated polarized cells to hyposmotic (Fig. 9 D) or hyperosmotic solutions (Fig. 9 E) elicited rapid changes in intracellular fluorescence, indicative of swelling and shrinkage, respectively, with no regulatory volume changes during the >10-min period of exposure to anisosmotic solution. These experiments confirm the results described in the preceding section, and argue against the possibility that the lack of volume regulatory responses results from artefacts related to microelectrode impalements.

To eliminate the possibility of an artifactual inability to measure a putative volume regulatory response, we exposed CAMP-stimulated cells to an isosmotic, high-K⁺ external solution ([K⁺] was raised from 2.5 to 67.5 mM, replacing Na⁺), at constant external [Cl−] (63.1 mM). The result, illustrated in Fig. 9 F, was rapid and reversible cell swelling, consistent with previous observations made with the TMA⁺ technique in the assembled epithelium (Cotton and Reuss, 1991). These results further strengthen the conclusion that, in the experimental conditions of these studies, osmotically swollen NGB epithelial cells do not undergo short-term regulatory volume decrease.

**DISCUSSION**

Schultz (1981; see also Schultz and Hudson, 1991) developed the important notion that changes in transport rate at one of the membrane domains of an epithelium must result in “matching” changes in transport rate at

**Figure 8.** Effects of exposure to hyposmotic solution on: (A) Changes in cell water volume assessed from [TMA⁺], in TMA⁺-loaded cells exposed to a hyposmotic bathing solution. The log of Δ(VTMA - Vcs) is directly proportional to [TMA⁺], and therefore inversely proportional to cell volume. There is no measurable cell volume regulation after osmotic swelling (interruption in the record denotes an ~2-min interval). (B) Changes in aCl after exposure to a hyposmotic bathing solution. The log of Δ(VCl - Vcs) is inversely proportional to aCl. For 5 min during osmotic swelling, there is a monotonic decrease in aCl, due to dilution. (C) Plot of aCl vs. aTMA⁺ during the first 5 min of exposure to hyposmotic solution (records in A and B). The linear relationship between both ion activities indicates that the rates of change are the same. The decreases in aCl and aTMA⁺ in response to a 34% reduction in osmolality (36 ± 2%, n = 8, and 31 ± 5%, n = 8, respectively) were not significantly different from each other.
the opposite membrane domain, so that in the steady state cell volume and composition remain near constant. This adaptive mechanism requires a signaling system between the cell membranes, i.e., intermembrane cross talk. The study of the mechanisms of cross talk between apical and basolateral membranes is of great importance to understand the regulation of salt and water transport in gallbladder and other epithelia. Parameters possibly involved in cross talk are cell volume, intracellular ionic activities, and membrane voltage.

One instance in which parallel changes in transport rates of apical and basolateral membranes occur in NGB epithelium is that resulting from the effect of cAMP. After an increase in cAMP levels, it has been demonstrated that an apical Cl\(^{-}\) conductance, not present under control conditions, develops and dominates the cell membrane conductances (Copello et al., 1993; Heming et al., 1994; Reuss and Altenberg, 1995). This results in net Cl\(^{-}\) loss across the apical membrane (Cotton and Reuss, 1991), because intracellular Cl\(^{-}\) is above the value predicted from electrochemical equilibrium as a consequence of the operation of the apical membrane Cl\(^{-}\)/HCO\(_3\) exchanger (Reuss, 1988). Garvin and Spring (1992) have also suggested that cAMP elevation results in a change in the dominant mechanism for Na\(^{+}\) and Cl\(^{-}\) transport across the apical membrane: from Na\(^{+}/H^{+}\) and Cl\(^{-}/HCO_{3}^{-}\) exchanges to Na\(^{+}\)-Cl\(^{-}\) cotransport. Further, Dausch and Spring (1994) have proposed that protein kinase C activation causes the opposite effect. Regardless of these possibilities, the increase in apical membrane G\(_{Cl}\) causes membrane depolarization and activation of voltage-sensitive apical membrane maxi-K\(^{+}\) channels (Cotton and Reuss, 1991; Reuss, 1991). The parallel increases in apical membrane G\(_{Cl}\) and G\(_{K}\) cause net KCl efflux, intracellular Cl\(^{-}\) and K\(^{+}\) contents fall, the cells shrink (Cotton and Reuss, 1991), and transepithelial salt and water transport decrease. The latter result indicates that net transport across the basolateral membrane must also be reduced. Our starting hypothesis was that cAMP causes a reduction in G\(_{Cl}\). Hence, we assessed the effects of changes in cell water volume on basolateral membrane Cl\(^{-}\) and K\(^{+}\) conductances.

**Cell Swelling Stimulates and Cell Shrinkage Inhibits the Basolateral Membrane K\(^{+}\) Conductance**

Our results show that cell swelling causes a selective increase in basolateral membrane conductance, attributable to an increase in K\(^{+}\) conductance. This effect is teleologically appropriate for cell volume regulation, inasmuch as it would facilitate K\(^{+}\) loss from the swollen cells.

The opposite experimental perturbation, i.e., cell shrinkage by exposure to a hyperosmotic solution,
caused depolarization of both cell membranes, an increase in basolateral membrane resistance, and a decrease in the relative \( G_{Cl} \). These effects are opposite to those elicited by exposure to hyposmotic solution and indicate that cell shrinkage decreases \( G_{K} \). Again, this effect is teleologically appropriate, i.e., net \( K^+ \) efflux across the basolateral membrane would decrease in a cell that has undergone shrinkage.

The elevation in \([Ca^{2+}]_i\), after exposure to a hyposmotic solution could be solely or in part responsible for the increase of \( G_{K} \), inasmuch as this conductance is activated by maneuvers expected to elevate \([Ca^{2+}]_i\) (Bello-Reuss et al., 1981). Activation of nonselective cation channels, followed by \( Ca^{2+} \) entry and activation of \( Ca^{2+} \)-activated \( K^+ \) channels, has been demonstrated in other epithelia (Christensen, 1987). Our measurements demonstrated that \([Ca^{2+}]_i\) does rise during cell swelling; chelation with BAPTA prevents the elevation in \([Ca^{2+}]_i\), but not the membrane hyperpolarization. Although this result could be interpreted to indicate that \([Ca^{2+}]_i\), plays no role in the activation of \( G_{K} \), we cannot rule out that, in the presence of BAPTA, \([Ca^{2+}]_i\), rose in a small compartment, undetectable by our method. An alternative to \( Ca^{2+} \) activation is that the basolateral \( K^+ \) channels are activated by stretch.

**Cell Swelling Inhibits the Basolateral Membrane \( Cl^- \) Conductance**

Cell swelling reduced the relative \( G_{Cl} \) to a value not different from zero, indicating that the basolateral membrane \( Cl^- \) conductance decreases. This effect is contrary to expectations, inasmuch as it is opposite to the effect of cell swelling on \( G_{K} \) and would, per se, tend to prevent \( K^+ \) efflux via conductive pathways. The effect of cell swelling on \( G_{Cl} \) could be indirect, since it occurs during membrane hyperpolarization. We found that membrane hyperpolarization per se reduces \( G_{Cl} \), as indicated by basolateral solution \( Cl^- \)-substitution experiments at three membrane voltages. Atypical osmosensitivity of \( Cl^- \) channels has been observed in other cell types (Chesnoy-Marchais and Fritsch, 1994). It is possible that cell swelling and membrane hyperpolarization have separate effects on \( G_{Cl} \), but the membrane voltage changes appeared to dominate under the present experimental conditions.

**Implications of the Results for Cell Volume Regulation**

Osmotic swelling experiments yielded the expected increase in the total basolateral membrane conductance. The unexpected feature was that changes in \( G_{Cl} \) do not parallel the changes in \( G_{K} \), because the basolateral membrane \( Cl^- \) conductance is voltage sensitive. Hence, it is possible that the effects of cell volume (e.g., via membrane stretch) are directly exerted on \( G_{K} \) and that the resulting change in membrane voltage is responsible for the change in \( G_{Cl} \).

Our cell volume measurements suggest that NGB epithelial cells do not regulate their volume after exposure to anisomotic solution. Our measurements of intracellular \([TMA^+] \) and \( aCl \) are in apparent contradiction with the results of Spring and associates (Persson and Spring, 1982; Larson and Spring, 1984; Furlong and Spring, 1990), which suggested that the epithelial cells from NGB undergo regulatory volume decrease from hyposmotic swelling by basolateral exit of \( K^+ \) and \( Cl^- \), probably via basolateral membrane channels (Furlong and Spring, 1990). In contrast, our studies suggest that there is no measurable short-term cell volume regulation in NGB epithelial cells undergoing osmotic swelling. The experiments on isolated, polarized cells strengthen our conclusions in that they rule out artifacts resulting from \( TMA^+ \) loading and microelectrode impalement as possible explanations for the lack of cell volume regulation. Further, measurements of cell volume changes in isolated polarized cells in which apical membrane \( G_{Cl} \) was activated by cAMP showed that although these cells do not regulate rapidly their volume after osmotic swelling (data not shown), they undergo fast, sizable swelling after elevation of medium \([K^+]_o \), i.e., under isomotic conditions. This again indicates that our results cannot be attributed to a methodological inability to detect cell swelling in isomotic conditions. The explanation for the differences between our results and those of Spring and associates (e.g., Furlong and Spring, 1990) is not clear.

**Implications of the Results for Cross Talk**

Our results indicate that cell swelling causes rapid and reversible changes in basolateral membrane \( Cl^- \) - and \( K^+ \)-channel activities. The demonstration that \( G_{K} \) is elevated by cell swelling supports the notion that cell volume is a signal responsible for cross talk between apical and basolateral membranes. However, under these circumstances \( G_{Cl} \) not only does not increase, but in fact decreases. This would not be the expected response if the cell had been swollen by increased solute entry across the apical membrane. Under these conditions, the expected cross talk response would be to increase solute exit across the basolateral membrane. It is in principle possible that net solute exit is activated by cell swelling, but not via conductive pathways. A pathway for basolateral membrane \( Cl^- \) efflux in NGB epithelium is KCl cotransport (Corcia and Armstrong, 1981; Reuss, 1981). Activation or inactivation of this mechanism during cell swelling and cell shrinkage, respectively, would fulfill the requirements of intermembrane cross talk, but would also result in cell volume regulation, and hence this possibility is not supported by our results.
The present observations suggest that $G_{K^+}$ is regulated mainly or exclusively by $V_m$ changes, although a minor role of cell volume is possible. The dominant role of membrane voltage suggests that this regulatory mechanism is designed to preserve intracellular Cl$^{-}$ content instead of cell volume, i.e., there is an inverse relationship between electrical driving force and electrolytically permeability. Because of the hyperpolarization elicited by activation of $G_{K^+}$ after cell swelling, $G_{K^+}$ falls, and this prevents Cl$^{-}$ loss and tends to maintain aCl$^{-}$ closer to control levels. The need for this mechanism from the point of view of cell homeostasis is not obvious; cell Cl$^{-}$ content ends up being preserved at the expense of cell volume. In other cell types, intracellular [Cl$^{-}$] has been shown or claimed to modulate membrane transporters (e.g., Robertson and Foskett, 1994) and regulators, such as protein kinases (Treharne et al., 1994). In NGB epithelium, in addition to effects derived from these “messenger” roles of intracellular Cl$^{-}$, a likely consequence of a decrease in intracellular [Cl$^{-}$] is a fall in intracellular pH. The apical membrane of this epithelium expresses a highly active anion (Cl$^{-}$/HCO$_3^-$) exchanger (Reuss, 1988). A fall in aCl$^{-}$ at constant [Cl$^{-}$] in the apical bathing solution enhances Cl$^{-}$ influx and HCO$_3^-$ efflux across the apical membrane, which would cause cell acidification. In conclusion, osmotic swelling of NGB epithelial cells increases $G_{K^+}$, but reduces $G_{Cl^+}$, and hence there is no appreciable short-term regulatory volume decrease. If cell swelling is involved in cross talk between apical and basolateral membranes, then the effects of the increase in cell volume are not sufficient to provide an effective negative-feedback mechanism to restore cell volume to control levels, suggesting a more complex adaptive mechanism.

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