Electrophysiological Effects of Basolateral [Na\(^+\)] in *Necturus* Gallbladder Epithelium

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**ABSTRACT** In *Necturus* gallbladder epithelium, lowering serosal [Na\(^+\)] ([Na\(^+\)]\(_s\)) reversibly hyperpolarized the basolateral cell membrane voltage (\(V_{cl}\)) and reduced the fractional resistance of the apical membrane (\(fR_{ap}\)). Previous results have suggested that there is no sizable basolateral Na\(^+\) conductance and that there are apical Ca\(^{2+}\)-activated K\(^+\) channels. Here, we studied the mechanisms of the electrophysiological effects of lowering [Na\(^+\)]\(_s\), in particular the possibility that an elevation in intracellular free [Ca\(^{2+}\)] hyperpolarizes \(V_{cl}\) by increasing gK\(^+\). When [Na\(^+\)]\(_s\) was reduced from 100.5 to 10.5 mM (tetramethylammonium substitution), \(V_{cl}\) hyperpolarized from \(-68 \pm 2\) to a peak value of \(-82 \pm 2\) mV (\(P < 0.001\)), and \(fR_{ap}\) decreased from 0.84 \pm 0.02 to 0.62 \pm 0.02 (\(P < 0.001\)). Addition of 5 mM tetraethylammonium (TEA\(^+\)) to the mucosal solution reduced both the hyperpolarization of \(V_{cl}\) and the change in \(fR_{ap}\), whereas serosal addition of TEA\(^+\) had no effect. Ouabain (10\(^{-4}\) M, serosal side) produced a small depolarization of \(V_{cl}\) and reduced the hyperpolarization upon lowering [Na\(^+\)]\(_s\), without affecting the decrease in \(fR_{ap}\). The effects of mucosal TEA\(^+\) and serosal ouabain were additive. Neither amiloride (10\(^{-4}\) or 10\(^{-3}\) M) nor tetrodotoxin (10\(^{-6}\) M) had any effects on \(V_{cl}\) or \(fR_{ap}\) or on their responses to lowering [Na\(^+\)]\(_s\), suggesting that basolateral Na\(^+\) channels do not contribute to the control membrane voltage or to the hyperpolarization upon lowering [Na\(^+\)]. The basolateral membrane depolarization upon elevating [K\(^+\)], was increased transiently during the hyperpolarization of \(V_{cl}\) upon lowering [Na\(^+\)]. Since cable analysis experiments show that basolateral membrane resistance increased, a decrease in basolateral Cl\(^-\) conductance (gCl\(^-\)) is the main cause of the increased K\(^+\) selectivity. Lowering [Na\(^+\)]\(_s\) increases intracellular free [Ca\(^{2+}\)], which may be responsible for the increase in the apical membrane TEA\(^+\)-sensitive gK\(^+\). We conclude that the decrease in \(fR_{ap}\) by lowering [Na\(^+\)]\(_s\), is mainly caused by an increase in intracellular free [Ca\(^{2+}\)], which activates TEA\(^+\)-sensitive maxi K\(^+\) channels at the apical membrane and decreases apical membrane resistance. The hyperpolarization of \(V_{cl}\) is due to increases in: (a) apical membrane gK\(^+\), (b) the contribution of the Na\(^+\) pump to \(V_{cl}\), (c) basolateral membrane K\(^+\) selectivity (decreased gCl\(^-\)), and (d) intraepithelial current flow brought about by a paracellular diffusion potential.

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INTRODUCTION

Ion movements across the apical membrane of *Necturus* gallbladder epithelium have been extensively characterized (for review, see Reuss, 1989), but only partial information is available on the ion pathways across the basolateral membrane. Basolateral Cl⁻ extrusion is via both electroneutral KCl cotransport (Cocchia and Armstrong, 1983; Reuss, 1983) and a conductive mechanism (Stoddard and Reuss, 1988b, 1989a). If present, basolateral gHCO₃⁻ seems to be small, since decreasing basolateral solution [HCO₃⁻] from 10 to 1 mM at constant CO₂ decreases intracellular pH (pHᵢ) by only 0.03 units in 3 min, and increasing [HCO₃⁻] to 50 mM at constant pH does not reduce the depolarization of basolateral membrane voltage (Vₑᵣ) produced by elevating serosal [K⁺] (Stoddard and Reuss, 1989b).

Basolateral Na⁺ extrusion is via the Na⁺ pump (Reuss et al., 1979; Rose and Nahrwold, 1980; Ericson and Spring, 1982; Reuss, 1989); it is unclear whether there are pathways for inward Na⁺ movement across the basolateral membrane (see Weinman and Reuss, 1984). Decreasing basolateral [Na⁺] ([Na⁺]ᵣ) in gallbladders incubated in 10 mM HCO₃⁻/1% CO₂ hyperpolarizes Vₑᵣ (Stoddard and Reuss, 1989b). This hyperpolarization is by itself consistent with a sizable basolateral gNa⁺, but estimations of gK⁺ and gCl⁻ indicate that these alone could account for the basolateral conductance under control conditions (Stoddard and Reuss, 1989b). In addition, partial gNa⁺ of the basolateral membrane must be small because the zero-current voltage of the basolateral membrane (Eₑᵣ) (=−70 mV) is far removed from the Na⁺ equilibrium potential (Eᵣₑ⁺) (=60 mV; Reuss and Weinman, 1979; Weinman and Reuss, 1984; Reuss, 1989).

Previous results indicate that there are Ca²⁺-activated K⁺ channels at the apical and basolateral membranes (Bello-Reuss et al., 1981; García-Díaz et al., 1983; Stoddard and Reuss, 1988b, 1989b; Reuss, 1989; Segal and Reuss, 1990a). Here we study the mechanisms of the electrophysiological effects of lowering [Na⁺]ᵣ, in particular the possibility that an elevation in intracellular free [Ca²⁺]ᵣ hyperpolarizes Vₑᵣ by increasing gK⁺.

METHODS

Mudpuppies (*Necturus maculosus*) purchased from Kons Scientific Co., Inc. (Germantown, WI) or Nasco Biologicals (Ft. Atkinson, WI) were maintained in aquariums at 5–10°C. Anesthesia was accomplished by immersion of the animals 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). Tissues were mounted serosal side up, a patch of subepithelial connective tissue was removed by dissection (Stoddard and Reuss, 1989b), and impalements with microelectrodes were done across the basolateral membrane. Bathing solutions contained (mM): 90 NaCl, 10 NaHCO₃, 2.5 KCl, 1.8 CaCl₂, 1.0 MgCl₂, and 0.5 Na₂HPO₄, gassed with 1% CO₂/99% air, pH 7.66. Low-Na⁺ solutions were prepared by isomolar replacement of Na⁺ with tetramethylammonium (TMA⁺) or N-methyl-d-glucammonium (NMDG⁺); for high K⁺ solutions, Na⁺ was replaced with K⁺ mole by mole. In some experiments 5 mM tetraethylammonium (TEA⁺) was added, replacing Na⁺ or TMA⁺ mole by mole.
Electrophysiological Techniques

Transepithelial voltage ($V_{m}$, referred to the mucosal bathing solution, i.e., the bottom half-chamber) and cell membrane voltages (apical = $V_{m}$, basolateral = $V_{o}$, referred to the adjacent bathing solutions) were measured as previously described (Reuss and Finn, 1975a, b; Altenberg et al., 1990). The ground electrode was an Ag-AgCl pellet separated from the mucosal bathing solution by a short Ringer-agar bridge. The serosal bathing solution electrode was a flowing, saturated KCl bridge in series with a calomel half-cell. Hence, corrections for liquid junction potentials upon changes in the serosal solution were not required. The transepithelial resistance, $R_{t}$ ($\Delta V_{m}/I$), and the apparent fractional resistance of the apical membrane, $R_{a}$ ($\Delta V_{m}/\Delta V_{m} = R_{a}/(R_{a} + R_{b})$), where the subscripts a and b denote apical and basolateral membranes, respectively, were determined from the voltage deflections at 600 ms after the onset of a DC pulse of 50 μA/cm² and 2-s duration, applied across the tissue through Ag-AgCl electrodes. The voltage deflections were corrected for series resistances.

To estimate changes in the apical ($R_{a}$) and basolateral ($R_{b}$) membrane resistances produced by lowering [Na⁺], we used two-point cable analysis (Petersen and Reuss, 1985; Stoddard et al., 1990). Two cells were simultaneously impaled with microelectrodes; current ($I_{0}$) was injected through one of the microelectrodes and the cell membrane voltage changes ($\Delta V_{e}$) were measured in the second cell before, during, and after reducing [Na⁺]. Results obtained at several interelectrode distances were pooled and normalized to a Bessel function, choosing the parameters that yield the average equivalent resistance of $R_{a}$ and $R_{b}$ in parallel ($R_{e}$) and also $R_{a}$ under the same conditions (gallbladders incubated in 10 mM HCO₃⁻/1% CO₂; see Stoddard and Reuss, 1988a). By this procedure, the normalized distance (x) assigned to each measurement is retained for the analysis of all data obtained from the same pair of impalements. The values of $\Delta V_{e}$ under experimental conditions were fit to the Bessel function $K_{0}$ by a nonlinear, least-squares routine. The experimental values of $A$ and the space constant ($\lambda$) were determined and used to calculate $R_{e}$ [$R_{e} = R_{a}R_{b}/(R_{a} + R_{b})$] according to Frömter (1972):

$$R_{e} = 2\pi A\lambda^{2}/I_{0}$$

(1)

$R_{a}$ and $R_{b}$ were calculated from $R_{e}$ and $fR_{e}$; $I_{0}$ varied between 7 and 20 nA, and $\Delta V_{e}$ was normalized to an $I_{0}$ of 10 nA (Stoddard and Reuss, 1988a).

Intracellular Na⁺ activity (aNa⁺) and pH (pH) were measured with ion-sensitive microelectrodes. Intracellular conventional and pH-sensitive microelectrodes and double-barreled Na⁺-selective microelectrodes were constructed and calibrated as previously described (Stoddard et al., 1989a; Altenberg et al., 1990). Validation of impalements was as described before (Weinman and Reuss, 1984; Altenberg et al., 1990). Intracellular pH was measured with simultaneous impalements with single-barreled pH-sensitive and conventional microelectrodes (Weinman and Reuss, 1982; Altenberg et al., 1990). Intracellular Na⁺ was measured with double-barreled microelectrodes.

Fluorescence Techniques

Attempts to load the epithelial cells of Necturus gallbladder with the acetoxymethyl (AM) ester form of the Ca⁺⁺-sensitive dyes fura-2, fluo-3, and quin-2 were unsuccessful. We tested cell loading with different dye concentrations from 5 to 100 μM, by mucosal, serosal, or bilateral exposure, varying exposure times from 0.5 to 4 h using 2 mM probenecid, and subjecting the preparation to hypotonic shock during exposure to the free acids of fura-2 or fluo-3. None of these procedures worked. Finally, we microinjected individual cells of the preparation with dextran-bound fura-2 (see below). The gallbladder was pinned to a cork ring, serosal side up,
the connective tissue was dissected away, and then the tissue was mounted mucosal side up in
the modified Ussing chamber used for microelectrode studies (Altenberg et al., 1990). Glass
pipettes with internal fiber, similar to those used to measure cell membrane voltages, were
back-loaded with 2–3 μl of a solution containing 1.7 or 5 mM dextran-bound fura-2 (10,000
mol wt; Molecular Probes, Inc, Eugene, OR) in 100 mM KCl. The tip was filled and then
broken to an outer diameter of ~ 1 μm by pressing the connective tissue near the edge of the
preparation. Well-dissected areas were selected and cells were injected manually by pressure
using a plastic syringe (Graessmann and Graessmann, 1983). The glass pipette was withdrawn
either when the cell diameter increased by ~ 10% or when the nucleus became clearly visible by
an increase in contrast between nucleus and cytoplasm (Graessmann and Graessmann, 1983).
In each preparation, 5–30 cells were microinjected. The plastic ring with the gallbladder was
removed from the modified Ussing chamber (Altenberg et al., 1990) and then placed in a
Leiden chamber (Leiden microincubator; Medical Systems Corp., Greenvale, NY) sealed at
the bottom with a glass coverslip 110 μm thick. A droplet of control Ringer solution was placed on
the coverslip, and the tissue, mucosal side down, was secured in place with dental wax. The
serosal solution was replaced by gravity at a rate of ~ 20 ml/min, while a static layer of fluid
50–100 μm thick remained in contact with the apical surface of the epithelium. Fluorescence
determinations were carried out with a Deltascan system (Photon Technology International
Inc., South Brunswick, NJ). The light source was a 75-W xenon lamp chopped between two
monochromators set at wavelengths of 340 ± 4 and 380 ± 4 nm. Monochromatic light, carried
by fiber optics, was reflected with a dichroic mirror (405 nm; Omega Optical Inc., Brattleboro,
VT). An inverted microscope (Nikon Diaphot; Nikon, Tokyo, Japan) and a high numerical
aperture objective (NA = 1.3, 40x, Nikon #78820) were employed. Light emitted from a
single cell was filtered with a band-pass filter (510 ± 20 nm, Omega Optical) and measured
with a photomultiplier (Photon Technology International Inc.). Data were acquired at 1-s
intervals. The microinjected cells appeared homogeneously fluorescent under microscopic
observation at 400x. There was no evidence of dye leak. Experiments were started 1–2 h after
the microinjection, but the dye remained inside the cells for at least 4 h. At the end of the
experiments 10–20 μM ionomycin was added to the control Ringer to obtain saturating free
[Ca^{2+}], and then 1–2 mM MnCl\textsubscript{2} was added, in the continuous presence of ionomycin, to
quench the dye. Free [Ca^{2+}]), was estimated according to
\[
\text{free } [\text{Ca}^{2+}] = \frac{(R - R_{\text{min}})/(R_{\text{max}} - R)}{K_D(S_{\text{a}}/S_{\text{b}})}
\]
where \(K_D\) (190 ± 12 nM) is the apparent dissociation constant of the Ca\textsuperscript{2+}-dextran-bound
fura-2 complex, and \(S_{\text{a}}/S_{\text{b}}\) is the ratio of fluorescence at 380 nm at 0 Ca\textsuperscript{2+} and at saturating
Ca\textsuperscript{2+} levels (10.5 ± 0.7). \(R_{\text{max}}\) (18.9) and \(R_{\text{min}}\) (0.74) are the 340/380 nm wavelength ratios at
saturating and 0 free [Ca\textsuperscript{2+}]. Calibration solutions contained (mM): 120 KCl, 20 NaCl, 1.0
MgCl\textsubscript{2}, and 10 HEPES; these were titrated to pH 7.35 with KOH. The Ca\textsuperscript{2+} buffer EGTA (Fluka
Chemical Corp., Ronkonkoma, NY) was used at a concentration of 5 mM (Tsien and Rink,
1981; Harrison and Bers, 1989), and CaCl\textsubscript{2} was added to each solution to obtain free [Ca\textsuperscript{2+}]'s of
\(10^{-9}\) to \(10^{-5}\) M.

The microinjections did not result in significant membrane damage. Cell membrane
voltages, \(\bar{V}_m\), and apical membrane K\textsuperscript{+} selectivity were similar in injected and noninjected cells
from the same tissue.

**Statistical Analysis**

Results are given as mean ± SEM or SD, as indicated. 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 Lowering [Na⁺], on Membrane Voltages and fRₐ

Fig. 1 illustrates the effects of lowering [Na⁺], from 100.5 to 10.5 mM (TMA⁺ replacement) on transepithelial and cell membrane voltages and fRₐ. Decreasing [Na⁺], caused a serosa-positive change in Vₑₑ which reached a plateau in 1–2 min.

This voltage change is due to a paracellular biionic potential (Pₐ ≫ P_TMA). The changes in Vₑₑ and Vₑₑ were more complex and could be arbitrarily divided into three phases, a, b, and c (see Fig. 1). Both Vₑₑ and Vₑₑ initially depolarized by 0.1–4.2 mV (phase a). Over the next 10–20 s Vₑₑ and Vₑₑ hyperpolarized (phase b). In all cases the hyperpolarization of Vₑₑ was larger than that of Vₑₑ and persisted for the duration of
the experiment (phase c), although in most cases it decreased slowly toward control values (see also Figs. 2, 5, and 6). During phase c \( V_{mc} \) was always depolarized.

The small initial depolarization of \( V_{mc} \) (phase a) is an artifact. The ensuing changes in membrane voltages are complex. The serosa-positive paracellular diffusion potential depolarizes \( V_{mc} \) and hyperpolarizes \( V_{mc} \) because of intracellular current flow; the change in \( V_{mc} \) is larger than in \( V_{mc} \) because most of the transcellular electrical resistance is located at the apical membrane (\( R_a \) is 0.84 in control and 0.62 in low-Na\(^+\) Ringer; see Table I). Consistent with this notion, after the initial 20-30 s the change in \( V_{mc} \) resembles that of \( V_{mc} \), while \( V_{mc} \) appears to be largely independent of \( V_{mc} \). However, the hyperpolarization of \( V_{mc} \) during phase b is too large and the concomitant change in \( V_{mc} \) is in the wrong direction to be explained by the paracellular diffusion potential. Therefore, these voltage changes must result from changes in the ion transport properties of one or both cell membranes. The effects of decreasing \([\text{Na}^+]\) on membrane voltages are summarized in Table I.

| \( V_{mc} \) | \( V_{mc} \) | peak \( \Delta V_{mc} \) | \( V_{mc} \) | \( \Delta R_a \) | \( R_e \) |
| mV | mV | mV | mV | \( \Omega \cdot \text{cm}^{-2} \) |
|---|---|---|---|---|---|
| Control | 0.0 ± 0.1 | -68 ± 2 | -68 ± 1 | 0.84 ± 0.02 | 152 ± 6 |
| Low [Na\(^+\)] | 13.3 ± 0.7\* | -78 ± 2\* | -14.2 ± 0.9\* | -62 ± 1\* | 0.62 ± 0.02\* | 209 ± 9\* |
| Recovery | 0.1 ± 0.1 | -69 ± 2 | -69 ± 1 | 0.85 ± 0.02 | 150 ± 7 |

\( V_{mc} \) is the transepithelial voltage, \( V_{mc} \) and \( V_{mc} \) are the apical and basolateral membrane voltages, respectively, \( \Delta R_a \) is the fractional resistance of the apical membrane, and \( \Delta R_a \) is the transepithelial resistance. Values are means ± SD of \( n = 67 \) tissues, except for \( R_e \) (\( n = 65 \)) and \( \Delta R_a \) (\( n = 64 \)). [Na\(^+\)] was reduced from 100.5 to 10.5 mM (TMA\(^+\) substitution). Peak \( \Delta V_{mc} \) is the peak hyperpolarization of \( V_{mc} \) 15-25 s after reducing [Na\(^+\)]. All the other parameters in low [Na\(^+\)] were determined at 1 min after the ionic substitution. Recovery values were determined 3-8 min after reexposure to control Ringer. \*\( P < 0.001 \) compared with control and recovery periods. This table includes the control data from all experiments performed.

As shown in Fig. 1 and summarized in Table I, reducing [Na\(^+\)] decreases \( \Delta R_a \) reversibly. Since \( R_e \) is voltage sensitive (Garcia-Diaz et al., 1983; Stoddard and Reuss, 1988b) the depolarization of \( V_{mc} \) may contribute to the change in \( \Delta R_a \). The magnitude of the apparent change in \( \Delta R_a \) depends on the polarity of the transepithelial current pulses (Stoddard and Reuss, 1988b), but \( \Delta R_a \) decreases with both hyperpolarizing and depolarizing pulses. Further, in ~10% of the experiments in Table I, \( \Delta R_a \) was reduced at a time when \( V_{mc} \) was still hyperpolarized. In most of the experiments TMA\(^+\) was used to replace Na\(^+\), but similar results were obtained using NMDG\(^+\).

\(^1\) The artifact results from the subtraction of \( V_{mc} \) (an average measurement of the voltage across the entire preparation) from \( V_{ms} \) (the voltage of a single cell in a "patch" of the gallbladder with virtually no adjacent subepithelial tissue). The average change in \( V_{ms} \) is less than the change ideally expected in the vicinity of the impaled cell because of subepithelial diffusive delays in the rest of the preparation. Supporting this interpretation, the small depolarization of \( V_{mc} \) disappears when the ground electrode is positioned in the serosal bath, allowing for direct measurement of \( V_{mc} \).
The changes in $V_o$, upon lowering $[Na^+]$, from 100.5 mM to 70.5, 40.5, 25.5, or 10.5 mM were studied in four tissues (Fig. 2). The magnitudes of the peak hyperpolarization of $V_o$ and the decrease in $IR_o$ were proportional to the extent of the reduction in $[Na^+]$.

The hyperpolarization of $V_o$ could be due, in principle, to an increase in $gK^+$ at the apical and/or basolateral membranes, to the presence of a conductive pathway for $Na^+$ at the basolateral membrane, to a decrease in basolateral $gCl^-$, and/or to stimulation of the $Na^+$ pump. The decrease in $IR_o$ could be explained by a decrease in $R_o$, an increase in $R_b$, or a combination of both. The experiments below were aimed to ascertain the mechanisms of the initial hyperpolarization of $V_o$ (phase b) and of the decrease in $IR_o$ produced by lowering $[Na^+]$. In addition, we explored the possibility that an elevation in free $[Ca^{2+}]$ could play a role in the electrophysiological effects of reducing $[Na^+]$.

**Effects of TEA**

To explore the possible involvement of the TEA$^+$-sensitive apical membrane conductance in the response to lowering $[Na^+]$, we carried out the experiments illustrated in Fig. 3A and summarized in Table II. As shown, TEA$^+$ reduces the hyperpolarization
FIGURE 3.
of $V_c$ and abolishes the decrease in $R_c$ upon reducing $[Na^+]$. These results indicate that a TEA$^+$-sensitive apical membrane conductance is the main contributor to the initial fall in $R_c$ and accounts for a sizable portion of the hyperpolarization of $V_c$ elicited by lowering $[Na^+]$. It must be pointed out that since TEA$^+$ increases $R_c$ (see below), the intraepithelial current flow is reduced, as well as the contribution of this current to the hyperpolarization of $V_c$; for the same reason, in TEA$^+$ the magnitude of the depolarization of $V_{mc}$ increases (see Fig. 3 A).

Inasmuch as the change in $V_c$ is not abolished by mucosal exposure to TEA$^+$, we studied the possibility of a contribution of a TEA$^+$-sensitive basolateral $g_K$ to the $V_c$ hyperpolarization produced by lowering $[Na^+]$. To avoid apical membrane effects of serosal TEA$^+$, we carried out these experiments with 5 mM TEA$^+$ on the mucosal solution. In this series, lowering $[Na^+]$ in the presence of TEA$^+$ reduced $R_c$ from $0.85 \pm 0.02$ to $0.82 \pm 0.02$ ($n = 6$). Although this change was significant ($P < 0.05$), its magnitude was small compared with that observed in the absence of TEA$^+$ (Table I).

### Table I

<table>
<thead>
<tr>
<th>Condition</th>
<th>$V_{mc}$</th>
<th>$V_c$</th>
<th>peak $\Delta V_c$</th>
<th>$V_{mc}$</th>
<th>$R_c$</th>
<th>$R_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$-0.2 \pm 0.2$</td>
<td>$-66 \pm 2$</td>
<td>$-66 \pm 2$</td>
<td>$0.84 \pm 0.02$</td>
<td>182</td>
<td>15</td>
</tr>
<tr>
<td>Low $[Na^+]$</td>
<td>$15.8 \pm 1.4^*$</td>
<td>$-76 \pm 2^*$</td>
<td>$-15.4 \pm 1.8^*$</td>
<td>$-60 \pm 2^*$</td>
<td>$0.58 \pm 0.02^*$</td>
<td>250</td>
</tr>
<tr>
<td>Control</td>
<td>$0.2 \pm 0.2$</td>
<td>$-68 \pm 2$</td>
<td>$-69 \pm 3$</td>
<td>$0.87 \pm 0.03$</td>
<td>193</td>
<td>18</td>
</tr>
<tr>
<td>TEA$^+$</td>
<td>$-0.6 \pm 0.2^*$</td>
<td>$-68 \pm 2$</td>
<td>$-68 \pm 2$</td>
<td>$0.91 \pm 0.01$</td>
<td>188</td>
<td>16</td>
</tr>
<tr>
<td>TEA$^+$/Low $[Na^+]$</td>
<td>$16.2 \pm 1.4^*$</td>
<td>$-71 \pm 2^*$</td>
<td>$-10.5 \pm 1.7^*$</td>
<td>$-55 \pm 3^*$</td>
<td>$0.90 \pm 0.01^*$</td>
<td>273</td>
</tr>
</tbody>
</table>

Values are means ± SEM of $n = 16$ except for control vs. TEA$^+$ ($n = 11$). For abbreviations and experimental protocol see Table I and Fig. 4, respectively. Low $[Na^+]$ denotes low $[Na^+]$ Ringer (10.5 mM). TEA$^+$ (5 mM) was added to the mucosal solution (TEA$^+$) 10 min before lowering $[Na^+]$. All the other parameters were determined after 1 min of exposure to low $[Na^+]$ Ringer. $^*P < 0.05$ or better compared with the preceding condition; $^1P < 0.05$ compared with the effect of lowering $[Na^+]$, under control conditions.

Fig. 3 B shows that addition of 5 mM TEA$^+$ to the serosal solution produces a small depolarization of cell membrane voltages ($\Delta V_c = 1.2 \pm 0.4$ mV, $P < 0.05$; $\Delta V_{mc} = 1.8 \pm 0.4$ mV, $n = 6$, $P < 0.02$), suggesting that a small TEA$^+$-sensitive basolateral $g_K$ is present under control conditions. Lowering $[Na^+]$, in the continuous presence of serosal TEA$^+$ causes a hyperpolarization of $V_c$, not significantly different from that in its absence. These results rule out that a basolateral membrane...
TEA⁺-sensitive gK⁺ contributes to the change of $V_\alpha$ upon lowering [Na⁺]. The small changes in $V_{sm}$ produced by application of mucosal (Table II) and serosal TEA⁺ (not shown) are most probably the result of paracellular bionic potentials.

**Effects of Ouabain**

In Necturus gallbladder epithelium bathed with 10 mM HCO₃⁻/1% CO₂, exposure to $10^{-4}$ M ouabain on the serosal side depolarizes cell membrane voltages by $\approx 3$ mV in $\approx 15$ s (Baerentsen et al., 1982; Stoddard and Reuss, 1989a), suggesting that the Na⁺ pump is electrogenic and/or that its inhibition leads to a rapid change in transmembrane ionic gradients, i.e., a fall in $E_K$ (Stoddard and Reuss, 1989a). Since the activity of the Na⁺ pump is increased by decreasing extracellular [Na⁺] (Garrahan and Glynn, 1967; Nakao and Gadsby, 1989), the Na⁺ pump could contribute to the hyperpolarization of $V_\alpha$ upon lowering [Na⁺]. Therefore, we tested this possibility by measuring the ouabain-induced depolarization of $V_\alpha$ under control conditions and upon lowering [Na⁺]. Fig. 4 shows that $10^{-4}$ M ouabain depolarizes $V_\alpha$ by a few millivolts. When [Na⁺] was reduced after 1 min of exposure to ouabain (in the continuous presence of the steroid), the peak hyperpolarization of $V_\alpha$ was significantly less than in the absence of ouabain. In contrast, the change in $fR_\alpha$ was not affected (Table III). To confirm the significance of the small effect of ouabain on the peak hyperpolarization of $V_\alpha$, we compared the ouabain-induced depolarization in control and low-Na⁺ media. In 11 experiments, lowering [Na⁺] hyperpolarized $V_\alpha$ as described above. After 2 min, a 1-min addition of $10^{-4}$ M ouabain to the low-[Na⁺] Ringer depolarized $V_\alpha$ by $\approx 3$ mV. The depolarization was smaller than that observed under control conditions and upon lowering [Na⁺].

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Effects of serosal exposure to $10^{-4}$ M ouabain on the responses to lowering serosal [Na⁺]. (Left) Control. (Right) Exposure to ouabain from 1 min before lowering [Na⁺]. Traces are from the same cell. Ouabain depolarized $V_\alpha$ (see amplified trace, top right) and reduced the hyperpolarization of $V_\alpha$ upon lowering [Na⁺].
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$V_\alpha$ from $-74 \pm 2$ to $-69 \pm 2$ mV ($\Delta V_\alpha = 5.0 \pm 1.5$ mV, $P < 0.01$). In the same tissues, addition of ouabain to the control Ringer depolarized $V_\alpha$ less than in low-[Na⁺], Ringer, from $-71 \pm 2$ to $-69 \pm 2$ mV ($\Delta V_\alpha = 1.3 \pm 0.4$ mV, $P < 0.05$ compared with low-[Na⁺], Ringer). In these experiments, the 1-min exposure to ouabain was reversible as assessed from the absence of continuous depolarization that was observed with longer exposure times.

The effects of ouabain and mucosal solution TEA⁺ appear to be produced by different mechanisms, since TEA⁺ prevents the effect of lowering [Na⁺], on $I_R$, whereas ouabain does not. To test this hypothesis directly, the combined effects of both agents were studied. In 10 experiments apical solution TEA⁺ alone reduced the peak hyperpolarization of $V_\alpha$ by 25 ± 4% (4.7 ± 1.7 mV), whereas apical TEA⁺ and ouabain reduced the voltage change significantly more, by 44 ± 8% (8.2 ± 2.6 mV, $P < 0.05$ compared with TEA⁺ alone).

### Table 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>$V_{m}$</th>
<th>$V_\alpha$</th>
<th>Peak $\Delta V_\alpha$</th>
<th>$V_{m}$</th>
<th>$I_R$</th>
<th>$R_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0 ± 0.1</td>
<td>-71 ± 2</td>
<td>-71 ± 2</td>
<td>0.79 ± 0.03</td>
<td>136 ± 9</td>
<td></td>
</tr>
<tr>
<td>Low [Na⁺],</td>
<td>14.3 ± 1.3*</td>
<td>-78 ± 2*</td>
<td>-12.4 ± 2.2*</td>
<td>-64 ± 2*</td>
<td>0.61 ± 0.02*</td>
<td>191 ± 19*</td>
</tr>
<tr>
<td>Control</td>
<td>-0.2 ± 0.1</td>
<td>-71 ± 2</td>
<td>-72 ± 2</td>
<td>0.78 ± 0.03</td>
<td>137 ± 10</td>
<td></td>
</tr>
<tr>
<td>Ouabain</td>
<td>-0.2 ± 0.1</td>
<td>-70 ± 2*</td>
<td>-71 ± 2*</td>
<td>0.78 ± 0.03</td>
<td>141 ± 10</td>
<td></td>
</tr>
<tr>
<td>Ouabain/low [Na⁺],</td>
<td>14.7 ± 1.4*</td>
<td>-75 ± 2*</td>
<td>-10.0 ± 2.5*</td>
<td>-61 ± 2*</td>
<td>0.64 ± 0.02*</td>
<td>199 ± 19*</td>
</tr>
</tbody>
</table>

Values are means ± SEM of $n = 11$ tissues. For abbreviations and details on experimental protocol see Table 1 and Fig. 5, respectively. Ouabain (10⁻⁴ M) was added to the serosal solution and after 1 min [Na⁺], was reduced in the continued presence of ouabain. Peak $\Delta V_\alpha$ is the peak change of $V_\alpha$ 15–25 s after reducing [Na⁺], or adding ouabain as indicated. All the other values were measured 1 min after reducing [Na⁺]. *$P < 0.05$ compared with the preceding condition; †$P < 0.05$ or better compared with the effect of lowering [Na⁺], in the control condition.

### Effects of Amiloride and Tetrodotoxin

To test for a possible contribution of an amiloride-sensitive basolateral membrane Na⁺ conductance to the $V_\alpha$ hyperpolarization by lowering [Na⁺], we evaluated the effects of reducing [Na⁺], in the presence and absence of 10⁻⁵ M amiloride on the serosal side. At this concentration, amiloride is a highly specific blocker of epithelial Na⁺ channels (Benos, 1982, 1988). Fig. 5A shows that amiloride did not affect membrane voltages and did not change the $V_\alpha$ and $I_R$, responses to lowering [Na⁺].

Tetrodotoxin (TTX) blocks Na⁺ channels in excitable cells and recently TTX-sensitive Na⁺ channels have been described in epithelia (Fain and Farahbakhsh, 1989). Therefore, we tested for their possible existence and potential role in the hyperpolarization of $V_\alpha$ upon lowering [Na⁺]. Fig. 5B shows that serosal addition of 10⁻⁶ M TTX did not affect $V_\alpha$ or its response to lowering [Na⁺].
Effects of Lowering [Na⁺], on the Vₐ Response to Changes in Serosal Solution [K⁺]

Since basolateral gK⁺ seems to be increased by elevating cytosolic free [Ca²⁺] (Bello-Reuss et al., 1981), we explored the possible contribution of an increase in the partial gK⁺ of the basolateral membrane to the hyperpolarization of Vₐ. This was tested by measuring the depolarization of Vₐ produced by a 10-fold increase in serosal solution [K⁺]. To prevent changes in apical membrane gK⁺, 5 mM TEA⁺ was added to the apical side. Under these conditions, fRₐ remains high (see Table II) and Vₐ approaches Eₐ (see Reuss and Finn, 1975b; Stoddard et al., 1990). In these experiments, [Na⁺]ₐ was first reduced from 100.5 to 10.5 mM and then serosal [K⁺]...
Figure 6. Effects of lowering $[\text{Na}^+]$, on the membrane voltage changes upon elevating serosal solution $[\text{K}^+]$. 5 mM TEA$^+$ was present throughout in the mucosal solution to minimize the changes in $I_R$ (see text). (A) Serosal $[\text{K}^+]$ was increased from 2.5 to 25 mM at the peak of the hyperpolarization of $V_{cs}$. (B) The experimental protocol was similar to that in A, but serosal $[\text{K}^+]$ was elevated 2 min after reducing $[\text{Na}^+]$. Note that the depolarization of $V_{cs}$ upon increasing $[\text{K}^+]$ is greater at the peak hyperpolarization than 1.5 min later, when $V_{cs}$ is closer to the control value.

was increased from 2.5 to 25 mM, either at the peak hyperpolarization (usually 15–25 s after lowering $[\text{Na}^+]$) or once $V_{cs}$ had repolarized to a value close to that in control Ringer ($\sim$ 2 min after lowering Na$^+$). Fig. 6 shows that the $V_{cs}$ depolarization upon increasing serosal $[\text{K}^+]$ was larger at the peak of $V_{cs}$ hyperpolarization.
compared with the value obtained 2 min after reducing [Na⁺]. In four tissues, the depolarization of $V_m$ produced by increasing [K⁺] at the peak of the hyperpolarization was 33 ± 5 mV, a value significantly larger than that obtained 2 min after lowering [Na⁺], (20 ± 3 mV, $P < 0.05$). In four additional experiments no difference was found between the depolarization of $V_m$ produced by elevating serosal [K⁺] to 25 mM in control conditions and 2 min after lowering [Na⁺], ($\Delta V_m = 30 \pm 2$ and $30 \pm 3$ mV, respectively).

**Effects of Lowering [Na⁺], on Cell Membrane Resistances**

At the basolateral membrane, $g_{K^+}$ and $g_{Cl^-}$ are the main conductances (Stoddard and Reuss, 1989). To determine which was the dominant change in conductance responsible for the changes in selectivity shown above, we performed two-point cable analysis experiments (see Methods). The trace in Fig. 7A shows the changes in $V_m$ produced by exposure to low-[Na⁺] Ringer. 11 such observations at varying distances in seven tissues were pooled and normalized to average results under the same conditions (see Methods). The voltage deflections ($\Delta V_m$) under control conditions, 20 and 180 s upon reducing [Na⁺], are shown in Fig. 7B with their corresponding fits to the Bessel function $K_0$ (Frömter, 1972). The trace in Fig. 7A shows that $\Delta V_m$ initially fell, later increased above control, and finally returned slowly to control levels upon...
TABLE IV
Effects of Lowering [Na⁺], on Apical (Rₐ) and Basolateral (Rₜ) Membrane Resistances, and the Equivalent Resistance of the Apical and Basolateral Membrane Resistances in Parallel (Rₑ)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Low Na⁺</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 s</td>
<td>180 s</td>
<td></td>
</tr>
<tr>
<td>Rₐ, Ωcm²</td>
<td>7,150</td>
<td>3,680</td>
<td>2,810</td>
</tr>
<tr>
<td>Rₜ, Ωcm²</td>
<td>1,070</td>
<td>1,810</td>
<td>2,590</td>
</tr>
<tr>
<td>Rₑ, Ωcm²</td>
<td>930</td>
<td>1,210</td>
<td>1,350</td>
</tr>
<tr>
<td>fRₑ</td>
<td>0.87</td>
<td>0.67</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Data were obtained during continuous impalements across the basolateral membrane before, during, and after exposure to 10.5 mM [Na⁺], (7 tissues, 11 measurements). Values of Rₑ were calculated from the fit of the two-dimensional cable equation to the data (see text). Control data were fit to a curve characterized by \( A = 2.1 \text{ mV} \) and \( \lambda = 266 \mu \text{m} \). The distance from the current injection site was thus normalized for each impalement. \( Rₐ \) and \( Rₜ \) were calculated from \( Rₑ \) and \( fRₑ \). \( fRₑ \) values were taken from the experiments in Table I.

Reexposure to control Ringer. The changes in \( \Delta V \) were \(-28 \pm 5\% (P < 0.001) \) and \( 20 \pm 5\% (P < 0.005) \) 20 and 180 s after lowering [Na⁺], respectively. From \( Rₑ \) and \( fRₑ \), \( Rₐ \) and \( Rₜ \) were estimated according to: \( Rₐ = Rₑ/(1 - fRₑ) \), and \( Rₜ = Rₑ/fRₑ \). The observed decrease in \( Rₑ \) (see Table IV) supports our conclusion of a role for apical maxi K⁺ channels in the response to lowering [Na⁺]. As indicated above, at the peak of \( Vₜ \), hyperpolarization \( \Delta V \) is reduced, \( \lambda \) is increased from 266 to 408 μm, and parameter \( A \) is reduced from 2.1 to 1.2 mV. The latter effect suggests an increase in cell-to-cell coupling resistance, perhaps due to the increase in free [Ca²⁺]i (De Mello, 1987; see below).

Effect of Lowering [Na⁺], on Intracellular Free [Ca²⁺]

Changes in intracellular free [Ca²⁺] (free [Ca²⁺]i) were determined in six cells from different gallbladders microinjected with 1.7 mM dextran-bound fura-2 (see Methods). Shortly after lowering [Na⁺], the 340/380-nm excitation ratio rises and reaches a peak at 38 ± 5 s. Thereafter, the ratio returns to lower values, but always remains above control levels. Two examples are presented in Fig. 8. In four of the six cells 340/380 nm ratio were observed after the initial peak (left trace). For additional details see text.

![Figure 8](image-url)
studied the ratio underwent a second peak or actually oscillated. Based on the calibration in vitro of dextran-bound fura-2 and using a viscosity correction factor of 20% (Rink, 1988), free [Ca\(^{2+}\)], increased from 41 ± 13 nM to a peak value of 81 ± 13 nM (P < 0.005), and remained above control 180 s after lowering [Na\(^{+}\)], (54 ± 12 nM, P < 0.005). After 1–3 min in control Ringer, free [Ca\(^{2+}\)], was similar to the levels determined before reducing [Na\(^{+}\)], (41 ± 13 nM). In addition to the known limitations of the method (Grynkiewicz et al., 1985; Rink, 1988), we acknowledge several problems with our free [Ca\(^{2+}\)], estimations: (a) The calculated free [Ca\(^{2+}\)], 41 ± 13 nM, is lower than the values reported using Ca\(^{2+}\)-sensitive microelectrodes (Palant and Kurtz, 1987). (b) The preparation exhibits considerable autofluorescence when excited with ultraviolet light. This was somewhat reduced by dissecting away most of the subepithelial connective tissue, but autofluorescence remained a problem; the signal to autofluorescence ratio under control conditions was similar at 340 and 380 nm, and averaged 2.6 ± 0.3. This ratio could be increased by microinjecting more dextran-bound fura-2 (5 mM, see Methods), but under these conditions the 340/380 nm excitation ratio peaked later (64 ± 11 s, n = 6, P < 0.05 compared with microinjection of 1.7 mM dextran-bound fura-2) and no oscillations were observed, suggesting that Ca\(^{2+}\) was buffered by the microinjected dye. (c) The estimated changes in free [Ca\(^{2+}\)], upon adding 10–20 μM ionomycin were less than expected. The 340/380 nm excitation ratio increased from 0.91 ± 0.10 to 1.73 ± 0.23, which corresponds to a free [Ca\(^{2+}\)], of 170 ± 37 nM (n = 10). This result suggests that ionomycin does not equilibrate intra- and extracellular free [Ca\(^{2+}\)] and cannot be used to perform an in situ calibration of the Ca\(^{2+}\)-sensitive dye. Because of the uncertainty on the actual free [Ca\(^{2+}\)], levels, we emphasize only the qualitative response to lowering [Na\(^{+}\)],.

As indicated in Methods, only one side of the tissue could be superfused. Inasmuch as the Necturus gallbladder epithelium has a high paracellular permeability, it is possible that changes in the [Na\(^{+}\)] in the static mucosal fluid layer can be responsible for the increase in free [Ca\(^{2+}\)]. Therefore, in two experiments we superfused the mucosal side, keeping a static thin layer of solution on the serosal surface. Under these conditions, lowering mucosal [Na\(^{+}\)] produces a late (> 50 s latency), and very slow increase in free [Ca\(^{2+}\)], of 12 nM at 180 s after lowering [Na\(^{+}\)]. These results indicate that the rapid elevation in free [Ca\(^{2+}\)], upon lowering [Na\(^{+}\)], is specific for the serosal side.

**Effects of Lowering [Na\(^{+}\)], on aNa, and pH\(_{i}\)**

Lowering [Na\(^{+}\)], might produce changes in intracellular Na\(^{+}\) activity (aNa) and/or pH, that could be responsible for the increases in apical gK\(^{+}\) and Na\(^{+}\) pump activity (Eaton et al., 1984; Oberleithner et al., 1988; Copello et al., 1991). If there are significant basolateral membrane Na\(^{+}\) pathways sensitive to [Na\(^{+}\)], then aNa should be reduced. In fact, as illustrated in Fig. 9A, lowering [Na\(^{+}\)], reduces aNa. After 3 min of exposure to low Na\(^{+}\), aNa fell by 1.8 ± 0.3 mM (P < 0.02, see Table V). Fig. 9B depicts the effect of lowering [Na\(^{+}\)], on pH\(_{i}\). Intracellular pH fell over 3 min by 0.06 ± 0.02 (P < 0.02, see Table V). The mechanisms responsible for these changes in aNa, and pH\(_{i}\), were not studied further, but decreases in aNa, and pH\(_{i}\), would inhibit
the Na⁺ pump and the cell acidification would reduce, not increase gK⁺ (Eaton et al., 1984; Oberleithner et al., 1988; Copello et al., 1991).

**DISCUSSION**

These studies show that reducing [Na⁺], results in complex changes in membrane voltages in *Necturus* gallbladder epithelium. Regardless of some differences from
tissue to tissue, attributable to the several mechanisms involved, a pattern is evident. Lowering \([\text{Na}^+]_s\) increases free \([\text{Ca}^{2+}]_i\), hyperpolarizes \(V_m\), decreases \(R_n\), and increases \(R_b\).

### Evidence against Basolateral Na\(^+\) Channels

Stoddard and Reuss (1989b) calculated that in *Necturus* gallbladder epithelial cells bathed with 10 mM HCO\(_3^-\)/1% CO\(_2\) Ringer solution, gCl\(^-\), and gK\(^+\) contribute \(\approx\) 50% each to the basolateral membrane conductance. Further, \(E_{n_a}\) is far removed from \(E_{n_s}\), suggesting that if a basolateral Na\(^+\) membrane conductance exists, it is very small (Stoddard and Reuss, 1989b). The experiments with pharmacological inhibitors of Na\(^+\) channels support this idea. Neither amiloride nor TTX had any effects on cell membrane voltages or on the hyperpolarization of \(V_m\) elicited by lowering \([\text{Na}^+]_s\). We conclude that it is unlikely that basolateral Na\(^+\) channels contribute to the membrane voltage under control conditions, or to the hyperpolarization of \(V_m\) produced by lowering \([\text{Na}^+]_s\).

#### Table V

<table>
<thead>
<tr>
<th></th>
<th>(V_m)</th>
<th>(V_a)</th>
<th>aNa(_i)</th>
<th>pH(_i)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-0.2 ± 0.3</td>
<td>-65 ± 4</td>
<td>11.2 ± 3.1</td>
<td>7.37 ± 0.03</td>
</tr>
<tr>
<td>Low ([\text{Na}^+]_s)</td>
<td>18.4 ± 1.0*</td>
<td>-80 ± 4*</td>
<td>9.3 ± 2.8*</td>
<td>7.31 ± 0.03*</td>
</tr>
<tr>
<td>Recovery</td>
<td>-0.4 ± 0.3</td>
<td>-68 ± 4</td>
<td>10.9 ± 2.8</td>
<td>7.36 ± 0.03</td>
</tr>
</tbody>
</table>

Values in low \([\text{Na}^+]_s\), Ringer were determined after 3 min of reducing \([\text{Na}^+]_s\) from 100.5 to 10.5 mM (TMA\(^+\) substitution). Recovery values were measured 4–8 min after reexposure to control Ringer. aNa\(_i\) and pH\(_i\) were measured in different tissues (n = 4 and n = 7, respectively). Voltage values were not different between both series of experiments and were pooled. Values are means ± SEM. *P < 0.05 or better compared with control and recovery periods. For abbreviations and experimental protocols see Table I and Fig. 9, respectively.

### Mechanism of the Decrease in \(fR_n\)

Lowering \([\text{Na}^+]_s\) produces a dramatic decrease in \(fR_n\). The two-point cable analysis experiments indicate that \(R_n\) decreases and \(R_b\) increases. The decrease in \(R_n\) is the dominant mechanism, at least initially. The contribution of the increase in \(R_b\) is evident at 20 s and much greater at 180 s after lowering \([\text{Na}^+]_s\). The decrease in \(fR_n\) upon lowering \([\text{Na}^+]_s\) is substantially reduced by 5 mM TEA\(^+\), suggesting that the change in \(fR_b\) is brought about by activation of apical membrane maxi K\(^+\) channels (Segal and Reuss, 1990a, b). The simplest explanation is that when serosal solution Na\(^+\) is partially replaced by TMA\(^+\) or NMDG\(^+\), the serosa-positive paracellular diffusion potential causes apical membrane depolarization and hence activates the apical maxi K\(^+\) channels. However, two arguments indicate that depolarization of \(V_m\) is not the main mechanism accounting for the decrease in \(fR_n\): First, the fall in \(fR_n\) is also observed in experiments in which \(V_m\) is near control values, or even hyperpolarized at the time of the \(fR_n\) measurements (see Results). Second, when similar \(V_m\)
Depolarizations are produced by lowering serosal solution pH or increasing serosal [K⁺], a much smaller decrease in fRₘ is observed (i.e., 0.05 ± 0.02 by lowering HCO₃⁻, n = 6 [data not shown], and 0.19 ± 0.03 by lowering [Na⁺], in 10 mM HCO₃⁻-Ringer [see Stoddard and Reuss, 1989b]). Elevations of cytosolic free [Ca²⁺] also activate apical membrane maxi K⁺ channels (Segal and Reuss, 1990a) and increase apical membrane gK⁺ (Bello-Reuss et al., 1981; García-Díaz et al., 1983). Since lowering [Na⁺], increases free [Ca²⁺], it seems reasonable to conclude that the latter is the main cause of the initial decrease in Rₘ. The depolarization of Vₘ may contribute to the fall in Rₘ, especially when free [Ca²⁺] is below peak values (Fig. 1, phase c).

In several cell types, lowering external [Na⁺] raises free [Ca²⁺], by reducing Ca²⁺ efflux through the Na⁺/Ca²⁺ exchanger (Grinstein and Erlij, 1978; see also Chase, 1984 and Lorenzen et al., 1984) and/or by Ca²⁺ release from intracellular stores (Smith et al., 1989). We have not studied the mechanism of the elevation in free [Ca²⁺], but it was recently reported that replacing serosal solution Na⁺ with NMDG⁺ in Necturus gallbladder produced an increase in free [Ca²⁺], qualitatively similar to the change reported here (Dillard and Finn, 1991). Based on its dependence on the extracellular [Ca²⁺], the early rise in free [Ca²⁺] was thought to be due to release from intracellular stores, and the sustained elevation to Ca²⁺ influx (Dillard and Finn, 1991).

Mechanism of the Hyperpolarization of Vₘ

The present results indicate that the mechanism of the hyperpolarization of Vₘ by lowering [Na⁺], is complex and that the relative contributions of different factors vary with time. The late phase of the hyperpolarization of Vₘ (phase c) is mainly caused by: (a) the fall in fRₘ and the elevation in intraepithelial current flow (due to both the change in Vₑ and the decrease in transcellular resistance), and (b) the increase in TEA⁺-sensitive apical gK⁺. This conclusion is based on the effects of apical TEA⁺, which reduces the plateau hyperpolarization of Vₘ by ~70% while reducing the peak hyperpolarization by only ~30%. The mechanisms of the peak hyperpolarization of Vₘ (phase b) are discussed below.

The TEA⁺-sensitive apical K⁺ channels (via increases in Eₑ and intraepithelial current flow, and a decrease in fRₘ) account for about one-third of the hyperpolarization of Vₘ; therefore, their activation clearly is not the only mechanism involved. Addition of ouabain during exposure to low [Na⁺], medium caused a larger depolarization than under control conditions. An increased contribution of the Na⁺ pump current to Vₘ is expected even in the absence of pump stimulation because of the increase in Rₘ, but stimulation of the pump (Nakao and Gadsby, 1989) could also contribute to the hyperpolarization of Vₘ. During exposure to low-[Na⁺], in the continuous presence of mucosal TEA⁺, the K⁺ selectivity of the basolateral membrane increases transiently. The increase in basolateral K⁺ selectivity, together with an increase in Rₘ, strongly suggests that a decrease in basolateral gCl⁻ is the dominant mechanism of the change in K⁺ selectivity. The possibility of a concomitant increase in basolateral gK⁺, brought about by the elevation in free [Ca²⁺], seems reasonable since A23187 and cyanide increase basolateral gK⁺ in Necturus gallbladder epithelial cells (Bello-Reuss et al., 1981). 2 min after lowering [Na⁺], basolateral K⁺ selectivity
returns to control and $R_b$ is even greater than at the peak of the hyperpolarization of $V_c$ (Table IV), suggesting that, at the basolateral membrane, both $g_{K^+}$ and $g_{Cl^-}$ are reduced. The mechanism of the late fall in basolateral $g_{K^+}$ is unknown. The effects of low [Na$^+$], on basolateral membrane ionic conductances could be related to a decrease in cell volume produced by KCl efflux (via apical maxi K$^+$ channels and basolateral Cl$^-$ channels). However, preliminary results indicate that there are no measurable changes in cell volume 20 s after lowering [Na$^+$]. Alternatively, the decrease in basolateral $g_{Cl^-}$ may be due to the hyperpolarization of $V_c$, or the increase in free [Ca$^{2+}$], Cl$^-$ channels inactivated by hyperpolarization and elevation in free [Ca$^{2+}$], have been described (Akaike, 1990; Weiss and Magleby, 1990), but the properties of the Cl$^-$ conductive pathway(s) at the basolateral membrane of *Necturus* gallbladder epithelium are not known.

### Summary and Conclusions

Lowering [Na$^+$], produces a marked decrease in $fR_a$ and hyperpolarization of $V_c$. The dominant effect accounting for the decrease in $fR_a$ is a rapid reduction in $R_a$ due to activation of TEA$^+$-sensitive maxi K$^+$ channels at the apical membrane; there is also the contribution of an increase in $R_b$, which becomes more important at later times. The increase in free [Ca$^{2+}$]$_i$ seems to play a central role in the activation of apical membrane maxi K$^+$ channels. Several factors participate in the hyperpolarization of $V_c$: (a) activation of the TEA$^+$-sensitive maxi K$^+$ channels responsible for the decrease in $fR_a$, (b) increase in the ouabain-sensitive fraction of $V_c$, (c) transient TEA$^+$-insensitive increase in the K$^+$ selectivity of the basolateral membrane mainly due to a decrease in basolateral $g_{Cl^-}$, and (d) increase in intraepithelial current flow due to the serosa-positive change in $V_{in}$.

We thank J. Copello, S. Lewis, and S. Weinman for their comments on a preliminary version of the manuscript, K. Dawson and B. Perry for technical assistance, and L. Durant for secretarial help.

This work was supported by National Institutes of Health grant DK-38734.

Original version received 2 July 1991 and accepted version received 17 October 1991.

### References


ALTENBERG ET AL. Effects of Basolateral [Na+] in Necturus Gallbladder Epithelium


