Anion-sensitive Sodium Conductance in the Apical Membrane of Toad Urinary Bladder

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ABSTRACT Membrane potentials and the electrical resistance of the cell membranes and the shunt pathway of toad urinary bladder epithelium were measured using microelectrode techniques. These measurements were used to compute the equivalent electromotive forces (EMF) at both cell borders before and after reductions in mucosal Cl⁻ concentration ([Cl]ₘ). The effects of reduction in [Cl]ₘ depended on the anionic substitute. Gluconate or sulfate substitutions increased transepithelial resistance, depolarized membrane potentials and EMF at both cell borders, and decreased cell conductance. Iodide substitutions had opposite effects. Gluconate or sulfate substitutions decreased apical Na conductance, whereas iodide replacements increased it. When gluconate or sulfate substitutions were brought about in the presence of amiloride in the mucosal solution, apical membrane potential and EMF hyperpolarized with no significant changes in basolateral membrane potential or EMF. It is concluded that: (a) apical Na conductance depends, in part, on the anionic composition of the mucosal solution, (b) there is a Cl⁻ conductance in the apical membrane, and (c) the electrical communication between apical and basolateral membranes previously described is mediated by changes in the size of the cell Na pool, most likely by a change in sodium activity.

Singer and Civan (1971) showed that Cl substitutions in the solutions bathing toad urinary bladder resulted in significant changes in mucosa-to-serosa sodium flux and in the electrical parameters of the tissue. The ability of anions to modify Na transport was related to the position of the anion in the lyotropic series. However, it could not be established whether the anions were acting on sodium entry through the apical membrane or upon active Na extrusion at the basolateral side (Singer and Civan, 1971). In addition, it has been concluded from studies of transepithelial fluxes of isotopes that the movements of Cl⁻ across the tissue are passive and paracellular (Saito et al., 1974; Finn and Bright, 1978) and from experiments involving ³⁶Cl equilibration between cellular and extracellular compartments that little or no chloride enters the cell from the mucosal medium (MacKnight, 1977). On the other hand, an active mucosa-to-serosa Cl flux was found when K was removed from the
serosal medium (Finn et al., 1967) and when maneuvers that included removing Na or adding ouabain or amiloride to the solutions bathing toad urinary bladders (Soboslai et al., 1977) were carried out; thus, at least under those conditions, Cl⁻ presumably enters the cells. We have recently shown that the apical electromotive force in toad urinary bladder is a direct function of sodium selectivity, indicating that the apical membrane is permeable to ions other than Na⁺ (Narvarte and Finn, 1980). In this paper, we report the effect of mucosal chloride substitutions in the mucosal medium on the electrical parameters of toad urinary bladder. Our results show that there is a Cl⁻ conductance in the mucosal membrane and that apical sodium conductance is sensitive to the anionic composition of the mucosal medium. They also suggest that the communication between apical and basolateral membranes previously described (Reuss and Finn, 1975b) is mediated by changes in cell Na activity or some other function of the size of the sodium transport pool.

**METHODS**

Mexican toads (*Bufo marinus*) were obtained from the Charles P. Chase Co. (Miami, Fla.), the Pet Farm (Miami, Fla.), or the Jacques Well Co. (Rayne, La) and kept in running tap water. Bladders were excised and mounted mucosal side upward in a Lucite chamber, as previously described (Reuss and Finn, 1974), with Ringer's solution bathing both sides of the tissue. A nylon mesh was used to support the serosal side of the bladder, and a small negative pressure was applied to the lower half of the chamber to change continuously the bathing solution and keep the tissue against its support.

**Solutions**

Standard Ringer's solution had the following composition (mM): NaCl 110, KCl 2.5, NaHCO₃ 2.4, CaCl₂ 0.9, glucose 5.5, pH about 8.5, gassed with room air. Cl was replaced with sulfate, gluconate, or iodide. Sucrose was used to keep constant osmolality in sulfate replacements. Amiloride (a gift from Merck, Sharp and Dohme Research Laboratories, West Point, Pa.) was dissolved in the Ringer's solution to a final concentration of 10⁻⁵ or 10⁻⁴ M.

**Electrical Measurements**

(a) Transepithelial potential (Vₑₑₑ) and resistance (Rₑₑₑ). Vₑₑₑ was measured with a high-impedance electrometer connected to both bathing media with Ag-AgCl pellets and agar-Ringer bridges. Corrections for liquid junction potentials were made. Rₑₑₑ was measured from the change in Vₑₑₑ produced by a transepithelial DC pulse of 2.5 to 17 μA/cm².

(b) Cell membrane potential. Apical (Vₐₐₐ) and basolateral (Vₑₑₑ) membrane potentials were measured with glass microelectrodes prepared by pulling glass tubing (1 mm o.d., 0.6 mm i.d.) threaded with fiber glass and filled with 4 M potassium acetate. The microelectrodes were beveled to tip resistances of 10–30 MΩ and tip potentials were <5 mV. The impalements were performed with mechanical or motor-driven micromanipulators under visual control with an inverted, phase-contrast microscope (Leitz, Wetzlar, W. Germany) at × 200. The criteria for successful impalements were as previously described (Reuss and Finn, 1974). Potentials were measured with M-4 electrometer probes (W-P Instruments, Inc., New Haven, Conn.) and displayed on a
storage oscilloscope (Tektronix, Inc., Beaverton, Oreg.). An Ag-AgCl electrode connected to the serosal solution by means of a short agar-Ringer bridge was grounded and used as reference.

(c) Cell membrane and shunt ($R_s$) resistances. The resistances of the cell membranes (apical, $R_a$; basolateral, $R_b$) and $R_s$ were computed from the values of $R_t$ and $R_a/R_b$ before and after the addition of amiloride to the mucosal solution. The ratio $R_a/R_b$ is calculated from the ratio $\Delta V_{mc}/\Delta V_{es}$ upon passing a transepithelial DC pulse. Assuming that the only effect of amiloride is to increase the resistance of the apical membrane, $R_a$, $R_b$, and $R_s$ can be calculated as previously described (Reuss and Finn, 1974 and 1975a; Finn and Reuss, 1975; Narvarte and Finn, 1980). Apical Na conductance was calculated as $g^{Na}_a = g_a - g_a^s$, where $g_a$ and $g_a^s$ are the conductance of the apical membrane before and after the addition of amiloride to the mucosal solution, respectively; for this measurement a saturating concentration of amiloride must be used in order to reduce $g^{Na}_a$ to zero, avoiding any contribution of $g^{Na}_a$ to $g_a^s$ that would exist if smaller concentrations of amiloride were used.

(d) Calculation of the equivalent electromotive forces (EMFs) generated at the cell membranes and the shunt. The cell membrane and the shunt can each be represented by a battery (EMF) in series with an equivalent resistor. Each EMF corresponds to the potential that would be measured across a given membrane or the shunt if it were the only element in the circuit. With the values of $V_{mc}$, $V_{es}$, $R_a$, $R_b$, and $R_s$, apical ($V_a$) and basolateral ($V_b$) EMFs can be computed using equations published previously (Narvarte and Finn, 1980). $V_a$ was assumed to be zero, because bulk solution concentrations were the same in both media under control conditions, and shunt permeability to sulfate, iodide, and gluconate was assumed to be equal to that of CI. The finding that symmetrical substitutions give results identical to those of mucosal substitutions indicates that the last assumption does not introduce an important error. For convenience, all potentials and EMFs will be presented with the mucosal solution as reference. In this way, when the polarity of the tissue is the one observed in control conditions (cell positive to the mucosal and negative to the serosal medium), all potentials and EMFs have a positive sign.

(e) Experimental protocol. Impalements were begun 60 min after the bladder had been mounted, when both $V_{mc}$ and $R_t$ were stable. The values of $R_t$, $R_a/R_b$, $V_{mc}$, and $V_{es}$ were measured (in at least five cells in each condition) before and after amiloride was added to the mucosal solution; the medium was then replaced with an experimental solution, and the sequence was repeated. The first impalement was usually performed within 1 min after the change in solution. The experiments were performed under open circuit conditions.

(f) Statistics. All results are expressed as means ± SE. Differences between means were analyzed with Student's $t$ test.

**RESULTS**

Fig. 1 shows the time-course of $V_{mc}$ before and after reductions in mucosal CI$^-$ concentration ([Cl]$m$). When gluconate or sulfate replaced CI$^-$, the change in $V_{mc}$ was in the depolarizing direction, whereas the opposite was true with iodide substitutions. The changes in $V_{mc}$ were fast and reversible, reaching, as shown on the upper trace, a steady state within 1 min. In some preparations, there was a small decline after the peak change; it was not a general observation, and most of the tissues behaved as in the other experiments shown in the figure. Changes in $R_t$ and membrane potentials (see below) followed the same time-course illustrated in Fig. 1.
Fig. 2 shows the changes in transepithelial electrical parameters and EMF when [Cl]_m was reduced to 12 mM. Gluconate and sulfate substitutions gave identical results and were combined. It is clear that the direction of the change depends on the substitute used. With gluconate or sulfate, there is an increase in $R_t$, with depolarization of $V_m$ and both EMFs, whereas iodide substitutions elicit the reverse responses. To rule out the possibility that the results obtained
with gluconate or sulfate substitutions were explained by reduction in Ca activity in those solutions (Christoffersen and Skibsted, 1975; Kenyon and Gibbons, 1977), [Ca++] was increased to 4.5 mM in low-Cl- solutions. The changes in electrical parameters obtained in three bladders under those experimental conditions were not significantly different from the ones reported in Fig. 2.1

Table I shows that the changes in $V_m$ illustrated in Fig. 1 are explained by changes at both cell borders; it can be seen that with sulfate or gluconate substitutions membrane potentials and EMFs at both apical and basolateral membranes depolarize, whereas the opposite is true (and the changes are smaller) for iodide replacements. The different effects on $R_t$ are explained by changes in cellular conductance in opposite directions. Iodide substitutions cause increases in apical ($g_a$) and basolateral ($g_b$) conductances, but when gluconate or sulfate is used, the conductance at both cell borders decreases. It

Results similar to those shown in Fig. 2 were obtained with symmetrical replacements in both media. We have reported (Narvarte and Finn, 1979) that Cl- reductions in the serosal fluid cause transient hyperpolarization of both membranes and a decrease in $R_t$, regardless of the substitute used. Furthermore, the decrease in $R_t$ is largely due to an increase in $g_a^{Na}$. Therefore, the fact that bilateral Cl- substitutions gave results identical to those of mucosal Cl- reductions indicates that: (a) the iodide-induced enhancement of $g_a^{Na}$ observed with mucosal substitutions is not additive to the serosal effect and may imply a common site of action, and (b) the inhibition of $g_a^{Na}$ caused by mucosal gluconate or sulfate substitutions cannot be overcome by serosal replacements.
is also shown in Table I that with both types of anionic substitutions, shunt conductance decreases. As Fig. 3 illustrates, the changes in EMFs brought about by gluconate of sulfate substitutions are not larger the lower the [Cl]_m (or the higher the concentration of the substitutes); in fact, the tendency of the relationship (if any) is in the opposite direction. The changes in V_m and membrane potentials showed the same lack of dependency on [Cl]_m, i.e., the

|                | V_m' | V_m | V_a | g_m | g_a | g_s | n  |
|----------------|------|-----|-----|-----|-----|-----|----|
| 114 mM [Cl]_m | 25.4 | 34.4| 35.7| 40.5| 0.22| 0.37| 0.03| 4  |
| ±6.1          | ±5.7 | ±5.6| ±4.9| ±0.06| ±0.12| ±0.01|    |
| 12 mM [Cl]_m  | 31.6 | 40.0| 38.6| 44.2| 0.32| 0.44| 0.02|    |
| (Iodide)      | ±5.6 | ±5.8| ±3.8| ±4.7| ±0.09| ±0.11| ±0.01|    |
| Δ              | 6.2  | ±1.1| ±1.3| ±0.04| ±0.01| ±0.20| ±0.07| ±0.01|
| P              | <0.002| >0.05| >0.20| >0.10| <0.05| <0.01|    |
| 114 mM [Cl]_m | 38.4 | 33.9| 42.9| 46.3| 0.41| 0.50| 0.07|    |
| ±3.7          | ±4.4 | ±3.3| ±3.6| ±0.06| ±0.11| ±0.01|    |
| 12 mM [Cl]_m  | 21.1 | 28.7| 30.1| 38.9| 0.36| 0.35| 0.05| 10 |
| (Sulfate or gluconate) | ±3.4 | ±4.1| ±3.0| ±3.2| ±0.07| ±0.07| ±0.01|    |
| Δ              | ±7.3 | ±4.2| ±12.8| ±7.4| ±0.05| ±0.15| ±0.02| ±0.01|
| P              | <0.001| <0.01| <0.01| <0.01| <0.05| <0.01| <0.01| 0.01|

Δ = value in 12 mM [Cl]_m - value in 114 mM [Cl]_m.

Figure 3. Effects of [Cl]_m on apical (bar) and basolateral (crosshatched bar) EMFs. [Cl]_m was reduced to 60, 12, or 0 mM using sulfate or gluconate as substitutes. The effect of Cl⁻ substitutions on EMFs is shown as ΔEMF = EMF in low [Cl]_m - EMF in 114 mM [Cl]_m. Each value represents the mean ± SEM of at least eight bladders.

changes in V_m, V_mc, and V_cs were not larger the lower the [Cl]_m. On the other hand, the changes in R_t were larger the lower the [Cl]_m, as was also the case with R_a.

We have shown (Finn, 1974; Narvarte and Finn, 1980) that the changes in V_m and V_a brought about by reducing mucosal Na⁺ concentration are a
direct function of the baseline $V_{ma}$ or $V_a$. Those relationships are explained by the fact that baseline $V_a$ is a function of apical Na$^+$ selectivity, so that the higher the baseline $V_a$, the larger the apical Na$^+$ selectivity and the larger the change in $V_{ma}$ and $V_a$ brought about by the change in Na concentration (Narvarte and Finn, 1980). To investigate whether a similar relationship is present when $[Cl]_m$ is reduced, we plotted the change in $V_a$ brought about by sulfate or gluconate substitutions against the baseline $V_a$ before the change in solution. As is shown in Fig. 4 there is a direct relationship between $\Delta V_a$ and $V_a$, i.e., the larger the baseline $V_a$ the larger the depolarization of $V_a$ after a reduction in $[Cl]_m$.

![Figure 4](image_url)

**Figure 4.** Relationship between $V_a$ and $\Delta V_a$. Baseline apical EMF (ordinate) is plotted as a function of the change in $V_a$ brought about by lowering $[Cl]_m$ from 114 mM to 60, 12, 0 mM by the use of gluconate or sulfate as substitutes. ●, 0 mM $[Cl]_m$, sulfate; ○, 0 mM $[Cl]_m$, gluconate; ▲, 12 mM $[Cl]_m$, sulfate; □, 12 mM $[Cl]_m$, gluconate; △, 60 mM $[Cl]_m$, sulfate; ■, 60 mM $[Cl]_m$, gluconate. $\Delta V_a = V_a$ in low $[Cl]_m - V_a$ in 114 mM $[Cl]_m$.

Because the changes in membrane potentials and EMFs after reductions in $[Cl]_m$ were accompanied by changes in $g_a$ in the same direction, the different effects of the substitutes on the electrical parameters can be explained by opposite effects of the replacing anions on apical Na conductance ($g_a^{Na}$), and, hence, on $g_a$ and $V_a$. Table II shows $g_a^{Na}$ when $[Cl]_m$ was reduced to 12 mM. It can be seen that $g_a^{Na}$ increased with iodide substitutions by $38 \pm 6\%$, whereas the change with gluconate or sulfate substitutions was in the opposite direction and nonsignificant; that is, $g_a^{Na}$ decreased by $11 \pm 9\%$. Furthermore, none of the substitutions led to a significant change in nonsodium conductance. In the gluconate or sulfate substitutions, $g_a^{Na}$ was measured using a nonsaturating concentration of amiloride ($10^{-5}$ M). Under those conditions, the value of
$g^{Na}_{a}$ will be underestimated (see Methods). This can explain the finding of a nonsignificant change in $g^{Na}_{a}$ with gluconate or sulfate substitutions; i.e., the possibility exists that gluconate or sulfate replacements elicited changes in $g^{Na}_{a}$ that we could not measure accurately enough because of the underestimation of $g^{Na}_{a}$. To test this hypothesis we repeated those experiments in five bladders using $10^{-4}$ M amiloride. When $[Cl]_m$ was reduced to zero by replacing it with sulfate or gluconate, $g^{Na}_{a}$ decreased from $0.12 \pm 0.02$ to $0.08 \pm 0.02$ mS/cm$^2$, a change of $34 \pm 10\%$ ($P < 0.05$). On the other hand, $g_{a}$ went from $0.06 \pm 0.01$ to $0.05 \pm 0.01$ ($P > 0.20$).

Because the effect of Cl substitutes on $g^{Na}_{a}$ will mask any effect that changes in apical Cl EMF ($E^{Cl}_{a}$) would elicit on the electrical parameters of the tissue (see Discussion), it is important to study the effect of $[Cl]_m$ reductions on $V_a$ under conditions where $g^{Na}_{a}$ is reduced to zero. To accomplish this, measurements were made comparing the electrical parameters in $114$ mM $[Cl]_m$ plus amiloride with those in $0$ mM $[Cl]_m$ plus amiloride, using gluconate or sulfate as substitutes. These results are illustrated in Fig. 5. When $g^{Na}_{a}$ is blocked by amiloride, mucosal Cl$^-$ replacements by gluconate or sulfate caused $V_{mc}$ and $V_a$ to increase, as would be expected if passive diffusion of Cl occurs across the apical membrane. $V_a$ increased from $-29.0 \pm 8.0$ to $-14.9 \pm 5.1$ mV ($P < 0.05$).

### Table II

| Cl Substitutes | $g^{Na}_{a}$ | $g_{a}$ | $g^{Na}_{a} - g_{a}$ | $n$ |
|---------------|-------------|---------|---------------------|----|
| 114 mM $[Cl]_m$ | $0.22 \pm 0.06$ | $0.06 \pm 0.01$ | $0.16 \pm 0.05$ | 4  |
| $12$ mM $[Cl]_m$ | $0.32 \pm 0.09$ | $0.08 \pm 0.02$ | $0.23 \pm 0.08$ | 4  |
| Iodide | | | | |
| $\Delta$ | $0.10 \pm 0.04$ | $0.02 \pm 0.01$ | $0.07 \pm 0.03$ | 4  |
| $P$ | $<0.05$ | $>0.05$ | $<0.05$ | 4  |
| 114 mM $[Cl]_m$ | $0.41 \pm 0.09$ | $0.11 \pm 0.02$ | $0.30 \pm 0.07$ | 10 |
| $12$ mM $[Cl]_m$ | $0.36 \pm 0.08$ | $0.10 \pm 0.02$ | $0.26 \pm 0.06$ | 10 |
| Sulfate or gluconate | | | | |
| $\Delta$ | $-0.05 \pm 0.02$ | $-0.01 \pm 0.01$ | $-0.04 \pm 0.02$ | 4  |
| $P$ | $<0.05$ | $>0.80$ | $>0.05$ | 4  |

$\Delta = value$ in $12$ mM $[Cl]_m$ $- value$ in $114$ mM $[Cl]_m$.

When either a saturating concentration of amiloride is added to or Na$^+$ is removed from the mucosal solution bathing urinary bladders obtained from some subspecies of toads, $V_{ma}$, $V_{mc}$, and short circuit current become negative (Ludens and Fanestil, 1972; Ziegler et al., 1976), and the potential profile of the tissue changes from a “stair-step-like” to a “well” type, i.e., the cell becomes negative with respect to both bathing media (Reuss and Finn, 1974; Sudou and Hoshi, 1977). The reversed polarity of the tissue has been attributed to H$^+$ secretion into the mucosal fluid (Ludens and Fanestil, 1972). This change in polarity was also observed in the tissues exposed to amiloride in our studies. Therefore, to describe the results illustrated in Fig. 5, the term “increase” in PD is used to mean that the cell becomes less negative with respect to the mucosal solution.
NARVARTE AND FINN  Na Conductance in Toad Urinary Bladder Apical Membrane 77

Whereas $V_{mc}$ went from $-21.0 \pm 4.6$ to $-11.6 \pm 2.3$ mV ($P < 0.05$), $V_{mc}$ went from $-22.5 \pm 4.6$ to $-12.4 \pm 5.5$ mV. The magnitude of the increase in $V_a$ and $V_{mc}$ in the presence of amiloride varied considerably from tissue to tissue; e.g., the range of $\Delta V_a$ was 3-27 mV with sulfate or gluconate as substitutes, and 6-26 mV when iodide replaced $\mathrm{Cl}^-$, although the direction of the change was always the same, no matter which replacement anion was used. As Fig. 5 also shows, when $[\mathrm{Cl}]_m$ reductions were carried out in the presence of amiloride, no significant changes were found in $V_a$ and $V_b$, despite the marked changes in $V_{mc}$ and $V_a$. When gluconate or sulfate replaced $\mathrm{Cl}^-$ in

![Figure 5](image-url)

**Figure 5.** Effects of reductions in $[\mathrm{Cl}]_m$ on membrane potentials and EMFs with (crosshatched bar) and without (bar) amiloride in the mucosal solution. The changes in $V_{mc}$, $V_a$, $V_b$, and $V_c$ brought about by replacing $\mathrm{Cl}^-$ with sulfate or gluconate in the mucosal solution are shown for bladders incubated under control conditions and for tissues exposed to $10^{-4}$ M amiloride on the mucosal side. Each value is the mean $\pm$ SEM of at least six preparations. $\Delta V = V$ in low $[\mathrm{Cl}]_m - V$ in 144 mM $[\mathrm{Cl}]_m$.

The presence of amiloride, $V_a$ went from $5.5 \pm 2.3$ to $7.3 \pm 1.6$ mV ($P > 0.20$), and $V_b$ from $5.6 \pm 1.7$ to $6.8 \pm 1.9$ mV ($P > 0.20$). When iodide was the substitute, with amiloride present, $V_a$ went from $12.8 \pm 1.7$ to $8.9 \pm 1.5$ mV ($P > 0.20$), and $V_b$ from $12.3 \pm 1.7$ to $8.8 \pm 1.5$ mV ($P > 0.20$). It has been shown (Reuss and Finn, 1975 b; Finn and Reuss, 1978; Narvarte and Finn, 1980) that $V_a$ (and $V_b$) change within milliseconds after changes in $V_{mc}$ and $V_a$ induced by alterations in mucosal Na$^+$ concentration or by the addition of amiloride to the mucosal solution. The fact that changes in $V_a$ and $V_b$ parallel the changes in $V_{mc}$ and $V_a$ in the present experiments only when amiloride was absent (and no anion-dependent alterations in mucosal Na entry were likely to occur) supports the hypothesis that the changes in basolateral
potential are the result of changes in the activity of the Na pump probably secondary to the changes in cell Na activity (Narvarte and Finn, 1980).

**DISCUSSION**

**Apical Membrane Effects**

The changes in electrical parameters elicited by reductions in \([Cl]_m\) depended on the substitutes used to replace Cl. Iodide substitutions caused hyperpolarization of \(V_{ms}\) and a decrease in \(R_t\); depolarization of \(V_{ms}\) and increased \(R_t\) were the findings with gluconate or sulfate replacements. Singer and Civan (1971) showed that Cl replacements in the mucosal medium of toad urinary bladder caused changes in \(V_{ms}\), \(R_t\), short circuit current, and mucosa-to-serosa Na flux that were related to the position of the Cl substitute in the lyotropic series. Iodide increased both short circuit current and active Na flux, whereas the opposite was true with sulfate. They concluded that anions have important roles in determining the electrical properties of toad bladder and that their effects on Na transport may be mediated by interaction with one or more positively charged sites with relatively weak field strength. However, they could not localize the effects of anions to either membrane. More recently, somewhat similar findings have been reported in rabbit colon (Turnheim et al., 1977). These authors found that sulfate and certain organic anions were able to stimulate active Na transport when the basolateral Na pump mechanism was not saturated. The most likely mechanism of action of those anions was a selective decrease in the resistance of the amiloride-sensitive Na entry step at the mucosal membrane. Our microelectrode studies show that the changes in \(V_{ms}\) observed with Cl substitutions are explained by effects on both cell membranes. With iodide substitutions we found hyperpolarization of membrane potentials and EMFs, whereas the opposite was true for gluconate or sulfate replacements. The apical membrane in this tissue has the same polarity as the apical Na EMF \(E^{Na}_a = RT/F \ln([Na]_m/[Na]_c)\), i.e., the cell is positive to the mucosal solution (Narvarte and Finn, 1980; Rick et al., 1978). Because cell Cl concentration is low (Rick et al., 1978), \(E^{Cl}_a\) has a polarity opposite to that of \(V_{ms}\), \(V_a\), and \(E^{Na}_a\). If a finite Cl conductance exists in that membrane, \([Cl]_m\) reductions will decrease \(E^{Cl}_a\) and hyperpolarize \(V_a\), assuming no changes in ionic conductances. However, the changes in membrane potentials and EMF were accompanied by changes in tissue conductances. Shunt conductance decreased with all substitutions, indicating that either Cl permeability through the shunt pathway is higher than the shunt permeability to any of the other anions or that Cl somehow controls shunt conductance to cations. The changes in cell conductance, on the other hand, depended on the anion used to replace Cl; iodide substitution increased cell conductance, whereas the opposite was the finding with sulfate or gluconate replacements. We have shown (Narvarte and Finn, 1980) that \(V_a\) is a direct function of \(g^{Na}_a/g^a\). Thus, if no changes occur in \(g^a\), changes in \(g^{Na}_a\) will make \(V_a\) vary in the same direction. We found that anionic substitutions caused changes in apical sodium conductance; the direction and magnitude of these changes can readily explain the effects of Cl replacement on short circuit current and in
mucosa-to-serosa Na flux reported by Singer and Civan (1971). Fig. 3 indicates that the effect of Cl− substitutions on $V_a$ is complex; the fact that the depolarization seen with gluconate or sulfate substitutions is not larger the lower $[\text{Cl}]_m$ suggests that it cannot be explained by a reduction in $g_a^{\text{Na}}$ alone, but that a decrease in $[\text{Cl}]_m$ probably results in hyperpolarization of $V_a$ that partially counteracts the substitute-dependent depolarization. If there is a finite Cl conductance in the apical membrane, Cl reductions should make the cell more positive with respect to the mucosal solution. Because the inhibitory effect of gluconate or sulfate substitutions on $g_a^{\text{Na}}$ would mask any hyperpolarizing effect of low Cl solutions, we performed Cl substitutions with amiloride present in the mucosal solution to obliterate apical sodium conductance. Under those conditions, $V_a$ and $V_{mc}$ increased, i.e., the direction of the change was opposite to that observed when $g_a^{\text{Na}}$ was allowed to vary. The effect of low Cl− solutions on $V_a$ in the presence of amiloride strongly supports the existence of a finite Cl− conductance in the apical membrane. Therefore, the effect of mucosal Cl− substitutions on apical EMF is the result of changes in both $g_a^{\text{Na}}$ and $E_a^{\text{Cl}}$. Throughout our circuit analysis, we have assumed that all ionic resistances are ohmic, i.e., voltage independent. However, it has recently been suggested that this is not the case (Fuchs et al., 1977; Thompson et al., 1979). If that is also true for toad urinary bladder, the effect of anions on Na conductance might be mediated by changes in the electric field in the membrane elicited by the different anions. We do not have any data regarding that hypothesis, except that solutions of different ionic strength gave similar results (gluconate and sulfate plus sucrose), suggesting that the effect of anions was not related to changes in surface charge of the membrane. It is interesting that, even though we found no relationship between $R_a$ or $g_a^{\text{Na}}$ and $V_a$, and a direct one between $g_a^{\text{Na}}/g_a^z$ and $V_a$ (Narvarte and Finn, 1980), we also observed a direct relationship between $V_{mc}$ and $g_a^{\text{Na}}$, which suggests that $g_a^{\text{Na}}$ may be potential dependent in this tissue.³

Both the polarity and the concentration gradient of Cl across the apical membrane favor passive entry of Cl− into the cell in control conditions (Rick et al., 1978); the permeation of Cl− through the apical membrane may then well represent a passive entry step of the active mechanism that transports Cl− from mucosa to serosa in this tissue (Finn et al., 1967; Soboslai et al., 1977). However, no relationship can be established between apical Cl− conductance and active Cl− transport at the present time.

The data in Figs. 3 and 4 indicate that changes in $V_a$ are not significantly different with the three Cl− concentrations used. Because baseline $V_a$ is a direct function of apical sodium selectivity $g_a^{\text{Na}}/g_a^z$ (Narvarte and Finn, 1980), and because no significant changes in $g_a^z$ were found in the present experiments, the relationship in Fig. 4 can be explained if the higher the baseline $g_a^{\text{Na}}$ the greater is the change in $g_a^{\text{Na}}$ brought about by gluconate or sulfate substitutions. The implication of this is that in bladders with low baseline $V_a$ (and hence low $g_a^{\text{Na}}/g_a^z$) the change in $g_a^{\text{Na}}$ after gluconate or sulfate substitutions will be smaller than in bladders with higher baseline $V_a$; thus, the

³ Narvarte, J., and A. L. Finn. Unpublished observations.
hyperpolarizing effect due to the change in $E^c_{\text{Cl}}$ will be more evident, as is illustrated by three tissues in Fig. 4 that hyperpolarized after the replacement of Cl in the mucosal solution.

**Basolateral Membrane Effects**

We have proposed (Narvarte and Finn, 1980) that the changes in basolateral electrical parameters elicited by reductions in mucosal Na concentration can be explained by changes in rheogenic Na extrusion caused by a decrease in the size of the Na transport pool, most likely signalled by a decrease in cell Na activity brought about by the Na substitutions. A similar mechanism can be invoked to explain the changes in basolateral electrical parameters found after mucosal anion substitutions. When no amiloride was present in the mucosal solution, reductions in mucosal Cl concentration caused changes in basolateral membrane electrical parameters in the same direction as those recorded at the mucosal membrane, i.e., hyperpolarization of $V_{ca}$ and $V_b$ with increased $g_{Na}$ in iodide substitutions and depolarization of $V_{ca}$ and $V_b$ and decreased $g_{Na}$ with gluconate or sulfate. Inasmuch as we showed that iodide substitutions are associated with increases in $g_{Na}$ and that the opposite is true for gluconate or sulfate, an increase in the entry of Na is likely to occur in the former case, and a decrease in the latter. The changes in the size of the sodium pool caused by the alterations in Na entry will increase (iodide) or reduce (sulfate or gluconate) the basolateral Na pump rate leading to changes in electrical parameters, as discussed previously (Narvarte and Finn, 1980). This explanation is strongly supported by the results of the experiments in which [Cl]m reductions were performed with amiloride present in the mucosal solution. Under those conditions no changes in Na entry were likely to occur, and no significant changes in $V_b$ or $V_{ca}$ were observed, despite the fact that $V_a$ and $V_{mc}$ changed significantly. These results strongly support the hypothesis that the communication between apical and basolateral membranes previously described (Reuss and Finn, 1975 b; Finn and Reuss, 1978; Narvarte and Finn, 1980) is mediated by changes in cell Na activity.

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REFERENCES

Christoffersen, G. R. J., and L. H. Skibsted. 1975. Calcium ion activity in physiological salt solutions: influence of anions substituted for chloride. *Comp. Biochem. Physiol.* 52A:317–322.

Finn, A. L. 1974. Transepithelial potential difference in toad urinary bladder is not due to ionic diffusion. *Nature (Lond.)* 250:492–496.

Finn, A. L., and J. Bright. 1978. The paracellular pathway in toad urinary bladder: permselectivity and kinetics of opening. *J. Membr. Biol.* 44:67–83.

Finn, A. L., and L. Reuss. 1975. Effects of changes in the composition of the serosal solution on the electrical properties of the toad urinary bladder epithelium. *J. Physiol. (Lond.)* 250:541–558.

Finn, A. L., and L. Reuss. 1978. Electrical interaction between apical and basolateral cell
membranes in toad urinary bladder epithelium: evidence for rheogenic sodium extrusion. *In Membrane Transport Processes*, Vol. 1. J. F. Hoffman, editor. Raven Press, New York. 229-241.

**FINN, A. L., J. S. HANDLER, and J. ORLOFF.** 1967. Active chloride transport in the isolated toad bladder. *Am. J. Physiol.* 213:179-184.

**FUCHS, W., E. HVIID-LARSEN, and B. LINDEMAN.** 1977. Current-voltage curve of sodium channels and concentration dependence of sodium permeability in frog skin. *J. Physiol. (Lond.)* 267:137-166.

**KENN, J. L., and W. R. GIBBONS.** 1977. Effects of low-chloride solutions on action potentials of sheep cardiac Purkinje fibers. *J. Gen. Physiol. 70*:635-660.

**LUDENS, J. H., and D. D. FANESTIL.** 1972. Acidification of urine by the isolated urinary bladder of the toad. *Am. J. Physiol.* 223:1338-1344.

**MACKNIGHT, A. D. C.** 1977. Contribution of mucosal chloride to chloride in toad bladder epithelial cells. *J. Membr. Biol.* 36:55-63.

**NARVATE, J., and A. L. FINN.** 1979. Transient enhancement of Na⁺ transport by Cl⁻ reduction on the serosal side of toad urinary bladder: evidence for rheogenic Na⁺ extrusion. *Fed. Proc.* 38:962.

**NARVATE, J., and A. L. FINN.** 1980. Microelectrode studies in toad urinary bladder epithelium. Effects of Na concentration changes in the mucosal solution on equivalent electromotive forces. *J. Gen. Physiol.* 75:323-344.

**REUSS, L., and A. L. FINN.** 1974. Passive electrical properties of toad urinary bladder epithelium. Intercellular electrical coupling and transepithelial cellular and shunt conductances. *J. Gen. Physiol.* 64:1-25.

**REUSS, L., and A. L. FINN.** 1975a. Effects of changes in the composition of the mucosal solution on the electrical properties of the toad urinary bladder epithelium. *J. Membr. Biol.* 20:191-204.

**REUSS, L., and A. L. FINN.** 1975b. Dependence of serosal membrane potential on mucosal membrane potential in toad urinary bladder. *Biophys. J.* 15:71-75.

**RICK, R., A. DORGE, A. D. C. MACKNIGHT, A. LEAF, and K. THURAU.** 1978. Electron microprobe analysis of the different epithelial cells of toad urinary bladder. Electrolyte concentrations at different functional states of transepithelial sodium transport. *J. Membr. Biol.* 39:257-271.

**SAITO, T., P. D. LIEF, and A. ESSIG.** 1974. Conductance of active and passive pathways in the toad bladder. *Am. J. Physiol.* 226:1265-1271.

**SINGER, I., and M. M. CIVAN.** 1971. Effects of anions on sodium transport in toad urinary bladder. *Am. J. Physiol.* 221:1019-1026.

**SOBOSLAI, G. B., M. McTIGUE, and M. W. WEINER.** 1977. Mechanism of active chloride transport by urinary bladder of the colombian toad. *Am. J. Physiol.* 233:F421-F427.

**SUDDU, K., and T. HOSHI.** 1977. Mode of action of amiloride in toad urinary bladder. An electrophysiological study of the drug action on sodium permeability of the mucosal border. *J. Membr. Biol.* 32:115-132.

**THOMPSON, S. M., Y. SUZUKI, and S. G. SCHULTZ.** 1979. Current-voltage relations of the active Na-transport pathway in the rabbit colon. *Fed. Proc.* 38:1061.

**TURNHEIM, K., R. A. FRIZZELL, and S. G. SCHULTZ.** 1977. Effects of anions on amiloride-sensitive, active sodium transport across rabbit colon, in vitro. Evidence for "Trans-inhibition" of the Na entry mechanism. *J. Membr. Biol.* 37:63-84.

**ZIEGLER, T. W., D. D. FANESTIL, and J. H. LUDENS.** 1976. Influence of transepithelial potential difference on acidification in the toad urinary bladder. *Kidney Int.* 10:279-286.