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Influence of Mucosal Sodium Concentration on Electrical Resistances and Potentials of Toad Bladder (40824)[1]

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The urinary bladder of the toad is a single layer of epithelial cells which lie on a basement membrane supported by thin connective tissue stroma (1, 2). The apical membrane of the cell faces the lumen of the bladder and the basal portion of the basal-lateral membrane lies on the basement membrane. The lateral portion of the basal-lateral membrane borders the intercellular space. Contiguous cells are joined at the zonula occludens. This is a physiologically tight junction with electrical resistance of 10,000–12,000 $\Omega \cdot cm^2$ (3, 4). The bladder actively transports sodium from mucosal to serosal surface, and maintains large transepithelial Na^+ concentration gradients (5). Active Na^+ transport gives rise to transepithelial electrical potential which is the sum of apical and basal-lateral potentials. Previous studies have shown that reduction of mucosal NaCl concentration from 100 to 11 m*M* increases the junctional electrical resistance 2.5-fold but has no effect on cellular resistance (6). The transepithelial potential is also reduced. This is the result of decrease in both apical and basal-lateral potentials. Membrane potentials and resistances have not been studied at concentrations of mucosal Na^+ less than 11 m*M*. This study was undertaken to delineate the upper limits of paracellular resistance. A priori, these were taken to occur at mucosal Na^+ of 1 m*M*. Under certain experimental conditions, serosal to mucosal flux occurs through the active cellular path (7–9). Thus, maintenance of a serosal: mucosal concentration gradient of 100:1 might require increase in cellular resistance. The second experimental objective was to investigate this possibility. The third purpose of the study was to delineate epithelial and membrane potential changes at mucosal $Na^+ < 10$ m*M*. At these concentrations, it would seem possible that change in the epithelial potential might be primarily the result of change of apical potential. The results showed that reduction of mucosal Na^+ from 10 to 1 m*M* produced a twofold increment in shunt resistance and no significant change in cellular resistance. The decrease in epithelial potential was the result of equal decrements of apical and basal-lateral potentials.

Methods. Bufo marinus were obtained from Mogul-Ed Corporation, Oshkosh, Wisconsin. They were kept at room temperature, given free access to water and fed with *Tenebrio* larvae. Bladders were removed after pithing the brain and spinal cord. They were mounted horizontally in a modified Ussing lucite chamber with mucosal surface upward. Edge damage to the bladder from clamping the hemichambers (10) was avoided by separation of bladder from clamping surfaces with Silastic silicone rubber gaskets (RTV, General Electric Corp.). The mucosal hemichamber was open from above to allow entrance of a microelectrode. Hemichamber volume was 2 ml. Ringers gassed with 100% O_2 at 20°C flowed through the lower closed serosal hemichamber by a siphon effect so as to keep the bladder firmly against a supporting tantalum mesh. Gassed Ringers flowed by gravity across the upper mucosal hemichamber. A flow of 2–3 ml/min was achieved by clamps on inflow and outflow tubing. Inflow was to the bottom of each hemichamber and outflow from the top. Mucosal inflow tubing was connected to seven reservoirs with various Na^+ concentrations. Thus by clamp application and re-

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lease, mucosal solution Na^+ concentration could be changed by a rapid flow-through system. Amphibian Ringers contained 100 m*M* Na^+, 4 m*M* K^+, 1.7 m*M* Ca^{2+}, 0.8 m*M* Mg^{2+}, 107 m*M* Cl^-, 1 m*M* HPO_4^{2-}, and 10 m*M* glucose. Na^+ concentration was reduced either by substitution of choline for Na^+, or isosmolal substitution of NaCl with sucrose.

Addition of sucrose to 11 m*M* Na^+ mucosal solution up to isosmolality has no effect on bladder resistances (6). Preliminary experiments in 10 bladders showed no effect of mucosal Cl^- concentration *per se* on transepithelial and membrane potentials in the absence of mucosal Na^+. Concentrations were 100, 20, 10, and 1 m*M*. This was accomplished by isosmolal substitution of SO_4^{2-} for Cl^-. Choline was substituted for Na^+. Potentials responded to $[Na^+]$ only, and did not seem to be affected by other ionic strengths. Results with choline substitutions were identical to those obtained with sucrose replacement.

Microelectrodes were made from cleaned capillary glass tubing and pulled to a fine tip (<1 μm) by a modification of the Nastuk glass puller. They were filled with methanol by gentle vacuum boiling. Methanol was displaced by distilled water, which in turn was displaced by filtered 3 *M* KCl. Tip resistance was 10–20 MΩ and electrodes that had tip potentials >3 mV were discarded.

Calomel electrodes were inserted into the outflow tubing of each hemichamber. The electrodes were connected to a Grass amplifier polygraph and to two microelectrode amplifiers. The microelectrode was connected to the microelectrode amplifiers by a KCl agar bridge, 3 *M* KCl reservoir, and a calomel electrode. Outputs from the amplifiers were delivered to the polygraph. Thus, continuous recordings of three potentials were obtained: epithelial (ψ_t), apical (ψ_a—microelectrode to mucosal solution), and basal lateral (ψ_b—microelectrode to serosal solution).

When ψ_t became stable, the microelectrode was lowered into mucosal solution and the potential microelectrode to serosal solution was compared at 10–12 points in mucosal hemichamber to ψ_t measured by calomel electrodes. These values were always identical to ψ_t and hence eliminated the possibility of edge damage (10). Cell impalement was accomplished with a Leitz micromanipulator under visualization through a Zeiss stereomicroscope with transmitted light. Cell polarity was always positive to mucosal solution and negative to serosal solution. Adequacy of the rather complicated circuitry was demonstrable by $\psi_a + \psi_b = \psi_t$. Through silver–silver chloride electrodes in mucosal and serosal hemichambers, 1 sec pulses of 11.3 $\mu A \cdot cm^{-2}$ were delivered to the bladder from an external current source. Criterium for satisfactory impalement was demonstration of voltage deflections of ψ_a and $\psi_b <$ voltage deflection of ψ_t. This was the most precise of the criteria utilized by Reuss and Finn (3).

The siphon effect of serosal solution flow and the supporting tantalum mesh enabled recording of stable and acceptable potentials for up to 90 min. Similar temporal stability is demonstrable in frog skin (11). Voltage values were determined 15 min after a rapid flow through of mucosal solution. Preliminary experiments showed that there was no further decrease in transepithelial and membrane potentials after 15 min. Microelectrode impalement was performed immediately after each mucosal solution change. Subsequently two more impalements were performed at each mucosal $[Na^+]$, and the three potentials and voltage divider ratios were averaged at each mucosal $[Na^+]$. Potentials of 10 bladders were studied at 100, 20, and 7 m*M* Na^+. Eleven bladders were studied at 10 m*M* Na^+, 8 at 3 m*M* and 12 at 1 m*M*. It has been suggested that impalement of cell from the mucosal side may give rise to a microelectrode shunt of the luminal membrane (12), which would affect both apical potential and resistance. In *Necturus* bladder, this theoretical objection has been overcome by impalement from serosal side (13, 14). In 5 toad bladders mounted with serosal surface upward and with satisfactory ψ_t and R_t, we found it impossible to make cell impalements through the serosal surface.

Transepithelial resistance (R_t), apical resistance (R_a), basal-lateral resistance (R_b)

and shunt resistance (R_s) were measured by methods described previously (3, 4). Shunt resistance was calculated from

$$R_s = \frac{R_t \cdot R'_t \cdot (a' - a)}{R_t \cdot (a' + 1) - R'_t \cdot (a + 1)} \quad [1]$$

R_t was calculated from the voltage deflection ($\Delta\psi_t$) and current density (11.3 μA · cm^{-2}). $a = \Delta\psi a/\Delta\psi b = R_a/R_b$. R'_t and a' are experimental values determined with amiloride (1.25 · 10^{-4} *M*) in mucosal solution. After experimental determination of R_t and R_a/R_b and calculation of R_s, apical and basal-lateral resistances were calculated from the equivalent circuitry of toad bladder.

$$R_t = \frac{(R_a + R_b)\, R_s}{R_a + R_b + R_s} \quad [2]$$

Resistances were determined in 10 bladders at 100 and 10 m*M* mucosal Na^+, 7 at 7 m*M*, 5 at 3 m*M*, and 6 at 1 m*M*.

Results. The response of bladder potentials to decreasing mucosal Na^+ concentrations is shown in Fig. 1. When mucosal Na^+ was reduced from 100 to 10 m*M*, ψ_t decreased from 33 to 22.2 mV. ψ_a decreased from 16 to 11.5 mV and ψ_b from 15 to 8.6. When mucosal Na^+ was reduced to 1 m*M*, ψ_t decreased to 4.8, ψ_a to 2.7, and ψ_b to 2.9. The decrease in apical and basal-lateral potentials was approximately equal at each concentration. At low mucosal Na^+ concentrations, a primary effect on ψ_a was not demonstrable.

The effect of mucosal Na^+ concentration on bladder resistances is shown in Fig. 2. As previously demonstrated, lowering the mucosal Na^+ from 100 to 10 m*M* produced over a twofold increase in shunt resistance 10,424 to 23,543 ohm·cm^2. When mucosal Na^+ was decreased to 1 m*M*, there was an enormous further increase in R_s to 41,030 ohm·cm^2. There was no appreciable change in cellular resistance ($R_a + R_b$). At 100 mM Na^+, mean cellular *R* was 4098 ± SE 574 ohm·cm^2. At 1 m*M* Na^+, cellular resistance was 5099 ± 513 ohm·cm^2. Thus the increase in R_t from 2749 at 100 m*M* mucosal Na^+ to 4657 at 1 m*M* mucosal Na^+ was due to the increase in shunt resistance. The mean increase in R_a when mucosal Na^+ was reduced from 100 to 10 m*M* was 280 ohm·cm^2 ± SE 392. This was not statistically significant. The *P* value was 0.50. The increase in R_a when mucosal Na^+ was decreased from 10 to 1 m*M* was 817 ± 230 ohm·cm^2. In comparison to the enormous increase in R_s, this was very slight. In actuality it was of no statistical significance as *P* was >0.01.

Discussion. The urinary bladder of the toad transports sodium from the mucosal to

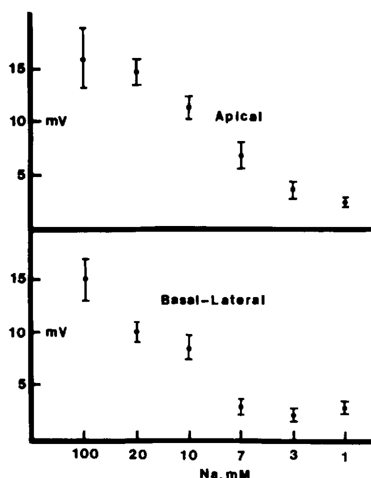

FIG. 1. Apical (ψ_a) and basal-lateral (ψ_b) potentials at six mucosal Na^+ concentrations. Epithelial potential (ψ_t) was $\psi_a + \psi_b$. Values are mean ± SE of 10 bladders studied at 100, 20, and 7 m*M*, 11 at 10 m*M*, 8 at 3 m*M*, and 12 at 1 m*M* mucosal Na^+.

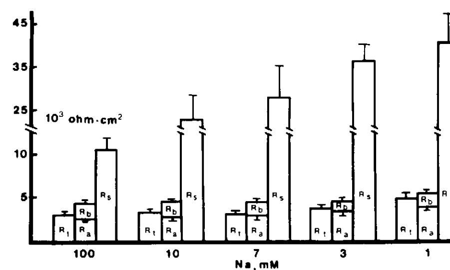

FIG. 2. Epithelial, membrane and shunt resistances at five mucosal Na^+ concentrations. Values are means ± SE of determinations in 10 bladders at 100 and 10 m*M*, 7 at 7 m*M*, 5 at 3 m*M*, and 6 at 1 m*M* mucosal Na^+. R_t = epithelial resistance. R_a = apical membrane resistance. R_b = basal-lateral membrane resistance. R_s = shunt or junctional resistance.

the serosal surface by an active process located at the basal-lateral border of the epithelial cell (5, 15). Activity of the basal-lateral sodium pump is dependent on the metabolic state of the tissue (16) and the delivery of Na^+ (17, 18). Transport creates an electrical gradient with serosal polarity positive. Sodium penetration of the apical cell border is believed to be a passive process, and probably occurs down an electrochemical gradient (15, 19). In the open circuit state, microelectrode studies have shown the epithelial cell polarity positive to the mucosal solution (20, 21). This suggests Na^+ permselectivity of the apical membrane. Thus, apical potential should be a direct function of mucosal Na^+ concentration. In addition to an apical membrane EMF, ψ_a may also contain an IR drop, dependent on basal-lateral pump activity and Na^+ penetration from mucosal solution. Basal-lateral membrane potential is most likely the result of activity of the sodium pump with some contribution from K^+ and Cl^- gradients (19, 22).

Toad bladder is a tight junctional epithelium. Epithelial resistance is about 3000 ohm·cm² (3, 4), although values as high as 75,000 ohm·cm² have been reported (10). Cellular resistance is 5700 to 7000 $\Omega \cdot cm^2$ and is the sum of the apical and basal-lateral resistances in series. In parallel with cell resistance is the shunt or junctional resistance. In toad bladder, this is quite high and ranges between 10,000 to 12,000 $\Omega \cdot cm^2$. Presumably the physiological purpose of the high resistance epithelial junctions is to limit Na^+ back flux. At 100 mM mucosal NaCl, $J^{Na^+}m \rightarrow s$ is 13–14.8 times greater than $J^{Na^+}s \rightarrow m$ (23, 24). Reduction of mucosal NaCl from 100 to 11 mM increases shunt resistance 2.5-fold, but has no effect on cellular resistance (6). Thus, the tightening of the shunt with low mucosal Na^+ would facilitate Na^+ transport by reducing $J^{Na^+}s \rightarrow m$. The sodium conductance of the tight junction is also influenced by mucosal osmolality. Increase in osmolality with either salt or urea will decrease shunt resistance (6) and augment $J^{Na^+}s \rightarrow m$ (7, 25), and thereby limit net Na^+ transport.

The present experiments were undertaken to investigate the upper limits of paracellular junctional resistance. These were postulated to occur at about mucosal Na^+ of 1 mM, and would be required to maintain a maximum physiological concentration gradient of 100:1. As there is some cellular pathway $J^{Na^+}s \rightarrow m$ under certain conditions (8, 9) and a small passive basal lateral Na^+ conductance (22), it was important to eliminate any role of increased cellular resistance in the maintenance of the gradient. The study showed an enormous increase in junctional resistance with mucosal Na^+ of 1 mM, which was approximately fourfold that at 100 mM. There was no increase in cellular resistance.

The results may have been influenced by a high conductance paramicroelectrode shunt of the apical membrane (12). Such methodological artefact would fail to demonstrate an actual increase of voltage divider ratio ($\Delta\psi_a/\Delta\psi_b = R_a/R_b = a$). The high conductance artefactual shunt of apical membrane would minimize or eliminate an increase in $\Delta\psi_a$. From Eq. [1] then an increment in R_t with little or no change of a leads to increase in R_s with little change in R_a and R_b. On the other hand, a change in a will reflect significant changes in cellular resistance (Eqs. [1] and [2]). It seems unlikely however that the postulated microelectrode shunt influenced the results, which correlate well with those from isotopic flux studies. These showed that paracellular conductance was a direct function of $s \rightarrow m$ Na^+ and Cl^- flux, and that both conductance and flux varied directly with mucosal Na^+ and epithelial potential (8, 25, 26, 27, 28). Thus low mucosal Na^+ increased paracellular resistance. Significant $J^{Na^+}s \rightarrow m$ in the cellular pathway is only demonstrable with epithelial potentials of 93–150 mV (8, 28). This suggests considerable rectification of Na^+ flux in the cellular pathway, a condition which would not require increases in cellular membrane resistances to maintain a large concentration gradient at low epithelial potential.

The magnitudes of both epithelial potential and short-circuit current of toad bladder have been shown to be directly related to the sodium concentration of mucosal fluid

(17, 18). Mucosal Na^+ concentration appears to be the major determinant of apical potential. However, mucosal Na^+ concentration was also the major determinant of the basal-lateral potential. With decreasing mucosal Na^+, the decrease in basal-lateral potential was almost identical to that of apical potential. There seems to be a close linkage of apical and basal-lateral potentials and they appear to be functions of each other. Even at 1 m*M* mucosal Na^+, independence of basal-lateral potential from apical potential was not demonstrable. This data suggests modification of the transport model of Koefed-Johnsen and Ussing for frog skin (23) and that of Leb *et al.* for toad bladder (24). The voltage changes at the apical membrane with change in mucosal Na^+ are less than those expected for a pure Na^+ sensitive electrode. To some extent ψ_a is influenced by current flow through the intercellular shunt, as is ψ_b. A factor other than active Na^+ transport that may affect ψ_b is basal-lateral K^+ diffusion potential.

The major decrease in apical potential occurred when mucosal Na^+ was reduced from 10 to 1 m*M* (Fig. 1). From 100 to 10 m*M* mucosal Na^+, the decrease in apical potential was 4.5 mV, while that from 10 to 1 m*M* was 8.8. These data are very similar to that of the effect of decreasing mucosal Na^+ concentration on tissue sodium content, short-circuit current and mucosal to serosal sodium flux. Frazier *et al.* (17, 18) have shown that the maximum decrease in these parameters occurs when the mucosal Na^+ is decreased from 20 to 0.4 m*M*. Decrease of flux, short-circuit current and tissue sodium occurred at a slower rate when mucosal Na^+ was decreased from 114 to 20 m*M*. There is also a similarity of the effect on apical potential to the Na^+ conductance in the outer resistive membrane of frog skin. The decrease in Na^+ conductance in this tissue when external sodium concentration is reduced from 10 to 1 m*M* is much greater than that observed when external Na^+ is reduced from higher concentrations to 10 m*M* (29, 30). Expressed differently, the change in potentials, conductance, Na^+ transport and tissue Na^+ will be less between 10 and 100 m*M* mucosal Na^+ than between 1 and 10 m*M*. This may be the result of Na^+ binding by apical membrane between 10 and 100 m*M*, and consequent closure of sodium channels.

Summary. This study was undertaken to delineate the upper limits of paracellular resistance in toad bladder, and the effect of mucosal $[Na^+] < 10$ m*M* on cellular resistance and epithelial, apical, and basal-lateral potentials. These parameters were determined at mucosal Na^+ of 100, 20, 10, 7, 3, and 1 m*M*. There was an enormous increase in paracellular or junctional resistance which, at 1 m*M* Na^+, was over fourfold that determined at 100 m*M*. There was no effect on cellular resistance. A primary effect of low mucosal Na^+ on apical potential independent of effect on basal-lateral potential was not demonstrable. Although apical membrane potential responds to mucosal $[Na^+]$, the response is less than that seen with pure Na^+ sensitive electrode. The response of basal-lateral potential to mucosal $[Na^+]$ suggest that, to some considerable extent, ψ_b may be the result of Na^+ pump activity. However, basal-lateral K^+ diffusion potentials may contribute to ψ_b and both membrane potentials may be influenced by shunt current flow. The data also support the concept that Na^+ penetration of apical and basal-lateral membranes is unidirectional, from mucosal solution to cell to serosal solution. Shunt resistance varies inversely with epithelial potential and mucosal Na^+. This is probably required to maintain large transepithelial concentration gradients.

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