BRIEF COMMUNICATIONS

DEPENDENCE OF SEROSAL MEMBRANE POTENTIAL ON MUCOSAL MEMBRANE POTENTIAL IN TOAD URINARY BLADDER

LUIS REUSS and ARTHUR L. FINN

From the Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

Ussing's model for sodium transport across epithelia assumes the existence of two barriers in series; the outer barrier behaves as a sodium electrode and the inner barrier as a potassium electrode (Koefoed-Johnsen and Ussing, 1958); furthermore, these membranes are supposed to be independent of one another. Although experimental determinations of the changes in transepithelial potential across frog skin and toad urinary bladder after ionic substitutions agree with this view (Koefoed-Johnsen and Ussing, 1958; Gatzy and Clarkson, 1965; Leb et al., 1965), microelectrode experiments have shown that, in the steady state, unilateral changes in the composition of the bathing solution alter the potential difference across both barriers in frog skin (Cereijido and Curran, 1965) and *Amphiuma* distal tubule (Wiederholt and Giebisch, 1974). However, an alternative explanation for these observations would be that significant alterations in intracellular ionic activities took place. Accordingly, it has been proposed that fast changes in the composition of the outer bathing solution in frog skin or toad urinary bladder produce changes in the transepithelial potential by altering only the potential drop across the outer barrier (Lindemann and Gebhardt, 1973).

The present experiments were designed to study the alterations in potential profile across the toad urinary bladder epithelium during changes of the composition of the mucosal bathing solution. Urinary bladders from Colombian toads were studied as previously described (Reuss and Finn, 1974). The solutions employed were standard amphibian Ringer (in millimoles per liter, NaCl 109, $NaHCO_3$ 2.4, $CaCl_2$ 0.9, KCl 2.5, glucose 5.5), potassium-Ringer (equimolar K-for-Na substitution), or Ringer plus 10^{-5} or 10^{-4} M amiloride. Short current pulses were passed transepithelially in order to measure the total transepithelial resistance and the ratio of the apical to basal-lateral membrane resistances. The mucosal solution was changed by gravity superfusion through a glass pipette with the microelectrode in a cell.

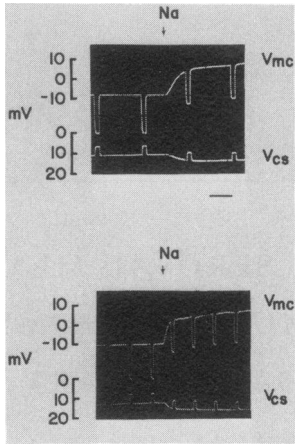

FIGURE 1 Effect of an increase in mucosal sodium concentration on the potentials across the apical and basal-lateral membranes. The bladder was exposed to K-Ringer on the mucosal side and then superfused with standard Ringer (at the arrow). V_{mc} = apical membrane potential (cell – mucosal solution); V_{cs} = basal-lateral membrane potential (serosal solution – cell). Transepithelial pulses: 6 $\mu A/cm^2$, 100 ms (upper records); 7 $\mu A/cm^2$, 100 ms (lower records). Calibration: 0.5 s (upper records), 1 s (lower records).

Fig. 1 shows a typical record of apical (V_{mc}) and basal-lateral (V_{cs}) membrane potentials when the mucosal solution was changed from potassium-Ringer to standard Ringer. After this change the transepithelial resistance decreases and the ratio $\Delta V_{mc}/\Delta V_{cs}$ (equal to the ratio of resistances of the membranes) also decreases. These observations are consistent with the known sodium permselectivity of the apical membrane. V_{mc} was negative in potassium-Ringer (cell negative to the mucosal solution), and as expected, became positive as the mucosal sodium concentration increased. However, note that V_{cs} increased almost simultaneously with the change in V_{mc}. Faster records show that the delay is about 10 ms.

Fig. 2 shows a typical record of membrane potentials before and after the sudden addition of amiloride to the mucosal solution. As expected, the transepithelial resistance increases immediately and the voltage divider ratio across the cellular pathway ($\Delta V_{mc}/\Delta V_{cs}$) also increases. These observations and the fall of V_{mc} are consistent with a reduction in apical sodium conductance produced by amiloride. However, V_{cs} falls 10–20 ms after the initial change of V_{mc}.

If the only pathway across the epithelium were the transcellular pathway, the two-membrane hypothesis would predict no change in serosal membrane potential as a consequence of rapid changes in mucosal membrane potential. However, if there is a paracellular shunt, the potential generated across one membrane influences the potential measured across the other. The lower the resistance of the shunt (R_s), the higher this contribution (Schultz, 1972). According to the known potential profile across this epithelium (two positive steps in the M to S direction) an increase in V_{mc} in the presence of a low resistance paracellular shunt would by itself generate an outward (S to M)

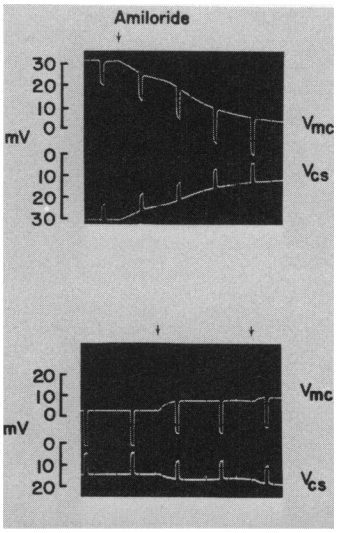

FIGURE 2 Effect of amiloride on the potentials across the apical and basal-lateral membranes. Bladder exposed to standard Ringer. Amiloride was added by superfusion (at the arrow) to a final concentration of about $2 \cdot 10^{-5}$ M (upper tracings). The lower tracings show reversibility of the amiloride effect when its concentration was reduced by successive additions of fresh Ringer solution (arrows). Abbreviations as in Fig. 1. Transepithelial pulses: 6 $\mu A/cm^2$, 100 ms. Calibration: 0.5 s.

shunt current and a reduction in V_{cs}. Conversely, a depolarization of the apical membrane should hyperpolarize the basal-lateral membrane. (This is the reverse of that seen in Figs. 1 and 2, where both potentials changed in the same direction).

That this is the case is shown by the experiment illustrated in Fig. 3. The "shunt resistance" was reduced by placing a variable resistor (R_o) in parallel with the tissue. It was possible to reduce and reverse the changes in V_{cs} after the same experimental maneuvers when R_o was reduced below a critical level. Similar results are obtained when the resistance of the shunt is decreased by hyperosmolal mucosal solutions. Note that the magnitude of the potential changes is considerably smaller than those shown in Fig. 1. This can also be explained by the presence of the shunt.

These experiments demonstrate that changes in V_{cs} take place in the opposite direction to what would be predicted if the mechanism were a low resistance shunt pathway. The alternative remains that the change in mucosal solution from potassium-Ringer to standard Ringer, and the addition of amiloride, alter both V_{mc} and R_s in the same direction (K-to-Na Ringer increases and amiloride decreases both). However, that such changes in R_s take place is highly unlikely because of two reasons: first, the changes in total transepithelial resistance are in both cases in the opposite direction (Figs. 1 and 2) to the necessary change in shunt resistance, and second, the passive permeability coefficients for Na and K are $1.30 \cdot 10^{-7}$ and $1.45 \cdot 10^{-7}$ cm $\cdot$ s^{-1}, respectively (Finn and Hutton, 1974), indicating a low permselectivity of the shunt pathway.

In summary, the potentials across the apical and basal-lateral membranes of the toad

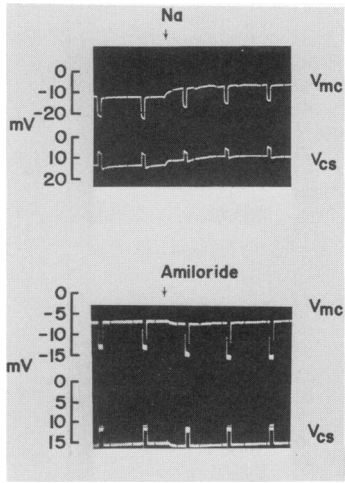

FIGURE 3 Effect of an increase in mucosal sodium concentration and the addition of amiloride on the potentials across the apical and basal-lateral membranes in an externally "shunted" bladder. A parallel resistor (800 Ω) was connected to both solutions, reducing the transepithelial resistance from 6,700 to 720 Ω · cm^2. Then, the same experiments illustrated in Figs. 1 and 2 were performed. Note that the direction of the change of V_{cs} is reversed in both cases. Transepithelial pulses: 20 $\mu A/cm^2$, 100 ms (upper records); 15 $\mu A/cm^2$, 100 ms (lower records). Calibration: 0.5 s.

urinary bladder epithelial cell change almost simultaneously when the composition of the mucosal solution is changed or amiloride is added. This effect cannot be explained by the presence of a low resistance transepithelial shunt pathway or by changes in the resistance of the shunt produced by the experimental maneuvers. The fast time course and the magnitude of the change in potential indicate that it cannot be due to changes in intracellular ionic concentrations mediated by diffusion.

Thus, one must conclude that there is some kind of signal that allows the membrane potentials to follow each other so closely. The mechanism whereby such a signal is generated and received is not yet known. The participation of the microtubular system in this response seemed possible, and colchicine has been shown to disrupt microtubules and modify the hydroosmotic response of this tissue to vasopressin (Taylor et al., 1973). However, up to 4 h after the addition of $2 \cdot 10^{-4}$ M colchicine, the observations documented in Figs. 1, 2, and 3 remained unchanged.

In any event, these experiments make it clear that one can no longer assume that the effect of a drug or other perturbation is at only one border of the cells, since alterations at the other border secondary to signal generation of the type described may take place.

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