ILLUMINATIONS

Transepithelial sodium transport across frog skin

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AMPHIBIAN SKIN SERVES as an extra renal organ for the regulation of ion transport across the epithelium (2). Ion channels and transporters present in the epithelium of frog skin assist in the regulation of the same. These channels and transporters are necessary for the maintenance of intracellular ionic concentrations, osmotic concentration and cell integrity, and function (4).

Figure 1A depicts a transverse section of frog skin, which consists of the following two layers: the superficial epidermis and the deeper dermis. The principal cells (P-cells) form the superficial epidermis and the deeper dermis. The principal cells (P-cells) form the principal and intercalated cells of the vertebrate kidney (6, 9). These cells are necessary for the maintenance of intracellular ionic concentrations, osmotic concentration and cell integrity, and function (4).

The apical membrane contains epithelial sodium channels (ENaC) for transport of Na+ (7, 8). The serosal membrane has Na+-K+-ATPase pump and ATP-gated K+ channels (KATP channel). From the water in ponds, Na+ passively enters the cell through ENaC on the apical membrane, down its electrochemical gradient. Na+-K+-ATPase pump present on the serosal surface pumps out excess Na+ and maintains the intracellular Na+ concentration. K+ enters the cells in exchange of the Na+ pumped out. These K+ ions get recycled to the serosal side via KATP channels. Tolbutamide is a blocker of KATP channels (3, 17). Cl⁻ movement across the epithelium is mediated via paracellular pathways and MRCs (1, 12). An imbalance in the transport of Na+ and Cl⁻ produces a voltage difference or a potential difference across the epithelium (10, 16). Hence, outside of the apical side is negative with respect to the serosal side, thereby making the frog skin epithelium a polarized membrane. This ionic transport system, in an isolated ventral abdominal skin of a frog, facilitates the transport of Na+ from the apical side to the serosal side (13), as depicted in Fig. 1B. Thus the aim of this model is to demonstrate the role of epithelial apical and serosal membranes in the transport of Na+ ions through channels and transporters.

MATERIALS AND METHODS

The study was carried out after receiving the approval of the institutional animal ethics committee. The species selected for this study was Rana hexadactyla, which was obtained from a local animal vendor. Electrophysiological methods were used to measure voltage difference across the frog skin epithelium. Materials used in this study include an Ussing chamber, frog skin, chloride-coated silver electrodes, and a multimeter.

A modified Ussing chamber was used for these experiments. The chamber was divided into two portions using two Perspex half plates. One Perspex half plate was fixed, whereas the other was removable. Both of the plates had a central opening area, the size of which was ~1 cm² (Fig. 2A). This model has been used to study ionic transport across the epithelium (18).

Chemicals. Ouabain, tolbutamide, MgCl2, glucose, and HEPES were obtained from Sigma Chemical USA; amiloride from Micro Laboratories (Bangalore, India); NaCl and KCl from SD Fine Chemicals; and CaCl2 from Merck (Mumbai, India).

Frogs were anesthetized with ether and then pithed. The ventral abdominal skin was dissected out and rinsed with aerated normal Ringer (NR) solution with the composition as follows (in mM): NaCl 115.0; KCl 2.5; CaCl2 1.0; MgCl2 1.0; HEPES 3.5; glucose 10.0; pH 7.35–7.4. The dissected skin was spread onto the removable Perspex plate, the extra portions were trimmed off, and it was inserted into the gutter, the gap meant for holding it (Fig. 2B).

Experimental setup to measure the voltage difference as an ionic transport. After the frog skin was mounted in the Ussing chamber, the apical and serosal surfaces were bathed in NR. Two Ag-AgCl electrodes were placed on either side of the skin. The recording electrode was placed on the apical side, whereas the reference electrode was placed on the serosal side, which was grounded (13). The electrodes were connected to a digital multimeter, and the voltage difference across the epithelium was read out as a digital value (Fig. 3).

The experiment was carried out only if the resting voltage difference was recorded to be above ~10 mV. Subsequently, the next intervention was done and voltage recorded until a steady state was achieved.

Channel and transporter inhibitors used in the study. Amiloride, an ENaC blocker, was added on the apical side of frog skin epithelium and a final concentration of 100 μM was obtained (4). Ouabain, an inhibitor of the active transporter Na+-K+-ATPase pump, was added on the serosal surface, and a final concentration of 10 μM was achieved (4). Tolbutamide, an inhibitor of KATP, was added onto the serosal side, and a final concentration of 100 μM was obtained (17). In these experiments, different preparations had to be used for different inhibitors because these were not washable.

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Statistical analysis. All values are expressed as means ± SE; n = 5 animals. Statistical significance for changes in voltage were estimated by Wilcoxon signed-rank test. Probability values of P < 0.05 are considered significant. The asterisks shown in Figs. 4–6 denote the significant blocking effect of inhibitors.

RESULTS

Experiment 1: Effect of amiloride, an ENaC blocker. NR solution on both sides of the frog skin membrane, the apical and the serosal, produced a control voltage difference of −45.8 ± 4.1 mV (mean ± SE). The application of amiloride (100 μM) on the apical side led to a decrease in voltage difference to −6.9 ± 2.1 mV within 10 min (Fig. 4).

Experiment 2: Effect of ouabain, a Na⁺-K⁺-ATPase pump blocker. NR solution on both sides of the frog skin membrane, the apical and the serosal, produced a control voltage difference of −43.0 ± 9.2 mV (means ± SE). Treatment of the serosal side with 10 μM ouabain led to a decrease in the voltage difference to −8.3 ± 4.1 mV within 60 min (Fig. 5).

Experiment 3: Effect of tolbutamide, a KATP channel blocker. NR solution on both sides of the frog skin membrane, the apical and the serosal, produced a control voltage difference of −34.7 ± 12.6 mV (mean ± SE). Treatment of the serosal side with 100 μM tolbutamide decreased the voltage difference to −8.4 ± 6.1 mV within 60 min (Fig. 6).

DISCUSSION

The voltage difference arises due to the difference between the membrane voltages of the apical membrane and the serosal membrane due to Na⁺ transport. The increase in voltage...
difference signifies either a depolarization of the apical membrane or a hyperpolarization of the serosal membrane (Fig. 1B). The contribution of Cl– ion is minimal, and the cause for the voltage difference is the considerable amount of Na+ transport (which is unaccompanied by Cl–) and reaches equilibrium at a particular voltage (10, 16).

These experiments demonstrate that Na+ transport in frog skin epithelium is dependent on ENaC present on the apical membrane and on Na+–K+–ATPase pump with KATP channel present on the serosal membrane. Experiment 1 showed that the voltage difference decreased while blocking the apical ENaC with amiloride (Fig. 4), and in experiment 2 the voltage difference across frog skin decreased on blocking Na+–K+–ATPase pump with ouabain on the serosal side (Fig. 5). The results of these two experiments hence demonstrate that the voltage difference across frog skin arises as a result of Na+ sequestration across the epithelium. The blocking of ENaC or Na+–K+–ATPase pump essentially abolishes this difference in voltage (5, 11). Cl– movement across the frog skin can be activated by serosa-positive voltage, and this movement can be inhibited when Na+ transport is blocked (14).

Experiment 3 showed that the voltage difference across frog skin decreased on blocking KATP channel with tolbutamide on the serosal side (Fig. 6). This demonstrates that the KATP channel has a prominent role in the prevention of accumulation of K+ inside the epithelium, thereby maintaining an electrical gradient for Na+ entry across the apical membrane.

K+ conductance would increase by increasing transepithelial Na+ transport and decrease either by inhibiting Na+ transport.
transport by blocking of ENaC or Na\(^+\)-K\(^+\)-ATPase pump, or with the K\(_\text{ATP}\) channel blocker tolbutamide.

The concept of voltage difference results from transepithelial Na\(^+\) transport, and this has been demonstrated by the marked decrement in voltage difference associated with the amiloride, ouabain, and tolbutamide treatment, which are inhibitors of the ENaC, the Na\(^+\)-K\(^+\)-ATPase pump, and K\(_\text{ATP}\) channel, respectively.

**Conclusion.** This model demonstrates the role of apical membrane Na\(^+\) entry through ENaC and serosal membrane Na\(^+\)-K\(^+\)-ATPase pump activity. The K\(_\text{ATP}\) channel is essential for the prevention of K\(^+\) accumulation inside the epithelium and hence for the maintenance of an electrical gradient for Na\(^+\) entry across the apical membrane. This simple method can be used to teach the physiology of Na\(^+\) transport in undergraduate and graduate courses.

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