INTRACELLULAR MICROELECTRODE ANALYSIS OF THE MEMBRANE CONDUCTIVE PROPERTIES OF CULTURED SQUALUS ACANTHIAS RECTAL GLAND CELLS

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We have previously shown that cultured shark rectal gland (SRG) tubular epithelium can be studied using the Ussing short-circuit current (Isc) technique (Valentich and Forrest MDIBL Bull.26:91-94, 1986). Monolayer cultures exhibit characteristic Isc responses to agonists and inhibitors of secondary active chloride secretion. In this report we describe the basic electrophysiologic properties of the apical and basolateral membranes of cultured SRG cells and their response to the chloride secretagogue, forskolin, an adenylate cyclase stimulator.

Intracellular electrical potentials were determined using SRG cells grown as monolayers on Millipore Millicell-CM membranes coated with a dried film of type 1 collagen. Cultures were prepared as described previously (Valentich and Forrest, Ibid.) All cultures used in this study were 4-6 weeks old. Immediately before use, membranes were cut from the Millicell and mounted, apical surface up, between two halves of a Plexiglass chamber. The chamber allowed both surfaces of the monolayer to be superfused separately with shark Ringer. The monolayer was either short-circuited or open circuited with a voltage clamp that automatically compensated for fluid resistance. Microelectrodes were prepared from glass capillary tubing and backfilled with 0.5 M potassium acetate. Electrodes had resistances of 60-80 MΩ when filled and the tips immersed in 0.5 M potassium chloride. Cells were impaled and the electrical potential difference across the apical membrane (V^a) monitored with a high impedance electrometer and referenced to the apical solution. The fractional resistance of the apical membrane, fR^a, was determined from changes in V^a and transmonolayer potential (V^{ab}) produced by bipolar transepithelial current pulses.

Superfusing the basolateral surface of SRG monolayers with forskolin (10^{-6}M) increased Isc and, under open-circuit conditions, V^{ab} (Fig. 1, lower trace). The increase in Isc and V^{ab} is consistent with an increase in the rate of rheogenic transcellular chloride secretion. Forskolin also caused V^a to depolarize by an average of 23mV (n=4 monolayers) and decreased the fR^a dramatically from 0.90 to 0.20 (n=4 monolayers), consistent with an increase in the conductance of the apical membrane. The upper trace in Figure 1 illustrates these effects. Superfusing the apical surface with low chloride Ringer (10-fold reduction) under non-stimulated conditions had little effect on V^a and fR^a. When monolayers were exposed to forskolin, low chloride Ringer depolarized V^a by an average of 27mV (n=2 monolayers) and caused a modest increase in fR^a. Taken together, these

data indicate that forskolin increased the chloride conductance of the apical membrane, leading to an increased Isc and presumably increased chloride secretion. The identical mechanism has been described by Greger et al. for the microperfused SRG tubule (Pflugers Arch. 402:376-384, 1984). However, SRG cultures are more responsive to forskolin than perfused tubules since we find a large change in fR^a following forskolin stimulation which, when used alone, has only minimal effects on isolated SRG tubules (Greger et al. Ibid.). The small Isc in the presence of a large change in fR^a probably results from low intracellular chloride activity and a high cellular resistance relative to the native SRG tubule. Nevertheless, our results demonstrate that cultured SRG monolayers respond to forskolin with qualitatively similar changes in membrane properties as native tubules.

We have also evaluated, in part, the conductive properties of the basolateral membrane of SRG monolayers. A 10-fold increase in basolateral Ringer potassium concentration caused an average 40mV (n=2 monolayers) depolarization of V^a. The depolarization of V^a reflects a large depolarization (approximately 40mV) of the basolateral membrane potential. V^a depolarized because the apical and basolateral membranes are electrically coupled via the paracellular shunt pathway. Superfusing the basolateral surface with Ringer containing 5mM barium caused V^a to depolarize by an average of 18mV (n=3 monolayers) and decreased fR^a from 0.88 to 0.56. These data are consistent with the basolateral membrane being potassium conductive and support the microperfused tubule results of Greger and Schlatter (Pflugers Arch. 402:63-75, 1984).

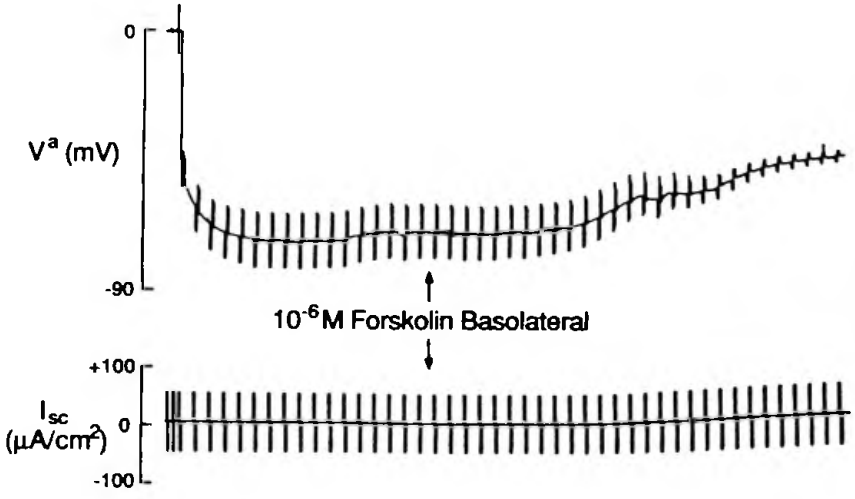

FIGURE 1

Typical record illustrating the effect of forskolin on apical membrane potential (V^a), fractional resistance and short circuit current (Isc) of a SRG monolayer culture.

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