IMPEDANCE ANALYSIS REVEALS CHANGES IN APICAL AND BASOLATERAL MEMBRANE RESISTANCE IN SHARK (SQUALUS ACANTHIAS) RECTAL GLAND CULTURES.

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The experiments described here were designed to test the hypothesis that alternating current (AC) Impedance Analysis can provide an assay for changes in the conductances of the apical and basolateral membranes of epithelial cell layers. AC impedance analysis or "alternating current spectroscopy" is based on the notion that an epithelial cell layer can be represented as the series arrangement of two parallel RC circuits, representing the apical and basolateral membranes respectively. The AC impedance of any electrical network is defined as $Z(\omega)$ where $Z(\omega) = V(\omega)/I(\omega)$ and ω is the angular frequency, $2\pi f$. The magnitude of $Z(\omega)$ is frequency dependent because of the membrane capacitance. The presence of the membrane capacitance requires that $Z(\omega)$ be represented as a complex number, i.e. as the sum of a real and an imaginary part. One way of representing impedance data is to plot the imaginary part of $Z(\omega)$, denoted here as X, versus the real part of $Z(\omega)$, denoted here as R, for different values of ω. For a parallel RC circuit this so called "Nyquist Plot" has the form of a semi circle. A series arrangement of two RC circuits representing two membranes in series can have the form of two semi circles, if the values of C_m (membrane capacitance) for the two membranes are sufficiently different and the ratio of apical to basolateral membrane resistance is in the range of about 0.2 to 5.

We measured the AC impedance of rectal gland cell layers cultured on permeable supports as described by Valentich and Forrest (M.D.I.B.L. Bull. 26:91,1986) using the protocol described by Margineanu and Van Driessche (J. Physiol. 427:567, 1990). The experiments were designed to compare the Nyquist Plots for four conditions: (1) Unstimulated cultures which were not secreting Cl and which were expected to behave as a single (apical) membrane of very high resistance. (2) Maximally stimulated (1 μM forskolin) cultures which were secreting Cl and which were expected to exhibit greatly reduced apical membrane resistance. (3) Stimulated cultures treated with serosal barium to decrease basolateral K conductance and, hence, increase basolateral membrane resistance. (4) Stimulated cultures treated with mucosal diphenylamine-2-carboxylate (DPC), a compound which blocks single Cl channels in rectal gland cells, to decrease apical Cl conductance, corresponding to an increase in apical membrane resistance.

Figures 1-3 illustrate the Nyquist Plots obtained by determining $Z(\omega)$ for values of f ranging from 0.05 Hz to 5.5 kHz. Curves were fit to the data points using software designed in the laboratory of Dr. Willy Van Driessche, Leuven, Belgium. Figure 1 shows the plot for an unstimulated culture which appears as a single semi circle as expected if the apical membrane resistance is much greater than basolateral. Maximal stimulation of Cl secretion by 1 μM forskolin (I_{sc} ~100 $\mu A/cm^2$) was associated with a Nyquist Plot which can be resolved into two semi circles, assumed provisionally to represent the apical (left) and basolateral (right) membranes. Note that the apical (left) semi circle is much smaller in size than the right (basolateral) as expected if forskolin leads to a marked decrease in apical membrane resistance. This result is consistent with the notion that the activation of Cl secretion is accompanied by a massive increase in apical Cl conductance.

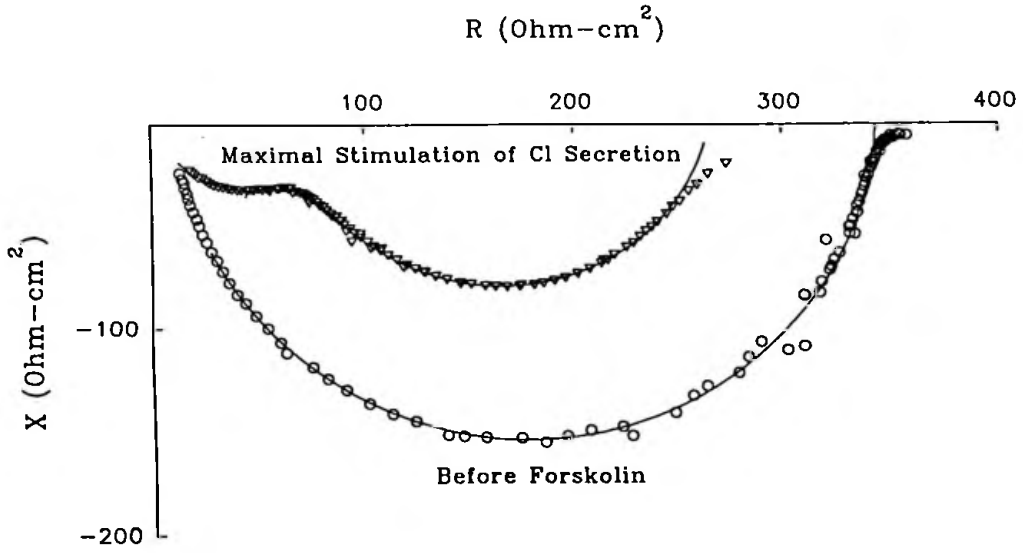

Figure 1

Figure 2 shows the effect of serosal Ba^{2+}, a selective blocker of K channels. Addition of Ba^{2+} decreased I_{SC} (not shown) and produced a marked alteration in the Nyquist Plot, namely an increase in the size of the basolateral semi circle as expected for a selective increase in basolateral membrane resistance. The decline of I_{SC} is also expected because blocking K exit will indirectly attenuate Cl secretion (Dawson and Richards, Am. J. Physiol. 259:C181-C195, 1990).

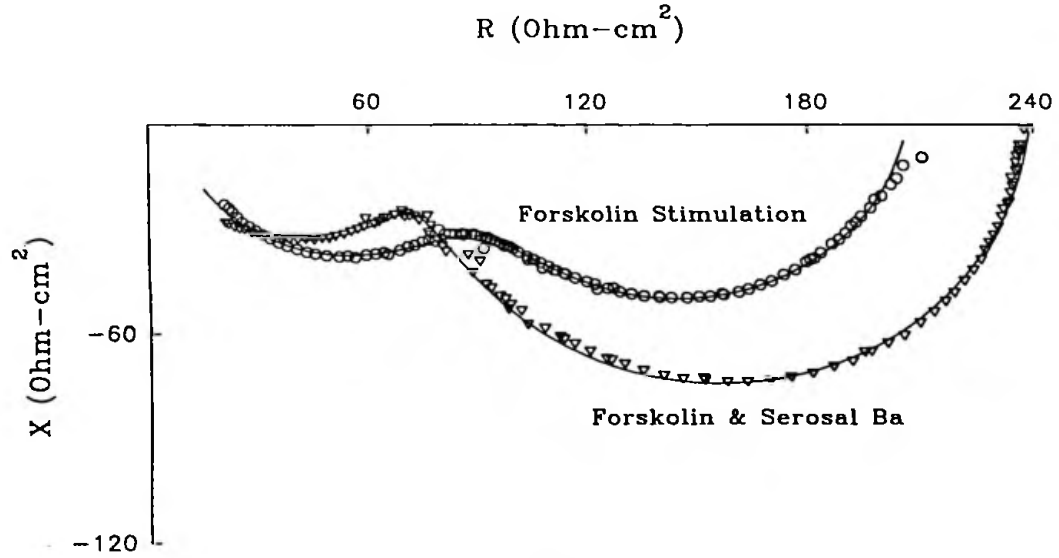

Figure 2

Figure 3 shows the effect of DPC on Cl secretion in a maximally stimulated (1 μM forskolin) rectal gland culture. DPC decreased I_{SC} (not shown) as expected if Cl exit from the cell was reduced. Accordingly, the apical (left) semi-circle on the Nyquist Plot was increased in size as expected for a selective increase in apical membrane resistance.

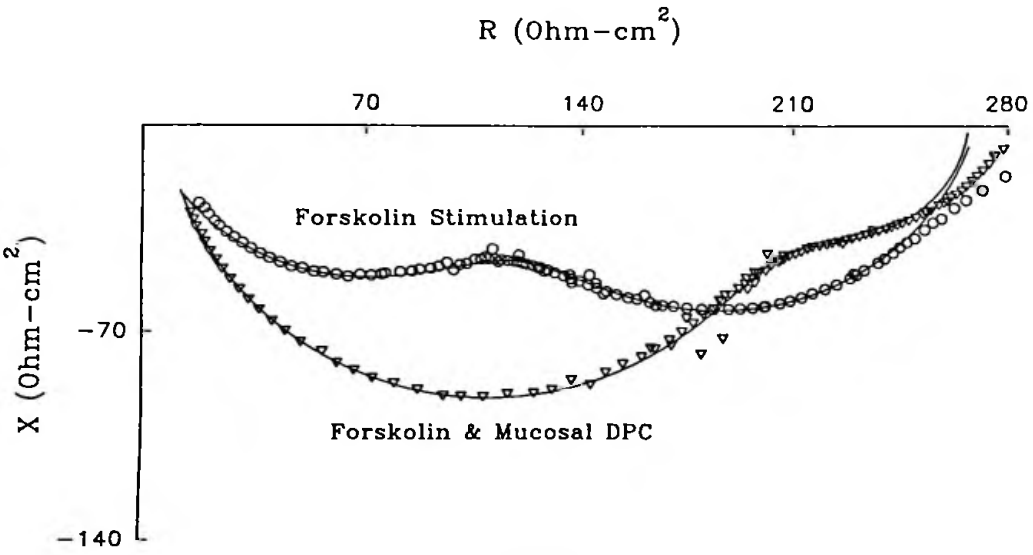

Figure 3

The results of these studies suggest that AC impedance analysis can provide a non-invasive assay for changes in apical and basolateral membrane resistance which accompany changes in ion transport. This type of result suggests that the technique may offer insight into, not only the resistance but also the capacitance of a series membrane system. Changes in membrane capacitance may be related to changes in membrane area due to exocytotic events associated with the insertion of channels into the plasma membrane. (This work was supported by NIH and the Michigan Heart Association.)