# Capacitance measurements of electrically isolated membranes of *Squalus acanthias* rectal gland primary culture monolayers

Sarah E. Decker[1,2], Ali Poyan Mehr[1,2], Michael B. Butterworth[2,3], Raymond A. Frizzell[2,3], and John N. Forrest, Jr. [1,2]

[1] Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06510

[2] Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672

[3]Department of Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260

Capacitance measurements have been used to characterize proteins in electrically isolated individual membranes of epithelia [1,2,5,6]. Unlike heterologous expression systems, capacitance measurements evaluate protein function in intact, native tissues. The technique has been used by Van Driessche and colleagues to examine the insertion of epithelial sodium channels (ENaC) into apical membranes of cultured toad kidney (A6) cells)[2], and by Butterworth et al. [1] who demonstrate that cyclic AMP stimulates apical ENaC insertion from a recycling channel pool in cultured mouse cortical collecting duct cells.

Electrical isolation of apical and basolateral membranes is achieved by selective permeabilization of the opposing membrane with ionophores during simultaneous measurement of transepithelial short circuit current ($I_{sc}$), conductance ($G_T$), and capacitance ($C_T$). Nystatin is added to either side to permeabilize the membrane and effective permeabilization is monitored by changes in $I_{SC}$, $G_T$, and $C_T$. To interpret the $C_T$ measurements, the epithelium is modeled as a series of RC circuits corresponding to the apical and basolateral membranes, shunted by a paracellular resistance (Figure 1). Because total capacitance changes are the result of changes in apical capacitance alone, $C_T$ recordings are assumed to reflect changes in $C_A$ [1,2,6]. We have evaluated this technique for studies of shark rectal gland epithelial cells in primary culture.

Shark rectal gland tubular epithelial cells were isolated as previously described[4], cultured on CoStar Transwell Clear filter supports, and mounted in a modified Ussing chambers. The bathing ringers solution for SRG cells is composed of 270 mM NaCl, 6 mM KCl, 3 mM $MgCl_2$, 5 mM $CaCl_2$, 20 mM $NaHCO_3$, 350 mM urea, 5 mM glucose. Chambers were constantly gassed with 95% $O_2$/5% $CO_2$ at 18°C, maintaining the pH at 7.5. Solution changes and chamber washes were performed by means of a five fold volume exchange through a peristaltic pump connected to the base of each chamber. The voltage clamp and recording system to acquire data were designed and constructed by W. Van Driessche (Katholieke Universiteit, Leuven, Belgium) and have been described in detail previously[1,2,6]. In brief, hardware for $C_T$ measurements uses two Digital Signal Processing (DSP) boards (Model 310B; Dalanco Spry) with one board recording transepithelial conductance ($G_T$) and short-circuit current ($I_{sc}$) and the second, $C_T$. Continuous $C_T$ values are calculated from imposed voltage sine waves of frequencies 2, 2.7, 4.1, 5.4, and 8.2 kHz. Impedance analysis is performed at steady states by simultaneously imposing 78 sine waves to the command input of the voltage clamp and analyzing as previously described[1,2,7].

$$\frac{1}{C_T} = \frac{1}{C_A} + \frac{1}{C_B}$$

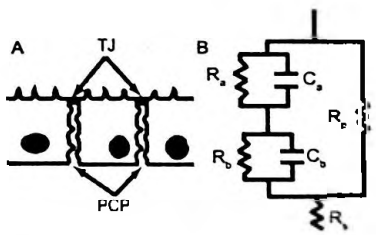


Figure 1. Epithelium modeled as a series of RC (resistance and capacitance circuis showing tight junctions (TJ) and paracellular pathway (PCP). (Figure modified from Kreindler *et al.*, 2005)

We found that most SRG epithelial cell monolayers CoStar Transwell Clear filter supports had adequate resistances (> 1000 Ω cm$^2$) and were well suited for use in this system. In these monolayers we achieved consistent permeabilization of the apical membrane using 140 $\mu$M nystatin. This is illustrated in Figure 2, panel A, yielding Nyquist plots similar to those observed in CCD epithelia by

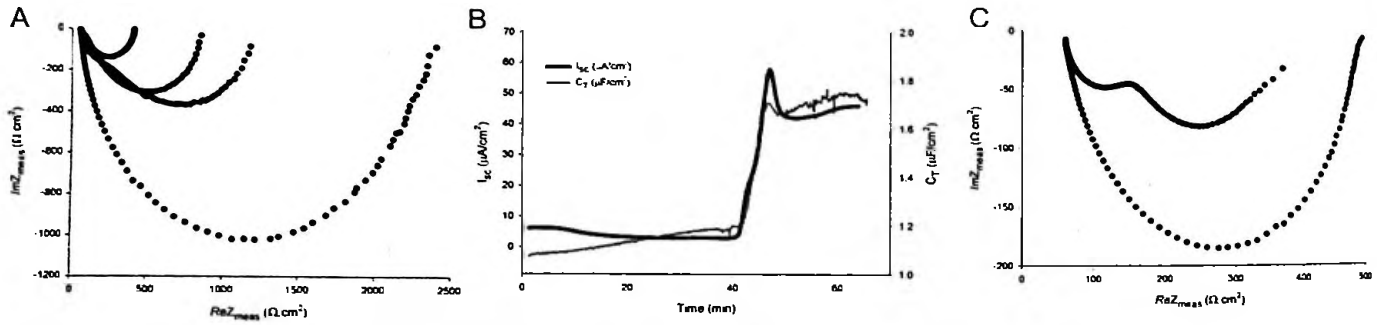


Figure 2. A: Nyquist plots from a SRG epithelium to which increasing concentrations of apical nystatin was added. From an unpermeabilized state, a single impedance locus (one semicircle) transitioned through two loci until only one locus corresponding to the basolateral membrane was observed. B: Forskolin + IBMX added at t=40 min. elicits a simultaneous increase in Isc and capacitance in SRG epithelial cell monolayers. C: Nyquist plots from a SRG epithelium showing the effect of forskolin. From an unpermeabilized state, a single impedance locus (one semicircle) transitioned to two loci, as two membranes were resolved.

Butterworth, *et al.*[1] (a single impedance locus transitioning to one smaller locus corresponding to the basolateral membrane). In early experiments in rectal gland monolayers (Figure 2, Panels B-C), we found that forskolin 10 $\mu$M and IBMX 100 $\mu$M increased $C_T$, consistent with our previous immunohistochemical studies showing insertion of CFTR channels into the apical membrane[4].

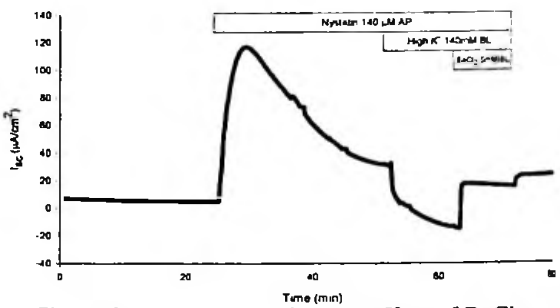


Figure 3. Isc trace showing the effect of $BaCl_2$ on basolateral $K^+$ conductance in SRG epithelial cell monolayers.

After permeabilization of the apical membrane with nystatin, we were able to study the basolateral potassium conductance. The corresponding current, which was achieved by imposing a potassium gradient across this membrane (apical 140 mM K, luminal 4 mM) is sensitive to inhibition by $BaCl_2$ (Figure 3).

We believe this technique will be an important new tool in elucidating the role of basolateral and apical membrane proteins in intact SRG epithelial cell monolayers.


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