cAMP stimulates CFTR Trafficking in Shark (*Squalus acanthias*) Rectal Gland

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The shark rectal gland is a model for active chloride secretion across epithelial tissues. Chloride exits the cells through cAMP/PKA activated apical membrane chloride channels (Greger et. al., Pfluegers Arch. 402:63-75, 1984) that represent the shark homologue of the cystic fibrosis transmembrane conductance regulator (CFTR, see Marshall et al., J. Biol. Chem. 266:22749-54, 1991). Single channel recordings demonstrate that increased cellular cAMP activates a small linear Cl^- channel (~5pS) that resembles human CFTR in its biophysical and pharmacological properties (Devor et al., Am. J. Physiol. 268:C70-9, 1995). The Cl current (I_{Cl}) stimulated by cAMP activation may arise not only from a phosphorylation-induced increase in the open probability (P_o) of membrane-resident CFTR, but also from an increase in the number (N) of plasma membrane CFTR Cl channels, due to their regulated insertion into plasma membrane. CFTR trafficking was associated with an increase in the electrical capacitance of the plasma membrane (C_m) in oocytes expressing human CFTR (Takahashi et al., Am. J. Physiol. 271:C1887-94, 1996), and the resulting increase in plasma membrane area was confirmed by morphometry. To determine whether shark CFTR undergoes regulated trafficking, we used *xs* oocytes expressing shark CFTR and determined C_m during stimulation by cAMP agonists. In addition, epithelial impedance and capacitance measurements were performed using primary cultures of shark rectal gland to study shark CFTR trafficking in polarized epithelial monolayers.

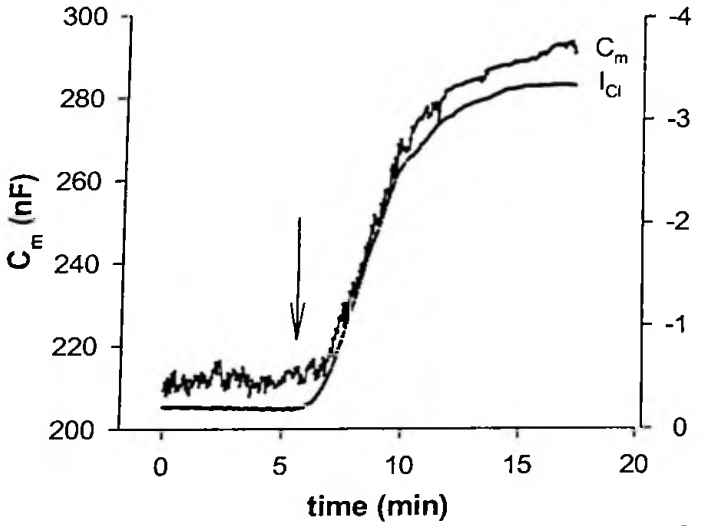

Fig. 1 The time course of C_m and I_{Cl} during stimulation by an agonist cocktail (10μM forskolin and 1 mM IBMX). The arrow indicates cocktail addition.

cRNA for oocyte expression was prepared directly from shark rectal gland (SRG) cDNA, produced by RT-PCR. Oocyte isolation, cRNA injection, current (I_{Cl}) and capacitance (C_m) recordings were performed as described (Takahashi et al. Am. J. Physiol. 271:C1887-94, 1996). Oocytes were injected with 15 ng shark CFTR cRNA in 50 nl water, and recordings were performed 2-3 days after cRNA injection.

A typical time course of C_m and I_{Cl} during stimulation of Xenopus oocytes expressing shark CFTR by the agonist cocktail

(10μM forskolin and 1 mM IBMX) is shown in Fig. 1. CFTR stimulation increased I_{Cl} and C_m in parallel. To determine the relation of C_m and I_{Cl} to the level of CFTR stimulation, we varied the IBMX concentration in the stimulation cocktail. Figure 2 shows that the dose dependence of C_m and I_{Cl} is similar. This confirms the close correlation between stimulation of CFTR Cl current and membrane insertion, observed previously for human CFTR (Takahashi et al. Am. J. Physiol. 271:C1887-94, 1996). There are no obvious differences in the current and capacitance responses of human and shark CFTR.

To extrapolate our findings to the intact shark rectal gland epithelium, we performed transepithelial impedance and capacitance measurements using primary cultures of shark rectal gland. Capacitance in the oocyte system reflects CFTR trafficking at the single cell level, whereas the monolayer impedance measurements examine the combined resistive and capacitative properties of the epithelial series membranes. SRG monolayer cultures were prepared as previously described (Valentich, J. Tissue Culture Methods 13:149-162, 1991). The epithelium can be modeled as a lumped capacitance and resistance (RC) circuit (Schifferdecker and Fromter, Pflugers Arch. 377:125-133, 1978). The transepithelial impedance measurements were performed under short-circuit conditions (V_t = 0), using a current waveform composed of a sequential series of ten sinusoidal (4 Hz - 8 kHz) currents. The epithelial capacitance was calculated on-line at two frequencies (4 and 8 kHz). The methods were previously described (Margineanu and Van Driessche, J. Physiol. 427:567-581, 1990).

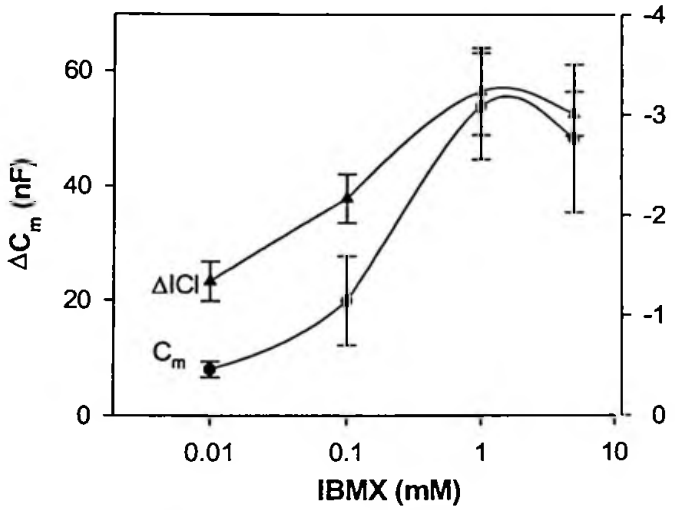

Fig. 2 IBMX dose-response relation for C_m and I_{Cl}. Values are means ± SE (n=6).

Figure 3A shows a representative example of an impedance plot of the SRG epithelium. The impedance locus is described by a simple semicircle with a small central depression due to the complex dielectric behavior of biological membranes. After stimulation by 10 μM forskolin, the impedance locus splits into two semicircles due to the difference in the time constants ($\tau = R \bullet C$) of the individual apical and basolateral membranes, as shown in Figure 3B, C and D. During stimulation by 10 μM forskolin, the apical membrane resistance decreases (B), then undergoes a secondary increase as the current continues to rise (C); see I_{sc} trace in Fig.4. Addition of 0.5 mM Ba^{2+} into the serosal solution, to block basolateral K channels, increased the basolateral membrane resistance, and this was reflected by the shift of the impedance plot corresponding to the basolateral membrane, as shown in Figure 3D. Taken together, the data of Figure 3 are consistent with a cAMP-dependent stimulation of the apical CFTR Cl conductance; this causes the apical membrane semicircle to become distinct from that describing

the basolateral membrane. The effect of Ba^{2+} in Figure 3D identifies the right-most semicircle as the impedance locus of the basolateral membrane.

Correlation of these properties with the transepithelial short circuit current (I_{sc}) corroborates the impedance results. As shown in Figure 4, cAMP increased the I_{sc}, consistent with stimulation of Cl secretion. The initial I_{sc} stimulation corresponds to the large decrease in apical membrane resistance depicted by the impedance data of Figure 3B. There is a pause in the current rise during stimulation by forskolin. According to the Nyquist plot Figure 3C, at this time the apical membrane resistance continues to decrease while the basolateral membrane

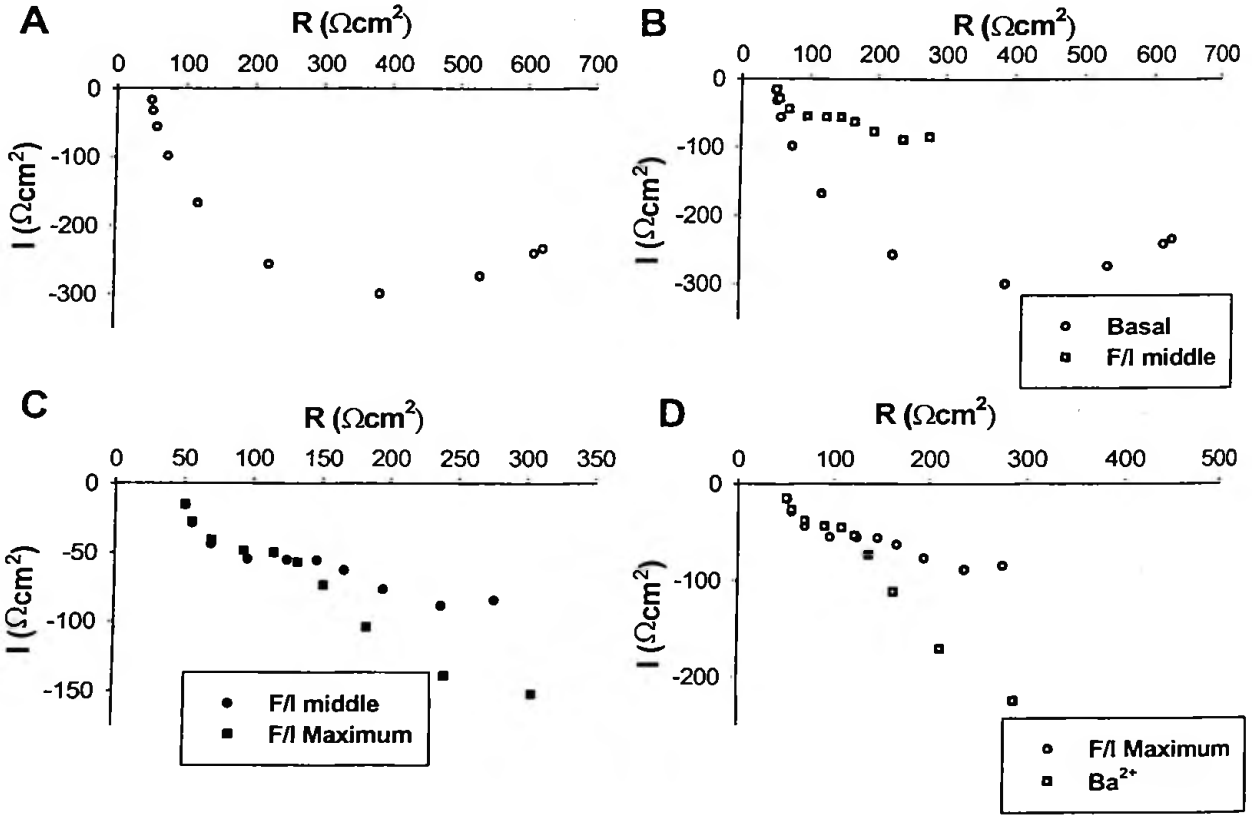

Fig. 3 Impedance locus of SRG epithelium under A. Basal condition; B. 10 μM forskolin stimulation, comparing data obtained at the current pause to basal conditions; C. 10 μM forskolin stimulation, comparing data at maximal current stimulation to data obtained during the current pause; D. 0.5 mM Ba^{2+} added to the serosal side, compared with data at maximal current stimulation.

resistance increases. This behavior is consistent with the findings of Xiao et al. (Life Science 60: 2231-2243, 1997), who described an inhibitory effect of cAMP on the inwardly rectifying K+ current in cultured and fresh SRG cells; this K current was blocked by Ba^{2+}. The I_{sc} pause is possibly caused by a delayed inhibition of K channels by cAMP in the basolateral membrane. The increase in epithelial capacitance observed during cAMP stimulation, as shown in Figure 4,

suggests that insertion of vesicles containing CFTR into the apical membrane is an important component of this stimulatory effect.

In summary, capacitance measurements from oocytes expressing CFTR and from SRG epithelia suggest that cAMP increases apical membrane Cl conductance, at least in part, by stimulating CFTR insertion into the apical membrane. Supported by a grant from the NIH (DK 35818).

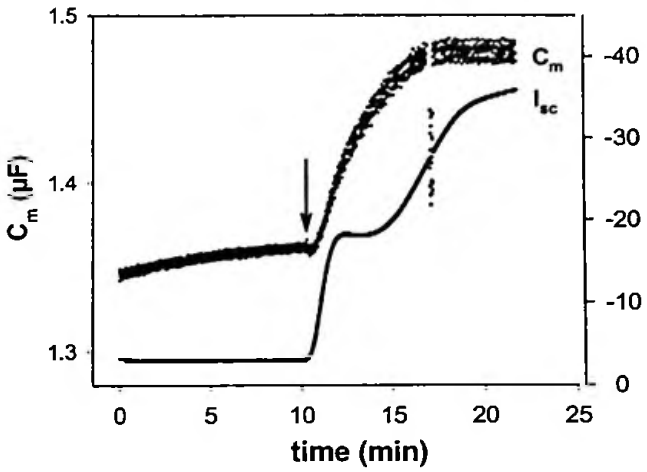

Fig. 4 Time course of transepithelial short-circuit current (I_{SC}) and transepithelial capacitance C_m (at 4kHz) . The arrow indicates the time when 10 μM forskolin was added.