cAMP-MEDIATED ACTIVATION OF Cl^- CURRENTS IN PRIMARY CULTURES OF SPINY DOGFISH (Squalus acanthias) RECTAL GLAND

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Rectal gland tubules of the spiny dogfish secrete Cl^- in response to agonists which stimulate an increase in cAMP (Forrest et al., J. Clin. Invest. 72:1163-1167, 1983). An increase in the apical membrane Cl^- conductance was shown to be the seminal event in this Cl^- secretory process (Greger et al., Pflugers Arch. 402:376-384, 1984). Greger and his colleagues have previously demonstrated the existence of two distinct Cl^- channels in the apical membrane of shark rectal gland using single-channel patch-clamp techniques; a large (50 pS) channel (Pflugers Arch. 409:114-121, 1987) as well as a small (11 pS) channel (Pflugers Arch. 409:122-125, 1987), both of which exhibited linear current-voltage (I-V) relationships. This larger channel could be activated by cAMP in the cell-attached configuration (Pflugers Arch. 403:446-448, 1985) and was regulated by ATP and cAMP-dependent protein kinase (PKA) in excised, inside-out patches (Comp. Biochem. Physiol. 90A (4):733-737, 1988). These results led the authors to speculate the large conductance Cl^- channel was responsible for Cl^- secretion across the apical membrane of dogfish rectal gland.

The gene responsible for the cystic fibrosis (CF) defect has been identified (Rommens et al., Science 245:1059-1065, 1989). It encodes the cystic fibrosis transmembrane conductance regulator (CFTR), which has been shown to function as a cAMP/PKA-activated Cl^- channel in complementation studies (Drumm et al., Cell 62:1227-1233, 1990; Rich et al., Nature 347:358-363, 1990), heterologous expression systems (Anderson et al., Science 251:679-682, 1991; Rommens et al., PNAS 88:7500-7504, 1991), and planar lipid bilayers (Bear et al., Cell 68:809-818, 1992). This CFTR-mediated channel was shown to have a linear I-V relationship with a conductance of 8-10 pS (Berger et al., J. Clin. Invest. 88:1422-1431, 1991). Recently, a dogfish homolog of human CFTR was identified and shown to have 72% homology with human CFTR at the protein level (Marshall et al., J. Biol. Chem. 266:22749-22754, 1991). This overall similarity between human and dogfish CFTR has led us to re-assess the identity of the cAMP-mediated Cl^- conductance in rectal gland cells using whole-cell and cell-attached patch-clamp techniques and primary cultures of dogfish rectal gland (Valentich and Forrest, Am. J. Physiol. 260:C813-C823, 1991).

Figure 1A shows the currents stimulated by forskolin (10 μM) and 8-(4-chlorophenylthio)-adenosine 3':5'-cyclic monophosphate (cpt-cAMP; 400 μM) during whole-cell patch-clamp recording. cAMP stimulated an inward current (downward deflection) when the cell was voltage-clamped to E_K (-94 mV), while no outward current was stimulated during voltage-clamp to E_{Cl} (0 mV). Upon removal of agonist the current returned to baseline. This result is consistent with the activation of a Cl^- conductance with no change in K^+ conductance. The I-V relationship for the cAMP-stimulated current is nearly linear, showing only slight inward rectification (Fig. 1C). A linear I-V relationship has previously been described for Cl^-

currents induced by cAMP-mediated agonists in secretory epithelia (Cliff and Frizzell, PNAS 87:4956-4960, 1990).

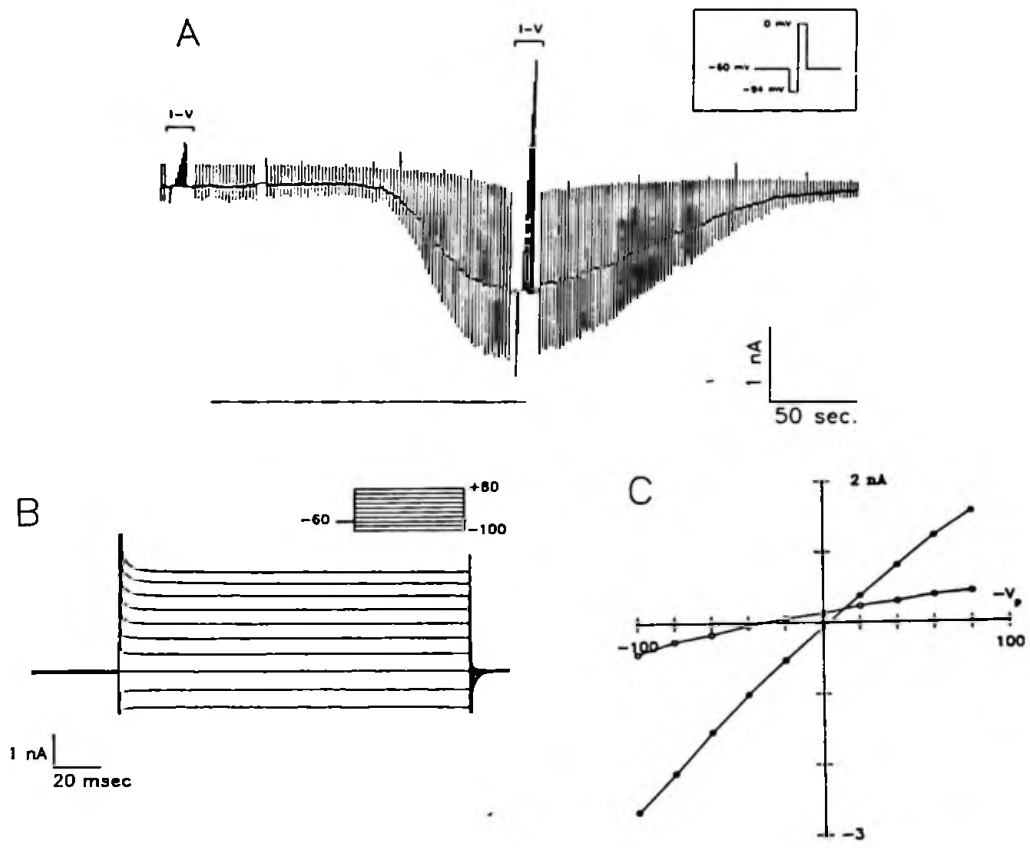

Figure 1. A. Whole-cell current response to 10 μM forskolin and 400 μM cpt-cAMP (solid line). The cell was held at -60 mV and pulsed to E_K (-94 mV) and E_{Cl} (0 mV) for 200 msec at 2 sec intervals (see inset). The pulse protocol for determining the I-V relationship (see inset in B) was run at the indicated times. B. Overlay of cell currents during the I-V pulse protocol after stimulation (from panel A). C. I-V relation before (open circles) and after (filled circles) stimulation. Currents were recorded during the steady-state. The pipette solution contained (in mM) 275 NMDG-Cl, 350 urea, 3 $MgCl_2$, 1 EGTA (free $Ca^{2+} = 100$ nM), and 20 HEPES. The bath solution contained (in mM) 275 NaCl, 350 urea, 3 $MgCl_2$, 5 $CaCl_2$, 5 glucose, 20 HEPES. The pH of both solutions was adjusted to 7.4 with NaOH.

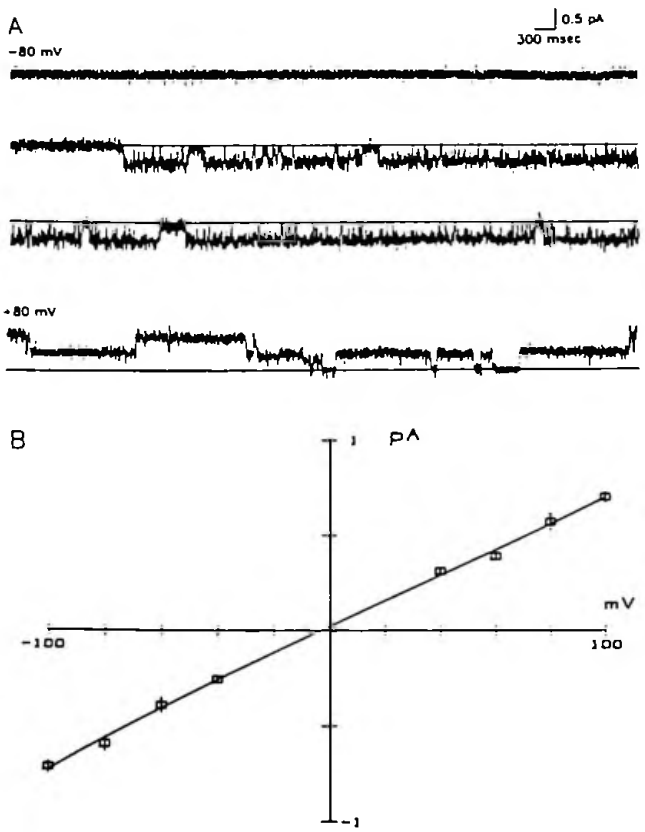

Figure 2. A. Cell-attached activation of Cl^- channels in a previously quiescent patch. The top 3 traces are a continuous recording; there is a 2 sec gap between the -80 mV and +80 mV records. Records were filtered at 200 Hz. Solutions are the same as in Fig. 1 legend. B. I-V relation for channels activated in A.

Figure 2A illustrates the single-channel events activated by forskolin (10 μM) and cpt-cAMP (400 μM) during cell-attached recording from a previously quiescent patch. The inward events at -80 mV (inside negative) must be due to the flow of Cl^- from the cell to the pipette since there are no permeant cations in the pipette (see Fig. legend). At negative potentials, open channel currents are characterized by many brief transitions toward the zero-current baseline; this flickering behavior is less pronounced at positive potentials (Fig. 1A). This kinetic behavior is similar to that reported previously for human CFTR (Cliff et al., Am. J. Physiol. 262:C1154-C1160, 1992). This channel is characterized by a linear I-V relationship with a single-channel conductance of 6 pS (Fig. 2B). Activation of a 4-6 pS Cl^- channel has been observed in 7 additional cells in which channels were not observed prior to stimulation. The large 50 pS Cl^- channel similar to that previously described by Greger et al. (Pflugers Arch. 409:114-121, 1987) and La et al. (Am. J. Physiol. 260: C1217-C1233, 1991) has not been observed in these cells. The reason for this disparity between our results and those previously reported is unclear. However, recent complementation studies (Egan et al. Nature. 358: 581-584, 1992) have demonstrated that expression of CFTR in CF bronchial cells results in both the expression of a small linear chloride channel as well as restoring the ability of PKA to activate an outwardly rectifying Cl^- channel. Whether DFTR may have multiple functions as well will require further studies.

These preliminary results suggest that increased cellular cAMP in dogfish rectal gland activates a small linear Cl^- channel which exhibits biophysical properties similar to those previously described for the Cl^- channel associated with expression of human CFTR. Additional experiments should help elucidate whether the structural differences between DFTR and CFTR at the protein level (e.g. PKA and PKC consensus sequences) result in functional differences in channel regulation. (Supported by NIH DK38518, DK08810, DK34208 & AHA 92011310).