EVIDENCE FOR AN ATP CONDUCTIVE PATHWAY INDUCED BY CYCLIC AMP IN RECTAL GLAND CELLS FROM THE SHARK SQUALUS ACANTHIAS

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Adenosine triphosphate (ATP) is an essential intracellular metabolite whose extracellular role in cell function is increasingly recognized (Dubyak & El-Moatassim, Am. J. Physiol. 265: C577-C606, 1993). Although the non-cytolytic release of ATP into the extracellular milieu has been documented in a variety of cell types, the molecular mechanisms involved in this movement from the intra- to extracellular compartment are largely unknown. Recent studies from our laboratory demonstrated that the cystic fibrosis transmembrane conductance regulator (CFTR), the molecule responsible for the cystic fibrosis phenotype in human epithelial cells, not only is a cAMP-inducible, chloride-selective ion channel as has been previously demonstrated (Rich et al., Nature 347: 358-363, 1990), but also is capable of conducting ATP as the charge carrier (Reisin et al., J. Gen. Physiol. 100: 33a, 1992 and Cantiello et al., J. Biol. Chem., in press, 1994). This is in agreement with recent evidence from our laboratory indicating that another member of the ATP-binding cassette (ABC) family of proteins, P-glycoprotein, is also able to conduct ATP (Abraham et al., Proc. Natl. Acad. Sci. USA, 90: 312-316, 1993). These findings indicate that ATP may serve as a substrate for electrodiffusional movement by suitable transport proteins that behave as novel nucleotide channels. Because the ATP-conductive mode of CFTR bears several functional similarities to its putative role as a chloride channel, namely activation by cAMP, inhibition by diphenylamine-2-carboxylate (DPC), and non-rectifying properties, a general functional characteristic of the ABC family of proteins, in particular CFTR analogs, may be their novel nucleotide-conductive capabilities.

The shark rectal gland (SRG) of the spiny dogfish shark Squalus acanthias is a paradigmatic secretory epithelial model that has aided understanding the molecular steps associated with salt and water movement and the secretory response. Original studies by Greger and co-workers demonstrated the presence of at least two different apical and anion-selective channels in the cells lining this epithelium (Greger et al., Pflugers Arch., 409: 114-125, 1987). One of those channels may be DFTR, a homologue of CFTR present in SRG cells (Marshall et al., J. Biol. Chem. 266: 22749-22754, 1991), thus indicating that the secretory response of this epithelium might share functional similarities to those of human secretory epithelia. In this report we used patch-clamp techniques to determine the presence of an endogenous ATP conductive pathway in SRG cells in culture.

To determine whether cAMP activation of SRG cells could induce an ATP-conductive pathway, whole-cell ATP currents were determined with symmetrical ATP (200 mM) before (basal conditions) and after the addition of cpt-cAMP (500 μM). The unstimulated whole-cell ATP conductance was linear in symmetrical MgATP with a whole-cell conductance $\gamma_{(+)}$ of 2.09 ± 0.11 nS (mean ± SEM, n=4) versus $\gamma_{(-)}$ 3.10 ± 0.08 nS (n=4). The conductance for negative potentials, $\gamma_{(-)}$ was 48.3% higher ($p<0.01$). In 6 out of 12 experiments, addition of cpt-cAMP increased the whole-cell conductance to 5.53 ± 0.22 nS (n=6) and 11.6 ± 0.89 (n=6) for positive and negative holding potentials, respectively. This indicates an increase of 157% ($p<0.05$) and 274% ($p<0.001$), respectively. The change in reversal potential was consistent with an increase in the anionic conductance. This was also determined by dilution potentials. Thus, cAMP activation induces an ATP-conductive pathway that, under symmetrical conditions rectified in the direction of ATP efflux.

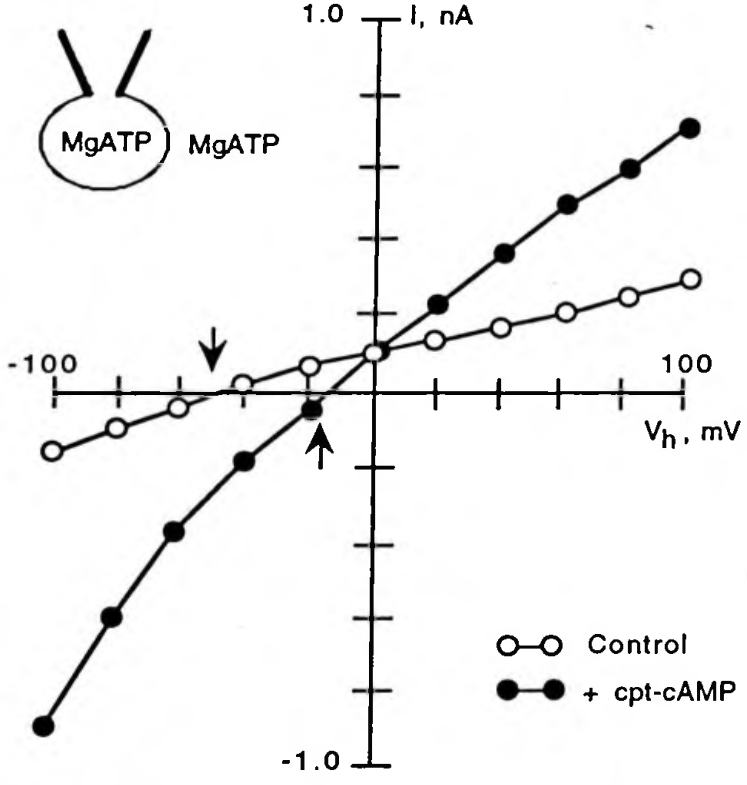

Figure 1: Effect of cAMP stimulation on ATP currents under symmetrical ATP conditions. Whole-cell currents were obtained in symmetrical 200 mM MgATP before (open circles) and after addition of 500 μM cpt-cAMP (filled circles). The experimental points are the average of 4-6 experiments under either condition. Reversal potentials are indicated by the arrows. Whole-cell ATP currents were obtained as previously reported (Abraham et al., Proc. Natl. Acad. Sci. USA, 90: 312-316, 1993).

The ABC family of transporters are capable of electrodiffusional movement of ATP as we have demonstrated for P-glycoprotein and CFTR. This raises the possibility that ATP movement may be implicated in signal transduction mechanisms associated with the regulation of ion channels (Dubyak & El-Moatassim, Am. J. Physiol. 265: C577-C606, 1993). The data for P-glycoprotein and CFTR were generated in cells induced to express the protein at greater than physiological levels. The data in the present study provide evidence that endogenous levels of DFTR might also share functional similarities to those observed with CFTR.

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