ATP RELEASE MEDIATED BY HUMAN AND SHARK HOMOLOGS OF THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR

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Recent studies from our laboratory have directly implicated the human cystic fibrosis transmembrane conductance regulator (CFTR) in the transport of ATP using several techniques, including patch clamping (Reisin, et al., *J. Biol. Chem.* 269: 20584-20591, 1994), luminometry (Prat, et al., *Am. J. Physiol.* 270: C538-C545, 1996), and reconstitution of purified protein in planar lipid bilayers (Cantiello, et al., *Am. J. Physiol.*, In Press, 1998). Furthermore, we recently determined the presence of an ATP-permeable pathway in cultured shark rectal gland (SRG) cells. Interestingly, SRG cells also express shark CFTR, a protein highly homologous to human CFTR (Marshall, et al., *J. Bioil. Chem.* 266: 22749-22754, 1991). Therefore, in this report, we initiated a functional characterization of the ATP permeability properties of both CFTR homologs using a combination of luminometry techniques and the *Xenopus* oocyte expression system.

Oocytes from *Xenopus laevis* were harvested and injected with 10-15 ng of mRNA of either the human (hCFTR) or shark (sCFTR) homolog of CFTR. The oocytes were then maintained at room temperature in a "Frog Ringer" solution containing, in mM, 98 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, pH 7.4.

ATP release was determined using a modified luciferin-luciferase assay as previously described (Abraham, et al., Proc. Nat. Acad. Sci. USA 90: 312-316, 1993). A single oocyte was placed in a plastic cuvette containing 0.5 ml of frog Ringer and 0.1 ml of the luciferin-luciferase assay mix (Analytical Luminescence Laboratories, Ann Arbor, MI). Basal ATP release was determined as photon release using a luminometer (Monolight 2010, Analytical Luminescence Labs), which was followed for 2-3 min prior to addition of 10 μM forskolin (Sigma, St. Louis, MO) to the cuvette. ATP release was again followed for up to 10 minutes after cAMP stimulation with forskolin. To determine the amount of ATP released from the oocytes, known concentrations of ATP were titrated with the assay mix to obtain a calibration curve. Using this curve, relative light units (RLU's) could be translated to molar concentrations of ATP.

Oocytes expressing hCFTR showed both low and high ATP release states. The "low" release group had a basal ATP release of 1.3 ± 0.1 pmoles ATP/cell (n=3, Fig. 1). This ATP release was increased 1,476% after addition of forskolin (18.6 ± 6.9 pmoles ATP/cell, n=3, p<0.1). The "high" release group had a basal release of 11.7 ± 1.3 pmoles ATP/cell (n=5), which only increased 270% after addition of forskolin (31.6 ± 4.1 pmoles ATP/cell, p< 0.01).

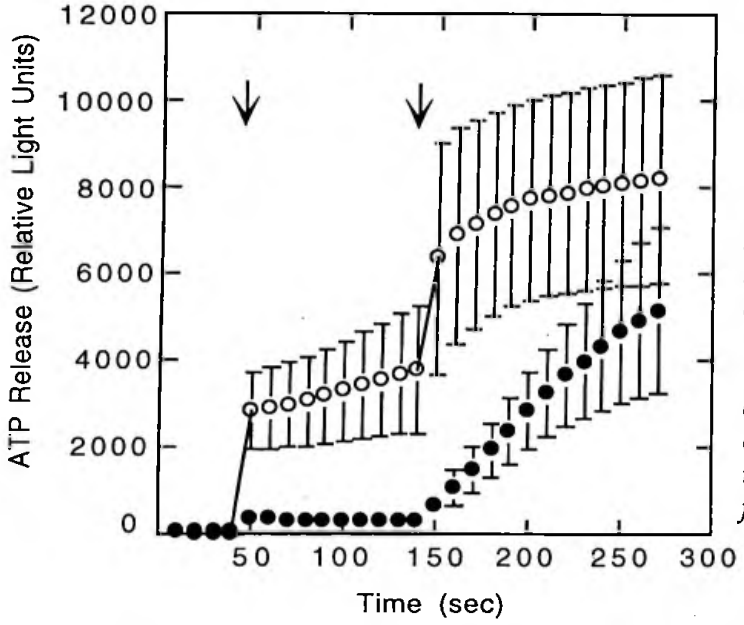

Fig. 1. ATP release from oocytes expressing human CFTR. The filled symbols represent the "low" release state, and open symbols indicate the "high" release state. Data are the mean ± sem of 3 and 5 experiments, respectively. The first arrow indicates the point at which the oocyte was placed in the cuvette, and the second indicates the addition of forskolin (10 μM).

Oocytes expressing sCFTR showed a high basal ATP release (7.5 ± 1.1 pmoles ATP/cell, n=4, Fig. 2) when placed in the cuvette. Addition of forskolin increased ATP release by 509% (45.7 ± 7.7 x pmoles ATP/cell, n=4, p<0.01).

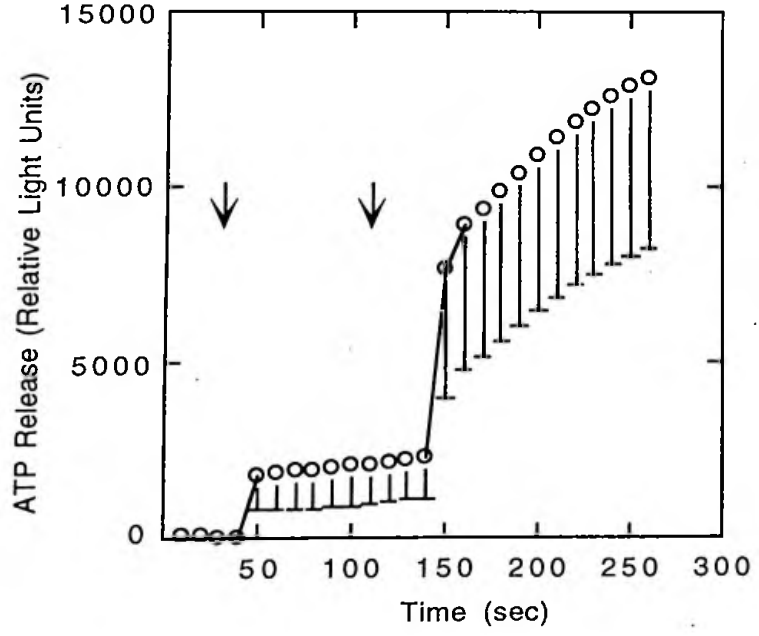

Fig. 2. ATP release from oocytes expressing sCFTR. Data are the mean ± sem of 4 experiments. The first arrow indicates addition of the oocyte to the cuvette, and the second arrow indicates addition of forskolin (10 μM).

In 5 out of 6 experiments, control oocytes injected with water failed to respond to addition of forskolin (data not shown). In addition, forskolin did not induce a significant increase in ATP release in oocytes used 6 days after injection (data not shown). This may be due to a reduced expression of protein over time. In addition, the "low" release state observed with hCFTR, which did not reach statistical significance after stimulation with forskolin (Fig. 1), may be due to a low expression of the protein. The data indicate that both human and shark CFTR are associated with a cAMP-stimulated ATP release, and that the spontaneous ATP release in the "high" release state may be dependent on a certain level of activation of hCFTR prior to addition of forskolin. Further experiments will be required,however, to correlate ATP release with protein expression.

The data in this report are in agreement with the recent demonstration that hCFTR is indeed an ATP-permeable channel (Cantiello, et al., *Am. J. Physiol.* 274: In Press), and support the hypothesis that human and shark CFTR share this common functional feature. The present data are the first demonstration that sCFTR is indeed capable of eliciting the transmembrane release of ATP, and allows the postulation that at least one of the electrodiffusional ATP pathways recently described in cultured SRG cells (Cantiello, et al., *Am. J. Physiol.* 272: C466-C475, 1997) may be attributed to sCFTR.

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