5' AMP-activated protein kinase regulates chloride secretion and is phosphorylated in the dogfish shark (*Squalus acanthias)* rectal gland under hypoxic conditions and co-immunoprecipitates with CFTR

Rugina I. Neuman[1,5,6], Daniel J. Pfau[2,5,6], Dhariyat Menendez[3,5,6], Kathryn Peters[4,6], Raymond A. Frizzell[4,6], Lawrence H. Young[5] John N. Forrest, Jr[5,6]
[1]Erasmus University Medical Center, Rotterdam, Netherlands, [2]Fordham University, Bronx, NY 10548
[3]Case Western University, Cleveland, OH 44106
[4] Department of Cell Biology University of Pittsburgh, Pittsburgh PA 15224
[5]Department of Medicine, Yale University School of Medicine, New Haven, CT 06510
[6]Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672

AMP Kinase (AMPK) is a metabolic regulator that is phosphorylated in several mammalian tissues under hypoxic stress. We find that AMPK is present in the shark rectal gland, is phosphorylated under hypoxic conditions and co-immunoprecipitates with CFTR in lysates of rectal gland cells.

AMP-activated protein kinase (AMPK) is an important metabolic-sensing serine/threonine kinase and regulator of a variety of cellular processes. AMPK exists as a heterotrimer consisting of α-catalytic subunits, regulatory β-subunits and γ-subunits[1] and is sensitive to metabolic stressors, such as glucose deprivation, hypoxia and ischemia. These conditions lead to an increase in the AMP:ATP ratio, by inhibiting ATP production, which in turn leads to activation and phosphorylation of the AMPK α-subunit.[1] In mammalian cells, AMPK interacts with cystic fibrosis transmembrane conductance regulator (CFTR), as indicated by a yeast two-hybrid screen[2]. CFTR is a member of the ATP-binding cassette family (ABC) of transporters and is an ATP-gated chloride (Cl^-) channel[3]. The rectal gland of the spiny dogfish (*Squalus acanthias*) (SRG) is an excellent model to study epithelial chloride transport through CFTR[4]. We hypothesized that AMPK is present in the gland and is phosphorylated when the gland is exposed to severe hypoxia.

Shark rectal gland perfusion studies demonstrated significantly lower chloride secretion (μEq/h/g; Y axis) under hypoxic conditions (nitrogen perfusion) as compared to controls (Fig 1).

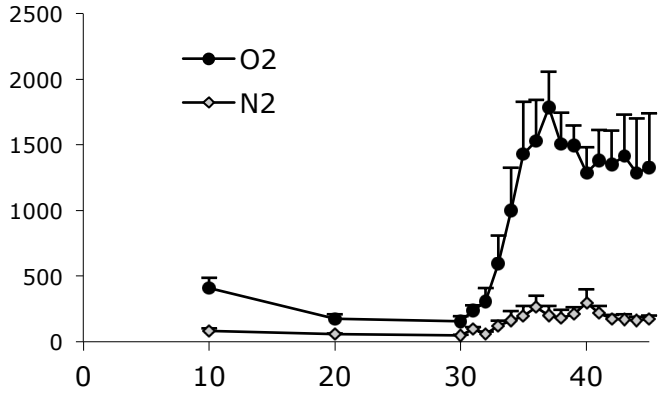

Figure 1. The conditions were created by bubbling shark Ringer's perfusate with either 99% N_2 and 1% CO_2 or 99% O_2 and 1 CO_2. Stimulation of chloride secretion was induced by adding forskolin (adenylyl cyclase activator, 1 μM) and IBMX (PDE inhibitor, 100 μM) to the perfusate at 30 min. Glands were immediately snap frozen after perfusion and used for Western blot analysis (Fig 2).

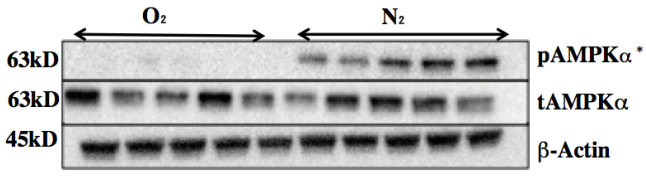

Figure 2. Immunoblot analysis of AMPKα phosphorylation during hypoxia. Phosphorylated AMPKα was significantly greater in hypoxic glands (n = 5) than in normoxic glands (n = 5; $P < 0.0001$; paired *t*-test). Rabbit anti-AMPKα (t AMPKα) and rabbit anti-phospho-AMPKα (pAMPKα) were obtained from Cell Signaling (Boston, MA); rabbit anti-phospho-AMPKα detects AMPKα only when phosphorylated at threonine172 in the catalytic domain.

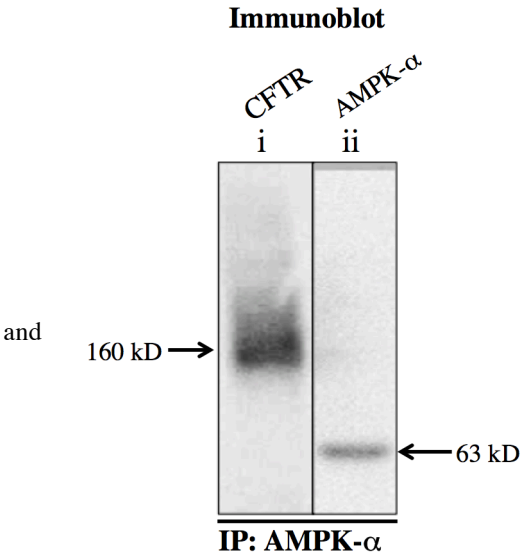

Figure 3. Interaction between CFTR and AMPK in shark rectal gland. Immunoblots of immunoprecipitated AMPKα from shark rectal gland probed with either CFTR 596 antibody (lane i) or AMPKα antibody (lane ii). These data strongly suggest that AMPK CFTR co-interact.

Taken together, these experiments demonstrate that 1) AMPKα is present in the SRG; 2) AMPKα is phosphorylated under hypoxic conditions when chloride secretion is marked reduced; and 3) AMPK co-immunoprecipitates with CFTR, which further suggests CFTR and AMPKα are tightly bound and interact in a physiologically relevant manner in rectal gland cells.

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1. **Hallows KR**. Emerging role of AMP-activated protein kinase in coupling membrane transport to cellular metabolism. *Curr. Opin. Nephrol. Hy.* 14:464-471, 2005.
2. **Hallows KR, Raghuram V, Kemp BE, Witters LA, Foskett JK**. Inhibition of cystic fibrosis transmembrane conductance regulator by novel interaction with the metabolic sensor AMP-activated protein kinase. *J. Clin. Invest.* 105:1711-1721, 2000.
3. **King JD, Jr., Fitch AC, Lee JK, McCane JE, Mak DO, Foskett JK**, et al. AMP-activated protein kinase phosphorylation of the R domain inhibits PKA stimulation of CFTR. *Am. J. Physiol. Cell Physiol.* 297:C94-101, 2009.
4. **Forrest JN, Jr**. Cellular and molecular biology of chloride secretion in the shark rectal gland: regulation by adenosine receptors. *Kid. Intl.* 49:1557-1562, 1996.