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 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. In mammalian cells, AMPK interacts with cystic fibrosis transmembrane conductance regulator (CFTR), as indicated by a yeast two-hybrid screen. CFTR is a member of the ATP-binding cassette family (ABC) of transporters and is an ATP-gated chloride (Cl−) channel. The rectal gland of the spiny dogfish (Squalus acanthias) (SRG) is an excellent model to study epithelial chloride transport through CFTR. 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% N₂ and 1% CO₂ or 99% O₂ and 1% CO₂. 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 and 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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