The chloride secretory response to hypoxia in the rectal gland of the spiny dogfish, *Squalus acanthias:* Is 5'AMP kinase (AMPK) involved?

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The spiny dogfish shark (*Squalus acanthias*) rectal gland (SRG) is an ideal model for studying the relationship between hypoxia and ion transport across rectal gland tubules through the cystic fibrosis transmembrane conductance regulator (CFTR). In mammalian tissues the enzyme 5' adenosine monophosphate-activated protein kinase (AMPK) regulates ATP production and consumption, and should be activated when a cell is under metabolic stress, such as hypoxia. To initiate an understanding of the possible role of AMPK in the SRG, we induced hypoxia by perfusing the gland with shark Ringer's solution bubbled with nitrogen and compared this to normal shark Ringer's bubbled with oxygen.

The shark rectal gland (SRG) is a tubular epithelial organ in the dogfish shark that carries out secondary active chloride transport. Because it is composed of a single epithelial cell type, a single artery, vein and duct and is a record holder among epithelia at secreting salt, the perfused shark rectal gland is an ideal model for studying chloride transport[3]. As shown in Figure 1, chloride enters uphill across the cotransporter and is secreted across the apical membrane through the cystic fibrosis transmembrane conductance regulator (CFTR) when cAMP is elevated. Na and K ions enter through the co-transporter and exit through the basolateral sodium pump and K channels. cAMP, when elevated, activates PKA which phosphorylates the regulatory domain of the CFTR channels.

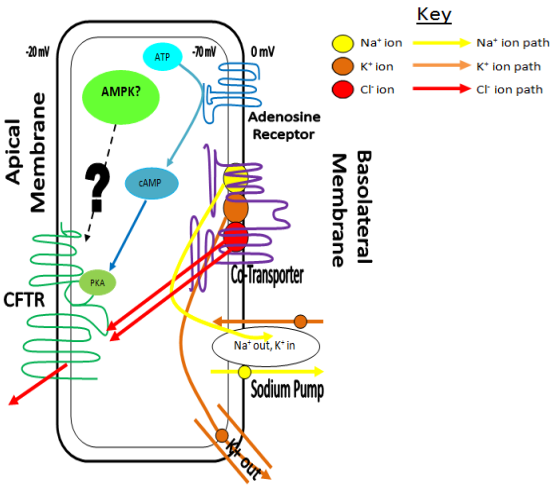

Figure 1. Shark rectal gland cell model.

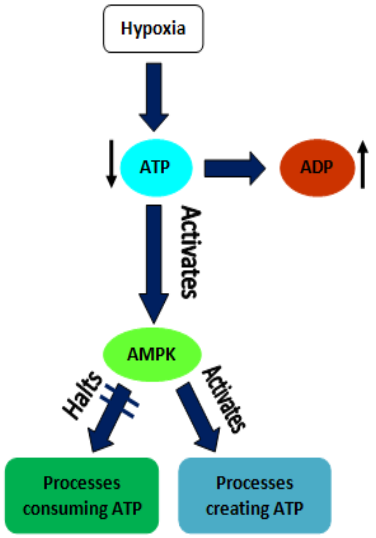

Figure 2. Postulated effect of hypoxia on AMPK in epithelia.

Adenosine monophosphate-activated protein kinase (AMPK) is found in mammalian epithelial cells, but it is unknown whether it is present in shark rectal gland. In mammalian species AMPK regulates pathways involved in cell cycle, apoptosis, transcription, inflammation, and ion transport[1,2]. AMPK inhibits CFTR in mammalian tissues[5]. Since CFTR plays a major role in chloride secretion of the SRG, if CFTR is inhibited at any point, chloride secretion will fall. AMPK is activated when there is a disruption in the ADP:ATP ratio within the cells, for example, under hypoxia (Figure 2). In this condition, ATP levels fall, and ADP levels rise. In response to the depletion of ATP, AMPK deactivates processes that consume ATP, while maintaining processes that create ATP (Fig 2)[5]. We hypothesize that under hypoxic conditions in the shark rectal gland, AMPK if present, is activated, deactivating the ATP consuming process of chloride secretion.

We thus perfused glands under both hypoxia and normal conditions. Under hypoxia conditions (Fig 3) chloride secretion was markedly limited but increased after oxygen was introduced in the second hour.

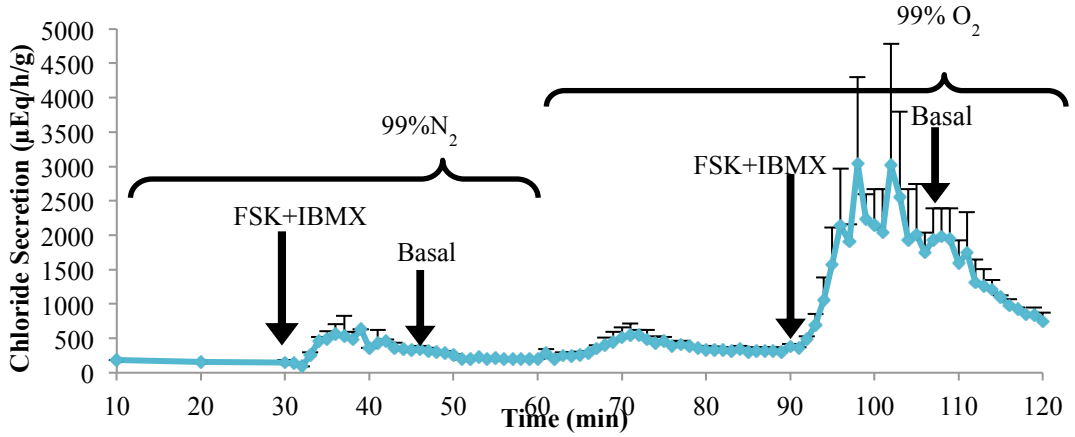

Figure 3. Graph of two experiments in which the perfusate was bubbled for 60 min with N_2, and then 60 minutes with O_2 (glucose 20 μM and 1% CO_2 in all perfusates); values are mean ± SEM.

We found that hypoxia markedly diminishes chloride secretion in response to secretagogues (Forskolin 1 μM and IBMX 100 μM) in shark rectal gland, and this effect was reversible. The presence of AMPK in the shark rectal gland is established in the abstract by van Kalmthout in this Bulletin.

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