Lack of evidence that endogenous fatty acids can support chloride secretion in the rectal gland of *Squalus acanthias*

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All cells require fuel to sustain their morphological integrity and execute their functions. This report shows that fatty acids within the rectal gland cells do not fuel the cells neither in the presence nor in the absence of glucose. Thus, chloride secretion by the rectal gland cells can be entirely supported by glucose metabolism.

When isolated rectal glands are perfused without an external source of energy they continue to secrete chloride albeit at a reduced rate.[1] Although the rate is reduced, it is still a significant, amounting to about forty percent of that in the presence of an exogenous fuel such as glucose.[1] This observation led to the conclusion that the rectal gland has an endogenous source(s) of energy capable of partially sustaining the secretion of chloride.[2] Because the rectal gland cells contain glycogen, it is natural to think that the endogenous source of energy is glucose derived from the hydrolysis of glycogen.[2] It is also possible that the rectal gland cells have other sources of energy capable of supporting the secretion of chloride. One such source could be the oxidation of fatty acids. Lipid bodies have been identified in the elasmobranch rectal glands.[3] It is likely then that fatty acids are available as an energy source for the rectal gland cells. To test for this possibility, we used mercaptoacetate to inhibit the oxidation of fatty acids.[4]

Isolated rectal glands of *S. acanthias* were perfused through their single artery by gravity at 16˚C and 40 mm Hg pressure with oxygenated shark Ringer's solution containing 350 mM urea and with and without 5 mM glucose in a single pass perfusion. Venous effluent and duct fluid were collected separately from PE-90 catheters placed in the vein and duct of the gland. Collections were made every ten minutes. Chloride was measured using a Buchler-Cotlove chloridometer (Labconco, Kansas City, MO). Chloride secretion was calculated from the chloride concentration in the duct fluid, the volume of the fluid, the collection time, and the weight of the gland and expressed as μEq per gram of gland per hour. Glucose levels of the rectal gland tissue were measured using a Cobas Mira analyzer using glucose oxidase. Statistical analysis was done using Student's *t* test.

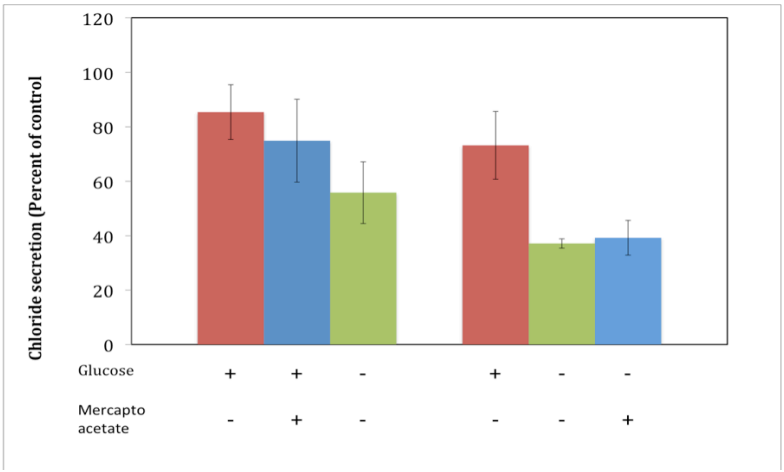

Figure 1. Mercaptoacetate has no effect on the secretion of chloride in the absence or presence of glucose. Glands perfused with glucose are depicted by the red columns; glands perfused without glucose are depicted by the green columns; glands perfused with mercaptoacetate depicted by the blue columns. The secretion of chloride in the presence of glucose was the same with and without mercaptoacetate. Mercapto-acetate did not reduce the secretion further after the removal of glucose. Values are mean ± SEM. N = 7 for control, 8 for no glucose, and 5 for mercaptoacetate experiments.

Figure 1 shows the effect of mercaptoacetate on chloride secretion in glands perfused with (left hand side) and without glucose (right hand side). During the initial thirty minutes of perfusion the glands were perfused with 5 mM glucose. At the end of the third collection period the solution was changed to one containing 5 x 10^{-4} M or 10^{-3} M mercaptoacetate. After an additional thirty minutes of perfusion the solution was changed to one

containing mercaptoacetate but without glucose. There was no difference between the two concentrations of mercaptoacetate, and the results were pooled together. In another series of experiments (right hand side), glands were perfused first with glucose, then without glucose and finally mercaptoacetate was added to the perfusion; again no statistically significant change in chloride secretion was observed.

A possible explanation for the failure of mercaptoacetate to inhibit the secretion of chloride is that there is always enough glucose within the cells in the glands, even during perfusion without glucose. Therefore, tissue glucose was measured in an acid extract of rectal glands at the end of the perfusions, at a time when the glands had been perfused without glucose for thirty minutes and were still actively transporting chloride. The concentration of glucose in the glands was 0.99±0.18 μM per gram of tissue water (mean ± SEM n= 5). The level of glucose in separate control rectal glands perfused with 5 mM glucose was 1.47±0.15 μM/g of tissue water (n = 6). Since the latter glands were perfused with 5 mM glucose and the extracellular space of the glands is 30%, the difference in the concentration of glucose between the two sets of glands can be almost completely accounted for by the glucose in the extracellular space in the glands perfused with glucose.

Mercaptoacetate inhibits the initial step in the beta oxidation of long chain fatty acids and therefore, should prevent their use as a source of fuel. In the present series of experiments, we used mercaptoacetate at concentrations of 5 x 10^{-4} and 10^{-3} M. Mercaptoacetate had no effect on the secretion of chloride either in the presence of glucose or in its absence. Glands perfused without glucose still had significant amounts of glucose even after thirty minutes of perfusion without glucose, likely the result of the hydrolysis of glycogen. In fact, the amount of glucose was similar in magnitude to that of glands perfused with glucose. However, the failure of mercaptoacetate to alter the secretion of chloride should not be taken as evidence that endogenous fatty acids cannot support the metabolic needs of the gland cells at all, because the gland cells apparently contained enough glucose to support their needs. Another caveat that must be considered is that we did not measure fatty acid metabolism directly and thus we only assume, but did not demonstrate, an inhibitory action of mercaptoacetate in the rectal gland.

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2. **Kinne R, Spokes KC, Silva P.** Glycogen measurement in the rectal gland of *Squalus acanthias*. *Bull. Mt Desert Isl. Biol. Lab*. 2011, 50:26-27.
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