The regulation of intestinal bicarbonate secretion by marine teleost fish
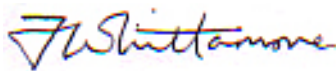
Volume 1 (of 2)

Submitted by Jonathan Mark Whittamore, to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Biological Sciences in December 2008.

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Signature   *J W Whittamore*   Date   **10th December 2008**

Acknowledgements

Abstract

In seawater, drinking is a fundamental part of the osmoregulatory strategy for teleost fish, and presents a unique challenge. The intestine has an established role in osmoregulation, and its ability to effectively absorb fluid from imbibed seawater is crucial to compensating for water losses to the surrounding hyperosmotic environment. Alongside solute-linked water transport (driven by NaCl cotransport), intestinal bicarbonate (HCO_3^-) secretion also benefits fluid absorption directly (*via* apical Cl^-/HCO_3^- exchange), and indirectly through the formation of calcium carbonate ($CaCO_3$) thus removing the osmotic influence of Ca^{2+} within the gut fluid. For the European flounder (*Platichthys flesus*), elevated luminal Ca^{2+} has proven to be a specific, potent stimulator of HCO_3^- secretion both *in vitro* and *in vivo* where these actions are presumably modulated by an extracellular Ca^{2+}-sensing receptor (CaR). The focus of this work was to learn more about how intestinal HCO_3^- secretion is regulated, the role of Ca^{2+}, and more specifically the CaR. To achieve this, *in vitro* 'gut sac' experiments investigated how luminal Ca^{2+} influenced HCO_3^- secretion, and associated ion and fluid transport. Contrary to expectation, increasing Ca^{2+} from 5 to 20 mM did not stimulate HCO_3^- secretion. In an attempt to elucidate the role of $CaCO_3$ precipitation in fluid absorption, and further explore the physiological implications of HCO_3^- secretion, the intestine was perfused *in vivo* with salines containing varying concentrations of Ca^{2+} (10, 40 and 90 mM). The production and secretion of HCO_3^-, in addition to $CaCO_3$ formation increased accordingly with Ca^{2+}, and was associated with a dramatic 25 % rise in the fraction of fluid absorbed by the gut. Additional *in vitro* experiments, utilising the Ussing chamber, helped establish some of the characteristics of intestinal HCO_3^- secretion by the euryhaline killifish (*Fundulus heteroclitus*), but was unresponsive to elevated mucosal Ca^{2+}. Further attempts to potentiate the activity of the CaR, and application of the receptor agonists gadolinium (Gd^{3+}) and neomycin, failed to produce responses consistent with the effect of Ca^{2+} observed previously, either *in vitro* or *in vivo*. With no evidence supporting a direct role for an extracellular, intestinal CaR in HCO_3^- secretion it was argued that secretion would be principally regulated by two factors, the ability of the epithelia to generate high levels of intracellular HCO_3^- and the rate of $CaCO_3$ formation.

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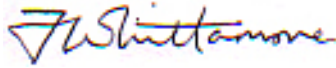# The regulation of intestinal bicarbonate secretion by marine teleost fish

Volume 2 (of 2)

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