

**THE CONSEQUENCES OF INTESTINAL BICARBONATE
SECRETION ON ION, OSMOTIC AND ACID-BASE REGULATION
IN THE EURYHALINE EUROPEAN FLOUNDER**

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Introduction

Marine teleost fish are hypo-osmotic to the medium they inhabit. To avoid dehydration they drink the external seawater, absorb the majority of the imbibed volume within the intestine, and the remainder is excreted as rectal fluid. However, the rectal fluid is both alkaline (pH 8.4-9.0) and rich in the basic HCO_3^- and CO_3^{2-} ions (50-150 mM; Walsh et al., 1991; Wilson et al., 1996; 1999). This bicarbonate-rich environment causes the precipitation of imbibed calcium and magnesium as insoluble carbonates that are subsequently excreted along with the rectal fluids. The removal of these divalent cations by precipitation possibly serves two important functions: 1) to minimise the absorption of calcium (and its subsequent costly excretion via the kidney), and 2) to reduce the osmolality of intestinal fluid thus promoting water absorption into the blood. The importance of these roles is suggested by the linear increase of intestinal bicarbonate secretion with external salinity (and hence with drinking rate and passage of divalent ions into the intestine). Changes in intestinal bicarbonate secretion rates (i.e. net base excretion) will presumably have additional consequences for whole animal acid-base balance. The aim of the present study was to quantify how changes in bicarbonate secretion and calcium precipitation within the intestine impact a) the uptake of calcium and water from the intestine, and b) whole animal acid-base balance.

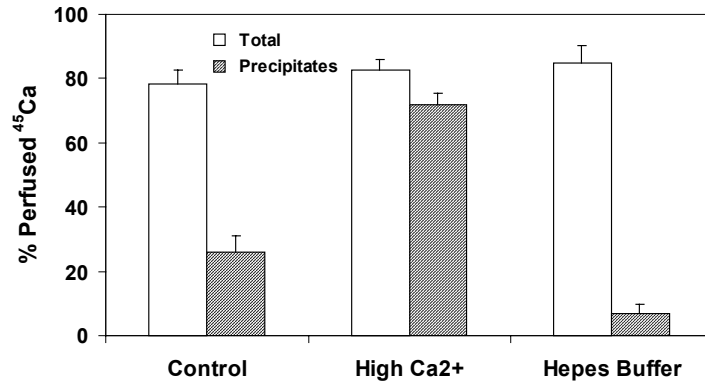
Materials and Methods

European flounder (*Platichthys flesus*), were obtained from commercial fishing in the northern part of the Sound of Copenhagen, Denmark, and held in a re-circulating artificial seawater system at $13\pm 1^\circ\text{C}$. Fish were then prepared for *in situ* perfusion of the intestine according to the method of Grosell *et al.* (1999). Following surgery flounder ($n=21$) were placed in individual flux chambers and the intestine perfused at $\sim 3.8 \text{ ml kg}^{-1} \text{ h}^{-1}$ with one of 3 different gut salines designed to stimulate or decrease bicarbonate secretion and/or calcium precipitation; 1) Control saline (with 5 mM Ca^{2+}), 2) High calcium saline (20 mM Ca^{2+}), 3) Buffered Saline (5 mM Hepes , $\text{pH } 7.5$), all with matched osmolality (310 mOsm kg^{-1}). Rectal fluids were continuously collected via catheters during 72 h of perfusion. Salines also contained ^{45}Ca to trace the absorption versus precipitation of calcium. Fluxes of acid-base relevant ions via the intestine and non-intestinal routes (gills, skin, kidney) were measured over 11 or 22 h periods during perfusion. All values are expressed as means \pm standard error.

Results

Perfusion of the intestine with 20 mM (high Ca^{2+}) instead of 5 mM calcium (controls) resulted in a 7-fold increase in the amount of precipitated carbonate and a 57% increase in the total intestinal bicarbonate secretion rate (sum of HCO_3^- equivalents in rectal fluid and precipitates). Perfusion with Hepes-buffered saline did not affect the overall bicarbonate excretion rate, but halved the proportion that was excreted as carbonate precipitates. There were no significant differences in the $[\text{Ca}^{2+}]$ in rectal fluid of fish from the 3 treatments (means ranged from 2.4 ± 0.4 to $3.6\pm 0.6 \text{ mM}$). Regardless of the perfusate $[\text{Ca}^{2+}]$, the % recovery of ^{45}Ca in the rectal fluids was very high ($\sim 80\%$) and virtually identical in all three treatments (Fig.1). The similar % recovery from the high Ca^{2+} saline was mainly due to large increase in precipitation of ^{45}Ca as calcium carbonate (70%) rather than recovery in rectal fluid (Fig. 1). The osmolality of both rectal fluid and plasma was significantly reduced in fish perfused with the high Ca^{2+} saline, but net water absorption by the intestine was unaffected by any treatment. Blood pH and ammonia excretion via non-intestinal routes were also unaffected by perfusion with the different salines.

Fig. 1 : % of the ^{45}Ca in the intestinal salines recovered in the rectal fluid and carbonate precipitates of flounder with intestines perfused over 72 h *in situ*.



Conclusions

The large stimulation of total bicarbonate secretion by the intestine when calcium was elevated in the perfusion saline indicates that precipitation of calcium as carbonate may be a primary role of the bicarbonate secretion process. This is supported by acute *in vitro* experiments showing that bicarbonate secretion is stimulated very rapidly (within minutes) in response to elevation of calcium but not magnesium (see parallel presentation in this session by Grosell & Wilson). The reduced rectal fluid and plasma osmolality caused by high Ca^{2+} saline confirms the potential for an important role of bicarbonate secretion in osmoregulation. The up-regulation of bicarbonate secretion is very effective at precipitating at least 80% of the calcium entering the intestine, regardless of the initial concentration. Precipitation therefore also represents an important process in calcium homeostasis of marine teleosts by minimising absorption of imbibed calcium.

The stimulation of total intestinal bicarbonate secretion during perfusion with high calcium saline amounts to a simultaneous elevation of the net excretion of base via this route. This must have consequences for the acid-base balance of the whole animal. However, as no blood-acid-base disturbance was observed, whole animal acid-base balance must be maintained by the excretion of an equivalent

amount of excess acid via non-intestinal routes. The net excretion of NH_4^+ via non-intestinal routes was not significantly altered, therefore an increase in the branchial excretion of other acidic equivalent ions (or decrease in excretion of basic equivalent ions) is likely responsible for maintaining acid-base homeostasis.

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