

Na⁺,K⁺-ATPase and NKCC (Na⁺:K⁺:2Cl⁻ COTRANSPORTER)

EXPRESSION IN GLASS EELS (*ANGUILLA ANGUILLA*)

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Introduction

The European eel (*Anguilla anguilla*) has a facultative catadromous life history and spawns in the Sargasso Sea. Following a long trans-Atlantic migration, the leptocephalus larvae metamorphose to take on the eel-like body form once they reach the continental shelf. This glass-eel stage migrates into freshwater making the important transition from hypo-osmoregulation to hyper-osmoregulation. Despite the economical importance of the glass eel fishery (1000€/kg) to aquaculture and significance of this freshwater transition to river system recruitment, relatively little attention has been directed at the osmoregulatory physiology of this life history stage (e.g. Birrell et al. 2000). In fish in general, the gill is the most important effector organ in ion regulation. The diametric shift in ionic regulatory strategy from active ion excretion in seawater to ion uptake in freshwater would involve the realignment of ion transport processes in the gill.

Two key ion transport proteins associated with seawater ion regulation are Na⁺,K⁺-ATPase and the Na⁺:K⁺:2Cl⁻ cotransporter (NKCC) (McCormick 1995; Pelis et al. 2001). Na⁺,K⁺-ATPase is the driving force for the elimination of Cl⁻ in conjunction with the NKCC and a Cl⁻ channel protein in seawater fishes and is also involved in Na⁺ uptake in freshwater fishes although levels of activity tend to be lower in freshwater fishes.

The regulation of Na⁺,K⁺-ATPase activity has been shown to be under endocrine control (McCormick 1995). The steroid hormone cortisol has been shown to increase levels of Na⁺,K⁺-ATPase and NKCC in the gill as well as the number of branchial mitochondria-rich cells (MRCs) and has been called the “seawater hormone” although it does have similar effects in freshwater salmonids. In the present study, we compared branchial Na⁺,K⁺-ATPase and NKCC expression in glass-eels adapted to freshwater and seawater. Also, the effect of the exogenous cortisol and cortisol receptor blockade (RU-486) was studied in each group.

Material & Methods

Glass eels (*Anguilla anguilla* L.) were collected from the mouth of the River Minho (February 2002) and transported to the laboratory where they were kept in holding tanks containing either freshwater or seawater (32‰). The water was well aerated and temperature kept constant at 15°C, and 20% of the tank volume was changed every third day. No attempt was made to feed animals during holding. Animals were randomly separated into groups of ~25 animals in enclosed floating baskets within each holding tank. Animals were anaesthetized and given intra-peritoneal injections of either coconut oil (CO; 0.5μl·0.1g bw); CO+5μg·g⁻¹ cortisol (F); CO +50μg·g⁻¹ F; or +50μg·g⁻¹ RU-486 (Sigma Chemical Co.). After 10 days animals were sampled. Some animals were prepared for immunohistochemistry (Wilson et al. 2000), while others had the gill basket excised for ion transport protein quantification. Fish length, wet weight, % water, and sodium were also measured.

Na⁺,K⁺-ATPase activity was measured using a microassay (see McCormick et al. 1995). Protein level expression of Na⁺,K⁺-ATPase and NKCC were measured by immunoblotting using the antibodies α5 and T4 respectively (DSHB, U.Iowa). Branchial MRC labelling was also quantified by immunohistochemistry in tissue sections (intensity and area of labelled cells; SigmaScan 1.0, Jandel Scientific).

Data are presented as mean ± SEM. Statistical differences between sample groups were determined using one and two way ANOVAs followed by the post hoc SNK test (SigmaStat, Jandel Scientific). The fiducial limit was set at 0.05.

Results and Discussion

Branchial Na⁺,K⁺-ATPase activity and protein level expression (immunoblot) were significantly greater in seawater adapted glasseels (Fig.1). In freshwater glasseels, cortisol increased expression in a dose dependent manner which remained unchanged in RU486 treated animals. NKCC expression was approximately 5-6 fold higher in seawater animals

(data not shown). No treatment effect was observed unlike in Atlantic salmon (Pelis et al. 2001).

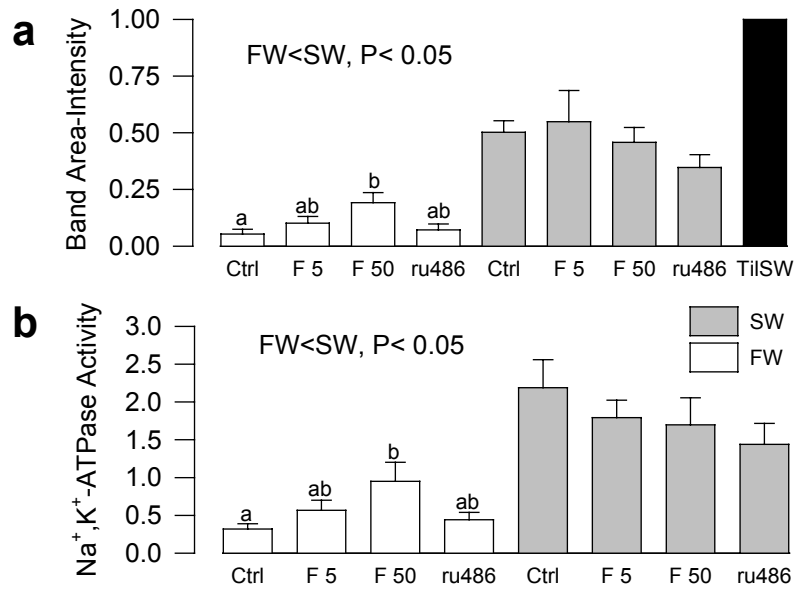


Figure 1. Branchial Na^+, K^+ -ATPase (a) activity and (b) protein level expression in freshwater and seawater adapted glass eels. Bars sharing like characters are not significantly different.

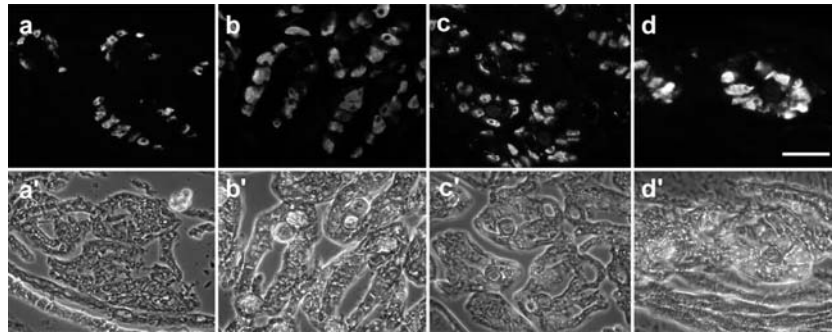


Figure 2. Paired immunofluorescence and phase contrast images showing the distribution of (a,b) Na^+, K^+ -ATPase and (c,d) NKCC in the gills of (a,c) freshwater and (b,d) seawater adapted glass eels. Scale bar = $50\mu\text{m}$.

Na^+, K^+ -ATPase and NKCC were preferentially localized to cells predominantly in the filament epithelium (Fig2a,b and c,d, respectively). This co-localization has been seen in a number of species (salmon, Pelis et al. 2001; tilapia, turbot, JM Wilson unpublished). Unexpectedly, the intensity of Na^+, K^+ -ATPase cell labeling was greater in FW vs SW although the smaller size (area) of the cells resulted in no significant difference in cell area-intensity. No effect of cortisol treatment was detected. Seawater NKCC labelled cells were larger and had higher cell-area intensity than in freshwater glasseels.

There were no differences in body parameters (length, wet weight, water content) of glass eels acclimated to freshwater or seawater, although FW glass eels did have significantly lower sodium levels as would have been expected.

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