SEAWATER TRANSFER AND CONFINEMENT: EFFECTS ON BRANCHIAL NA-K-ATPASE ACTIVITY IN CHLORIDE AND PAVEMENT CELLS OF ATLANTIC SALMON SMOLTS.

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EXTENDED ABSTRACT ONLY - DO NOT CITE

In teleost fish the gills are the major organ involved in ion regulation and maintenance of hydromineral balance. This is essential given the hypo- and hyperosmolar conditions fish are facing in fresh and sea water respectively. This challenge is even more extreme for fish migrating between fresh and sea water like e,g, salmon. The general concept of ionregulation involves active accumulation of ions in freshwater with Na^+ absorption via the pavement cells. In seawater fish excrete ions mostly Na^+ and Cl^- via the chloride cells. The Na-K-ATPase is the enzyme playing a crucial role in the functionality of these osmoregulatory cells. It is well established that cortisol, which in fish has both mineralo- and corticosteroid functions and it's synthetic analogue dexamethasone play a crucial role in the modulation of Na-K-ATPase activity (Wendelaar Bonga, 1997). In addition it has been shown by means of catalytic histochemistry that dexamethasone regulates Na-K-ATPase activity differently in pavement and chloride cells of the European eel under fresh or sea water conditions respectively (Marsigliante et al., 2000). Cortisol in fish is the main endproduct of the hypothalamic-pituitary-interrenal axis which co-ordinates the stress response in fish (Wendelaar Bonga, 1997).

The aim of this study was to investigate the effects of net-confinement, an aquacultural relevant handling process, known to evoke a stress response in fish, hence resulting in increased plasma cortisol levels and seawater transfer (for 1 and 7 days) in combination with confinement, on gill Na-K-ATPase activity. In particular this study aimed to compare whole gill homogenate measurements of Na-K-ATPase activity with the catalytic histochemistry technique which specifically allows to study the distribution

Na-K-ATPase activity between chloride or the pavement cells in gill cryosections and also study expression of Na-K-ATPase related protein expression by means of immunohistochemistry using an antibody frequently used to determine fish gill Na-K-ATPase protein expression (Nolan et al., 1999; Wilson et al., 2000).

To determine whether confinement really induced a stress response in these fish plasma cortisol levels were measured by means of a radio immuno assay and indeed plasma cortisol levels in all groups of confined fish (day 0 = freshwater control, day 1 and 7 after sea water transfer) were significantly higher than plasma cortisol levels measured in fish sampled at rest. Whole gill homogenate Na-K-ATPase activity was increased in sea water fish when compared to fresh water animals and confinement resulted in an increase in enzyme activity in all groups. Catalytic histochemistry revealed that the confinement induced increase of Na-K-ATPase activity in the gills of freshwater fish was due to an increase in pavement cell related enzyme activity whereas in salmon transferred to seawater this increase in Na-K-ATPase activity was mostly observed in the chloride cells and this shift of enzyme activity from one cell type to the other progressed with duration of sea water transfer. Normal immunohistochemistry only revealed an increase in Na-K-ATPase related protein expression in chloride cells of seawater transferred fish, but under no conditions could any positive signal be detected in the pavement cells of any of the fish.

It is concluded that Atlantic salmon like European ell during seawater transfer shift Na-K-ATPase activity from the pavement to the chloride cells, which is in agreement with the two different roles attributed to this two cells types. The results obtained by means of normal immunohistochemistry, however, clearly show that this technique is not sufficient to describe and understand the mechanisms involved in seawater acclimatisation of fish.

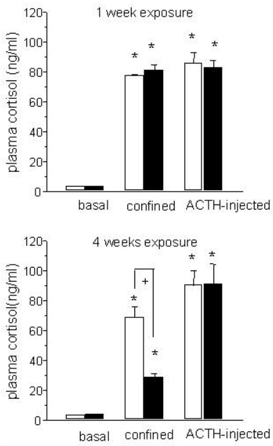

Figure 1: Effect of dietary B(a)P on the primary stress response in turbot. Data for trout and tilapia are published (Quabius et al. 2000) Environmental Toxicology and Chemistry Vol 12 p 2892 - 2899. Open bars represent control fish and black bars represent fish receiving the B(a)P containing diet (n=8 +/- SEM). Asterisks indicate significant differences from basal sample, and pluses indicate significant differences due to the B(a)P exposure.

Figure 1:FW gill Na-K-ATPase – effect of confinement. The upper picture shows the catalytic Na-K-ATPase activity in the gills of confined Salmon smolts, the lower picture shows the results obtained immunohistochemistry.

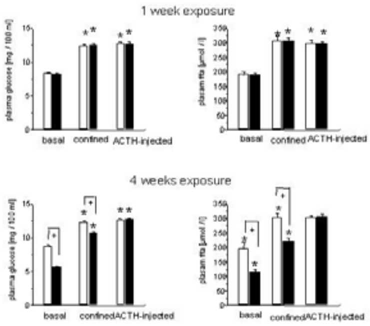

Figure 2: Effect of dietary B(a)P on secondary stress responses in turbot. Data for trout and tilapia are published (Quabius et al. 2000) Environmental Toxicology and Chemistry Vol 12 p 2892 - 2899. Open bars represent control fish and black bars represent fish receiving the B(a)P containing diet (n=8 +/- SEM). Asterisks indicate significant differences from basal sample, and pluses indicate significant differences due to the B(a)P exposure.

Figure 2 :SW gill Na-K-ATPase – effect of confinement. The upper picture shows the catalytic Na-K-ATPase activity in the gills of confined Salmon smolts, the lower picture shows the results obtained immunohistochemistry

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