SPECIFIC AND NON-SPECIFIC ACTIONS OF STRESSORS IN FISH:

EFFECTS OF A LOW HIERARCHICAL POSITION

ON BRANCHIAL STRUCTURE

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Introduction

The stress response of teleost fishes is comparable to that of other vertebrates and includes enhanced release of catecholamines and cortisol, a rise of plasma glucose and lactate, drop in blood plasma pH, and stimulated cardiac output and gas exchange as the most prominent early effects of these hormones, and reduction of growth, reproduction and disease resistance in the longer term. Typical for fish is the rapid disturbance of hydromineral balance during stress. The latter is, partially or mainly, caused by the increased perfusion of the gills, and increased permeability of the cellular and/or paracellular routes (see several authors in Pickering, 1981; Barton and Iwama, 1991). These are all non-specific effects of stressors, mainly connected with the actions of catecholamines and cortisol. In addition, a stressor may produce effects that are specific for this stressor or for a group of related stressors. Examples are the inhibition of the Ca^{2+}-ATPase activity already at very low concentrations of cadmium, and of Na^{+}/K^{+}-ATPase by low levels of copper.

These metals also produce structural damage to the gills, including increased rates of apoptosis of chloride cells and pavement cells and swelling of intercellular spaces in the branchial epithelia (Wendelaar Bonga and Lock, 1992; Pratap and Wendelaar Bonga, 1993). Whether these effects are specific or non-specific stressor effects is unclear. One approach to distinguish between specific and non-specific effects of stressors on the gills is the comparison of the effects of heavy metals and other toxic stressors, known to be able to affect branchial tissue components directly, with those of non-toxic stressors that have no direct action on the gill epithelia, such as a low position in the social hierarchy (submissiveness). We present some data on blood and gill parameters that can be used as stress indicators, and a description of branchial ultrastructure of dominant and submissive tilapia. The results are compared with those of our earlier studies on the effects of toxic metals on fish.

Materials and Methods

Male *O. mossambicus* of about 20 g body weight were kept in tap water (0.8 mM Ca^{2+}) in 100 l aquaria, in groups of 8 males and 4 females. The males rapidly established an hierarchical order. Fish were sampled at the start of the experiment (controls, day 1) and 14 days later (the 2 most dominant and submissive males per group). They were lightly anesthesized in 0.2% phenoxyethanol. Filaments of the second branchial arches were prepared for electron microscopy. Chloride cell densities (n/mn^2), branchial Na^+/K^+-ATPase and plasma osmolarity, Na^+ and cortisol concentrations were determined as described by Wendelaar Bonga et al. (1990).

Results

Plasma parameters. Plasma osmolarity, Na^+, Cl^-, and Ca^{2+} decreased and plasma cortisol increased significantly in the submissive fish when compared to controls and dominant fish (Table 1).

Opercular chloride cells and branchial Na^+/K^+-ATPase activity. The number of chloride cells per unit surface area of opercular epithelium (which reflects the density of branchial chloride cells, Wendelaar Bonga et al., 1990) increased significantly in the submissive fish when compared to controls and dominant fish. The specific Na^+/K^+-ATPase activity of the gills was similar in controls, dominant fish and submissive fish (Table 2).

Table 1
Plasma osmolality and plasma Na^+, Ca^{2+} and cortisol levels; means $\pm$ SD of 8 (controls and dominant fish) or 7 (submissive fish); $*P < 0.01$.

	Osmolality mosmol/kg	Na^+ mmol/l^{-1}	Ca^{2+} mmol/l^{-1}	Cortisol ng/ml^{-1}
controls	324 $\pm$ 10	142 $\pm$ 7	2.36 $\pm$ 0.07	19 $\pm$ 8
dominant	322 $\pm$ 7	145 $\pm$ 11	2.42 $\pm$ 0.11	11 $\pm$ 6
submissive	284 $\pm$ 9 *	116 $\pm$ 9 *	2.07 $\pm$ 0.08 *	63 $\pm$ 12 *

Ultrastructure of the gills. Controls. The branchial lamellae of tilapia are covered by one or two layers of - smooth-surfaced - pavement cells, which separate the blood sinuses from the water. Leucocytes are only occasionally found in the blood sinuses (in particular neutrophils) and are very scarce in the lymphoid spaces (leucocytes and lymphocytes). Many chloride cells are present at the epithelium between the lamellae. Intercellular spaces are small. Most chloride cells are mature cells, with shallow apical crypts in contact with the water, an extensive tubular system, and many mitochondria. They are occasionally accompanied by an accessory or replacement chloride cell. Apoptotic necrotic or apoptotic chloride cells or pavement cells - characterised by cytoplasmic and mitochondrial swelling, or cytoplasmic densification, respectively (Wendelaar Bonga et al., 1990) are scarce and less than 2% (necrotic cells) or about 6% (apoptotic cells) of the total number of chloride cells (Table 2).

In dominant fish, the ultrastructure of the branchial lamellae was similar to that of the controls. The percentage of apoptotic and necrotic chloride cells was also similar. In submissive fish, enlargement of the lymphoid spaces in the lamellar epithelium and the intercellular spaces in the filament epithelium was common. Several pavement cells of the filament epithelium showed many small electron transparent secretory vesicles. The number of apoptotic pavement cells (not shown) and chloride cells (Table 2) increased dramatically. Chloride cells with characteristics of immature replacement cells were significantly more frequent than in control or dominant fish. The lymphoid spaces of the lamellae and the intercellular spaces of the filament epithelium were infiltrated by many macrophages, and lymphocytes. A substantial part of the lymphocytes were apoptotic.

Table 2

Numerical density of the chloride cells (number of cells per mm^2 of epithelial surface area) in the opercular epithelium, as determined after DASPEI staining (means $\pm$ SD of 12 fish per group), specific Na^+/K^+-ATPase in the gills, after 35 days (means $\pm$ SD of 7 fish per group), and percentage of apoptotic chloride cells in the gills (means $\pm$ SD of 6 fish per group); $^*p < 0.01$.

	Chloride cells $\#.mm^{-2}$	Na^+/K^+-ATPase $\mu mol\ P_i.h^{-1}$ per mg prot.	Apoptosis %
controls	113 ± 14	54.3 ± 6.2	6.2 ± 2.4
dominant	95 ± 9	44.1 ± 5.8	5.3 ± 2.1
submissive	228 ± 18 *	59.3 ± 7.6	19.8 ± 4.7 *

Discussion

Plasma parameters. The observed reduction of plasma osmolarity and ion levels in the submissive fish is typical for freshwater fish stressed by physical, chemical or social stressors, including rapid temperature changes, toxic chemicals, handling, confinement (Wendelaar Bonga and Lock, 1992). The high circulating cortisol levels we observed in the submissive fish further point to stress in the submissive tilapia.

Increased opercular and branchial chloride cell numbers have been reported after exposure to heavy metals, acid water with and without aluminium. This effect has been interpreted as a compensatory response to the increased passive ion fluxes and reduction in plasma electrolytes as a result of negative effects of the chemicals on ion transport mechanisms and structural integrity of the gills. The present results show that a submissive position also can lead to a dramatic increase in opercular chloride cells numbers.

Branchial structure. The major changes observed in the submissive fish were increased apoptosis and necrosis of chloride cells and pavement cells, extension of the lymphatic spaces in the lamellae and of the intercellular spaces in the epithelium of the primary lamellae, and penetration of many leucocytes in these spaces. These changes have also been reported for the gills of Mozambique tilapia exposed to rapid seawater transfer, acid water with aluminium, cadmium or copper, and brown trout exposed to acid water with aluminium, (see review in Wendelaar Bonga and Lock, 1993). The present findings show that the increased incidence of cell death in these studies cannot exclusively be ascribed to the immediate toxic actions of the metals or the high proton concentration on these fish. At least part of the elevated rate of cell death must be considered as non-specific stress effects. The cortisol levels of the fish of the above mentioned studies were all elevated and we suggest that these initiate apoptosis of branchial cells. We have shown that high cortisol concentrations can induce apoptosis of branchial cells in vitro (primary cultures of branchial pavement cells of trout; N. Bury, unpublished) as well as in vivo (dietary administration of cortisol to tilapia;

S.E. Wendelaar Bonga, unpublished). Induction of apoptosis of trout skin pavement cells has been reported following a single cortisol containing meal (Iger et al., 1995). Increased apoptosis was associated with increased mitotic activity, indicating accelerated aging of these cells. In this study cortisol administration also induced apoptosis of lymphocytes present in the intercellular spaces of the skin epithelium, which may represent a sign of immuno-suppression by the hormone. Increased lymphocyte apoptosis was also observed in the intercellular spaces of the gills in the present study.

References

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