REGULATION OF PITUITARY-INTERRENAL ACTIVITY
IN TILAPIA ACCLIMATING TO LOW pH CONDITIONS

P.H.M. Balm, A. Lamers and S.E. Wendelaar Bonga

ABSTRACT

This study deals with the regulation of pituitary-interrenal axis activity in the freshwater teleost Oreochromis mossambicus (tilapia) exposed to low environmental pH for 2 or 5 days. The stimulation of the cortisol-producing interrenal cells which occurs under these stressful conditions was not reflected in increased plasma cortisol levels. However, increased sensitivity of the interrenal cells of 5 days acid treated tilapia to 2 pituitary secretagogues could be demonstrated in vitro. First to ACTH and secondly to a product with alpha-MSH immunoreactivity, which has been detected in tilapia plasma, and was purified from pars intermedia tissue. An electron microscopic survey demonstrated the concurrent activation of both the ACTH and MSH cells of fish acclimating to the low pH stress. Future studies will have to determine the relative contribution of ACTH and alpha-MSH to the interrenal hyperactivity under these conditions.

INTRODUCTION

Water acidification greatly affects freshwater fish faunas. Studies on the effects of environmental acidification go back some seventy years (Wells 1915). Since then, the progressively devastating effects of acid pollution on freshwater fish populations (recently reviewed by Howells et al -1983- and Muniz -1984-) only urged the need for more fundamental research. Most of the original observations on low pH effects were obtained from field studies but the current understanding of the complexity of the low pH syndrome necessitates further laboratory experiments during which factors like water pCO₂ (Neville 1979), ambient calcium (Graham and Wood 1981, McDonald 1983 a, McDonald et al 1983, McWilliams 1982), and aluminium concentrations (Muniz and Leivestad 1980, Driscoll 1985) can be controlled.

Much of the research regarding the effects of low pH on teleosts has been focussed on the regulation of electrolyte status and acid-base balance of the animals (reviews by Fromm 1980, McDonald 1983 b, and Heisler 1984). There are only very few reports indicating adaptive behaviour of teleosts in response to low pH conditions (Ashcom 1979, McWilliams 1980, McDonald 1983 a, McDonald et al 1983, Wendelaar Bonga et al 1984). The study of McWilliams
on brown trout was the first to show restoration of initially depressed plasma Na⁺ concentrations under low pH conditions (pH 6.0). Wendelaar Bonga et al (1984) reported the same phenomenon after abrupt transfer of Oreochromis mossambicus (tilapia) to pH 4.0, thereby illustrating the existence of adaptive regulation to low pH in this species. Very little information on the nature of the regulation exists, but endocrine factors are likely involved.

The present study deals with the regulation of pituitary-adrenal (-interrenal) axis activity in tilapia exposed to sublethal levels of acidification. Cortisol, the major corticosteroid in these fish (Balm 1986), has been demonstrated to possess both potent mineralocorticoid and glucocorticoid activities. In addition, prolonged activation of the pituitary-interrenal axis in fish has been interpreted as a mechanism involved in adaptation of the organisms to stressful conditions (Donaldson 1981). Although traditionally ACTH is considered the pituitary factor regulating interrenal function in fish, recent studies on the adrenal cortex of higher vertebrates have suggested a role for α-MSH in this respect (Hinson et al 1985). Therefore in the present study, which compares interrenal cell function in control and low pH treated tilapia, special attention was paid to ACTH and α-MSH effects.

MATERIALS AND METHODS

Experiments were performed with sexually mature female tilapia from our laboratory stock. Water was acidified gradually by adding sulfuric acid by means of a flow through system. The reduction in water pH usually took 24 h. Pituitary tissues were fixed for electron microscopy as described by Wendelaar Bonga and van der Meij (1981). Headkidneys were incubated in vitro applying a microsuperfusion technique. For further details on experimental procedures see Balm (1986). Data are presented as means ± SEM. Differences between control and experimental groups were analyzed by means of the Student's t-test for unpaired observations.
RESULTS

Blood parameters

Exposure of tilapia to pH 3.5 for 48 h led to negligible reductions in plasma osmolality and plasma Na⁺ concentrations (table 1), whereas significant increases in haematocrit values and plasma K⁺ concentrations were observed.

<table>
<thead>
<tr>
<th></th>
<th>controls (n=6)</th>
<th>2 days pH 3.5 (n=6)</th>
<th>p</th>
</tr>
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<tr>
<td>haematocrit (%)</td>
<td>33.1 ± 1.5</td>
<td>36.8 ± 1.1</td>
<td>&lt; 0.01</td>
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<tr>
<td>plasma osmolality</td>
<td>330 ± 5</td>
<td>325 ± 8</td>
<td>n.s.</td>
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<tr>
<td>K⁺ conc. (mM)</td>
<td>5.5 ± 0.4</td>
<td>7.7 ± 0.3</td>
<td>&lt; 0.05</td>
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<tr>
<td>Na⁺ conc. (mM)</td>
<td>137 ± 4</td>
<td>131 ± 2</td>
<td>n.s.</td>
</tr>
<tr>
<td>cortisol (μg‰)</td>
<td>6.4 ± 1.2</td>
<td>5.7 ± 1.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>glucose (mg‰)</td>
<td>46 ± 5</td>
<td>318 ± 34</td>
<td>&lt; 0.001</td>
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Table 1 Blood parameters of control and low pH treated tilapia.

Figure 1 Tankwater NH₄⁺ concentrations upon acidification of the tank (t=0 h). At t=24 h the water pH was 3.3 ± 0.1 and remained at that level for the remaining 24 h. Values are means of 4 separate experiments. The insert shows the rate of NH₄⁺ production between t=24 h and t=48 h against the total body weight of the animals in the tank.
Plasma cortisol levels remained unchanged, but the low pH treated fish were strongly hypoglycaemic. The reduction in water pH resulted in an almost immediate stimulation of whole body $\text{NH}_3^+ - \text{NH}_4^+$ efflux (measured as $\text{NH}_4^+$ efflux; figure 1).

In vitro cortisol production

A five days low pH regime resulted in an increased sensitivity of the cortisol producing interrenal cells to ACTH stimulation (table 2). Basal in vitro cortisol release rates were not significantly different, but the tissue from acid treated tilapia was stimulated significantly more by 0.6 nU ACTH than control tissue.

<table>
<thead>
<tr>
<th>cortical release rate (pg/ min. mg headkidney)</th>
<th>controls</th>
<th>5 days pH 3.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>basal</td>
<td>2.7 ± 0.3</td>
<td>4.4 ± 0.8</td>
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<tr>
<td>stimulated by ACTH</td>
<td>20.8 ± 0.1</td>
<td>48.0 ± 4.0</td>
</tr>
<tr>
<td>% increase due to ACTH</td>
<td>685 ± 84</td>
<td>1033 ± 132</td>
</tr>
</tbody>
</table>

headkidney cortisol content (pg/ mg headkidney) 10.4 ± 0.7 15.1 ± 0.5 p ≤ 0.001

Table 2 Stimulation of in vitro cortisol release by ACTH. Interrenal tissue of tilapia was superfused in vitro until basal release rates were reached (4 h in vitro). ACTH (0.6 nU) was administered as a five minute pulse. The values for ACTH stimulated cortisol release were measured 20 minutes thereafter, and represent maximum release rates reached. The presented data are from 4 individual superfusions per experimental group. Each superfusion contained headkidney tissue from 2 fish. Tissue cortisol contents were quantified at the end of the superfusions.

A comparable effect was observed when interrenal cells from control and low pH treated (5 days, pH 3.5) tilapia were challenged with 5 nM of a product with α-MSH immunoreactivity (‘peak IV’), purified from tilapia pituitaries. In contrast, the low pH treatment appeared to result in a decreas-
controls | 5 days pH 3.5
---|---
peak II (50 nM) | 535 ± 72 | 207 ± 53 | - 61% (n=3, p ≤ 0.02)
peak IV (5 nM) | 111 ± 38 | 260 ± 22 | + 134% (n=3, p ≤ 0.02)

Table 3  Stimulation of cortical release by α-MSH peptides during in vitro superfusion. After 4 hours in vitro superfusion interrenal cells of controls and low pH exposed tilapia were challenged for 30 minutes with 2 products displaying α-MSH immunoreactivity. These products tested were purified from tilapia pituitary pars intermedia tissue by HPLC procedures (see Wandelar Bonga and Balm 1987 for details). Results are presented as area under the curve in arbitrary units.

...and sensitivity of the interrenal cells to 50 nM of a second form of α-MSH (‘peak II’).

**Activation pituitary ACTH and αMSH cells upon water acidification**

Figure 2 compares electron micrographs from the pituitary ACTH regions from control tilapia and low pH treated fish. ACTH cells of tilapia from the latter experimental group appeared more active than controls, as illustrated by the presence of large Golgi complexes and increased amounts of rough endoplasmatic reticulum (RER) in the cells of the acid exposed fish.

![Electron micrographs of pituitary ACTH cells of control and low pH (five days pH 3.5) treated tilapia. 7000 x.](image)
The electron micrographs of figure 3 show a comparable effect of water acidification on the pars intermedia MSH cells. The most striking features of the MSH cells of the low pH treated tilapia, when compared to MSH cells of controls, were the degranulation of the cells and the higher amounts of RER in these cells, both signs of increased cellular activity.

Figure 3  Electron micrographs of pituitary pars intermedia MSH cells. Low pH: 5 days pH 3.5. 7000 x.

DISCUSSION

Fish experiencing environmental acidification, whether under field or laboratory conditions, can be considered stressed. The intensity and types of stress involved probably are important factors determining first of all the degree of acclimation to the osmoregulatory disturbances associated with water acidification and, ultimately, survival. To witness this, our (unpublished) experiments demonstrated that the net loss of body ions from the fish during experimental acidification was enhanced when the fish were stressed mildly for just 10 minutes. The data presented in this paper indicate that tilapia, when adapted gradually to pH 3.5, were able to prevent the drop in plasma osmoles, which was maximal 2 days after acute acidification (Wendelaar Bonga et al 1984). The fact that we could not measure a decline in plasma Na\(^+\) concentration does not necessarily imply that these animals did not lose sodium to the water. It serves as an indication that
fish are better able to compensate for the osmoregulatory problems which come with water acidification if they experience as little (additional) stress as possible.

Despite the unchanged plasma cortisol levels after 48 h pH 3.5, we were able to demonstrate that the interrenal cells of low pH treated tilapia were still highly activated 5 days after the onset of the acidification (Balm et al. 1986). Since experimental stress was minimized, we conclude that cortisol is involved in the compensatory regulation of the fish to osmoregulatory imbalances which occur during acidification. Apparent signs of the activation of the interrenal cells were the observed hyperglycaemia and the concomitant increase in NH$_4^+$ efflux. Whether these glucocorticoid actions of cortisol or its mineralocorticoid properties (Johnson 1973, Balm 1986) dominate under these conditions remains to be established.

The increased ACTH sensitivity of the interrenal cells which occurred after several days at low pH probably is an important factor maintaining interrenal cell activity in these fish. These cells in vivo rely on constant stimulatory control, as demonstrated by the absence of appreciable cortisol storage by the cells. On top of the increased ACTH sensitivity of the interrenal cells the pituitary ACTH cells of low pH tilapia also appeared stimulated, which indicates the activation of the pituitary-interrenal axis under these conditions. Generally ACTH is considered the predominant pituitary factor regulating at least interrenal function (Donaldson 1981). The results presented in this chapter indicate that factors from a second pituitary cell type, which also derives its products from proopiomelanocortin, might also be involved in the regulation of cortisol production under low pH conditions. These MSH cells were also more active in appearance in low pH tilapia than in control fish and, analogous to the aforementioned ACTH effect, the response of the interrenal cells to their product ('peak IV') was enhanced in low pH treated fish. We assume that this latter effect is more relevant than the observed diminished reaction of the interrenal cells to the second form of α-MSH ('peak II'), since we subsequently could not detect significant amounts of peak II in tilapia plasma, in contrast to peak IV (data not shown). Presumably the forms of the hormone merely differ in their degree of acetylation.

Having shown the simultaneous activation of two pituitary cell types in tilapia acclimating to low pH conditions, one matter unsolved is the relative contribution of ACTH and α-MSH to the observed stimulation of cortisol production associated with water acidification. It is generally accepted that POMC derived peptides from ACTH cells exert several actions on the adrenal cortex of higher vertebrates, including trophic effects (Lowry 1984). An interes-
tling aspect of the depicted situation in tilapia under low pH conditions lies in the fact that α-MSH is also derived from POMC, which lends further support to the concept of this pro-hormone being a "multiple adrenal hormone precursor" (Lowry 1984). Although this concept was originally based only on studies on higher vertebrates, it now appears that fish acclimating to low pH conditions may serve a useful model to verify the idea for the lower vertebrates.

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