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The effect of water acidification on prolactin cells and pars intermedia PAS-positive cells in the teleost fish Oreochromis (formerly Sarotherodon) mossambicus and Carassius auratus

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Summary. Although exposure to acid water (pH 3.5) induces severe and prolonged reduction in plasma osmolarity and total plasma calcium concentration in tilapia (Oreochromis mossambicus) and goldfish (Carassius auratus), the responses of the hypophysial cells are clearly different. In tilapia, the size of the rostral pars distalis of the pituitary gland is enlarged as a result of the increase in size and number of prolactin cells. The pars intermedia PAS-positive (PIPAS) cells are not noticeably changed. Conversely, in goldfish, prolactin cells are unaffected, whereas the number of enlarged PIPAS cells increases markedly. Stimulation of prolactin secretion may be responsible for the partial restoration of plasma osmolarity and calcium levels observed in tilapia after two weeks exposure to acid water. Prolactin cells apparently play a role in the adaptation to acid stress by countering osmoregulatory disturbances. Goldfish show no restoration of plasma osmolarity during the course of the experiment. Plasma calcium levels tend to increase. Although prolactin may have an osmoregulatory function in goldfish under steady state conditions, goldfish prolactin cells do not seem to participate in the physiological adaptation to environmental changes that disturb water and ion homeostasis. The function of PIPAS cells in tilapia remains unclear and is apparently unconnected with ion regulation. The observations on these cells in goldfish are consistent with the hypercalcemic activity suggested for them.

Key words: Teleost pituitary gland – Prolactin cells – Pars intermedia PAS-positive cells – Acid water

Exposure of fish to acid water leads to severe ion losses through gills and kidneys, and several authors consider osmoregulatory disturbances the primary cause of death at lethal pH levels (Muniz and Leivestad 1980; Milligan and Wood 1982; McDonald 1983a, b). However, many fish can survive acid water from pH 3.5 or 4.0 (Muniz and Leivestad 1980; Harvey 1980). Natural populations of trout are known that have developed tolerance to acid stress (McWilliams 1982). In the acid tolerant fish gill permeability to ions is significantly less affected by low pH of the water than in trout from more alkaline water (McWilliams 1982). In the cichlid freshwater fish Oreochromis mossambi-
teleost species, do not respond to environmental osmolarity or calcium content but to background colour (Van Eys 1980; Van Eys and Wendelaar Bonga 1984). In goldfish however, the PIPAS cells show a dramatic increase in size and number when the calcium concentration of the water is reduced, and therefore they have been termed calcium-sensitive cells (Oliverneau et al. 1980b). Although this name may be appropriate for goldfish PIPAS cells, as these cells have only low affinity for the PAS-staining procedure, for convenience we will continue to refer to them as PIPAS cells. There is little doubt that these cells are homologous to the PIPAS cells of tilapia, or those of the molly Poecilia latipinna, for which the name PIPAS cells was first introduced (Ball and Batien 1981). We have studied the effects of acid water on Oreochromis mossambicus and Carassius auratus to determine whether it stimulates prolactin cells in tilapia, and PIPAS cells in goldfish.

Materials and methods

Sexually mature male tilapia (Oreochromis mossambicus), of 12–15 g body weight, were obtained from laboratory stock. Immature goldfish (Carassius auratus), varying in weight from 7 to 10 g, were purchased from a commercial fish dealer. The fish were kept in well-aerated glass aquaria with tap water (Ca$^{2+}$: 0.8 mM; Na$^{+}$: 1.9 mM; K$^{+}$: 0.05 mM; Cl$^{-}$: 3.1 mM; Mg$^{2+}$: 0.2 mM; 9 mOsmol/l; pH: 7.5), at 20°C for goldfish or 25°C for tilapia, and with a daily light period of 12 h. On the first day of the experimental period of 2 weeks (day 0) the pH of the water was reduced from 7.5 to 4.5, by adding H$_2$SO$_4$ (analytical grade). On days 2 and 4, it was reduced to pH 4 and 3.5, respectively. The pH was monitored daily and adjusted by titration with H$_2$SO$_4$. The final total SO$_4^{2-}$ concentration of the water never surpassed 2 mM. The water was replaced every third day to remove nitrogenous wastes. During the experiment, with the exception of the first 3 days, the control fish (kept in normal tap water) and the acid-exposed fish were fed on Tetramin tropical fish food. No deaths occurred during the experiment.

On days 1, 7 and 14, groups of 6 fish of both species were anesthetized in tap water or acidified tap water, containing 0.2% methoxyethanol. Blood was collected from the cut end of the caudal peduncle and plasma was collected by centrifugation into heparinized microhematocrit tubes. The osmolarity of fresh plasma samples was determined in a Vogel micro-osmometer. Total plasma calcium was determined using a Sigma total calcium kit, as described earlier (Wendelaar Bonga and Van der Meij 1980, 1981) and goldfish (Leatherland 1972).

In tilapia, the rostral pars distalis of control fish (pH 7.3) formed a prominent part of the pituitary gland, comprising 31 ± 5% of its total volume. Exposure of the fish to acid water for 14 days led to a marked increase in the size of the rostral pars distalis, to 47 ± 6% of the total volume of the pituitary gland. Since the rostral pars distalis was the only part of the pituitary gland that changed in size during acid exposure (Wendelaar Bonga et al. 1984), the total volume of the prolactin cells more than doubled whereas in goldfish, the granules of the MSH cells, but not those of the PIPAS cells, stained slightly metachromatically. This identification was verified by examination of sections of the same cells using the light and electron microscope. The cell and nuclear areas were measured light microscopically, by projecting the sections on an X-Y tablet connected to a Kontron Digiplan morphometrical analyser. Only cell profiles containing nuclei were measured. For determination of the nuclear areas only nuclear profiles showing nucleoli were taken into account. Forty cells and nuclei of each cell type were measured per fish. The volume of isolated rostral pars distalis of tilapia was determined volumetrically as described earlier (Wendelaar Bonga et al. 1984). In goldfish, the rostral pars distalis is relatively small, and therefore its volume was reconstructed from morphometrical measurements of the rostral pars distalis area in serial sections of the pituitary glands. In goldfish, prolactin cells are confined to the rostral pars distalis of the pituitary glands, in goldfish, these cells have only low affinity for the PAS-staining procedure, for convenience we will continue to refer to them as PIPAS cells.
Fig. 2. Prolactin cells of control tilapia; Ga Golgi area. × 10600

Fig. 3. Prolactin cell of tilapia exposed for 14 days to acid water; ger granular endoplasmic reticulum; Ga Golgi area. × 10600
during the two-week experimental period. Cell and nuclear area were increased significantly (Fig. 1; P < 0.001 and < 0.01, respectively, compared to the control values determined at day 0). The increase in cell volume, as calculated from the increase in cell area, was about 50%: this implies that the growth of the rostral pars distalis was not only accounted for by cell growth but also by cell proliferation. The ultrastructure of the cells showed notable changes already after 24 h at pH 3.5 (results not shown here). The Golgi areas were enlarged and showed many signs of formation of secretory granules, whereas the amount of granules present in the cytoplasm was reduced. This indicates that the increase in the release of the granule contents surpassed the increase in granule formation. After 14 days in acid water, the granular endoplasmic reticulum and Golgi areas increased significantly after 14 days in acid water (Fig. 4; P < 0.001). The calculated mean cell volume had increased five-fold compared to control fish. In the latter group (Fig. 9) PIPAS cells showed many secretory granules, some strands of granular endoplasmic reticulum and small Golgi fields, whereas after 24 h in acid water the granular endoplasmic reticulum was extended, the Golgi areas showed many signs of granule formation, and the number of secretory granules had decreased. After 14 days in acid water the cells showed a marked extension of the granular endoplasmic reticulum (Fig. 10). Well-developed Golgi areas could be observed. Secretory granules were still scarce, this indicated that the contents of the granules were released shortly after formation of the granules in the Golgi areas.

2. Plasma osmolarity and total plasma calcium

Following 24 h exposure to acid water, the osmolarity of the blood plasma was reduced significantly (tilapia: P < 0.05; goldfish: P < 0.001). Plasma calcium levels showed a similar reduction in both species (P < 0.001; Fig. 11). Six days later, a further decrease was noticed for both parameters. In tilapia plasma osmolarity and plasma calcium had decreased to around 85% of the control values. In goldfish the reduction was even more pronounced, i.e., to 73% and 78%, respectively. On day 14 some restoration to control levels was noticeable in tilapia. Plasma osmolarity and calcium concentration were significantly higher than at day 7 (P < 0.01; Fig. 12), although the values for both parameters were still below control levels as observed at day 0. In goldfish no restoration was observed for plasma osmolarity. The mean plasma calcium concentration at day 14 was slightly, but not significantly, higher than the low values found at day 7.

Discussion

Exposure of tilapia and goldfish to water at pH 3.5 led to drastic reduction in plasma osmolarity and total calcium concentration. This was especially marked in the goldfish. In the pituitary glands, however, conspicuous differences were observed between the species. Whereas in tilapia the prolactin cells were stimulated and the PIPAS cells remained unchanged, the reverse occurred in goldfish. These observations provide further evidence that in teleosts endocrine responses to osmoregulatory disturbances show fundamental species-specific differences.

Prolactin cells

In tilapia, as in many other teleost species, prolactin cells are stimulated when the fish are transferred from seawater

Conformed to earlier observations on normal freshwater fish (Van Eys 1980; Van Eys and Wendelaar Bonga 1984). In contrast, signs of cellular activation were very conspicuous in the PIPAS cells of goldfish. The cells were more numerous than in control fish and cellular and nuclear areas had increased significantly after 14 days in acid water (Fig. 4; P < 0.001). The calculated mean cell volume had increased five-fold compared to control fish. In the latter group (Fig. 9) PIPAS cells showed many secretory granules, some strands of granular endoplasmic reticulum and small Golgi fields, whereas after 24 h in acid water the granular endoplasmic reticulum was extended, the Golgi areas showed many signs of granule formation, and the number of secretory granules had decreased. After 14 days in acid water the cells showed a marked extension of the granular endoplasmic reticulum (Fig. 10). Well-developed Golgi areas could be observed. Secretory granules were still scarce, this indicated that the contents of the granules were released shortly after formation of the granules in the Golgi areas.

Fig. 4. Total cell area (total length of bars) and nuclear area (dense part of bars) of prolactin cells and PIPAS cells of control goldfish (pH 7.5) and of goldfish exposed for 14 days of acid water (pH 3.5); means ± S.D.; n = 6

Fig. 5. Prolactin cell (PRL) of control goldfish, surrounded by stellate cells (sc); Ga Golgi area. × 10600

Fig. 6. Prolactin cell of goldfish exposed for 14 days to acid water; Ga Golgi area; sc stellate cell. × 10600

Fig. 7. PIPAS cells of control tilapia; Ga Golgi area. × 10600

Fig. 8. PIPAS cell of tilapia exposed for 14 days to acid water; Ga Golgi area; MS1 MSH cell; sc stellate cell. × 10600
Fig. 9. PIPAS cells of control goldfish; Ga Golgi area; MSH MSH cells. × 9500

Fig. 10. PIPAS cells of goldfish exposed for 14 days to acid water; Ga Golgi area; ger granular endoplasmic reticulum. × 9500
to freshwater. This stimulation of prolactin secretion is connected with the low osmolarity and low calcium and magnesium concentration of freshwater (Wendelaar Bonga and Van der Meij 1980, 1981; Wendelaar Bonga et al. 1983). The drop in plasma electrolytes that occurs after transfer of fish from seawater to freshwater, or from normal freshwater to low-calcium freshwater, is primarily caused by branchial and urinary ion losses and branchial osmotic water uptake across the gill surface. In tilapia and some other teleost fish prolactin is known to reduce the permeability of the gills to ions (Ogawa et al. 1973; Wendelaar Bonga and Van der Meij 1978; Dharmamba and Maetz 1972) and the osmotic water permeability of the gills (Ogawa et al. 1973; Wendelaar Bonga and Van der Meij 1981; Ogasawara and Hirano 1984). Stimulation of prolactin secretion is therefore an expected response of fish to loss of plasma electrolytes.

Acid exposure leads to similar osmoregulatory disturbances as does transfer to low-calcium water. The drop in plasma electrolytes is also likely to be caused primarily by an increase in the branchial permeability to water and ions (McWilliams 1980, 1982; McDonald 1983a, b; Wendelaar Bonga et al. 1984). The marked activation of the prolactin cells occurring in tilapia can therefore be interpreted as a response that will contribute to the restoration of water and ion homeostasis. Enhanced prolactin secretion may thus have effected the rise in plasma osmolarity and calcium concentration observed in tilapia during the second week of acid exposure. Almost complete restoration of plasma electrolyte levels has been observed in tilapia after three weeks of exposure to less severe acidity (pH 4; Wendelaar Bonga et al. 1984).

In goldfish from normal freshwater, prolactin cells are smaller in size and lower in number than in tilapia. The rostral pars distalis is also relatively small and comprises only 20% of the total pituitary gland, as compared to about 30% in freshwater tilapia. Goldfish are less euryhaline than tilapia and are unable to survive full strength seawater. Unlike tilapia, goldfish may not show increased secretion of prolactin following reduction in osmolarity or calcium content of the water. Transfer to distilled water supplemented or not with calcium does not noticeably affect the prolactin cells in goldfish even though plasma osmolarity or plasma calcium content are reduced considerably (Olivereau et al. 1980a, 1982a). The present data show that exposure to acid water likewise does not affect the prolactin cells in this species. Nevertheless, the marked drop in plasma osmolarity and calcium content that occurred in goldfish indicates that the effects of low pH on water and ion balance are essentially similar to those in tilapia. The prolactin cells in goldfish are not activated by osmoregulatory disturbances that lead to marked stimulation of the prolactin cells in tilapia. This implies that in goldfish prolactin has no osmoregulatory function. However, physiological evidence points to the contrary. For goldfish as well as for tilapia and many other species it has been demonstrated that injection of mammalian prolactin can partially restore the low plasma electrolyte levels that result from hypophysectomy (Dharmamba et al. 1967; Dharmamba 1970; Lah-lou and Sawyer 1969; Lahlou and Giordan 1970). In both the species studied here, prolactin reduces branchial osmotic water permeability as determined in vitro on isolated gills (Ogawa et al. 1973; Wendelaar Bonga and Van der Meij 1981), and stimulates the proliferation of the epidermal mucocytes (Ogawa 1970; Wendelaar Bonga and Meis 1982). These data suggest that its function with respect to water and ion regulation is similar in tilapia and goldfish. It appears therefore that the most important difference between the two species is the fact that in tilapia prolactin cells become activated following environmental changes that lead to osmoregulatory disturbances, whereas in goldfish these cells lack the capacity to show such an adaptive response. This may be the main reason that in tilapia, but not in goldfish, plasma osmolarity is partially restored during the second week of acid exposure.

**PIPAS cells**

In tilapia, the PIPAS cells are not noticeably influenced by exposure to acid water. This supports our conclusion, based on earlier observations, that in this species these cells are not implicated in the control of water and ion balance. The PIPAS cells of tilapia appeared to be active in fish on a neutral background, more active in fish kept on a black background or in complete darkness, and inactive in fish on a white background. No effects of variation of osmolarity or calcium concentration of the water could be observed (Van Eys 1980; Van Eys and Wendelaar Bonga 1984).

In the goldfish, a relationship has been demonstrated between the calcium concentration of the water and the number and size of the PIPAS cells. In a series of papers it has been shown that these cells are stimulated when goldfish are transferred to distilled water, a response that can be prevented by supplying calcium ions to the water (Olivereau et al. 1980b, 1981). Our data show that exposure to water of pH 3.5 induces a similar increase in cell and nuclear areas of the PIPAS cells as in fish transferred to distilled water (Olivereau et al. 1980b). The reduction induced in plasma total calcium levels is also similar after both treatments (Olivereau et al. 1982a). The activation of the PIPAS cells under exposure to acid water is therefore consistent with the suggestion of Olivereau et al. (1980b) that these cells produce a hypercalcemic factor. The PIPAS cells in goldfish do not respond to the osmolarity or the magnesium concentration of the water (Olivereau et al. 1982b). The function of these cells must therefore be different from that of the prolactin cells in tilapia, even though prolactin has hypercalcemic effects in tilapia and in some other teleosts (Pang et al. 1973; Pang 1981; Wendelaar Bonga and Flik 1982).

Until now the evidence in favor of a hypercalcemic function for the PIPAS cells is indirect. In goldfish, cell (Anguilla anguilla) and killifish (Fundulus heteroclitus), but not in some other species, the PIPAS cells become activated after reduction of the calcium concentration of the water (Olivereau et al. 1980a; Ball et al. 1982). Fast-acting hypercalcemic substances have been demonstrated in cod pituitary extracts and these may originate from the PIPAS cells (Pang and Yee 1980). Our observation showing that plasma calcium levels in goldfish tend to increase at the end of the period of exposure to acid water, when the PIPAS cells have become highly active, is a further indication that these cells secrete a hypercalcemic hormone in this species.

**Plasma osmolarity and calcium**

In both species examined plasma osmolarity and total calcium levels are markedly reduced within 24 h in acid water.
In goldfish, the reduction is more severe than in tilapia. Such low levels as occurred in goldfish have been observed in tilapia exposed acutely to water of pH 3, but the fish died within a few days (Wendelaar Bonga et al. 1984); thus, goldfish are more tolerant than tilapia of low plasma electrolyte levels.

In tilapia, plasma osmolarity and calcium concentration increased significantly during the experiment. No restoration of osmolarity did occur in goldfish, whereas calcium increased only slightly. Thus, although both species can survive acute exposure to severe acid stress, the mechanisms involved in survival may be different. In tilapia, a strongly euryhaline freshwater fish, there are signs of restoration of water and ion balance. This may be connected with the observed stimulation of prolactin secretion. In the less euryhaline freshwater fish, where no response of the prolactin cells was noticeable, survival seems to be the result of tolerance of low plasma electrolyte levels. In a study on hypophyscetomized goldfish, Lahrou and Sawyer (1969) have come to a similar conclusion. In freshwater fish removal of the pituitary leads to severe electrolyte losses and many fish, including tilapia (Dharmamba et al. 1967; Dharmamba 1970), die rapidly. Goldfish are among those species of fish that can survive for a long time in a hypophyscetomized condition, even though plasma electrolyte levels remain extremely low. Lahrou and Sawyer (1969) have concluded that survival in hypophyscetomized goldfish is related to high tolerance of low plasma electrolyte levels rather than to a special ability of the fish to maintain plasma ion homeostasis in the absence of the pituitary gland.

References


Olivereau M, Olivereau J, Aimar C (1982a) Influence of deionized water supplemented or not with different ions on prolactin cell activity and osmotic regulation in the goldfish. Comp Biochem Physiol 71 A:11-16


Van Eys GJJM (1980) Structural changes in the pars intermedia of the cichlid teleost *Sarotherodon mossambicus* as a result of background adaptation and illumination. II. The PAS positive cells. Cell Tissue Res 210:171-179


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