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Effect of cadmium on prolactin cell activity and plasma electrolytes in the freshwater teleost *Oreochromis mossambicus*

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Exposure of tilapia to a sublethal cadmium (Cd) concentration (10 μg Cd/l) led after 4 days to a rapid and substantial decrease of plasma total Ca and a small drop in plasma osmolarity and Na^+. This ionic disturbance was only transitory and control levels were reached after 14 days. Prolactin is implicated in this recovery since prolactin cell activity increased markedly during the first 4 days. The gradually decreasing activity of prolactin cells thereafter points to induction of a second mechanism sustaining the resistance of fish to Cd. In spite of the pronounced hypocalcemia, no apparent signs of demineralisation of bony tissues were observed. Since administration of prolactin to tilapia is known to exert a hypercalcemic action by increasing the net branchial Ca^{2+}-uptake, it is concluded that restoration of Cd-induced hypocalcemia is connected with the observed stimulation of prolactin cell activity.

Key words: Cadmium; Tilapia; Teleost fish; Ion balance; Calcium

INTRODUCTION

Exposure of freshwater fish to cadmium (Cd) in the water could lead to a disturbance of the calcium (Ca) balance (Roch and Maly, 1979; Giles, 1984; Yamawaki et al., 1986; Pratap et al., 1989). This hypocalcemic effect is apparently caused mainly by inhibition of Ca^{2+}-uptake via the gills (Verbost et al., 1987; Reid and McDonald, 1988). Indeed, symptoms of Cd poisoning in fish such as hyperexcitability, rhythmic muscle contraction, and tetanic body movements (Pascoe and Mattey, 1977; Roch and Maly, 1979; Larsson et al., 1981) strongly resemble those of hypocalcemia (Doneen, 1976).

In contrast to lethal Cd concentrations whereby the blood Ca-level is permanently disturbed, and which is associated with a complete disruption of the ion- and water

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balance, a specific but transitory drop in plasma Ca can often be seen following exposure of fish to sublethal Cd concentrations (Giles, 1984; Yamawaki et al., 1986; Pratap et al., 1989). Most attention to explain this recovery of Cd-induced hypocalcemia has been focused on the role of metal-binding proteins sequestering intracellular Cd (Webb, 1979; Klaverkamp et al., 1984). However, since water and ion balance in fish are under endocrine control, hormones are also expected to play a role in counter-acting Cd-induced ionic disturbances. In this respect the pituitary hormone prolactin should be mentioned, since it is with cortisol one of the main osmoregulatory hormones in freshwater fish (Loretz and Bern, 1982). Prolactin has hypercalcemic actions in teleost fish. Injections of prolactin or ectopic transplants of prolactin lobes induce hypercalcemia in different species of fish (Pang, 1981; Wendelaar Bonga et al., 1984; Flik et al., 1986). Conversely, hypophysectomized fish become hypocalcemic, exhibiting severe tetanic convulsions (Pang et al., 1973; Doneen, 1976). It seems reasonable therefore to predict that Cd-induced hypocalcemia is off-set, at least initially, by an increase of prolactin release. Involvement of prolactin is furthermore indicated since this hormone is known to promote activity and proliferation of mucus cells (Marshall, 1979; Wendelaar Bonga and Meis, 1981), and increased mucus secretion has been observed in Cd-exposed fish (Varanasi and Markey, 1978). After establishing the level of Cd which induces a transitory change in plasma osmolarity, we studied the effect of this sublethal Cd concentration on prolactin cell activity and on some physiological parameters controlled by this hormone: plasma Na^+ and total Ca. In addition, potentially exchangeable calcium and phosphate stores of different bony tissues were measured. This study is part of an investigation into the role of hormones in the acclimation of fish to cadmium.

MATERIALS AND METHODS

Animals Freshwater male tilapia, *Oreochromis mossambicus* of 16 to 25 g body weight were obtained from our own laboratory stock. Fish were acclimated in 100-l aquaria on a 12-h photoperiod for at least three weeks before the start of the experiment. Aquaria contained circulating filtered Nijmegen tap water (main ion concentration in nmol/l: Ca, 0.8; Na, 1.9; K, 0.05; Cl, 3.1; Mg, 0.2; pH 7.5) of 28°C. Animals were daily fed with Tetramin tropical fish food up till 24 h before sacrifice.

Experimental design To establish the effect of Cd on the plasma osmolarity, we exposed in a preliminary experiment tilapia to Cd of 1, 10, 100 and 1000 μg Cd/l. The Cd concentration causing a transitory effect on the osmolarity (and total plasma Ca) was selected for further experimentation. Experiments were started after addition of a predetermined aliquot from a stock 1 mg Cd/ml solution of $Cd(NO_3)_2$ (RCB, Brussels) to the water. Concentrations were daily monitored by atomic absorption spectrophotometry (Video II, Thermo Jarrell Ash USA) at 228.8 nm, and

adjusted if necessary. Detection limit was 0.5 μg Cd/l. Fish were exposed to Cd for 2, 4, 14 and 35 days. To assure that Cd-exposed and control fish were of identical holding history, a control group was taken concurrently with each experimental group.

Sacrifice Fish were killed at indicated time intervals by spinal dissection, body weight recorded and blood plasma collected. Pituitaries were quickly excised for electron microscopical examination, and scales, operculum and vertebral bone removed for the analysis of calcium and phosphate content.

Blood plasma parameters Total plasma osmolarity was determined with a micro-osmometer (Vogel, Giessen, FRG). Plasma sodium was determined by flame photometer (Model IV Auto-Analyzer, Technicon). Plasma total calcium was determined spectrophotometrically by the cresolphthalein complexone method (Sigma).

Prolactin cell activity Pituitary glands of control and Cd-exposed fish were fixed for electron microscopy as described by Wendelaar Bonga and Van der Meij (1980), dehydrated and embedded in Spurr's resin. Ultrathin sections were examined under a Philips 301 electron microscope. For quantitative evaluation of prolactin cells, randomly selected samples of cell profiles at least totalling 20 cells per animal, were analyzed. Electron micrographs of these cells were scanned using a Kontron Digiplan integration equipment with magnetostriction tablet. The fractional volumes (relative to cytoplasmic volume) of granular endoplasmic reticulum (GER), Golgi apparatus (Ga), and secretory granules (GRAN) were determined. For details see Wendelaar Bonga and Van der Meij (1980).

Tissue Ca and PO_4 analyses Three types of bone samples viz. scales, operculum and vertebral bone were taken from each fish. Samples of 10 scales each were taken from both sides at the lateral line region directly posterior to the gill chamber. A sample of opercular bone ($\pm$ 0.5 cm^2) was taken, from which skin and connective tissue were removed by rubbing with tissue paper until it became transparent. A sample of vertebrae at the caudal end was removed and cleaned from adhering soft tissues. All bony samples were dried overnight at 90°C and dry weights determined to the nearest 0.01 mg. The dried samples were subsequently dissolved in 0.5 ml concentrated HNO_3 and the sample volume brought to 5 ml with distilled water. Calcium and phosphate concentrations were measured by Inductively Coupled Plasma Atomic Emission Spectrometer (Plasma IL200, Thermo Electron, USA) and expressed as mmol/g dry weight.

Statistics Differences between Cd-exposed and control groups were tested for significance by analysis of variance (ANOVA) and Student's *t* test. Tests were two-sided at the 5% significance level.

RESULTS

Plasma osmolarity Exposure of tilapia to 1000 μg Cd/l resulted in a high acute mortality. After 2 days, a significant drop ($P < 0.01$) in plasma osmolarity was

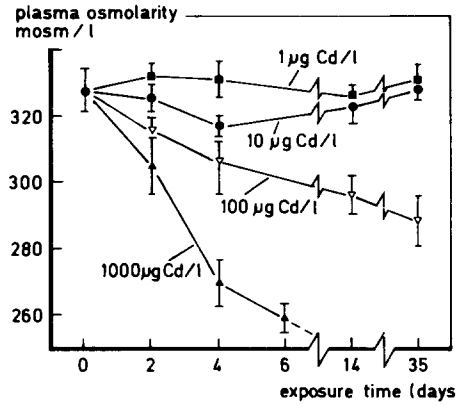


Fig. 1. Plasma osmolarity of tilapia after exposure for 2, 4, 14 and 35 days to indicated cadmium concentrations. Data are mean ± SE of 8 observations; at 1 000 µg Cd/l all fish died within 8 days.

observed (Fig. 1). After 4 days when the first fish started to die, plasma osmotic values had further dropped in the remaining fish and symptoms of Cd-poisoning such as erratic body movements, tetanic seizures and hyper-excitability were noticeable. At this point the experiment was terminated as the remaining fish became moribund. Exposure of fish to 100 µg Cd/l resulted initially in a low mortality; < 15% died during the first 2 wk. However, a significant drop ($P < 0.01$) in plasma osmolarity became already apparent after 48 h and continued until the end of the experiment (Fig. 1). Of the remaining fish more than 70% died in the following weeks, most of them in week 5. Plasma total Ca levels were also severely affected, reaching levels as low as 45% of control values (1.6 ± 0.3 µmol/l Ca).

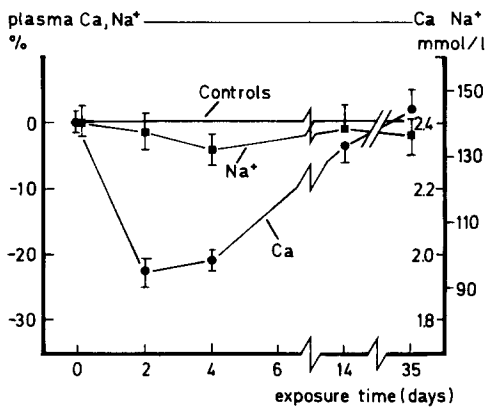


Fig. 2. Plasma total Ca and Na^+ after exposure to 10 µg Cd/l at indicated time; mean ± SE of 8 fish indicated.

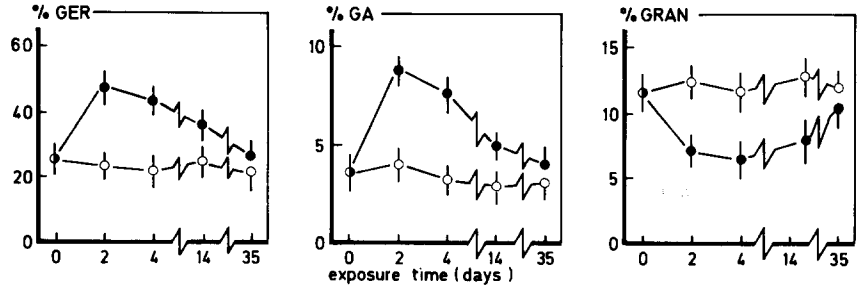


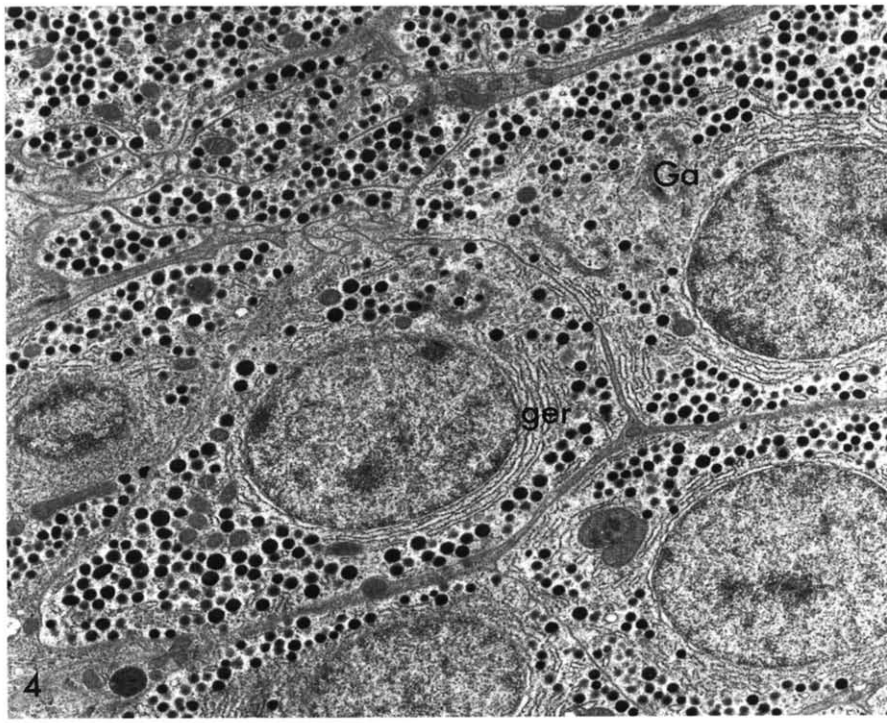
Fig. 3. Morphometrical data of prolactin cells of control tilapia (○) and of fish exposed to 10 μg Cd/l (●). Volumes of granular endoplasmic reticulum (Ger), Golgi area (Ga) and hormone-containing granules (Gran) are expressed as percentage of cytoplasmic volume. Mean ± SE of 5 fish.

Moribund fish exhibited similar symptoms of Cd-poisoning as observed in tilapia exposed to 1000 μg Cd/l. At 10 μg Cd/l mortality was not significantly different from controls, and no symptoms of Cd poisoning were observed during the experimental period. However, a small drop in plasma osmolarity was observed during the first few days, leading to a slight but significant ($P < 0.05$) change in plasma osmolarity at day 4, which had disappeared at day 14 (Fig. 1). At 1 μg Cd/l, no mortality or changed plasma osmotic values were noticeable. A Cd concentration of 10 μg Cd/l, which was considered sublethal but led to a transitory drop of the plasma osmolarity, was selected for further experiments.

Plasma Na^+ and plasma total Ca Exposure of tilapia to 10 μg Cd/l led to a small but significant drop ($P < 0.05$ after 4 days) in plasma Na^+, which had completely disappeared in the following weeks (Fig. 2). Plasma total Ca levels showed a pronounced decrease of 23% and 21% ($P < 0.01$) after respectively 2 and 4 days of exposure. This initial drop was followed by a rapid recovery, resulting in Ca-levels not significantly different from control levels in the following weeks (Fig. 2).

Prolactin cell morphometry Morphometrical and ultrastructural examination of prolactin cells of fish exposed for different times to 10 μg Cd/l showed marked changes, indicative for enhanced prolactin release (Figs. 3–5). During the first week of exposure, the extent of granular endoplasmic reticulum (GER) and Golgi area (Ga) had significantly ($P < 0.01$) increased, while a marked ($P < 0.01$) drop in hormone-containing granules was observed (Fig. 3). This difference is further illustrated in the electron micrograph of prolactin cells from control fish (Fig. 4) and fish exposed to Cd for 4 days (Fig. 5). Increased prolactin cell activity appeared transitory; no significant differences between control and Cd-exposed fish were observed after 35 days.

Figs. 4 and 5. Prolactin cells of control tilapia (Fig. 4) and of fish exposed for 4 days to 10 μg Cd/l (Fig. 5). Cd-exposed fish show more granular endoplasmic reticulum (Ger), more extensive Golgi area (Ga) and less hormone containing granules (Gran); 11 000 ×.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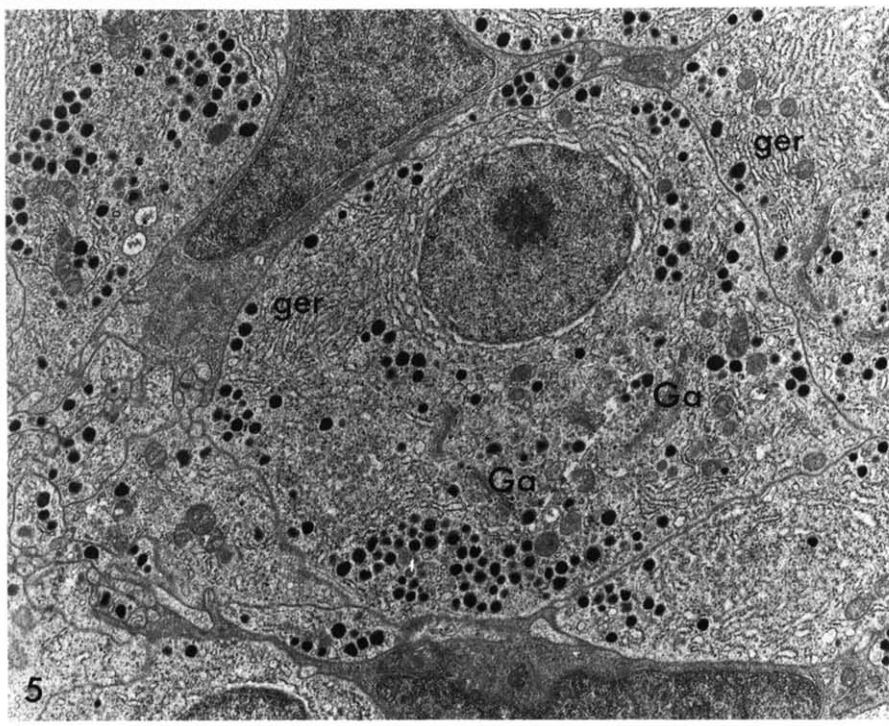
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TABLE I

Calcium (Ca) and phosphate (PO_4) content (mmol/g dry weight) and Ca/PO_4 ratio in scales, operculum and vertebrae of control tilapia (*Oreochromis mossambicus*) and of fish exposed to 10 μg Cd/l for 2 and 14 days.

	Control fish			Cd-exposed fish		
	Ca	PO_4	Ca/PO_4	Ca	PO_4	Ca/PO_4
2 Days						
Scales	3.91 ± 0.40	2.64 ± 0.25	1.48 ± 0.01	3.83 ± 0.31	2.59 ± 0.20	1.48 ± 0.02
Operculum	4.93 ± 0.22	3.26 ± 0.14	1.51 ± 0.01	5.01 ± 0.18	3.31 ± 0.12	1.52 ± 0.01
Vertebrae	3.72 ± 0.14	2.55 ± 0.11	1.46 ± 0.02	3.41 ± 0.12	2.31 ± 0.19	1.47 ± 0.02
14 Days						
Scales	3.74 ± 0.23	2.51 ± 0.14	1.49 ± 0.03	3.55 ± 0.95	2.43 ± 0.41	1.43 ± 0.13
Operculum	4.29 ± 0.34	2.65 ± 0.16	1.51 ± 0.03	4.19 ± 0.16	2.81 ± 0.11	1.49 ± 0.02
Vertebrae	3.58 ± 0.45	2.37 ± 0.39	1.51 ± 0.06	3.84 ± 0.31	2.62 ± 0.18	1.47 ± 0.05

Values are expressed as mean ± SE of 10 observations.

Tissue Ca and PO_4 analysis The content of Ca and PO_4 as well as the Ca/PO_4 ratio of scales, operculum and vertebral bone in controls and Cd-exposed fish are shown in Table I. No alteration in Ca and PO_4 content or their ratio is observed in any of the tissues examined during the experimental period of 5 wk (only data of fish after 2 and 14 days of exposure are shown).

DISCUSSION

Exposure to 1 000 μg Cd/l proved to be lethal to tilapia. Observations by others on the same and different species of fish have shown Cd-concentrations at this level to be critical (Morgan, 1976; McCarty et al., 1978; Majewski and Giles, 1981; Pascoe et al., 1986). Although no acute toxicity was observed at 100 μg Cd/l during the first week of exposure, the gradual reduction of plasma osmolarity leading to a high mortality in the following weeks reflects the inability of the fish to regain proper water and ion homeostasis. This disturbance in osmoregulation at Cd-concentrations of this level is a probable consequence of gill and kidney damage as frequently reported for Cd-intoxicated fish species (Gardner and Yevich, 1970; Stromberg et al., 1983; Karlsson-Norrgren et al., 1985). The Cd-induced injuries are apparently of such serious nature that normal mechanisms of regulation are incapable of restoring the ion balance.

Except for a small but significant drop in plasma osmolarity after 4 days of exposure to 10 μg Cd/l, no further changes in plasma total osmolarity were observed, indicating an intact osmoregulation. In contrast, a serious and specific drop in plasma total Ca-level was observed after 2 days of exposure. This rapid induction of hypocalcemia by sublethal concentrations of Cd has also been shown in rainbow

trout (Roch and Maly, 1979; Giles, 1984), flounder (Larsson et al., 1981), carp (Koyama and Itazawa, 1977; Yamawaki et al., 1986), and tilapia (Pratap et al., 1989). It reflects a specific reduction of Ca^{2+}-uptake via the gills as demonstrated in rainbow trout (Verbost et al., 1987; Reid and McDonald, 1988). At lethal Cd-concentrations branchial Ca^{2+}-uptake in these fish has even been shown to be completely inhibited (Verbost et al., 1987). Severe hypocalcemia became also apparent in our tilapia exposed to 100 μg Cd/l and above where plasma Ca-levels in moribund fish had dropped with almost 45% (1.6 $\pm$ 0.3 mmol Ca/l).

Since Ca is of crucial importance in maintaining integrity and stability of gill epithelial cell membranes (Wendelaar Bonga et al., 1983), as well as to the development of action potentials in muscle and nerve cells (Prosser, 1973), a pronounced alteration of plasma Ca concentration will severely affect these processes. In mammals for example, decreased plasma Ca-concentrations are known to cause neuromuscular hyperexcitability and cramp conditions, so-called hypocalcemic tetanus (Ganong, 1975). It is quite likely therefore that severe hypocalcemia is the major cause for the tetanic body movements and loss of blood ions as observed in Cd-intoxicated tilapia. Similar neuromuscular effects of Cd have been observed in minnows (Bengtsson et al., 1975), flounder (Larsson et al., 1981), and rainbow trout (Roch and Maly, 1979; Giles, 1984). Indeed, Roch and Maly (1979) considered extreme hypocalcemia as the primary cause of death of Cd-intoxicated freshwater fish.

In contrast to fish exposed to 100 μg Cd/l and above, only a transitory disruption of the Ca-balance was observed in tilapia exposed to 10 μg Cd/l. This phenomenon has also been reported for rainbow trout (Giles, 1984), carp (Yamawaki et al., 1986) and tilapia (Pratap et al., 1988), indicating a successful process of detoxification or acclimation. The question as to which biochemical and physiological mechanisms are primarily responsible for this restoration of plasma Ca-levels is rather unclear. Although detoxification by Cd-binding proteins such as metallothioneins (MT) undoubtedly plays a crucial role in sustaining long term acclimation to Cd (Webb, 1979; Klaverkamp et al., 1984), we propose that prolactin is initially involved in counteracting Cd-induced hypocalcemia in fish. Our arguments are the following: prolactin is with cortisol one of the main osmoregulatory hormones in fish. Prolactin has been implicated in the maintenance of plasma electrolyte levels, mainly by controlling permeability of the gill epithelium (Clark and Bern, 1980). This action of prolactin has also been demonstrated for tilapia (Dharmamba and Maetz, 1972; Wendelaar Bonga et al., 1983). Moreover, in tilapia and other freshwater fish, prolactin has a specific action on Ca-metabolism. It has been shown to promote hypercalcemia in various species of fish, mainly via increased net uptake rate of Ca^{2+} via the gills (Pang et al., 1973; Wendelaar Bonga and Flik, 1982; Flik et al., 1986). A response of prolactin to Cd-induced hypocalcemia is therefore expected. Our results show that hypocalcemia is indeed related with increased activity of prolactin cells. One of the most striking features observed was a degranulation of prolactin cells, which together with the increased volumes of granular endoplasmic reticulum

(GER) and Golgi area (GA) is considered as a reliable indicator of prolactin release (Wendelaar Bonga et al., 1984). Moreover, these changes were most pronounced at the time (day 4) of severest hypocalcemia supporting the role of prolactin as hypercalcemic hormone. Other than stimulating the active Ca^{2+}-uptake via the gills, prolactin may also exert its hypercalcemic action via remobilization of Ca from exchangeable Ca-stores. Bone demineralization has been shown in Cd-exposed carp (Koyama and Itazawa, 1977; Muramoto, 1981) and has been interpreted as a mechanism to restore plasma Ca-levels. We have no evidence in support of a similar mechanism taking place in tilapia since no decrease in Ca and PO_4 content of exchangeable Ca-stores was observed. Even in tilapia exposed to a lethal Cd concentration of 1 000 μg Cd/l, which resulted in severe hypocalcemia, no changes in Ca and PO_4 content of bony tissues were apparent (results not shown). The reason for this discrepancy between carp and tilapia is a likely result of the different bone structure between these fish. In contrast to carp, which possesses cellular bone, tilapia has acellular bone, which by its absence of enclosed osteocytes is dead tissue and therefore unavailable for cellular calcium mobilization (Moss, 1965). Since freshwater fish take up most of their calcium required for growth and Ca-homeostasis directly via their gills (Flik et al., 1985), a process stimulated by prolactin (Flik et al., 1986), an increased branchial net Ca-flux via increased prolactin secretion appears the most obvious explanation for the observed restoration of plasma Ca-levels.

To our knowledge the effect of Cd on prolactin cell activity is restricted to one other study by James and Wigham (1986). They could not find any consistent effect on prolactin cell activity in rainbow trout after Cd treatment via intraperitoneal injection, water exposure or in vitro incubation of pituitaries with cadmium. This discrepancy with our results is probably due to the extremely high Cd-concentration used in their study (up to 500 times the 96-h LC_{50} value), which could block any adaptive response. An analogous observation was reported in acid-stressed tilapia in our laboratory, where strong activation of prolactin cells was blocked after lethal pH levels were reached (Wendelaar Bonga et al., 1987).

One of the remarkable observations in this study was that the increased prolactin cell activity did not continue during the entire Cd-exposure period. If Cd continues to exert its hypocalcemic action one would expect a permanently elevated level of prolactin release to counter-act this effect. That this did not happen points to the induction of a second mechanism like increased capacity to sequester intracellular Cd by metal-binding proteins in the gills. Whether induction of these proteins is directly stimulated by prolactin or indirectly through actions of cortisol is currently investigated.

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