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EFFECTS OF WATER-BORNE CADMIUM ON PLASMA CORTISOL AND GLUCOSE IN THE CICHLID FISH
OREOCHROMIS MOSSAMBICUS

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Abstract—1. Freshwater cichlids Oreochromis mossambicus (tilapia) were exposed to 10 μg Cd/l in ambient water for 2, 4, 14 and 35 days. Plasma cortisol and glucose levels were determined to evaluate if cadmium induced a typical stress response in these fish.
2. Exposure to cadmium for 2, 4 and 14 days elicited a significant elevation of plasma cortisol levels.
3. A significant hyperglycemia occurred on days 2 and 4 in cadmium-exposed fish.
4. During long-term exposure to cadmium (35 days), the plasma cortisol and glucose levels returned to control values. This recovery after 35 days indicates the ability of tilapia to adapt to low cadmium concentrations in the ambient water.

INTRODUCTION
Cadmium is a widely-occurring industrial heavy metal pollutant that is well-known for its toxic effects on aquatic organisms. In fish, the metal has adverse effects on growth and reproduction and causes osmoregulatory stress (Roch and Maly, 1979; Giles, 1984; Klaverkamp and Duncan, 1987; Verboss et al., 1987; Reid and McDonald, 1988; Pratap et al., 1989).

Frequently, plasma corticosteroid and glucose levels are used as indicators to determine the magnitude of stress responses in vertebrates, including fish (Silbergeld, 1974; Mazeaud et al., 1977; Donaldson et al., 1984; Pickering, 1981). In fish, the major corticosteroid is cortisol, which has mineralo- as well as glucocorticoid properties (Donaldson, 1981). Increased cortisol levels are considered a primary stress response. They have been observed along with increased nuclear diameter and cell size of the corticosteroid-producing cells of the interrenal gland in fish exposed to industrial pollutants such as sanitary landfill leachate (McBride et al., 1979), butoxyethanol ester of 2,4-dichlorophenoxy acid (McBride et al., 1981), or acid water (Brown et al., 1984). Elevated plasma glucose levels, probably caused by cortisol and considered a secondary stress response, have been reported for fish exposed to water pollutants, water acidification, thermal handling or crowding stress (Thomas et al., 1981; Brown et al., 1984; Barton and Schreck, 1987; Pickering and Steward, 1984). Several heavy metals have also been reported to stimulate interrenal activity and plasma corticosteroid and glucose levels in fish. These studies include the exposure of rainbow trout to chromium (Hill and Fromm, 1968), and of sockeye salmon to copper (Donaldson and Dye, 1975). Reports on the stress response of fish to cadmium are scarce, and the available information is not consistent. Schreck and Lorz (1978) were unable to detect increased cortisol levels in cadmium-exposed rainbow trout. Inconsistent effects were reported for the same species by Jambs and Wigham (1986). The reported variation was high and exposure times were limited. Nevertheless, one might conclude that the effects of cadmium on cortisol and interrenal activity differ from those of other heavy metals and water pollutants in that it does not elicit a typical stress response. The objective of this study was to verify this conclusion by investigating the influence of cadmium on plasma cortisol and glucose levels. The effects on these parameters of sublethal, environmentally relevant concentrations of water-borne cadmium were analyzed after exposure periods varying from 2 to 35 days.

MATERIALS AND METHODS
Freshwater laboratory stock of male Oreochromis mossambicus with a body weight of 16.2 ± 4.8 g (mean ± SD) were used in the present study. Fish were maintained in 1001 aquaria with continuous aeration and circulation of filtered water (Eheim pumps 1021), pH 7.4, at 28°C on a 12L:12D photoperiod. They were fed Tetramin tropical fish food.

Fish were divided into four groups, of which two were kept in experimental tanks and two served as controls. Fish maintenance and water quality were as described by Pratap et al. (1988). All fish were acclimatized for two weeks in experimental tanks before starting the experiment. All sides of the experimental and control tanks were screened with dark grey polyethylene foil with small observation windows to minimize disturbance of the fish. Each aquarium was subdivided into two compartments by a frame of fine nylon mesh, with 3 to 4 fish in each compartment. Experimental fish were exposed to 10 μg Cd/l. The cadmium was...
administered to the water in the aquaria from a stock solution of 1000 µg Cd/l (Cd(NO₃)₂; RCB, Bruxelles). Cadmium concentration in the water was monitored daily in all tanks, with a Video 11 frameless atomic absorption spectrophotometer (Thermo Jarrell Ash, USA), and adjusted if necessary.

Fish were sampled after 2, 4, 14 and 35 days, and the experiment was repeated twice. Each time a control group was sampled along with the cadmium-exposed fishes. All fish were starved for 24 hr before sacrifice. To exclude the influence of the reported daily rhythmic changes in plasma cortisol levels (Pickering and Pottinger, 1983; Nichols and Weisbart, 1984), all fish were sacrificed at 09.30 a.m. Each time a group of fish was rapidly netted with minimal disturbance, and immediately stunned by a blow on the head and blood was taken by severance of the caudal peduncle. The plasma was obtained after centrifugation in heparinized microhaematocrit tubes at 12,000 g for 5 min. The plasma samples were stored at −30°C for the determination of glucose and cortisol. Plasma glucose was measured using Glucose GOD-Perid enzymatic method kit from Boehringer, FRG.

Cortisol was determined by radioimmunoassay (RIA) according to Vecsei (1974) and De Man et al. (1980). Plasma samples (5 µl) and cortisol standards (50 µl) were incubated with 200 µl of ¹²⁵I-cortisol and antiserum buffer at 37°C for 30 min, and subsequently at 4°C for 1 hr. Following incubation at 4°C, bound and free cortisol were separated by adsorption of the free fraction of dextran-coated charcoal. The suspension was mixed with the contents of the tubes by shaking them simultaneously. After 6 min at 4°C, the tubes were centrifuged at 9000 g for 10 min at 4°C. The supernatant was decanted in counting vials and after adding 4 ml scintillation cocktail (Aqua Luma, Lumac, Belgium) radioactivity was determined. All samples were assayed in triplicate. The intra-assay coefficient of variaton was 7.6% and interassay variation was avoided by measuring all samples in the same assay. All experimental results are given as means ± SD and the data were subjected to analysis of variance and statistical significance was set at 5%.

RESULTS

Visual observations through aquarium windows of Oreochromis mossambicus exposed to cadmium for up to a period of 35 days did not reveal any symptoms of abnormal behaviour. No mortality occurred during the experiment.

Cortisol

Exposure of tilapia to cadmium in ambient water caused a significant rise in plasma cortisol levels (Fig. 1). After 2 days, in the cadmium-exposed fish the plasma cortisol titers were significantly elevated (130 ng/ml) compared to the controls (12 ng/ml; P < 0.001). In the following two weeks a gradual decline in the plasma cortisol of cadmium exposed tilapia was observed. On day 4, cortisol titers decreased to 111.5 ng/ml. After 14 days the plasma cortisol level had further reduced to 88.3 ng/ml though it was still statistically significantly higher than the control values (P < 0.001). A sharp decline was evident by 35 days in the cadmium-exposed fish, when the cortisol levels were restored to normal values and not significantly different from those observed in the control fishes. Though care was taken to avoid any disturbance during the experiment, in fish of control groups cortisol titers were increased on days 4 and 14 (47.6 ng/ml, and 33.8 ng/ml) when compared to day zero values.

Glucose

In cadmium-exposed fishes, a transitory hyperglycemia was observed (Fig. 2). A significant elevation in plasma glucose was evident on days 2 and 4 in cadmium-exposed fish. The highest plasma glucose levels occurred on day 2 (80.4 mg%, P < 0.001), followed by a decline on day 4 (45.3 mg%, P < 0.01). On day 14, a recovery from the hyperglycemic condition was observed, with no significant difference in the plasma glucose levels between the cadmium-exposed and control groups. On further exposure to cadmium for a period of 35 days, no significant changes occurred in the plasma glucose levels. No significant changes were observed between the control groups.

DISCUSSION

Exposure to cadmium appears to elicit a transient stress response in Oreochromis mossambicus. Significantly elevated plasma cortisol titers were evident on days 2, 4 and 14, while increased plasma glucose levels were observed on days 2 and 4. The association of increased plasma cortisol and glucose levels is frequently observed following exposure of fish to water pollutants or other stressors, and the relationship most likely is causal: the primary response (elevated cortisol) leads to the secondary (elevated glucose) via stimulation by cortisol of gluconeogenesis (Leach and Taylor, 1980, 1982; Pickering, 1981).
Cadmium and cortisol in a teleost fish

Our results contrast with earlier reports on the relationship between cadmium and cortisol. Schreck and Lorz (1978) were unable to find a clear effect on plasma cortisol of Coho salmon exposed to cadmium, at water concentrations (4–12 mg/l) a factor of 100 higher than in our experiments. In their experiment the absence of significant difference with the controls may be connected with the high cortisol levels in the control fish, which might have been caused by the experimental conditions or capture stress (cf. Sumpter et al., 1986). In a study on trout, James and Wigham (1986) reported minor and time-dependent changes in plasma cortisol levels in response to water-borne cadmium. Exposure to 0.05 mg/l for 24 hr significantly increased plasma cortisol, whereas a decrease was found after 72 hr. Exposure to 0.1 mg/l resulted in a decrease after 6 hr, a rise after 24 hr, and no effect after 96 hr and the control levels varied substantially. In the present investigation, although utmost care was taken to avoid disturbance, the finding of elevated plasma cortisol in the un­treated tilapia on day 4 and 14 suggested that the experimental conditions could have caused some increase in interrenal activity. Nevertheless, our data clearly show a marked stimulation by cadmium of plasma cortisol of Coho salmon exposed to cadmium, contaminated lake were significantly lower than cortisol values of pike from a methylmercury­contaminated lake (Lockhart and Lutz, 1977) showed no significant difference with laboratory controls or the finding of elevated plasma cortisol in the un­significant difference with the controls. Recovery of plasma cortisol and glucose levels observed in the present study, and the recovery of plasma calcium and magnesium levels reported earlier (Pratap et al., 1989) suggest that tilapia develop tolerance to sublethal concentrations of ambient cadmium on prolonged exposure. Recovery of plasma electrolyte levels has been observed in rainbow trout exposed to cadmium (Giles, 1984) and copper (Lauren and McDonald, 1987). Tolerance or adaptation may be partially caused by hormonal action and partially by the production of metallothioneins. Such metal binding proteins are induced by exposure to many heavy metals, including cadmium (Goering and Klaassen, 1983). Probably the role of hormones in the adaptation to stressors such as cadmium is limited to the first weeks of the exposure period, when the production of metallothioneins is insufficient for adequate protection of the fish.

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