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Exogenous Steroids Enhance the Copper Resistance of Tilapia Larvae (*Oreochromis mossambicus*)

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Su-Mei Wu, Am-Ni Deng, Yi-Ying Chou, and Leang-Shin Wu (2005) Exogenous steroids enhance the copper resistance of tilapia larvae (*Oreochromis mossambicus*). *Zoological Studies* **44**(3): 373-381. In this study, we attempted to evaluate the effects of exogenous steroids on copper resistance and the relationship among steroids, Na^+/K^+-dependent adenosine triphosphatase (Na^+-K^+ ATPase), and ionic homeostasis after challenge with copper toxicity in tilapia larvae (*Oreochromis mossambicus*). Tilapia larvae were reared on artificial feed containing 0 (control), 50 (low-dose), 125 (middle-dose), and 250 mg/kg (high-dose) cortisol or progesterone for 10 d before Cu^{2+} (1 mg/L) challenge for 72 h. The results indicated that both cortisol and progesterone significantly increased the activity of Na^+-K^+-ATPase, while progesterone was more effective than cortisol treatment at increasing the survival rates in larval fish after Cu^{2+} challenge. Upon larval exposure to waterborne Cu^{2+}, the whole-body Na^+ content was retained for a prolonged period. Larvae were treated with cortisol and progesterone to determine their effects on Cu^{2+} resistance and the resultant cation retention. Results for the effect of progesterone were clearer than those of cortisol. On the other hand, cortisol levels significantly increased after 10 d of exogenous administration of cortisol. While larval aldosterone levels increased, the cortisol content was not enhanced after 10 d of oral progesterone treatment. We suggest that in addition to progesterone's involvement in copper resistance mechanisms in tilapia larvae by ion retention, aldosterone might also effectively enhance this mechanism. http://zoolstud.sinica.edu.tw/Journals/44.3/373.pdf

Key words: Steroids, Na^+-K^+-ATPase, Copper, Aldosterone.

The toxicity of sublethal Cu^{2+} concentrations to adult fish, its accumulation within tissues, and its impacts on physiological mechanisms have been well studied in some fish (Sorensen 1991, Dethloff 1999) including tilapia (Dang et al. 1999). Evidence shows that Cu^{2+} toxicity also disrupts ion homeostasis and growth in juvenile and adult fish (Nussey et al. 1996, Svecevicius and Vosyliene 1996, Taylor et al. 1996, Li et al. 1998, Perschbacher and Wurts 1999, Priya et al. 1999, McGeer et al. 2000). In addition, the toxic effects of sublethal concentrations of Cu^{2+} on larvae have also been reported. Whole-body Na^+-K^+ and Ca^{2+} contents of larvae significantly decreased following Cu^{2+} exposure beginning at up to 96 h (Wu et al. 2003). Secondarily, Na^+/K^+-dependent adenosine triphosphatase (Na^+-K^+ ATPase)-specific activity was found to be very sensitive in fish exposed to waterborne Cu^{2+} (Li et al. 1998, de Boeck et al. 2001). Free Cu^{2+} has been shown to bind covalently to SH groups of Na^+-K^+-ATPase, thus interfering with conformational changes of the protein (Kone et al. 1990). Na^+-K^+-ATPase-specific activation was found to have significantly decreased following 4 h of Cu^{2+} treatment and also to have decreased to about 65% after 96 h of Cu^{2+} exposure in carp (de Boeck et al. 2001).

Na^+-K^+-ATPase is known to be present in embryos of oviparous species and was also demonstrated to be involved in the osmoregulation of embryos incubated in seawater (Conte et al. 1991). Exogenous cortisol significantly reduces

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the mortality of tilapia larvae upon seawater (SW) challenge, indicating improvement in the hypo-osmoregulatory ability (Hwang and Wu 1993). Even though Na^+-K^+-ATPase activity measurements were given in those papers, exogenous cortisol demonstrated induction of 14%~65% increases in gill Na^+-K^+-ATPase activity in adult tilapia (Dange 1986), and has been shown to significantly increase gill Na^+-K^+-ATPase activity in seawater-adapted fish (Miguel et al. 2002). Lin et al. (1999) showed that treatment of tilapia larvae with cortisol enhances their hypo-osmoregulation and ouabain binding levels, and suggested that this may be achieved by increasing the Na^+-K^+-ATPase of the yolk-sac epithelia. Therefore, it may be expected that the increased Na^+-K^+-ATPase activity may enhance resistance of larvae to Cu^{2+} challenge with administration of exogenous steroids.

In fact, cortisol is involved in both glucocorticoid and mineralocorticoid functions in teleosts (Mazeaud et al. 1977). Cortisol responds to any stressors that interfere with ionic stability or carbohydrate metabolism. These stressors also include heavy metal pollution, but not salinity challenge. For instance, plasma cortisol levels increased in Cu^{2+}-exposed fish and might be interpreted as cortisol-associated ionic homeostasis after ions were disturbed by Cu^{2+} exposure (Pelgrom et al. 1995). However, Lin et al. (1999) found that a significant effect of cortisol on the whole-body Na^+ content in larvae occurred as early as 4~8 h after transfer to SW. It would be interesting to know whether or not ionic regulation through the cortisol response occurs in the early stage of Cu^{2+} exposure.

Progesterone is the precursor of mineralocorticoid steroid biosynthesis; the end product includes corticosterone, cortisol, and aldosterone in mammals (Agarwal and Mirshahi 1999). Aldosterone was first reported in bony fish, which also produce corticosterone and cortisol (Henderson et al. 1975), but the pathway of steroid biosynthesis in fish is not clear. Generally, cortisol has been shown to increase the Na^+ influx in freshwater rainbow trout (Perry and Laurent 1989) and the Na^+ efflux in seawater-adapted eels (Mayer et al. 1967). The physiological role of aldosterone also involves Na^+ and K^+ homeostasis and increases basilar Na^+-K^+-ATPase activity, although the presence of aldosterone in teleost fish still remains doubtful. The general consensus is that most fishes do not produce this steroid (Henderson and Kime 1987, Joss et al. 1999). However, recent evidence indicates that aldosterone or mineralocorticoid-like hormones have physiological functions in a few teleost fish. Law et al. (2001) found that exogenous mineralocorticoid aldosterone decreases the phagocytosis index in tilapia (*Oreochromis niloticus* x *O. aureus*) leukocytes. Colombe et al. (2000) cloned a mineralocorticoid-like receptor in rainbow trout (*Oncorhynchus mykiss*) and found that it has clear homology with human aldosterone receptor cDNA sequences. Goldfish (*Carassius auratus*) have been found to contain small amounts of aldosterone (Norris 1997). These facts encouraged us to investigate the roles of exogenous cortisol and progesterone in the enhancement of the survival rates in tilapia larvae upon exposure to waterborne Cu^{2+}. Furthermore, it was of interest to explain the relationship between steroids and ionic homeostasis after Cu^{2+}-exposure.

MATERIALS AND METHODS

Fish

Mature adult tilapia (*O. mossambicus*) from the Mariculture Research Center of the Taiwan Fisheries Research Institute, Tilapia were reared in 182-L glass aquariums using plastic chips for gravel. Each tank was supplied with dechlorinated, circulated, and aerated local tap water (FW) at 26~28°C under a photoperiod of 12~14 h. Fish were fed commercial fish food pellets. Larvae from the same brood were incubated under the same conditions as above. Larval tilapia at 0.5~0.7 cm in total

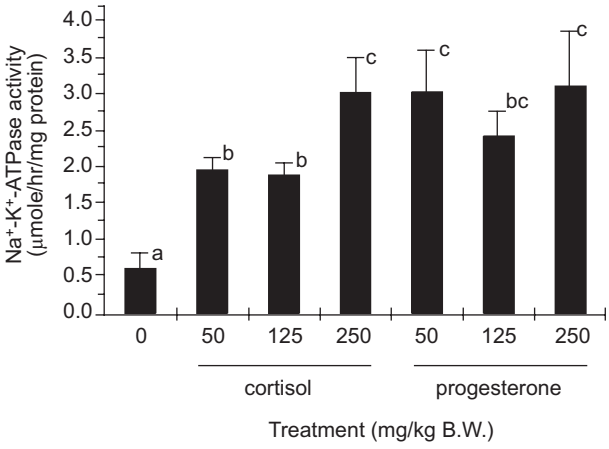

Fig. 1. Comparison of Na^+-K^+- activated adenosine triphosphatase in tilapia larvae after 10 d of treatment with steroid hormones (n = 3~5). Different superscripts indicate a significant difference among treatments ($p < 0.05$, ANOVA analysis with Tukey's comparisons).

length and 0.012~0.017 g in body weight were used in the present study.

Artificial feed containing glucocorticoids

Cortisol (hydrocortisone) and progesterone (Sigma, St. Louis, MO) were mixed with artificial dried feed, and the mixture was stored at -20°C until administration. According to our previous study (Wu et al. 2002), 4 doses of 0 (control), 50 (low-dose), 125 (middle-dose), and 250 mg/kg (high-dose) of both cortisol and progesterone were selected for use in the following experiments.

Experimental media

Completely dried $CuSO_4$ (Merck, Darmstadt, Germany) was dissolved in double-deionized water to prepare the 1000 mg/L Cu^{2+} stock solution. This stock solution was diluted to the desired concentrations with local tap water as described in Wu et al. (2000), and the Cu^{2+} test media were prepared at concentrations within 0 and 1 mg/L according to the experimental designs (see below). During all experiments, the test containers used were cleaned with HNO_3 and thoroughly rinsed with double-deionized water before use. The medium in the test containers was changed daily, and the variance in Cu^{2+} concentrations measured less than 5% within 24 h. The Cu^{2+} concentration in local tap water was less than 0.01 mg/L (data provided from a routine report by the Taiwan Water Supply Corporation, Chiayi, Taiwan). Other parameters of the exposure media (including local tap water) were a total hardness of 146.6 ± 5.6 mg/L; DO (dissolved oxygen) of 7.5 ± 0.5 mg/L; Na^+ of 35.6 ± 0.3 mg/L; K^+ of 3.3 ± 0.1 mg/L; Ca^{2+} of 30 ± 2.3 mg/L; Mg^{2+} of 20.2 ± 0.2 mg/L; and pH of 8.2 ± 0.3~8.7 ± 0.2.

Whole-body Na^+-K^+-ATPase activity

Five larvae were pooled together for 1 sample, and 3~5 samples were collected from the same treatment. Samples were suspended in the homogenization solution (100 mM imidazole-HCL buffer (pH 7.6), 5 mM Na_2EDTA, 200 mM sucrose, and 0.1% sodium deoxycholate). Na^+-K^+-ATPase activity was assayed in a reaction mixture (100 mM imidazole-HCL buffer (pH 7.6), 125 mM NaCl, 75 mM KCl, 7.5 mM $MgCl_2$, and 5 mM Na_2ATP). The reaction was run at 37°C for 30 min, and then stopped by the addition of 0.2 ml of 30% cold trichloroacetic acid. The inorganic phosphate concentration was determined by the method of Peterson (1978). Total protein was determined by the method of Lowry et al. (1951), using crystalline bovine albumin as the standard. The enzyme activity of Na^+-K^+-ATPase was defined as the difference between the inorganic phosphate liberated in the presence and absence of 0.5 mM ouabain in the reaction mixture. Each sample was assayed in triplicate. All chemicals were purchased from Sigma Biochemical (St. Louis, MO, USA).

Cortisol and aldosterone extraction

The methods for steroid extraction followed Hwang and Wu (1993). Briefly, 10 frozen larvae were placed in a 1.5 ml Eppendorf centrifuge tube. Homogenizer containing 0.6 ml 0.1 M phosphate buffer with gelatin (PBSG, pH 7.0) was homogenized on ice with a motorized Teflon pestle at 1200 rpm for 20 strokes. The homogenate solution was transferred to a 10 ml glass test tube and mixed with 3 ml ether by vortexing at 1200 rpm for 5 min after rinsing the homogenizer with 0.5 ml PBSG. The test tube was quickly frozen in a -80°C deep freezer, and then the ether solution including steroid hormones was transferred to a new tube and evaporated to dryness at 42°C for 2~3 h. The final combined extract was reconstituted with 0.6 ml ELISA assay buffer (PBSG) for ELISA (enzyme-linked immunosorbent assay) of cortisol or aldosterone. The recovery rates, using $^3H^+$-labeled steroids as indicators, was about 85%, and this indicated that most (greater than 85%) of the cortisol and progesterone was dissolved in PBSG. All data were calibrated for recovery rates.

ELISA for cortisol

Cortisol was determined by ELISA, which followed the general protocol of Chou (1999). Briefly, diluted antiserum of cortisol was placed in a coated microtiter plate (96 wells) for 24 h at 4°C. Blocking buffer was reacted for 24 h at 4°C following 3 washes. Tissue extract and the standard solution were mixed with cortisol conjugate HRP (horseradish peroxidase); OPD (O-phenylenediamine; Sigma) was added for color development; and the results were measured at 490 nm with an automatic microtiter plate ELISA reader (Dynes MRX, Chantilly, VA, USA). The displacement curve for the serial dilutions of sample extracts was found to be parallel to that of the cortisol standard. The linear regression coefficient was determined using Microsoft Excel 97 SR-1 (1997;

Microsoft, Seattle, WA, USA) and for the logarithms of cortisol standard concentrations was 0.99, and the slope was -0.18. The coefficients of intra-assay and interassay variations were 1.5%~3.7% and 5.4%~6.3%, respectively.

ELISA for aldosterone

The methods of tissue extraction and aldosterone restoration were the same as for cortisol (see the above description). Aldosterone contents were determined using an aldosterone ELISA kit (Cayman Chemical, Ann Arbor, MI, USA). The specificity of the aldosterone antiserum with corticosterone was 0.11%, that of testosterone was 0.04%, while those of the other steroids (including 11-deoxy corticosterone, cortisone, estradiol, etc.) were all less than 0.01%.

Experiment 1. Effect of steroid hormones on Na^+-K^+-ATPase activation

Fifteen to 25 tilapia larvae were fed artificial feed mixed with cortisol or progesterone at doses of 0 (control), 50, 125, and 250 mg/kg of body weight at a rate of about 10% of body weight per day for 10 d. At the end of the rearing experiment, 5 larvae for 1 sampling, and 3~5 samples at the same treatment were collected. Whole-body Na^+-K^+-ATPase activity was measured.

Experiment 2. Effect of steroid hormones on Cu^{2+} resistance

Larvae were treated with cortisol or progesterone, using the same methods as described above. At the end of the rearing experiment, they were exposed to 1 ppm Cu^{2+}, and mortality was detected every 12 h until the 72nd hour. Twenty larvae were used for each determination, and 4 replicates (n = 4) were conducted for each treatment. We repeated the experiments with different broods of larvae.

Experiment 3. Effects of exogenous glucocorticoids on whole-body cortisol and aldosterone concentrations

Thirty larvae were treated with cortisol or progesterone, using the same methods described above. At the end of the treatment, 10 larvae for 1 sample and 3 or 4 samples were collected from the same treatment, and cortisol and aldosterone contents were measured.

Experiment 4. Effects of exogenous steroids on K^+, Na^+, and Ca^{2+} contents

Twenty larvae for 1 sample were treated with cortisol or progesterone after 10 d, and then exposed to 1 mg/L Cu^{2+} for 72 h, and their mortality was examined. In addition, whole-body K^+-Na^+ and Ca^{2+} contents of surviving larvae were respectively measured from 1 and 5~8 samples.

Statistical analysis

Data are presented as the mean ± SE. Statistical differences among treatments were analyzed using one-way ANOVA. Tukey's multiple-comparison or Dunnett's analysis was used to identify differences among treatments. Statistical significance was accepted for $p < 0.05$.

RESULTS

The Na^+-K^+-ATPase activation of tilapia larvae was found to have significantly increased by 3.2- and 4.7-fold, respectively, after cortisol and progesterone administration. The effect of exogenous steroids on Na^+-K^+-ATPase activation was higher with progesterone than with cortisol treatment (Fig.

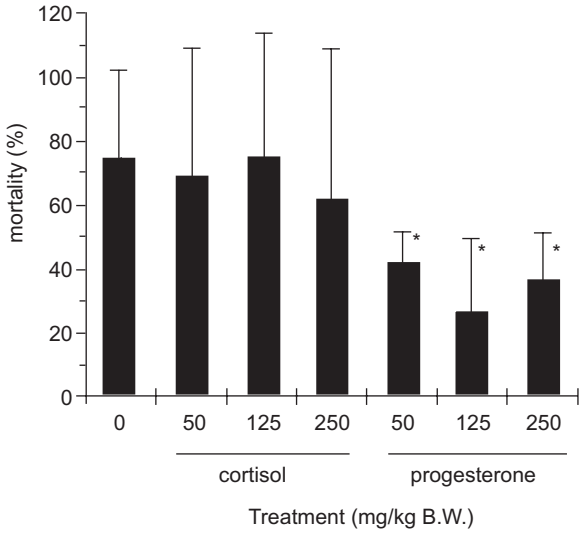

Fig. 2. Changes in mortality (%) of tilapia larvae after Cu^{2+} challenge (1 mg/L for 72 h). Tilapia were pretreated with 50, 125, or 250 mg/kg of cortisol or progesterone for 10 d and then were exposed to 1 mg/L Cu^{2+} for 72 h. Data (n = 4) were compared using one-way ANOVA analysis. A significant difference was found between the control and treatment groups (p = 0.026).

1). Administration of progesterone to tilapia larvae appeared to produce lower mortality (25%~41%) than in the control group (73%), after exposure to 1 mg/L Cu^{2+} for 72 h. However, administration of cortisol to tilapia larvae had no effect on the mortality (60%~73%) when compared with the control group after exposure to 1 mg/L Cu^{2+} for 72 h (Fig. 2). Cortisol contents in the whole body of larvae were found to have significantly increased with cortisol administration, and a dose-dependent response also appeared, although there was no evident change after exogenous progesterone administration (Fig. 3). Exogenous progesterone enhanced aldosterone in a dose-dependent manner (Fig. 4). We measured whole-body K^+ and Ca^{2+} levels after larvae were treated with cortisol or progesterone for 10 d and then exposed to 1 mg/L Cu^{2+} for 72 h. Data show that the amounts of K^+ and Ca^{2+} were higher with greater doses of administered progesterone compared to the control, but there was no significant change after any dose of administered cortisol. With respect to Na^+ contents, both cortisol and progesterone treatments were found to have significantly enhanced

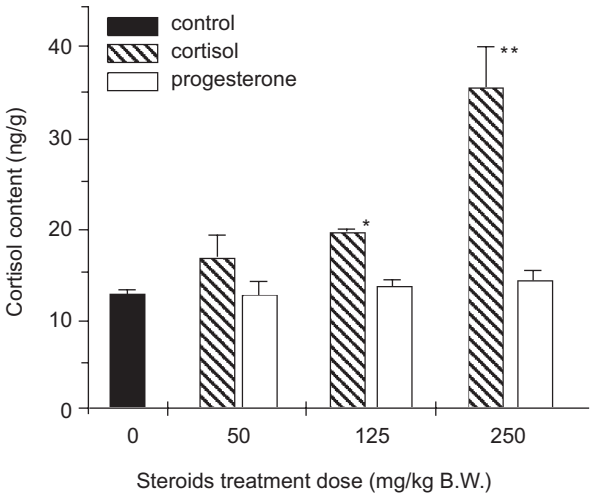

Fig. 3. Cortisol (ng/g body weight) content in tilapia larvae orally administered various doses of cortisol or progesterone for 10 d. Data were compared using one-way ANOVA with Dunnett's test analysis. * and ** indicate a significant difference between the control and treatment groups (n = 3) at p < 0.05 and < 0.01, respectively.

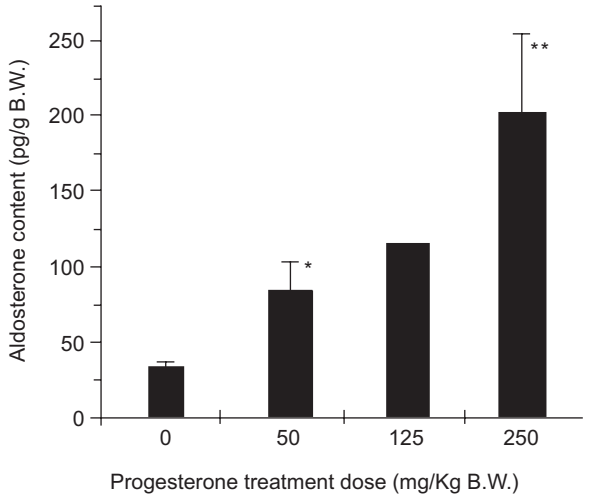

Fig. 4. Aldosterone content (pg/g body weight) in tilapia larvae orally administered various doses of progesterone for 10 d. Data were compared using one-way ANOVA with Dunnett's test analysis. * and ** indicate a significant difference between the control (n = 3) and treatment groups (low dose and high dose, both n = 4) at p < 0.05 and < 0.01, respectively. No standard error was given for the mid-dose treatment because its sample size was only 2.

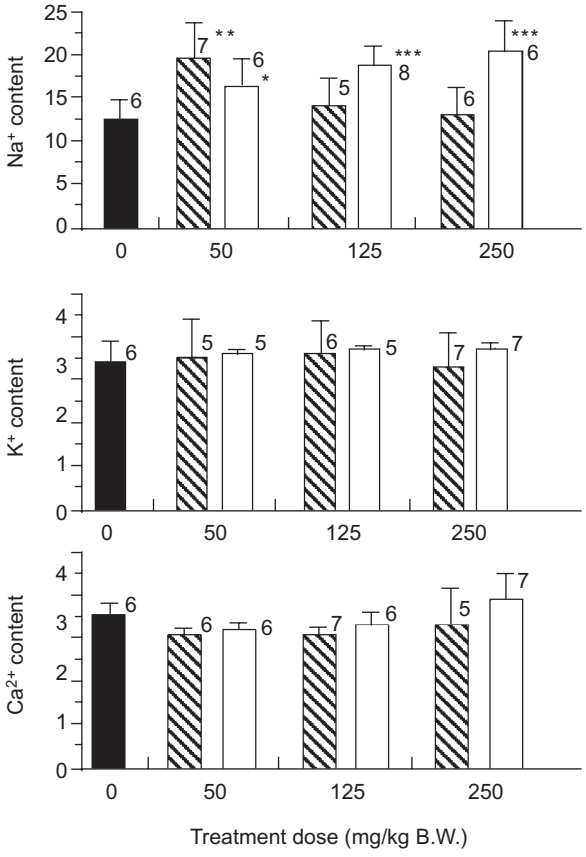

Fig. 5. Changes in Na^+, Ca^{2+} and K^+ contents (mg/g) in steroid-treated larval tilapia after exposure to 1 mg/L Cu for 72 h. Before the exposure to Cu, larvae were orally administrated various doses of cortisol (hatched columns) and progesterone (open columns), or no treatment was given (control, closed columns) for 10 d. Data (n = 5~8, labeled on the column side) were compared using one-way ANOVA with Dunnett's test analysis. * , **, and *** indicate significant differences between the control and treatment groups at p < 0.05, < 0.01, and < 0.001, respectively.

retention of Na^+ after Cu^{2+} exposure in larvae, but progesterone administration was also seen to be more effective than cortisol administration (Fig. 5).

DISCUSSION

Both cortisol and progesterone significantly increased Na^+-K^+ ATPase activity, while only progesterone enhanced the Cu^{2+} resistance of tilapia larvae. Our research design did not provide strong evidence to prove that cortisol and progesterone are involved in the Cu^{2+} detoxification mechanism, which occurs via regulation of Na^+-K^+-ATPase in tilapia larva. A previous paper reported that a significant effect of cortisol on whole-body Na^+ content of larvae occurred as early as 4~8 h after transfer to seawater, while no significant difference was found in the ouabain binding of yolk-sac epithelia between control and cortisol-treated larvae even 12 h after transfer (Lin et al. 1999). In addition a previous study reported that the Na^+ content dramatically changed upon 100 μg/L Cu^{2+} exposure for 1~7 h, with a significant increase at 3 h, and a quick recovery to control levels, then a significant decrease up to 12 h. Cortisol levels in tilapia larvae were also significantly increased at 3 h with 100 μg/L Cu^{2+} treatment (unpublished data). Therefore, we suggest that cortisol might also be involved in the early phase of Na^+ homeostasis after Cu^{2+} challenge, in accordance with cortisol activity during seawater adaptation. Generally, Na^+-K^+-ATPase is primarily located in the gills, brain, kidneys, and intestines of teleosts. This enzyme is involved in ion- and osmoregulation mechanisms in tilapia, and shows tissue specificity and developmental expression (Lee et al. 1998). This enzyme regulates active ion transport on its target tissue. Results of sodium pump activity observed from the whole-body analysis showed that both cortisol and progesterone were found to have significantly increased Na^+-K^+-ATPase activity, although only progesterone was effective at enhancing Cu^{2+} tolerance. Furthermore, there was greater evidence of cation retention with progesterone than with cortisol after oral steroids had been administered and larvae were exposed to Cu^{2+}. Three possible reasons were considered: (1) cortisol may be involved in ionic regulation during the early phase of Cu^{2+} exposure, (2) active transport is not necessary for ionic balance during Cu^{2+} exposure, and (3) some other end product of progesterone is more effective than cortisol at ionic regulation upon Cu^{2+} exposure in fish larvae.

The effect of progesterone on fish mortality upon Cu^{2+} challenge was found to be positive but dose independent (Fig. 2). On the other hand, despite there also being a 20%~40% variation for every test replication, progesterone still had a significant effect on mortality, and the tendencies of mortality appeared similar between treatments. These circumstances were examined in our previous study (Wu et al. 2002). Some possible reasons were considered: the sensitivity to Cu^{2+} might not have been uniform throughout due to the larval origin, which might have resulted from a maternal effect (Lin et al. 2000), or there may have been differences in the level of health of the larvae.

Stress response systems in fishes are mediated by the hypothalamo-pituitary-adrenocortical (HPA) axis, which is homologous to the HPA axis of mammals (Sumpter 1987). Circulating cortisol levels in fish rapidly rise in response to several environmental stresses including salinity challenge, anesthesia, handling stress, and high living density (Belloso et al. 1996, Tort et al. 1996). Furthermore, plasma cortisol levels have been found to significantly increase after both cadmium and copper exposure in rainbow trout (Gill et al. 1993, Tort et al. 1996, Dethloff et al. 1999). Pelgrom et al. (1995) also reported that exposure of tilapia (*O. mossambicus*) to Cd^{2+} or Cu^{2+} resulted in increased plasma cortisol levels. Therefore, the level of cortisol is considered to be a primary stress response, with its physiological functions during stress involving increased glucose, induced immunosuppression, increased Na^+-K^+-ATPase activity of the gills, and counteraction of the metal-induced disturbance of ion homeostasis. However, in this study, it was shown that exogenous cortisol administration to tilapia larvae was less helpful than progesterone for overcoming a Cu^{2+} environmental challenge in FW (Fig. 2). Although cortisol was shown to induce the sodium pump and enhance Na^+ retention in larvae upon administration of low oral doses of cortisol and Cu^{2+} exposure, respectively (Figs. 1, 5), endogenous cortisol significantly increased in larvae after cortisol administration, and this increase appeared to be dose dependent (Fig. 3). Therefore, excessive amounts of cortisol may cause a decrease in the ionic regulation function. A review of our earlier study indicates that Na^+, K^+, and Ca^{2+} significantly decreased after 96 h of exposure to 100 μg/L Cu^{2+} (Wu 2003). Obviously, survival tactics under the impact of heavy metal exposure in aquatic animals are complex and multidirectional. Perturbations in larvae were not limited to Na^+ retention or rises in

Na^+-K^+-ATPase activity after exogenous cortisol administration under ambient Cu^{2+} conditions. There appeared to be K^+ and Ca^{2+} imbalances as well as other factors, such as a timing effect as described above (Lin et al. 1999).

Progesterone is the precursor of steroid synthesis, and end products include cortisol, aldosterone, and estradiol. Generally, estradiol is considered important for use as a hormone in reproduction, but cortisol and aldosterone are related to ionic regulation. Therefore, they were measured in the present study. Cortisol is physiologically active in several classes of fishes; it appears to be the major corticosteroid in bony fishes; and it appears to regulate Na^+ fluxes (Perry and Laurent 1989). Cortisol treatment increases the activity of Na^+-K^+-ATPase in gills, gut epithelial cells, and the kidneys (Miguel et al. 2002). Aldosterone treatment can reduce renal and extrarenal Na^+ losses from lampreys (*Lampetra fluviatilis*) held in fresh water. It was also found that injection of aldosterone altered the electrolyte composition of body fluids with respect to Na^+ (Takei 1993). In mammals, aldosterone secretion is generally regulated by the levels of pituitary ACTH, kidney angiotensin II, and plasma ions (Agarwal and Mirshahi 1999). Decreasing the plasma Na^+ concentration may be a stimulus for further aldosterone secretion (Hadley 1996). These physiological functions remain unclear in fish. However, in this study, oral progesterone only increased Na^+ retention, but was found not to be very effective at enhancing K^+ and Ca^{2+} levels in the whole body after larvae were exposed to waterborne Cu^{2+} (Fig. 5). Furthermore, aldosterone levels increased after progesterone treatment (Fig. 4). We suggest that the effect of aldosterone on the homeostatic settings of ions in fish is similar to that in mammalian systems, particularly for Na^+, since Na^+ is the major body fluid electrolyte, and the ranges of enhancement between Na^+ and K^+ or Ca^{2+} can be compared. Rather than regulating the ion balance, aldosterone causes retention of Ca^{2+} and K^+ ions. Therefore, cortisol and aldosterone are related to the regulation of ions; yet when the effective dose of each is considered, aldosterone was found to be more effective in maintaining an ionic balance, while proving to be more effective in Cu^{2+} resistance.

Steroid biosynthesis is clear in neither fish nor mammals; on the other hand, adrenocortical cells of fish differ most from the general mammalian pattern of corticosteroid genesis with respect to some of the hormones produced. However, the general sequences for corticosteroid genesis are similar in all vertebrates with respect to the precursor-product relationships, with many of the same enzymes being involved (Norris 1997). Aldosterone, along with corticosterone, was first found in lungfish and amphibians, while cortisol is the major steroid secreted by teleost interregnal cells, but little convincing evidence exists for aldosterone production (Sangalang and Uthe 1994, cited in Jiang et al. 1998). However, aldosterone produced in mammals is stimulated by the rennin-angiotensin system, which is present in all of the spiny-rayed fish groups, and juxtaglomerular-like cells have been identified in several teleosts (Norris 1997). The fish-type ANG II shows significantly elevated plasma aldosterone levels without affecting corticosterone or cortisol levels (Joss et al. 1999). This may demonstrate that aldosterone has evolved in fishes. Cortisol levels in plasma are about 400-fold higher than those of aldosterone in goldfish (*Carassius auratus*) (Holmes and Phillips 1976, cited in Norris 1997), and the ratio of cortisol and aldosterone contents in tilapia larvae is also about 350-fold (compare with Figs. 3 and 4). Since aldosterone in animals has a half-life of only 20~30 min, the presence of aldosterone in teleost fish still remains doubtful. A more-sensitive method of quantifying aldosterone is needed to screen this hormone in most fishes. In this study, it appeared that cortisol significantly increased after exogenous cortisol treatment, and aldosterone levels significantly increased after progesterone treatment. A previous study showed that exogenous 17α-hydroxyprogesterone had no effect on SW adaptation. Survival rates were lower after SW challenge in tilapia larvae orally administered 11α-deoxycortisol than cortisol (Hwang and Wu 1993). We suggest that cortisol has not yet been induced by other exogenous steroids in tilapia larvae. This is the first study to measure aldosterone stores in tilapia using a commercial aldosterone ELISA kit. Further research is necessary, however, to confirm the mechanism of this system.

Summing up these results, we suggest that in addition to progesterone's involvement in copper resistance mechanisms in tilapia larvae by ion retention, aldosterone also effectively enhances this mechanism. Exogenous steroids enhance activation of the sodium pump, but this might have no effect on increasing the resistance of tilapia larvae to Cu^{2+}.

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