Effects of Cortisol and Salinity Challenge on Water Balance in Developing Larvae of Tilapia (*Oreochromis mossambicus*)

Li-Yih Lin[1]
Ching-Feng Weng[2]
Pung-Pung Hwang[1,2,*]
[1]Institute of Fisheries Science, National Taiwan University, Taipei, Taiwan, Republic of China; [2]Institute of Zoology, Academia Sinica, Nankang, Taipei, Taiwan, Republic of China

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ABSTRACT

Effects of exogenous cortisol on drinking rate and water content in developing larvae of tilapia (*Oreochromis mossambicus*) were examined. Both freshwater- and seawater-adapted larvae showed increases in drinking rates with development. Drinking rates of seawater-adapted larvae were about four- to ninefold higher than those of freshwater-adapted larvae from day 2 to day 5 after hatching. Seawater-adapted larvae showed declines in drinking rate and water content at 4 and 14 h, respectively, after immersion in 10 mg L^{-1} cortisol. In the case of freshwater-adapted larvae, the drinking rate decreased after 8 h of cortisol immersion, while the water content did not show a significant change even after 32 h of cortisol immersion. In a subsequent experiment of transfer from freshwater to 20 ppt (parts per thousand, salinity) seawater, immersion in 10 mg L^{-1} cortisol for 8–24 h enhanced the drinking rate in larvae at 4 h after transfer, but no significant difference was found in water contents between cortisol-treated and control groups following transfer. These results suggest that cortisol is involved in the regulation of drinking activity in developing tilapia larvae.

Introduction

Marine fish living in hypertonic environments have the ability to balance osmotic water loss by drinking seawater and to excrete excess ions by means of mitochondria-rich (MR) cells on the gill filaments (Eddy 1982). Several hormones have been suggested to control the regulating mechanisms for water balance (Hazon and Balment 1998). In teleosts, cortisol is one of the major corticosteroids and has both glucocorticoid and mineralocorticoid functions. Cortisol is thought to be a seawater-adapting hormone because it promotes salt excretion in hypoosmoregulating fish (Pickford et al. 1970; Richman et al. 1987; Madsen 1990). Previous studies indicated that this function might be achieved through stimulation of gill Na^+-K^+ ATPase activity and development of branchial MR cells (Madsen 1990; McCormick 1990; Ayson et al. 1995). Recently, exogenous cortisol was shown to stimulate drinking in salmon presmolt and rainbow trout juveniles, suggesting that cortisol is also involved in the regulation of another hypoosmoregulatory mechanism, water drinking (Fuentes et al. 1996).

Embryos and larvae of several teleosts whose gills or kidneys are poorly or underdeveloped have been found to be capable of maintaining constancy in ion concentrations and osmolality of their body fluids as well as adults do (Alderdics 1988). Recent studies have demonstrated the ion-regulation abilities of embryos and larvae, i.e., the active secretion of NaCl in seawater (SW) and absorption of Ca^{2+} in freshwater (FW) via skin MR cells (Guggino 1980*a*; Hwang and Hirano 1985; Hwang 1990; Hwang et al. 1994, 1999). Moreover, mechanisms of water balance, which are conducted through drinking activities, were also found to be developed in early developmental stages of teleosts, including embryos of killifish (Guggino 1980*b*), larvae of cod (Mangor-Jensen and Adoff 1987), halibut (Tytler and Blaxter 1988), rainbow trout (Tytler et al. 1990), and tilapia (Miyazaki et al. 1998). However, little is known about the hormonal control for these mechanisms of ion and water balance in early developmental stages of fish.

Previous studies have demonstrated the presence of cortisol in embryos and larvae whose internal glands have not yet developed (De Jesus et al. 1991; Hwang et al. 1992). Moreover, cortisol was suggested to play a positive role in hypoosmoregulation of larvae by enhancing the regulation of body osmolality and Na^+ content upon SW challenges (Hwang and Wu 1993; Lin et al. 1999) and to stimulate the development of yolk-sac MR cells (Ayson et al. 1995). However, the acting mechanism of cortisol in larval hypoosmoregulation is unclear. Since activation of water uptake is also critical for larval hypoosmoregulation, we hypothesize that cortisol is also involved in the regulation of water balance in developing larvae. In the developing larvae of some euryhaline teleosts, hypoosmoregulation mechanisms were stimulated within several hours after transfer from FW to SW (Hwang and Hirano 1985; Hwang

*To whom correspondence should be addressed; e-mail: zophwang@ccvax.sinica.edu.tw.

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1990; Lin et al. 1999). Moreover, some studies suggested short-term (several hours to 1 or 2 d) actions of cortisol on hypo-osmoregulation in teleosts upon SW challenges (Forrest et al. 1973; McCormick 1995; Lin et al. 1999; Marshall et al. 1999). Therefore, in this study, short-term effects of exogenous cortisol on water content and drinking rate in tilapia larvae adapted to FW or SW, or transferred directly from FW to SW, were examined.

Material and Methods

Fish

Mature tilapia (*Oreochromis mossambicus*) from the Tainan branch of the Fisheries Research Institute were reared in circulating FW at 26°–28°C under a photoperiod of 12L : 12D.

Fertilized eggs were retrieved from the mouth of females about 24 h before hatching. Embryos were incubated in aerated FW at the same conditions as adults. Part of the brood was adapted to 20 ppt (parts per thousand, salinity) SW for 1 d and then transferred to 32 ppt SW as SW larvae. During this transfer process, the embryos adapted well to 32 ppt SW with a mortality of <5%. The incubation water was changed daily to control water quality. All experiments were conducted on yolk-sac larvae, and no feeding occurred.

Measurement of Drinking Rate

Drinking rate of larvae was measured by using isotope-labeled dextran as a tracer following previous studies (Guggino 1980*b*; Mangor-Jensen and Adoff 1987; Tytler and Blaxter 1988).

The initial stock solutions of ^{14}C-dextran (SA 0.75 mCi mM^{-1}, Du Pont) were made by dissolving 250 μCi tracer in 4 mL double-deionized water, and then 1 mL of stock solution was added into 25 mL of aquarium water to give an exposure medium with an approximate activity of 2.5 μCi mL^{-1}. The larvae were incubated in the exposure medium at 27°C for 1 h. After exposure, the larvae were transferred with a wide-mouthed dropper to an isotope-free water bath for 3 min to remove most adhering tracer on the integument. Preliminary experiments, in which gut and the rest of the larval body were separated for radioactivity measurement, confirmed that the radioactivity of adhering tracer on the integument was only about 1.5- to twofold that of the background and could be neglected. Therefore, whole larva was rinsed, anesthetized on ice, and digested with tissue solubilizer (Soluene-350, Packard, Meriden, Conn.) in a counting vial at 50°C for 4 h. After solubilization, counting solution (Hionic-Fluor, Packard, Meriden, Conn.) was added to each vial. The radioactivities of both the incubation medium and the solubilized larvae were measured by liquid scintillation counter (LS 6500, Beckman, Fullerton, Calif.). The plot of radioactivity against time was linear within the first hour in both FW and SW larvae; therefore, 1 h of exposure time was chosen to determine the drinking rate in the following experiments. Drinking rate was calculated by the equation:

$$\text{Drinking rate} = (R - C)/(M \times T),$$

where R is the total radioactivity of the sample after accumulation of tracer for 1 h (dpm $larva^{-1}$), C is the background radioactivity obtained from the larva without exposure to tracer, M is the specific radioactivity of the exposure medium (dpm nL^{-1}), and T is the time of exposure (h). Drinking rate was expressed as nL $larva^{-1}$ h^{-1} in order to compare with the data of previous studies.

Measurement of Water Content and Body Size

Sampled larvae were rinsed with double-deionized water and then wiped off with filter paper. The dry weight of each larva was measured after drying in a 60°C oven until the weight was constant. Water content was calculated as wet weight minus dry weight. The total length and yolk-sac diameter were measured under a dissecting microscope equipped with a micrometer on the eyepiece.

Experiment 1: Effects of Various Doses of Cortisol on Water Balance in SW Larvae

Cortisol (hydrocortisone, 21-hemisuccinate, Sigma, St. Louis, Mo.) was dissolved in double-deionized water and then diluted with SW or FW to give different concentrations (0.1, 1, 5, and 10 mg L^{-1}) of immersing solutions. Preliminary experiments confirmed that immersion of 10 mg L^{-1} cortisol for 4–8 h resulted in about 10-fold increases in whole-body cortisol content of larvae (data not shown), similar to our previous study (Lin et al. 1999).

The experiment was designed to determine whether the effects of cortisol on larval water balance are dose related. Four-day-old SW larvae were immersed in cortisol solution for 24 h and then were sampled for measurements of body size, water content, and drinking rate.

Experiment 2: Effects of Various Times of Cortisol Immersion on Water Balance in SW and FW Larvae

This experiment was aimed to examine the time-course changes in various parameters of water balance in larvae treated with cortisol. SW and FW larvae were immersed in 10 mg L^{-1} cortisol solutions for various times: 2, 4, 8, 14, or 24 h for SW larvae and 8, 16, or 32 h for FW larvae, respectively. Then the larvae were sampled for measurements similar to those in experiment 1.

Table 1: Changes in drinking rate of freshwater and seawater larvae with development

Days after Hatching	FW Larvae		SW Larvae	
	nL h^{-1} mg^{-1}	nL h^{-1} $larva^{-1}$	nL h^{-1} mg^{-1}	nL h^{-1} $larva^{-1}$
2	1.7 ± .3	9.1 ± 1.7	7.4 ± .6	41.4 ± 3.4
5	7.1 ± 1.6	52.5 ± 11.8	60.8 ± 15.2	468.2 ± 117.0

Note. Values represent $\overline{X}$ ± SD ($n = 10$).

Experiment 3: Effects of Cortisol Immersion on Water Balance in Larvae Transferred from FW to SW

Based on the results of experiments 1 and 2, we hypothesized that cortisol alone might not be able to stimulate larval water drinking and considered that SW might be one of the necessary factors. FW larvae were immersed in 10 mg L^{-1} cortisol solution (in FW) for 8 h and then were directly transferred to 20 ppt SW (also containing 10 mg L^{-1} cortisol). The larvae were sampled for measurements similar to those in experiment 1 at 2, 4, 8, 16, or 24 h after transfer. Ten larvae were sampled for each test condition and for each sampling time in all the experiments.

Results

Drinking Rates of FW and SW Larvae

Drinking rates of FW and SW tilapia (*Oreochromis mossambicus*) larvae were measured on days 2 and 5 after hatching. Table 1 reveals dramatic differences in drinking rates between SW and FW larvae. Both FW and SW larvae showed increases in drinking rates with development. Drinking rates of SW larvae were about fourfold higher than those of FW larvae on day 2 after hatching, and this difference reached nearly ninefold on day 5 after hatching.

Water Balance in SW Larvae after 24 h of Cortisol Immersion

SW larvae treated with cortisol showed significant decreases in drinking rates at concentrations of 5 and 10 mg L^{-1} (Fig. 1). At concentrations of 5 and 10 mg L^{-1}, larvae also showed significant decreases in wet weight and water content (Table 2). The wet weight and water content of 10 mg L^{-1} cortisol-immersed larvae were about 5.8% and 9.2%, respectively, lower than those of control larvae (Table 2). The scale of the decrease in wet weight was equal to that of water content, indicating that the decreased proportion of wet weight was due to water loss. However, cortisol did not affect the other parameters, including dry weight, total length, and yolk-sac diameter of larvae.

Water Balance in SW and FW Larvae Treated with Cortisol for Various Times

Table 3 shows the effects of 10 mg L^{-1} cortisol on wet weight, water content, dry weight, and drinking rate of 4-d-old SW larvae after various times of cortisol immersion. The drinking rate of larvae decreased significantly after 4 h of cortisol immersion; however, significant decreases in water content and wet weight did not occur until 14 h of cortisol immersion (Table 3).

Significant decreases in drinking rates were also found in 4-d-old FW larvae after 8 h of cortisol immersion (Table 4). However, there were still no significant differences between control and cortisol-immersed groups in wet weight and water content even after 32 h of cortisol immersion (Table 4).

Effects of Cortisol Immersion on Water Balance in Larvae Transferred from FW to SW

Exogenous cortisol enhanced the drinking rate in larvae upon their transfer from FW to 20 ppt SW. Before transfer to SW

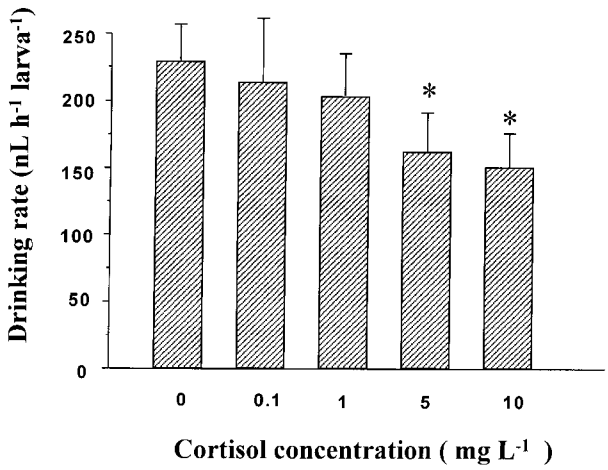

Figure 1. Changes in drinking rate of 4-d-old seawater larvae immersed in different concentrations of cortisol for 24 h. Student's *t*-test analysis was used to assess differences between control and cortisol-immersed groups for each parameter. Values represent $\overline{X}$ ± SD ($n = 10$). An asterisk indicates a significant difference from the control value at $P < 0.05$.

Table 2: Changes in body size and water content of 4-d-old seawater larvae immersed in 5 or 10 mg L^{-1} cortisol for 24 h

Cortisol Concentration	Wet Weight (mg)	Water Content (mg)	Dry Weight (mg)	Total Length (mm)	Yolk-Sac Diameter (mm)
0	9.01 ± .18	6.50 ± .34	2.51 ± .21	9.12 ± .41	1.72 ± .17
5	8.43 ± .22*	6.02 ± .18*	2.41 ± .24	ND	ND
10	8.49 ± .38*	5.90 ± .21*	2.59 ± .26	9.07 ± .21	1.73 ± .21

Note. All data were obtained from one brood of tilapia larvae. $\bar{X}$ ± SD ($n = 10$) are indicated. Student's *t*-test analysis was used to assess differences between control and cortisol-immersed groups for each parameter. ND = data not determined. An asterisk indicates that means are significantly different from the control ($P < 0.05$).

(at 0 h), the cortisol-treated group showed a lower drinking rate than the control. Both control and cortisol-treated groups increased their drinking rates right after transfer, but the rate of the cortisol-treated group turned out to be significantly higher than that of the control at 4 h after transfer to SW (Fig. 2). Drinking rates of both control and cortisol-treated groups reached the level of SW-adapted group 8 h after transfer (Fig. 2). However, water contents of transferred larvae of both control and cortisol-treated groups were lower than those of SW- and FW-adapted groups at 4 and 8 h after transfer and returned to levels of SW- and FW-adapted groups after 16 h (Fig. 3). Moreover, no significant difference was found in water contents between the control and cortisol-treated groups of transferred larvae during transfer (Fig. 3).

Discussion

This study found that exogenous cortisol affects water balance in tilapia (*Oreochromis mossambicus*) larvae adapted to either SW or FW and promotes larval water drinking upon transfer from FW to SW. These results suggest that cortisol may be involved in the mechanisms of water balance in the early developmental stages of teleosts.

Drinking activity is well developed in early stages of teleosts

Table 3: Changes in body size, water content, and drinking rate of 4-d-old seawater larvae immersed in 10 mg L^{-1} cortisol for various times

Immersion Time (h)	Wet Weight (mg)	Water Content (mg)	Dry Weight (mg)	Drinking Rate (nL h^{-1} $larva^{-1}$)
2:				
Control	7.58 ± .23	4.99 ± .14	2.59 ± .11	215.3 ± 31.5
Cortisol	7.67 ± .22	5.05 ± .14	2.62 ± .09	189.1 ± 37.3
4:				
Control	7.61 ± .28	5.21 ± .18	2.40 ± .18	227.3 ± 28.6
Cortisol	7.75 ± .21	5.19 ± .19	2.56 ± .10	194.5 ± 24.2*
8:				
Control	7.91 ± .21	5.30 ± .13	2.61 ± .13	198.1 ± 28.6
Cortisol	7.91 ± .23	5.29 ± .12	2.62 ± .13	145.7 ± 22.3*
14:				
Control	8.25 ± .34	5.62 ± .22	2.63 ± .16	218.3 ± 26.4
Cortisol	7.88 ± .39*	5.33 ± .27*	2.55 ± .16	188.1 ± 20.2*
24:				
Control	8.32 ± .26	5.88 ± .28	2.44 ± .16	274.2 ± 16.2
Cortisol	8.05 ± .21*	5.62 ± .19*	2.43 ± .08	214.5 ± 43.1*

Note. All data were obtained from one brood of tilapia larvae. $\bar{X}$ ± SD ($n = 10$) are indicated. Student's *t*-test analysis was used to assess differences between control and cortisol-immersed groups for each parameter. An asterisk indicates that means are significantly different from the control ($P < 0.05$).

Table 4: Changes in body size, water content, and drinking rate of 4-d-old freshwater larvae immersed in 10 mg L^{-1} cortisol for various times

Immersion Time (h)	Wet Weight (mg)	Water Content (mg)	Dry Weight (mg)	Drinking Rate (nL h^{-1} $larva^{-1}$)
8:				
Control	6.96 ± .42	4.81 ± .30	2.15 ± .17	86.4 ± 12.5
Cortisol	7.21 ± .26	5.04 ± .16	2.17 ± .13	43.6 ± 12.1*
16:				
Control	7.16 ± .37	5.06 ± .28	2.10 ± .14	109.3 ± 20.3
Cortisol	7.27 ± .27	5.13 ± .19	2.14 ± .12	45.7 ± 10.5*
32:				
Control	7.66 ± .41	5.63 ± .35	2.03 ± .21	156.3 ± 33.1
Cortisol	7.69 ± .18	5.65 ± .21	2.04 ± .13	128.4 ± 25.6*

Note. All data were obtained from one brood of tilapia larvae. $\overline{X} \pm$ SD ($n = 10$) are indicated. Student's t-test analysis was used to assess differences between control and cortisol-immersed groups for each parameter. An asterisk indicates that means are significantly different from the control ($P < 0.05$).

as described above (Guggino 1980*b*; Mangor-Jensen and Adoff 1987; Tytler and Blaxter 1988; Tytler et al. 1990; Miyazaki et al. 1998). During acute salinity challenges, tilapia larvae show profound changes in drinking rate, i.e., increased or decreased drinking rate within several hours while facing hypertonic or hypotonic challenges, to maintain constancy of their body fluids for normal development and survival (L.-Y. Lin, C.-F. Weng, and P.-P. Hwang, unpublished data). It is interesting and important to determine whether hormones regulate this rapid regulation in water balance upon salinity challenges. In this study, exogenous cortisol promotes drinking activity in larvae transferred from FW to SW. This finding supports our hypothesis that cortisol can promote hypoosmoregulation of larvae by upregulating drinking. FW-adapted salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) juveniles implanted with 80 mg kg^{-1} cortisol for 3 wk showed no significant changes in drinking but developed a greater drinking rate than did sham fish following transfer to SW (Fuentes et al. 1996). Administration of cortisol at 150 mg kg^{-1} in the diet for 8–12 d or immersion of cortisol at 10 mg L^{-1} for 1–5 d could increase the survival of tilapia larvae after transfer from FW to SW by diminishing the degree of increase in larval body osmolality or Na^+ content (Hwang and Wu 1993; Lin et al. 1999). In the paper by Ayson et al. (1995), immersion in 0.1–100 mg L^{-1} cortisol for 3–9 d stimulated the size and number of yolk-sac MR cells in FW tilapia larvae. Taken together, involvement of cortisol in the hypoosmoregulation of larvae acclimating to SW may include enhancement of water drinking and stimulation of ion regulation and development of MR cells.

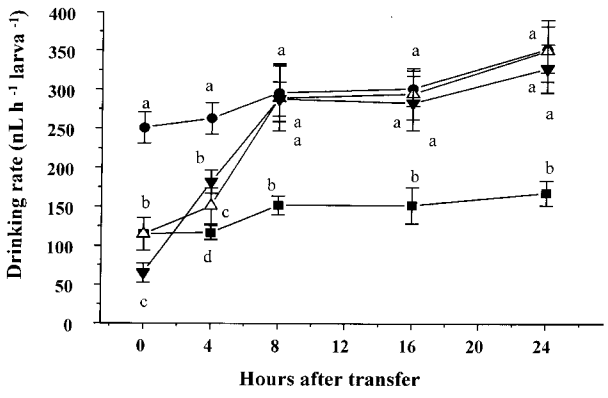

Figure 2. Changes in drinking rate of cortisol-treated freshwater (FW) larvae (*solid triangles*) and sham FW larvae (*open triangles*) after transfer to 20 ppt seawater (SW). Drinking rates of FW larvae (*squares*) and SW larvae (*circles*), which were kept in FW and SW, respectively, are also shown. Treatment of cortisol refers to experiment 3 of "Material and Methods." One-way ANOVA (Tukey's pairwise comparisons) analysis was used to assess differences among different groups at the same time. Values represent $\overline{X} \pm$ SD ($n = 10$). Different letters indicate a significant difference ($P < 0.05$).

For most previous studies, it generally took several days to weeks for the treatments of exogenous cortisol to induce stimulation of hypoosmoregulation (see review by McCormick 1995). Only a very few studies have so far discussed short-term (several hours to 1 or 2 d) actions of cortisol in teleosts (Forrest et al. 1973; Lin et al. 1999; this study). In a recent study on killifish (*Fundulus heteroclitus*), Marshall et al. (1999) argued that the dramatic change in cortisol level within 1–8 h after transfer from FW to SW is a major cue for target tissues to switch the expression of seawater-adaptive proteins, based on the time-course changes in blood cortisol and Na^+ (Marshall et al. 1999), as well as the expression of gill kfCFTR (a homologue of cystic fibrosis transmembrane conductance regulator) that is associated with the Cl^- secretion in gills (Singer

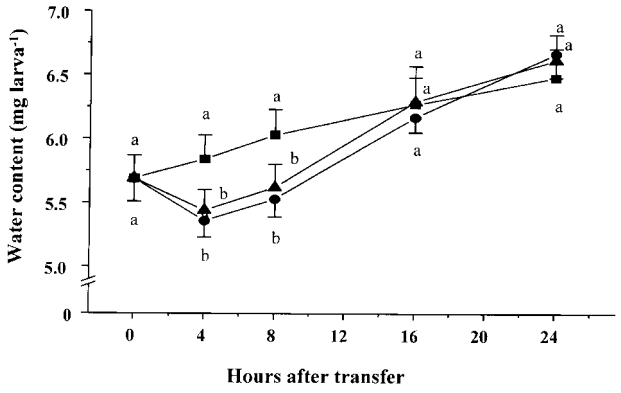

Figure 3. Changes in water content of cortisol-treated freshwater (FW) larvae (*triangles*) and sham FW larvae (*circles*) after transfer to 20 ppt seawater (SW). Water contents of FW-adapted larvae (*squares*), which were kept in FW, are also shown. Treatment of cortisol refers to experiment 3 of "Material and Methods." One-way ANOVA (Tukey's pairwise comparisons) analysis was used to assess differences among different groups at the same time. Values represent $\overline{X} \pm$ SD ($n = 10$). Different letters indicate a significant difference ($P < 0.05$).

et al. 1998). These rapid actions of cortisol on hypoosmoregulation mechanisms in teleosts are of physiological significance and should not be discounted, as McCormick (1995) indicated.

One more notable finding from this study is that the effect of cortisol was inhibitory on drinking in tilapia adapted well to either SW or FW. One might argue that the decrease in the drinking rate could be due to a developmental delay and not really an inhibitory effect of cortisol since cortisol was demonstrated to retard development and growth in tilapia larvae (Mathiyalagan et al. 1996). It seems not to be the case because dry weight, body length, and yolk-sac diameter, which, reflecting some degree of larval development, showed no significant changes in both SW- and FW-adapted larvae after 10 mg L^{-1} cortisol immersion for 24 h (this study; Lin et al. 1999). Interestingly, exogenous cortisol inhibited water drinking in both SW and FW tilapia larvae, and this resulted in a decrease in the water content (and thus, the wet weight) of SW larvae but did not affect that in FW ones. This suggests that, in contrast to SW larvae, drinking is not a crucial mechanism of water balance for FW larvae.

The inhibitory effect of cortisol on drinking is interesting and a noteworthy phenomenon, although its acting mechanism and physiological significance is unclear. The renin-angiotensin system has been well documented to play a major role in control of drinking in fishes, and angiotensin II is the main biologically active component of the renin-angiotensin system (Olson 1992; Perrot et al. 1992; Tierney et al. 1995). In mammalian systems, cortisol is able to inhibit the secretion of arginine vasopressin by a feedback mechanism (Liu et al. 1995) and to regulate the expression of central angiotensin II (Ryan et al. 1997), implying that an interaction between cortisol and drinking activity–linked hormones is possible. In this finding, additional exogenous cortisol appeared to be redundant for the tilapia larvae that were well adapted to FW or SW and even inhibited normal drinking activity probably through a putative feedback pathway. Our other data ("Experiment 3") and those of Fuentes et al. (1996), i.e., cortisol promoting water drinking upon transfer from FW to SW, also support this possible interaction between cortisol and drinking activity–linked hormones. This interaction should be a subject for future studies.

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