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Influence of cortisol, growth hormone, insulin-like growth factor I and 3,3′,5-triiodo-L-thyronine on hypoosmoregulatory ability in the euryhaline teleost *Fundulus heteroclitus*

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Abstract

The capacity of cortisol, ovine growth hormone (oGH), recombinant bovine insulin-like growth factor I (rbIGF-I) and 3,3′,5-triiodo-L-thyronine (T_3) to increase hypoosmoregulatory capacity in the euryhaline teleost *Fundulus heteroclitus* was examined. Fish acclimated to brackish water (BW, 10 ppt salinity) were injected with a single dose of hormone suspended in oil and transferred to seawater (SW, 35 ppt salinity) 10 days post-injection. Fish were sampled 24 h after transfer and plasma osmolality and gill Na^+, K^+-ATPase activity were examined. Transfer from BW to SW induced significantly increased plasma osmolality but not gill Na^+, K^+-ATPase activity. Cortisol (50 μg g^{-1} body weight) improved the ability to maintain plasma osmolality and to increase gill Na^+, K^+-ATPase activity. oGH (5 μg g^{-1} body weight) also increased hypoosmoregulatory ability and gill Na^+, K^+-ATPase activity. A cooperation between oGH and cortisol was observed in increasing hypoosmoregulatory ability but not in increasing gill Na^+, K^+-ATPase activity. rbIGF-I (0.5 μg g^{-1} body weight) alone was without effect in increasing salinity tolerance or gill Na^+, K^+-ATPase activity. rbIGF-I and oGH showed a positive interaction in increasing salinity tolerance, but not gill Na^+, K^+-ATPase activity. Treatment with T_3 (5 μg g^{-1} body weight) alone did not increase salinity tolerance or gill Na^+, K^+-ATPase activity, and there was no consistent significant interaction between cortisol and T_3 or between GH and T_3. The results confirm the classical role of cortisol as a seawater-adapting hormone and indicate an interaction between cortisol and the GH/IGF-I axis during seawater acclimation of *Fundulus heteroclitus*.

Introduction

The control of gill Na^+, K^+-ATPase and chloride cell function in teleost fishes involves a number of hypophysial and extrahypophysial hormones (see McCormick 1995). The role of cortisol in promoting seawater acclimation is clear. This hormone stimulates the development of gill chloride cells, gill Na^+, K^+-ATPase activity, expression of Na^+, K^+-ATPase α-subunit and increases salinity tolerance in numerous teleost species (Pickford et al. 1970; Foskett et al. 1981; Dange 1986; Madsen 1990a,b; Madsen et al. 1995; McCormick 1995, 1996). Stimulatory *in vitro* effects of cortisol on gill and opercular membrane Na^+, K^+-ATPase have been demonstrated in coho salmon (*Oncorhynchus kisutch*) and tilapia (*Oreochromis mossambicus*) (McCormick and Bern 1989; McCormick 1990).

In salmonid fishes, both growth hormone (GH) and insulin-like growth factor I (IGF-I) improve hypoosmoregulatory capacity. GH increases salinity tolerance through its stimulation of chloride cell number, gill Na^+, K^+-ATPase activity and expression of Na^+, K^+-ATPase α-subunit (Bolton et al. 1987; Sakamoto et al. 1993; Madsen et al. 1995; McCormick 1996). In

tilapia, treatment with GH increases opercular chloride cell density, gill Na^+, K^+-ATPase activity and salinity tolerance (Flik et al. 1993; Borski et al. 1994; Sakamoto et al. 1997). In the Nile tilapia, *Oreochromis niloticus*, recombinant tilapia GH did not affect the ability of fish in fresh water to acclimate to brackish water (Auperin et al. 1995). However, Xu et al. (1998) reported that treatment with recombinant eel GH enhanced seawater adaptation and stimulated the differentiation of chloride cells in Nile tilapia. In salmonid fishes, IGF-I has short-term effects on salinity tolerance and is a potential mediator of the long-term actions of GH in seawater acclimation (McCormick et al. 1991; Madsen et al. 1995; McCormick 1996).

The osmoregulatory role of thyroid hormones in teleosts is uncertain (Grau, 1987; McCormick 1995). Thyroxine (T_4) alone failed to increase gill Na^+, K^+-ATPase activity in some salmonid and non-salmonid species (Miwa and Inui 1985; Saunders et al. 1985; Dange 1986). However, in other experiments repetitive T_4 injections increase gill Na^+, K^+-ATPase activity and chloride cell number in Atlantic salmon, *Salmo salar* (Madsen and Korsgaard 1989) and rainbow trout, *Oncorhynchus mykiss* (Madsen 1990b). In addition, an interaction between T_3 and GH has been reported in seawater acclimation of brown trout, *Salmo trutta* and rainbow trout (Leloup and Lebel 1993). In tilapia, T_4 and cortisol act in synergy to increase gill Na^+, K^+-ATPase activity (Dange 1986).

The mummichog, *Fundulus heteroclitus*, is a euryhaline teleost that under natural conditions lives in an environment of varying salinity. This species has been used as a model to study mechanisms of osmoregulation in euryhaline fish (Wood and Marshall 1994). In a previous study, we demonstrated that short-term (48 h) treatment with GH and IGF-I increased hypoosmoregulatory capacity of mummichog (Mancera and McCormick 1998). Treatment with IGF-II and insulin, however, did not improve this capacity. In this study we examine the capacity of treatment with cortisol, GH, IGF-I and T_3 to improve salinity tolerance and stimulate gill Na^+, K^+-ATPase activity in mummichog. We have also examined the interaction of the GH/IGF-I axis with cortisol and T_3. The results are discussed in relation to the osmoregulatory functions of these hormones in teleost fishes.

Material and methods

Fish

Mummichog, *Fundulus heteroclitus*, (4–8 g body weight) were collected in the Connecticut river estuary and transferred to the S.O. Conte Anadromous Fish Research Center, Turners Falls, Massachusetts. They were acclimated for at least 2 weeks to BW (Instant Ocean, 10 ppt salinity) under simulated natural photoperiod and constant temperature (15 °C). They were maintained in a 60 l aquarium and 50% of the water was changed every 3 days. Fish were fed daily with commercial fish food (Tetra Standard Mix, Tetrawerke, Germany). They were fasted for 24 h before hormone injection and throughout the experiment. Experiments were conducted between May–June (Experiments 1, 2 and 3) and September–October (Experiments 5 and 6) of 1995 and May–June of 1996 (Experiment 4).

Experimental protocol

Fish were anaesthetized (100 mg l^{-1} MS-222, pH=7.0), weighed, injected intraperitoneally with vehicle or vehicle plus hormone and returned to BW. Hormones were suspended in vegetable oil as outlined in McCormick (1996) and injected intraperitoneally (10 μl g^{-1} body weight). After 10 days the fish were transferred to SW (35 ppt). Twenty four hour after transfer the fish were anaesthetized, weighed and sampled. Gill tissue was placed in 100 μl of ice-cold SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) and frozen at -80 °C. Blood was obtained by severing the tail and collecting the blood in ammonia heparinized microcapillary tubes. Capillary tubes were centrifuged at 3000 $\times$ g for 5 min and plasma stored at -80 °C.

Experiment 1. Treatment with: (a) cortisol (Sigma H-2882, hydrocortisone hemisuccinate) (50 μg g^{-1} body weight), (b) ovine GH (oGH, NIADDK-oGH-15, National Institutes of Health, Bethesda, MD, USA) (5 μg g^{-1} body weight) or (c) cortisol plus oGH.

Experiment 2. Treatment with: (a) oGH (5 μg g^{-1} body weight), (b) cortisol (50 μg g^{-1} body weight), (c) recombinant bovine IGF-I (rbIGF-I, Monsanto Corporation, St. Louis MO, USA) (0.5 μg g^{-1} body weight), (d) oGH plus rbIGF-I, or (e) cortisol plus rbIGF-I.

Experiment 3. Treatment with: (a) oGH (5 μg g^{-1} body weight), (b) cortisol (50 μg g^{-1} body weight), (c) 3,3′,5-triiodo-L-thyronine (T_3, Sigma T-2752) (5 μg g^{-1} body weight), (d) oGH plus T_3, or (e) cortisol plus T_3.

Experiment 4. Following the results of experiment 3, influence of T_3 and cortisol plus T_3 was tested again. Treatment with: (a) cortisol (50 μg g^{-1} body weight), (b) T_3 (5 μg g^{-1} body weight), or (c) cortisol plus T_3.

Experiment 5. Treatment with oGH (5 μg g^{-1} body weight) or cortisol (50 μg g^{-1} body weight), returned to BW for 11 days and sampled. This experiment was done to analyze the effect of oGH and cortisol without salinity change on plasma osmolality and gill Na^+, K^+-ATPase activity.

Experiment 6. Treatment with: (a) rbIGF-I (0.5 μg g^{-1} body weight) in oil, keep 10 days in BW, transfer to SW and sample 24 h post-transfer; (b) rbIGF-I (0.1 μg g^{-1} body weight) in saline every other day for 10 days ($n = 5$), fish keep in BW, transfer to SW and sample 24 h after transfer. This experiment was done to check the influence of rbIGF-I injection method (saline or oil vehicle) on plasma osmolality and gill Na^+, K^+-ATPase activity.

Analytical techniques

Na^+, K^+-ATPase activities were determined using the microassay method of McCormick (1993). Gill tissue was homogenized in 125 μl of SEI buffer with 0.1% deoxycholic acid then centrifuged at 2000 × g for 30 s. Duplicate 10 μl homogenate samples were added to 200 μl assay mixture with and without 0.5 mM ouabain in 96-well microplates at 25 °C and read at 340 nm for 10 min with intermittent mixing. Ouabain-sensitive ATPase activity was detected by enzymatic coupling of ATP dephosphorylation to NADH oxidation and expressed as μmol ADP mg protein^{-1} h^{-1}. The Pierce BCA Protein kit (Pierce, Rockford, IL, USA) was used with bovine albumin as standard. Both assays were run on a THERMOmax microplate reader using SOFTmax software (Molecular Devices, Menlo Park, CA, USA). Plasma osmolality was measured with a vapor pressure osmometer (Wescor 5500, Logan, UT, USA) and expressed as mOs kg^{-1}.

Table 1. Effect of a single injection of cortisol (50 μg g^{-1} body weight) or oGH (5 μg g^{-1} body weight) in oil after eleven days in BW (Experiment 5)

	Oil	Cortisol	oGH
Plasma osmolality (mOs kg^{-1})	315 ± 1.8	310± 1.2	313 ± 1.1
Gill Na^+, K^+-ATPase	5.4 ± 0.3	7.0 ± 0.3*	5.9 ± 0.1

Values are means ± SEM (n = 6-7 per group); asterisk indicates significant difference with respect to oil group (p <0.05, 1-way ANOVA).

Statistics

Significant differences among groups were tested by one- or two-way ANOVA, followed by the Student–Newman–Keuls multiple comparison test (SNK). Results were considered significantly different when p <0.05.

Results

Transfer of vehicle-treated mummichog from BW to SW for 24 h induced a significant plasma hyperosmolality in all experiments. Gill Na^+, K^+-ATPase activity also increased (6.0–6.8 μmol ADP mg protein^{-1} h^{-1}) but statistically significant differences were not observed relative to BW-acclimated fish (5.4–5.6 μmol ADP mg protein^{-1} h^{-1}).

Treatment with cortisol for 10 days reduced the typical plasma hyperosmolality and increased gill Na^+, K^+-ATPase activity following transfer to SW (Figures 1–4). Cortisol treatment without salinity change (Experiment 5) significantly increased gill Na^+, K^+-ATPase activity but had no effect on plasma osmolality (Table 1).

oGH treatment significantly reduced the plasma hyperosmolality and increased gill Na^+, K^+-ATPase activity, but these effects were not as great as in cortisol-treated fish (Figures 1–3). oGH treatment without salinity transfer had no effect on plasma osmolality and gill Na^+, K^+-ATPase activity (Table 1). Treatment with cortisol plus oGH resulted in significantly lower plasma osmolality after SW transfer than with either hormone by itself. However, this combined treatment did not increase gill Na^+, K^+-ATPase activity over cortisol or oGH treatment alone (Figure 1).

Treatment with rbIGF-I had no effect on plasma hyperosmolality and gill Na^+, K^+-ATPase activity

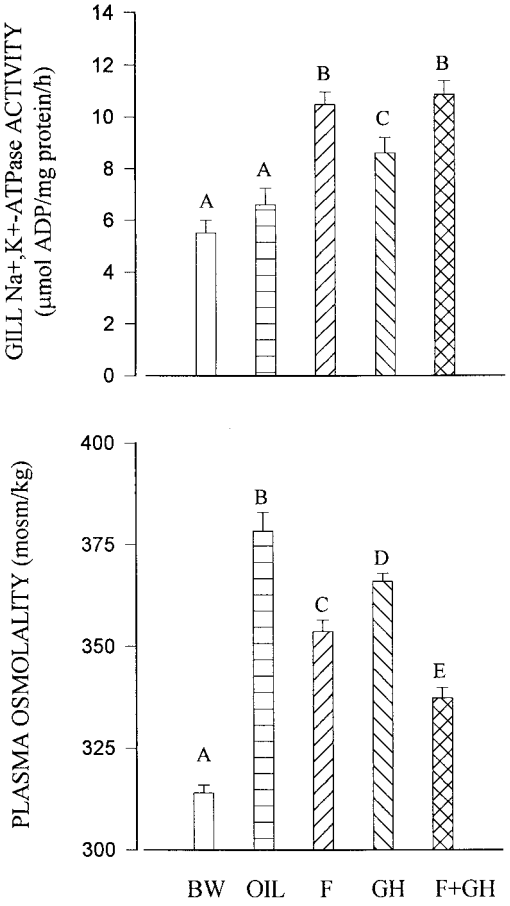

Figure 1. Gill Na^+, K^+-ATPase activity and plasma osmolality after a single oil (control), cortisol (50 μg g^{-1}), oGH (5 μg g^{-1}) or cortisol (50 μg g^{-1}) plus oGH (5 μg g^{-1}) injection. Fish were kept in BW (10 ppt) for 10 days before transfer to SW (35 ppt) for 24h (Experiment 1). Values are means ± SEM (n = 6–7 per group). Same letters indicate no statistically significant differences among groups (p <0.05, 1-way ANOVA, SNK test). There was a significant effect of GH and cortisol (p <0.05) on gill Na^+, K^+-ATPase activity and plasma osmolality, and a significant interaction only for plasma osmolality (p <0.05, two-way ANOVA).

after transfer to SW (Figure 2, Table 2). The effectiveness of rbIGF-I delivery by oil-injection relative to saline injection was tested (Experiment 6). With both systems, results were similar and rbIGF-I treatment did not affect plasma osmolality or gill Na^+, K^+-ATPase activity (Table 2). Treatment with rbIGF-I plus cortisol improved salinity tolerance and increased gill Na^+, K^+-ATPase activity to the same degree as cortisol-treatment alone (Figure 2). However, treatment with rbIGF plus oGH significantly reduced the plasma osmolality relative to either hormone by itself (Figure 2).

Table 2. Effect of a single oil injection of rbIGF-I (0.5 μg g^{-1} body weight) and 5 saline injections every other day of rbIGF-I (0.1 μg g^{-1} body weight). Fish were kept in BW for 10 days before being transfer to SW for 24 h (Experiment 6).

	Plasma osmolality (mOs kg^{-1})	Gill Na^+, K^+-ATPase (μmol ADP mg $protein^{-1}$ h^{-1})
BW	314 ± 1.1	5.5 ± 0.2
Saline	360 ± 3.2	6.8 ± 0.2
Saline IGF-I	359 ± 4.1	6.9 ± 0.2
Oil	361 ± 3.9	6.6 ± 0.3

Values are means ± SEM (n = 6-7 per group); there was no significant difference between control (saline or oil) and hormone-treated groups.

Treatment with T_3 had no effect on plasma osmolality and gill Na^+, K^+-ATPase activity (Figures 3 and 4). In the two experiments performed for testing the interaction between T_3 plus cortisol, Experiment 3 showed significantly higher gill Na^+, K^+-ATPase activity in the combined group relative to fish injected with either hormone alone, whereas there was no significant interaction in Experiment 4. In both experiments T3 plus cortisol reduced plasma osmolality to the same level as cortisol alone (Figures 3 and 4). In fish treated with T3 plus oGH, plasma osmolality and gill Na^+, K^+-ATPase activity changed to the same degree as in fish treated with oGH alone (Figure 3).

Discussion

Cortisol has an important role in seawater acclimation in many teleosts (McCormick 1995). Treatment with cortisol stimulates gill Na^+, K^+-ATPase activity, development of chloride cells and salinity tolerance (see Introduction). Our results demonstrate that cortisol improves gill Na^+, K^+-ATPase activity and salinity tolerance in BW-acclimated mummichog. These results agree with previous studies on osmoregulatory action of cortisol in this species (Pickford et al. 1970). The capacity of cortisol to increase gill Na^+, K^+-ATPase activity in mummichog in the present study was greater in fish transferred to SW than those that remained in BW. Treatment of mummichog with cortisol in BW resulted in gill Na^+, K^+-ATPase activity of 7.0 μmol ADP mg $protein^{-1}$ h^{-1} and those subsequently transferred to SW had 10.5 to 11.6 μmol ADP mg $protein^{-1}$ h^{-1}. In contrast to cortisol-injected fish, control fish exhibited no statistically significant increase in gill Na^+, K^+-ATPase activity following

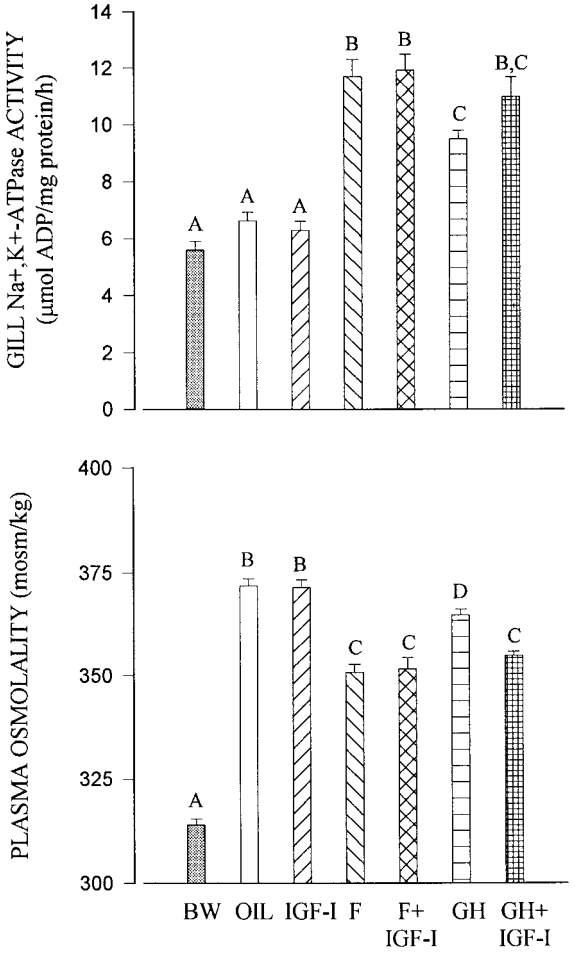

Figure 2. Gill Na^+, K^+-ATPase activity and plasma osmolality after a single oil (control), rbIGF-I (0.5 μg g^{-1}), cortisol (50 μg g^{-1}), oGH (5 μg g^{-1}), cortisol (50 μg g^{-1}) plus rbIGF-I (0.5 μg g^{-1}) or oGH (5μg g^{-1}) plus rbIGF-I (0.5 μg g^{-1}) injection. Fish were kept in BW (10 ppt) for 10 days before transfer to SW (35 ppt) for 24 h (Experiment 2). Values are means ± SEM (n = 6-7 per group). Same letters indicate no statistically significant differences between groups (p <0.05, 1-way ANOVA, SNK test). Two-way ANOVA indicated a significant effect of cortisol and GH (p <0.05), but not IGF-I, for gill Na^+, K^+-ATPase activity and plasma osmolality. There was no significant interaction of cortisol with IGF-I for gill Na^+, K^+-ATPase activity and plasma osmolality (p >0.1, two-way ANOVA). There was a significant interaction of GH with IGF-I for plasma osmolality (p <0.05) but not for gill Na^+, K^+-ATPase activity (p >0.1, two-way ANOVA).

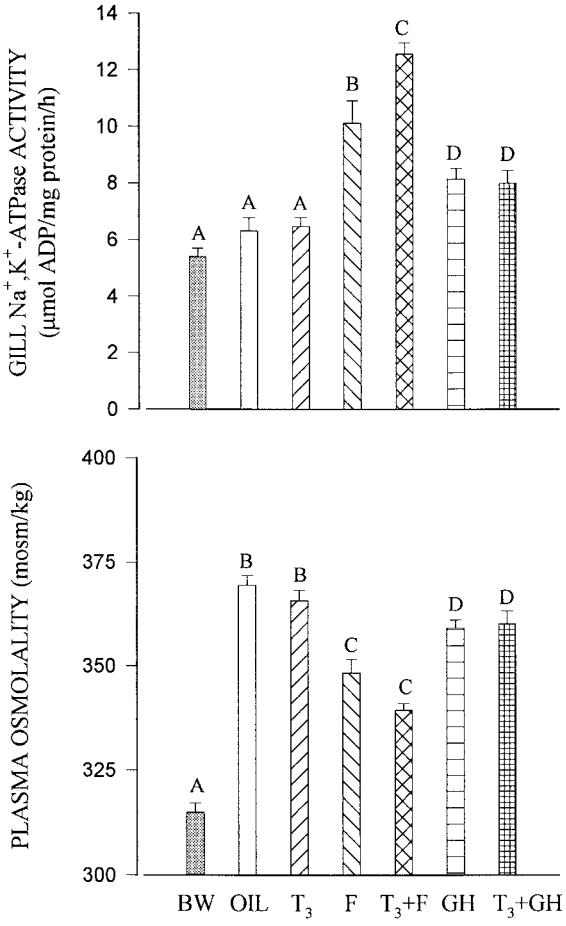

Figure 3. Gill Na^+, K^+-ATPase activity and plasma osmolality after a single oil (control), T_3 (5 μg g^{-1}), cortisol (50 μg g^{-1}), oGH (5 μg g^{-1}), cortisol (50 μg g^{-1}) plus T_3(5 μg g^{-1}) or oGH (5 μg g^{-1}) plus T_3 (5 μg g^{-1}) injection (Experiment 3). Fish were kept in BW (10 ppt) for 10 days before being transfer to SW (35 ppt) for 24 h (Experiment 3). Values are means ± SEM (n = 6-7 per group). Same letters indicate no statistically significant differences between groups (p <0.05, 1-way ANOVA, SNK test). Two-way ANOVA indicated a significant effect of cortisol and GH (p <0.05), but not T_3, for gill Na^+, K^+-ATPase activity and plasma osmolality. There was a significant interaction of T_3 with cortisol for plasma osmolality (p <0.05) but not for gill Na^+, K^+-ATPase activity (p >0.1, two-way ANOVA). There was no significant interaction of T_3 with GH for gill Na^+, K^+-ATPase activity and plasma osmolality (p >0.1, two-way ANOVA).

exposure to SW (5.4 *vs.* 6.0–6.8 μmol ADP mg protein^{-1} h^{-1}) (Figures 1–3, Table 1). This suggests that cortisol has promoted the capacity of gill Na^+, K^+-ATPase to respond to salinity changes, perhaps by increasing chloride cell number or size, or the capacity of these cells to respond to salinity or salinity-induced neuroendocrine factors. It is possible that the reduced impact of cortisol on fish in BW relative to SW was due to seasonal changes in responsiveness (experiments were conducted in spring and autumn), similar to the differences in responsiveness found in salmon. This seem unlikely, however, since we found no seasonal changes in gill Na^+, K^+-ATPase in BW or after transfer of control fish to SW.

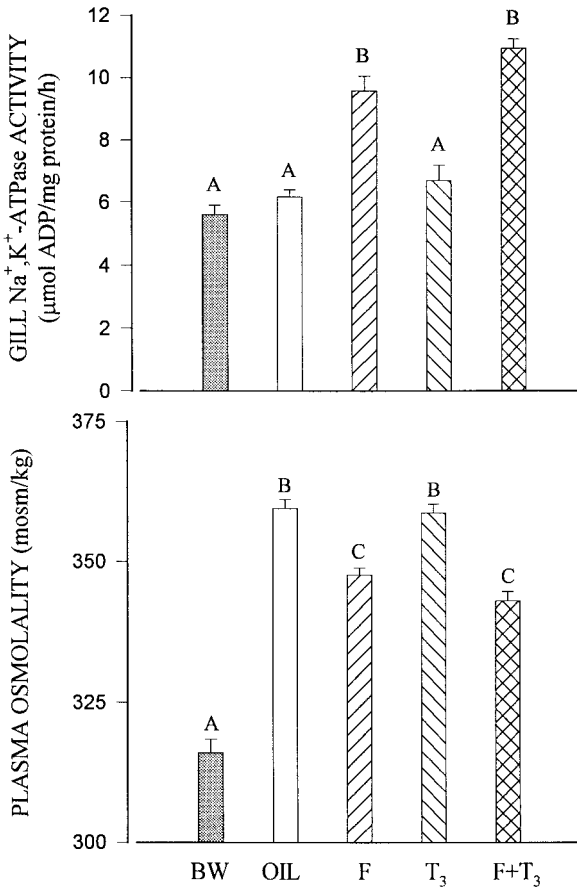

Figure 4. Gill Na^+, K^+-ATPase activity and plasma osmolality after a single oil (control), cortisol (50 μg g^{-1}), T_3 (5 μg g^{-1}), or cortisol (50 μg g^{-1}) plus T_3(5 μg g^{-1}) injection (Experiment 4). Fish were kept in BW (10 ppt) for 10 days before being transfer to SW (35 ppt) for 24 h (Experiment 3). Values are means ± SEM (n = 6-7 per group). Same letters indicate no statistically significant differences between groups (p <0.05, 1-way ANOVA, SNK test). For gill Na^+, K^+-ATPase activity and plasma osmolality, there was a significant effect of cortisol (p <0.05), but not T_3, and no significant interaction between cortisol and T_3 (p >0.1, two-way ANOVA).

The transfer of mummichog from hypoosmotic to hyperosmotic medium induces a rise in plasma osmolality (Jacob and Taylor 1983; Zadunaisky et al. 1995; present results). Foskett et al. (1981) studying electrophysiological activity of chloride cells in the opercular epithelium of tilapia suggested that salinity itself is necessary for activating ion secretion. Increases in osmolality of 50 mOsm kg^{-1}, similar to that seen after SW exposure in the present study, activates active chloride transport in the isolated opercular membrane of mummichog (Zadunaisky et al. 1995). Our finding also suggest a rapid activation of gill Na^+, K^+-ATPase activity in mummichog 24 h after tranfer from BW to SW but statistically significant differences were not observed. Although most teleosts require several days to increase gill Na^+, K^+-ATPase activity, previous studies have demonstrated rapid activation of this enzyme in mummichog (Towle et al. 1977; Mancera and McCormick unpublished data).

A role of the GH/IGF-I axis in osmoregulation has been demonstrated in salmonid fishes. Only recently has an osmoregulatory role for GH been established for non-salmonids (see Introduction). In mummichog, GH treatment for 48 h increases gill Na^+, K^+-ATPase activity and decreases plasma osmolality following SW transfer (Mancera and McCormick 1998). Present results show that long-term treatment with oGH also improves salinity tolerance and increases gill Na^+, K^+-ATPase activity after salinity transfer (Experiments 1, 2, 3). However, oGH treatment of fish without salinity change did not result in significant changes in plasma osmolality or gill Na^+, K^+-ATPase activity (Experiment 4). These different responses to GH in fish with and without salinity transfer suggest that similar to cortisol treated fish, there is an interaction of GH with other hormone/s or the rise in plasma osmolality that increases gill Na^+, K^+-ATPase activity in mummichog.

The pathway for this hypoosmoregulatory action of oGH in mummichog is not known. In addition to the direct actions of GH on osmoregulatory organs, IGF-I could carry out some or all of physiological actions of GH (somatomedin hypothesis, see below). Also, GH can increase the number of gill cortisol receptors in salmonid fishes (Shrimpton et al. 1995), which would explain the hypoosmoregulatory action of GH by itself and the positive cooperation between GH and cortisol (Madsen 1990a; McCormick 1996). In addition, GH also increases the response of the interrenal gland to ACTH and, hence, could increase endogenous secretion of cortisol (Young 1988).

Unlike results with salmonid species, our results do not show any positive interaction between GH and cortisol in increasing gill Na^+, K^+-ATPase activity in mummichog. However, these fish showed better salinity tolerance than fish treated with cortisol or oGH alone. It is possible that a maximal level of stimulation of gill Na^+, K^+-ATPase activity has occurred with cortisol treatment alone, thus precluding any further stimulation by GH. Alternatively, GH may act on other osmoregulatory organs (kidney, intestine) or other aspects of gill ion secretory capacity that do no involve gill Na^+, K^+-ATPase activity.

oGH has thyrotropic activity in intact and hypophysectomized mummichog, and treatment with this hormone increases serum T_4 levels (Grau and Stetson 1979). In the present study, treatment of mummichog with oGH would be expected to result in increased plasma thyroid hormones. GH has also been shown to increase T_4 to T_3conversion in trout (de Luze 1989). However, our results show that treatment with T_3 by itself did not increase hypoosmoregulatory capacity in this species (Experiments 3 and 4), and thus do not support the idea a thyrotropic action of GH is responsible for the hypoosmoregulatory action of this hormone.

In salmonid fishes, IGF-I may mediate some of the osmoregulatory actions of GH (McCormick et al. 1991; Sakamoto and Hirano 1993; Madsen et al. 1996; McCormick 1996). A clear hypoosmoregulatory effect of IGF in short-term (48 h) treatment has been shown in salmonids, but positive effect of this hormone has not been demonstrated with longer treatment (>7 d) (McCormick 1991, 1996; Madsen et al. 1996). Short-term treatment with rbIGF-I improves hypoosmoregulatory capacity in mummichog transferred from BW to SW (Mancera and McCormick 1998), whereas, long-term treatment with rbIGF-I did not alter hypoosmoregulatory capacity (present results). We cannot rule out the possibility that the oil system is not appropriate for administration of IGF-I. We examined the influence of injection method (saline or oil vehicle) of rbIGF-I on hypoosmoregulatory capacity (Experiment 6); the results were similar with both systems, and no change in plasma osmolality or gill Na^+, K^+-ATPase activity was observed. Long-term treatment with IGF-I may be problematic due to the negative feedback of IGF-I on growth hormone secretion (Blaise et al. 1995).

Cooperation between IGF-I and GH affects osmoregulatory physiology of salmonids (Madsen and Bern 1993). Mummichog treated with GH plus IGF-I showed significantly lower plasma osmolality than fish treated with either hormone alone. One possible mechanism for this interaction is the regulation of IGF-I binding proteins by GH, which could increase the efficacy of exogenous IGF-I (Jones and Clemmons 1995). The interaction of GH and IGF-I observed by Madsen and Bern (1993) were observed following *in vivo* GH treatment followed by *in vitro* IGF-I, suggesting that GH increases the capacity of gill to respond to IGF-I. A suggested mechanism for this interaction is a GH-dependent increase in the number of cells that respond to IGF-I, similar to that observed in mammalian cartilage (Green et al. 1985). The different pattern of plasma osmolality and gill Na^+, K^+-ATPase activity in fish treated with IGF-I plus GH, similar to that observed between GH and cortisol, also suggests a long-term interaction of IGF-I and/or GH on other aspect of ion transport by the gills or effects on other osmoregulatory organs.

The role of thyroid hormones on osmotic and ionic balance in teleosts is contradictory (Grau 1987; see Introduction). Knoeppel et al. (1982) using treatment with thiourea found that a functional thyroid is essential for maintenance of sodium and osmotic balance and for survival of mummichog in SW. In addition, SW-acclimated mummichog had higher levels of T_4 in serum compared to FW-acclimated fish (Grau 1987). These data suggest that in the euryhaline teleost mummichog thyroid hormones may be important for osmotic regulation in SW. In the present study T_3 alone failed to increase gill Na^+, K^+-ATPase activity and salinity tolerance. These data are in agreement with other studies finding no effect of thyroid hormones on gill Na^+, K^+-ATPase activity in several salmonids and tilapia (Miwa and Inui 1985; Saunders et al. 1985; Dange 1986). Although we cannot rule out the possibility that the T_3 administration was ineffective, the doses of T_3 used in the present study were based on previous studies which have found increased plasma titers and positive physiological effects (Madsen and Korsgaard 1989; McCormick, unpublished results). Other factors such as treatment duration, acclimation to brackish water and temperature may affect the ability to detect osmoregulatory actions of thyroid hormones. In any event, the lack of effect of thyroid hormones should not be considered definitive without further study.

Some data suggest that thyroid hormones have an osmoregulatory role through their synergism with other osmoregulatory hormones. In salmonids, a positive interaction between T_3 and GH has been reported in acclimation of brown and rainbow trout to seawater (Leloup and Lebel 1993). In the present study no positive interaction between T_3 and GH for increasing gill Na^+, K^+-ATPase activity and salinity tolerance was observed. Similarly, we could not find a consistent interaction between T_3 and cortisol for increasing salinity tolerance. In tilapia, a cooperation between T_4 and cortisol to increase gill Na^+, K^+-ATPase activity has been shown (Dange 1986), although the mechanism for this interaction is not currently known.

In summary, our results confirm the classical role of cortisol as a seawater-adapting hormone, and sup-

port a hypoosmoregulatory role of the GH/IGF-I axis in mummichog. We provide evidence for physiologically important interactions between GH and cortisol and between GH and IGF-I. An osmoregulatory role of T_3 in seawater acclimation of mummichogis not evident. Further work is necessary to determine the mechanism(s) of action of these hormones and their interaction.

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