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Evidence for Growth Hormone/Insulin-like Growth Factor I Axis Regulation of Seawater Acclimation in the Euryhaline Teleost *Fundulus heteroclitus*

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The ability of ovine growth hormone (oGH), recombinant bovine insulin-like growth factor I (rbIGF-I), recombinant human insulin-like growth factor II (rhIGF-II), and bovine insulin to increase hypoosmoregulatory capacity in the euryhaline teleost *Fundulus heteroclitus* was examined. Fish acclimated to brackish water (BW, 10 ppt salinity, 320 mOsm/kg H_2O) were injected with a single dose of hormone and transferred to seawater (SW, 35 ppt salinity, 1120 mOsm/kg H_2O) 2 days later. Fish were sampled 24 h after transfer and plasma osmolality, plasma glucose, and gill Na^+,K^+-ATPase activity were examined. Transfer from BW to SW increased plasma osmolality and gill Na^+,K^+-ATPase activity. Transfer from BW to BW had no effect on these parameters. rbIGF-I (0.05, 0.1, and 0.2 μg/g) improved the ability to maintain plasma osmolality and to increase gill Na^+, K^+-ATPase activity in a dose-dependent manner. oGH (0.5, 1, and 2 μg/g) also increased hypoosmoregulatory ability but only the higher doses (2 μg/g) significantly increased gill Na^+,K^+-ATPase activity. oGH (1 μg/g) and rbIGF-I (0.1 μg/g) had a significantly greater effect on plasma osmolality and gill Na^+,K^+-ATPase activity than either hormone alone. rhIGF-II (0.05, 0.1, and 0.2 μg/g) and bovine insulin (0.01 and 0.05 μg/g) were without effect. The results suggest a role of GH and insulin-like growth factor I (IGF-I) in seawater acclimation of *F. heteroclitus.* Based on these findings and previous studies, it is concluded that the capacity of the GH/IGF-I axis to increase hypoosmoregulatory ability may be a common feature of euryhalinity in teleosts.

The transfer of fish to a hyperosmotic environment disturbs osmoregulatory homeostasis and activates osmoregulatory organs. A number of hypophysial and extrahypophysial hormones, with short-term and long-term effects, are involved in this process (McCormick, 1995). An osmoregulatory role of growth hormone (GH) has been demonstrated for salmonid fishes. Long-term treatment with GH increases salinity tolerance, chloride cell numbers, gill Na^+,K^+-ATPase activity, and / or expression of Na^+,K^+-ATPase α-subunit in *Salmo salar, Salmo trutta, Oncorhynchus mykiss,* and *Oncorhynchus nerka* (Richman and Zaugg, 1987; Bolton *et al.,* 1987; Boeuf *et al.,* 1994; Madsen 1990a,b, 1995; Sakamoto *et al.,* 1993; McCormick, 1996). Also, a single injection of GH improves salinity tolerance in *O. mykiss* (Collie *et al.,* 1989; McCormick *et al.,* 1991) and *S. salar* (McCormick, 1996) within 48 h of hormone treatment.

In nonsalmonids the hypoosmoregulatory role of GH is uncertain. In fish acclimated to different salinities, morphology of GH-producing cells shows different patterns of activation depending on the species studied (Nishioka *et al.,* 1988). Long-term treatment with GH increases opercular chloride cell density in tilapia *Oreochromis mossambicus* (Flik *et al.,* 1993), gill Na^+,K^+-ATPase activity (Borski *et al.,* 1994; Sakamoto

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et al., 1997), and salinity tolerance (Sakamoto *et al.,* 1997). In contrast, GH appears to have no effect on acclimation of *Oreochromis niloticus* from fresh water to brackish water (Auperin *et al.,* 1995).

The somatomedin hypothesis suggests that GH stimulates the production and release of IGF-I predominantly from the liver, which carries out some or all of the physiological actions of GH (Green *et al.,* 1985; Holly and Wass, 1989). In salmonids, it has been reported that insulin-like growth factor I (IGF-I) improves hypoosmoregulatory capacity after short-term treatment and is a potential mediator of long-term actions of GH on seawater acclimation (McCormick *et al.,* 1991; Sakamoto *et al.,* 1993; Madsen *et al.,* 1995; McCormick, 1995, 1996).

IGF-II is another member of the insulin-like growth factor family (Cohick and Clemmons, 1993). In rainbow trout IGF-II mRNA has been detected in several organs, including osmoregulatory structures (gill and kidney), where levels of IGF-II mRNA are higher than those of IGF-I mRNA (Chen *et al.,* 1994). However, there is no specific information on the possible osmoregulatory role of IGF-II in salmonid or nonsalmonid teleosts.

The osmoregulatory role of insulin in teleosts is unclear. In *Oncorhynchus kisutch* an increase in plasma insulin levels during the process of smolting has been reported and may be involved in metabolic and/or osmoregulatory changes (Plisetskaya *et al.,* 1988). Unlike the effects of IGF-I, however, treatment of coho salmon with insulin did not improve hypoosmoregulatory ability (McCormick *et al.,* 1991). In the eel there is no apparent role for insulin in osmoregulation (Epple, 1987).

The mummichog, *F. heteroclitus,* is an intertidal, euryhaline teleost that lives in an environment of widely varying salinities. According to Wood and Marshall (1994), ''*Fundulus heteroclitus* (together with a few congeners) has been the single most important species contributing to our current understanding of salt transport in the gill of seawater.'' Although this species was used in some of the earliest studies on the endocrine control of ion transport (e.g., Pickford *et al.,* 1970), the roles of growth hormone and the insulin family of peptide hormones in seawater acclimation have not been examined. This study tests the capacity of GH, IGF-I, IGF-II, and insulin to improve salinity tolerance and stimulate gill Na^+,K^+-ATPase activity of *F. heteroclitus.*

MATERIALS AND METHODS

Fish

F. heteroclitus (4–8 g body wt) were collected in the Connecticut River estuary and transferred to the S.O. Conte Anadromous Fish Research Center, Turners Falls, MA. Fish were acclimated for at least 2 weeks to brackish water (BW) (Instant Ocean, 10 ppt salinity, 320 mOsm/kg H_2O) under natural photoperiod and constant temperature (15°C). They were maintained in 60-L aquaria and 50% of the water was changed every 3 days. Fish were fed daily with commercial fish food (Tetramix, Tetrawerke, Germany). They were fasted for 24 h before hormone injection and throughout the remainder of the experiment. Experiments were conducted between April–June (experiments 1, 2, and 3) and September–November (experiments 4 and 5) of 1995 and July–August of 1996 (experiments 6 and 7).

Hormones

Ovine GH (oGH; NIADDK-oGH-15) was obtained from the National Institutes of Health (Bethesda, MD). Recombinant bovine IGF-I (rbIGF-I) was provided by Monsanto Corp. (St. Louis, MO). Recombinant human IGF-II (rhIGF-II) was obtained from Peninsula Laboratories (IP 8004, Belmont, CA). Bovine insulin was obtained from Sigma Chemical Co. (I6634, St. Louis, MO). The vehicle for all hormones was saline solution. Fish received intraperitoneal injections of 10 μl/g body wt.

Experimental Protocol

To examine the effect of hormone treatment on hypoosmoregulation, the protocol of McCormick *et al.* (1991) was followed. Fish were anesthetized (100 mg/L MS-222, pH 7.0), weighed, injected intraperitoneally with vehicle or vehicle plus hormone, and placed back in BW. After 48 h fish were transferred to seawater (SW) (35 ppt, 1120 mOsm/kg H_2O) and 24 h after transfer the fish were anesthetized, weighed, and sampled. A gill filament biopsy was taken, 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 fin and collecting the blood in ammonia heparinized microcapillary tubes. The tube was centrifuged at 3000*g* for 5 min, and plasma was stored at −80°C.

The following experiments were conducted:

Experiment 1. Treatment with rbIGF-I (0.05, 0.1, and 0.2 µg/g body wt), rhIGF-II (0.05, 0.1, and 0.2 µg/g body wt), and oGH (0.25 and 0.5 µg/g body wt).

Experiment 2. Treatment with rbIGF-I (0.1 µg/g body wt), oGH (0.5 µg/g body wt), and rbIGF-I (0.1 µg/g) + oGH (0.5 µg/g).

Experiment 3. Treatment with bovine insulin (0.05 µg/g body wt). In preliminary experiments (results not shown) a single injection of 0.1 µg/g insulin resulted in death within 48 h of injection.

Experiment 4. Transfer from BW to BW and sample at 24 h posttransfer. The aim of this experiment was to examine the effect of physical transfer in the absence of salinity change on plasma osmolality and gill Na^+,K^+-ATPase activity.

Experiment 5. Treatment with rbIGF-I (0.2 µg/g body wt), kept three days in BW and sampled. This experiment was performed to analyze the effect of IGF-I on plasma osmolality and gill Na^+,K^+-ATPase activity without salinity change.

Experiment 6. Treatment with oGH (0.5, 1, and 2 µg/g body wt). Following the results of experiment 1, the effects of higher doses of oGH were examined.

Experiment 7. Treatment with rbIGF-I (0.1 µg/g body wt), oGH (1 µg/g body wt), and rbIGF-I (0.1 µg/g) + oGH (1 µg/g).

Analytical Techniques

Na^+,K^+-ATPase activities were determined using the microassay method of McCormick (1993). Gills were homogenized in 125 µl SEID (SEI buffer with 0.1% deoxycholic acid), then centrifuged at 3000*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 micromoles ADP/milligram protein/hour. Protein concentration was determined using a bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL) with bovine albumin as standard. Both assays were run on a THERMOmax microplate reader using SOFTmax software (Molecular Devices, Menlo Park, CA).

Plasma osmolality was measured with a vapor pressure osmometer (Wescor 5500, Logan, UT) and expressed as milliosmoles/kilogram. Plasma glucose was measured by enzymatic coupling with hexokinase and glucose-6-phosphate dehydrogenase (Stein, 1963) and expressed as millimolar.

Statistics

Significant differences among groups were tested by one-way ANOVA. Two-way ANOVA and the Student–Newman–Keuls multiple comparison test were used to test the significance of hormone combinations. Results were considered significantly different when $P < 0.05$.

RESULTS

Transfer of *F. heteroclitus* from BW to SW for 24 h significantly increased plasma osmolality. Gill Na^+,K^+-ATPase activity also increased but statistically significant differences were not observed. Fish transferred from BW to BW and sampled 24 h later did not differ from untransferred fish in plasma osmolality or gill Na^+,K^+-ATPase activity (Table 1).

A single injection of oGH (0.25, 0.5, 1, and 2 µg/g body wt) dose-dependently attenuated the rise in plasma osmolality following transfer to SW and increased gill Na^+,K^+-ATPase activity (Figs. 1 and 2). The reduced plasma osmolalities were statistically

TABLE 1
Effect of Transfer from BW to BW on Plasma Osmolality and Gill Na^+,K^+-ATPase

	BW	BW transfer
Plasma osmolality (mOsm/kg)	312 ± 1	311 ± 1
Gill Na^+,K^+-ATPase (µmol ADP/mg protein/h)	5.5 ± 0.1	5.6 ± 0.2

Note. There was no significant difference between groups. Values are means ± standard error (n = 6–7).

significant at the three higher doses (0.5, 1, and 2 μg/g body wt), and the increased gill Na^+,K^+-ATPase activities were statistically significant only at the highest dose (2 μg/g body wt). Treatment with this dose resulted in a 38% increase in enzyme activity over control. Treatment with oGH significantly increased plasma glucose levels at the two higher doses (1 and 2 μg/g body wt) (Fig. 3).

A single injection of rbIGF-I 48 h before transfer of fish from BW to SW reduced the increase in posttransfer plasma osmolality and increased gill Na^+,K^+-ATPase activity (Figs. 1 and 4). The effect of rbIGF-I (0.05, 0.1, and 0.2 μg/g body wt) on plasma osmolality and gill Na^+,K^+-ATPase activity was dose-dependent,

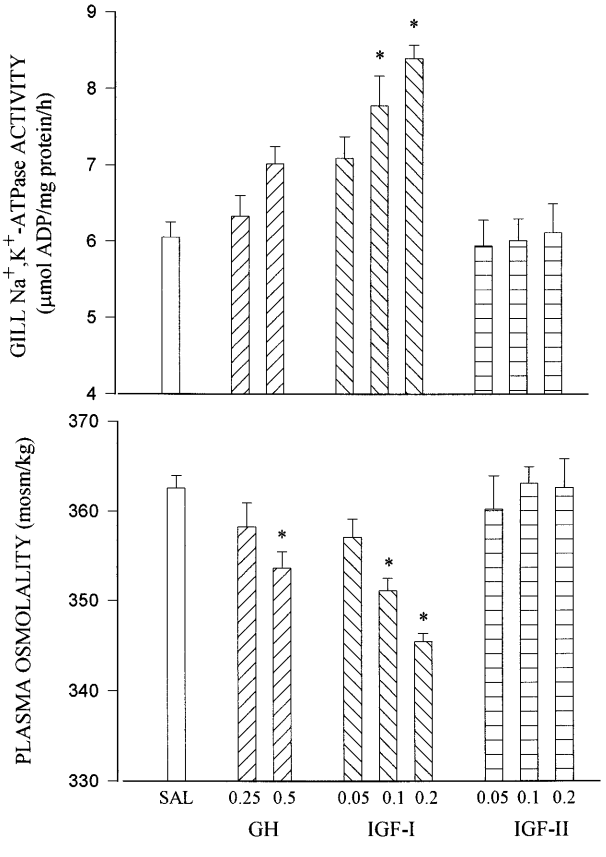

FIG. 1. Effect of a single injection of oGH, rbIGF-I, and rhIGF-II on gill Na^+,K^+-ATPase activity (top) and plasma osmolality (bottom). Fish were kept in BW for 2 days before transfer to SW for 24 h. In BW-adapted fish, gill Na^+,K^+-ATPase activity was 5.5 ± 0.2 μmol ADP/mg protein/h and plasma osmolality was 312 ± 1 mOsm/kg. Values are means ± standard error (n = 6–7). Asterisks indicate significant difference relative to the saline group ($P < 0.05$).

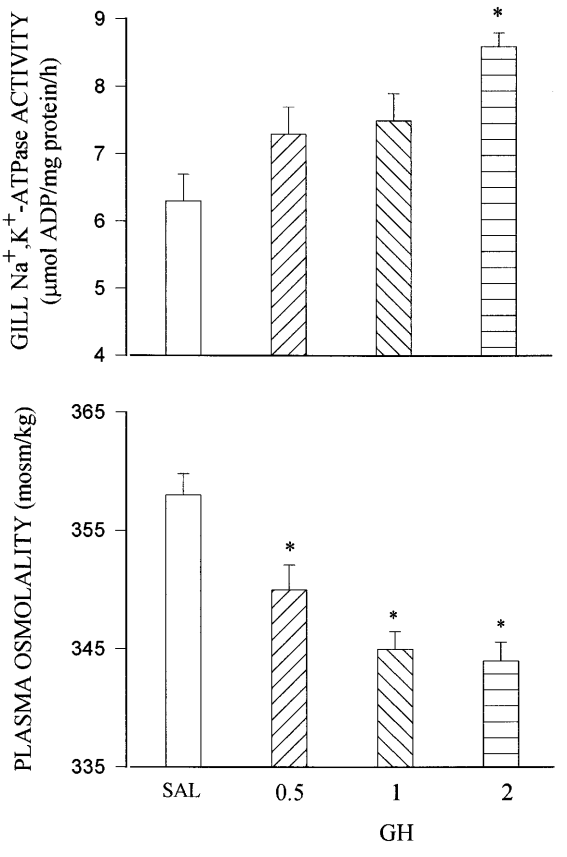

FIG. 2. Effect of a single injection of oGH (0.5, 1, and 2 μg/g body wt) on gill Na^+,K^+-ATPase activity (top) and plasma osmolality (bottom). Fish were kept in BW for 2 days before transfer to SW for 24 h. In BW-adapted fish, gill Na^+,K^+-ATPase activity was 5.5 ± 0.3 μmol ADP/mg protein/h and plasma osmolality was 317 ± 2 mOsm/kg. Values are means ± standard error (n = 5). Asterisks indicate significant difference relative to the saline group ($P < 0.05$).

and there were significant decreases with respect to control at doses of 0.1 and 0.2 μg/g body wt (Fig. 1). Treatment with rbIGF-I increased plasma glucose levels, but not significantly (Table 2). A single injection of rbIGF-I (0.2 μg/g body wt) without salinity change did not significantly affect plasma osmolality or gill Na^+,K^+-ATPase (Table 3).

Treatment with oGH (0.5 μg/g body wt) plus rbIGF (0.1 μg/g body wt) did not improve salinity tolerance with respect to IGF-I treatment alone (data not shown). Fish treated with oGH (1 μg/g) and rbIGF-I (0.1 μg/g) had higher gill Na^+,K^+-ATPase activity and lower plasma osmolality than treatment with either hormone

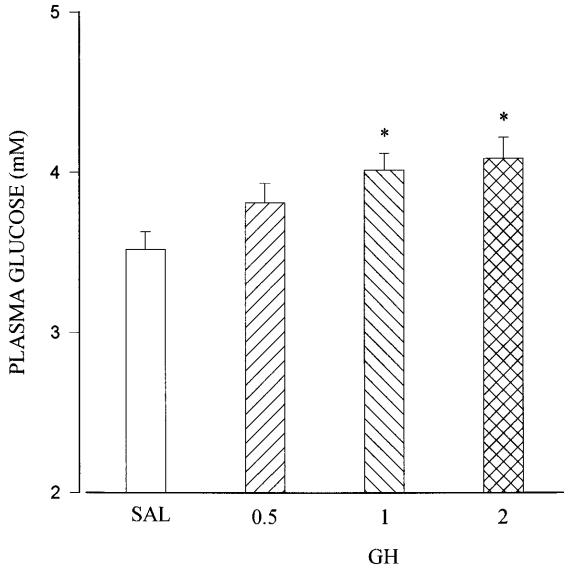

FIG. 3. Effect of a single injection of oGH (0.5, 1, and 2 μg/g body wt) on plasma glucose. Fish were kept in BW for 2 days before transfer to SW for 24 h. In BW-adapted fish, plasma glucose was 3.62 ± 0.12 mM. Values are means ± standard error ($n = 5$). Asterisks indicate significant difference relative to the saline group ($P < 0.05$).

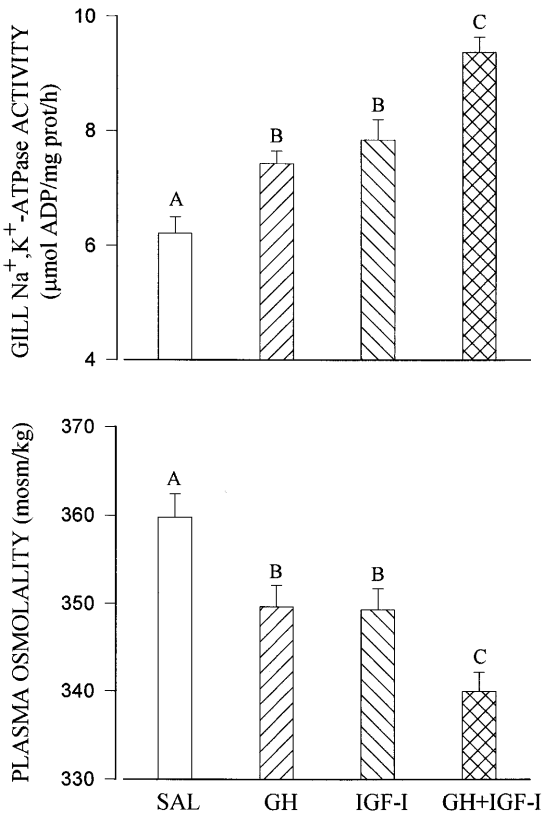

FIG. 4. Gill Na^+,K^+-ATPase activity (top) and plasma osmolality (bottom) after a single injection of saline, oGH (1 μg/g body wt), rbIGF-I (0.1 μg/g body wt), and rbIGF-I plus oGH. Fish were kept in BW for 2 days before transfer to SW for 24 h. In BW-adapted fish gill Na^+,K^+-ATPase activity was 5.7 ± 0.3 μmol ADP/mg protein/h and plasma osmolality was 318 ± 2 mOsm/kg. Values are means ± standard error ($n = 6$–7). Same letters indicate no differences among groups ($P < 0.05$).

alone (Fig. 4). Although two-way ANOVA found no significant interaction ($P > 0.5$), post hoc comparison indicated a significant difference of the combined hormones group from oGH and rbIGF-I alone ($P < 0.01$ for gill Na^+,K^+-ATPase activity; $P < 0.05$ for plasma osmolality).

Treatment with rhIGF-II (0.05, 0.1, and 0.2 μg/g body wt) had no effect on plasma osmolality and gill Na^+,K^+-ATPase activity after transfer to SW (Fig. 1). Plasma glucose levels in the rhIGF-II treated group were not different from those in the saline group (Table 2).

In preliminary experiments (results not shown), the effects of different doses of insulin (0.01, 0.05, and 0.1 μg/g body wt) on fish survival ($n = 3$ for each group) were tested. A single injection of insulin at its highest doses (0.1 μg/g) resulted in death within 48 h, whereas at lower doses (0.01 μg/g) no effects on plasma osmolality and gill Na^+,K^+-ATPase activity were observed. In experiment 3, insulin treatment (0.05 μg/g body wt, $n = 7$) did not decrease plasma osmolality after SW transfer, nor did it affect gill Na^+,K^+-ATPase activity (Table 4).

TABLE 2

Effect of a Single Injection of oGH, rbIGF-I, and rhIGF-II on Plasma Glucose Levels (mM)

Saline 3.27 ± 0.11	oGH (0.25 μg/g) 3.55 ± 0.25	oGH (0.5 μg/g) 3.57 ± 0.13
rbIGF-I (0.05 μg/g) 3.39 ± 0.25	(0.1 μg/g) 3.53 ± 0.16	(0.2 μg/g) 3.70 ± 0.24
rhIGF-II (0.05 μg/g) 3.28 ± 0.22	(0.1 μg/g) 3.27 ± 0.10	(0.2 μg/g) 3.13 ± 0.05

Note. There was no significant difference among saline and hormone-treated groups. Values are means ± standard error ($n = 6$–7). Fish were kept in BW for 2 days before transfer to SW for 24 h.

TABLE 3
Effect of a Single Injection of rbIGF-I (0.2 μg / g body wt) in Fish after 3 Days in BW (without SW Transfer) on Plasma Osmolality and Gill Na^+,K^+-ATPase

	Saline	rbIGF-I
Plasma osmolality (mOsm / kg)	315 ± 1	313 ± 2
Gill Na^+,K^+-ATPase (μmol ADP / mg protein / hour)	5.7 ± 0.2	6.4 ± 0.2

Note. There was no significant difference between groups. Values are means ± standard error (n = 6–7).

DISCUSSION

A dose-dependent osmoregulatory action of GH over 3 days of treatment of *F. heteroclitus* has been demonstrated. High doses of oGH (1 and 2 μg / g body wt) improved salinity tolerance and increased gill Na^+,K^+-ATPase activity. These results agree with those reported for tilapia *O. mossambicus*, in which GH treatment increased opercular chloride cell number, improved the ability to decrease plasma osmolality following transfer to SW, and stimulated gill Na^+,K^+-ATPase activity (Flik *et al.*, 1993; Borski *et al.*, 1994; Sakamoto *et al.*, 1997). There was, however, a differential response of plasma osmolality and gill Na^+,K^+-ATPase activity to oGH treatment: low doses of oGH increased salinity tolerance but only high doses of oGH increased gill Na^+,K^+-ATPase activity. If low doses of oGH have physiological effects on other osmoregulatory organs (kidney, intestine), this could explain the observed increase in salinity tolerance without increased gill Na^+,K^+-ATPase activity. In addition, other physiological actions of GH on gills (e.g., modification of permeability, activation of existing transporters, etc.) could explain these differences.

Effects of IGF-I on hypoosmoregulatory capacity following a single injection have been reported in salmonids (McCormick *et al.*, 1991; Madsen *et al.*, 1995; McCormick, 1996). In *F. heteroclitus* rbIGF-I significantly decreased plasma osmolality and increased gill Na^+,K^+-ATPase activity after the transfer from BW to SW in a dose-dependent manner. IGF-I has effects on osmoregulatory actions on mammalian kidney and toad urinary bladder (Kopple and Hirschberg, 1990; Cohick and Clemmons, 1993), but there are no reports of extrabranchial osmoregulatory actions of IGF-I in teleosts.

In striped bass (*Morone saxatilis*), a single injection of IGF-I just before transfer from freshwater (FW) to SW induced an unfavorable metabolic effect and an osmoregulatory imbalance in the fish (S. S. Madsen, personal communication). In the present study fish were adapted to BW (10 ppt salinity, 320 mOsm / kg H_2O) rather than the FW used for striped bass. In BW, plasma prolactin levels are likely to be low, whereas striped bass adapted to fresh water could have high prolactin levels. McCormick (1996) reported hypoosmoregulatory actions of a single injection of IGF-I in Atlantic salmon acclimated to 12 ppt salinity but no effect in freshwater-acclimated fish. Higher circulating levels of prolactin in FW fish could explain this difference. Antagonism between prolactin and GH on hypoosmoregulation has been reported in salmonids (Madsen and Bern, 1992). However, several studies indicate that sufficiently long-term treatment with GH (generally more than a week) overcomes the potential antagonism of high prolactin levels in FW and results in significant increases in salinity tolerance and gill Na^+,K^+-ATPase activity (Madsen, 1990b; Boeuf *et al.*, 1994; McCormick, 1996).

The transfer of *F. heteroclitus* from hypoosmotic to hyperosmotic medium induces a rise in plasma osmolality (Jacob and Taylor, 1983; Zadunaisky *et al.*, 1995; present results). After studying the electrophysiological activity of chloride cells in the opercular membrane of freshwater- and seawater-acclimated *O. mossambicus*, Foskett *et al.* (1981) suggested that salinity itself is necessary for activating ion secretion. Zadunaisky *et al.* (1995) showed that activation of chloride transport in opercular membrane of *F. heteroclitus* during rapid

TABLE 4
Effect of a Single Injection of Insulin (0.05 μg / g body wt) on Plasma Osmolality and Gill Na^+,K^+-ATPase

		BW-SW	
	BW	Saline	Insulin
Plasma osmolality (mOsm / kg)	315 ± 2	360 ± 3*	357 ± 3*
Gill Na^+,K^+-ATPase (μmol ADP / mg protein / hour)	5.4 ± 0.3	6.4 ± 0.3	6.5 ± 0.3

Note. Asterisks indicate significant difference relative to the BW group ($P < 0.05$). Values are means ± standard error (n = 6–7). Fish were kept in BW for 2 days before transfer to SW for 24 h.

acclimation to high salinity required an increase in plasma osmolality. The present results showed that oGH and rbIGF-I improved salinity tolerance and gill Na^+,K^+-ATPase activity after transfer from BW to SW, but there are no discernible effects without transfer to SW. It is interesting to note that in salmonids effects of GH and IGF-I treatment on gill Na^+,K^+-ATPase activity can be observed without transfer to high salinity (see McCormick, 1995). The results observed in *F. heteroclitus* suggest that exposure to SW promotes the actions of exogenous (and likely endogenous) growth hormone and insulin-like growth I. This effect could be due to SW-induced increases in a stimulatory factor (such as cortisol, see below) or decreases in an inhibitory factor such as prolactin.

Cortisol may play a role in the observed increases in gill Na^+,K^+-ATPase activity. In *F. heteroclitus* treatment with cortisol increases gill Na^+,K^+-ATPase activity in hypophysectomized fish (Pickford *et al.,* 1970). In this species, plasma cortisol levels rise during the transfer from FW to SW (Jacob and Taylor, 1983). In *S. salar,* it has been reported that GH and to a lesser extent IGF-I act synergistically with cortisol to increase gill Na^+,K^+-ATPase activity (McCormick, 1996). If such a synergy exists in *F. heteroclitus,* it could explain the different gill Na^+,K^+-ATPase activities observed in GH- and IGF-I-treated fish with and without exposure to high salinity.

The pathway for the osmoregulatory effects of GH and IGF-I in *F. heteroclitus* is not known. GH could act by itself on osmoregulatory organs or, according to the somatomedin hypothesis, IGF-I could mediate some or all of the physiological action of GH (see McCormick, 1995). McCormick *et al.* (1991) showed that rbIGF-I was more effective than oGH in reducing plasma osmolality following transfer of *O. mykiss* from BW (12 ppt) to SW (29 ppt). This difference also has been observed in *F. heteroclitus* treated with oGH or rbIGF-I after transfer from BW (10 ppt, 320 mOsm/kg H_2O) to SW (35 ppt, 1120 mOsm/kg H_2O). In *O. mykiss,* rbIGF-I stimulates gill Na^+,K^+-ATPase activity *in vitro* only in fish pretreated with oGH (Madsen and Bern, 1993). In salmonids, transfer to SW increases GH levels (Collie *et al.*, 1989; Boeuf *et al.,* 1989), and GH, in turn, could stimulate IGF-I production in the liver or osmoregulatory organs. GH could also sensitize chloride cells to exogenous and/or endogenous IGF-I, as suggested by previous investigators (McCormick *et al.,* 1991; Madsen and Bern, 1993). If plasma GH concentration increased during transfer to SW in *F. heteroclitus,* GH could result in a similar potentiation of the physiological actions of IGF-I. In accordance with this possibility, the present results show cooperation between oGH and rbIGF-I in improving gill Na^+,K^+-ATPase activity and salinity tolerance (Fig. 4).

Previous studies have shown that injection of insulin induces hypoglycemia in teleosts (Skyrud *et al.,* 1989; McCormick *et al.,* 1991). The lethal effect of higher doses of insulin observed in *F. heteroclitus* can be ascribed to hypoglycemia induced by this hormone. In brook trout (*Salvelinus fontinalis*) a single injection of rhIGF-I (2 μg/g body wt) results in hypoglycemia 24–72 h after injection (Skyrud *et al.,* 1989). However, rainbow trout (*O. mykiss*) treated with rbIGF-I (0.05 and 0.2 μg/g body wt) 48 h before transfer from BW (12 ppt) to SW (29 ppt) showed a clear plasma hyperglycemia 24 h after transfer (McCormick *et al.,* 1991). In the present study using a similar experimental protocol, plasma glucose levels were not significantly affected by rbIGF-I (Table 2). Similarly, fish treated with lower doses of oGH (0.25 and 0.5 μg/g body wt) did not show any change in plasma glucose levels (Table 2). However, higher doses of oGH resulted in increased plasma glucose (Fig. 3) in addition to a hypoosmoregulatory effect (Fig. 2). Similar effects of GH on plasma glucose have been found in *O. mykiss* and *O. mossambicus* and are due in part to stimulation of gluconeogenesis (Leung *et al.,* 1991; O'Connor *et al.,* 1993).

In *O. mykiss,* two distinct IGF cDNA sequences have been cloned from the liver (Chen *et al.,* 1994). Mammalian IGF-I has a 80% sequence homology with coho salmon IGF-I (Cao *et al.,* 1990), and the biological potency of mammalian and salmon IGF-I is nearly identical (Moriyama *et al.,* 1993). The amino acid sequence of rainbow trout IGF-II is similar to human IGF-II (78%); thus the use of human recombinant IGF-II should be valid in understanding the physiological actions of IGF-II in fish (Chan and Steiner, 1994). In *O. mykiss* levels of IGF-II mRNA in gill and other tissues (brain, kidney, muscle, spleen, and pylori) were higher than those of IGF-I mRNA, and a role for IGF-II has been suggested in these tissues (Chen *et al.,* 1994). The failure of rhIGF-II to improve hypoosmoregulatory capacity suggests that this hormone has no effect on monovalent ion secretion in *F. heteroclitus.* However,

several reasons could explain this negative effect of IGF-II treatment (see below) and it is possible that the experimental design was not appropriate to discern these effects.

In mammals IGF-I and IGF-II bind to their own receptors and also may bind, with low affinity, to the other receptors (LeRoith *et al.*, 1995). In fish IGF-I receptors have been characterized but it is not known to which type of receptor IGF-II peptide binds (IGF-I receptor and / or a specific receptor) (Elies *et al.*, 1996). It is possible that human IGF-II may not interact with *F. heteroclitus* IGF-II receptors. In addition, IGF-binding proteins (IGFBPs) occur in mammals. These proteins bind circulating IGF-I or IGF-II to affect their biological activities (Jones and Clemmons, 1995). IGFBPs have also been reported in fish (Kelley *et al.*, 1992; Siharath *et al.*, 1995). In these studies, IGF-I binding analysis was examined but there is no specific information about binding of IGF-II to IGFBPs in fish. However, rat IGF-II is about 50 times less potent than bovine IGF-I in stimulating sulfate uptake by eel cartilage (Duan and Hirano, 1990). A similar difference in IGF-I and IGF-II activity on osmoregulatory systems could explain the lack of effect of rhIGF-II treatment in the present study.

Salmonid fish provided some of the first information on the influence of the GH / IGF-I axis on osmoregulation in teleost fishes, and it was suggested that this effect was related to their anadromous life history in which seawater entry is associated with higher growth rates. The influence of the GH on hypoosmoregulation may, however, be restricted to salmonids. However, recent studies on tilapia (see Introduction) and the present study on *F. heteroclitus* indicate that this physiological action of GH is not restricted to salmonids or anadromous fishes and occurs in three widely separated families of teleosts (Salmonidae, Cyprinodontidae, Cichlidae). Although this represents a very small number of teleost species, it does suggest that the influence of the GH / IGF-I axis on osmoregulation may be more widespread than currently appreciated.

The present study suggests a role of GH and IGF-I, but not IGF-II and insulin, in seawater acclimation of *F. heteroclitus.* Long-term study of the osmoregulatory actions of GH, IGF-I, and other osmoregulatory hormones would be useful in advancing our understanding of the role and mechanism(s) of action of these hormones in osmotic regulation in *F. heteroclitus.* In addition, information on circulating plasma levels and tissue-specific expression of the insulin family of peptides during seawater acclimation of killifish and other species is necessary.

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