

# Effects of Growth Hormone and Insulin-like Growth Factor I on Salinity Tolerance and Gill Na<sup>+</sup>, K<sup>+</sup>-ATPase in Atlantic Salmon (*Salmo salar*): Interaction with Cortisol

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The potential roles of growth hormone (GH) and insulin-like growth factor I (IGF-I) in seawater (SW) acclimation of juvenile Atlantic salmon (*Salmo salar*) were examined. Compared to controls, fish in 12 ppt seawater given one or three injections (2–6 days) of GH (ovine, 0.2  $\mu\text{g} \cdot \text{g}^{-1}$ ) or IGF-I (recombinant bovine, 0.05–0.2  $\mu\text{g} \cdot \text{g}^{-1}$ ) had significantly greater salinity tolerance as judged by lower plasma sodium, osmolality, and muscle moisture content following transfer to 34 ppt. Single injections of GH and IGF-I in fish in fresh water failed to improve salinity tolerance following transfer to 25 ppt SW. Treatment of fish in 12 ppt with GH or IGF-I for 2–6 days did not increase gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity, but treatment with GH prevented decreases in gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity that occurred in controls following transfer to 34 ppt seawater. Fish in fresh water administered GH by implants (5.0  $\mu\text{g} \cdot \text{g}^{-1}$ ) or osmotic minipumps (0.5  $\mu\text{g} \cdot \text{g}^{-1}\text{day}^{-1}$ ) for 7–14 days had greater gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity and salinity tolerance than controls. IGF-I administered by implants (0.5–1.0  $\mu\text{g} \cdot \text{g}^{-1}$ ) or osmotic minipumps (0.1  $\mu\text{g} \cdot \text{g}^{-1}\text{day}^{-1}$ ) for 4–14 days did not increase salinity tolerance or gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity. Cortisol implants (50  $\mu\text{g} \cdot \text{g}^{-1}$ ) also increased gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity and salinity tolerance after 14 days, and in combination with GH had a synergistic effect. Although IGF-I and cortisol implants had no significant effect after 7 days, in combination they significantly increased gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity.

The results indicate that GH and cortisol can increase salinity tolerance and gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity of Atlantic salmon and together act in synergy. Although IGF-I can increase salinity tolerance in short-term treatments (2–6 days) in 12 ppt, it is less effective than GH in increasing salinity tolerance and gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity in long-term treatments (7–14 days) and in interacting with cortisol. © 1996 Academic Press, Inc.

The acquisition of salinity tolerance is an important developmental event in anadromous salmonids. In many salmonids increased salinity tolerance occurs as part of the parr–smolt transformation, a process preparatory for downstream migration and seawater entry. Substantial physiological and biochemical changes occur in the gill, gut, and kidney during the parr–smolt transformation that result in increased salinity tolerance (McCormick and Saunders, 1987; Hoar, 1988). Prominent among these are increases in gill Na<sup>+</sup>, K<sup>+</sup>-ATPase, an ion-translocating enzyme involved in sodium and chloride secretion by the gill.

Although the circulating levels of a number of hormones are altered during the parr–smolt transformation, growth hormone (GH) and cortisol have been most directly implicated in the control of hypoosmoregulatory ability. Salinity tolerance and gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity of Atlantic salmon are increased by treatment with exogenous growth hormone (Boeuf *et*

*al.*, 1990) and cortisol (Bisbal and Specker, 1991). Changes in circulating hormone levels and receptors also support a role of GH and cortisol in seawater acclimation of salmonids (see reviews by Specker, 1982; Sakamoto *et al.*, 1993; McCormick, 1995). Recent work with rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*O. kisutch*) suggests that insulin-like growth factor I (IGF-I) mediates at least some of the osmoregulatory actions of GH (McCormick *et al.*, 1991; Sakamoto and Hirano, 1993; Madsen and Bern, 1993). The present study was undertaken to further examine the influence of GH and IGF-I on salinity tolerance, to examine the interaction of the GH/IGF-I axis with cortisol, and to investigate gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity as a potential physiological target of these hormones.

## MATERIALS AND METHODS

### Animals

Juvenile Atlantic salmon (*Salmo salar*) were obtained from the White River National Fish Hatchery (Bethel, VT) and brought to the Anadromous Fish Research Center (Turners Falls, MA). Fish were maintained in 1-m-diameter tanks with fresh water at a flow rate of 4 liters  $\cdot$  min<sup>-1</sup> under natural photoperiod and fed to satiation twice daily (Zeigler Bros., Gardners, PA). In experiments conducted in fresh water, fish were maintained under the above conditions, with the exception that food was withheld throughout the study. In experiments in seawater, fish were held in similar tanks with recirculation, particle and chemical (activated charcoal) filtration, and continuous aeration. Salinity was adjusted with sea salt and checked with a sodium-sensitive electrode prior to introducing fish. Unless stated otherwise, all experiments were conducted with 25- to 70-g juveniles between September and December at 9–12°. For experiments in 12 ppt, fish were kept at this salinity for 3 days prior to injection.

### Hormones

Ovine GH (NIADDK-oGH-15) was obtained from the National Institutes of Health (Bethesda MD). Recombinant bovine IGF-I was provided by Monsanto

Corporation (St. Louis, MO). This recombinant mammalian hormone has nearly identical biological activity as recombinant salmon IGF-I in an *in vitro* assay for sulfate incorporation (Moriyama *et al.*, 1993). For saline injections the protocol outlined by (McCormick *et al.*, 1991) was followed. Hormones were dissolved in salmon Ringers' solution just prior to use. Fish were anesthetized (100 mg  $\cdot$  liter<sup>-1</sup> MS-222, pH = 7.0) and injected intraperitoneally (5  $\mu$ l  $\cdot$  g<sup>-1</sup> body weight). Administration of GH and IGF-I with osmotic minipumps (Model 1003D, Alza, Palo Alto, CA) followed the protocol of McCormick *et al.* (1992). Hormones were dissolved in salmon Ringers' solution and 100  $\mu$ l was loaded into each minipump. In experiments examining interactions of GH and IGF-I with cortisol, hormones were dissolved in 1:1 vegetable oil:shortening as outlined in (Specker *et al.*, 1994). Hormone suspensions were warmed to 40° and injected intraperitoneally (5  $\mu$ l  $\cdot$  g<sup>-1</sup> body weight).

### Analytical Methods

At the time of sampling fish were anesthetized as above and fork length to the nearest mm and weight to the nearest 0.1 g were recorded. Blood was drawn from the caudal vessels into a 1-cc ammonium heparinized syringe, centrifuged at 3000g for 5 min at 4°, and plasma aliquots were stored at -80°. Four to six gill filaments were severed above the septum, placed in 100  $\mu$ l of ice-cold SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3), and frozen at -80° within 30 min.

$\text{Na}^+$ ,  $\text{K}^+$ -ATPase activities were determined using the microassay method of McCormick (1993). Gill tissue was homogenized in 125  $\mu$ l of SEID (SEI buffer with 0.1% deoxycholic acid) and then centrifuged at 2000g for 30 sec. Duplicate 10- $\mu$ l homogenate samples were added to 200  $\mu$ l assay mixture with and without 0.5 mM ouabain in 96-well microplates at 25° 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  $\mu$ mol ADP  $\cdot$  mg protein<sup>-1</sup>  $\cdot$  hr<sup>-1</sup>.

Plasma glucose was measured by enzymatic coupling with hexokinase and glucose-6-phosphate dehydrogenase (Stein, 1963). Plasma osmolality was mea-

sured with a vapor pressure osmometer (Wescor 5500, Logan, UT). Plasma sodium, potassium, and calcium were measured using ion-selective electrodes (AVL 984-S, Roswell GA). Muscle moisture content was measured by dissecting and weighing 0.5–1.0 g of epaxial white muscle (just below and anterior to the dorsal fin) and then drying to a constant weight at 60°.

### Statistics

The nonparametric Kruskal–Wallis test was used to determine the statistical significance of single hormone treatments. Two-way analysis of variance of ranks was used to test the significance of hormone combinations. Dunnett's test of ranks was used for multiple comparisons to the control group. The Newman–Keuls test of ranks was used for pairwise comparison of individual treatments. Unless otherwise stated the probability for establishing statistical significance was  $P < 0.05$ .

## RESULTS

In initial experiments on the short-term effects of GH and IGF-I, juvenile Atlantic salmon were kept in 12 ppt for 3 days prior to injection; 48 hr after treatment fish were transferred to 34 ppt seawater and then

bled 24 hr later. A single injection of GH ( $0.2 \mu\text{g} \cdot \text{g}^{-1}$ ) or IGF-I ( $0.1\text{--}0.2 \mu\text{g} \cdot \text{g}^{-1}$ ) increased salinity tolerance as judged by lower plasma sodium concentrations relative to controls following transfer to 34 ppt seawater (Fig. 1) Gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity increased 20% after GH treatment and transfer to 34 ppt. Although IGF-I at the highest doses also increased gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity relative to controls (10–15%, Fig. 1), this effect was not statistically significant. IGF-I at the lowest dose ( $0.05 \mu\text{g} \cdot \text{g}^{-1}$ ) resulted in a significant decrease in gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity. Doses of IGF-I greater than  $0.2 \mu\text{g} \cdot \text{g}^{-1}$  resulted in aberrant swimming behavior, hypoglycemia, and death.

Fish in 12 ppt were given a total of three injections of GH ( $0.2 \mu\text{g} \cdot \text{g}^{-1}$ ) and IGF-I ( $0.1 \mu\text{g} \cdot \text{g}^{-1}$ ) every other day. There was no effect of these treatments on plasma osmolality, sodium, potassium, calcium, glucose, muscle moisture, or gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity in 12 ppt (Table 1). Following transfer to 34 ppt, however, multiple injections of GH and IGF-I resulted in significantly lower plasma osmolality, sodium, and calcium and higher muscle moisture content. As with a single injection, gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity of GH-treated fish was significantly higher than controls; IGF-I had an intermediate effect but was not significantly different from controls. Gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity and muscle moisture significantly decreased in controls after transfer to 34 ppt whereas all other variables increased ( $P < 0.05$ , Kruskal–Wallis test, Table 1).

TABLE 1  
The Effect of Three Injections of IGF-I ( $0.1 \mu\text{g} \cdot \text{g}^{-1}$ ) and GH ( $0.2 \mu\text{g} \cdot \text{g}^{-1}$ ), Every Other Day, on Physiological Parameters in 12 ppt and after 24 hr in 34 ppt

		Control	IGF-I	GH
Plasma osmolality (mosm/liter)	12 ppt	303 ± 3	305 ± 1	307 ± 2
	34 ppt	373 ± 6	360* ± 3	347* ± 2
Plasma sodium (mM)	12 ppt	163 ± 2	161 ± 1	164 ± 1
	34 ppt	200 ± 3	193* ± 2	184* ± 2
Plasma potassium (mM)	12 ppt	1.27 ± 0.10	1.27 ± 0.17	1.21 ± 0.10
	34 ppt	1.77 ± 0.15	1.38 ± 0.11	1.77 ± 0.16
Plasma calcium (mM)	12 ppt	1.55 ± 0.01	1.53 ± 0.03	1.59 ± 0.02
	34 ppt	1.95 ± 0.02	1.86* ± 0.01	1.84* ± 0.02
Plasma glucose (mM)	12 ppt	3.7 ± 0.2	4.8 ± 0.2	4.3 ± 0.1
	34 ppt	7.1 ± 0.3	7.3 ± 0.6	6.8 ± 0.5
Muscle moisture (%)	12 ppt	77.2 ± 0.2	77.2 ± 0.3	77.3 ± 0.2
	34 ppt	73.9 ± 0.3	75.0* ± 0.4	75.7* ± 0.2
Gill $\text{Na}^+$ , $\text{K}^+$ -ATPase ( $\mu\text{mol} \cdot \text{mgprot}^{-1} \cdot \text{h}^{-1}$ )	12 ppt	2.1 ± 0.2	1.9 ± 0.2	1.9 ± 0.2
	34 ppt	1.5 ± 0.1	2.0 ± 0.3	2.2* ± 0.2

Note. Values are means ± standard errors ( $n = 8\text{--}10$  per group). Asterisk indicates significant difference from the control group in 34 ppt ( $P < 0.05$ ).

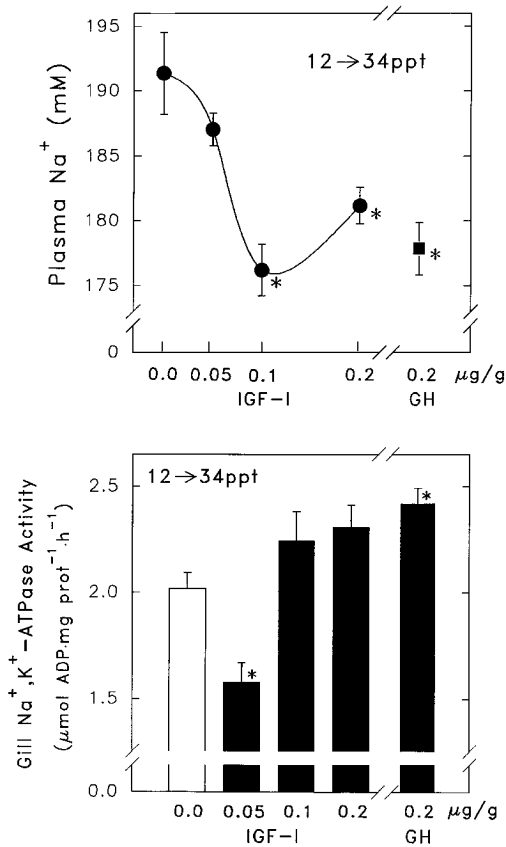


FIG. 1. Influence of a single injection of IGF-I or GH on plasma sodium (top) and gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity (bottom) in juvenile Atlantic salmon 24 hr after transfer to 34 ppt. Fish were held in 12 ppt for 3 days prior to hormone injection and then transferred to 34 ppt 48 hr after injection. Values are means  $\pm$  standard errors ( $n = 8$ –16 per group). Asterisk indicates significant difference from the control group ( $P < 0.05$ , Dunnett test).

To compare the response in fresh water to that in 12 ppt (above) a single injection of IGF-I ( $0.1 \mu\text{g} \cdot \text{g}^{-1}$ ) or GH ( $0.2 \mu\text{g} \cdot \text{g}^{-1}$ ) was administered in fresh water and fish were transferred to 25 ppt 48 hr later. Plasma sodium after 24 hr in seawater was  $206 \pm 3$  (control),  $199 \pm 4$  (GH), and  $198 \pm 2$  (IGF-I) and did not differ significantly among the groups. Multiple injection of GH ( $0.5 \mu\text{g} \cdot \text{g}^{-1}$ ) in fresh water (FW) over a 2-week period resulted in a doubling of gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity and a significant increase in salinity tolerance (Table 2). An identical injection protocol with IGF-I ( $0.15 \mu\text{g} \cdot \text{g}^{-1}$ ) did not affect gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity or salinity tolerance.

Administration of GH by osmotic minipump for 14 days in fish in fresh water resulted in significantly

greater salinity tolerance and gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity (Table 3). In contrast, IGF-I treatment had a significantly detrimental effect on salinity tolerance and no effect on gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity (Table 3).

Administration of GH and IGF-I in combination with cortisol was accomplished by suspending hormones in 1:1 vegetable oil:shortening. GH treatment ( $5 \mu\text{g} \cdot \text{g}^{-1}$ ) resulted in significant increases in gill Na<sup>+</sup>, K<sup>+</sup>-ATPase after 7 and 14 days of treatment. Cortisol significantly stimulated gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity only after 14 days of treatment. After both 7 and 14 days of treatment, GH and cortisol in combination resulted in significantly higher levels than those seen in controls ( $P < 0.05$ , Dunnett test) or with either hormone alone ( $P < 0.05$ , Newman-Keuls test, Fig. 2).

After 7 days IGF-I ( $0.5 \mu\text{g} \cdot \text{g}^{-1}$ ) and cortisol ( $50 \mu\text{g} \cdot \text{g}^{-1}$ ) did not individually increase gill Na<sup>+</sup>, K<sup>+</sup>-ATPase, but in combination resulted in significantly higher levels than seen in controls ( $P < 0.05$ , Dunnett test) or with either hormone alone ( $P < 0.05$ , Newman-Keuls test; Fig. 2). There was no significant effect of IGF-I at Day 14, nor was there a significant interaction with cortisol ( $P > 0.2$ , two-way ANOVA).

After 14 days of hormone treatment both GH and cortisol significantly decreased plasma sodium relative to controls following exposure to seawater ( $P < 0.05$ , two-way ANOVA, Fig. 3). There was no significant difference between the effect of GH and cortisol alone and that induced by combining both hormones ( $P > 0.1$ , Newman-Keuls test). After 14 days of treatment IGF-I by itself had no effect on salinity tolerance, nor was there a significant interaction with cortisol ( $P > 0.2$ , two-way ANOVA, Fig. 3).

## DISCUSSION

Previous work on brown trout (*Salmo trutta*) demonstrated that exogenous GH and cortisol could each increase gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity, chloride cell numbers, and salinity tolerance, and together these hormones act synergistically (Madsen, 1990). The present study indicates that a similar response to individual hormones and synergy occurs in Atlantic salmon. Cortisol can act directly on gill tissue to increase gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity, whereas *in vitro* actions of GH on gill tissue have not yet been demon-

**TABLE 2**  
The Effect of Six Injections of IGF-I ( $0.15 \mu\text{g} \cdot \text{g}^{-1}$ ) and GH ( $0.5 \mu\text{g} \cdot \text{g}^{-1}$ ), Every Other Day, on Physiological Parameters in Fresh Water (FW, 0 ppt) and after 48 hr in 30 ppt

		Control	IGF-I	GH
Plasma sodium (mM)	FW	157 ± 1	157 ± 1	154 ± 2
	30 ppt	193 ± 3	199 ± 5	162* ± 2
Plasma potassium (mM)	FW	1.07 ± 0.10	0.70* ± 0.06	1.10 ± 0.14
	30 ppt	2.09 ± 0.17	2.53 ± 0.17	1.50 ± 0.14
Plasma calcium (mM)	FW	1.53 ± 0.02	1.63 ± 0.05	1.51 ± 0.04
	30 ppt	1.36 ± 0.03	1.38 ± 0.04	1.12* ± 0.06
Muscle moisture (%)	FW	76.9 ± 0.1	76.7 ± 0.2	77.2 ± 0.1
	30 ppt	73.6 ± 0.3	73.3 ± 0.4	77.2* ± 0.2
Gill Na <sup>+</sup> , K <sup>+</sup> -ATPase ( $\mu\text{mol} \cdot \text{mg prot}^{-1} \cdot \text{h}^{-1}$ )	FW	1.7 ± 0.1	2.0 ± 0.2	3.4* ± 0.2
	30 ppt	1.6 ± 0.1	2.0 ± 0.2	2.5* ± 0.2

*Note.* Values are means ± standard errors ( $n = 8$  per group). Asterisk indicates significant difference from the control group in 30 ppt ( $P < 0.05$ ).

strated (McCormick, 1995). One possible route of action of GH is through IGF-I, which has been shown to increase gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity of coho salmon *in vitro* when preceded by *in vivo* GH treatment (Madsen and Bern, 1993). In the present study with Atlantic salmon *in vivo* administration of IGF-I by itself did not result in significant increases in gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity. Another possible pathway for GH to act on the gill is through changes in tissue responsiveness to cortisol. Shrimpton *et al.* (1995) found that GH treatment of coho salmon results in increased numbers of gill cortisol receptors, which may explain both the actions of growth hormone by itself and its synergy with cortisol.

The present study demonstrated an interaction between IGF-I and cortisol in the regulation of gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity (Fig. 2), though this interaction is clearly less effective than that between GH and cortisol. The mechanism for this interaction is unclear. Work with mammals suggests that several growth fac-

tors (including IGF-I) can interact with steroid receptors, even in the absence of steroid binding (Ignar-trowbridge *et al.*, 1993). Recent studies indicate that both GH and IGF-I affect the binding of cortisol to its receptor in gill tissue of Atlantic salmon (J. M. Shrimpton and S. D. McCormick, unpublished results).

As in previous studies on rainbow trout (McCormick *et al.*, 1991), a single injection of GH or IGF-I in Atlantic salmon held in 12 ppt increases their capacity to regulate ions after exposure to higher salinities. This effect of a single injection on salinity tolerance is not observed in fish maintained in fresh water. Collie *et al.* (1989) found that prior exposure to dilute seawater enhanced the ability of GH to increase salinity tolerance of rainbow trout. High circulating levels of prolactin in fresh water may have been responsible for the inefficacy of IGF-I and GH treatment in fresh water. Madsen and Bern (1992) reported an antagonism between the effects of prolactin and growth hormone on salinity tolerance of rainbow trout and brown trout (S.

**TABLE 3**  
The Effect of Treatment with IGF-I ( $0.1 \mu\text{g} \cdot \text{g}^{-1} \text{day}^{-1}$ ) and GH ( $0.5 \mu\text{g} \cdot \text{g}^{-1} \text{day}^{-1}$ ) Administered to Juvenile Atlantic Salmon in Osmotic Minipumps

	Control	IGF-I	GH
Plasma sodium (mM)	184 ± 2	200* ± 2	159* ± 1
Plasma potassium (mM)	3.22 ± 0.28	3.04 ± 0.13	2.91 ± 0.28
Plasma calcium (mM)	1.78 ± 0.03	1.91* ± 0.04	1.62* ± 0.02
Gill Na <sup>+</sup> , K <sup>+</sup> -ATPase ( $\mu\text{mol} \cdot \text{mg prot}^{-1} \cdot \text{h}^{-1}$ )	1.1 ± 0.1	1.3 ± 0.2	2.1* ± 0.2

*Note.* Fish were kept in fresh water for 14 days and then transferred to 30 ppt for 48 hr. Values are means ± standard errors ( $n = 6$  per group). Asterisk indicates significant difference from the control group ( $P < 0.05$ ).

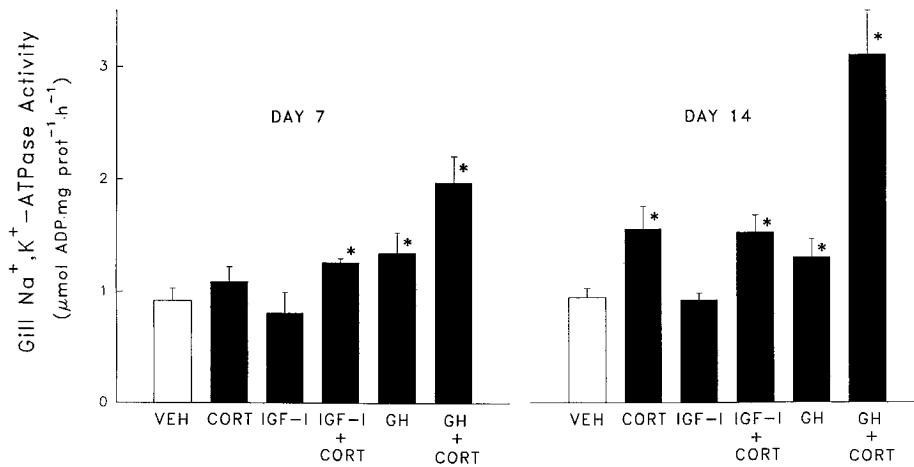


FIG. 2. Influence of GH ( $5 \mu\text{g} \cdot \text{g}^{-1}$ ), IGF-I ( $1 \mu\text{g} \cdot \text{g}^{-1}$ ), and cortisol ( $50 \mu\text{g} \cdot \text{g}^{-1}$ ) on gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity after 7 and 14 days of treatment. Hormones were suspended in 1:1 vegetable oil:shortening and injected into fish on Day 0. Fish were held in fresh water without feeding throughout the study. Values are means + standard errors ( $n = 6$  per group). Asterisk indicates significant difference from the control group ( $P < 0.05$ , Dunnett test). On Day 7, two-way ANOVA indicated a significant effect of cortisol ( $P = 0.005$ ), no effect of IGF-I, but an interaction between cortisol and IGF-I significant at the 94.5% confidence level. The combination of IGF-I and cortisol was significantly greater than either hormone alone ( $P < 0.05$ , Newman-Keuls test). On Days 7 and 14, two-way ANOVA indicated a significant effect of cortisol and GH ( $P < 0.05$ ), and the two hormones together were significantly greater than either hormone alone ( $P < 0.05$ , Newman-Keuls test). There was no significant effect of IGF-I on Day 14 and no significant interaction with cortisol ( $P > 0.1$ , two-way ANOVA).

*trutta*). Alternatively, other endocrine factors stimulated in 12 ppt may increase responsiveness to GH and IGF-I. Circulating levels of cortisol, GH, and IGF-I are all increased in salmonids during seawater acclimation (Young *et al.*, 1989; Sakamoto and Hirano, 1993; S. D. McCormick and S. Moriyama, unpublished results).

In Atlantic salmon kept in 12 ppt then transferred to 34 ppt seawater for 48 hr gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity was significantly higher in fish given GH than in controls (Fig. 1). Results from Table 1 indicate that this is the result of decreased gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity in control fish that does not occur in fish administered GH. Although similar results were obtained with IGF-I, the results were not statistically significant. These findings suggest that GH (and possibly IGF-I) may exert a type of "protective" effect on gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase following seawater exposure. It will be necessary to measure rates of synthesis and degradation of  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase to determine the mechanism of this effect. The relative importance of this protective effect is unclear, as a drop in gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity did not occur following transfer of controls from fresh water to 30 ppt (Table 2), although decreases did occur in the GH group. McCormick *et al.* (1989) observed decreases in gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity 1 and 2 days

after transfer of Atlantic salmon smolts from fresh water to seawater in spring. In the present study IGF-I at  $0.05 \mu\text{g} \cdot \text{g}^{-1}$  decreased gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity relative to controls (Fig. 1), further complicating interpretation of this effect.

Previous studies have demonstrated that GH treatment for a week or more can increase chloride cell number and gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity (Sakamoto *et al.*, 1993). Results of the present study indicate that GH (and IGF-I) can increase salinity tolerance 48 hr after injection, prior to increases in gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity. It may therefore be useful to introduce a concept of short-term and long-term actions of GH on osmoregulatory physiology of fish. In addition to their effects on chloride cell proliferation and gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase, the long-term effects of GH likely involve other osmoregulatory organs such as intestine and kidney. The possible rapid mechanism of actions of GH in ion regulation of fish have not been characterized. Relatively rapid actions of GH and IGF-I have been observed in mammalian renal physiology, including effects on renal plasma flow and glomerular filtration rate (Kopple and Hirschberg, 1990). IGF-I has been shown to stimulate sodium uptake in the toad urinary bladder within an hour of administration, independent of hemodynamic effects (Blazer-Yost and

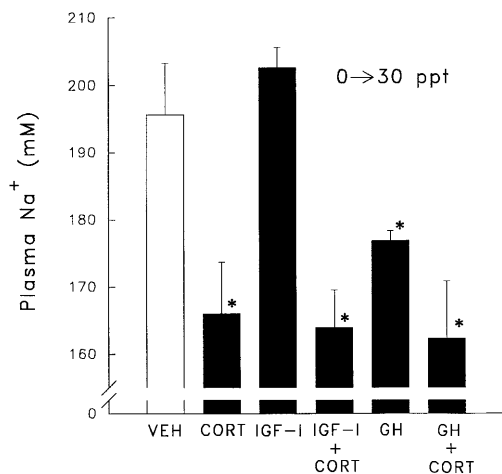


FIG. 3. Influence of GH ( $5 \mu\text{g} \cdot \text{g}^{-1}$ ), IGF-I ( $1 \mu\text{g} \cdot \text{g}^{-1}$ ), and cortisol ( $50 \mu\text{g} \cdot \text{g}^{-1}$ ) treatment for 14 days in fresh water on plasma sodium after transfer to 30 ppt for 48 hr. Hormones were suspended in 1:1 vegetable oil:shortening and injected into fish on Day 0. Fish were held in fresh water without feeding and then transferred to 30 ppt and sampled 48 hr later. Values are means + standard errors ( $n = 6$  per group). Asterisk indicates significant difference from the control group ( $P < 0.05$ , Dunnett test). Two-way ANOVAs indicated a significant effect of cortisol and GH ( $P < 0.05$ ) and no significant effect of IGF-I. The effect of GH and cortisol together was not significantly different from either hormone alone ( $P > 0.1$ , Newman-Keuls test).

Cox, 1988). Activation (e.g., phosphorylation) of existing proteins, including  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase, may account for some of the rapid actions of GH in fish. This kind of rapid *in situ* activation would not be detected by  $V_{\text{max}}$  measurements of gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity used in the present study. The protective effect of GH on  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase described above may also contribute to the short-term actions of these hormones.

None of the several methods of delivery of IGF-I used in the present study resulted in significant increases in gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity. One cannot eliminate the possibility that the methods of delivery were nonphysiological or somehow inappropriate for full *in vivo* function of IGF-I. For instance, binding proteins for IGF-I have been found in teleosts (Kelley *et al.*, 1992), and in mammals these proteins are important for full biological activity of IGF-I (Ballard *et al.*, 1993). However, osmotic minipumps as used in the present study have been used to demonstrate the ability of exogenous IGF-I to increase growth in coho salmon (McCormick *et al.*, 1992). In these studies growth of coho salmon was stimulated by IGF-I, whereas gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity was unaffected

(S. D. McCormick, unpublished results). Madsen and Bern (1993) found that *in vitro* stimulation of gill  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity by IGF-I was possible after *in vivo* treatment with GH, suggesting that responsiveness to IGF-I is GH-dependent. If it is assumed that physiological delivery occurred in the present studies, the results indicate that IGF-I can carry out the short-term hypoosmoregulatory actions of GH, but not all of the long-term effects. Such a conclusion is consistent with current understanding of growth regulation by GH and IGF-I. Although IGF-I carries out many growth-promoting actions of GH, GH still has important (direct) effects on target tissues, binding proteins, metabolism, and regulation of IGF-I itself (Green *et al.*, 1985; Isgaard, 1992). It remains to be determined to what extent the GH/IGF-I axes act in an analogous fashion on osmoregulatory physiology of teleosts.

Studies on rainbow trout pituitary cells in culture indicate that IGF-I is a potent inhibitor of GH release and acts additively with somatostatin (Perez-Sanchez *et al.*, 1992). In mammals negative feedback of IGF-I on GH release occurs at both the hypothalamus and the pituitary (Frohman *et al.*, 1992). IGF-I infusion in rats can inhibit pulsatile GH secretion (Abe *et al.*, 1983), although this has not been found in all studies (Carlsson *et al.*, 1989) and to date has not been examined in teleosts. It is possible that treatments with IGF-I in the present study resulted in lower circulating levels of GH, which might explain both the lack of effect of IGF-I and its negative impact on salinity tolerance when administered by osmotic minipump (Table 3). In future studies it would be useful to measure circulating GH levels following IGF-I administration.

The present study indicates that GH, IGF-I, and cortisol can each influence salinity tolerance of Atlantic salmon and lends support to a role for these hormones in the development of salinity tolerance during the parr-smolt transformation. Increased circulating levels of cortisol and GH have also been shown to occur during smolting of Atlantic salmon (Thorpe *et al.*, 1987; Prunet *et al.*, 1989), and liver IGF-I mRNA levels increase during smolting of coho salmon (Duan *et al.*, 1995). The interaction of these hormones has important implications for understanding environmental and endocrine control of the parr-smolt transformation in anadromous salmonids. It will be instructive to examine circulating levels of these hormones in the same study in order to determine how environmental

factors such as photoperiod and temperature may differentially affect these hormones and thereby influence the development of salinity tolerance during smolting. Simultaneous measurement of these hormones may also help point out deficiencies in artificial culture and release of smolts. The several hormones involved in smolting and their apparent complex interaction indicates that a number of environmental factors and stressors may affect the parr-smolt transformation by altering normal endocrine patterns and responses.

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