# Osmoregulatory actions of insulin-like growth factor-I in rainbow trout (*Oncorhynchus mykiss*)

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## ABSTRACT

The ability of insulin-like growth factor-I (IGF-I), insulin and GH to promote hypoosmoregulatory ability was examined in juvenile rainbow trout (*Oncorhynchus mykiss*). Following adaptation to 12 parts per thousand (p.p.t.) seawater for 5 days, fish were given a single injection of hormone or vehicle, then exposed to 29 p.p.t. for 24 h and examined for changes in plasma osmolarity, ions and glucose. Ovine GH (oGH; 0·2 μg/g) significantly improved the ability of rainbow trout to maintain plasma osmolarity and sodium levels following transfer to 29 p.p.t. seawater. Recombinant bovine IGF-I (0·01, 0·05 and 0·2 μg/g) also improved the hypoosmoregulatory ability of trout; the effect being dose-dependent and greater than that of oGH. Bovine insulin (0·01, 0·05 and 0·2 μg/g) had no statistically significant effect on plasma ions. The results indicate that IGF-I is a potential mediator of the action of GH in seawater adaptation of salmonids.
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## INTRODUCTION

Growth hormone (GH) stimulates both growth and seawater adaptability of several salmonid species (Komourdjian, Saunders & Fenwick, 1976; Clarke, Farmer & Hartwell, 1977; Miwa & Inui, 1985). Recently, Bolton, Collie, Kawauchi & Hirano (1987) and Collie, Bolton, Kawauchi & Hirano (1989) have demonstrated that the effect of GH on seawater adaptation of rainbow trout (*Oncorhynchus mykiss*) is independent of the growth-promoting actions of GH. The mechanism(s) by which GH exerts these osmoregulatory actions are not known.

Insulin-like growth factor-I (IGF-I) is an important mediator of the growth-promoting actions of GH in mammals (Daughaday & Rotwein, 1989), and possibly also in teleosts (Cao, Duguay & Plisetskaya *et al.* 1990). Growth hormone and IGF-I also affect electrolyte balance and kidney function in mammals (Hirschberg & Kopple, 1989). In the present study, we tested the possibility that IGF-I is a mediator of the osmoregulatory actions of GH in salmonids by examining whether recombinant bovine IGF-I increases the hypoosmoregulatory ability of rainbow trout.

## MATERIALS AND METHODS

### Fish

Juvenile rainbow trout (*Oncorhynchus mykiss*) were obtained from the Samegai Trout Hatchery (Siga prefecture, Japan) and reared in flow-through tanks supplied with fresh water at 13–15 °C and continually aerated. Lighting was by incident natural light and overhead fluorescent lights during the daytime only. Fish were fed an artificial diet (Masu No. 3, Oriental Kobo Kyogo, Japan) at approximately 1·5% body weight per day. Experiments were conducted between March and May.

### Hormones

Ovine GH (oGH; NIADDK-oGH-S12) was obtained from the National Institutes of Health, Bethesda,

 
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MD, U.S.A. Recombinant bovine IGF-I (rbIGF-I) was provided by the Monsanto Corporation (St Louis, MO, U.S.A.; Lot GTS-2) to Professor H. A. Bern (University of California, Berkeley, CA, U.S.A.). rbIGF-I content was greater than 95% as determined by high-pressure liquid chromatography, and contained no detectable IGF-II. Bovine insulin was obtained from Sigma Chemical Co. (108F-0547, St Louis, MO, U.S.A.). The vehicle for all hormones was salmon Ringers' solution (in mmol/l: 140 NaCl, 10 $NaHCO_3$, 2 $NaH_2PO_4$, 1 $MgSO_4$, 1 $CaCl_2$, 4 KCl) with 0·1% bovine serum albumin. Fish received intraperitoneal injections of 5 µl/g body weight.

### Experimental protocol

We have followed the protocol of Collie *et al.* (1989), who demonstrated that a single GH injection increases the hypoosmoregulatory ability of rainbow trout. Fish weighing between 11 and 21 g were randomly assigned to 30 litre static tanks containing 12 parts per thousand (p.p.t.) seawater (185 mmol Cl/l), prepared by diluting natural seawater (35 p.p.t.) with fresh water, and provided with continuous aeration and charcoal filtration. Water temperature was maintained at 14 °C throughout the experiment. Fish were fed for the first 3 days and starved thereafter. After 5 days in 12 p.p.t. seawater, fish were anaesthetized in 0·05% 2-phenoxyethanol, weighed, then injected intraperitoneally with vehicle or vehicle plus hormone, and returned to 12 p.p.t. seawater. Forty-eight hours after the injection they were transferred to identical 30 litre tanks containing 29 p.p.t. seawater (443 mmol Cl/l). Twenty-four hours later the fish were anaesthetized, and length and weight were measured. Then they were bled from the caudal vessels with (ammonia) heparinized syringes. Blood was immediately centrifuged and plasma stored at −80 °C.

### Analytical techniques

Plasma ion concentrations were determined by atomic absorption spectrophotometry (Hitachi 180-50). Plasma osmolarity was measured with a Wescor 5500 vapour pressure osmometer. Plasma glucose was determined by the hexokinase method (Schmidt, 1971). Statistical comparison of vehicle and single-dose hormone-treated groups was carried out using one-way analysis of variance (ANOVA). Statistical comparison of vehicle and hormone-treated groups at multiple doses was carried out using one-way ANOVA followed by Dunnett's test. The probability for establishing statistical significance was $\leqslant 0{\cdot}05$.

## RESULTS

Plasma osmolarity and sodium levels of rainbow trout exposed to 29 p.p.t. seawater for 24 h (Fig. 1) were substantially higher than for fish in 12 p.p.t. seawater. Compared with rainbow trout given a vehicle injection, a single injection of oGH (0·2 µg/g) significantly reduced plasma osmolarity and sodium level in rainbow trout transferred from 12 to 29 p.p.t. seawater (Fig. 1). Plasma calcium, magnesium and glucose levels were not affected by growth hormone treatment (Table 1).

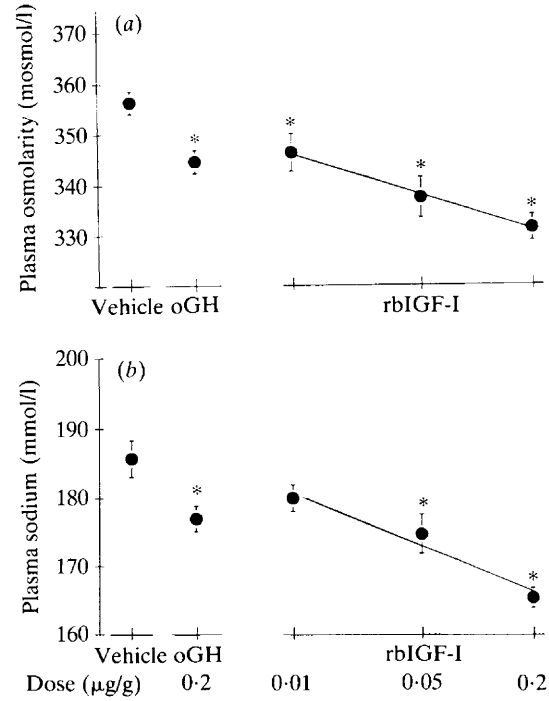


FIGURE 1. Effect of ovine GH (oGH) and recombinant bovine IGF-I (rbIGF-I; µg/g) on plasma (*a*) osmolarity and (*b*) sodium level in juvenile rainbow trout transferred from 12 to 29 parts per thousand (p.p.t.) seawater for 24 h. Fish were acclimated to 12 p.p.t. seawater and given a single intraperitoneal injection of either hormone or vehicle, and transferred to 29 p.p.t. seawater. Values are means ± S.E.M. ($n=10$). *$P<0{\cdot}05$ compared with control (one-way ANOVA followed by Dunnett's test). Regression lines include only the three doses of rbIGF-I (*a*: $r=0{\cdot}99$; *b*: $r=0{\cdot}98$).

A single injection of rbIGF-I resulted in a similar though more pronounced decrease in plasma osmolarity and sodium level (Fig. 1). Significant decreases relative to control were seen at doses of 0·05 and 0·2 µg/g; increased doses of rbIGF-I resulted in greater decreases in plasma osmolarity and sodium

TABLE 1. Effect of ovine GH (oGH) and recombinant bovine IGF-I (rbIGF-I) on plasma calcium, magnesium and glucose levels in rainbow trout transferred from 12 to 29 parts per thousand seawater for 24 h. Treatment was with a single intraperitoneal injection (μg/g) 48 h prior to transfer. Values are means ± S.E.M. ($n = 10$)

| | | | rbIGF-I | | |
|---|---|---|---|---|---|
| | **Control** | **oGH** (0·2) | 0·01 | 0·05 | 0·2 |
| Ca (mmol/l) | 3·0±0·1 | 3·2±0·1 | 3·6±0·1 | 3·5±01 | 3·3±0·1 |
| Mg (mmol/l) | 1·6±0·1 | 1·5±0·1 | 1·6±0·1 | 1·5±0·1 | 1·3±0·1 |
| Glucose (mmol/l) | 3·8±0·3 | 4·1±0·3 | 4·5±0·3 | 4·9±0·2* | 4·7±0·2* |

*$P < 0·05$ compared with control (one-way ANOVA followed by Dunnett's test).

TABLE 2. Effect of recombinant bovine IGF-I (rbIGF-I) and bovine insulin on mortality, plasma osmolarity, sodium, calcium, magnesium and glucose levels in juvenile rainbow trout transferred from 12 to 29 parts per thousand seawater for 24 h. Treatment was with a single intraperitoneal injection (μg/g) 48 h prior to transfer. Values are means ± S.E.M. ($n = 9$–10, except when mortalities occurred)

| | | | Bovine insulin | | |
|---|---|---|---|---|---|
| | **Control** | **rbIGF-I** (0·2) | 0·01 | 0·05 | 0·2 |
| Mortality | 0/10 | 0/9 | 0/10 | 3/9 | 7/10 |
| Osmolarity (mosmol/l) | 393±5 | 358±13* | 374±5 | 374±8 | 371±14 |
| Na (mmol/l) | 200±3 | 186±2* | 197±2 | 197±5 | 191±8 |
| Ca (mmol/l) | 3·3±0·1 | 2·9±0·2* | 3·5±0·1 | 3·3±0·1 | 3·3±0·1 |
| Mg (mmol/l) | 2·2±0·4 | 2·6±0·3 | 2·2±0·2 | 3·6±1·5 | 2·0±0·2 |
| Glucose (mmol/l) | 5·2±0·5 | 4·8±0·3 | 4·3±0·2 | 5·1±0·9 | 3·2±0·8 |

*$P < 0·05$ compared with control (one-way ANOVA followed by Dunnett's test).

levels, indicating a dose-dependent response (Fig. 1). Treatment with rbIGF-I resulted in an approximately two-fold greater decrease in plasma osmolarity and sodium levels than did oGH treatment at the same dose of 0·2 μg/g (14 versus 25 mosmol/l and 9 versus 20 mmol/l for oGH and rbIGF-I respectively).

Plasma calcium and magnesium levels were not significantly affected by rbIGF-I treatment (Table 1). Plasma glucose levels increased between 0·7 and 1·1 mmol/l (18–29%) relative to controls at all doses of rbIGF-I. These changes were statistically significant at the two higher doses.

There was no mortality in any of the control, oGH- or rbIGF-I-treated groups. Doses of rbIGF-I substantially higher than those used in the present experiments (> 0·4 μg/g) can result in mortality (S. D. McCormick, unpublished results).

A single injection of bovine insulin at the two highest doses (0·05 and 0·2 μg/g) resulted in significant mortality within 48 h after injection (Table 2), with higher mortality occurring at the higher dose. The increases in plasma osmolarity and in sodium, calcium and magnesium levels experienced by the control group as a result of transfer from 12 to 29 p.p.t. seawater were slightly greater in this experiment. As in the previous experiment, rbIGF-I (0·2 μg/g) significantly reduced plasma osmolarity and sodium levels relative to controls (Table 2); plasma calcium was also reduced.

Although there was some indication that plasma osmolarity was reduced by bovine insulin treatment, this effect was not statistically significant ($P = 0·07$, Table 2), and was much smaller in magnitude than the effect of rbIGF-I. Plasma levels of sodium ($P = 0·6$), calcium ($P = 0·09$), magnesium ($P = 0·4$) and glucose ($P = 0·2$) were not affected by insulin treatment.

## DISCUSSION

The cDNA of coho salmon IGF-I has recently been cloned (Cao *et al.* 1990) and the deduced amino acid sequence is identical with mammalian IGF-I in 56 of 70 residues. We have recently found that (recombinant) bovine IGF-I can increase the growth rate of coho salmon *in vivo* and increase sulphate incorporation by cartilage *in vitro* (S. D. McCormick, K. M. Kelley, P. I. Tsai, G. Young, R. S. Nishioka & H. A. Bern, unpublished results), the latter effect being ten times more potent than that of native coho salmon insulin. Although fish IGF-I is not currently available, the high sequence similarity of mammalian and

salmon IGF-I, and the ability to distinguish between mammalian insulin and IGF-I in a salmon bioassay indicate that exogenous treatment with mammalian IGF-I is a valid technique for exploring the function of IGF-I in fishes.

The present study confirms the previous findings of Bolton *et al.* (1987) and Collie *et al.* (1989), in which GH decreased plasma sodium levels during seawater adaptation of juvenile rainbow trout, and extends these findings to include plasma osmolarity. This effect of GH is highly specific, as salmon prolactin is ineffective under the same conditions (Bolton *et al.* 1987). In the present study, rbIGF-I was more effective than GH in reducing plasma osmolarity and sodium levels following exposure of rainbow trout to seawater (Fig. 1). The effect was strongly dose-dependent between 0·01 and 0·2 μg/g.

Control rainbow trout in the two experiments experienced different levels of absolute increase in plasma osmolarity and plasma sodium levels following transfer from 12 to 29 p.p.t. (Fig. 1 and Table 2). This may have been the result of different levels of stress, or some other uncontrolled factor, that varied between the experiments. Irrespective of these differences, however, rbIGF-I significantly reduced plasma osmolarity and sodium levels in both experiments, and the proportional effect of 0·2 μg rbIGF-I/g was approximately the same (Fig. 1 and Table 2).

Exposure of several salmonid species to seawater has been shown to result in transient (1–3 day) increases in circulating GH levels (Sweeting, Wagner & McKeown, 1985; Hasegawa, Hirano, Ogasawara *et al.* 1987; Sakamoto, Ogasawara & Hirano, 1990). It should be noted, however, that GH does not always increase following seawater adaptation of salmonids; Young, Björnnson, Prunet *et al.* (1989) and Rydevik, Borg, Haux *et al.* (1990) found no change in juvenile coho and Atlantic salmon respectively, and suggest that the lack of increase in GH may be related to the development of preparatory mechanisms for seawater entry that occur during the parr-smolt transformation. The turnover rate of GH is elevated for at least 4 days after transfer of adult rainbow trout from fresh water to seawater (Sakamoto *et al.* 1990). Increased GH (or GH turnover) induced by seawater exposure could result in increased circulating levels of IGF-I which could then act on distant osmoregulatory organs to induce increased hypoosmoregulatory ability. In this regard, Cao *et al.* (1990) have demonstrated that GH treatment of coho salmon results in increased liver IGF-I mRNA. Alternatively, GH could stimulate production of IGF-I at the target tissue where it would act in a paracrine or autocrine fashion.

In the present study, rbIGF-I was clearly more effective than bovine insulin in reducing plasma osmolarity and sodium levels after seawater exposure. Although exogenous insulin can affect electrolyte balance of eels (*Anguilla rostrata*), there is as yet no clear role of insulin in the osmoregulatory physiology of this species (Epple, 1987). Pancreatectomy results in poor seawater adaptation of eels, perhaps through an effect on intracellular amino acid content (Epple, 1987). Unfortunately there is little information on the possible osmoregulatory action of insulin in salmonids. Plasma insulin is known to increase during the parr-smolt transformation of coho salmon (Plisetskaya, Swanson, Bernard & Dickhoff, 1988), though such increases may be involved in the metabolic changes that occur at this stage of development. Blazer-Yost, Cox & Furlanetto (1989) found that both mammalian IGF-I and insulin stimulate sodium flux in toad urinary bladder, and that receptors specific for each hormone are present in the tissue.

After a bolus injection, free IGF-I in excess of that associating with binding proteins induces acute insulin-like effects in mammals (Froesch & Zapf, 1985), and probably in teleosts as well (Skyrud, Andersen, Aleström & Gautvik, 1989). Such an occurrence may explain the mortalities that occurred with doses of rbIGF-I greater than 0·4 μg/g. However, an insulin-like effect does not explain the results of lower doses, since IGF-I was clearly different from insulin in seawater-adapting actions and its effects on plasma glucose (rbIGF-I increased plasma glucose at low doses, whereas insulin tended to decrease it). Failure to observe a clear hypoglycaemic action of insulin in the present study may be the result of sampling 3 days after injection, a time-period that may be inappropriate for observing the acute actions of insulin on carbohydrate metabolism. This is supported by the observation that all mortality caused by exogenous insulin occurred within 2 days of injection.

Recent research with mammals suggests that IGF-I may mediate some (or all) of the renal actions of GH. GH treatment of hypophysectomized rats results in a 40% increase in kidney weight, a twofold increase in renal IGF-I mRNA and an increase in immunostainable IGF-I in the collecting duct (Rotwein, DeVol, Lajara *et al.* 1989). In addition to their potential effects on renal hyperplasia, GH and IGF-I can stimulate renal plasma flow and glomerular filtration rate in man (Hirschberg & Kopple, 1989). The effect of IGF-I occurs more rapidly than GH (as quickly as 20 min), consistent with the hypothesis that GH acts by stimulating the production and release of IGF-I.

Results of the present study suggest that IGF-I may be involved in seawater adaptation of salmonids. This should not be taken to indicate, however, that it is the sole mediator of the actions of GH in hypoosmoregulation, or that GH does not have direct effects of its own. GH-binding sites have been found in the gill and

kidney of salmonids (Fryer & Bern, 1979; Gray, Young & Bern, 1990; Yao, Niu, Le Gac & Le Bail, 1990; Sakamoto & Hirano, 1991). Cortisol has previously been shown to be important in seawater adaptation via the stimulation of gill $Na^+/K^+$-ATPase activity and chloride cell differentiation (Björnsson, Yamauchi, Nishioka *et al.* 1987; Richman & Zaugg 1987; McCormick & Bern, 1989; McCormick, 1990). Although relatively little is known of the functional relationship of GH and cortisol, there is some evidence that they are positively linked. Young (1988) found that GH enhanced the in-vitro response of coho salmon interrenal to adrenocorticotrophin, increasing cortisol production. Although GH is known to stimulate gill $Na^+/K^+$-ATPase activity *in vivo* (Björnsson *et al.* 1987; Richman & Zaugg, 1987), IGF-I does not appear to stimulate gill $Na^+/K^+$-ATPase activity either *in vitro* (McCormick, Dickhoff, Duston *et al.* 1991) or *in vivo* (S. D. McCormick, R. S. Nishioka & H. A. Bern, unpublished results). Further research may show that both cortisol and IGF-I mediate the effects of GH, and that all are important in the process of seawater adaptation of salmonids.

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