Prolactin Antagonizes the Seawater-Adaptive Effect of Cortisol and Growth Hormone in Anadromous Brown Trout (Salmo trutta)

Authors: Seidelin, Michel, and Madsen, Steffen S.

Source: Zoological Science, 14(2) : 249-256

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.14.249

ZOOLOGICAL SCIENCE **14**: 249–256 (1997)

Prolactin Antagonizes the Seawater-Adaptive Effect of Cortisol and Growth Hormone in Anadromous Brown Trout (*Salmo trutta*)

Michel Seidelin and Steffen S. Madsen

Institute of Biology, Odense University, Campusvej 55, DK-5230 Odense M, Denmark

ABSTRACT—Two experiments are described in which the interaction of prolactin, cortisol and growth hormone on hypoosmoregulation in the anadromous brown trout was studied. In experiment 1, fish at the postsmolt stage in freshwater (FW) were given four injections on alternate days of 5 μg cortisol (F)/g in combination with 0, 0.2, 1 or 2 μg ovine prolactin (oPRL)/g. Additional groups received 2 μg oPRL/g or saline as control. In experiment 2, FW parr were given four injections on alternate days with 5 μg F/g and 2 μg ovine growth hormone (oGH)/g in combination with 0, 0.2, 1 or 2 μg oPRL/g. Additional groups received 2 μg oPRL/g, 2 μg oGH/g, 5 μg F/g or saline as control. In both experiments, the fish were subjected to a 48 hr seawater (SW) challenge test 24 hr after the last injection. Muscle water content, plasma osmolality and ion levels, kidney and gill Na^+,K^+-ATPase activity were measured. In experiment 1, F-treated fish had better hypoosmoregulatory capacity than control fish as judged by a higher level of muscle water content and lower plasma osmolality after 48 hr in SW. All three doses of oPRL completely abolished this action of F. Gill Na^+,K^+-ATPase activity was stimulated by F and unaffected by oPRL at any dose. In experiment 2, oPRL impaired, whereas F and oGH (injected individually or together) improved performance in the 48 hr SW test relative to control fish, judged by plasma osmolality and muscle water content. Ovine PRL inhibited the combined action of F and oGH in a dose-related manner but could not completely counteract the combined effect of these hormones. F and oGH had additive stimulatory effects on gill Na^+,K^+-ATPase activity. This activation was counteracted by coinjection of oPRL in a dose-related manner but not below the level found in the F-treated fish. We conclude that exogenous oPRL inhibits the hypoosmoregulatory action of F. However, oPRL does not antagonize the F-dependent stimulation of gill Na^+,K^+-ATPase activity. The observed antagonism between PRL and F in SW-acclimation may therefore occur on a target different from gill Na^+,K^+-ATPase.

INTRODUCTION

In salmonids of the genera Salmo and Oncorhynchus, cortisol (F), growth hormone (GH), and prolactin (PRL) are believed to be key hormones in the development of seawater (SW)-tolerance during smoltification (e.g., Björnsson *et al.*, 1989; Young *et al.*, 1989). Both F and GH stimulate hypoosmoregulation by increasing gill chloride cell density and size and Na^+,K^+-ATPase activity *in vivo* (Richman and Zaugg, 1987; Madsen, 1990a,c; Bisbal and Specker, 1991; Almendras *et al.*, 1993; Boeuf *et al.*, 1994; Nonnotte and Boeuf, 1995). Gill Na^+,K^+-ATPase activity is stimulated by F *in vitro* but is unaffected by GH (McCormick *et al.*, 1991a). Madsen and Bern (1993) showed, however, that insulin-like growth factor I (IGF-I) may be the mediator for GH's stimulatory action *in vitro*.

PRL, on the other hand, is important for hyperosmoregulation in FW teleosts and is generally thought to regulate branchial ion-exchange by decreasing epithelial permeability (review by Hirano, 1986). PRL also decreases the ability of salmonids to hypoosmoregulate (Hasegawa *et al.*, 1986; Madsen and Bern, 1992; Boeuf *et al.*, 1994), and increases (*Salmo salar*: Boeuf *et al.*, 1994), decreases (*Oncorhynchus mykiss*: Madsen and Bern, 1992), or has no effect on (*Salmo trutta*: Madsen *et al.*, 1995) gill Na^+,K^+-ATPase activity. Recently, gill Na^+,K^+-ATPase α-subunit mRNA-levels were shown to increase in *S. trutta* after treatment with F, recombinant salmon (rs) GH or recombinant bovine (rb) IGF-I, whereas salmon (s) PRL had no effect (Madsen *et al.*, 1995). A few studies have emphasized the importance of endocrine interactions in the control of hypoosmoregulation in salmonids. Cortisol and the GH-IGF-I axis have additive effects on SW-tolerance and gill Na^+,K^+-ATPase activity *in vivo* (Björnsson *et al.*, 1987; Madsen, 1990b,c; McCormick, 1996), whereas GH and PRL have antagonistic effects on the development of hypoosmoregulatory ability (Madsen and Bern, 1992; Boeuf *et al.*, 1994). Interactions between PRL and F have not been described yet, and the aim of the present study was to investigate how oPRL affects the hypoosmoregulatory ability of *S. trutta* treated simultaneously with either F or F in

* Corresponding author: Tel. +45-66158600; FAX. +45-65930457.

combination with oGH.

MATERIALS AND METHODS

Fish maintenance

Immature anadromous brown trout of mixed sexes were obtained from the Haarkjaer Hatchery, Denmark, where they had been reared in ponds under natural photoperiod and temperature. For Experiment 1, 70 postsmolts, age 1+, weight 62.2 ± 1.5 g (mean ± SEM), were obtained in June 1995; for Experiment 2, 90 parr, age 0+, weight 24.7 ± 0.3 g (mean ± SEM), were obtained in September 1995. The fish were brought to Odense University and acclimated in 2000-l flow-through freshwater tanks (FW, 15°C), and artificial photoperiod (12:12, L:D). They were fed 2% body weight commercial trout pellets once daily. After 14 days in Experiment 1, and one month in Experiment 2 the fish were randomly sorted into groups of ten fish (7 and 9 groups in Exp. 1 and 2, respectively) and transferred to 400-l tanks. A group of untreated fish was left in one tank. Two groups were pooled in each of the other tanks and tagged by adipose fin clipping. Food was withheld from this day onwards.

Experimental protocol and hormone treatment

Hormone treatments were initiated four days after the fish were divided into groups. Prior to intraperitoneal injection, the fish were slightly anesthetized in 0.05% phenoxyethanol. All injections were repeated on alternate days in FW for a total of four injections per fish. The fish were then transferred to dilute seawater (SW, 15°C) 24 hr after the last injection and sampled after 48 hr. The salinity was 27.3 ppt and 24.8 ppt in Exp. 1 and 2, respectively. The following vehicle and hormones were used: saline (0.9% NaCl, 0.05% NaOH, 0.4% bovine serum albumin), oPRL (NIADDK-oPRL-19, Baltimore, MD), F (Na-hydrocortisone hemisuccinate, Sigma, St. Louis, MO), and oGH (NIADDK-oGH-15). All hormones were dissolved in vehicle immediately prior to injection. The fish were injected according to body weight with one of the following combinations and doses of hormones. *Experiment 1*: 1) none (untreated group), 2) saline, 3) 2 μg oPRL/g, 4) 5 μg F/g, 5) 5 μg F + 0.2 μg oPRL/g, 6) 5 μg F + 1 μg oPRL/g, 7) 5 μg F + 2 μg oPRL/g. The injection volume was 2 μl/g. *Experiment 2*: 1) none (untreated group), 2) saline, 3) 2 μg oPRL/g, 4) 2 μg oGH/g, 5) 5 μg F/g, 6) 5 μg F + 2 μg oGH/g, 7) 5 μg F + 2 μg oGH + 0.2 μg oPRL/g, 8) 5 μg F + 2 μg oGH + 1 μg oPRL/g, 9) 5 μg F + 2 μg oGH + 2 μg oPRL/g. The injection volume was 3 μl/g.

Sampling and analysis

The fish was stunned by a blow to the head, and blood was drawn from the caudal vessels into a heparinized syringe and immediately centrifuged at 5000 × g for 3 min. The fish was then decapitated. One second gill arch and the posterior part of the body kidney were dissected and placed in sucrose-EDTA-imidazole buffer (SEI; 300 mM sucrose, 20 mM EDTA, 50 mM imidazole, pH 7.3). All samples were immediately frozen in liquid nitrogen and stored at -80°C until analyzed. A piece of paraxial muscle was dissected and weighed for determination of muscle water content (MWC).

Plasma osmolality was analyzed on a micro vapor pressure osmometer (Wescor 5500, Logan, UT). Plasma sodium was determined by flame photometry (Instrumentation Laboratory 243, Italy). Plasma total calcium and total magnesium were analyzed by atomic absorption spectrophotometry (Perkin Elmer 2380, Mountain View, CA) in samples diluted with 0.1% La_2O_3. Plasma chloride was determined by coulometric titration (Radiometer CMT 10, Copenhagen). MWC was determined as percent weight loss after drying at 105°C for 3 days. Gill and kidney Na^+,K^+-ATPase activities were analyzed at 25°C by the method of McCormick (1993) using a plate reader (Ceres 900UV; Bio-Tek, Winooski, VT). Protein content was measured by the method of Lowry *et al.* (1951) modified for plate reader.

Statistics

Statistical differences among groups were analyzed using SYSTAT 5.03 (Systat, 1991, Evanston, IL). When necessary, transformation of data was done to meet the assumption of homogeneity of variances (Bartlett test). Differences were then analyzed by one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference Test ($P \leq 0.05$). Correlation analyses were performed on plasma osmolality versus muscle water content and osmolality versus gill Na^+,K^+-ATPase activity.

RESULTS

Experiment 1

Two saline- and one oPRL-injected fish died during hormone treatment in FW. After 48 hr in 27.3 ppt SW, plasma [Na^+], [Cl^-] (Fig. 1A, B) and osmolality (Fig. 2A) were affected similarly by hormone treatment. MWC (Fig. 2B) was inversely related to changes in plasma osmolality (linear correlation analysis: $r = 0.837$, $P < 0.001$). F treatment improved SW-acclimation (decreased plasma [Cl^-]), and oPRL treatment impaired SW-acclimation (increased plasma [Na^+], [Cl^-] and osmolality and decreased MWC). When coinjected with F, oPRL abolished the SW-adaptive effect of F. This effect was significant for plasma [Na^+], [Cl^-] and osmolality at the high dose of oPRL and for MWC at the low and medium doses of oPRL. Plasma total [Ca] and [Mg] (Fig. 1 C, D) were unaffected by hormone treatment.

Gill Na^+,K^+-ATPase activity (Fig. 2C) was stimulated by F but unaffected by oPRL treatment at any dose. Kidney Na^+,K^+-ATPase activity was significantly lower in the untreated control group than in the saline-injected control group (Fig. 2D) but otherwise unaffected by experimental treatment.

Experiment 2

A few fish died during the experiment but there was no clear relation to treatment. After 48 hr in 27.3 ppt SW, the saline-injected control fish had a poorer hypoosmoregulatory ability than the untreated control group as judged from a higher plasma osmolality and a lower MWC (Fig. 3A, B). As in Exp. 1, plasma osmolality and [Na^+] (Fig. 3C) were affected similarly by hormone treatment and MWC was negatively correlated with plasma osmolality (linear correlation analysis: $r = 0.956$, $P < 0.001$). As judged from plasma osmolality, [Na^+] and MWC, oGH and F treatment improved, and oPRL treatment impaired the overall hypoosmoregulatory performance. Ovine PRL antagonized the SW-adaptive effect of oGH+F treatment in a dose-related manner, with 1 and 2 μg oPRL/g being equipotent. Neither dose of oPRL was able to completely abolish the hypoosmoregulatory effect of oGH+F treatment.

Plasma total [Ca] (Fig. 3D) showed a different response to hormone treatment from plasma [Na^+] after 48 hr in SW. Treatment with F, oGH or oPRL alone did not alter plasma total [Ca]. The combined treatment of F and oGH, however, significantly decreased plasma total [Ca]. Ovine GH and F treatment significantly increased gill Na^+,K^+-ATPase activity (Fig. 3E), and when combined they had an additive stimulatory effect. Ovine PRL had no effect on gill Na^+,K^+-ATPase when

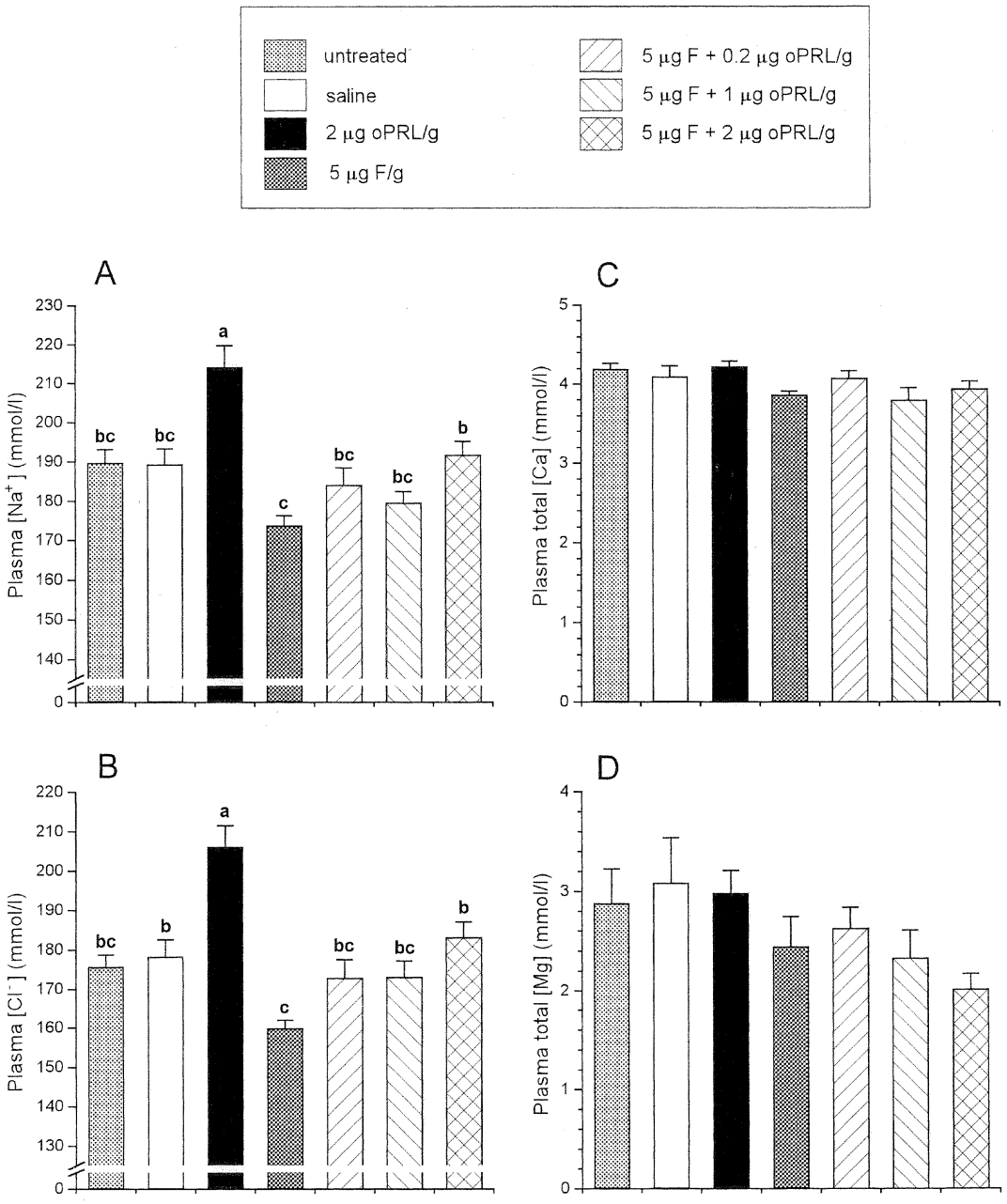

Fig. 1. Effect of various hormone treatments on plasma [Na^+] (**A**), [Cl^-] (**B**), total [Ca] (**C**) and total [Mg] (**D**) in *S. trutta* 48 hr after transfer to SW. Fish were given 4 injections of each dose in FW and transferred to 27.3 ppt SW one day after the last injection. Values are mean + SEM of 8-10 fish. Values with shared letters are not significantly different ($P > 0.05$). For plasma total [Ca] and total [Mg] none of the values is significantly different.

injected alone but counteracted the effect of oGH+F in a dose-related manner with maximal effect at 1 μg/g. In all the combined treatment groups, oPRL was unable to depress enzyme activity completely to the level of the saline-injected control.

In both experiments, plasma osmolality and gill Na^+,K^+-ATPase activity were nonlinear negatively correlated (Figs. 4A, B) and could be described by the following equations: Exp. 1: Osmolality = $356.3 + 553.7 * e^{-1.6*activity}$ ($r = 0.636$, $P < 0.001$). Exp. 2: Osmolality = $322.3 + 853.2 * e^{-1.3*activity}$ ($r = 0.793$, $P < 0.001$).

DISCUSSION

Several studies have shown that treatment with either F or GH improves SW-tolerance and hypoosmoregulation in salmonid teleosts. These effects were also evident in both of the present experiments (plasma [Na^+], [Cl^-] and osmolality and MWC; Figs. 1-3), and may be causally related to stimulated levels of gill Na^+,K^+-ATPase activity after F and GH treatment

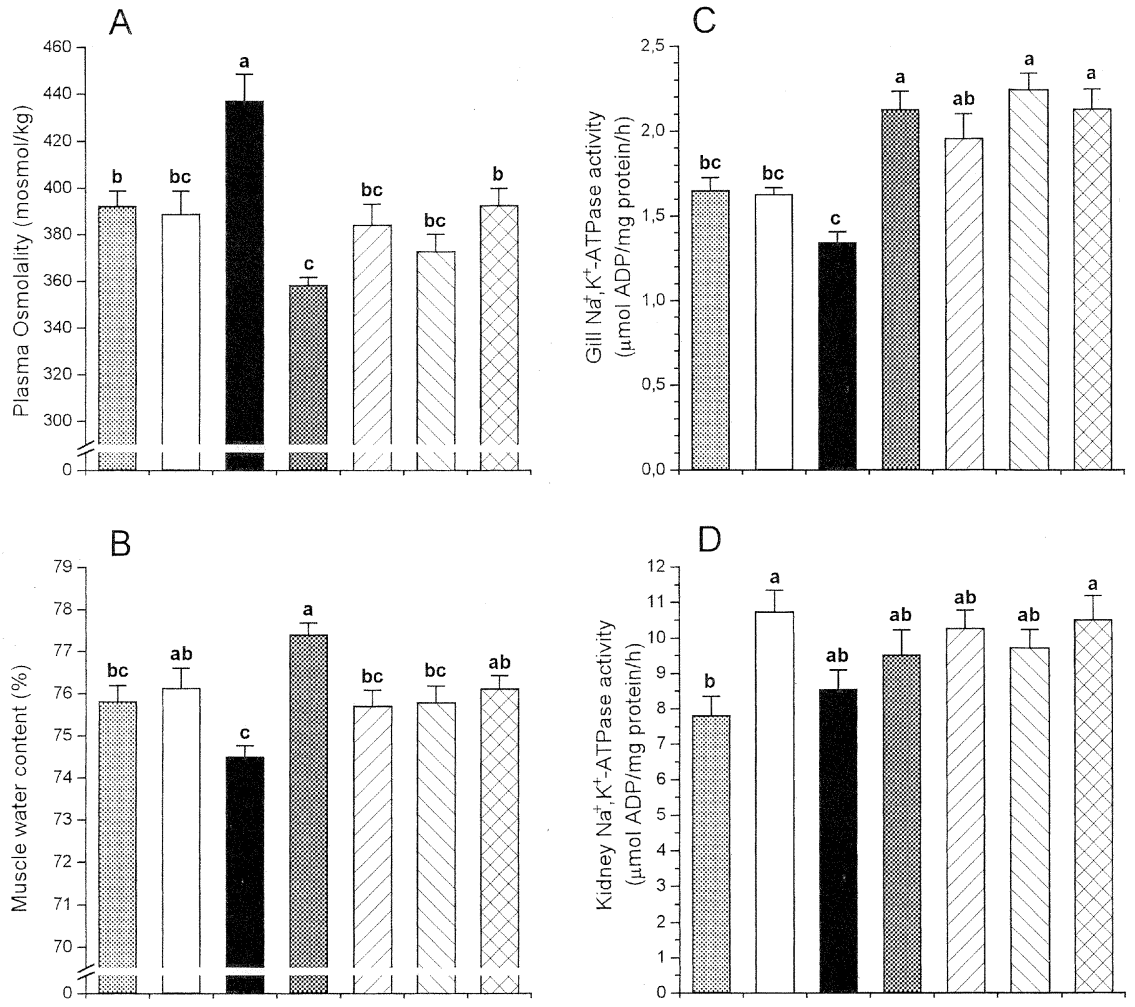

Fig. 2. Effect of various hormone treatments on plasma osmolality (**A**), muscle water content (**B**), gill (**C**) and kidney (**D**) Na^+,K^+-ATPase activity in *S. trutta* 48 hr after transfer to SW. The filling of each bar corresponds to the usage in Fig. 1. Fish were given 4 injections of each dose in FW and transferred to 27.3 ppt SW one day after the last injection. Values are mean + SEM of 8-10 fish. Values with shared letters are not significantly different ($P > 0.05$).

(Figs. 2C, 3E; Richman and Zaugg, 1987; Madsen, 1990a,c; Bisbal and Specker, 1991; Almendras *et al.*, 1993; Boeuf *et al.*, 1994; Nonnotte and Boeuf, 1995). This is supported by the nonlinear negative correlation between plasma osmolality and gill Na^+,K^+-ATPase activity observed in the present experiments (Fig. 4A, B). The correlation analyses further suggest that there may be a gill Na^+,K^+-ATPase threshold level (of about 2.5 µmol ADP/mg protein/hr) below which the brown trout cannot balance the influx of salt after SW-transfer with increased salt excretion. Treatment with oPRL impairs the ability of salmonids to hypoosmoregulate (Hasegawa *et al.*, 1986; Figs. 1-3) although in some studies these effects were less significant (Madsen and Bern, 1992; Boeuf *et al.*, 1994).

The use of ovine GH and PRL is justified by the fact that these hormones are known to bind specifically to their respective teleost receptors, although with lower affinity than the homologous hormones (oPRL: Auperin *et al.*, 1994; Prunet and Auperin, 1994; oGH: Yao *et al.*, 1991). Several studies have shown that the mammalian hormones induce qualitatively similar osmoregulatory effects as the teleost hormones. The receptor's lower affinity for the heterologous hormone is, however, reflected in the reduced potency compared to the native hormones (oPRL: Hasegawa *et al.*, 1986; oGH: Bouef *et al.*, 1994).

None of the present hormone treatments had a significant effect on kidney Na^+,K^+-ATPase activity when compared to the saline-injected control. This agrees with Madsen *et al.* (1995), who found no effect of F, rsGH, sPRL or rbIGF-I on kidney Na^+,K^+-ATPase activity and its α-subunit mRNA expression in *S. trutta*. Similarly, kidney Na^+,K^+-ATPase activity and mRNA levels were unaffected during SW-acclimation (*O. mykiss*: Jürss *et al.*, 1985; *S. salar*: McCormick *et al.*, 1989; *S. trutta*: Madsen *et al.*, 1995). Kidney Na^+,K^+-ATPase decreases during SW-acclimation in other teleosts (*F. heteroclitus*: Epstein *et al.*, 1969; *Chelon labrosus*: Gallis *et al.*, 1979) and the activity is stimulated by PRL and F in *F.*

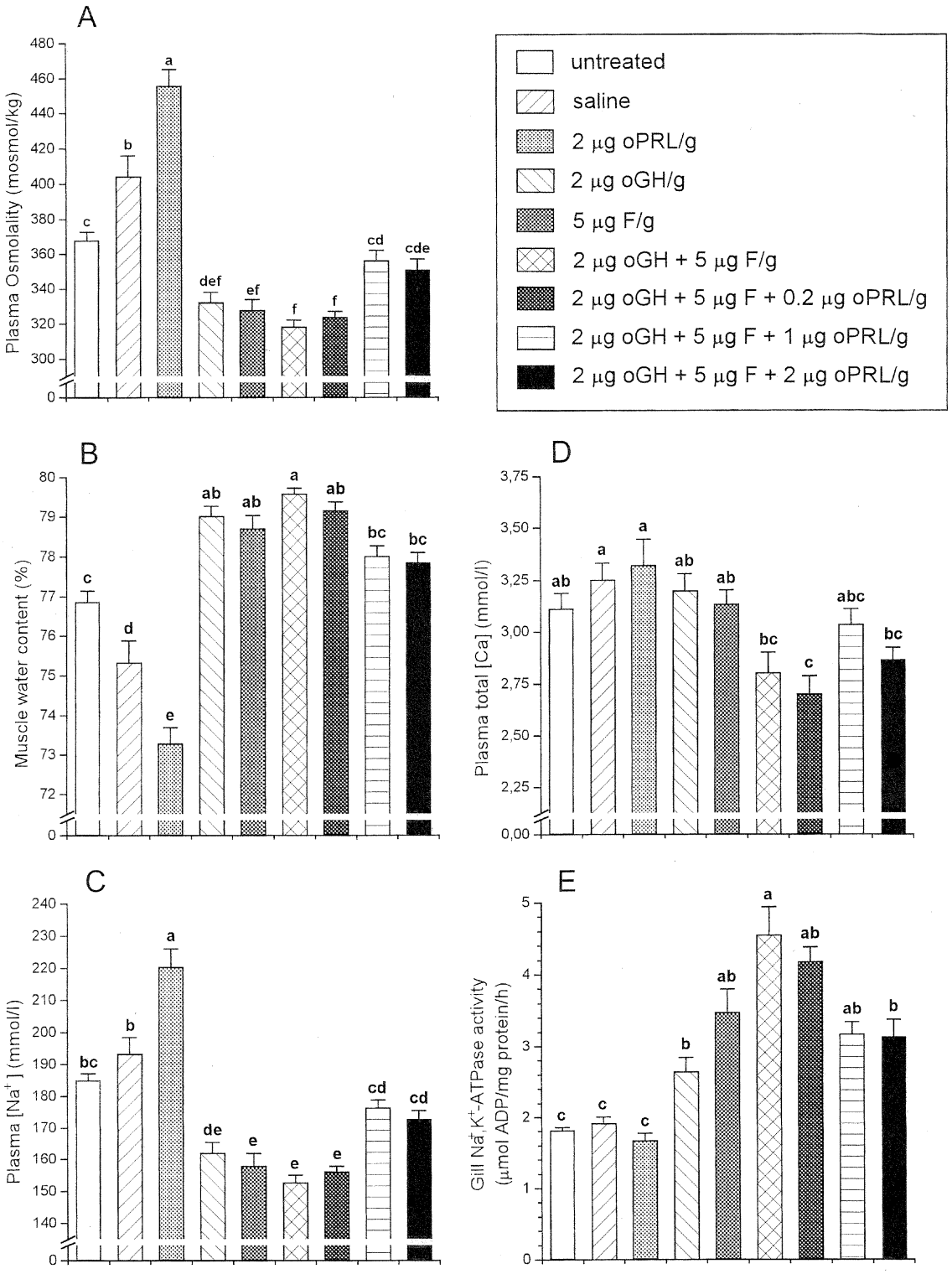

Fig. 3. Effect of various hormone treatments on plasma osmolality (**A**), muscle water content (**B**), Plasma [Na^+] (**C**), plasma total [Ca] (**D**) and gill Na^+,K^+-ATPase activity (**E**) in *S. trutta* 48 hr after transfer to SW. Fish were given 4 injections of each dose in FW and transferred to 24.8 ppt SW one day after the last injection. Values are mean + SEM of 7-10 fish. Values with shared letters are not significantly different ($P > 0.05$).

heteroclitus (Pickford *et al.*, 1970a,b) The adaptive role and the endocrine control of changes in kidney Na^+,K^+-ATPase activity in salmonids remain uncertain. Plasma total [Ca] (Figs. 1C and 3D) and [Mg] (Fig. 1D) showed little response to the experimental treatment. F, oGH or oPRL treatment had no effect on plasma total [Ca] after 48 hr in SW. This is in

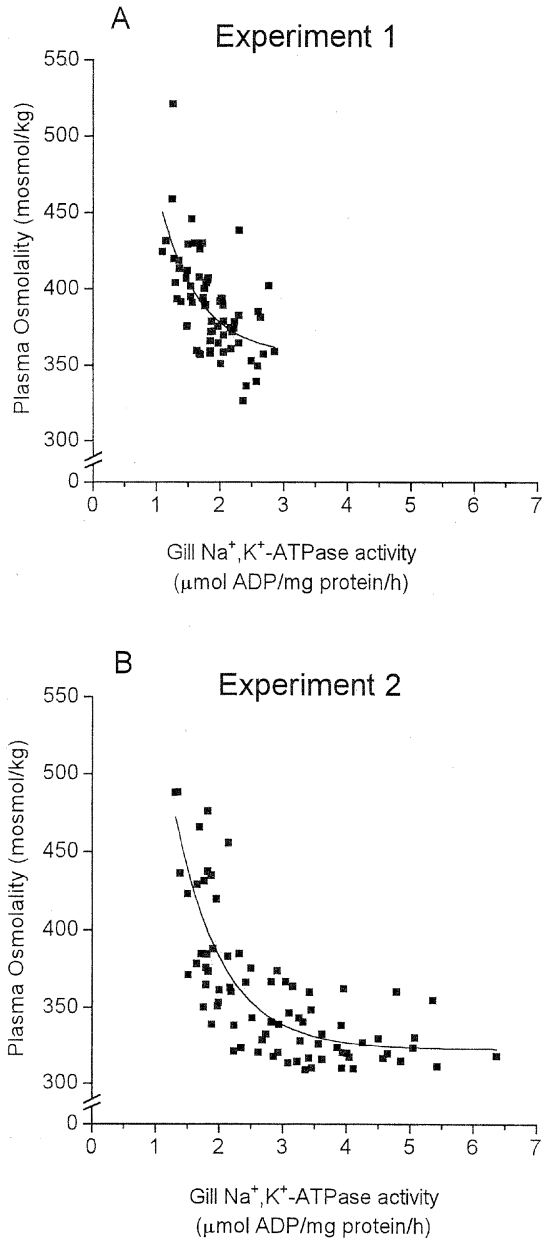

Fig. 4. Correlation between plasma osmolality and gill Na^+,K^+-ATPase activity in Experiment 1 (**A**) and Experiment 2 (**B**) in *S. trutta* 48 hr after transfer to SW. Each point represents one fish. Data from fish in all treatment groups were used. The fitted lines describe the nonlinear equations: Exp. 1: Osmolality = 356.3 + 553.7 * $e^{-1.6*activity}$ ($r = 0.636$, $P < 0.001$). Exp. 2: Osmolality = 322.3 + 853.2 * $e^{-1.3*activity}$ ($r = 0.793$, $P < 0.001$).

agreement with Madsen and Bern (1992) who found no changes in plasma total [Ca] in either oGH- or oPRL-injected *S. trutta* transferred to 25 ppt SW. In contrast, implants of oGH decreased plasma total [Ca] after transfer of *S. trutta* to 35 ppt SW (Almendras *et al.*, 1993). Bolton *et al.* (1987) found a hypocalcemic effect of oGH after transfer of *O. mykiss* to 80% SW, whereas PRL had no effect on plasma total [Ca]. In the present Exp. 2, combined F and oGH had a hypocalcemic effect, which is at variance with Madsen and Korsgaard (1991) who found no effect of coinjecting F and oGH in *S. trutta* on plasma total [Ca] after 2 days in 28.5 ppt SW. Calcium regulation is a complex process in fishes that may be controlled at various levels and by various hormones. GH, PRL and F have been reported as calcitropic hormones in *O. mykiss* and *Oreochromis mossambicus* (Flik and Perry, 1989; Flik *et al.*, 1993, 1994) and may affect either active or passive steps of transbranchial transport. The variable responses may therefore be due to differences in experimental protocols such as salinity, hardness of water, nutritive status and stress. The mode of action of PRL's anti-SW-adaptive effect is unknown. Madsen and Bern (1992) proposed that one possible mechanism may occur via inhibition of gill Na^+,K^+-ATPase activity. In the present study, oPRL treatment did not inhibit basal levels of gill Na^+,K^+-ATPase activity (Figs. 2C and 3E), yet PRL had clear hyperosmotic effects in both experiments (Figs. 1-3). One reason for the lack of effect of oPRL when injected alone may be that the gill Na^+,K^+-ATPase activity is at near-basal level during the fall in FW-acclimated brown trout, and thus may not be further decreased by hormonal manipulation. Madsen *et al.* (1995) also found a similar lack of effect of sPRL on both gill enzyme activity and mRNA expression in FW brown trout. Conversely, when gill Na^+,K^+-ATPase activity was increased by long-term SW acclimation in 0+ brown trout, a single injection of 0.2 μg oPRL/g significantly inhibited enzyme activity after 3 days (M. Seidelin, 1996, unpublished). Madsen and Bern (1992) found that GH-stimulated gill Na^+,K^+-ATPase activity could be depressed to control fish levels by simultaneous PRL treatment in *O. mykiss*, and a similar antagonism has been found in *S. trutta* (M. Seidelin, A. Heilskov and S.S. Madsen, 1996, unpublished). Thus it appears that gill Na^+,K^+-ATPase activity needs to be at a stimulated level in order for PRL to have an inhibitory action *in vivo*.

When *S. trutta* were treated with oPRL in combination with F, it antagonized the SW-adaptive effect of F on plasma osmolality, $[Na^+]$, $[Cl^-]$ and MWC (Figs. 1A, B and 2A, B). However, oPRL did not affect the stimulated gill Na^+,K^+-ATPase activity of F-treated fish (Fig. 2C). In this situation, an alternative route for the anti-SW adaptive effect of PRL must be sought. Other studies have shown that PRL may decrease branchial ion-permeability (Hirano, 1986), or impair *in situ* chloride cell function, as shown for opercular membrane chloride cells in *O. mossambicus* (Foskett *et al.*, 1982; Herndon *et al.*, 1991).

The lack of effect of oPRL on the F-stimulated level of gill Na^+,K^+-ATPase activity, led us to investigate the ability of oPRL to antagonize the combined effect of F and oGH on the development of hypoosmoregulatory ability (see Madsen 1990b,c). In Experiment 2, fish treated with either oGH, F or oGH+F in FW performed well in the 48 hr SW challenge test (Fig. 3). Plasma osmolality and MWC in these groups were comparable to the levels measured in *S. trutta* acclimated to FW (Madsen, 1990c) or to 25 ppt SW for 50 days (Madsen *et al.*, 1995). Ovine GH and F have clear additive effects on gill Na^+,K^+-ATPase activity (Fig. 3E; Björnsson *et al.*, 1987;

Madsen, 1990b,c). In the present study we did not find additive effects on plasma osmolality, [Na^+] or MWC (Fig. 3A, B, C), as reported by Madsen (1990b,c). One likely explanation is that the salinity used in the SW-challenge test may have been too low to reveal such effects. Recent experiments have pursued some possible mechanism for the interaction of F and GH. F treatment of salmonids generally reduces the cytosolic and increases the nuclear fraction of the gill corticosteroid receptor (CR) resulting in a general decrease in the total amount of CRs (e.g., Weisbart *et al.*, 1987; Shrimpton and Randall, 1994). GH treatment increases CRs in the gill (Shrimpton *et al.*, 1995) and may thereby increase the responsiveness of the gill to F. Exogenous GH has no direct effect alone or together with F on gill Na^+,K^+-ATPase activity (McCormick *et al.*, 1991a), and there is evidence that the interaction between F and GH at the level of gill Na^+,K^+-ATPase may be mediated by IGF-I. GH increases the hepatic and branchial production of IGF-I (Sakamoto and Hirano, 1993) and thus increases IGF-I levels in plasma (Moriyama, 1995). IGF-I stimulates hypoosmoregulatory ability (*O. mykiss*: McCormick *et al.*, 1991b) as well as gill Na^+,K^+-ATPase activity and mRNA levels *in vivo* (*S. trutta*: Madsen *et al.*, 1995). Furthermore, IGF-I has been shown to increase gill Na^+,K^+-ATPase activity *in vitro* when the gill epithelium had been pre-exposed to endogenous or exogenous GH *in vivo* (Madsen and Bern, 1993). Recently, it has been shown that IGF-I may increase the responsiveness of gill Na^+,K^+-ATPase activity to F in *S. salar* (McCormick, 1996).

As shown in Fig. 3, oPRL did not abolish the hypoosmoregulatory ability (plasma osmolality, [Na^+] and MWC) of the oGH+F-injected fish to the level of the saline-injected controls. Nor was gill Na^+,K^+-ATPase activity depressed below the level of the F-injected fish, which suggests that oPRL only counteracts the oGH-induced stimulation of gill Na^+,K^+-ATPase activity. This is supported in Exp. 1, where oPRL was unable to inhibit F-stimulated Na^+,K^+-ATPase activity. The molecular mechanism by which PRL antagonizes the effect of GH on gill Na^+,K^+-ATPase activity is not known. Competition between oPRL and oGH at the receptor level appears unlikely, since displacement studies show that the teleost GH-receptor has less than 1% of the affinity for oPRL that it has for GH (Gray *et al.*, 1990; Sakamoto and Hirano, 1991; Yao *et al.*, 1991). On the other hand, based on the similarity of their receptors, GH and PRL may interact at the postreceptor signalling level (review by Roupas and Herington, 1994). PRL may also alter the amount of IGF-I binding proteins (as has been shown *in vitro* in *Morone saxatilis* by Fukazawa *et al.*, 1995), thus possibly modifying the half-life of IGF-I in the plasma as well as changing the target orientation of IGF-I (review by Siharath and Bern, 1993).

Our finding that PRL is unable to inhibit the F-stimulated gill Na^+,K^+-ATPase activity may have relevance during the development of euryhaline capacity at smoltification. Typically, the plasma PRL-level decreases concomitantly with an increase in plasma F and GH just prior to development of maximum hypoosmoregulatory ability (e.g., Björnsson *et al.*, 1989; Young *et al.*, 1989). The decrease in plasma PRL may release the constraints on GH action at the level of the gill, which may be a prerequisite for increasing gill Na^+,K^+-ATPase activity to its maximum level. GH and F may have to act in concert to achieve this goal (Björnsson *et al.*, 1987; Madsen, 1990b,c; Experiment 2, this study).

In conclusion, in *S. trutta* oPRL inhibits the hypoosmoregulatory action of exogenously administered F. Ovine PRL does not affect the increase in gill Na^+,K^+-ATPase activity brought about by F, and the gill Na^+,K^+-ATPase enzyme may therefore not be the target for the antagonistic interaction of PRL and F in salmonid hypoosmoregulation.

ACKNOWLEDGMENTS

This study was supported by a grant from the Danish Research Council (11-9659-1). The National Hormone and Pituitary Program, the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Child Health and Human Development and the U.S. Department of Agriculture are acknowledged for the gift of pituitary hormones used in this study.

REFERENCES

Almendras JME, Prunet P, Boeuf G (1993) Responses of a non-migratory stock of brown trout, *Salmo trutta*, to ovine growth hormone treatment and seawater exposure. Aquaculture 114: 169–179

Auperin B, Rentier-Delrue F, Martial JA, Prunet P (1994) Characterization of a single prolactin (PRL) receptor in tilapia (*Oreochromis niloticus*) which binds both PRL_I and PRL_{II}. J Mol Endocrinol 13: 241–251

Bisbal GA, Specker JL (1991) Cortisol stimulates hypo-osmoregulatory ability in Atlantic salmon, *Salmo salar* L. J Fish Biol 39: 421–432

Björnsson BT, Yamauchi K, Nishioka RS, Deftos LJ, Bern HA (1987) Effects of hypophysectomy and subsequent hormonal replacement therapy on hormonal and osmoregulatory status of coho salmon, *Oncorhynchus kisutch*. Gen Comp Endocrinol 68: 421–430

Björnsson BT, Young G, Lin RJ, Deftos J, Bern HA (1989) Smoltification and seawater adaptation in coho salmon (*Oncorhynchus kisutch*): Plasma calcium regulation, osmoregulation, and calcitonin. Gen Comp Endocrinol 74: 346–354

Boeuf G, Marc AM, Prunet P, Le Bail P-Y, Smal J (1994) Stimulation of parr-smolt transformation by hormonal treatment in Atlantic salmon (*Salmo salar* L.). Aquaculture 121: 195–208

Bolton JP, Collie NL, Kawauchi H, Hirano T (1987) Osmoregulatory actions of growth hormone in rainbow trout (*Salmo gairdneri*). J Endocrinol 112: 63–68

Epstein FH, Manitius A, Weinstein E, Katz AI, Pickford GE (1969) Sodium- and potassium-activated adenosine triphospatase in kidneys of *Fundulus heteroclitus* adapted to fresh and salt water. Yale J Biol Med 41: 388–393

Flik G, Atsma W, Fenwick JC, Rentier-Delrue F, Smal J, Wendelaar Bonga SE (1993) Homologous recombinant growth hormone and calcium metabolism in the tilapia, *Oreochromis mossambicus*, adapted to fresh water. J Exp Biol 185: 107–119

Flik G, Perry S (1989) Cortisol stimulates whole body calcium uptake and the branchial calcium pump in freshwater rainbow trout. J Endocrinol 120: 75–82

Flik G, Rentier-Delrue F, Wendelaar Bonga SE (1994) Calcitropic effects of recombinant prolactins in *Oreochromis mossambicus*. Am J Physiol 266: R1302–R1308

Foskett JK, Machen TE, Bern HA (1982) Chloride secretion and

conductance of teleost opercular membrane: Effects of prolactin. Am J Physiol 242: R380–389

Fukazawa Y, Siharath K, Iguchi T, Bern HA (1995) *In vitro* secretion of insulin-like growth factor-binding proteins from liver of striped bass, *Morone saxatilis*. Gen Comp Endocrinol 99: 239–247

Gallis JL, Lassere P, Belloc F (1979) Freshwater adaptation in the euryhaline teleost, *Chelon labrosus*. I. Effects of adaptation, prolactin, cortisol and actinomycin-D on plasma osmotic balance and (Na^+-K^+)ATPase in gill and kidney. Gen Comp Endocrinol 38: 1–10

Gray ES, Young G, Bern HA (1990) Radioreceptor assay for growth hormone in coho salmon (*Oncorhynchus kisutch*) and its application to the study of stunting. J Exp Zool 256: 290–296

Hasegawa S, Hirano T, Kawauchi H (1986) Sodium-retaining activity of chum salmon prolactin in some euryhaline teleosts. Gen Comp Endocrinol 63: 309–317

Herndon TM, McCormick SD, Bern HA (1991) Effects of prolactin on chloride cells in opercular membrane from seawater-adapted tilapia. Gen Comp Endocrinol 83: 283–289

Hirano T (1986) The spectrum of prolactin action in teleosts. In "Comparative Endocrinology: Developments and Directions" Ed by CL Ralph, Alan R Liss, Inc, New York, pp 53–74

Jürss K, Bittorf T, Volker T (1985) Influence of salinity and ratio of lipid to protein in diets on certain enzyme activities in rainbow trout (*Salmo gairdneri* Richardson). Comp Biochem Physiol 81B: 73–79

Lowry OH, Roseborough NJ, Farr AL, Randall DJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 266–275

Madsen SS (1990a) Cortisol treatment improves the development of hypoosmoregulatory mechanisms in the euryhaline rainbow trout, *Salmo gairdneri*. Fish Physiol Biochem 8: 45–52

Madsen SS (1990b) Enhanced hypoosmoregulatory response to growth hormone after cortisol treatment in immature rainbow trout, *Salmo gairdneri*. Fish Physiol Biochem 8: 271–279

Madsen SS (1990c) The role of cortisol and growth hormone in seawater adaptation and development of hypo-osmoregulatory mechanisms in sea trout parr (*Salmo trutta trutta*). Gen Comp Endocrinol 79: 1–11

Madsen SS, Bern HA (1992) Antagonism of prolactin and growth hormone: Impact on seawater adaptation in two salmonids, *Salmo trutta* and *Oncorhynchus mykiss*. Zool Science 9: 775–784

Madsen SS, Bern HA (1993) *In vitro* effects of insulin-like growth factor-I on gill Na^+,K^+-ATPase in coho salmon, *Oncorhynchus kisutch*. J Endocrinol 138: 23–30

Madsen SS, Jensen MK, Nøhr J, Kristiansen K (1995) Expression of Na^+,K^+-ATPase in the brown trout, *Salmo trutta*: in vivo modulation by hormones and seawater. Am J Physiol 269: R1339–1345

Madsen SS, Korsgaard B (1991) Opposite effects of 17β-estradiol and combined growth hormone-cortisol treatment on hypo-osmoregulatory performance in sea trout presmolts, *Salmo trutta*. Gen Comp Endocrinol 83: 276–282

McCormick SD (1993) Methods for nonlethal gill biopsy and measurement of Na^+,K^+-ATPase activity. Can J Fish Aquat Sci 50: 656–658

McCormick SD (1996) Effects of growth hormone and insulin-like growth factor I on salinity tolerance and gill Na^+,K^+-ATPase in Atlantic salmon (*Salmo salar*) - interaction with cortisol. Gen Comp Endocrinol 101: 3–11

McCormick SD, Dickhoff WW, Duston J, Nishioka RS, Bern HA (1991a) Developmental differences in the responsiveness of gill Na^+-K^+-ATPase to cortisol in salmonids. Gen Comp Endocrinol 84: 308–317

McCormick SD, Moyes D, Ballantyne JS (1989) Influence of salinity on the energetics of gill and kidney of Atlantic salmon (*Salmo salar*). Fish Physiol Biochem 6: 243–254

McCormick SD, Sakamoto T, Hasegawa S, Hirano T (1991b) Osmoregulatory actions of insulin-like growth factor-I in rainbow trout (*Oncorhynchus mykiss*). J Endocrinol 130: 87–92

Moriyama S (1995) Increased plasma insulin-like growth factor-I (IGF-I) following oral and intraperitoneal administration of growth hormone to rainbow trout, *Oncorhynchus mykiss*. Growth Reg 5: 164–167

Nonnotte G, Boeuf G (1995) Extracellular ionic and acid-base adjustments of Atlantic salmon presmolts and smolts in fresh water and after transfer to sea water: The effects of ovine growth hormone on the acquisition of euryhalinity. J Fish Biol 46: 563–577

Pickford GE, Griffith RW, Torretti J, Hendlez E, Epstein FH (1970a) Branchial reduction and renal stimulation of (Na^+-K^+)-ATPase by prolactin in hypophysectomized killifish in fresh water. Nature Lond 228: 378–379

Pickford GE, Pang PKT, Weinstein E, Torretti J, Hendlez E, Epstein FH (1970b) The response of the hypophysectomized cyprinodont, *Fundulus heteroclitus*, to replacement therapy with cortisol: effect on blood serum and sodium potassium activated adenosine triphosphatse in the gills, kidney, and intestinal mucosa. Gen Comp Endocrinol 14: 524–534

Prunet P, Auperin B (1994) Prolactin Receptors. In "Fish Physiology vol XIII" Ed by NM Sherwood, TJ Shuttleworth, Academic Press, Inc, pp 367–390

Richman III HN, Zaugg WS (1987) Effects of cortisol and growth hormone on osmoregulation i pre- and desmoltified coho salmon (*Oncorhynchus kisutch*). Gen Comp Endocrinol 65: 189–198

Roupas P, Herington AC (1994) Postreceptor signaling mechanisms for growth hormone. Trends Endocrinol Metab 5: 154–159

Sakamoto T, Hirano T (1991) Growth hormone receptors in the liver and osmoregulatory organs of rainbow trout - characterization and dynamics during adaptation to seawater. J Endocrinol 130: 425–433

Sakamoto T, Hirano T (1993) Expression of insulin-like growth factor I gene in osmoregulatory organs during seawater adaptation of the salmonid fish: Possible mode of osmoregulatory action of growth hormone. Proc Natl Acad Sci USA 90: 1912–1916

Siharath K, Bern HA (1993) The physiology of insulin-like growth factor (IGF) and its binding proteins in teleost fishes. Proc Zool Soc, Calcutta, Haldane Comm Vol: 113–124

Shrimpton JM, Devlin RH, McLean E, Byatt JC, Donaldson EM, Randall DJ (1995) Increases in gill cytosolic corticosteroid receptor abundance and saltwater tolerance in juvenile coho salmon (*Oncorhynchus kisutch*) treated with growth hormone and placental lactogen. Gen Comp Endocrinol 98: 1–15

Shrimpton JM, Randall DJ (1994) Downregulation of corticosteroid receptors in gills of coho salmon due to stress and cortisol treatment. Am J Physiol 267: R432–438

Weisbart M, Chakraborti PK, Gallivan G, Eales JG (1987) Dynamics of cortisol receptor activity in the gills of the brook tout, *Salvelinus fontinalis*, during seawater adaptation. Gen Comp Endocrinol 68: 440–448

Yao K, Niu PD, Le Gac F, Lebail P-Y (1991) Presence of specific growth hormone binding sites in rainbow trout: characterization and dynamics during adaptation to seawater. J Endocrinol 130: 425–433

Young G, Björnsson BT, Prunet P, Lin RJ, Bern HA (1989) Smoltification and seawater adaptation in coho salmon (*Oncorhynchus kisutch*): Plasma prolactin, growth hormone, thyroid hormones, and cortisol. Gen Comp Endocrinol 74: 335–345

(Received November 11, 1996 / Accepted January 13, 1997)