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Effects of somatostatin on the growth hormone-insulin-like growth factor axis and seawater adaptation of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Growth hormone (GH) has been shown to contribute to the seawater (SW) adaptability of euryhaline fish both directly and indirectly through insulin-like growth factor-1 (IGF-1). This study examined the role of somatostatin-14 (SS-14), a potent inhibitor of GH, on the GH–IGF-1 axis and seawater adaptation. Juvenile rainbow trout (*Oncorhynchus mykiss*) were injected intraperitoneally with SS-14 or saline and transferred to 20 ppt seawater. A slight elevation in plasma chloride levels was accompanied by significantly reduced gill Na^+, K^+-ATPase activity in SS-14-treated fish compared to control fish 12 h after SW transfer. Seawater increased hepatic mRNA levels of GH receptor 1 (GHR 1; 239%), GHR 2 (48%), and IGF-1 (103%) in control fish 12 h after transfer. Levels of GHR 1 (155%), GHR 2 (121%), IGF-1 (200%), IGF-1 receptor A (IGFR1A; 62%), and IGFR1B (157%) increased in the gills of control fish 12 h after transfer. SS-14 abolished or attenuated SW-induced changes in the expression of GHR, IGF-1, and IGFR mRNAs in liver and gill. These results indicate that SS-14 reduces seawater adaptability by inhibiting the GH–IGF-1 axis.
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Keywords: Osmoregulation; Somatostatin; Growth hormone; Growth hormone receptor; Insulin-like growth factor

1. Introduction

Euryhaline teleosts rely upon a variety of internal signaling mechanisms to coordinate the physiological adjustments necessary to respond to changes in environmental salinity. Considerable research has shown that seawater adaptability is associated with alterations in numerous hormones, including increases in plasma concentrations of growth hormone (GH), insulin-like growth factor-1 (IGF-1), cortisol, and thyroid hormones accompanied by decreases in prolactin (PRL) (Young et al., 1989; McCormick, 2001; Evans, 2002).

Increasing attention has been given to the GH–insulin-like growth factor-1 (IGF-1) axis and its role in promoting readiness to osmoregulate in seawater. The role of GH in ion regulation appears separate from the growth-promoting effects of the hormone. Increases in plasma GH and IGF-1 following transfer to seawater are accompanied by increased mRNA expression of the hormones (Sakamoto et al., 1990; McCormick et al., 2000; Agustsson et al., 2001). A connection between

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IGF-1 mRNA production and GH was demonstrated in the gill and kidney of trout in response to increases in environmental salinity (Sakamoto and Hirano, 1993). Both GH and IGF-1 can increase salinity tolerance and gill Na^+, K^+-ATPase activity in several teleost species (McCormick et al., 1991; Mancera and McCormick, 1998), and a synergistic relationship between the two hormones in controlling gill Na^+, K^+-ATPase activity has been suggested by Madsen and Bern (1993).

Somatostatin (SS), originally discovered in 1973 (Brazeau et al., 1973), has numerous effects on the growth, development, reproduction, and metabolism of vertebrates (Sheridan, 1994; Sheridan et al., 2000; Sheridan and Kittilson, 2004; Nelson and Sheridan, 2005; Klein and Sheridan, in press). Given that SS inhibits the release of numerous pituitary factors, including GH and thyroid stimulating hormone, the possibility exists that SS also may play a role in seawater adaptability and osmoregulation. The aim of this study was to examine the effects of SS on the GH–IGF-1 axis during acclimation to seawater and to provide further insight into the role of SS on organismal growth and seawater adaptability.

2. Materials and methods

2.1. Animals

Juvenile rainbow trout of both sexes were obtained from Dakota Trout Ranch near Carrington, ND, USA and transported to North Dakota State University. Fish were maintained in well-aerated, 800-l circular tanks supplied with recirculated (10% make-up volume per day) dechlorinated municipal water at 12 °C with 12:12 L:D photoperiod. Fish were fed twice daily to satiety with AquaMax™ pellets (PMI Nutrition International, Brentwood, MO), except 24 h prior to and during experimental trials.

2.2. Experimental conditions

Fish (95.1 ± 1.5 g mean body weight) acclimated to freshwater were anesthetized with 0.05% (v/v) 2-phenoxyethanol in 20‰ (w/v, InstantOcean®, Aquarium Systems Inc., Mentor, OH), weighed, and injected intraperitoneally (10 μl/g body weight) with either somatostatin-14 (SS-14; 1×10^{-11} mol/g body weight; Sigma, St. Louis, MO) or saline (0.75%, w/v), and placed into 100-l circular experimental tanks. Experimental tanks were supplied with 20‰ seawater (100% turnover rate per 24-hour period) at 12 °C under a 12:12 L:D photoperiod. Fish were sampled at 0, 6, 12, 24, 48, and 72 h following injection and seawater transfer. Following anesthesia as described above, sampled fish were weighed and measured for length. Blood was collected from the severed caudal vessels and the plasma was separated from blood cells by centrifugation (11,000 g for 3 min) and stored at −80 °C for later analyses. One intact gill arch from each fish was placed in 1.5-ml microfuge tubes containing ice-cold SEI buffer (250 mM sucrose, 10 mM Na_2EDTA, 50 mM imidazole) and frozen on dry ice for later assay of Na^+, K^+-ATPase. Liver and gill filament samples were removed and placed in 2-ml microfuge tubes and immediately placed on dry ice and stored at −80 °C until later analysis of RNA.

2.3. Plasma chloride and Na^+, K^+-ATPase

Plasma chloride was determined by silver titration using a Buchler-Cotlove Chloridometer. Gill Na^+/K^+-ATPase activity was determined as described previously (McCormick, 1993).

2.4. Quantitative real-time PCR

Total RNA was extracted from frozen tissue samples by homogenization in the presence of 500 μl TRI Reagent® (Molecular Research Center Inc., Cincinnati, OH) based on manufacturer's protocol. Each RNA pellet was resuspended in 100 μl RNase free deionized water and quantified by ultraviolet (A_{260}) spectrophotometry.

RNA was reverse transcribed in 10 μl reactions using 200 ng total RNA (2 μl) and 8 μl TaqMan® reverse transcription reagents (which included 5.5 mM $MgCl_2$, 500 μM each dNTP, 2.5 μM oligo $d(T)_{16}$ primer, 0.4 U/μl RNase inhibitor, and 3.125 U/ml of MultiScribe reverse transcriptase) according to the manufacturer's protocol (Applied Biosystems, Foster City, CA). Reactions without reverse transcriptase were included as negative controls; no amplification was detected in negative controls.

mRNA levels of GH receptor 1 (GHR 1), GHR 2, IGF-1, IGF receptor 1a (IGFR1a), and IGFR1b were determined by real-time PCR using TaqMan® chemistry and an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems). Real-time reactions were carried out for samples, standards, and no-template controls in a 10-μl volume, containing 2 μl cDNA from the reverse transcription reaction, 5 μl TaqMan Universal PCR Master Mix, and 1 μl of each forward primer, reverse primer, and probe at concentrations optimized for the mRNA species to be measured. The gene-specific primer and probe sequences used for GHR 1, GHR 2, and β-actin have been reported previously (Slagter et al., 2004; Very et al., 2005). The gene-specific nucleotides used for IGF-1 quantification were as follows: forward 5′ GTGGACACGCTGCAGTTTGT 3′ (900 nM), reverse 5′ CATACCCCGTTGGTTTACTGAAA 3′ (900 nM), and probe 5′ FAM-AAAGCCTCTCTCTCCA 3′ (150 nM). The sequences used for IGFR1a were forward 5′ AGAGAA-CACATCCAGCCAGGTT 3′ (600 nM), reverse 5′ TCCTG-CCATCTGGATCATCTT 3′ (600 nM), and probe 5′ FAM-TGCCCCCGCTGAA 3′ (150 nM), and for IGFR1b were forward 5′ CCTGAGGTCACTACGGGCTAAA 3′ (600 nM), reverse 5′ TCAGAGGAGGGAGGTTGAGACT 3′ (600 nM), and probe 5′ FAM-ATCCGTCCCAGTCCT 3′ (150 nM). Cycling parameters were set as follows: 95 °C for 10 min and 45 cycles of 95 °C for 15 s and 60 °C for 1 min. Cross reaction was assessed by substituting alternate primer/probe sets in TaqMan assays for each standard; no amplification was observed under these conditions. Sample copy number was calculated from the threshold cycle number (C_T) and relating C_T to a gene-

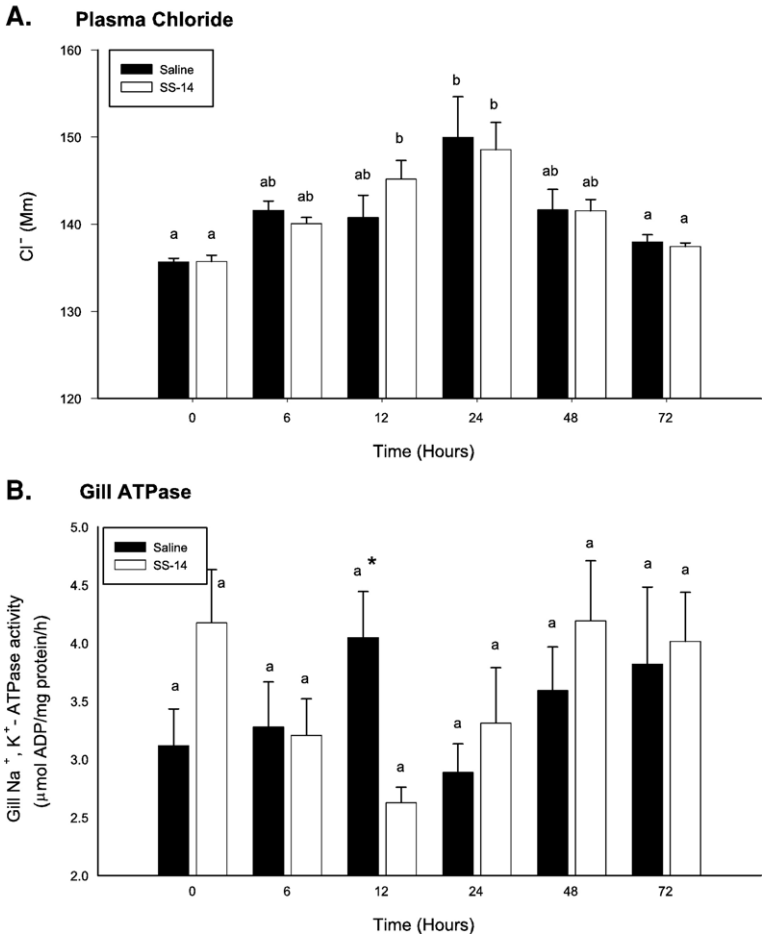

Fig. 1. Effects of somatostatin-14 (SS-14) on plasma chloride concentration and gill Na^+/K^+-ATPase activity in rainbow trout during acclimation to 20‰ seawater. Data are presented as mean±SEM. Within a treatment (saline or SS-14), groups with different letters are significantly different ($p<0.05$); * indicates a significant difference ($p<0.05$) between the control- and SS-14-treated groups.

specific standard curve (Very et al., 2005) followed by normalization to β-actin levels within each time group (Sakamoto and Hirano, 1993).

2.5. Statistical analyses

Data are expressed as mean±SEM. Statistical differences were analyzed by two-way ANOVA followed by post-hoc Bonferoni's test ($\alpha=0.05$) to examine differences between and among treatment groups. Statistics were performed on untransformed data using Sigma Stat (SPSS, Chicago, IL). Outliers were determined using Dixon's test ($\alpha=0.05$).

3. Results

3.1. Plasma chloride and gill Na^+, K^+-ATPase

Plasma chloride and gill Na^+, /K^+-ATPase were used as indicators of the ability to osmoregulate in increased salinity (Fig. 1). While plasma chloride showed a significant transitory increase compared to time 0 in both the saline- and SS-14-injected fish, reaching maximum levels 24 h after transfer, the temporal profiles of the two treatment groups were somewhat different. Notably, plasma chloride levels in the SS-14-injected fish were significantly elevated by 12 h after transfer, while those in the saline-injected fish were not. This significant change in SS-14-injected fish corresponded to the only significant difference between the saline- and S-14-injected groups in gill Na^+/K^+-ATPase (Fig. 1B).

3.2. GH–IGF-1 axis

The direct transfer of rainbow trout from freshwater to seawater resulted in significant increases in the levels of mRNA encoding GHRs (Fig. 2) and IGF-1 in trout liver (Fig. 3). Seawater transfer had the greatest effect on GHR 1, which increased 239% in control fish 24 h following seawater transfer; afterward, GHR 1 mRNA levels declined to near

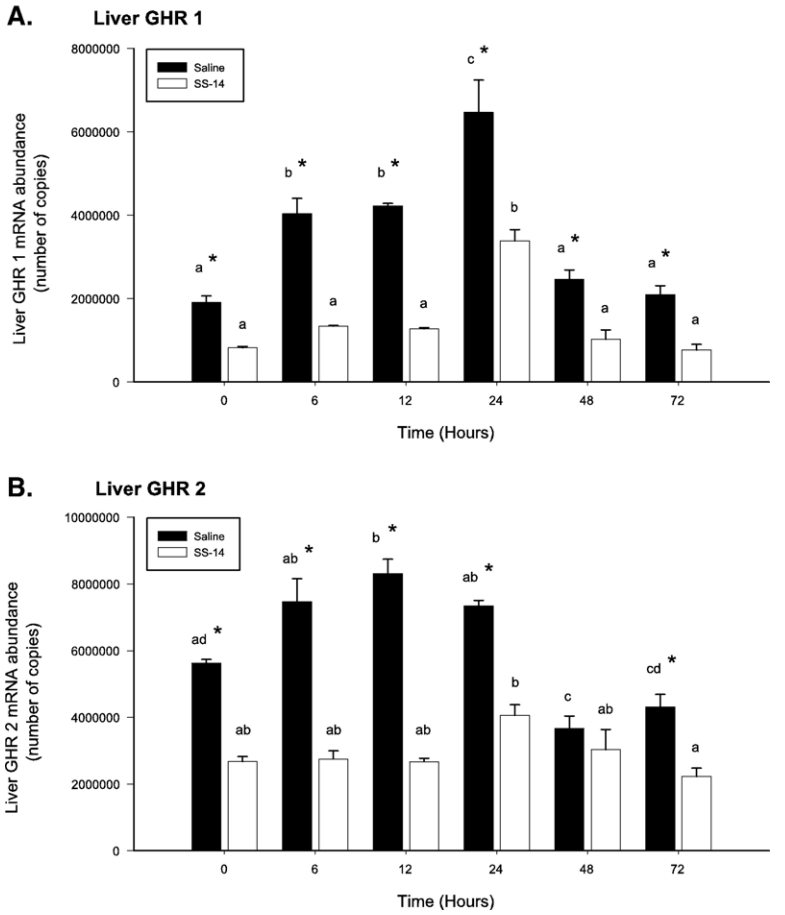

Fig. 2. Effects of somatostatin-14 (SS-14) on the abundance of (A) growth hormone receptor 1 (GHR 1) and (B) GHR 2 mRNA in the liver of rainbow trout during acclimation to 20‰ seawater. Data are presented as mean ± SEM. Within a treatment (saline or SS-14), groups with different letters are significantly different ($p<0.05$); * indicates a significant difference ($p<0.05$) between the control- and SS-14-treated groups.

those in fish in the initial group (Fig. 2A). Hepatic GHR 2 mRNA levels also increased in control fish in a transitory manner, increasing 48% 12 h after transfer (Fig. 2B). Seawater transfer similarly increased hepatic IGF-1 mRNA expression, where a maximum increase of 103% was observed in control fish 12 h after seawater transfer (Fig. 3). SS-14 injection

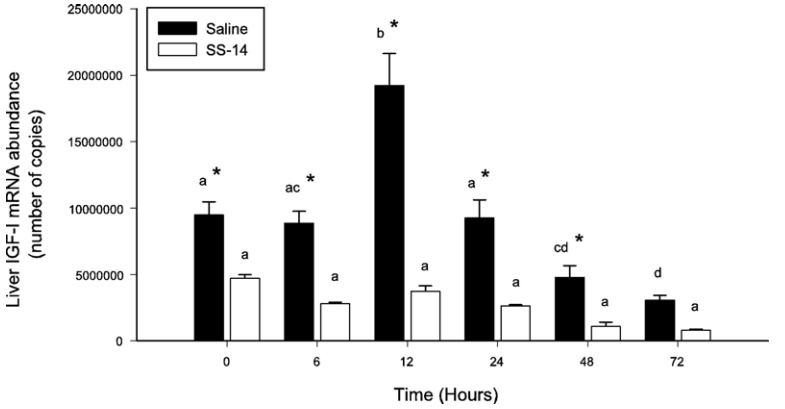

Fig. 3. Effects of somatostatin-14 (SS-14) on the abundance of insulin-like growth factor-1 (IGF-1) mRNA in the liver of rainbow trout during acclimation to 20‰ seawater. Data are presented as mean ± SEM. Within a treatment (saline or SS-14), groups with different letters are significantly different ($p<0.05$); * indicates a significant difference ($p<0.05$) between the control- and SS-14-treated groups.

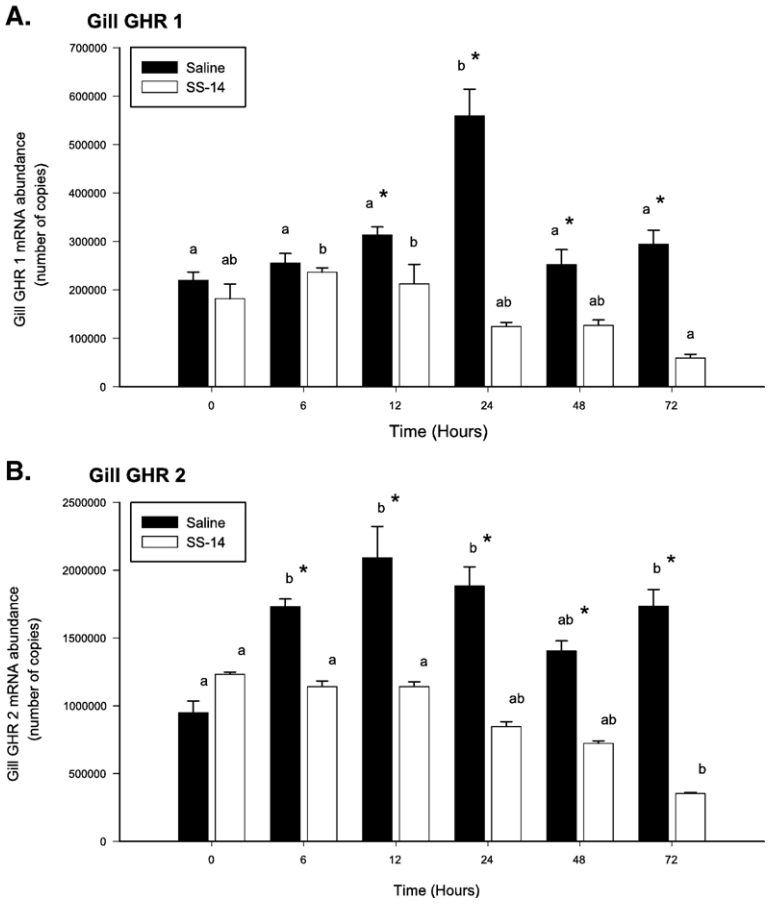

Fig. 4. Effects of somatostatin-14 (SS-14) on the abundance of (A) growth hormone receptor 1 (GHR 1) and (B) GHR 2 mRNA in the gill of rainbow trout during acclimation to 20‰ seawater. Data are presented as mean±SEM. Within a treatment (saline or SS-14), groups with different letters are significantly different ($p<0.05$); * indicates a significant difference ($p<0.05$) between the control- and SS-14-treated groups.

significantly inhibited the normal seawater-associated increase of GHR 1, GHR 2, and IGF-1 mRNAs in liver. Maximum attenuation of the seawater-induced increases in gene expression occurred 12 h post-transfer for all mRNA species (Figs. 2 and 3). The inhibitory action of SS on hepatic GHR expression was not as substantial after 24 h, especially in the

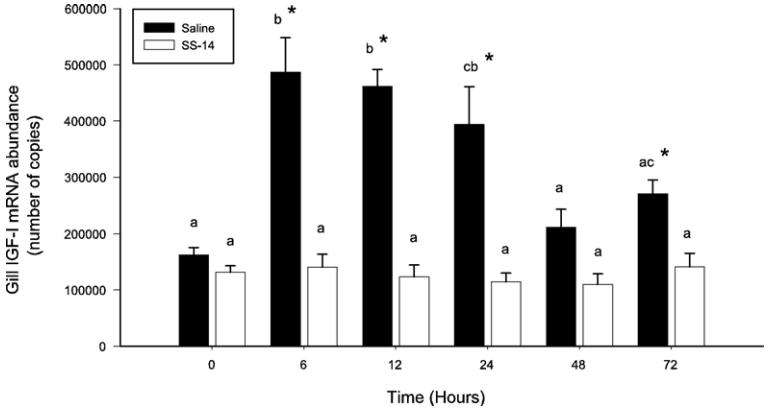

Fig. 5. Effects of somatostatin-14 (SS-14) on the abundance of insulin-like growth factor-1 (IGF-1) mRNA in the gill of rainbow trout during acclimation to 20‰ seawater. Data are presented as mean±SEM. Within a treatment (saline or SS-14), groups with different letters are significantly different ($p<0.05$); * indicates a significant difference ($p<0.05$) between the control- and SS-14-treated groups.

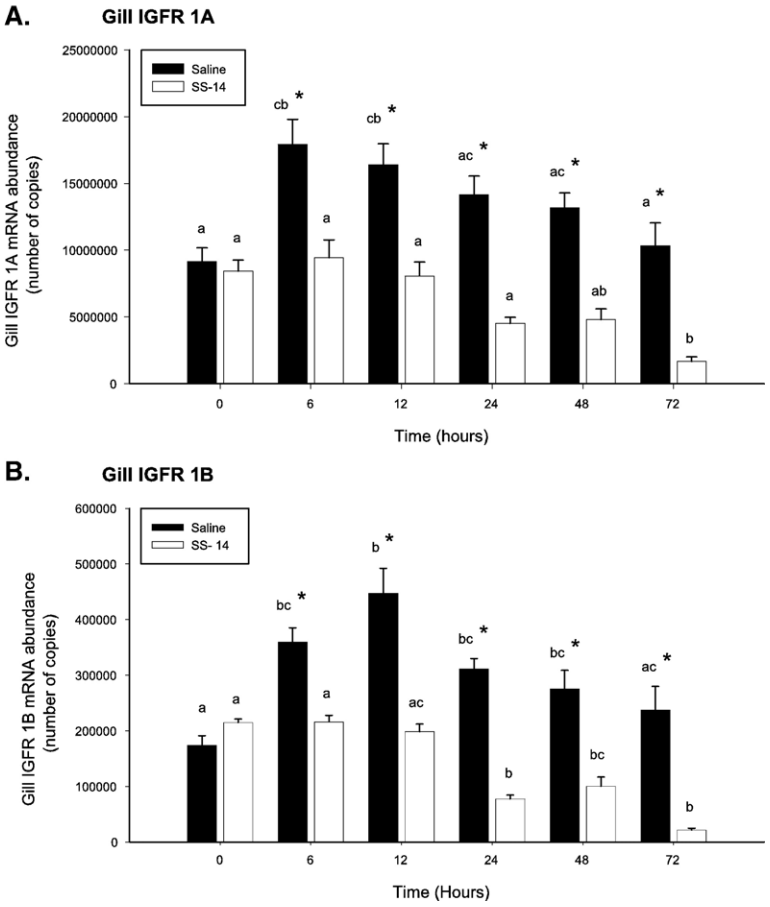

Fig. 6. Effects of somatostatin-14 (SS-14) on the abundance of (A) insulin-like growth factor receptor 1A (IGFR1A) and (B) IGFR1B mRNA in the gill of rainbow trout during acclimation to 20‰ seawater. Data are presented as mean±SEM. Within a treatment (saline or SS-14), groups with different letters are significantly different ($p<0.05$); * indicates a significant difference ($p<0.05$) between the control- and SS-14-treated groups.

case of GHR 1 (Fig. 2A), but mRNA levels remained significantly lower than in control fish. Hepatic IGF-1 levels in SS-14-injected fish remained depressed through the first 48 h, but not thereafter.

The patterns of expression of GH–IGF-1 axis components in the gill were similar to those in the liver. Seawater transfer increased gill GHR 1 expression by 151% and GHR 2 expression by 121% in control fish (Fig. 4). SS-14 injection ablated seawater-associated increases in GHR expression. Levels of IGF-1 mRNA in the gill of control fish rose significantly after transfer to seawater (Fig. 5). SS-14 completely abolished the seawater-associated increase in IGF-1 mRNA levels. Two forms of IGFR were detected, IGFR1A and IGFR1B. Direct transfer to seawater increased the expression of both IGFRs in the gill of control fish; IGFR1A levels rose rapidly and increased to a maximum of 62% of initial levels 6 h after transfer, whereas IGFR1B levels increased to a maximum level (157%) 12 h after transfer (Fig. 6). As was the case with liver GHRs and IGF-1, SS-14 abolished seawater-associated increases in the expression of gill IGFR1s. By later stages of the experiment (e.g., 72 h), levels of IGFR mRNAs in the gill of SS-14-injected fish declined compared to the initial sampling.

4. Discussion

This study indicates that transfer of rainbow trout to 20‰ seawater results in increased hepatic expression of GHR and IGF-1 mRNAs as well as in increased expression of GHR, IGF-1 and IGFR mRNAs in gill. In addition, SS-14 injection abolishes or attenuates seawater-associated increases in GHR, IGF-1 and IGFR mRNAs as well as retards seawater adaptability as assessed by Na^+, K^+-ATPase activity. These findings extend our knowledge of the control of osmoregulation and the role of SS in regulating the GH–IGF-1 axis.

Increases in GH, IGF-1, and cortisol have been well documented during the transition of euryhaline fish from freshwater to seawater (Thorpe et al., 1987; Young et al., 1989; McCormick, 2001; Evans, 2002). The

current findings extend our knowledge of GH–IGF-1 axis components in seawater adaptation in several ways. First, increased expression of GHR mRNAs occurred at the level of the liver and gill following seawater exposure. Such increased GHR synthesis should result in increased sensitivity of these tissues to GH. Second, expression of IGF-1 in liver and gill increased following seawater exposure. Seawater-associated increases in hepatic IGF-1 expression similar to those observed in the current study also were reported by Sakamoto and Hirano (1993) and would be consistent with increased plasma levels of IGF-1 observed following seawater transfer (Shepherd et al., 2005). Third, expression of IGFRs in the gill increased following seawater exposure. Such alterations would increase the sensitivity of this organ to IGF-1 and result in increased seawater adaptability (McCormick, 2001; Evans, 2002). This pattern of seawater-induced alterations in the GH–IGF-1 axis is consistent with previous observations that GH increases production of IGF-1 and IGFR mRNAs in gill and liver in rainbow trout (Biga et al., 2004) and salmon (Pierce et al., 2005).

The current findings suggest that SS may affect hypoosmoregulatory ability through a combination of direct and indirect mechanisms. Foskett et al. (1983) showed that SS-14 directly inhibited chloride transport across the tilapia opercular membrane, and a direct effect of SS-14 in trout is also possible. The indirect pathway operates through the abolishment or attenuation of seawater-associated changes in the expression of GH–IGF-1 axis components. Previously, it was reported that SS-14 injection prior to seawater transfer decreased plasma GH levels in coho salmon (Sweeting and McKeown, 1987). Reduced seawater adaptability following hypophysectomy can be partially rescued by GH (Bjornsson et al., 1987). Although exogenous GH stimulates increased salinity tolerance and gill Na^+, K^+-ATPase activity (Madsen and Bern, 1993), many of the effects of GH on osmoregulatory ability appear to be indirect through the production of IGF-1. For example, GH *in vitro* does not affect gill Na^+, K^+-ATPase, but IGF-1 does (Madsen and Bern, 1993; McCormick, 1995). While Na^+, K^+-ATPase was used as an indicator of hypoosmoregulatory ability, it is not the only protein associated with gill ion transport in chloride cells. Na^+/K^+/$2Cl^-$ co-transporter and cystic fibrosis transmembrane conductance regulator (CFTR) both are necessary for ion excretion under the current model of gill chloride function (McCormick, 1995, 2001; Marshall and Bryson, 1998; Sakamoto et al., 2001; Hirose et al., 2003; McCormick et al., 2003). Increased levels of mRNA in gill for the above-mentioned proteins have been documented with direct transfer to seawater (Scott et al., 2004). The elucidation of effects of SS on the regulation of CFTR and Na^+/K^+/$2Cl^-$ co-transporter proteins would be necessary for a more complete understanding of the osmoregulatory role(s) of SS. It also should be noted that the lack of pronounced effects on plasma chloride in the present study, despite suppression of GH–IGF-1 components, may stem from the presence of other factors involved in osmoregulation that were at least partially functional. For example, experimental evidence points to a synergism between cortisol and GH in promoting the ability to regulate gill Na^+, K^+-ATPase activity and co-transporter function (McCormick, 2001; McCormick et al., 2003). Taken together, these findings may explain why coho salmon pre-smolts displayed reduced seawater adaptability in association with elevated plasma SS (Sheridan et al., 1998).

The effects of SS on components of the GH–IGF-1 axis also provide insight into the role of SSs in regulating growth. The present findings suggest that SS-14, in addition to its inhibitory effects on pituitary GH release, also may affect other levels of the GH–IGF-1 axis (Very and Sheridan, 2002; Klein and Sheridan, in press). The extrapituitary actions of SS on GH sensitivity may help to explain reduced hepatic GH receptor number and depressed plasma IGF-1 in the face of elevated plasma SS observed in stunting salmonids (Very and Sheridan, 2002). Further research is necessary to provide information on the direct effects of SS on GHR, IGF-1, and IGFR mRNA levels. The ability of SS to modulate the effects of GH outside the pituitary at the level of target tissues contributes to our understanding of the multifaceted SS signaling system and how the hormone may coordinate osmoregulation with growth, development, reproduction, and metabolism.

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