Insulin and growth hormone stimulate somatostatin receptor (SSTR) expression by inducing transcription of SSTR mRNAs and by upregulating cell surface SSTRs

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Nelson, Laura E., and Mark A. Sheridan. Insulin and growth hormone stimulate somatostatin receptor (SSTR) expression by inducing transcription of SSTR mRNAs and by upregulating cell surface SSTRs. Am J Physiol Regul Integr Comp Physiol 291: R163–R169, 2006.—This study examined the effects of insulin (INS) and growth hormone (GH) on mRNA and functional expression of somatostatin receptors (SSTRs). Rainbow trout liver was used as a model system to evaluate the direct effects of INS and GH on mRNA expression of three SSTR subtypes characterized previously from this species: SSTR1A, SSTR1B, and SSTR2. INS and GH directly stimulated steady-state levels of all SSTR mRNAs in a concentration- and time-dependent manner; however, the pattern of expression was hormone and SSTR subtype specific. INS stimulated SSTR2 expression to a greater extent than SSTR1A or SSTR1B expression, whereas GH stimulated SSTR2 and SSTR1B expression to a similar extent, with SSTR2 and SSTR1B expression being more responsive to GH than SSTR1A. Whether INS- or GH-stimulated SSTR expression resulted from altered rates of transcription and/or changes in mRNA stability also was investigated. Formation of nascent SSTR transcripts in nuclei isolated from rainbow trout hepatocytes was significantly stimulated by INS and GH. Neither INS nor GH, however, affected the stability of SSTR mRNAs. Functional expression of SSTRs was studied in Chinese hamster ovary (CHO-K1) cells stably transfected with SSTR1A or SSTR1B. Surface expression of functional SSTRs was stimulated by INS and GH. These findings indicate that INS and GH stimulate SSTR expression by regulating transcription of SSTR mRNAs and by increasing functional SSTRs on the cell surface, and they suggest that regulation of SSTRs may be important for the coordination of growth, development, and metabolism of vertebrates.

Insulin-like growth factor I (IGF-I), regulate the synthesis and release of SS (3, 7, 9, 14, 19, 32). The various actions of SSs are initiated through the specific binding of SSs to membrane-bound G protein-coupled receptors that are linked to several different effector pathways. Five SS receptors (SSTRs) are known in mammals and four are known in fish. Variant forms of several SSTRs also exist: SSTR2A and SSTR2B in humans, SSTR3A, SSTR3B, SSTR5A, SSTR5B, and SSTR5C in goldfish, and SSTR1A and SSTR1B in trout (19, 23). As was the case with SS genes, phylogenetic analysis suggests that SSTR genes appear to have arisen from a series of gene duplication events (19).

Numerous structural features, including the presence/locaton of select amino acids on various domains and the extent/position of glycosylation, affect the ligand-binding characteristics of the various SSTRs (23). After binding, the SS ligand-receptor complex may become internalized, which results in the desensitization of target cells (6). Binding analysis in rainbow trout indicates preferential binding of SSTR1A and SSTR1B to the various PPSS products (10). The distribution of SSTRs may underlie tissue-specific responses of SSs. Despite the importance of SSs in the growth, development, and metabolism of organisms (19, 23, 29, 32), relatively little has been reported regarding the regulation of SSTR expression and biosynthesis. Recently, Slagter et al. (30), using the rainbow trout model system, showed that fasting increased SSTR1 and SSTR2 expression. In addition, in vivo administration of INS, GH, and IGF-I to rainbow trout regulated SSTR mRNA expression in a tissue- and subtype-specific manner (31). However, the mechanisms by which alterations in steady-state levels of SSTR mRNA occur and the regulation of recruitment and activation of functional SSTRs on cellular membranes have yet to be determined.

In this study, we used rainbow trout to evaluate further the regulation of SSTR expression. Given the importance of the interplay among GH, INS, and SSs in regulating growth and metabolic processes (26, 29, 32), we focused on the influence of GH and INS on SSTR regulation. Our hypothesis was that INS and GH regulate mRNA and functional expression of SSTRs. We examined the direct effects of INS and GH on hepatic SSTR expression and determined whether changes in the steady-state levels of SSTR mRNAs result from altered rates of RNA transcription and/or from altered mRNA stability. We also used Chinese hamster ovary (CHO-K1) cells...
stably transfected with rainbow trout SSTR1A and SSTR1B to evaluate the effects of INS and GH on the functional expression of SSTRs.

METHODS

Experimental animals. Juvenile rainbow trout (Oncorhynchus mykiss) of both sexes were obtained from Dakota Trout Ranch (near Carrington, ND) and transported to North Dakota State University. They were maintained in 800-liter circular tanks supplied with recirculated (10% make-up volume per day) dechlorinated municipal water at 14°C under a 12:12-h light-dark photoperiod. Fish were fed PMI AquaMax Grower (Brentwood, MO) to satiety twice daily, except 24–36 h before experimentation. Animals were acclimated to laboratory conditions for ≥4 wk before experimentation.

Tissue culture and steady-state mRNA levels. The direct effects of salmonid INS (sINS) and salmonid GH (sGH) on steady-state levels of SSTR mRNAs were determined in liver tissue removed from rainbow trout (O. mykiss) and incubated in vitro. Fish were anesthetized with 0.05% (vol/vol) 2-phenoxyethanol (Sigma) and measured (weight and length), and their livers were removed and prepared for culture as described previously (12). Briefly, livers were perfused with 0.75% (wt/vol) saline (14°C) until cleared of blood, cut into ~1-mm³ pieces, placed in 24-well culture plates (~5–6 pieces per well), and preincubated (14°C, 100% O₂, shaken at 100 rpm with a gyratory shaker) for 1 h in 1 ml of Hank's medium [in mM: 137 NaCl, 5.4 KCl, 4 NaHCO₃, 1.7 CaCl₂, 0.8 MgSO₄, 0.5 KH₂PO₄, 0.3 Na₂HPO₄, 10 HEPES, and 4 glucose, with 0.24% (wt/vol) BSA, pH 7.6]. After preincubation, the medium was removed, the pieces were washed gently with 1 ml of fresh Hank's medium, and 900 µl of an appropriate hormone test solution were added to each well. Test solutions were isosmotic and consisted of basal medium (control), sINS (generously donated by E. Plisetskaya, University of Washington), or sGH (GroPep, Adelaide, Australia). After incubation for up to 24 h under the same conditions described for preincubation, the medium was removed and the pieces were immediately frozen on dry ice. Liver pieces were stored at −80°C until RNA extraction and quantification by real-time PCR, as described by Slagter et al. (30).

Analysis of SSTR mRNA transcription and stability. Transcription of SSTR mRNAs was assessed by quantification of nascent transcripts in nuclei isolated from rainbow trout hepatocytes. Hepatocytes were isolated essentially as described previously (18). Briefly, after anes-

RESULTS

Steady-state mRNA levels. The direct effects of sINS and sGH on SSTR expression were determined in pieces of rainbow trout liver incubated in vitro. Basal (control) levels of SSTR1A, SSTR1B, and SSTR2 were 569 ± 48 copies of mRNA/µg total RNA, 939 ± 104 units, and 4,695 ± 334 units, respectively; basal levels did not change significantly over the course of the incubation. Treatment of liver tissue with sINS and sGH significantly altered steady-state SSTR mRNA expression. sINS significantly stimulated SSTR steady-state mRNA levels in a dose-dependent manner, with the maximal response at 100 ng/ml (~20 nM; Fig. 1A). sINS stimulated expression of SSTR2 mRNAs to a greater extent than SSTR1A and SSTR1B. SSTR1A was least affected by sINS, with the only significant increases observed at 100 and 1,000 ng/ml. At 100 ng/ml, steady-state levels were increased by 45, 246, and
359% for SSTR1A, SSTR1B, and SSTR2, respectively, compared with controls.

sINS also directly stimulated steady-state SSTR1A, SSTR1B, and SSTR2 mRNA levels in a time-dependent manner (Fig. 1B). Significant changes in mRNA levels of each receptor subtype were apparent after 3 h of incubation at 100 ng/ml. Maximum expression for each mRNA species was observed after 6 h; thereafter, steady-state mRNA levels declined. The rate of responsiveness of SSTR2 expression to sINS was greater than that of SSTR1A or SSTR1B, whereas the responsiveness of SSTR1A and SSTR1B expression to sINS was similar. After 6 h, SSTR1A, SSTR1B, and SSTR2 mRNA levels were 247, 126, and 96% higher, respectively, than control.

sGH significantly stimulated steady-state SSTR mRNA levels in a dose- and time-dependent manner. sGH incrementally stimulated SSTR2 and SSTR1B expression to a similar extent, with SSTR2 and SSTR1B expression being more responsive to sGH than SSTR1A expression, but with SSTR2 expression increasing the most at the highest concentration of sGH (1,000 ng/ml; Fig. 1C). Expression of SSTR1A and SSTR1B peaked at 100 ng/ml (~4 nM); at this concentration, the levels of SSTR1A, SSTR1B, and SSTR2 increased 73, 217, and 236%, respectively, over levels observed in controls. Addition of sGH at 100 ng/ml caused the steady-state SSTR mRNA levels to rise rapidly (Fig. 1D). The rate of responsiveness of all SSTRs to sGH was similar at 6 h. Maximal expression of SSTR1A occurred at 6 h and then declined. Maximal expression of SSTR2 and SSTR1B, which increased 269 and 223%, respectively, over controls, was observed after 12 h.

**Rates of SSTR mRNA transcription.** To determine whether sINS- and sGH-induced increases in steady-state SSTR mRNA levels resulted from alterations in the rates of SSTR transcription, nascent mRNA transcripts were evaluated by nuclear run-on assays. Treatment of rainbow trout hepatocytes with sINS and sGH modulated the rates of transcription of SSTR mRNAs. At 100 ng/ml, sINS significantly increased the rates of transcription of SSTR1A, SSTR1B, and SSTR2 after 6 h of incubation (Fig. 2A). The effect of sINS was notably less on SSTR1A than on SSTR1B and SSTR2, with SSTR1A increasing 53%, SSTR1B increasing 124%, and SSTR2 increasing 127% over the controls. Treatment with sGH (100 ng/ml) resulted in increases similar to those seen with sINS after 6 h, with the transcription rates of SSTR1A increasing 61%, SSTR1B increasing 117%, and SSTR2 increasing 145% over controls (Fig. 2B).

**SSTR mRNA stability.** To determine whether sINS- and sGH-induced increases in the steady-state levels of SSTR mRNAs resulted from alterations in the rates of SSTR mRNA degradation, the stability of mRNAs was evaluated by decay curves. The half-life of each SSTR mRNA species in rainbow trout hepatocytes was ~12 h. After sINS incubation (100 ng/ml), the half-life of each SSTR subtype was not significantly different from those in controls (Fig. 3, A–C). SSTR mRNA levels similarly decreased in all subtypes when incubated in sGH at 100 ng/ml and were not significantly different from controls (Fig. 3, D–F). Taken together, these results indicate that the stability of SSTR mRNAs was unaffected by the presence of sINS or sGH.

**SSTR functional expression.** To determine whether sINS- and sGH-induced increases in SSTR mRNA expression lead to alterations in the functional expression of SSTRs, surface binding of SS was evaluated in CHO-K1 cells stably transfected with SSTR1A or SSTR1B (Fig. 4). sINS and sGH
maximum of 197% compared with controls (Fig. 4). sINS at 1,000 ng/ml increased incrementally to a concentration-dependent manner. The surface expression of SSTR1A in cells treated with sINS at 100 ng/ml increased surface expression of SSTR1B in a time-dependent manner (Fig. 4B). sINS at 1,000 ng/ml increased surface expression of SSTR1B to a maximum of 178% (Fig. 4). sINS at 1,000 ng/ml increased surface expression of SSTR1B to a maximum of 195% (Fig. 4). In cells treated with sGH at 100 ng/ml, surface expression of SSTR1A increased to a maximum of 147% compared with controls, whereas sGH at 10 ng/ml significantly increased surface expression of SSTR1B; thereafter, surface expression remained constant (Fig. 4C).

Exposure of SSTR-expressing CHO-K1 cells to sINS and sGH produced time-dependent increases in SSTR1A and SSTR1B expression. Significant upregulation of SSTR1A was apparent after 6 h and continually increased over the 24 h of treatment (Fig. 4B). Maximum expression was observed at 24 h, when sINS-treated cells demonstrated a 246% increase in SSTR1A surface expression, whereas sGH upregulated surface expression of SSTR1A by 219% compared with controls (Fig. 4B). sINS and sGH also upregulated the surface expression of SSTR1B in a time-dependent manner (Fig. 4D). sINS treatment incrementally increased SSTR1B expression over 24 h, whereas sGH upregulated SSTR1B expression until 18 h, when a significant decline in surface expression was observed. The response to sINS appeared within 6 h, whereas the effects of sGH became significant after 12 h. At 24 h, sINS-treated cells demonstrated a maximal increase of 195% in the surface expression of SSTR1B; maximal upregulation of SSTR1B expression by 107% was observed after 18 h of sGH treatment, with only a 47% increase after 24 h compared with controls.

**DISCUSSION**

This study demonstrated that GH and INS regulate the expression of SSTRs. Incubation of rainbow trout liver tissue in vitro revealed that sINS- and sGH-induced increases in steady-state mRNA levels are direct and rapid and occur at concentrations in the physiological range in the plasma of fish and other vertebrates (26, 32). Additionally, these changes in steady-state SSTR mRNA levels were due to changes in rates of transcription, and not to alterations in SSTR mRNA stability. Using stably transfected CHO-K1 cells, we found that INS and GH promote the recruitment of functional SSTR proteins to the cell surface. These findings support our original hypothesis and suggest that regulation of SSTRs may be important for the coordination of growth, development, and metabolism of vertebrates.

sINS and sGH differentially stimulate the expression of hepatic SSTR mRNAs, as evidenced by the differential patterns of SSTR expression elicited by the hormones during the in vitro incubation. INS stimulated the expression of all SSTR subtypes, with SSTR2 stimulated to a greater extent than SSTR1A or SSTR1B. The responsiveness to sINS was the least with SSTR1A. sGH also increased the expression of all SSTR subtypes, but, in contrast to the pattern observed with sINS, the level of responsiveness was similar for SSTR2 and SSTR1B, and the responsiveness of both was greater than the responsiveness of SSTR1A to sGH. Differential expression of SSTR mRNAs also was observed in rainbow trout implanted with INS and GH (31); however, the nature of the pattern was somewhat different from that observed in the present study: INS in vivo did not affect hepatic SSTR1A, and GH in vivo did not affect hepatic SSTR2. The difference between the two studies may, in part, result from indirect actions of INS and GH mediated by unknown factor(s) in vivo. Nevertheless, the differential patterns of expression observed between the various receptor subtypes suggest that the hormones elicit their effects through multiple pathways and that the mechanisms regulating the individual SSTR subtypes may be working independently. Because SS isoforms display differential binding to SSTRs and because SSTR subtypes may be linked to the cell surface. These findings support our original hypothesis and suggest that regulation of SSTRs may be important for the coordination of growth, development, and metabolism of vertebrates.

This study extends our knowledge of the regulation of SSTR expression. Previous studies in goldfish, rainbow trout, and mammals reported the effects of other hormones, including 17β-estradiol, glucocorticoids, SS, and GH-releasing hormone (GHRH) on SSTR expression (4, 16, 19, 21, 22, 31). For example, in rainbow trout, in vivo INS treatment reduced SSTR1B expression in the optic tectum, increased SSTR2...
expression in the pancreas, and increased SSTR1B and SSTR2 expression in the liver (31). In some cases, the results appear contradictory. For example, in vitro treatment of rat pituitaries with GHRH significantly increased SSTR1 and SSTR2 mRNA levels (21), whereas a trend toward reduced SSTR1 and SSTR2 expression was observed in pig pituitary cultures in response to acute GHRH treatment (16). The collective findings of the present work and previous reports suggest a species-, tissue-, and subtype-specific regulation of SSTR expression.

The INS- and GH-induced increases in SSTR mRNA expression resulted from increased rates of transcription, and not from altered SSTR mRNA stability. This conclusion is supported by the following observations. The inability of sINS or sGH to alter degradation of SSTR mRNAs supports the notion that sINS- and sGH-stimulated SSTR expression does not involve changes in RNA stability. The formation of nascent transcripts of SSTR mRNAs in nuclei of rainbow trout hepatocytes was significantly stimulated by sINS and sGH. Notably, sINS- and sGH-induced changes in transcription were consistent with observed alterations in steady-state mRNA levels. Previous studies have observed the in vivo effects of INS treatment on the rates of transcription of SSTR mRNA in the pancreas of rats. INS injections increased the number of SSTR5 transcripts in the pancreas, encouraging speculation that INS directly regulates SSTR5 gene activation (17). To our knowledge, no other experiments have examined the factors that regulate the rates of transcription of SSTR mRNA, but other studies have examined transcriptional regulation of the GH and INS receptors (25, 33).

The mechanisms by which INS and GH induce changes in SSTR transcription are unknown. Presumably, such changes involve the promoter regions of SSTR genes, as well as certain transcription factors. The promoter regions of SSTR genes in rats, mice, and humans have been studied, but there is little information on the SSTR genes of fish (20). Interestingly, researchers have identified a transcription factor in rats that acts at a specific binding site, designated Pit-1, which is hypothesized to genetically control SSTR1. Other transcription factors for SSTR2, SSTR3, and SSTR5 have been identified in mammals as well (1, 20). More information about the molec-
ular mechanisms that affect SSTR expression is needed to fully elucidate the regulation of the SS signaling system.

INS and GH stimulate the functional expression of SSTR1A and SSTR1B. This conclusion is supported by the observation that surface expression of SSTRs, as assessed by specific binding of radiolabeled SS-14, was significantly enhanced in the presence of INS and GH. These findings indicate that not only do INS and GH promote the transcription of SSTR mRNAs, they also stimulate the synthesis of SSTRs and their recruitment to the cell surface. Binding analyses of various SSTRs have been performed in numerous species of fish and mammals (5, 23, 24). Analysis of fish SSTRs expressed in mammalian cell lines revealed saturable, high-affinity binding that is selective for specific SS peptides (10, 15). There is a paucity of information on the regulation of SSTR binding. Nutritional state and various metabolic hormones (e.g., thyroid-stimulating hormone, triiodothyronine, and thyroxine) have been reported to alter the binding of SSTRs (2, 13, 24). In addition, surface binding of SS-14 to rat SSTR subtypes expressed in GH4CL cells showed varied responses to 17βestradiol and testosterone treatment (2).

The regulation of SSTR by INS and GH has important physiological ramifications. By stimulating the mRNA and functional expression of SSTRs, INS and GH increase the sensitivity of cells to SSs. An increase in the sensitivity of cells to SS would initiate a series of metabolic and growth counter-regulatory measures that would result in a shift away from the anabolic/growth-promoting actions of GH and INS (e.g., cellular uptake of amino acids, elevated protein synthesis, and production of IGF-I) (26, 32) toward a pattern of catabolism. At the level of the pancreas, SS inhibits INS release (29). At the level of the liver, enhanced sensitivity to SS would promote its catabolic actions (e.g., glycogenolysis and lipolysis) (29). In addition, at the liver and other sites (e.g., gill), SS decreases GH binding and IGF-I production and results in lower plasma IGF-I levels and reduced growth (32). INS- and GH-induced changes in SSTR expression appear to be coordinated with increased PPSS expression (19). The increased production of SS would then be able to bind to SSTRs, the surface expression of which was previously increased by the presence of INS and GH. Because of the physiological properties of INS and GH, it is not surprising that they differentially regulate SSTRs (26, 29, 32). For example, despite the involvement of both factors in growth, in some situations (e.g., fasting) their actions diverge (e.g., GH becomes catabolic) (32); differential interaction among INS, GH, SSTRs, and, ultimately, SSs may underlie such divergent conditions (via selective suppression of anabolic or growth-promoting pathways and/or selective activation of catabolic or growth-inhibiting pathways).

In summary, the findings of this study indicate that INS and GH stimulate the mRNA and functional expression of SSTRs. Such regulation may be important for the control of numerous physiological processes, including the coordination of growth, development, and metabolism. Future work is needed to identify the effector pathways through which hormones regulate the transcription of SSTR genes and the recruitment of functional SSTRs to the cell surface and to elucidate the means by which tissue- and subtype-specific regulation of SSTR expression occurs.

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Fig. 4. Effects of INS and GH on functional expression of SSTR. CHO-K1 cells stably transfected with SSTR1A (A and B) or SSTR1B (C and D) were incubated for 12 h with 1–1,000 ng/ml sINS or sGH (A and C) or for 6–24 h with sINS (100 ng/ml) or sGH (100 ng/ml; B and D). Whole cell specific binding was measured using [125I]Tyr11-SS-14. Values are means ± SE (n = 8). For a given hormone treatment, groups with different letters (a, b, c) are significantly different from each other. *Significantly different from control (P < 0.05).
REFERENCES


