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

Laura E. Nelson and Mark A. Sheridan

Department of Biological Sciences, North Dakota State University, Fargo, North Dakota

Submitted 26 October 2005; accepted in final form 31 January 2006

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 was also 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.

Address for reprint requests and other correspondence: M. A. Sheridan, Dept. of Biological Sciences, North Dakota State Univ., Fargo, ND 58105 (e-mail: Mark.Sheridan@ndsu.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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 gyrotary 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 fresh medium and 100 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 ~8°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 anesthetia with 0.05% (vol/vol) 2-phenoxyethanol and transaction of the spinal cord, livers (~6–8 per experiment) were perfused in situ at a rate of 1 ml/min with cold (14°C) medium A (in mM: 136.9 NaCl, 5.4 KCl, 0.81 MgSO₄, 0.44 KH₂PO₄, 0.33 Na₂HPO₄, 10 HEPES, 5.0 NaHCO₃, and 5 EGTA, pH 7.63) during continuous exposure to 100% O₂. Once cleared of blood, the perfusate was changed to medium B (medium A with 0.6 mg/ml collagenase). After 20–30 min, the livers were removed and placed in a petri dish on ice, and the cells were separated using sterile razor blades and ice-cold medium A. The pieces were gently passed through a 73-μm nylon screen, and the resulting cells were collected by centrifugation (100 g for 3 min at 14°C). The cells were washed once in medium A and three times in medium C (medium A with 1.5 mM CaCl₂ and 2% defatted BSA, pH 7.63) and, finally, resuspended in incubation medium [medium C with 4 mM glucose, 2 ml of 50× MEM amino acid mix/100 ml (GIBCO), and 1 ml of 100× nonessential amino acid mix/100 ml (GIBCO)]. The viability of the cells as assessed by trypan blue dye exclusion was ≳90% for all experiments. The isolated cells (~8 × 10⁷/ml) were preincubated (14°C, 100% O₂, shaken at 100 rpm with a gyrotary shaker) for 2 h to allow “metabolic recovery” before experimentation.

For transcription assays, isolated cells (~2 × 10⁷/sample) were placed in 50-ml culture flasks in the presence or absence of 100 ng/ml sINS (~20 nM) or sGH (~4 nM) and incubated (14°C, 100% O₂, shaken at 100 rpm with a gyrotary shaker) for up to 24 h. Nuclei were isolated as described by Greenberg (11). Once isolated, the nuclei were stored at ~8°C in glycerol storage buffer [10 mM Tris·HCl, 5 mM MgCl₂, 0.1 mM EDTA, and 40% (vol/vol) glycerol, pH 8.3]. The run-on assay was conducted as described previously (27). Briefly, nuclei were thawed and mixed with run-on buffer reagents [25 mM Tris·HCl, pH 8.0, 12.5 mM MgCl₂, 750 mM KCl, ATP, GTP, and CTP at 1.25 mM each, 0.6% (wt/vol) Sarkosyl, and 100 μCi of [³²P]UTP (Amersham Life Science, Arlington Heights, IL)] in 2-ml microcentrifuge tubes. The samples were incubated for 60 min (30°C), DNase I was added, and incubation continued for 15 min. RNA was immediately extracted (27), and labeled nuclear transcripts were hybridized to specific SSTR cDNA probes previously immobilized on nylon membranes by use of a slot-blot apparatus. Blots were quantified by phosphor imaging, as described previously (8).

To examine the stability of SSTR mRNAs, isolated hepatocytes (2 × 10⁶/ml) were placed in 24-well plates, as described previously (8). After incubation with actinomycin D (5 μg/ml) for 30 min, the cells were collected and incubated in the presence or absence of 100 ng/ml INS or GH for up to 24 h. Then the cells were collected, and RNA was extracted and analyzed by quantitative real-time PCR as described by Slagter et al. (30).

Transfection and analysis of functional expression. Chinese hamster ovary (CHO-K1 wild-type) cells (America Type Culture Collection, Rockville, MD) were maintained and stably transfected with rainbow trout SSTR1A and SSTR1B, as described previously (10). For the experiment, 5 × 10⁵ cells successfully transfected with SSTR1A and SSTR1B were seeded in each well of 24-well culture plates. They were incubated (37°C, 5% CO₂, 95% humidity) overnight in complete medium [Ham’s F-12K supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.1% (vol/vol) amphotericin B (Fungizone)]. Cells were washed three times with binding medium (Ham’s F-12K without fetal calf serum or antibiotics), and incubation was started by addition of binding medium (control) or binding medium plus hormone (INS or GH at 100 ng/ml). At various times, surface binding of [³²P]Tyr¹¹-SS-14 (74 Tbq/mmol; Amersham Pharmacia Biotech, Piscataway, NJ) to whole cells was determined as described previously (10).

Statistical analysis. Quantitative data are expressed as means ± SE. Results from experiments were evaluated for statistical differences by ANOVA followed by Student-Newman-Keuls multiple range test. Differences were considered significant at P < 0.05. All statistics were performed using SigmaStat (SPSS, Chicago, IL). For ease of comparison, data are presented as percent change (final level – initial level/initial level × 100); statistics were performed on untransformed data.

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 ± 84 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 mRNA increasing 236% and 217%, 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). Insulin (sINS) at 1,000 ng/ml increased surface expression of SSTR1A in a concentration-dependent manner. The surface expression of SSTR1A in cells treated with sINS at 1,000 ng/ml increased incrementally to a maximum of 178% (Fig. 4A). 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 SSTR1B expression 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 different cellular responses (23, 28), differential regulation of SSTRs may influence the sensitivity and response of cells to SSs.

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, and significantly increased surface expression of SSTR1B; there-
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-

---

**Fig. 3.** Effects of INS and GH on stability of SSTR mRNAs. Half-life of SSTR1A, SSTR1B, and SSTR2 was determined in rainbow trout hepatocytes incubated in the presence or absence of 100 ng/ml sINS (A–C) or 100 ng/ml sGH (D–F). Actinomycin D (5 μg/ml), an inhibitor of RNA synthesis, was added to cultures 30 min before time 0, total RNA was extracted, and SSTR mRNA was quantified using real-time PCR. Values are means ± SE (n = 8).
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 PSSS 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.

ACKNOWLEDGMENTS

We thank Jun-Yang Gong, Jeff Kittilson, and Laura Luick for technical assistance.
REFERENCES


30. Slagter RJ, Kittilson JD, and Sheridan MA. Somatostatin receptor subtype 1 and subtype 2 mRNA expression is regulated by nutritional state in rainbow trout (Oncorhynchus mykiss). Gen Comp Endocrinol 139: 236–244, 2004.

