Korean J Physiol Pharmacol
Vol 7: 79–84, April, 2003

Modulation of Pituitary Somatostatin Receptor Subtype (sst1-5) mRNA Levels by Growth Hormone (GH)-Releasing Hormone in Purified Somatotropes

Seungjoon Park[1], Heesoon Park[1], Mina Lee[1], Sookjin Sohn[1], Eunhee Kim[1], Jeechang Jung[1], Lawrence A. Frohman[2], and Rhonda D. Kineman[2]

[1]Department of Pharmacology, Kyunghee University School of Medicine, Seoul 130–701, Korea; [2]Department of Medicine, Section of Endocrinology and Metabolism, University of Illinois at Chicago, Chicago, IL 60612, USA

We have previously reported that expression of the somatostatin receptor subtypes, sst1-5, is differentially regulated by growth hormone (GH)-releasing hormone (GHRH) and forskolin (FSK), *in vitro*. GHRH binds to membrane receptors selectively located on pituitary somatotropes, activates adenylyl cyclase (AC) and increases sst1 and sst2 and decreases sst5 mRNA levels, without significantly altering the expression of sst3 and sst4. In contrast FSK directly activates AC in all pituitary cell types and increases sst1 and sst2 mRNA levels and decreases sst3, sst4 and sst5 expression. Two explanations could account for these differential effects: 1) GHRH inhibits sst3 and sst4 expression in somatotropes, but this inhibitory effect is masked by expression of these receptors in unresponsive pituitary cell types, and 2) FSK inhibits sst3 and sst4 expression levels in pituitary cell types other than somatotropes. To differentiate between these two possibilities, somatotropes were sequentially labeled with monkey anti-rat GH antiserum, biotinylated goat anti-human IgG, and streptavidin-PE and subsequently purified by fluorescent-activated cell sorting (FACS). The resultant cell population consisted of 95% somatotropes, as determined by GH immunohistochemistry using a primary GH antiserum different from that used for FACS sorting. Purified somatotropes were cultured for 3 days and treated for 4 h with vehicle, GHRH (10 nM) or FSK (10 μM). Total RNA was isolated by column extraction and specific receptor mRNA levels were determined by semi-quantitative multiplex RT-PCR. Under basal conditions, the relative expression levels of the various somatostatin receptor subtypes were sst2 > sst5 > sst3=sst1 > sst4. GHRH treatment increased sst1 and sst2 mRNA levels and decreased sst3, sst4 and sst5 mRNA levels in purified somatotropes, comparable to the effects of FSK on purified somatotropes and mixed pituitary cell cultures. Taken together, these results demonstrate that GHRH acutely modulates the expression of all somatostatin receptor subtypes within GH-producing cells and its actions are likely mediated by activation of AC.

Key Words: GHRH, Forskolin, Somatostatin receptor, Somatotrope, cAMP, Adenylyl cyclase

INTRODUCTION

Somatostatin (SRIF), a hypothalamic tetradecapeptide, is a physiologic inhibitor of growth hormone (GH) release from anterior pituitary somatotropes. It is also present in other peripheral tissues such as the gastrointestinal tract and the pancreas, where it regulates islet cell functions, gastrointestinal cell functions and immune cell functions (Reisine, 1995). SRIF acts through specific membrane-bound, high affinity, G protein-coupled receptors, termed SRIF receptors. Five SRIF receptor subtype genes have been cloned; sst1-sst5, encoding six proteins, sst1, sst2A, sst2B, sst3, sst4, and sst5 (Patel, 1999). In the rat anterior pituitary, all SRIF receptor subtypes are known to be expressed (Kumar et al, 1997; O'Carrol & Krempels, 1995), and sst2 and sst5 appear to be the dominant subtypes in the rat somatotropes (Day et al, 1995; Mezey et al, 1998) and the human somatotropes (Greenman & Melmed, 1994a,b; Panetta & Patel, 1994).

We have recently observed that GHRH can mediate the expression of the SRIF receptor subtypes (sst1-5), *in vivo* and *in vitro* (Park et al, 2000). Specifically, GHRH (10 nM, 4h) stimulates sst1 and sst2 expressions and decreases the expression of sst5, but had no significant effect on sst3 and sst4 expressions. Treatment of primary rat pituitary cell cultures with forskolin (FSK; 10 μM) mimicked the effects of GHRH on sst1, sst2 and sst5 expression. In addition, FSK decreased sst3 and sst4 mRNA levels. Since SRIF

Corresponding to: Seungjoon Park, Department of Pharmacology, Kyunghee University School of Medicine, #1 Hoiki-dong, Dongdaemun-gu, Seoul 130-701, Korea. (Tel) 02-961-0913, (Fax) 02-968-0560, (E-mail) sjpark@khu.ac.kr

ABBREVIATIONS: SRIF, somatostatin; GH, growth hormone; GHRH; growth hormone-releasing hormone; FSK, forskolin; sst, somatostatin receptor; GHRH-R, GHRH receptor; GHS-R, GH secretagogue receptor.

receptors are expressed by multiple pituitary cell types (Day et al, 1995; O'Carroll & Krempels 1995; Panetta & Patel, 1995; Mezey et al, 1998), two possibilities can account for the differential effects of GHRH and FSK: 1) GHRH inhibits sst3 and sst4 expressions in soma totropes, but this effect is masked by expression of these receptors in unresponsive pituitary cell types, or 2) FSK inhibits sst3 and sst4 expression levels in pituitary cell types other than somatotropes. In the present study, in order to differentiate between these possibilities we examined the effects of GHRH and FSK on SRIF receptor subtype mRNA levels in mixed pituitary cell cultures and purified somatotropes.

METHODS

Purification of somatotropes by fluorescent-activated cell sorting (FACS)

Somatotropes were purified from freshly isolated monodispersed rat pituitary cells, using an adaptation of the protocol reported by Wynick et al. (1990a,b), which was originally developed to purify both lactotropes and soma totropes from rat pituitary cell cultures. This procedure is based on the fact that in ligand-stimulated pituitary cells a large portion of the secreted hormone coats the surface of the cells, and therefore represents a cell-type specific target. In each experiment, four male rat pituitaries were enzymatically dispersed, and the recovered cells ($10\sim12\times10^6$) were incubated for 1h at 37C, to allow for recovery from the dispersion. Pituitary cells ($7\sim8\times10^6$) were subsequently incubated with monkey anti-rat GH antiserum (1 : 1000) in the presence of GHRH (10 nM) for 1h at 4C. Cells were then washed and incubated with biotinylated goat anti-human IgG (1 : 250; Vector Laboratories) for 30 min and subsequently washed and incubated (at 4C) with streptavidin-PE (15 ng/1×10^6 cells, Becton & Dickinson Pharmingen, Franklin Lakes, NJ) for 30 min. A separate aliquot of cells ($1\sim2\times10^6$) was treated as described above, except that primary antibody was replaced with normal serum to quantify nonspecific signal intensity. Cells were subsequently filtered through a 20 μm nylon mesh to insure single cells for FACS. After filtering, approximately 80% of the starting cell number was recovered. The fluorescent signal intensity of a small aliquot (0.02×10^6) of cells, previously treated with primary antibody (specific) or without primary antibody (non specific), was assessed using an Epics Elite ESP FACS (Beckman Coulter, Fullerton, CA). Within each set of cells processed, the nonspecific signal was determined and used to gate primary antibody-treated cells ($4\sim5\times10^6$) during sorting. Approximately 50~60% of cells were discarded in the flow-through due to signal intensities in the nonspecific range. Final cell recovery after FACS was $0.5\sim1\times10^6$ each of somato trope-enriched and somatotrope-depleted populations. Fig. 1A shows representative scatter plots of the nonspecific fluorescent signal (upper left panel; cells processed in the absence of 1st antibody) and the GH-specific fluorescent signal (upper right panel; cells processed in the presence of 1st antibody) in unsorted cells. Cells with signals greater than nonspecific were considered as somatotrope-enriched (GH+, lower right panel) and those with signals less than the nonspecific signal were considered as GH-depleted (GH−, lower left panel).

To test the purity of the sorted cells, somatotrope-enriched and somatotrope-depleted populations were plated

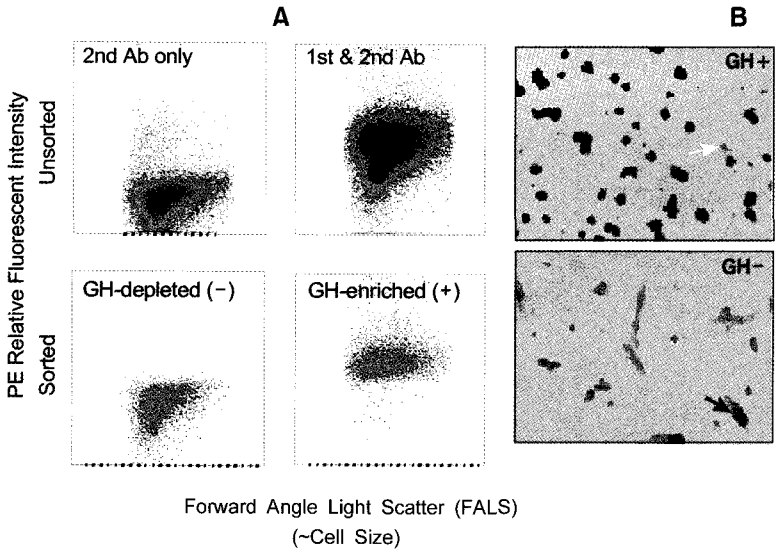

Fig. 1. An Epics Elite ESP FACS (Beckman Coulter) was used to sort monodispersed rat pituitary cells labeled sequentially with monkey anti-rat GH sera (1st antibody), biotinylated goat anti-human serum (2nd antibody) and strepavidin-PE. (A) Upper panels show scatter plots of fluorescent intensity of unsorted cells processed in the absence of 2nd antibody (left panel; nonspecific signal) or in the presence of 1st and 2nd antibody (right panel; specific and non-specific signal). The nonspecific signal was used to gate cells for sorting. Cells with signals greater than nonspecific were considered somatotrope-enriched (GH+, lower right panel) and those with signals less than the nonspecific signal were considered somatotrope-depleted (GH−, lower left panel). (B) GH immunocytochemical (ICC) staining of the GH+ (upper panel) and GH− (lower panel) populations showing somatotrope-enriched cells are >95% GH-immunopositive, where only ~5% of the GH-depleted cells stained positive for GH. The black arrow indicates a GH positive cell in a somatotrope-depleted cell culture.

on culture well slides (Becton & Dickinson) and incubated overnight in serum-containing media. The following day, cultures were washed and processed for GH immuno cytochemistry as previously described (Kineman et al, 1996) with the exception that the primary antibody used was from a species different from that used for cell sorting (rabbit anti-rat GH, 1 : 10,000 Lot #AFP5672099; provided by Dr. AF Parlow, NHPP, NIDDK). GH ICC demonstrated somatotrope-enriched cells were >95% GH-immuno positive (Fig. 1B, upper panel), where only ~5% of the GH-depleted cells stained positive for GH (Fig. 1B, lower panel). To control the potential "carry over" effect of the FA CS labeling antibody, controls without primary antibody were used in the ICC reaction and all were negative (data not shown).

Cell culture, RNA extraction and multiplex RT-PCR

Unsorted and FACS-sorted cells were cultured for 3 days in DMEM/10% HS. Media were then replaced with serum-free media containing GHRH (10 nM) or FSK (10 μM). Cells were incubated for 4h and total RNA was subsequently extracted using Strategene's Total RNA Microprep Kit (Stratagene, La Jolla, CA), according to the manufacturer's instructions. This kit is designed to efficiently extract and purify total RNA from a small number of cells. In brief, cells were lysed and protein was denatured using a guanidine thiocyanate-containing buffer. Following cell lysis, the suspension was mixed with ethanol and applied to a fiber matrix column to bind the RNA. The column was washed and DNase was added, followed by an additional wash to remove DNase and impurities, and the RNA was then eluted from the fiber matrix with 30 μl of 10 mM Tris-HCl, pH 7.5.

RNA was reverse transcribed with random hexamer priming. The resultant cDNA was PCR amplified using primers specific for rat SRIF receptor subtypes (sst1-5) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDN A in the presence of [α-^{32}P]dCTP, as previously reported by our laboratory (Park et al, 2000). To measure the relative levels of pituitary GHRH receptor (GHRH-R) and GH secretagogue receptor (GHS-R) mRNA, multiplex RT-PCR was performed using primers specific for rat GHRH-R, GHS-R, and GAPDH. The rat GHRH-R primers consisted of a forward primer, 5'-AGG GGC TGT GAA AAG GGA CTG-3' and reverse primer, 5'-CAG TGT CCT CAA AAG CCA GTT-3' designed using GenBank L01407 (PCR product size, 545 bp). The rat GHS-R primers consisted of a forward primer, 5'-TTC GCC ATC TGC TTC CCT CTG-3' and a reverse primer, 5'-TGT CTG CTT GTG GTT CTG GTC-3' designed using GenBank U94321 (PCR product size 354 bp). Validation reactions were performed to determine the PCR conditions and primer concentrations that would yield noncompetitive and specific amplification for each PCR product (data not shown). Based on these preliminary experiments, 0.1 μM GHRH-R primers, 0.6 μM GHS-R primers and 0.05 μM GAPDH primers were used and the following cycle profile applied: 1 cycle of 95C for 10 min; 27 cycles of 95C 30 sec, 60C 1min, 72C 1 min; 1 cycle of 72C 10 min. PCR products were separated on polyacrylamide-8M urea gels. Gels were dried and exposed to a phosphorimage screen. Band intensity was evaluated by image analysis software.

Statistical analysis

All data are expressed as mean±SEM. Comparisons between groups were made by Student's t-test. $P < 0.05$ was considered significant. All comparisons were made between samples electrophoresed on the same gel.

RESULTS

Comparison of the relative expression levels of GH regulatory receptors in somatotrope-enriched and somatotrope-depleted cultures

The relative expression of the SRIF receptor subtypes

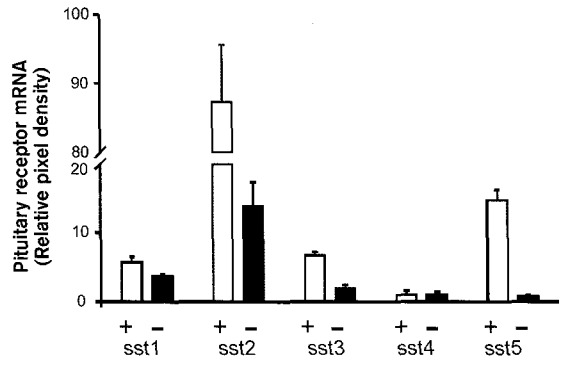

Fig. 2. Relative expression levels of SRIF receptor subtype in somatotrope-enriched (+) and somatotrope-depleted (−) cultures. Somatotropes were FACS purified as described in the legend to Fig. 1 and plated at 4×10^4 cells/well in α-MEM/10% horse serum. After 3 days of culture, total RNA was extracted and receptor mRNA levels were determined by multiplex RT-PCR. Values shown are mean±SEM (n=4 wells) of signal intensity for each receptor subtype adjusted by GAPDH (a housekeeping gene). Similar differences in the relative receptor mRNA levels in somatotrope-enriched and somatotrope depleted cultures were observed in two independent experiments.

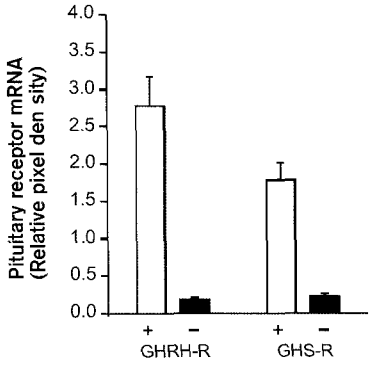

Fig. 3. Relative expression levels of GHRH-R and GHS-R in somatotrope-enriched (+) and somatotrope-depleted (−) cultures. Somatotropes were FACS purified as described in the legend to Fig. 1 and plated at 4×10^4 cells/well in α-MEM/10% horse serum. After 3 days of culture, total RNA was extracted and receptor mRNA levels were determined by multiplex RT-PCR. Values shown are mean±SEM (n=4 wells) of signal intensity for each receptor subtype adjusted by GAPDH (a housekeeping gene). Similar differences in the relative receptor mRNA levels in somatotrope-enriched and somatotrope depleted cultures were observed in four independent experiments.

(sst1-5) in FACS purified somatotrope-enriched and somatotrope-depleted cultures is illustrated in Fig. 2 (representative example of 2 independent experiments). Sst2 and sst5 were expressed at levels 5~9 fold higher in somatotrope-enriched cultures than in somatotrope-depleted cultures. Fig. 3 represents the relative expression levels of GHRH-R and GHS-R in FACS purified pituitary cultures (representative of 4 independent experiments). Somato trope-enriched cultures expressed high levels of GHRH-R mRNA when compared to somatotrope-depleted cultures, where the GHRH-R mRNA levels of somatotrope-depleted relative to somatotrope-enriched cultures was 6%. This result agrees with the ICC data that showed 5% GH positive cells in somatotrope-depleted cultures. GHS-R mRNA levels of the somatotrope-enriched population were also higher than those of somatotrope-depleted cultures.

Effect of GHRH and FSK on SRIF receptor subtype mRNA levels in mixed pituitary cell cultures and somatotrope-enriched cultures

The effects of GHRH and FSK on SRIF receptor subtype mRNA levels in mixed pituitary cell cultures are presented in Fig. 4. GHRH (10 nM) increased sst1 and sst2 mRNA levels to 171% ($P<0.05$) and 133% ($P<0.01$) of vehicle-treated control values, respectively. In contrast, GHRH decreased sst5 mRNA levels to 47% of controls ($P<0.01$). Sst3 and sst4 mRNA levels were not significantly altered by acute GHRH treatment. FSK (10 μM) increased sst1 and sst2 mRNA levels to 175% ($P<0.05$) and 144% ($P<0.05$) of vehicle-treated control values, respectively, while FSK decreased sst3, sst4, and sst5 mRNA levels to 26%, 61%, and 39% of controls ($P<0.01$).

The effects of GHRH and FSK on SRIF receptor subtype mRNA levels in somatotrope-enriched cultures are presented in Fig. 5. GHRH (10 nM) and FSK (10 μM) stimulation of somatotrope-enriched cells increased sst1 and sst2 mRNA levels and decreased sst3, sst4 and sst5 mRNA levels, similar to the effect of FSK on SRIF receptor expression in mixed pituitary cell cultures.

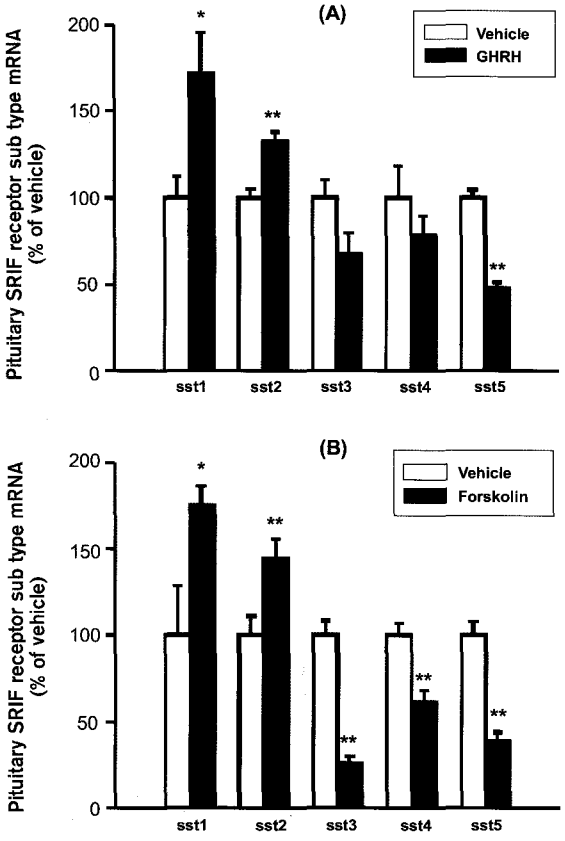

Fig. 4. Effects of GHRH (A) and forskolin (B) on SRIF receptor subtype mRNA levels in mixed pituitary cell cultures. Mixed (unsorted) pituitary cell cultures were incubated in the absence (vehicle) or presence of GHRH (10 nM) or forskolin (10 μM). Following a 4-h incubation, cells were extracted and SRIF receptor subtype (sst1-5) mRNA levels were determined by multiplex RT-PCR. Sst1-5 mRNA levels were adjusted by GAPDH and expressed as percent of vehicle-treated controls. Values represent mean±SEM (n=4 wells/treatment group). *, $P<0.05$; **, $P<0.01$.

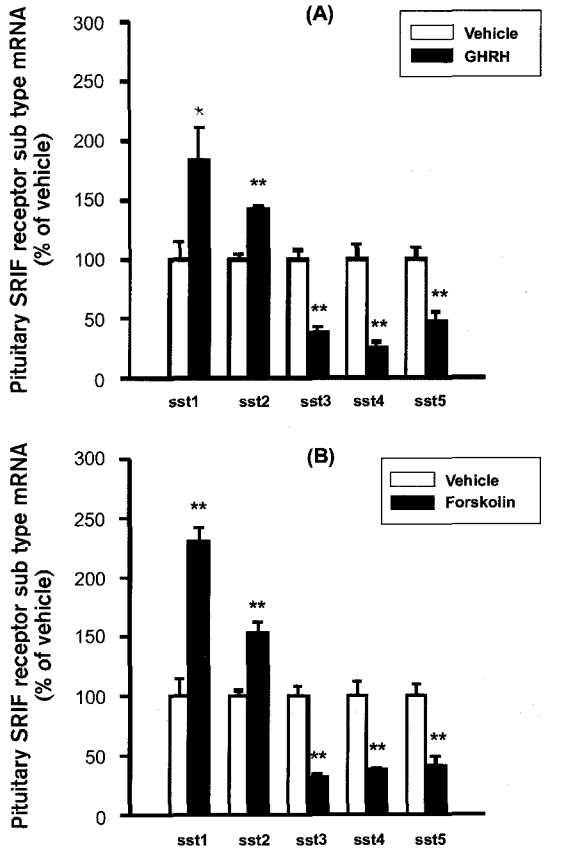

Fig. 5. Effects of GHRH (A) and forskolin (B) on SRIF receptor subtype mRNA levels in FACS purified somatotrope-enriched cultures. Somatotrope-enriched cultures were incubated in the absence (vehicle) or presence of GHRH (10 nM) or forskolin (10 μM). Following a 4-h incubation, cells were extracted and SRIF receptor subtype (sst1-5) mRNA levels were determined by multiplex RT-PCR. Sst1-5 mRNA levels were adjusted by GAPDH and expressed as percent of vehicle-treated controls. Values represent mean±SEM (n=4 wells/treatment group). *, $P<0.05$; **, $P<0.01$.

DISCUSSION

In the present study, we used FACS purified soma totrope-enriched cultures in order to determine the direct effect of GHRH on SRIF receptor subtypes mRNA levels within the GH-producing cells. The results of the present study demonstrate that GHRH acutely modulates the expression of all SRIF receptor subtypes within GH-producing cells and its actions are likely mediated by activation of the cAMP-intracellular signal transduction pathway.

As we previously reported (Park et al, 2000), pituitary sst1, sst2, and sst5 mRNA levels are regulated both *in vivo* and *in vitro* by GHRH. The effect of FSK mimicked that of GHRH on sst1, sst2, and sst5 mRNA and, in addition, FSK reduced sst3 and sst4 expression. Limiting our interpretation of changes in cell function in mixed pituitary cell cultures is the fact that it is not possible to determine if the effects of any given treatment were due to specific changes in somatotrope function or the effects represented alterations in other pituitary cell subtypes (*i.e.* lactotropes, thyrotropes, corticotropes and gonadotropes), since all pituitary cell types express variable levels of each SRIF receptor subtype (Day et al, 1995; O'Carroll & Krempels, 1995; Panetta & Patel, 1995; Mezey et al, 1998). To circumvent this problem, we used FACS to obtain somatotrope-enriched populations for *in vitro* studies. Treatment of purified somatotropes with GHRH and FSK increased sst1 and sst2 mRNA levels and decreased sst3, sst4, and sst5 mRNA levels, similar to the effects of FSK on mixed pituitary cell cultures. These results indicate that GHRH inhibits sst3 and sst4 mRNA levels in somatotropes, but this inhibitory effect is masked by expression of these receptors in unresponsive pituitary cell subtypes

It has been reported that expression of pituitary receptors can be rapidly regulated by homologous and heterologous ligand stimulation (Aleppo et al, 1997). Specifically, GHRH can decrease the expression of its own receptor, GHRH-R, both *in vitro* and *in vivo* and this effect can be mimicked *in vitro* by FSK. Although the pituitary is made up of multiple cell types, the inhibitory actions of GHRH on its own receptor expression are likely mediated by stimulation of the cAMP-intracellular signaling pathway within somatotropes. This assumption is based on the fact that the GHRH-R has been shown to be exclusively expressed by GH-producing cells of the normal pituitary (Lopes et al, 1997; Morel et al, 1999; Oka et al, 1999).

These results, taken together with previous findings, demonstrate that GHRH can rapidly regulate the expression of SRIF receptors, in addition to mediating the production of its own receptor. Within the context of normal physiology, we might speculate that rapid ligand-mediated changes in receptor synthesis are critical to maintain cell surface receptors at appropriate concentrations to temper intermittent hypothalamic signals and thus could contribute to pulsatile GH release. Although this logic fits with the GHRH-induced inhibition of GHRH-R and stimulation of sst2, it does not fit with the GHRH-induced decrease in sst5, where both sst2 and sst5 are considered to be the primary mediators of SRIF-induced inhibition of GH release (Shimon et al, 1997a,b; Rohrer et al, 1998). The differential regulation of sst2 and sst5 suggest that these receptors may have diverged in the rat to play distinct roles in somatotrope regulation.

Somatotropes express more sst2 and sst5 than any other SRIF receptor subtypes, as assessed by *in situ* hybridization and immunocytochemistry (Day et al, 1995; Kumar et al, 1997; Mezey et al, 1998; O'Carroll & Krempels, 1995; Panetta & Patel, 1995). In the current report, we examined the relative expression of the SRIF receptor subtypes (sst1-5) in FACS purified somatotrope-enriched and somatotrope-depleted cultures. The expression level of sst2 and sst5 in somatotrope-enriched cultures was higher than that of somatotrope-depleted cultures, consistent with reports using *in situ* hybridization (Day et al, 1995; O'Carroll & Krempels, 1995; Panetta & Patel, 1995, Mezey et al, 1998). Our observations, taken together with those of others, suggest that sst2 and sst5 are the dominant SRIF receptor subtype in the rat pituitary. We also examined the relative expression levels of GHRH-R and GHS-R in FACS purified pituitary cultures. Consistent with the exclusive expression of GHRH-R within the pituitary somatotrope (Lopes et al, 1997; Morel et al, 1999; Oka et al, 1999), somato trope-enriched cultures expressed high levels of GHRH-R mRNA, when compared to somatotrope-depleted cultures. The somatotrope-enriched population also expressed high levels of GHS-R, consistent with work of Smith *et al.* (1997), demonstrating that GHS-R mRNA is predominately localized to GH-producing cells.

In summary, these results demonstrate that GHRH acutely modulates the expression of all somatostatin receptor subtypes within GH-producing cells, and its actions are likely mediated by activation of AC. GHRH showed stimulatory action on sst1 and sst2 mRNA levels and inhibitory action on sst3, sst4, and sst5 mRNA levels. The differential modulation of these SRIF receptor sub types suggests that they exert independent and highly selective roles in the modulation of pituitary function.

ACKNOWLEDGMENT

This work was supported by grant No. (R01-2001-000-00127-0) from the Basic Research Program of the Korea Science & Engineering Foundation.

REFERENCES

Aleppo G, Moskal SFI, DeGrandis PA, Kineman RD, Frohman LA. Homologous down-regulation of growth hormone-releasing hormone receptor mRNA levels. *Endocrinology* 138: 1058–1065, 1997

Day R, Dong W, Panetta R, Kracier J, Greenwood MT, Patel YC. Expression of mRNA for somatostatin receptor (sstr) types 2 and 5 in individual rat pituitary cells. A double labeling in situ hybridization analysis. *Endocrinology* 136: 5232–5235, 1995

Greenman Y, Melmed S. Heterogeneous expression of two somatostatin receptor subtypes in pituitary tumors. *J Clin Endocrinol Metab* 78: 398–403, 1994a

Greenman Y, Melmed S. Expression of three somatostatin receptor subtypes in pituitary adenomas: evidence for preferential SSTR5 expression in the mammosomatotroph lineage. *J Clin Endocrinol Metab* 79: 724–729, 1994b

Kineman RD, Aleppo G, Frohman LA. The tyrosine hydroxy lase-human growth hormone (GH) transgenic mouse as a model of hypothalamic GH deficiency: growth retardation is the result of a selective reduction in somatotrope numbers despite normal somatotrope function. *Endocrinology* 137: 4630–4636, 1996

Kumar U, Laird D, Srikant CB, Escher E, Patel YC. Expression

of the five somatostatin receptor (SSTR1-5) subtypes in rat pituitary somatotropes: quantitative analysis by double-label immunoflourescence confocal microscopy. *Endocrinology* 138: 4473–4476, 1997

Lopes MBS, Gaylinn BD, Thorner MO, Stoler MH. Growth hormone-releasing hormone receptor mRNA in acromegalic pituitary tumors. *Am J Pathol* 150: 1885–1891, 1997

Mezey E, Hunyady B, Mitra S, Hayes E, Liu Q, Schaeffer J, Schonbrunn A. Cell specific expression of the sst2A and sst5 somatostatin receptors in the rat anterior pituitary. *Endocrinology* 139: 414–419, 1998

Morel G, Gallego R, Boulanger L, Pintos E, Garcia-Caballero T, Gaudreau P. Restricted presence of the growth hormone-releasing hormone receptor to somatotropes in rat and human pituitaries. *Neuroendocrinology* 70: 128–136, 1999

O'Carroll AM, Krempels K. Widespread distribution of somatostatin receptor messenger ribonucleic acids in rat pituitary. *Endcorinology* 136: 5224–5227, 1995

Oka H, Kameya T, Sato Y, Naritaka H, Kawano N. Significance of growth hormone-releasing hormone receptor mRNA in non-neoplastic pituitary and pituitary adenomas: a study by RT-PCR and in situ hybridization. *J Neurooncol* 41: 197–204, 1999

Panetta R, Patel YC. Expression of mRNA for all 5 human somatostatin receptor (hSSTR1-5) in pituitary tumors. *Life Sci* 56: 333–342, 1995

Park S, Kamegai J, Johnson TA, Frohman LA, Kineman RD. Modulation of pituitary somatostatin receptor subtype (sst1-5) messenger ribonucleic acid levels by changes in the growth hormone axis. *Endocrinology* 141: 3556–3530, 2000

Patel YC. Somatostatin and its receptor family. *Front Neuroendocrinol* 20: 157–198, 1999

Reisine T. Somatostatin. *Cell Mol Neurobiol* 15: 597–614, 1995

Rohrer SP, Hayes E, Berk SC, Hutchins SM, Shen D-M, Xiong Y, Parma R, Foor P, Mitra SW, Degrado SJ, Shu M, Klopp JM, Cai SJ, Blake A, Chan WW, Pasternak A, Yang L, Patchett A, Smith RG, Chapman KT, Schaeffer JM. Rapid identification of subtype-selective agonists of the somatostatin receptor through combinatorial chemistry. *Science* 282: 737–740, 1998

Shimon I, Taylor JE, Dong JZ, Bitonte RA, Kim S, Margan B, Coy DH, Culler MD, Melmed S. Somatostatin receptor subtype specificity in human fetal pituitary cultures. *J Clin Invest* 99: 789–798, 1997a

Shimon I, Yan X, Taylor JE, Weiss MH, Culler MD, Melmed S. Somatostatin receptor (SSTR) subtype selective analogues differentially suppress in vitro growth hormone and prolactin in human pituitary adenomas: novel potential therapy for functioning pituitary tumors. *J Clin Invest* 100: 2386–2392, 1997b

Smith RG, Van Der Ploeg HT, Howard AD, Feighner SD, Cheng K, Hickey GJ, Wyvratt MJ. Peptidomimetic regulation of growth hormone secretion. *Endocr Rev* 18: 621–645, 1997

Wynick D, Critchley R, Venetikou MS, Burrin JM, Bloom SR. Purification of functional lactotrophs and somatotrophs from female rats using fluorescence-activated cell sorting. *J Endocrinol* 126: 269–274, 1990a

Wynick D, Venetikou MS, Critchley R, Burrin JM, Bloom SR. Flow cytometric analysis of functional anterior pituitary cells from female rats. *J Endocrinol* 126: 261–268, 1990b