

Regulation of Somatostatin and Growth Hormone-Releasing Hormone Gene Expression in the Rat Brain

Philip Zeitler, Linda Vician, Julie A. Chowen-Breed, Jesús Argente, Gloria S. Tannenbaum, Donald K. Clifton, and Robert A. Steiner

We have studied the regulation of somatostatin (SS) and growth hormone-releasing hormone (GHRH) gene expression in the brain of the laboratory rat. We report that hypophysectomy in the adult male reduces SS mRNA in cells of the periventricular nucleus (PeN), while GH reverses this effect. We demonstrate that cellular levels of SS mRNA in the PeN are higher in male compared to female animals. We report that castration reduces cellular levels of GHRH mRNA and SS mRNA in the arcuate nucleus and PeN, respectively, and that testosterone reverses this effect through an androgen receptor-dependent mechanism. Finally, we present a theoretical model to explain the generation of the ultradian rhythm in GH secretion, which implicates the reciprocal interaction between GH feedback and the transcriptional regulation of the SS and GHRH genes and the kinetics of these relationships.

© 1990 by W.B. Saunders Company.

THE SECRETION of growth hormone (GH) is regulated by the interplay of the stimulatory effects of growth hormone-releasing hormone (GHRH) and the inhibitory effects of somatostatin (SS). These neuropeptides are synthesized in neurons within the hypothalamus, released from axon terminals in the median eminence into the hypophyseal portal system, and transported to the pituitary where they act on somatotropes through specific membrane receptors. The primary source of median eminence SS is from neurons in the periventricular nucleus (PeN),¹ while the primary source of GHRH is from neurons in the arcuate nucleus (ARC).²

Because of the importance of these neuropeptides in the regulation of GH secretion, attention has focused on the regulation of the synthesis and release of GHRH and SS. Much has been learned about SS and GHRH physiology from indirect techniques involving antipeptide antibodies, measurement of brain peptide content, intracerebroventricular infusions, and cell culture; however, these approaches provide only limited information about the cellular physiology of SS and GHRH neurons and how the biosynthetic capacity of these neurons changes over the course of development or as a function of alterations in the hormonal milieu.

In situ hybridization histochemistry permits the identification and quantification of target mRNAs within individual cells in the context of an intact anatomical circuitry. With this approach, a radiolabeled complementary RNA or DNA probe is applied to thin frozen sections of brain tissue and allowed to hybridize specifically with the cellular RNA. The

tissue is then dipped in photographic emulsion, resulting in the formation of autoradiographic silver grain clusters above those cells containing the mRNA of interest. The number of silver grains can be quantified with the aid of computer-assisted image analysis to give a measure of mRNA content. Using this technique, the investigator can assess the synthetic capacity of specific anatomical or functional subpopulations of neurons.

Here, we describe the application of in situ hybridization histochemistry to the study of SS and GHRH cellular physiology in the hypothalamus of the rat. In particular, we discuss the use of this technique to provide insight into the mechanism of GH feedback on the peptides controlling its secretion; the regulation of SS and GHRH synthetic capacity by sex steroids and the effect of gender on the expression of these peptides; and finally, we discuss a theoretical model for the cellular basis of the ultradian oscillation in SS and GHRH synthetic capacity and argue that a putative transcriptional component underlies the observed ultradian rhythms in SS, GHRH, and GH secretion.

GROWTH HORMONE FEEDBACK OF SOMATOSTATIN mRNA

Considerable evidence suggests that GH feeds back at the level of the hypothalamus to stimulate SS secretion.³⁻⁶ However, the mechanism of this stimulation has not been completely described. The possibility that GH stimulates synthesis of SS has been suggested by the observation that hypothalamic SS peptide content declines following hypophysectomy, while subsequent GH replacement reverses this decline.^{7,8}

To test the hypothesis that GH exerts its feedback effect on hypothalamic SS, at least in part, through an increase in SS mRNA content, we examined the effect of hypophysectomy and GH replacement on the level of pre-proSS mRNA in the periventricular nucleus of the male rat.⁹ Hypophysectomized (HPX; $n = 6$) and sham-operated ($n = 7$) adult male rats were fitted with minipumps and administered either bovine GH (bGH; 200 $\mu\text{g}/\text{d}$) or vehicle. After 5 days, the animals were killed by decapitation and the brains subjected to in situ hybridization with an ³⁵S-labeled cRNA probe complementary to pre-proSS mRNA.

As shown in Fig 1, HPX rats had significantly lower grains/cell than sham-operated animals (125.9 ± 10.1 v 195.5 ± 31.4 ; $P < .01$). Treatment with bGH reversed the decline in SS

From the Departments of Obstetrics and Gynecology, Physiology and Biophysics, Zoology, and the Population Center for Research in Reproduction, University of Washington, Seattle, WA; Departments of Pediatrics, Neurology, and Neurosurgery, McGill University and the Montreal Children's Hospital Research Institute, Montreal, Quebec, Canada.

Supported by National Institutes of Health Grants No. HD-12629 and HD-07329 and Grant No. MT-6837 from the Medical Research Council of Canada.

Address reprint requests to Robert A. Steiner, PhD, Department of Physiology and Biophysics, SJ-40, University of Washington, Seattle, WA 98195.

© 1990 by W.B. Saunders Company.
0026-0495/90/3909-2014\$03.00/0

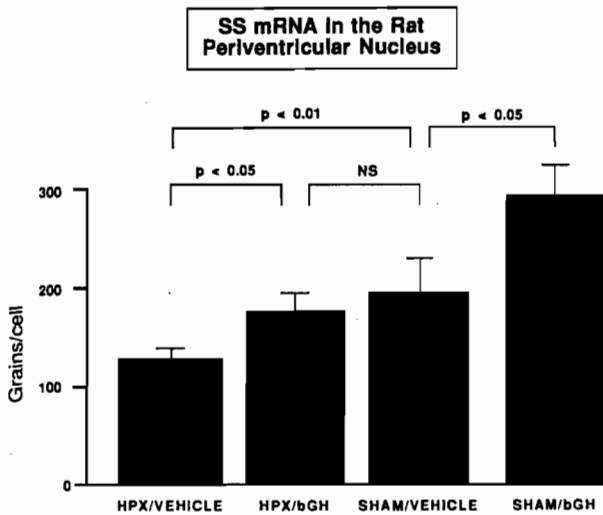


Fig 1. Relative content of SS mRNA in periventricular neurons of hypophysectomized (HPX/vehicle), hypophysectomized and GH-treated (HPX/bGH), sham-operated (sham/vehicle), and sham-operated-GH treated (SHAM/bGH) male rats. Values presented are mean grains/cell \pm SEM. (Modified from *Endocrinology* 122:586, 1988.)

mRNA signal and maintained a level not statistically different from the sham-operated animals. Furthermore, treatment of sham-operated animals with bGH increased grains/cell above that of the untreated intact animals (290.0 ± 28.8 v 195.5 ± 31.4 ; $P < .05$).

These results indicate that GH can influence the capacity of periventricular neurons to synthesize SS and complement previous studies of changes in SS content. Taken together, these findings indicate that GH influences the level of both the synthesis and secretion of SS, and suggest that GH participates in the regulation of its own secretion through a feedback loop involving SS gene expression.

Chomczynski et al¹⁰ have demonstrated that hypophysectomy stimulates an increase in GHRH mRNA, an effect that is reversed by replacement with GH. These observations are the converse of, and consistent with, our description of the feedback effects of GH on SS mRNA and help to complete our understanding of the short-loop feedback of GH on its own regulation by the hypothalamus.

SEXUAL DIMORPHISM IN SOMATOSTATIN mRNA

The secretory pattern of growth hormone exhibits a pronounced sexual dimorphism.¹¹ Adult male rats display high amplitude GH pulses superimposed on low, or undetectable, baseline plasma GH levels, whereas adult females have low amplitude pulses superimposed on a plasma baseline that is elevated relative to males. Since the baseline GH level appears to be largely a function of the release of SS into the hypophyseal portal system, we hypothesized that there is sexual dimorphism in the amount of SS released from the hypothalamus and that this difference would be reflected in a gender difference in the biosynthetic capacity of somatostatinergic neurons.

To test this hypothesis, we compared the level of pre-proSS mRNA in adult male rats and adult female rats at the time of proestrus.¹² On the afternoon of proestrus, between 2:00 and 3:00 PM, females ($n = 4$) and males ($n = 4$) were paired and processed for in situ hybridization.

As shown in Fig 2, male rats had a significantly greater number of grains/cell in somatostatinergic neurons of the PeN than females on the day of proestrus (210 ± 7 v 158 ± 5 ; $P < .005$). There was no significant difference between the sexes in pro-SS mRNA content of frontal cortex somatostatinergic neurons.

Thus, we conclude that there is a sexual dimorphism in the biosynthetic capacity of SS neurons involved in GH regulation. This sexual dimorphism in hypothalamic SS mRNA content may explain, at least in part, the observed gender differences in the GH secretory profile. Since there are also marked differences in the amplitude of GH peaks between males and females, we are presently analyzing the content of GHRH mRNA in the brains used in these experiments.

SEX STEROIDS AND SOMATOSTATIN GENE EXPRESSION

There is extensive evidence for an important role for androgens in regulating baseline GH levels^{11,13,14} in adult and juvenile rats. It is likely that these androgen effects are mediated, at least in part, by their effects on SS synthesis and secretion.

To explore the effect of androgens on the expression of SS mRNA in the adult male rat, we compared pre-proSS mRNA levels in intact and castrated rats and in castrated rats replaced with testosterone.¹² Male rats were anesthetized with ether and either castrated or sham-castrated ($n = 3$). Immediately after operation, the castrated rats received a Silastic capsule, which was either empty ($n = 3$) or contained crystalline testosterone ($n = 3$). Four days after surgery, animals were killed

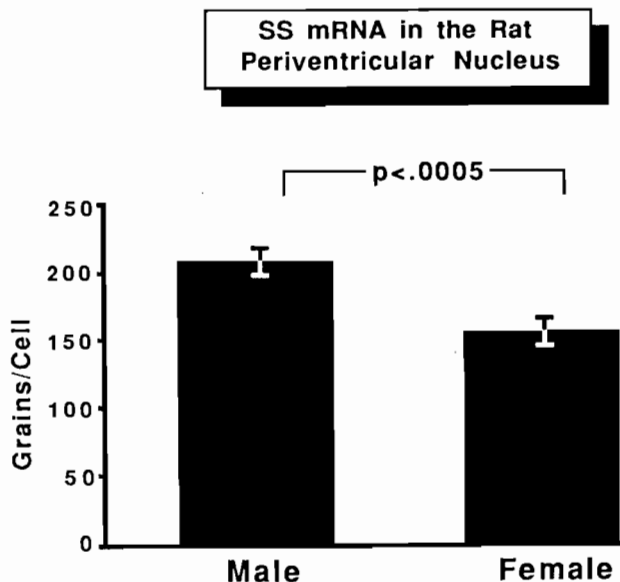


Fig 2. A comparison of SS mRNA content in periventricular neurons of adult male and female rats. Values presented are mean grains/cell \pm SEM (Modified from *Endocrinology* 125:357, 1989.)

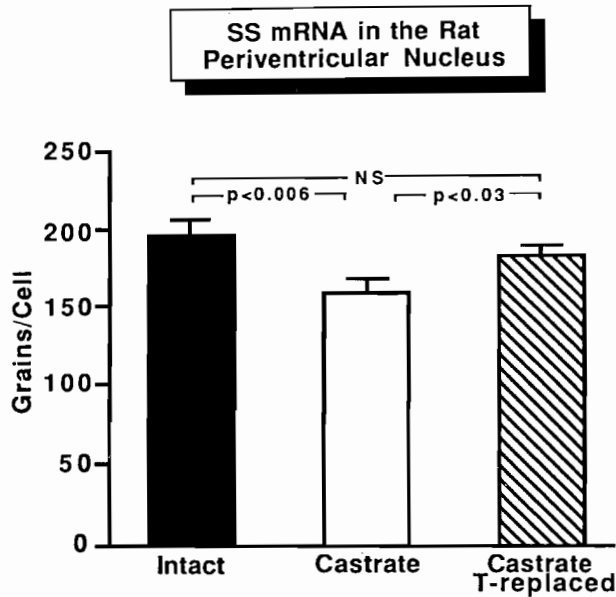


Fig 3. Relative content of SS mRNA in periventricular neurons of intact, castrated, and castrated testosterone-replaced adult male rats. Values presented are mean grains/cell \pm SEM. (Modified from *Endocrinology* 125:357, 1989.)

by decapitation, and their brains were processed for in situ hybridization as described above.

As shown in Fig 3, SS mRNA signal was significantly reduced in castrated animals compared with sham-operated controls (159 ± 6 v 195 ± 3 grains/cell; $P < .05$). Replacement with testosterone at the time of castration prevented the castration-induced decline in SS mRNA content (182 ± 4 grains/cell). Preliminary studies suggest that these effects of testosterone are mediated directly through an androgen receptor-dependent pathway, as opposed to aromatization and activation of the estrogen receptor.¹⁵

These data suggest that testosterone suppresses GH baseline levels through a stimulatory effect on SS gene expression and, presumably, secretion. Thus, the sexually dimorphic patterning of GH secretion and the gender difference in SS mRNA levels in the periventricular nucleus described above may be attributable, at least in part, to the influence of androgens on SS gene expression.

THE TRANSCRIPTIONAL OSCILLATOR MODEL

Considerable indirect evidence suggests that the GH secretory pattern in the adult male rat results from an interplay of ultradian SS and GHRH secretion from the hypothalamus, nearly 180° out of phase with one another.¹⁶ However, the mechanism underlying the generation of the ultradian rhythms of these and other neuropeptides remains unknown. A wide variety of oscillatory systems have been described and serve as potential models for understanding episodic neuroendocrine secretion.¹⁷ However, the majority of these systems are characterized by high-frequency oscillations, with periods on the order of milliseconds to seconds and do not readily provide for the relatively long period of the GH secretory pattern.

Recently, there has been great interest in the control of the cell cycle and the role of the protein "cyclin" in this process.¹⁸ The present understanding of the role of cyclin in the generation of low-frequency oscillations in this system may provide a useful model for understanding neuroendocrine rhythms. Briefly, the synthesis of cyclin during interphase leads to an accumulation of the protein. When the threshold is reached, cyclin converts inactive "maturation promoting factor" (MPF) to the active form. Active MPF has many actions that ultimately lead to mitosis and initiate the cell cycle progression. However, MPF also functions to induce cyclin degradation and promotes return to interphase. In the absence of cyclin, MPF returns to the inactive form and the process begins anew, with a frequency that is dependent on the rate of synthesis of cyclin. The frequency of such an oscillator would appear to be dependent on the rate of protein synthesis and, thus, on the biosynthetic capacity of the cell for the driving protein.

We propose that, by analogy with cyclin, the low-frequency oscillations in the GH secretory system are subserved by a transcriptional and/or translational step. Figure 4 outlines the basic components of a putative GH/SS/GHRH pulse oscillator. This model consists of an ensemble of SS neurons, GHRH neurons, somatotropes, and the feedback interactions among them. The feedback relationships proposed in this scheme have all been described in vitro or in vivo.^{3-10,19-23} As pictured here, the proposed model is an excitation-inhibition circuit, which will oscillate between SS release and GHRH release, thereby producing peaks and troughs in GH serum levels.

According to the "transcriptional oscillator" scheme, "readily releasable" peptide (SS or GHRH) may be exhausted during a period of increased neuronal activity, thereby causing

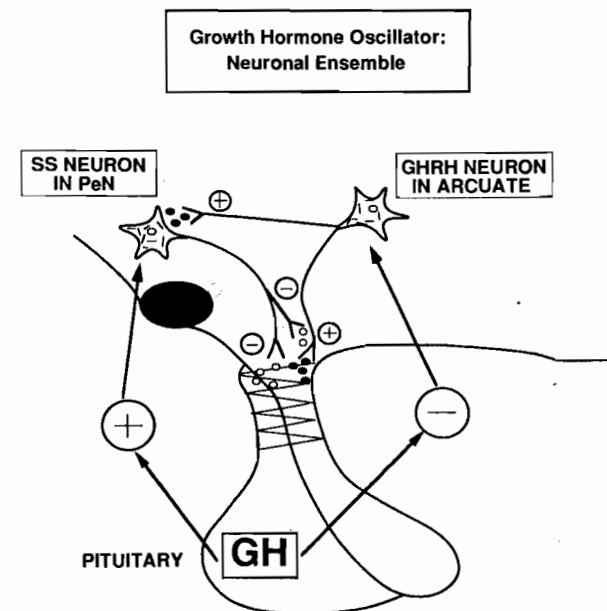


Fig 4. The transcriptional oscillator: schematic representation of the neuronal components and feedback relationships involved in the generation of pulsatile GH secretion.

"fatigue" and a subsequent period of secretory quiescence. During quiescence, synthesis of the peptide is increased under the influence of GH feedback and, when a critical point is reached, the next active period is initiated.

This "transcriptional oscillator" model for generation of the GH rhythm predicts that the rate of peptide synthesis for both SS and GHRH would be greatest at that time in the cycle when their respective release is lowest. Thus, we would expect that SS mRNA content and peptide synthesis would be greatest during the peak of GH release, when SS secretory activity is relatively quiescent, ie, when the neuron is in the process of repleting its store under the influence of elevated

serum GH and increased GHRH activity in advance of the next period of SS activity (subsequent GH nadir). The opposite would be expected for GHRH. Preliminary results exploring the cellular content of SS mRNA and GHRH mRNA in animals killed at the time of presumptive GH peaks and troughs are consistent with this deduction.²⁴ We are actively engaged in examination of this model.

ACKNOWLEDGMENT

The authors would like to thank Pam Kolb and Emilia Kabigting for their excellent technical assistance.

REFERENCES

1. Urman S, Kaler L, Critchlow V: Effects of hypothalamic periventricular lesions on pulsatile growth hormone secretion. *Neuroendocrinology* 41:357-362, 1985
2. Sawchenko PE, Swanson LW, Rivier J, et al: The distribution of growth-hormone-releasing factor (GRF) immunoreactivity in the central nervous system of the rat: An immunohistochemical study using antisera directed against rat hypothalamic GRF. *J Comp Neurol* 237:100-115, 1985
3. Sheppard MC, Kronheim S, Pimstone BL: Stimulation by growth hormone of somatostatin release from the rat hypothalamus in vitro. *Clin Endocrinol* 9:583-586, 1978
4. Berelowitz M, Firestone SL, Frohman LA: Effects of growth hormone excess and deficiency on hypothalamic somatostatin content and release and on tissue somatostatin distribution. *Endocrinology* 109:714-719, 1981
5. Robbins RJ, Leidy JW, Landon RM: The effects of growth hormone, prolactin, corticotropin, and thyrotropin on the production and secretion of somatostatin by hypothalamic cells in vitro. *Endocrinology* 117:538-543, 1985
6. Chihara K, Minamitani N, Kaji H, et al: Intraventricularly injected growth hormone stimulates somatostatin release into rat hypophysial portal blood. *Endocrinology* 109:2279-2281, 1981
7. Baker BL, Yen YY: The influence of hypophysectomy on the stores of somatostatin in the hypothalamus and pituitary stem. *Proc Soc Exp Biol Med* 151:599-602, 1976
8. Patel YC: Growth hormone stimulates hypothalamic somatostatin. *Life Sci* 24:1589-1594, 1979
9. Rogers KV, Vician L, Steiner RA, et al: The effect of hypophysectomy and growth hormone administration on pre-prosomatostatin messenger ribonucleic acid in the periventricular nucleus of the rat hypothalamus. *Endocrinology* 122:586-591, 1988
10. Chomczynski P, Downs TR, Frohman LA: Feedback regulation of growth hormone (GH)-releasing hormone gene expression by GH in rat hypothalamus. *Mol Endocrinol* 2:236-241, 1988
11. Jansson J-O, Eden S, Isaksson O: Sexual dimorphism in the control of growth hormone secretion. *Endocrine Rev* 6:128-150, 1985
12. Chowen-Breed J, Steiner RA, Clifton DK: Sexual dimorphism and testosterone-dependent regulation of somatostatin gene expression in the periventricular nucleus of the rat hypothalamus. *Endocrinology* 125:357-362, 1989
13. Jansson J-O, Frohman LA: Differential effects of neonatal and adult androgen exposure on the growth hormone secretory pattern in male rats. *Endocrinology* 120:1551-1557, 1987
14. Bardin CW, Bullock LP, Sherins RJ, et al: Androgen metabolism and mechanism of action in male pseudohermaphroditism: A study of testicular feminization. *Rec Prog Horm Res* 29:65-109, 1973
15. Argente J, Chowen-Breed J, Steiner RA, et al: Testosterone stimulates somatostatin gene expression by activation of androgen receptors and not through aromatization to estradiol. Program of the International Symposium on Somatostatin, Montreal, Quebec, Canada, August 1989, p A45
16. Tannenbaum GS, Ling N: The interrelationship of growth hormone (GH)-releasing factor and somatostatin in generation of the ultradian rhythm of GH secretion. *Endocrinology* 115:1952-1957, 1984
17. Rapp PE: An atlas of cellular oscillators. *J Exp Biol* 81:281-306, 1979
18. Murray AW, Kirschner MW: Cyclin synthesis drives the early embryonic cell cycle. *Nature* 339:275-280, 1989
19. Lumpkin MD, Samson WK, McCann SM: Effect of intraventricular growth hormone-releasing hormone on growth hormone release: Further evidence for ultrashort loop feedback. *Endocrinology* 116:2070-2074, 1985
20. Aguila MS, McCann SM: Stimulation of somatostatin release in vitro by synthetic growth hormone-releasing factor by a non-dopaminergic mechanism. *Endocrinology* 117:762-765, 1985
21. Richardson S, Twente S, Audhya T: GHRF causes biphasic stimulation of SRIF secretion from rat hypothalamic cells. *Am J Physiol* 255:E829-832, 1988
22. Plotsky PM, Vale WW: Patterns of growth hormone-releasing factor and somatostatin secretion into the hypophysial-portal circulation of the rat. *Science* 230:461-463, 1985
23. Tannenbaum GS, McCarthy GF, Beaudet A: Inhibitory role of somatostatin on growth hormone-releasing factor within the hypothalamus. Program of the 71st Annual Meeting of the Endocrine Society, Seattle, WA, 1989, p A36
24. Zeitler PS, Tannenbaum GS, Steiner RA: Ultradian variation in growth hormone-releasing hormone and somatostatin gene expression in the hypothalamus of the male rat. Program of the International Symposium on Somatostatin, Montreal, Quebec, Canada, 1989, p A46