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Ultradian oscillations in somatostatin and growth hormone-releasing hormone mRNAs in the brains of adult male rats

(gene expression/hypothalamus/*in situ* hybridization/biological rhythm)

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ABSTRACT **In the adult male rat, growth hormone (GH) secretion is characterized by an ultradian rhythm generated by the rhythmic interplay of the stimulatory effects of GH-releasing hormone (GHRH) and the inhibitory effects of somatostatin (Ss). Although considerable evidence indicates that GHRH and Ss are secreted in reciprocal 3- to 4-hr rhythms, the mechanism underlying the rhythmic secretion of these two neuropeptides is unknown. We tested the hypothesis that the rhythmic and reciprocal oscillations in secretion of Ss and GHRH are associated with parallel changes in synthesis and that this would be reflected by coincident oscillations in levels of the respective mRNAs. In the first experiment, Ss mRNA was significantly greater in the periventricular nucleus of animals sacrificed at the time of a presumed peak in the GH rhythm than in animals sacrificed at the time of a presumed trough; this variation was limited to the anterior third of this nucleus. Conversely, GHRH mRNA content throughout the arcuate nucleus was significantly greater at the time of a GH trough. In the second experiment, groups of animals were sacrificed during two consecutive cycles. In this set of animals, Ss mRNA content was 40% greater ($P < 0.005$) during peak GH concentrations, whereas GHRH mRNA content was 42% greater ($P < 0.005$) during the GH trough. This difference persisted when the two cycles were analyzed separately. The findings that the cellular mRNA content for Ss and GHRH varies in a reciprocal manner with the presumed secretion of these neuropeptides suggest that, like secretion, the synthesis of Ss and GHRH also varies rhythmically. The occurrence of this rhythm suggests a model for a transcriptional oscillator that may subserve the generation of this and possibly other neuroendocrine rhythms.**

In the adult male rat, the secretion of growth hormone (GH) is characterized by an endogenous ultradian rhythm. High-amplitude secretory bursts occur at regular 3.3-hr intervals and are separated by intervening troughs during which basal GH levels are undetectable (1). The patterning of GH secretion is generated by the interplay of the stimulatory effects of GH-releasing hormone (GHRH) and the inhibitory effects of somatostatin (Ss) (for review, see ref. 2). In the rat, these two neuropeptides are released in reciprocal 3- to 4-hr cycles and act upon the pituitary somatotropes to generate the ultradian rhythm of GH secretion (3, 4). The primary source of median eminence Ss is from neurons in the periventricular nucleus (PeN) (5), whereas the primary source of GHRH is from neurons in the arcuate nucleus (Arc) (6).

Although it is known that the rhythmic oscillation of GH secretion is governed by Ss and GHRH, the mechanisms underlying the rhythmic discharge of Ss and GHRH neurons remain to be elucidated. On the one hand, these discharge patterns could reflect the intrinsic properties of the peptidergic neurons themselves. Alternatively, it is conceivable that the oscillations reflect interactions among the elements of a feedback loop involving GH and its effects on synthesis and secretion of Ss and GHRH (7, 8). Using a colony of photoperiodically entrained adult male rats, we tested the hypothesis that the rhythmic and reciprocal oscillations in secretion of Ss and GHRH would be associated with parallel changes in synthesis and that this would be reflected by coincident oscillations in levels of the respective mRNAs. To accomplish this, we measured cellular levels of Ss mRNA and GHRH mRNA by *in situ* hybridization and compared these values between groups of animals sacrificed at times of peaks and troughs of plasma GH levels. We present evidence for an ultradian rhythm in the cellular content of these two mRNA species.

MATERIALS AND METHODS

Animals and Accommodations. Adult male Sprague–Dawley rats were purchased from Charles River Breeding Laboratories and housed under the auspices of the Animal Care Centre of Montreal Children's Hospital Research Institute. Animals were kept individually housed in an isolated room under a rigidly controlled 12-hr light, 12-hr dark cycle (lights on at 0600 hr) in a temperature- (22° ± 1°C) and humidity-controlled environment. The animals were given free access to rat chow (Ralston-Purina, St. Louis) and tap water, and they were handled once daily. After two weeks of acclimatization to the lighting cycle, the rats were sacrificed by rapid decapitation. The brains were immediately removed onto dry ice, rapidly frozen, and stored intact at −80°C until processed for *in situ* hybridization histochemistry.

Experimental Design. In the initial experiment, cellular levels of Ss mRNA in the PeN and frontal cortex (FC) and GHRH mRNA in the Arc and ventromedial (VMH) nucleus were measured and compared between animals killed at two different time points: 1100 hr ($n = 4$) and 1300 hr ($n = 4$). These times were chosen because they reflect typical peak and trough periods of GH secretion, respectively, in rats maintained under these photoperiodic conditions as previously documented in this laboratory (1, 3).

In the second experiment, a similar experimental design was used. However, animals were killed at times corresponding to two consecutive cycles of GH peaks and troughs. Thus, animals were killed at 0900 hr (trough 1), 1100 hr (peak 1),

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Abbreviations: GH, growth hormone; GHRH, GH-releasing hormone; Ss, somatostatin; PeN, periventricular nucleus; Arc, arcuate nucleus; VMH, ventromedial nucleus of the hypothalamus; FC, frontal cortex.

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1300 hr (trough 2), and 1500 hr (peak 2). In addition, in this experiment, trunk blood was collected at the time of sacrifice, and the plasma was stored at −20°C until RIA for rat GH. Only those animals whose GH level agreed unambiguously with their presumptive peak ($n = 4$) or trough ($n = 6$) assignment were used for further analysis. Plasma GH values >80 ng/ml (range: 87–347 ng/ml) were considered as peaks, whereas values <40 ng/ml (range: 1.2–37 ng/ml) were categorized as troughs.

Tissue Preparation. Before cutting, brains were allowed to equilibrate in the cryostat (−15°C), blocked, and embedded in OCT (Tissue-Tek, Elkhart, IN). Coronal sections (20 μm) were thaw-mounted onto poly(L-lysine)-coated slides. Brain sections were collected, beginning rostrally at the decussation of the anterior commissure and continuing caudally to the point at which the third ventricle splits. Tissue slices were stored at −80°C in air-tight boxes until processed for *in situ* hybridization.

Probe Preparation. *Ss cRNA.* Hybridization histochemistry for Ss mRNA was done by using a cRNA probe complementary to pre-pro-Ss mRNA. The preparation of this probe has been described in detail (7). Briefly, a 340-base-pair (bp) Bgl II–Sma I fragment, subcloned from the plasmid vector EV142 (9) into the transcription vector pSP64 (Promega Biotec), was transcribed *in vitro* with uridine [α-[^{35}S]thio]triphosphate (NEN) by using SP6 polymerase. This fragment consists of 44 bases of 5′ untranslated sequence and the bases coding for the first 98 amino acids of pre-pro-Ss. Transcription of this fragment at a UTP concentration of 50 μM, with 20% radiolabeled UTP, yields a probe with a specific activity of ≈2.8 × 10^8 dpm/μg. The identity and integrity of the transcripts were verified by PAGE against known standards. After purification, the transcript was hydrolyzed in 100 mM bicarbonate buffer (pH 10.2) to yield fragments of ≈150 bases in length.

GHRH cRNA. Hybridization histochemistry for GHRH mRNA was done by using an RNA probe complementary to rat GHRH-43. The original plasmid prGHRF-2 was provided by Kelly Mayo (Neurobiology Department, Northwestern University) (10). Preparation of this probe has been described in detail elsewhere (11). Briefly, a 198-bp *Eco*RI–*Hin*dIII fragment, containing the entire GHRH-43 coding sequence and a portion of coding sequence for the associated 3′ peptide, was subcloned into the transcription vector pGEM4 (Promega Biotec). Complementary RNA probes were transcribed *in vitro* by using the T7 promoter present on the plasmid vector. The transcription reaction was done at 50 μM UTP, with 20% [α-[^{35}S]thio]UTP (NEN), yielding a final probe specific activity of ≈5.5 × 10^8 dpm/μg. The identity and integrity of the transcripts were verified by PAGE against known standards.

***In Situ* Hybridization.** *In situ* hybridization was done as described (7, 11). Briefly, slices were fixed in 4% paraformaldehyde and pretreated with 0.25% acetic anhydride in 0.1 M triethanolamine for 10 min. After slides were rinsed in 2× standard saline citrate, probe (0.6 μg/ml·kb) was applied in 60 μl of hybridization buffer/50% (vol/vol) formamide. The slides were covered with Parafilm, sealed with rubber cement, and incubated overnight in moist chambers. Incubations were done at 45°C for Ss mRNA and at 56°C for GHRH mRNA. On the following day, slides were treated with RNase A and were rinsed in a series of washes of increasing stringency. The final wash was in 0.1× standard saline citrate without formamide at 60°C for Ss mRNA and at 66°C for GHRH mRNA. Slides were dehydrated in alcohols and air-dried.

After hybridization, slides were dipped in Kodak NTB-2 emulsion (43°C) diluted 1:1 with 600 mM ammonium acetate. Slides were allowed to air-dry for 30 min, followed by further drying in moist chambers at room temperature for 45 min. Slides were then stored in dessicant-containing, light-tight boxes at 4°C for 6 days. Slides were developed in Kodak D-19 developer and counterstained with cresyl violet.

Anatomical Matching of Sections. To ensure matched anatomical representation for all animals, both the PeN and the Arc were divided into three areas of approximately equal length by reference to the rat brain atlas of Paxinos and Watson (12). Four tissue sections from each area from each animal were included in the analysis (12 slices per animal) of each neuropeptide mRNA. For the Arc these areas are as follows: (*i*) beginning rostrally with the appearance of the Arc and GHRH-positive cells (12, plate 26) and continuing caudally 0.6 mm (12, plate 29); (*ii*) continuous with area *i* and continuing caudally 0.5 mm to the appearance of the dorsomedial nucleus (12, plate 31); (*iii*) continuous with area *ii* and continuing caudally 0.5 mm to the splitting of the hypothalamic third ventricle (12, plate 33), which corresponds to the disappearance of GHRH-positive cells.

For the PeN, these areas are as follows: (*i*) beginning rostrally at the decussation of the anterior commissure (12, plate 21) where Ss-positive cells first appear in the PeN and continuing caudally 0.6 mm to the appearance of the PeN (12, plate 24); (*ii*) continuous with area *i* and continuing caudally 0.8 mm to the disappearance of the PeN (12, plate 26); (*iii*) continuous with area *ii* and continuing caudally 0.6 mm to the disappearance of the PeN (12, plate 29).

Image Analysis. Slides selected for analysis as described above were assigned a random 3-letter code and analyzed in alphabetical order by an operator unaware of the experimental group to which the animal belonged. The automated image processing system consists of a Data Cube IVG-128 video acquisition board (DataCube, Peabody, MA) attached to an IBM AT computer. Video images were obtained by a Dage model 65 camera (Dage-MTI, Michigan City, IN) attached to a Zeiss photomicroscope (Zeiss, New York) equipped with a 40× epi-illumination dark-field objective.

Grain clusters were identified by the operator as areas of labeling unambiguously above the low background signal under dark-field illumination (see refs. 7 and 11). If the cluster was associated with a single cresyl violet-stained nucleus under light-field illumination and free from artifact, it was considered a message-positive cell and was analyzed by automated grain analysis as described (13). The grain-analysis system determined the number of specific grains associated with each cell (total grains minus nonspecific grains). All grain clusters in the PeN, VMH, and Arc that could be resolved as single cells were analyzed and assigned to an anatomic area.

Plasma Growth Hormone Assay. Plasma concentrations of GH were determined in duplicate by double-antibody RIA with materials supplied by the National Institute of Diabetes and Digestive and Kidney Diseases. The average GH values were determined relative to the rat GH reference preparation. The standard curve was linear between 0.62 and 320 ng/ml. The intra- and interassay coefficients of variation were 5.1 and 5.0%, respectively, for duplicate samples of pooled plasma containing a mean GH concentration of 19.9 ng/ml.

Statistical Analysis. The mean number of grains per cell for each individual animal was determined from the analysis of 150–200 cells per animal. The mean grains per cell for each animal was then used to determine the mean ± SEM for each experimental group. The n in all statistical analyses refers to the number of animals in the group. For experiment 1, mean grains per cell between groups was compared by Student's t test. For experiment 2, data collected during the first GH peak were placed in one group, and data from the first GH trough were placed in another group. These two groups formed the first data set. Data from the second GH peak and trough were grouped similarly and formed the second data set. The data were then analyzed by two-way analysis of variance (data set vs. group) (14). This analysis allowed us to

test not only for significant peak–trough differences (group effect) but also for consistency in peak–trough values over two consecutive peak–trough cycles (group vs. data set interaction).

RESULTS

Experiment 1. Ss mRNA signal in the PeN was significantly greater in animals killed at the time of a presumptive peak (209 ± 11 grains per cell) than at the time of a trough (185 ± 3; $P < 0.05$). However, this variation was restricted to the most rostral PeN (area *i*) where the Ss mRNA signal in neurons from animals killed at the time of a GH peak (263 ± 10 grains per cell) was 22% greater than in animals killed at the time of a trough (216 ± 6 grains per cell; $P < 0.01$) (Fig. 1*A*). Ss mRNA signal in more caudal areas of the PeN and in cells of the FC showed no evidence of temporal variation. (See Fig. 1*A*).

GHRH mRNA signal was 24% greater in animals killed at the time of a presumptive GH trough (147 ± 7 grains per cell) than at the time of a presumptive peak (118 ± 5 grains per cell; $P < 0.01$) (Fig. 1*B*), which was evident throughout the entire extent of the Arc. In contrast, GHRH mRNA signal in the VMH did not vary between GH peak and trough animals. (See Fig. 1*B*).

Experiment 2. As in the previous experiment, when GH peak and trough animals from sequential cycles were combined, Ss mRNA signal in the most rostral PeN was 40% greater in animals killed at the time of a peak in GH secretion (241 ± 10 grains per cell) than at the time of a trough (171 ± 9 grains per cell; $P < 0.005$). Ss mRNA signal in cells of the caudal PeN and FC did not vary significantly between peak and trough animals. Serum GH levels were approximately 10-fold higher in peak vs. trough animals. (See Fig. 2.)

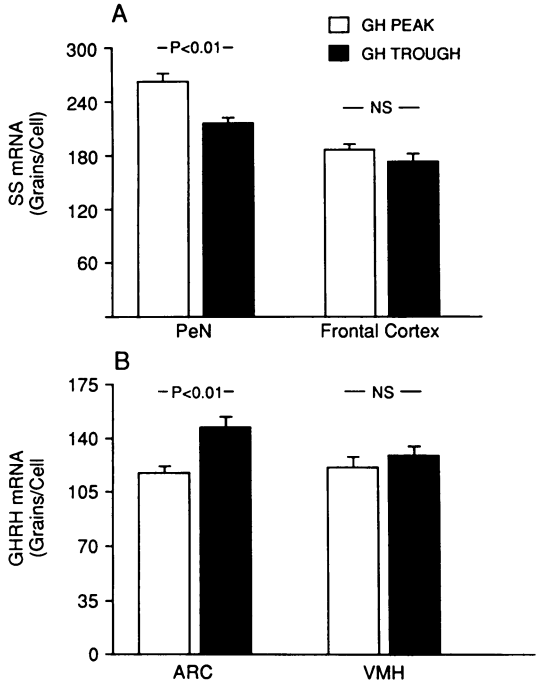

FIG. 1. Relative amounts of Ss mRNA and GHRH mRNA, as reflected by grains per cell, at the time of a presumptive peak (1100 hr; $n = 4$; open bars) and trough (1300 hr; $n = 4$; closed bars) in the GH secretory cycle in adult male rats. (*A*) Ss mRNA in the rostral (area *i*) PeN and in the FC. In the PeN, the cellular content of Ss mRNA was significantly greater during the time of a peak compared with a trough in GH secretion, whereas in the FC there were no significant differences between peaks and troughs. (*B*) GHRH mRNA in Arc and VMH. In Arc, GHRH mRNA content was significantly greater during the trough compared with the peak of GH secretion, whereas in VMH there were no significant differences (NS) between peaks and troughs. Values are given as means plus SEMs.

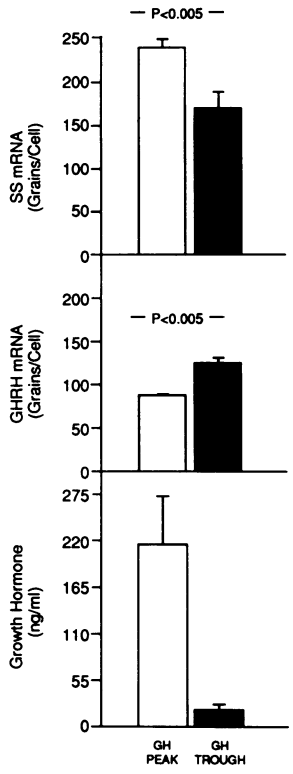

FIG. 2. Relative amounts of Ss mRNA and GHRH mRNA, as reflected by grains per cell and serum GH at the time of peaks (1100-hr and 1500-hr peaks considered together; $n = 4$; open bars) and troughs (0900-hr and 1300-hr troughs considered together; $n = 6$; closed bars) in the GH secretory cycle in adult male rats. (*Top*) Ss mRNA in the rostral PeN. The content of Ss mRNA was significantly greater during a peak compared with a trough in serum GH levels. (*Middle*) GHRH mRNA in Arc. GHRH mRNA was significantly greater during a trough compared with a peak in GH serum levels. (*Bottom*) Serum GH concentrations at secretory peaks and troughs. Values are given as means plus SEMs.

Conversely, GHRH mRNA signal in the Arc was 42% greater in animals killed at the time of a trough in plasma GH level (125 ± 7 grains per cell) than in animals killed at the time of a peak (88 ± 2 grains per cell; $P < 0.005$). As in the previous experiment, this difference was found only within the Arc, whereas there was no difference in GHRH mRNA signal in cells of the VMH between peak and trough groups. For both GHRH mRNA in the Arc and Ss mRNA in the PeN, the peak–trough patterns were not significantly different when the first peak–trough set was compared with the second peak-trough set. (See Figs. 2 and 3.)

When the small number of Ss mRNA-positive cells located in the Arc (≈30) analyzed as a separate anatomical area, there was also a marked difference in mRNA signal between combined GH peak and trough animals, with the peak animals (158 ± 21 grains per cell) having 50% greater mRNA signal than trough animals (105 ± 6 grains per cell; $P < 0.02$).

DISCUSSION

We had originally hypothesized that neuropeptide synthesis and, hence, cellular mRNA levels would exhibit an ultradian rhythm, which would be in-phase with the secretory activity of Ss and GHRH neurons. We confirmed the existence of an ultradian rhythm in cellular mRNA levels, suggesting that neuropeptide synthesis oscillates. Because cellular mRNA content reflects both the rate of synthesis and degradation, the results are consistent with an interpretation focusing on changes in transcription, message stability, or both. The rhythm in cellular message content was not coincident with presumed secretion. This result suggests that synthesis and secretion are out-of-phase with one another, but neither the precise temporal relationship nor the functional interactions between synthesis and secretion can be deduced from these studies.

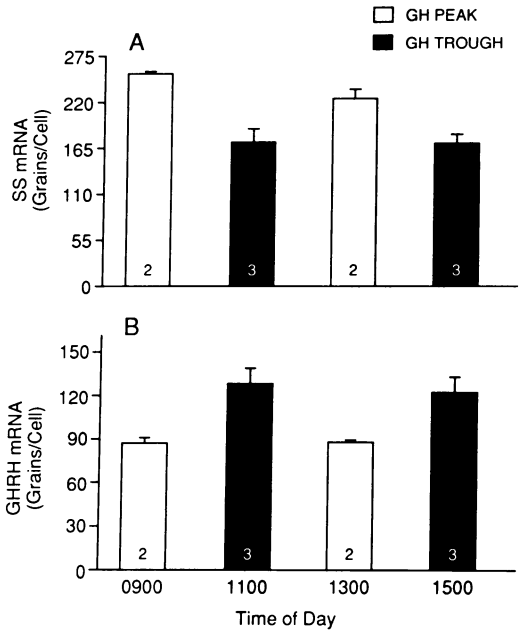

FIG. 3. Relative amounts of Ss mRNA and GHRH mRNA, as reflected by grains per cell, during sequential peaks (open bars) and troughs (closed bars) in the ultradian GH secretory rhythm. (*A*) Ss mRNA in rostral PeN. (*B*) GHRH mRNA in Arc. Values are given as means plus SEMs. Number of animals in each group is shown within each bar (≈150–200 cells analyzed per animal).

Although the difference in Ss mRNA signal between peak and trough animals in the PeN taken as a whole was small in both experiments, a larger difference was found in the rostral segment of the nucleus. The Ss mRNA signal in the more caudal areas was not significantly different between the experimental groups; thus, it would appear that the ultradian variation is predominantly limited to the rostral portion of the PeN. This restriction is consistent with anatomic evidence showing that those Ss neurons that project to the median eminence and have hypophysiotropic actions have cell bodies confined to the rostral portion of the PeN (15). It was also interesting to observe that the ultradian variation in Ss mRNA content was particularly marked in the small population of Ss neurons located in the Arc because these neurons are believed to provide the preponderance of Ss innervation to GHRH perikarya and dendrites (16, 17).

When considering the magnitude of the differences observed in these experiments, it is important to bear in mind that the exact relationship between grains per cell and mRNA copy number is uncertain. A recent investigation from this laboratory comparing grains per cell and number of Ss mRNA copies in transfected cells suggests that differences in grains per cell may underestimate actual differences in mRNA copy number, at least for Ss (18). Thus, the changes in grain counts observed here may reflect larger changes in Ss and GHRH mRNA copy number.

Oscillations in the cellular content of other neuropeptide mRNA species have been reported. Uhl and Reppert (19) described a circadian rhythm in vasopressin mRNA content in the suprachiasmatic nucleus, accompanied by a circadian variation in polyadenylate tail-length of the mRNA (20). Recently, preliminary evidence has been presented for oscillations in mRNA content of other neuropeptides known to be secreted in a rhythmic fashion. Both corticotropin-releasing factor mRNA in the rat PeN and pro-opiomelanocortin mRNA in the Arc of female rats have been shown to have a diurnal rhythm (21, 22). Changes in Ss and GHRH mRNA content occur with a time course that is much more rapid than that reported for these other neuropeptide mRNA rhythms; this result is expected because the rhythms in Ss and GHRH peptide secretion are also more rapid than those seen for the secretion of vasopressin, corticotropin-releasing factor, or pro-opiomelanocortin. We infer that changes in steady-state levels of these mRNAs are required to meet hour-to-hour fluctuations in demand for synthesis and secretion.

The oscillations may reflect the independent, intrinsic activity of one or both of the neuropeptide cell populations or the ensemble properties of their interacting neural network. Alternatively, the oscillations may result from the feedback effect of plasma GH (or insulin-like growth factors) on the expression of the two neuropeptide genes. The results of this study are consistent with the latter hypothesis that GH exerts feedback effects on Ss and GHRH mRNA content. Earlier reports from this laboratory and others have demonstrated the existence of GH feedback on levels of GHRH and Ss mRNA in the male rat, at least on a long-term basis (7, 8). The time-course of this feedback has not been established; however, there is precedent in the literature for the occurrence of rapid changes in neuropeptide mRNA in mammals. Proenkephalin mRNA content increases whereas prodynorphin mRNA content decreases, in rat hippocampal granule cells within 7 hr of electrical stimulation of the dentate gyrus *in vivo* (23). In addition, dramatic changes in tyrosine hydroxylase, dopamine β-hydroxylase and neuropeptide Y mRNA content in the thoracic ganglia of humans have been reported to occur in <1 hr after electrical preganglionic stimulation (24). Pro-opiomelanocortin mRNA in the pituitary increased significantly 60 min after insulin-induced hypoglycemia in rats (25), whereas mRNA for corticotropin-releasing factor in the hypothalamus increased in as little as 30 min after hypoglycemia (26). Similarly, neurotensin mRNA increased within 30 min of haloperidol administration to rats (27). Rapid feedback effects of GH on Ss and GHRH neurons acting within a similar time frame could account for the ultradian variations in message levels we report here.

This ultradian rhythm in neuropeptide mRNA content may be an epiphenomenon associated with a passive response to changes in GH milieu and without major physiologic significance. Alternatively, the occurrence of such a rhythm may reflect the operation of a transcriptional oscillator that is a vital component in the generation of this and other neuroendocrine secretory patterns. One model for such an oscillator is shown in Fig. 4. The model consists of an ensemble of interacting Ss and GHRH neurons with a feedback circuit involving GH. This reverberating circuit will oscillate between Ss release and GHRH release and thereby produce peaks and troughs of GH secretion at a frequency dependent on the properties of the components. The model supposes that some critical element within Ss and GHRH cells becomes depleted during periods of neuronal activity and neuropeptide discharge, thereby causing waning of secretion and eventual quiescence. During their respective and alternating periods of secretory quiescence, accumulation of Ss and GHRH mRNAs are maximal, but out-of-phase, by virtue of their differential response to the prevailing GH milieu. Thus, maximal secretory discharge of Ss leads to a trough in GH secretion, which, in turn, promotes maximal GHRH mRNA synthesis and accumulation, as well as a concomitant inhibition of Ss mRNA accumulation. However, as the Ss discharge wanes, GH release is disinhibited and, in the face of increased GHRH secretion, a peak of GH secretion ensues. Subsequently, the elevated plasma GH promotes renewed accumulation of Ss mRNA and inhibition of the GHRH gene. Once the transcriptional process delivers "threshold" levels of Ss, secretion of this peptide begins anew. With this increased Ss secretion, in conjunction with waning GHRH release, GH secretion diminishes, and a

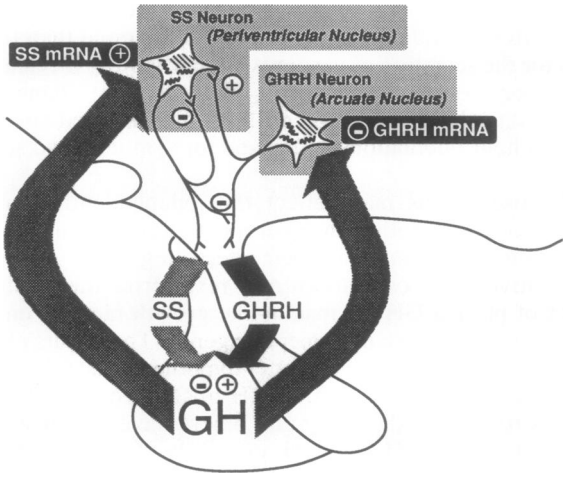

FIG. 4. A proposed model for a neuroendocrine ultradian transcriptional oscillator. According to this model, Ss inhibits and GHRH stimulates the release of GH from the pituitary. In turn, GH stimulates transcription of the Ss-encoding gene and simultaneously inhibits transcription of the GHRH-encoding gene. Axon collaterals from Ss and GHRH cells form the anatomical basis of reciprocal inhibition in the secretory activity between these two systems. Frequency of pulsatile GH release is determined by the time constants associated with the reciprocal effects of GH on Ss and GHRH gene transcription and the delay associated with secretory fatigue (see text for a more detailed description of this model).

plasma trough develops. The model supposes an identical, but out-of-phase, cycle of GHRH transcription and release.

One advantage of such a model is that it provides a mechanism by which a neural circuit could exhibit oscillatory behavior with a period in the range of hours, rather than in the second or millisecond range typical of most neural oscillators. In this case, GH and interconnections between Ss and GHRH neurons provide the feedback necessary to establish oscillatory activity, and the rates of GHRH and Ss transcription provide a sufficient lag (or time constant) to limit the oscillations to once every 3 hr. Another advantage of the model is that it is consistent with previous anatomical investigations, which suggest that hypothalamic GHRH and Ss neurons are in direct synaptic communication with one another (16, 17, 28–30). Although the functional state of these synapses has not been directly determined, numerous physiological studies indicate that these two neuropeptides exert central effects on one another (for review, see ref. 2). Although the model depicted in Fig. 4 presents one set of potential interactions between Ss and GHRH neurons, the model will generate oscillations with a variety of different combinations of positive and negative interactions, as well as without these Ss–GHRH interactions as a consequence of GH feedback. The role of such intrahypothalamic interactions in generating the GH secretory pattern, as well as the details of such connections, is amenable to further investigation.

In summary, we have demonstrated that Ss mRNA and GHRH mRNA content in the hypothalamus of the adult male rat varies between peaks and troughs of the GH secretory rhythm. This observation suggests that the biosynthetic activity of Ss and GHRH neurons varies as a function of the phase of GH secretion. The physiological significance of this rhythm in neuronal biosynthetic capacity is a matter for speculation. It may be an epiphenomenon associated with a passive response to changes in the GH milieu or varying secretory demands and without major significance. Alternatively, the occurrence of such a rhythm may indicate the operation of a transcriptional oscillator that subserves the generation of the ultradian rhythms in Ss and GHRH secretion and, subsequently, of GH secretion. The properties of such an oscillator could provide important insights into the operation of this and other neuroendocrine oscillators.

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