Growth Hormone Inhibits Its Own Secretion by Acting on the Hypothalamus through Its Receptors on Neuropeptide Y Neurons in the Arcuate Nucleus and Somatostatin Neurons in the Periventricular Nucleus

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Abstract. GH secretion is regulated by hypothalamic somatostatin and GH-releasing factor. It has been postulated that GH feeds back on the hypothalamus and regulates its own secretion. We focused our attention on the action of GH in the hypothalamus in relation to GH secretion. Adult male rats were used throughout the studies, and the observation was made in conscious rats. Systemic administration of human GH induced c-fos gene expression, a marker of neuronal activity, in the hypothalamic arcuate nucleus (ARC) and the periventricular nucleus (PeV) in hypophysectomized male rats. The major cells in which c-fos gene expression was induced were neuropeptide Y (NPY) neurons in the ARC and somatostatin neurons in the PeV. GH receptor mRNA was demonstrated to be present in these neurons by in situ hybridization. The injection of a small dose of rat GH into the ARC or PeV inhibited GH secretion, whereas microinjection of IGF-I into these nuclei did not. Intracerebroventricular injection of NPY suppressed GH secretion, and this effect was abolished by anterolateral deafferentation of the medial basal hypothalamus (MBH), a procedure which disrupts the somatostatinergic input to the MBH. Taken together, these findings suggest that GH acts on NPY neurons in the ARC and somatostatin neurons in the PeV through GH receptor, and the activation of these neurons augments somatostatin release and inhibits GH secretion.

Key words: GH, Somatostatin, Neuropeptide Y, GH receptor, Feedback regulation

GH secretion is regulated by hypothalamic somatostatin (SS) and GH-releasing factor (GRF), and it is markedly episodic. These hypophyseotropic hormones are also under the regulatory control of various neuropeptides, neurotransmitters, amines, nutrients, metabolites and others. GH itself is one of the important factors in regulating GH secretion. As with the classical hormonal systems, GH is presumed to exert feedback regulation on its own secretion [1]. Earlier studies have shown that GH feeds back on the hypothalamus and regulates pituitary GH secretion by stimulating SS secretion and/or by inhibiting GRF release [2–10]. Insulin-like growth factor-I (IGF-I), a primary mediator of GH actions, could also participate in the feedback regulation of GH. Accumulating evidence indicates that IGF-I regulates GH secretion at the level of the pituitary gland [11–13]. Concerning the action of IGF-I in the hypothalamus, however, there have been conflicting reports [4, 13–15].

The site of action of GH in the hypothalamus and the involvement of IGF-I in the autocrine regulation of GH are two subjects to be elucidated.
for further understanding of the central regulation of GH secretion. Cloning of the GH receptor (GHR) has provided new tools to study the localization of GHR synthesis in the hypothalamus [16–20]. Although this will generate much greater understanding of the possible site of action of GH, another functional approach with the observation of the c-fos gene expression induced by GH has given us an insight into the mechanism of autoregulation [21, 22]. In many cells including neuroendocrine cells, the synthesis of nuclear protooncogene products such as c-fos is an early response to a wide range of hormones and growth factors [23]. The observation of blood GH levels in response to GH itself or IGF-I is also important, because the secretion of GH is very episodic and the secretory pattern of GH is considered as one of the determinants of growth [24].

The purpose of our studies was to clarify the site of action of GH in the hypothalamus and to elucidate the mechanism of the autoregulation of GH. We studied 1) the cellular distribution of GHR mRNA in the rat hypothalamus, 2) the induction of expression of the c-fos gene in the hypothalamus by systemic administration of GH, and 3) the effect of microinjection of GH or IGF-I into the hypothalamic nuclei on GH secretion. Along with the evidence presented, it is possible that neuropeptide Y (NPY) is involved in the autoregulation of GH. We also studied 4) the role of NPY in the regulation of GH secretion.

### Cellular Distribution of GHR mRNA in the Rat Hypothalamus

Cloning of GHR established that GHR was the product of a gene that directed the synthesis of two different peptides, GHR and GH binding protein (GHBP) [16, 25]. The principal GHBP corresponds in sequence to the extracellular GH-binding domain of the GHR. In rats, Northern blotting analysis with cRNA probe for the extracellular domain of GHR revealed that the GHR gene was abundantly expressed in the liver, where the 1.2 kilobases (kb) transcript corresponding to GHBP mRNA predominated over the 4.5 kb transcript corresponding to GHR mRNA. The two transcripts are presumed to arise from the alternative splicing of the GHR gene [25]. Analysis of the hypothalamic tissue demonstrated both transcripts, and what appeared to be unique to the GHR gene expression in the hypothalamus relative to the liver was that the 4.5 kb transcript predominated over the 1.2 kb transcript [18]. This indicates that the pattern of splicing the initial transcript of the GHR gene is different in the hypothalamus from that in the liver in rats. We have shown that the 4.5 kb transcript of the GHR gene is preferentially processed postnatally in the rat hypothalamus [26].

We performed in situ hybridization with cRNA probe corresponding to the extracellular domain of GHR in the rat hypothalamus, and demonstrated that GHR mRNA-containing cells resided in the arcuate nucleus (ARC), the periventricular nucleus (PeV), supraoptic nucleus, the ventrolateral region of the ventromedial nucleus and the paraventricular nucleus [17, 18, 27]. GHR mRNA-containing cells were scattered throughout the ARC (Fig. 1A). NPY is an important orexigenic neuropeptide, and it may transduce the metabolic and nutritional state to the brain [28]. Cell bodies of NPY neurons in the hypothalamus are localized to the ARC [29] and NPY has an inhibitory influence on GH secretion [30, 31]. To determine whether NPY neurons in the ARC express the GHR gene, we used double label in situ hybridization for GHR and NPY mRNAs in the male rat (Fig. 1B). It was demonstrated that 95% of NPY mRNA-containing cells in the ARC expressed the GHR gene, and there was no significant regional variation through the rostral-caudal parts of the ARC [19].

Other investigators have demonstrated that SS cells in the PeV express the GHR gene [17], and the GHR gene is also expressed in a small subset of GRF cells in the ARC [32]. These findings suggest that the action of GH on the hypothalamus is mediated by GHRs expressed on SS and NPY neurons. But it should be kept in mind that the GHR gene has been demonstrated to be expressed in other cells in the hypothalamus and in cells in extrahypothalamic areas, which suggests that these cells may also be important targets for the central action of GH. It has also been shown that expression of the GHR gene in the ARC is subject to regulation by GH, since hypophysectomy reduced it in male rats and administration of
exogenous GH increased it in dwarf male rats [27].

C-fos Gene Expression Induced by GH in the Hypothalamus

The neuronal expression of the protooncogene c-fos can serve as a marker of neural activity [23]. If GH acts on the hypothalamus through functional GH receptors, the expression of the c-fos gene would be expected to be induced in the activated neurons in the hypothalamus. To identify the activated cells in the brain responding to GH, we have studied the c-fos gene expression in the rat brain induced by systemic administration of recombinant human GH (rhGH) by hybridization histochemistry [21]. Adult male Wistar rats were hypophysectomized 10 days before rhGH administration and received cortisone acetate (0.5 mg/kg BW) and L-T4 (20 µg/kg BW) daily. Four international units (1.33 mg) of rhGH were given intravenously through an indwelling right atrial cannula. Vehicle was administered to control animals. The rhGH treatment was accompanied by expression of the c-fos gene in the ARC of the hypothalamus (Fig. 2A). The accumulation of the c-fos mRNA was transient, reaching maximum values at 60 min and decreasing thereafter to reach control levels within 120 min after rhGH injection. In control animals, c-fos gene expression was not detected in the ARC. When rhGH was administered twice at 40 min intervals, c-fos gene expression was induced in the PeV as well as the ARC 40 min after the second rhGH injection. Throughout the studies, c-fos mRNA was not detected other than in the ARC and PeV. In the ARC, distribution of the cells expressing the c-fos gene appeared to overlap with that of NPY and partially with that of SS mRNA-containing cells, but it clearly differed from the distribution of GRF mRNA-containing cells. In the PeV, the distribution of the cells expressing the c-fos gene was comparable to that of SS mRNA-containing cells.

To further ascertain the distribution and characterize the neurons expressing the c-fos gene in response to rhGH administration, hypothalamic sections, 6 µm in thickness, were processed by double-label in situ hybridization with 35S-labeled c-fos cRNA probe and digoxigenin-labeled NPY or SS cRNA probe. In the ARC 65% of the c-fos gene-expressing cells were NPY neurons (Fig. 2B). In the PeV, 60% of the c-fos gene-expressing cells were SS neurons [22].

We have obtained direct evidence that systemic administration of GH can influence the neuronal activities of the cells in the ARC including NPY neurons and those in the PeV including SS neurons. As shown above, NPY neurons in the ARC and SS neurons in the PeV contain GHR mRNA. It is
suggested that these neurons are the primary functional targets of GH in the hypothalamus.

The Effects of Microinjection of GH or IGF-I into the Hypothalamic Nuclei on GH Secretion

Our data suggest the direct influence of GH on the hypothalamus, but by no means rule out the possibility that the central effects of GH are mediated through intermediary factors, such as IGF-I. To determine whether GH acts directly or indirectly through IGF-I on the hypothalamus to regulate its own secretion, rat GH or human IGF-I was injected directly into a defined area of the hypothalamus, and the blood GH profile was observed in conscious male rats [33]. The direct microinjection is the way to eliminate the systemic effects of GH by inducing metabolic or hormonal effects, some of which are mediated by IGF-I.

In the rats given 0.5 μg of rat GH into the ARC or PeV bilaterally, GH secretion was inhibited, and the inhibition lasted for 12 h (Fig. 3). During the period of inhibition, the duration and amplitude of GH pulses were decreased and the episodic secretion of GH appeared irregularly compared to the vehicle-injected control rats. Because the blood GH profile did not change in control rats given the

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Fig. 2. C-fos gene expression in the ARC induced by systemic administration of rhGH. A: c-fos mRNA observed by in situ hybridization with emulsion autoradiography 60 min after rhGH administration under dark field illumination. C-fos mRNA-containing cells were distributed in the ventromedial region of the ARC [21]. B: double-label in situ hybridization with digoxigenin-labeled NPY cRNA probe and 35S-labeled c-fos cRNA probe. The arrowhead indicates a representative cell double-labeled for both NPY and c-fos mRNAs. The arrow indicates a representative cell that appears to contain only NPY mRNA [22].

Fig. 3. Effect of GH injection into the hypothalamic nuclei on the amount of GH secretion estimated by the area under the curve of the blood GH levels (AUC). The AUC was analyzed every 6 h (n=6) and expressed as the percentage ± SEM of the AUC during 6 h before GH administration (0900–1500 h). Note that the GH secretion was significantly inhibited for 12 h (1500–2100 h, 2100–0300 h) by the injection of rat GH into the ARC (ARC) or PeV (PeV), but not by the injection of rat GH into the lateral hypothalamus (LH). *: P<0.01 compared with the AUC during control period.
vehicle or those given rat GH into the lateral hypothalamus, the effects of rat GH injection into the ARC or PeV were surmised to be site-specific. To test whether GH acts indirectly on the hypothalamus through the generation of IGF-I, the effects of microinjection of IGF-I were observed in conscious male rats. When 0.1 μg of recombinant human IGF-I was injected into the ARC or PeV bilaterally, the blood GH secretory pattern was not affected. The data indicate that GH, but not IGF-I, acts on the cells in the ARC and the PeV or in their vicinity to inhibit its own secretion.

The effects of rat GH injection into the PeV can be interpreted as the results of direct stimulation of SS neurons. A pituitary portal blood sampling study showed that intracerebroventricular injection of GH increased the SS level in the pituitary portal blood [3]. It was also reported that the suppression of endogenous GH secretion after systemic injection of GH was reversed by passive immunization against SS [9]. These data support stimulation of SS attributable to negative feedback by GH. Interestingly, the inhibitory effects on GH secretion induced by injection of rat GH into the ARC were very similar to those induced by injection into the PeV [33]. This suggests that the mechanisms were the same, despite the different sites of injection. One possible mechanism is stimulation of NPY neurons in the ARC, which in turn stimulate SS neurons in the PeV. Taken together with the results of our previous studies, it is suggested that GH acts directly on NPY neurons in the ARC and SS neurons in the PeV, and these cells play pivotal roles in the autoregulation of GH secretion.

Role of NPY in the Regulation of GH Secretion

The physiological significance of NPY neurons in relation to the neural regulation of GH secretion has not been established. NPY neurons in the ARC project to the PeV, and synaptic connections between NPY axons and SS neurons in the PeV have been demonstrated in rats [34]. We observed that the injection of 5 μg of NPY into the third ventricle was followed by immediate inhibition of the blood level of GH that lasted 3–4 h, whereas the administration of the vehicle did not affect the GH secretory profile in conscious male rats (Fig. 4A, B) [30]. These findings are compatible with observations by other investigators that the central administration of NPY inhibits GH secretion, and that the peptide stimulates the release of SS from hypothalamic tissue [31]. In addition, the ARC also contains the densest NPY-immunoreactive varicose fibers staining in the hypothalamus, with coarse fibers more concentrated in its ventrolateral parts, a distribution which is different from the location of immunoreactive cell bodies [35]. GRF neurons are present in the ventrolateral region of the ARC.

These findings led us to hypothesize that NPY may inhibit GRF neurons and thus participate in the feedback regulation of GH secretion. We observed the effect of NPY on the blood level of GH in adult male rats subjected to anterolateral deafferentation of the medial basal hypothalamus (ALC) [30]. ALC was performed to eliminate the influence of hypophyseotropic SS from the PeV, while leaving a functional system producing GRF. In the rat with ALC, the blood level of GH displayed small irregularly occurring fluctuations, instead of the usual high bursts produced every 3 h, but the baseline level of GH was higher than

![Fig. 4. Effect of intracerebroventricular administration of vehicle (A, C) or NPY (B, D) in sham-operated rat (A, B) and in rats with anterolateral deafferentation of the medial basal hypothalamus (ALC) (C, D). In the sham-operated rat, administration of NPY suppressed GH secretion, but not in the rat with ALC [30].](image-url)
that in the sham operated control rats (Fig. 4C). The administration of 5 \( \mu \)g of NPY into the third ventricle of the rat with ALC did not inhibit GH secretion as compared to the vehicle-administered control rat with ALC (Fig. 4D). It is reasonable to assume that the abolished inhibitory effect of NPY on GH secretion in ALC rats is a result of the disruption of SS input to the median eminence and the pituitary stalk, so that NPY does not influence the activities of GRF neurons in the ARC.

Conclusions

The distribution and characterization of GHR mRNA-containing cells in the hypothalamus and functional anatomy of GH-responding cells by the observation of c-fos expressing cells have provided new insights into the mechanism of autoregulation of GH through the hypothalamus. We provided data indicating that GH has an inhibitory influence on its own secretion, and NPY neurons in the ARC and SS neurons in the PeV are the primary target cells of blood-borne GH. Along with the previous data, our understanding of the autoregulation of GH is summarized by a scheme (Fig. 5). The mechanism of autoregulation of GH through the hypothalamus is complicated, and the functional interactions of many factors attributable to the mechanism should be elucidated further.

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References

AUTOFEEDBACK REGULATION OF GH


