Review

Gene Regulation of Growth Hormone-Releasing Hormone and Its Receptor

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Introduction

GH is a potent anabolic hormone and most of its biological action is mediated by insulin-like growth factor (IGF)-I. The GH-IGF-I axis is physiologically controlled by the hypothalamic releasing and inhibitory hormones, GH-releasing hormone (GRH) and somatostatin (SRIF) [1, 2]. Hypothalamic GRH stimulates both secretion and gene expression of GH through G protein-coupled receptors, GRH receptors, causing activation of cyclic AMP and A-kinase systems in the somatotropes [1, 2]. Many humoral factors affect GH secretion and some of their effects might be caused by changes in GRH and its receptor status. Blood glucose levels are known to alter GH secretion [3–10]. GH responses to hyperglycemia are similar among species, while GH responses to hypoglycemia differ between some species [3–10]. In rats, both hyper- and hypoglycemia inhibit pulsatile GH secretion [6, 7]. Evidence suggests that glucosensitive neurons, "glucoreceptors", are present in the lateral hypothalamic area of the hypothalamus in primates [4] and rats [11, 12], although they have not yet been identified. Previous *in vitro* studies have indicated that changes in the glucose concentration alter hypothalamic GRH [13, 14] and SRIF [13–15] secretion from rat and mouse hypothalmic fragments, but it is unclear whether these *in vitro*

observations reflect physiological regulation of GRH and SRIF. To better understand the glucose modification of hypothalamic GRH and SRIF, introduction of *in vivo* systems seems preferable. In this review, the changes in hypothalamic GRH and SRIF mRNA levels are described when hyper- or hypoglycemia is induced in conscious rats. Further, the effects of chronic hyperglycemia on these mRNA levels in streptozotocin-induced diabetic mice are reviewed mainly based on our findings.

Glucocorticoids (GC) exhibit diverse effects on GH secretion depending upon experimental conditions [16]. *In vitro*, GC enhance basal and GRH-induced GH release from cultured pituitary cells [17–19]. GC also appear to elicit both transcriptional and posttranscriptional influences on the GH gene [20–24]. These genomic actions by GC on the GH gene might be involved in the GC-induced increase in basal GH secretion. On the other hand, it has previously been reported that dexamethasone (Dex) increases GRH binding sites in rat somatotrope membrane [25]. This suggests that Dex-induced enhancement of GH responsiveness to GRH is due at least in part to the increase in GRH receptors in somatotropes. Further, the increase in GRH receptors caused by GC might be due to the genomic action of GC on GRH receptor gene as reported in many GC target genes including the GH gene [20–24, 26]. To elucidate the steroid regulation of GRH receptor gene, we therefore examined the effects of Dex on pituitary GRH receptor mRNA levels in cultured rat anterior pituitary cells. The expression of GRH receptor mRNA was found to be relatively low and a highly-sensitive method was required to assess the change [27]. We further introduced the

competitive reverse-transcription (RT)-polymerase chain reaction (PCR) method [28] to quantify GRH receptor mRNA levels in cultured pituitary cells.

Glucose Modificatin of Hypothalamic GRH and SRIF

Hypothalamic GRH and SRIF secretion in vitro

We had established a perifusion system with mouse hypothalamic fragments to study hypothalamic GRH and SRIF secretion [14, 29]. In this system, lowering the glucose concentration in the medium simultaneously stimulated both GRH and SRIF secretion [14]. The glucopenia-induced GRH and SRIF secretion were blocked by pyruvate, suggesting that a lack of pyruvate is responsible for glucopenia-induced GRH and SRIF secretion from the hypothalamus. In general, the lack of pyruvate leads to depletion of ATP, causing membrane depolarization and subsequent release of stored peptides from various types of neurons [30, 31]. It is therefore unclear whether GRH and SRIF neurons themselves respond to glucopenia or receive neuronal signals from the glucoreceptors. In addition, it is not precluded the possibility that glucopenia is a non-specific stimulation for hypothalamic peptides [32, 33]. To further investigate the physiological effects of hypoglycemia on hypothalamic GRH and SRIF, an *in vivo* system was used. Since the amount of hypothalamic peptide remains constant and does not reflect dynamic changes, mRNA levels are measured [34, 35]. We therefore used conscious rats exposed to hypo- or hyperglycemia and measured hypothalamic GRH and SRIF mRNA levels in the hypothalamus [36].

Effects of hypo- and hyperglycemia on GH secretion in conscious rats

Spontaneous GH secretion is episodic, occurring at about 3-h intervals in conscious male rats in a euglycemic state [36, 37]. Insulin-induced hypoglycemia almost completely eliminated GH surges in conscious rats (Fig. 1). These results are not due to the effects of insulin itself, since pulsatile GH secretion was not affected by insulin administration when a euglycemic clamp method

was applied to the rats [36]. Although severe general hypoglycemia induced by insulin administration may cause non-specific stress in rats, central glucopenia induced by icv injection of a competitive glucose analogue, 2-deoxy-D-glucose (2-DG), exhibited similar inhibition of pulsatile GH secretion in conscious rats [38]. This suggests that central glucopenia is primarily responsible for GH suppression caused by insulin-induced hypoglycemia. On the other hand, marked hyperglycemia induced by multiple injections of high doses of glucose partially inhibited GH surges in conscious rats (Fig. 1). Taken together, it is assumed that acute and severe changes in blood glucose levels inhibit spontaneous GH secretion in the rat.

Effects of hypo- and hyperglycemia on hypothalamic GRH and SRIF mRNA levels

After the exposure of conscious rats to hypo- and hyperglycemia, hypothalamic GRH and SRIF mRNA levels were examined with Northern blot analysis. Hyperglycemia significantly increased both GRH and SRIF mRNA levels in the rats [36] (Fig. 2). Hypoglycemia, however, increased only hypothlamic SRIF mRNA levels. These mRNA changes are consistent with the observation that inhibition of GH surges was more prominent in hypoglycemia than in hyperglycemia. The weaker
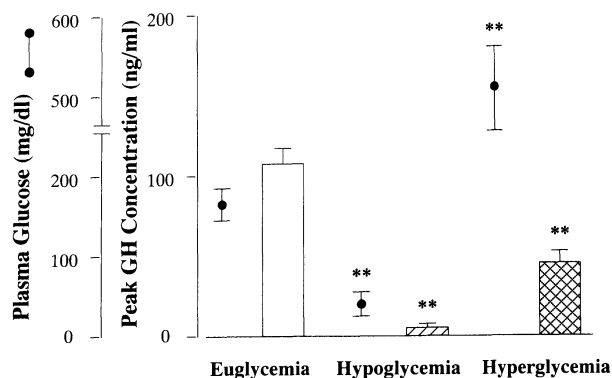

Fig. 1   Effects of hypo- and hyperglycemia on spontaneous GH secretion in conscious rats. Hypo- and hyperglycemia were induced in the rats by multiple injections of insulin (every 1 h) and 50% glucose (every 20 min), respectively. Adapted from Murao *et al.* (ref. 36; *Endocrinology* 134: 418-423, 1994).
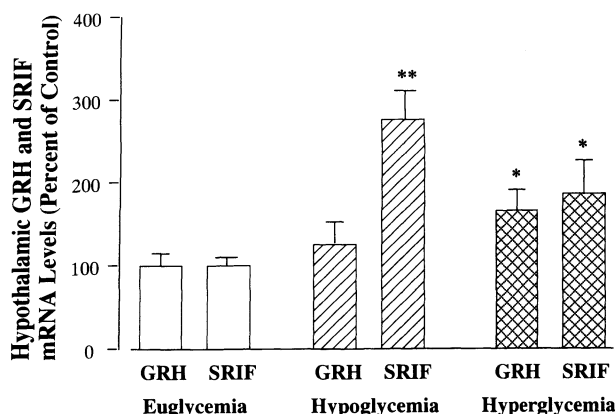
Fig. 2. Effects of hypo- and hyperglycemia on hypothalamic GRH and SRIF mRNA levels in rats. Adapted from Murao *et al.* (ref. 36; *Endocrinology* 134: 418–423, 1994).
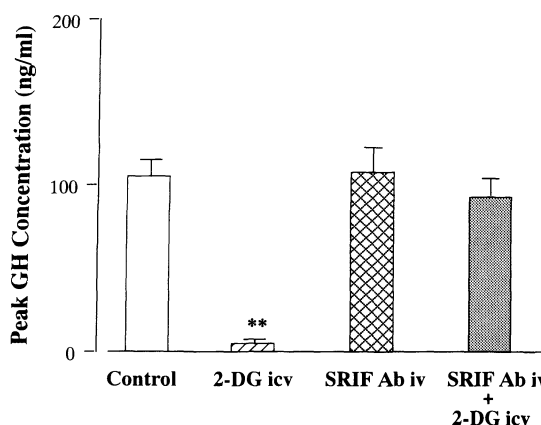

Fig. 3. Effects of a single icv injection of 2-DG on GH secretion in conscious rats with or without SRIF Ab. The SRIF Ab was iv administered 1 h before the icv injection of 2-DG. Adapted from Sato *et al.* (ref. 38; *J Neuroendocrinol* 7: 801–805, 1995).

inhibition of GH secretion in hyperglycemia is considered due to the co-activation of hypothalamic GRH and SRIF. Measurement of hypothalamic GRH and SRIF secretion in the rat hypophysial-portal circulation might provide us the direct evidence of their responses to hypo- and hyperglycemia, but it includes technical difficulties [39]. Instead of measuring GRH and SRIF secretion, we used SRIF antiserum to cancel the effects of endogenous SRIF secretion in rats receiving icv injection of 2-DG [38]. Pretreatment with SRIF antibody (SRIF Ab) completely reversed 2-DG-induced GH suppression (Fig. 3), suggesting that central glucopenia stimulates hypothalamic SRIF secretion. Our results indicate that acute changes in blood glucose levels stimulate hypothalamic SRIF gene expression in the rat. Hypothalamic GRH mRNA levels are stimulated only when exposed to severe hyperglycemia. *In vitro* studies [13, 14] have indicated that glucopenia stimulates both GRH and SRIF secretion from hypothalamic fragments. We did not observe any significant increase in hypothalamic SRIF mRNA levels *in vitro* in rat hypothalamic fragments exposed to low glucose (Sato M, unpublished data). Although changes in mRNA levels are not always in accordance with changes in peptide release, *in vitro* observations so far reported may not reproduce *in vivo* events.

Effects of insulin-induced hypoglycemia on hypothalamic c-fos expression

Insulin-induced hypoglycemia induced c-fos protein expression in the parvocellular division of the paraventricular nucleus (PVN) in rats [40]. This implies that neurons of CRH are activated by acute hypoglycemia [41–43]. Interestingly, the c-fos induction was also observed in the periventricular nucleus (PeN) and the lateral hypothalamic area (LH). The PeN contains SRIF neurons [44] and the LH is considered to contain glucose sensitive neurons (glucoreceptors) [4, 11, 12]. It is therefore assumed that both SRIF neurons and glucoreceptors are activated by insulin-induced hypoglycemia in rats. Furthermore, *in situ* hybridization showed that SRIF mRNA signals were increased in the PeN after insulin-induced hypoglycemia (Niimi M and Sato M, unpublished data). These results support the notion that SRIF neurons are activated by insulin-induced hypoglycemia.

GH secretion and hypothalamic GRH mRNA levels in diabetic mice

To investigate the effects of chronic hyperglycemia, we used streptozotocin (STZ)-induced diabetic mice [45]. Blood glucose levels
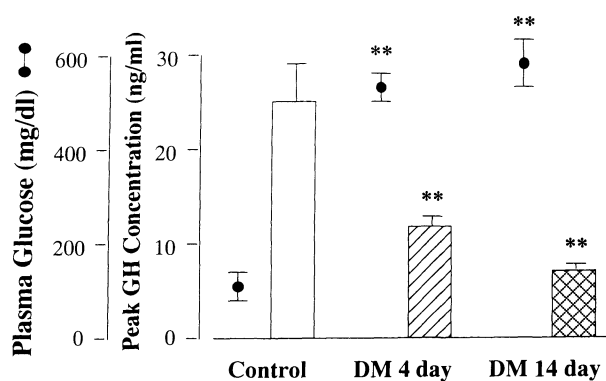
Fig. 4.   Plasma glucose levels and GH secretion in
conscious diabetic mice. Diabetes mellitus (DM)
was induced in the mice by ip injection of
streptozotocin (STZ). GH secretion was examined 4
and 14 days after STZ injection. Adapted from
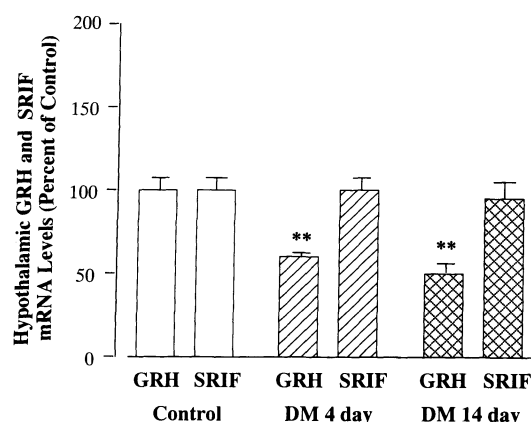Murao *et al.* (ref. 45; *Endocrinology* 136: 4498–4504,
1995).

Fig. 5.   Hypothalamic GRH and SRIF mRNA levels
in diabetic mice. Adapted from Murao *et al.*
(ref. 45; *Endocrinology* 136: 4498–4504, 1995).

Table 1.   Summary of the results for glucose modification of
hypothalamic GRH and SRIF in rodents

	GRH mRNA	SRIF mRNA	GH secretion
Hyperglycemia			
acute	↑	↑ *	↓
chronic	↓ ↓	→	↓ ↓
Hypoglycemia	→	↑ ↑	↓ ↓

*Secretion of SRIF appears predominant over GRH secretion
in acute hyperglycemia (ref. 36).

were greatly increased 4 days after STZ injection
and the severe hyperglycemia persisted for 14 days.
Spontaneous GH secretion was gradually impaired
in accordance with the persistence of
hyperglycemia (Fig. 4). Hypothalamic GRH mRNA
levels were decreased 4 days after STZ injection in
diabetic mice with only partially inhibitied GH
secretion (Fig. 5). The decrease in hypothalamic
GRH mRNA levels persisted for up to 14 days. In
contrast, hypothalamic SRIF mRNA levels were not
altered in any of the diabetic mice. These results
indicate that chronic hyperglycemia primarily
impairs hypothalamic GRH gene expression. The
impairment of GRH synthesis seems crucial to
suppression of GH secretion under diabetic
conditions. Similarly in rats, hypothalamic GRH
mRNA levels have been reported to be decreased
during chronic diabetes [46].

Table 1 summarizes our results on glucose
modification of hypothalamic GRH and SRIF in
rodents. Acute changes in blood glucose levels
preferentially activate SRIF neurons, and, as a
result, they stimulate both gene expression and
secretion of hypothalamic SRIF. Probably
hypoglycemia activates glucosensitive neurons
(glucoreceptors) in LH which send the neuronal
signals to SRIF neurons in the PeN. Hypothalamic
GRH neurons are less involved in these
physiological signals. Meanwhile, the mechanisms
whereby severe hyperglycemia increases both GRH
and SRIF gene expression are unclear. The

glucoreceptors seem little involved in these
responses, because the glucosensitive neurons
should be inactivated by hyperglycemia [12]. In a
state of chronic hyperglycemia such as diabetes
mellitus, hypothalamic GRH and SRIF gene
expression seems to be independent of acute
regulatory mechanisms. Under such pathological
conditions, hypothalamic GRH gene expression is
impaired, probably due to metabolic imbalance, as
is the case during starvation [47].

Steroid Regulation of Pituitary GRH Receptor Gene Expression

Expression of pituitary GRH receptor mRNA and its quantification

GRH receptor cDNA was originally cloned by
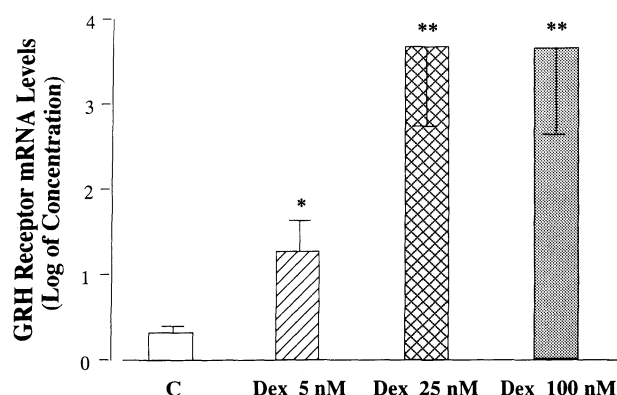Mayo in the man and rat [48]. As with other G-

Fig. 6. Effects of Dex on GRH receptor mRNA levels in cultured rat anterior pituitary cells. The cells were treated with 5, 25, and 100 nM Dex for 24 h. Adapted from Tamaki *et al.* (ref. 51; *J Neuroendocrinol* 8: 475–480, 1996).
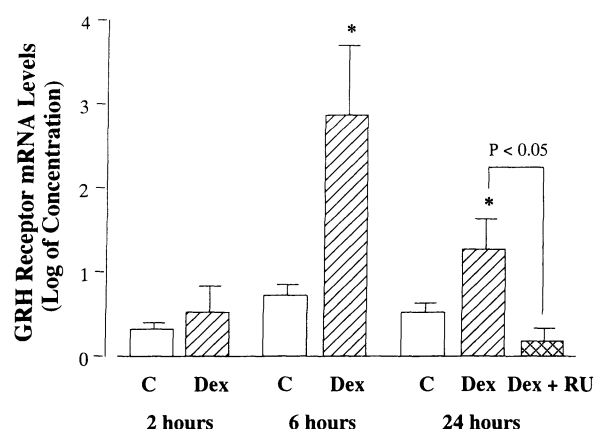

Fig. 7. Time-dependent effects of Dex on GRH receptor mRNA levels in cultured rat anterior pituitary cells. The cells were treated with 5 nM Dex for 2, 6, and 24 h in the presence or absence of RU 38486 (RU). Adapted from Tamaki *et al.* (ref. 51; *J Neuroendocrinol* 8: 475–480, 1996).

protein coupled receptors, expression of the receptor mRNA for GRH is relatively low even in the anterior pituitary [27]. Conventional Northern blot analysis cannot detect these low-abundant mRNAs when only a small sample of total RNA is available, so that a highly-sensitive method is required. PCR is extremely sensitive and has been used to detect GRH receptor mRNA in a variety of extrapituitary tissues in rats [27], but the standard PCR is unsuitable for quantitative analyses because of the exponential nature of the method. Competitive RT-PCR is one of the best methods for quantitative PCR analysis available [49, 50], but it requires synthesis of an RNA competitor, a complicated and time-consuming task [49, 50]. We have recently reported a novel method for preparing RNA competitors by utilizing a non-specific PCR product [28]. We have also established a competitive RT-PCR system for quantifying GRH receptor mRNA levels [51]. By this method, GRH receptor mRNA levels can easily be quantified even with total RNA obtained from cultured pituitary cells.

Effects of dexamethasone on GRH receptor mRNA levels

Primary culture of rat anterior pituitary cells was used to examine the effects of dexamethasone (Dex) on GRH receptor mRNA levels. Cultured pituitary cells were treated with Dex for 24 h and competitive

RT-PCR was carried out with a small amount of total RNA extracted from the treated cells [51]. Dex increased GRH receptor mRNA levels in a dose-depentent manner with the maximal effect obtained at a 25 nM concentration (Fig. 6). Time-course experiments showed that at least 6 h was required to obtain a significant increase in GRH receptor mRNA levels (Fig. 7). A glucocorticoids (GC) receptor-specific antagonist, RU 38486, significantly blocked the Dex-induced increase in GRH receptor mRNA levels (Fig. 7). These results indicate that Dex increases GRH receptor mRNA levels through GC receptors located in the rat somatotropes [52]. GC activates many genes at the transcriptional level [53]. Ligand-activated GC receptor homodimers bind to characteristic DNA response elements (GC response elements: GREs) [54]. Although GREs have not yet been identified in the GRH receptor gene, direct interaction between Dex and GRH receptor genes seems unlikely in Dex-induced enhancement of GRH receptor mRNA levels. If direct interaction was to occur, GRH receptor mRNA levels would change more rapidly [55, 56]. Dex may enhance the stability of GRH receptor mRNAs as demonstrated in several other genes [24, 55, 56]. Further studies are required to elucidate the molecular mechanisms whereby GC enhance GRH receptor mRNA expression in somatotropes.

Diverse effects of GC on GH secretion in vitro and in vivo

Our study showed that Dex increases GRH receptor gene expression in rat somatotropes. This finding agrees with a previous observation by Seifert *el al.* [25] that Dex increased GRH binding sites in the rat pituitary membrane. Many studies have shown that GC enhance GH responses to GRH *in vitro* [17–19]. Taken together, these findings suggest that enhancement of GH responsiveness to GRH by GC is due at least in part to stimulatory actions on GRH receptor gene in rat somatotropes. In contrast, in vivo actions of GC are diverse, varying with experimental conditions [16]. Evidence suggests that chronic GC excess inhibits GH secretion chiefly through hypersecretion of hypothalamic SRIF [16, 59–61]. The stimulatory effects of GC on GRH receptor and enhancement of GH responses to GRH might be overcome by strongly-activated SRIF tone. Meanwhile, GC deficiency has been known to impair GH secretion [62]. To investigate the effect of endogenous GC on pituitary GRH receptor mRNA levels, we prepared rats for adrenalectomy. In adrenalectomized rats, pituitary GRH receptor mRNA levels were decreased, returning to normal with Dex replacement (Ohyama T and Sato M, unpublished data). These findings imply that physiological levels of endogenous GC stimulate pituitary GRH receptor gene expression. The steroidal regulation of GRH receptor plays an important role in maintaining normal secretion of GH *in vivo*.

Regulation of Hypothalmic GRH and Pituiary GRH Receptor Gene Expression by Various Humoral Factors

Various humoral factors other than blood glucose and GC regulate gene expression of hypothalamic GRH and pituitary GRH receptor. Their actions are summarized in Table 2. Feedback regulation of GH secretion is mediated by both GH and IGF-I [1, 2, 63, 64]. Peripheral GH inhibits hypothalamic GRH mRNA [34, 35] and stimulates hypothalamic SRIF mRNA [65] due to its direct actions on hypothalamic GRH [66, 67] and SRIF neurons [68–70], respectively, whereas IGF-I inhibits hypothalamic GRH mRNA and stimulates hypothalamic SRIF mRNA in rats only by central administration [35]. Since IGF-I is produced locally in the hypothalamus [71], central IGF-I may be important in the regulation of hypothalamic GRH and SRIF gene expression [72]. Feedback regulation of GRH receptor mRNA by GH and IGF-I is unknown. Pituitary GRH receptor mRNA levels have been reported to be decreased by GH administration [73] and overexpression of GH in the hypothalamus [66]. These effects are probably due to the changes in hypothalamic GRH secretion.

GH secretory patterns are influenced by gonadal steroids [74], at least in part, through modification of hypothalamic GRH and SRIF gene expression. Hypothalamic GRH mRNA levels are increased by testosterone [75, 76] and decreased by estrogen [77]. Both gonadal steroids stimulate hypothalamic SRIF mRNA levels [77–80]. Although there is no report showing the regulation of GRH receptor by gonadal steroid hormones, one report suggests that GRH

Table 2. Summary of the actions of various humoral factors regulating GRH/SRIF mRNA and GRH receptor (GRH Re) mRNA levels in rodents

	GRH mRNA	SRIF mRNA	GRH Re mRNA	GH secretion
GH	↓ (34, 35)	↑ (65)	↓ (66, 73)	↓ (63)
IGF-I	↓ (35)	↑ (35)	NR	↓ (63, 64)
Testosterone	↑ (75, 76)	↑ (78, 79, 80)	NR	↑ (74)
Estrogen	↓ (77)	↑ (77, 78)	↓ (81)	↓ (74)
Thyroid hormone	↓ (84, 85, 86)	↑ (86)	↑ (86, 87)	↑ (83)

Effects of IGF-I and testosterone on pituitary GRH Re mRNA levels have not been reported (NR) to date. The numbers given in parentheses indicated the reference number of quoted papers.

receptor mRNA levels may be negatively regulated by estrogen [81]. GH secretion is known to be impaired by either abnormally high [82] or low levels of thyroid hormones [83]. Effects of thyroid hormones on hypothalamic GRH and SRIF mRNA levels seem to be secondary to the changes in GH secretion [84–86], although a direct action of thyroid hormones on pituiary GRH receptor mRNA levels may exist [87].

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