Hypothalamic Growth Hormone-Releasing Factor (GRF) Regulates Its Own Receptor Gene Expression In Vivo in the Rat Pituitary

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GH-releasing factor (GRF) is the hypothalamic peptide that specifically stimulates both synthesis and secretion of pituitary GH [1]. GRF is released into the hypophyseal-portal vessels under control by a variety of neurotransmitters and neuropeptides. After reaching the pituitary, GRF binds to its specific receptors [1] on somatotrophs and generates bursts of GH secretion episodically [2]. Earlier ligand-binding and functional studies in rats have shown that GRF down-regulates its own receptors in the pituitary [3, 4], but the mechanisms of down-regulation remain to be fully determined. We investigated how GRF regulated its own receptors at the gene expression level and report here evidence indicating that GRF receptor mRNA levels are up- or down-regulated in vivo in the absence or presence of GRF, respectively.

Materials and Methods

In all experiments we used 3-month-old conscious male Sprague-Dawley rats. In the first experiment, 3 groups of rats were injected with normal rabbit serum (NRS) or 0.25 and 1.0 ml of rat GRF antiserum [5, 6] at 0830 h through chronic atrial catheters. The total injection volume was adjusted to 1.0 ml per rat with NRS. The second experiment was designed to examine the effect of the depletion of noradrenaline, a neurotransmitter thought to stimulate GRF release, in 3 groups of animals. Group 1 received two ip injections of vehicle saline, one at 1700 h the day before the experiment, and the other at 0830 h on the day of the experiment. Following the same time schedules, Group 2 were given saline followed by diethyldithiocarbamate (DDC, 750 mg/kg), a dopamine β-hydroxylase inhibitor (Sigma Chemical Co., St. Louis, MO), and Group 3 received two injections of α-methyl-p-tyrosine (αMPT, 250 mg/kg), a tyrosine hydroxylase inhibitor (Sigma Chemical Co.). In the third experiment, the rats were injected ip with saline or DDC at 0830 h. The DDC-treated rats were then injected sc with either human GRF(1-44)-NH2 (10 μg/kg; Sumitomo Pharmaceutical Co., Ltd., Osaka, Japan) or 0.25% BSA in vehicle saline 3 times at 3.5-h intervals, i.e. at 0830, 1200, and 1530 h, which corresponded to the time of onset of spontaneous GRF-dependent GH pulses in our laboratory [2, 5]. The control rats received vehicle sc 3 times. All rats were killed by decapitation at 1630 h, and the anterior pituitaries were obtained for extraction of RNA as described [7]. Pituitary GRF receptor and β-actin mRNA levels were determined by RNase protection assays as described [8, 9]. The intensity of GRF receptor mRNAs were expressed relative to the β-actin mRNA levels in each sample. The results are presented as the means ± SEM. Two-way analysis of variance followed by Duncan’s multiple range test were used for the statistical analysis. A P value of <0.05 was considered to be significant.
Results

Figure 1 shows the protected mRNA fragments of GRF receptor and β-actin in the anterior pituitaries of rats passively immunized with rat GRF antiserum or NRS. The administration of antiserum increased GRF receptor mRNA levels in a dose-related manner. The mean values for GRF receptor mRNA in 0.25 and 1.0 ml antiserum-treated groups were 264 ± 27% (P<0.01) and 382 ± 41% (P<0.01) of the control NRS-treated levels (n=5 rats/group). As shown in Fig. 2, both DDC and αMPT treatment also caused a significant increase in GRF receptor mRNA to 313 ± 32% (P<0.01) and 294 ± 42% (P<0.01), respectively, of the control levels, which were not statistically different in the two groups (n=9 rats/group). As shown in Fig. 3, the increase in GRF receptor mRNA levels induced by DDC treatment (P<0.001 vs. control) was significantly reversed by 87 ± 15% by the repeated administration of GRF (P<0.01 vs. DDC group, n=10 rats/group).

Discussion

The continuous exposure of target tissues to their trophic hormones results in lessening their sensitivity or responsiveness to these hormones [10]. This phenomenon, referred to as down-regulation, is thought to represent an adaptive response to prolonged exposure to receptor agonists. Down-regulation is also true of GRF receptors. The addition of GRF in vitro, or the continuous infusion of GRF in vivo, down-regulates the GRF-binding capacity of the rat pituitary [3, 4], but the mechanisms of down-regulation are complex and presumably include changes in receptor kinetics such as internalization, cytoplasmic degradation,
REGULATION OF GRF RECEPTORS BY GRF

We wished to examine whether down-regulation of GRF receptors by GRF takes place at a gene expression level. Our first strategy was to observe the effect of eliminating endogenous GRF. With highly specific antiserum to rat GRF [5, 6], we demonstrated that immunoneutralization of circulating GRF noticeably up-regulated pituitary GRF receptor mRNA levels. This finding strongly indicates that endogenous GRF is continuously down-regulating gene expression of its own receptors under physiological condition. The up-regulation of GRF receptor mRNA levels was also obtained by the inhibition of noradrenaline synthesis. There is abundant evidence that activation of noradrenergic α-adrenoceptors facilitates GRF release. α-adrenoceptor agonists, clonidine and guanfacine, stimulate GRF release [2, 11], whereas α-adrenoceptor blocker, phenoxybenzamine, completely inhibits GRF-dependent, spontaneous GH pulses [12], as do DDC and αMPT [13, 14]. The up-regulation of GRF receptor gene expression in the DDC-treated rats was reversed by replacement with GRF, which was given 3 times at 3.5-h intervals, corresponding to the times of onset of physiological GRF-dependent GH surges [2, 5]. The sc dose of GRF was chosen on the basis of the results from pilot experiments to be 10 μg/kg, a dose ten times higher than that previously used for intravenous injection [5, 6]. This finding demonstrates that exogenous GRF can down-regulate its own receptor gene expression.

Our results indicate that increased or reduced GRF receptor mRNA accumulation is at least one important mechanism in up- or down-regulation, respectively. Of great importance is the finding that the GRF receptor mRNA levels were noticeably up-regulated in the absence of GRF, which suggests that the down-regulatory mechanism is operating under physiological conditions. Changes in GRF receptor expression could result in altered binding of the ligand, as our receptor probe was designed to recognize the N-terminal extracellular region [8], presumed to represent the ligand-binding domain [1]. Therefore, the regulation of GH secretion at the pituitary GRF receptor level would play an important role in the control of the hypothalamic GRF-pituitary GH axis.

Fig. 3. Effects of DDC alone, and in combination with synthetic GRF, on pituitary GRF receptor (GRF-R) mRNA levels. Four representative bands of GRF receptor and β-actin mRNA are shown in each group. Means ± SEM GRF-R mRNA levels, obtained from three separate experiments, are shown as columns, with the control value being 100%. n=10 animals/group. *P<0.001 vs. control; **P<0.01 vs. DDC alone.

References


