Gender Difference in Pituitary Growth Hormone-Releasing Factor (GRF) Receptor Gene Expression in the Rat

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GH-releasing factor (GRF) is the hypothalamic peptide that stimulates both synthesis and secretion of GH. The GRF signal is transduced to somatotroph cells through pituitary GRF receptors and generates episodic bursts of GH secretion into the bloodstream. Secretion of GH is highly sexually dimorphic, particularly in the rat [1]. In female rats, GH surges are of lower amplitude and occur more irregularly and frequently, whereas GH troughs are higher than those in male rats. GRF appears to play a critical role in sexual dimorphism in GH secretion, because pulsatile GH secretion does not occur in the absence of GRF in either male or female rats [2, 3]. We here report the first evidence of marked sexual dimorphism in pituitary GRF receptor gene expression in the rat. To determine the functional significance of this finding, we also examined how gender difference affects the ability to release GRF, an endogenous ligand for GRF receptors.

Materials and Methods

Anterior pituitaries and hypothalami were obtained from adult Sprague-Dawley rats. Pituitary total RNA was extracted by guanidine thiocyanate. After reverse transcription, cDNA fragments corresponding to rat GRF receptor and β -actin were amplified by polymerase chain reaction (PCR). The

PCR products of 262-base pair (bp) GRF receptor and 188-bp rat β -actin were subjected to cloning and sequencing [4]. After linearization of the plasmids with *Hind* III, antisense cRNA probes were synthesized in the presence of T7 RNA polymerase. RNase protection assays for the quantitation of mRNA levels were carried out as described [5]. The intensity of GRF receptor mRNA was normalized by that of β -actin mRNA. Hypothalamic incubation was performed as described [6, 7], using 12 male and 12 female rats in each of three separate experiments. Hypothalamic fragments were incubated under basal and depolarized (50 mM K⁺) conditions. GRF in the media and tissues was measured by a highly specific RIA [6, 7].

Results

Figure 1 shows the protected fragments of GRF receptor and β -actin mRNA in the anterior pituitaries of male and female rats. It was apparent that the GRF receptor mRNA levels in female rats were much lower than those in male rats. The mean value for GRF receptor mRNA normalized to β -actin mRNA band in female rats ($n=12$) was only 15.4 ± 2.3 % ($P<0.001$) of that in male rats ($n=12$). Figure 2 shows *in vitro* secretion of GRF from male and female rat hypothalami (left panel). The depolarizing stimulus-induced release of GRF (open columns) was significantly ($P<0.01$) smaller in female than in male rats (30.5 ± 2.4 vs. 45.5 ± 3.0 pg/hypothalamus/30 min, number of observations = 6 each), as was the basal GRF secretion (closed columns) ($P<0.025$). Hypothalamic GRF content (right panel) was similarly less in female than in male rats (611 ± 62 vs. 861 ± 33 pg/hypothalamus, $P<0.01$).

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Fig. 1. Pituitary GRF receptor mRNA levels in adult male and female rats. Protected mRNA bands of GRF receptor and β -actin in four representative rats of both sexes are shown. Results of three separate experiments are combined, and the means $\pm$ SEM levels of GRF receptor mRNA are shown by columns with the male value being 100% ($n=12$ for both male and female rats). * $P<0.001$ vs. male rats.

Discussion

The results of the present study demonstrate for the first time that female rats express a noticeably lower level of pituitary GRF receptor mRNA than male rats. We also demonstrated that female rats had a decreased capacity to release GRF compared with male rats. The combined reduction in GRF receptor gene expression and GRF-releasing capacity in female rats provide a molecular and physiological basis for a part of the sexual difference in GH secretion; spontaneous, GRF-dependent GH surges are of much lower amplitude in female than in male rats [1–3].

GRF receptors belong to the G protein-coupled receptor family [8, 9] and their mRNA levels are down-regulated by GRF [10]. However, this down regulation cannot account for the very low levels of GRF receptor mRNA observed in female rats, as they had lower, not higher, ability to release the ligand GRF than male animals. Female rats exhib-

Fig. 2. GRF-releasing capacity (left panel) and hypothalamic GRF peptide content (right panel) in male and female rats. Results represent one of three independent experiments and are presented as the means $\pm$ SEM for six observations per group. Closed and open columns in the left panel indicate basal and 50 mM K^+ -stimulated release of GRF, respectively. * $P<0.025$, ** $P<0.01$ vs. male rats.

it GRF-dependent GH surges *in vivo* of about a third to half the height [2, 3], or synthetic GRF-induced GH pulses *in vitro* of only a third the amplitude [11], of those that male rats do. This difference is greater in magnitude than the relative female to male value that we observed in the GRF-releasing capacity (67%) or the GRF content (71%) and in GRF mRNA levels (45 or 79%) reported by others [12, 13]. Compared with these parameters for the GRF system, the level of pituitary GRF receptor mRNA showed the greatest gender difference, with the female level being only 15% of the male value. This finding suggests that the regulation of GH secretion by GRF receptors plays an important role at least in sexually dimorphic expression of GH secretion and thus would be of physiological significance.

The decrease in both GRF release and receptor gene expression in the female rat, along with the reports of others [12, 13], indicates that the combined diminished tone in the synthesis, secretion and hypophyseal action of GRF is characteristic of the feminine GRF system. The underlying mechanism for the sexual dimorphism of pituitary GRF receptor gene expression is unknown at present, but may involve gonadal steroids secreted after puberty or may be associated with sexual differentiation of the brain.

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