Endocrine Journal 1996, **43** (1), 115–118

NOTE

Continuous Stimulation of Gonadotropin-Releasing Hormone (GnRH) Receptors by GnRH Agonist Decreases Pituitary GnRH Receptor Messenger Ribonucleic Acid Concentration in Immature Female Rats

HIDEYA SAKAKIBARA, MICHIYOSHI TAGA, MARIO IKEDA, KEIKO KUROGI AND HIROSHI MINAGUCHI

Department of Obstetrics and Gynecology, Yokohama City University School of Medicine, Yokohama 236, Japan

Abstract. Although it is well recognized that continuous administration of gonadotropin-releasing hormone agonist (GnRHa) induces pituitary desensitization, the precise molecular mechanism of this phenomenon is still unclear. To test the hypothesis that pituitary gonadotroph desensitization is mediated by a change in GnRH receptor (GnRH-R) gene expression, the GnRH-R mRNA concentration was analyzed in immature female rats during GnRHa treatment. Northern blot hybridization was used to determine the GnRH-R mRNA concentration several times after an injection of TAP-144-SR, a slow releasing GnRHa. The GnRH-R mRNA readings were 92.7 ± 9.5%, 49.9 ± 5.0%, 35.7 ± 2.3% and 73.8 ± 5.7% (Mean ± SD) compared to each control value at 1, 2, 4 and 8 weeks, respectively, after a single injection of 0.94 mg TAP-144 SR. These changes in GnRH-R mRNA coincided with the changes in gonadotropin secretion and LH-β mRNA in response to GnRH in the results of our previous report. The present results indicate that the reduction of the number of pituitary GnRH-R sites induced by continuous stimulation with GnRHa is regulated at a transcriptional level.

Key words: GnRH receptor, GnRH analog, Desensitization, Pituitary gland, mRNA

(*Endocrine Journal* **43**: 115–118, 1996)

THE SECRETION of LH and FSH by pituitary gonadotropes is regulated by gonadotropin-releasing hormone (GnRH). The responsiveness of gonadotropes to GnRH varies according to conditions and depends on the hormonal milieu. The variation also appears to correlate, to some extent, with the number of GnRH receptors (GnRH-R) in pituitary gonadotropes. It is well recognized that while pulsatile stimulation by a low concentration of GnRH induces an increase in GnRH-R activation, continuous GnRH stimulation causes its desensitization. In fact, the ability of GnRH agonists (GnRHa) to induce hypogonadotropic hypoestrogenism (desensitization) has come to be clinically used in treating such steroid hormone dependent diseases as endometriosis, leiomyoma, precocious puberty, and breast cancer, but the mechanisms underlying the desensitization of the pituitary gland to GnRH are still unclear.

GnRH-R cDNAs have recently been cloned in the mouse [1], rat [2] and man [3], and GnRH-R was shown to be a G-protein coupled receptor with seven transmembrane domains. The acquisition of GnRH-R cDNA enables us to examine the receptor regulation at a transcriptional level.

In this study, to clarify the molecular basis of the inhibitory action of continuous GnRHa stimulation on the gonadal axis, we examined the change in pituitary GnRH-R mRNA during GnRHa treat-

Received: February 16, 1995
Accepted: September 28, 1995
Correspondence to: Dr. Michiyoshi TAGA, Department of Obstetrics and Gynecology, Yokohama City University School of Medicine, 3–9 Fukuura, Kanazawa-ku, Yokohama 236, Japan

ment in immature female rats.

Materials and Methods

Tissue collection and RNA extraction

A total of 50 immature female Sprague-Dawley rats (21 days old, 70–90 g) were injected s.c. with a sustained-release formulation of GnRHa (TAP-144-SR, Takeda Chemical Ind., Ltd.) at a dose of 0.94 mg/rat. Control rats were injected with vehicle alone. This dosage of TAP-144-SR was chosen based on our previous related study [4]. TAP-144-SR is a depot formulation of a GnRHa, D-Leu^6/Pro^9-ethylamide GnRH, in copolymers composed of lactic acid and glycolic acid which allows constant release of the GnRHa over 4 weeks [5]. Five rats in each group were sacrificed by decapitation at 0, 1, 2, 4 and 8 weeks after the single injection of GnRHa or vehicle. To avoid the affect of LH surge on the GnRH-R mRNA concentration, rats were sacrificed in the morning. Anterior pituitary glands were rapidly removed and immediately frozen in liquid nitrogen.

Total RNA was prepared from pituitary glands by an acid guanidinium thiocyanate-phenol-chloroform procedure [6]. The amounts of total RNA were estimated by optical density.

Hybridization probe

GnRH-R probe was synthesized by a reverse transcriptase-polymerase chain reaction (RT-PCR). Sense primer (5'-GTGACCGTGACTTTCTTCC-3'; nucleic acid number 115–133) and antisense primer (5'-ACGACAAAGGAGGTAGCGAA-3'; 811–830) were made according to the mouse GnRH-R cDNA sequence [1]. PCR was carried out with cDNA made from mouse pituitary total RNA as a template. PCR product (716 bp) was extracted from the gel after electrophoresis. The extracted fragment was labeled with ^{32}P by a multiprime labeling system (Amersham).

Human β-actin cDNA probe purchased from Amersham was also labeled with ^{32}P and used as a hybridization probe to serve as an internal control.

Northern blot hybridization

Twenty μg of total RNA from each sample was denatured and subjected to electrophoresis and diffusion blotting onto a nylon membrane. Each blot was sequentially hybridized with mouse GnRH-R and human β-actin cDNA probes. The blots were washed and subjected to autoradiography, and the band densities were quantitatively determined in an imaging analyzer BAS 2000 (Fuji Co., Japan).

Data analysis

Densities of hybridized bands are presented as the mean arbitrary densitometric units (ADU) in the GnRHa treatment group and the control group each week. After the amount of total RNA in each sample was standardized within each blot by correcting the GnRH-R mRNA concentration according to that of β-actin mRNA, data are also presented as the ratio of the mean standardized concentration in the GnRHa treatment group/the mean standardized concentration in the control group. They are subjected to a one-way analysis of variance followed by *t*-test to assess the statistical significance.

Results

Figure 1 illustrates the results of Northern blot hybridization with GnRH-R cDNA, of the anterior pituitaries from the immature rats treated with the single injection of TAP-144-SR or vehicle for respective durations. Figure 2 is a conversion of the results in Fig. 1. The density of Northern blot was measured in an imaging analyzer. As shown in Fig. 2 (A), the mean ADU for GnRH-R mRNA were 15.9 ± 1.8, 18.2 ± 1.8, 9.3 ± 2.4, 6.4 ± 1.3, and 12.0 ± 1.1 (Mean ± SD) at 0, 1, 2, 4, 8 weeks after TAP-144-SR injection, respectively, whereas those of control rats injected with vehicle alone were 15.9 ± 1.8, 19.3 ± 2.5, 13.1 ± 1.4, 15.7 ± 2.3, 19.2 ± 2.5 at corresponding weeks. There was slight but not significant change in the mean ADU for β-actin mRNA during treatment with TAP-144-SR or vehicle (Fig. 2 (B)). When GnRH-R mRNA concentrations were internally standardized by correcting them according to the β-actin mRNA concentrations, they were 92.7 ± 9.5%, 49.9 ± 5.0%, 35.7 ± 2.3%, and 73.8 ± 5.7% (Mean ± SD) at 1, 2, 4, 8 weeks after TAP-144-SR injection, respectively, compared to the values for control rats. GnRH-R mRNA gradually decreased after the single injec-

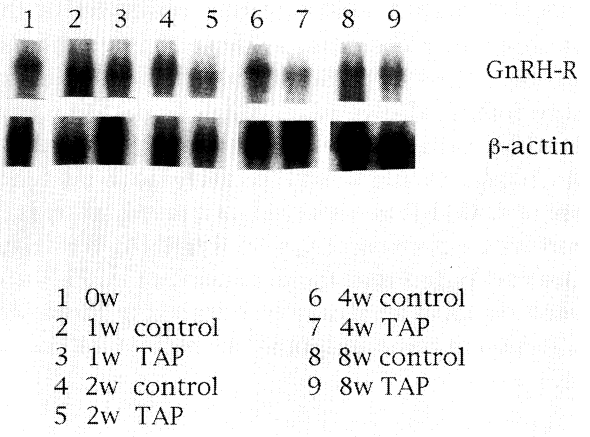

Fig. 1. Northern blot hybridization of rat pituitary GnRH-R mRNA during the treatment with TAP-144-SR or vehicle injection. Twenty μg of total RNA, which was extracted from the rat pituitary gland at 0 (lane 1), 1 (lane 3), 2 (lane 5), 4 (lane 7) and 8 weeks (lane 9) after vehicle injection or 1 (lane 2), 2 (lane 4), 4 (lane 6) and 8 weeks (lane 8) after TAP-144-SR injection, was hybridized to ^{32}P labeled GnRH-R probe (upper panel) and β-actin probe (lower panel).

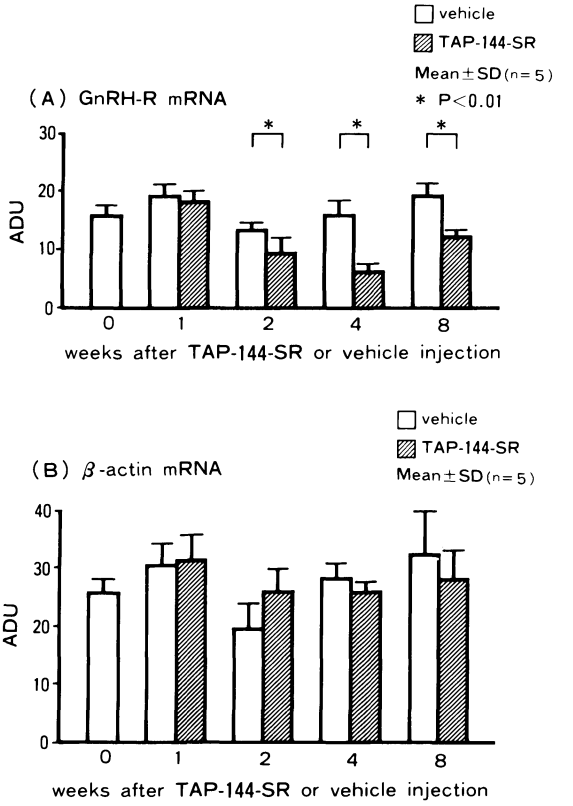

Fig. 2. Changes in GnRH-R mRNA (A) and β-actin mRNA (B) concentrations at 0, 1, 2, 4, 8 weeks after TAP-144-SR or vehicle injection. Data are presented as arbitrary densitometric units (ADU). *t*-test for independent samples was used to assess the statistical significance.

tion of TAP-144-SR, and was the lowest at 4 weeks after the injection. At 8 weeks the amount of GnRH-R had risen significantly, but still had not returned to the initial concentration.

Discussion

GnRH, a key hormone in the regulation of reproduction, stimulates gonadotropin secretion by binding to a specific GnRH-R in the pituitary gland. Because GnRH-R density in pituitary gonadotrophs varies in response to the hormonal environment, the analysis of GnRH-R is essential for the understanding of gonadotropin secretion. The regulation of the number of receptors could occur at various levels, including the alteration of receptor synthesis, degradation, and/or recycling [7].

The inhibitory action of GnRHa on gonadal function is widely utilized for the treatment of estrogen-dependent diseases and is basically thought to be due to a reduction of gonadotropin secretion. The precise molecular mechanism of this inhibition remains to be delineated. The reduction of GnRH-R by GnRHa is believed to involve the internalization and degradation of the agonist-occupied receptor [8], since desensitization of gonadotropes is likely to result, in part, from loss of the cell surface receptor, but whether GnRH-R synthesis is decreased by GnRHa remains unknown. The recent cloning of GnRH-R cDNA [1–3] allowed us to examine the GnRH-R mRNA concentration in various hormonal environments. Bauer-Dantoin *et al.* [7] reported dynamic regulation of the GnRH-R mRNA concentration in the anterior pituitary gland during the rat estrous cycle with 3-fold increase in GnRH-R mRNA at the LH surge. And the regulation of GnRH-R mRNA expression was also described in the sheep [9, 10], but there is no report concerning a change in pituitary GnRH-R mRNA during GnRHa treatment. In this study, we demonstrated that GnRH-R mRNA had gradually decreased 1, 2 and 4 weeks after the single injection of long-acting GnRHa and we observed partial recovery of GnRH-R at 8 weeks. The inhi-

bition of GnRH-R mRNA at 2 and 4 weeks after GnRHa treatment seems to be a direct effect of GnRHa on the pituitary gland not via sex steroid action because the concentrations of serum gonadotropin and estrogen were markedly reduced in these periods [4]. Previously, in a similar experimental design, we reported the effect of GnRHa on the GnRH-R concentration, serum LH, pituitary LH, LH-β mRNA, and LH secretory response to GnRH at 1, 2 and 4 weeks after the treatment with the single injection of TAP-144-SR in immature female rats [4, 11]. According to the results of these experiments, the pituitary GnRH-R concentration, LH response to GnRH, and LH-β mRNA concentration had significantly decreased 2 and 4 weeks after the TAP-144-SR injection, while those of control rats had gradually increased during sexual maturation. The profile of the changes in the GnRH-R mRNA concentration observed in the present study is similar to those in our previous results. The correlation between these 2 sets of experiments allows us to consider that the pituitary desensitization by continuous administration of GnRHa, during which the decrease in the number of GnRH-R is observed, is caused, at least in part, by the decrease in GnRH-R mRNA. It is therefore concluded that the decrease in GnRH-R at a transcriptional level may be involved in the desensitization of pituitary gonadotroph by GnRHa.

Acknowledgements

The authors wish to thank Dr. L.M. Mertz, NICHD, NIH, for the GnRH-R primers.

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