Response of GH, ACTH, TSH and IGF-I to Gonadotropin Releasing Hormone Stimulation in Ovariohysterectomized Rats

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Abstract

GnRH is not only a primary regulator of gonadal function but also a possible factor which affects other pituitary cells out of gonadotrophs. Recently, it has been reported that GnRH might induce the release of GH, but the effects on GH and other pituitary hormones of GnRH still remained controversial. In this study, OHE was performed in 30-week-old female Sprague Dawley rats as experimental groups. Intact female rats were taken as control group. GnRH stimulation at different weeks (20, 24, and 30) after OHE was examined in all rats. Evaluation of the circulating GH, IGF-I, ACTH, TSH concentrations at different time points with commercial ELISA kits was carried out. Results indicated that the GH levels after stimulation in all OHE groups tended to be lower than the control group but without significant difference. In all groups, the IGF-I levels at 30 min after GnRH stimulation were significantly lower than the basal IGF-I levels. The levels of ACTH at 30 min after GnRH administration were significantly higher (p<0.05) than the basal ACTH levels in all groups. Furthermore, both the basal and stimulated ACTH levels in all OHE groups were significantly higher (p<0.05) than the control ones. The TSH levels after GnRH stimulation in all OHE groups tended to be higher than the control ones but without significant difference. Thus, GnRH administration could induce GH, ACTH and TSH secretion but reduce IGF-I levels in ovariohysterectomized rats. The body weight increment after neutralization in female rats is probably accomplished with the elevation of IGF-I and ACTH.

Keywords: ACTH, GH, GnRH, IGF-I, rat, TSH

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Introduction

The increased prevalence of obesity in companion animals is getting problematic in veterinary practice as obesity correlates with diabetes mellitus, cardiorespiratory disease, neoplasia, and anesthetic complications (Colliard et al., 2006; German, 2006). Neutralization has been considered as one of the main risk factors of obesity (Martin et al., 2006a). Obesity is associated with multiple endocrine alterations in the concentration of circulating hormones (Martin et al., 2006b). In humans and animals, obesity is characterized by markedly decreased growth hormone (GH) secretion and production (Iwakura et al., 2007). Furthermore, low circulating GH levels and obese subjects have a blunted response to GH stimuli, which is reversible after losing weight (Williams et al., 1984). GH has been assumed to be an effective adjunct to standard regimens in the treatment of obesity recently (Munzer et al., 2009).

Gonadotropin-releasing hormone (GnRH) is a hypothalamic decapeptide with an undoubted role as a primary regulator of gonadal function in controlling the release of gonadotrophins. However, GnRH is inapposite to affect pituitary cells other than gonadotrophs differently in various species, for instance, GH release. However, the peroration of GnRH-induced GH release still remained controversial (Skinner et al., 2009). At present, there seem to be no specific reports referring to the relationship between GH and/or other pituitary hormones, GnRH action, and obesity after neutering. The aim of this study was to investigate the effects of GnRH stimulation on GH and other pituitary hormone levels in ovariohysterectomized rats.

Materials and Methods

Animals: Thirty-nine female Sprague Dawley (SD) rats (30 weeks old, 328.31 ± 4.97 g) were obtained from Animal Disease Diagnostic Center, National Chung Hsing University. All rats were housed under a 12:12 hr light-dark cycle (lights on at 8 AM, lights off at 8 PM), and at a constant temperature between 19 and 23 °C. During the entire study, all rats had ad libitum access to food and water, and received humane care in conformity with Institutional Animal Care and Use Committee (IACUC).

Experimental design: All the rats (n = 39) were divided into two groups: intact rats were taken as control group (n = 10), and ovariohysterectomized (OHE) rats as OHE group (n = 29). The OHE group was subdivided into three subgroups: 20 weeks after OHE group (n = 9), 24 weeks after OHE group (n = 10) and 30 weeks after OHE group (n = 10). Body weight (BW) and food intake of all groups were measured and recorded every week. Blood samples were collected before GnRH stimulation (0 min) and 5, 15, 30 min after GnRH stimulation. GH, ACTH, TSH, IGF-I concentrations were evaluated by commercial EIA or ELISA kits. At the end of the experiment, all the rats were sacrificed with humanism.

Ovariohysterectomy: All rats in the experimental groups underwent surgical removal of the ovaries and uteruses when the rats were 30 weeks old. During operation, all rats were anesthetized with a cocktail of zolazepam/tiletamine (Zoletil 50®, 50 mg/ml, Virbac, Carros cedex, France), xylazine (Rompun®, 23.32 mg/ml, Bayer, Leverkusen, Germany) and atropine sulfate (1 mg/ml, TAIYU, HsinChu, Taiwan) by the dosage of 0.1 ml/200 g.

GnRH stimulation test and blood sample collection: The rats were anesthetized with the same method as hereinbefore. An arterial catheter was filled with sterilized saline, and arterial catheter placement was performed with the left cervical artery. Blood sample was collected into a tube containing EDTA and an Eppendorf tube as 0 min (basal level) samples. Then, 50 ng/kg GnRH (CYSTORELIN®, Merial, Duluth, U.S.A.) was injected into the arterial catheter (Cooper et al., 2000). Blood samples were drained into the EDTA-coated tubes and Eppendorf tubes 5, 15, and 30 min after GnRH administration as 5, 15, and 30 min samples. After each blood sample collection, the arterial catheter was refilled with sterilized saline to avoid blood coagulation and some of the blood was discarded before the next blood collection.

Variant body weight ratio % (VarBW%) determination: In order to present the degree of body weight variation, variant ratio of body weight per week was calculated as VarBW% based on individual average body weight before OHE on the 27th, 28th, and 29th weeks.

Variant food intake ratio % (VarFD%) determination: In the same way, variant ratio of food intake per week was calculated as VarFD% based on individual average food intake before OHE on the 28th and 29th weeks.

Measurement of GH, IGF-I, ACTH, and TSH levels: The blood samples in tubes containing EDTA and Eppendorf tubes were centrifuged at 2500 rpm for 10 min. Plasma and serum samples were collected and kept at -70 °C until assay. GH concentrations were determined by commercial rat EIA kits (SPI bio, France). IGF-I concentrations were measured with commercial ELISA kits (ALPCO Diagnostics, U.S.A.). ACTH concentrations were measured with commercial human/ mouse/ rat ELISA kits (MD Bioproducts, Switzerland). TSH concentrations were measured with commercial rat ELISA kits (ALPCO Diagnostics, NIH, U.S.A.).

Statistical analysis: All data in the experiment were expressed as mean ± S.E.M. Statistical analysis was performed using SAS 9.2 software (SAS, U.S.A.). Differences in all results between the four groups were assessed by Mann-Whitney U test. Differences in all results within group were assessed by Wilcoxon test. Significance was set at P-value < 0.05 for all tests. Correlation coefficient (r) was calculated for body weight, food intake, plasma GH, plasma ACTH, serum TSH, and serum IGF-I concentrations before and after GnRH stimulation tested by Spearman correlation.
Results

The body weights of all rats increased gradually during this experiment. During the week ovariohysterectomy was performed, the body weights of the ovariohysterectomized rats were significantly lower than the body weights of the intact rats. Later on, the body weights of the ovariohysterectomized rats were significantly higher than the body weights of the intact rats starting on the 35th week until the end of experiment (Fig 1A). The VarBW% of all rats had a tendency to the body weights. During the week of ovariohysterectomy surgery, the VarBW% of the ovariohysterectomized rats was significantly lower than the VarBW% of the intact rats. After that, the VarBW% of the ovariohysterectomized rats was significantly higher than the VarBW% of the intact rats starting on the 32nd week until the end of experiment (Fig 1B). The food intakes of the ovariohysterectomized rats were slightly lower than the food intakes of the control group, but were not significantly different. After surgery, the food intakes of the ovariohysterectomized rats were significantly higher than the food intakes of the control group during the 32nd to 37th week. In the terminal period, the food intakes of both control and OHE groups were trending concurrent (Fig 2A). The VarFD% of the ovariohysterectomized rats was significantly lower (p<0.05) than the VarFD% of the control rats. After surgery, the VarFD% of the ovariohysterectomized rats was significantly higher than the VarFD% of the control rats during the 32nd to 35th week. In the terminal period, the VarFD% of both control and OHE groups were trending concurrent (Fig 2B). The GH levels at 0, 5, 15 and 30 min of all groups are shown in Figure 3. The basal GH level of the control group was higher than that of all OHE groups without significant difference. After GnRH stimulation, it was shown that the GH concentrations at 30 min of all groups were higher than those at 0 min without statistical significance (Fig 3). The concentrations of IGF-1 at 0, 5, 15 and 30 min of all groups are shown in Figure 4. The basal IGF-1 level of the 24 weeks after OHE group was significantly lower than the 20 weeks and 30 weeks after OHE ones. In each group, the IGF-1 levels at 30 min were significantly lower than at 0 min (Fig 4). The ACTH levels at 0, 5, 15 and 30 min of all groups are shown in Figure 5. The ACTH concentrations at 15 and 30 min were both significantly higher than those at 0 min (Fig 5). The concentrations of TSH at 0, 5, 15 and 30 min of all groups are shown in Figure 6. The TSH concentrations at 30 min of all groups were significantly higher than those at 0 min (Fig 6). At the end of experiment, the body weights showed significantly negative correlation with the GH concentrations at 0 min (r = -0.322, p < 0.05) (Fig 7); but significantly positive correlation with IGF-1 concentrations at 0 min (r = 0.346, p < 0.05) (Fig 8A) and significantly positive correlation with ACTH at 0 min (r = 0.372, p < 0.05) (Fig 8B), at 5 min (r = 0.398, p < 0.05), at 15 min (r = 0.429, P < 0.05), at 30 min (r = 0.391, p < 0.05). The IGF-I concentrations at 0 min showed significantly positive correlation with the TSH concentrations at 0 min (r = 0.468, p < 0.05).

Discussion

Sex hormones, especially estrogen, are the important regulators of energy intake and metabolism (Jeusette et al., 2005; Zoran, 2010). The three major naturally occurring estrogens in females are estrone (E1), estradiol (17β-estradiol, E2), and estriol (E3). 17β-estradiol (E2) is produced by the ovaries with the aromatization of androstenedione to estrone (E1), followed by the conversion to E2. E2 has the most estrogenic effect potency which is about 10 times as estrone and about 80 times as estriol (Astwood, 1938; Mauvais-Jarvis, 2011). Loss of sex hormones after neutering results in hormonal changes and metabolic rate reduction. In our study, although the food intakes and VarFD% of the OHE group were intensely increased for few weeks after the surgery, which gradually tended to the curve of the control group, at the end both were not different from those of the control group. The body weights and VarBW% of the ovariohysterectomized rats remained much higher than those of the intact rats throughout the study. A report showed that both male and female mice that lack the aromatase, which as a result could not

![Figure 1](image-url) (A) The change of body weights. (B) The change of variant body weight ratio % (VarBW%). All rats body weights and VarBW% were gradually increase in the experiment. *, p < 0.05.
synthesize E2, developed obesity without showing either hyperphagia or reduced energy expenditure, but rather exhibited reduced spontaneous physical activity and a decrease in lean body mass (Jones et al., 2000). It may elucidate our results that the body weight increase after neutering is more dependent on spontaneous physical activity rather than food intake or energy expenditure. Our observation that body weights negatively correlated with basal GH levels correspond with previous investigations which documented that obese subjects were characterized by markedly decreased GH production and secretion (Veldhuis et al., 1991; Argente et al., 1997), and obese subjects also had a blunted response to GH stimulus (Williams et al., 1984). In addition, the half-life, amplitude, and pulsatility of GH are decreased in obesity, and GH levels and body mass index (BMI) are within a negative correlation (Scacchi et al., 1999).

Our data showed that the basal IGF-I levels and body weights were within a positive correlation, which is in accord with the results of some studies (Hochberg et al., 1992; Nam et al., 1997). However, some authors reported decreased levels of IGF-I in obesity (Marin et al., 1993; Maccario et al., 1999), while others found normal levels (Rasmussen et al., 1994; Frystyk et al., 1999). Thus, the IGF-I concentrations in obese subjects still remained divergent. Estrogens may play an important role in the modulation of GH/IGF-I axis during adult life, according to observations that mean GH levels were higher in women than men (Weissberger et al., 1991; Ho et al., 1996). There is emerging evidence that estrogens modulate GH action independently of secretion through effects exerted on the liver. Oral exogenous estrogen administration

Figure 2  (A) The change of food intake. (B) The change of variant food ratio % (VarFd%). The food intake and VarFd% of OHE group rat were significantly increased few weeks after ovariohysterectomy surgery. * p < 0.05.

Figure 3  The concentrations of GH at different time before and after GnRH stimulation test. The GH levels of 30 min were higher than 0 min GH levels in control, 24 wks, and 30 wks groups, but there were no significant differences. The GH level of 5 min was significantly lower than 0 min GH level in 20 wks group. The comparison with basal level presented as percentage above the column.

Figure 4  The concentrations of IGF-I at different time before and after GnRH stimulation test. In each group, the IGF-I levels of 30 min were significantly lower than 0 min IGF-I levels. The comparison with basal level presented as percentage above the column. * p < 0.05.
other than transdermal administration inhibits hepatic IGF-I synthesis and increases GH secretion through reduced feedback (Weissberger et al., 1991; Leung et al., 2004). It may explain why the higher GH levels accompanied lower IGF-I levels in the intact female rats of this study.

Gonadally intact female rats are insensitive to the feedback effects of 50% corticosterone pellets on stress induced ACTH secretion and that ovariectomy increased the sensitivity to steroid feedback (Young, 1996). Similarly, women are less sensitive to dexamethasone feedback during the luteal phase, when estrogen and progesterone levels are high, than during the early follicular phase, when both estrogen and progesterone are very low (Altemus et al., 1997). There is evidence that both estrogen and progesterone may play a role in the relative resistance to glucocorticoid feedback in females (Rousseau et al., 1972; Ferrini et al., 1995). Our observation that the ACTH levels of ovariohysterectomized rats were much higher than those of the intact rats was in accord with these studies. However, several studies suggest that estradiol plays a role in enhanced stress responses in female rats, based on increased HPA axis responses to stress when ovariectomized rats are treated with estradiol (Viau and Meaney, 1991; Carey et al., 1995).

Figure 5  The concentrations of ACTH at different time before and after GnRH stimulation test. In all OHE groups, the ACTH levels of 15 min were significant higher than 0 min ACTH levels. And the ACTH levels of 30 min were significant higher than 0 min ACTH levels in each group. The comparison with basal level presented as percentage above the column. *, p < 0.05.

Figure 6  The concentrations of TSH at different time before and after GnRH stimulation test. The TSH level of 15 min was significantly higher than 0 min TSH level in control group. In all groups, the TSH levels of 30 min were significantly higher than 0 min TSH levels. The comparison with basal level presented as percentage above the column. *, p < 0.05.

The food intake in rats suffering from chronic stress may be related to the expression of pro-opiomelanocortin (POMC) mRNA and corticotropin-releasing hormone (CRH) function increment (Makino et al., 1999). The pituitary ACTH-producing cell hyperplasia may occur in massively obese patients (Shimizu et al., 2010). Previous data showed that normal mice bearing ACTH-secreting tumors developed obesity, hyperphagia, and hyperinsulinemia (Hausberger, 1961).

GnRH administration affects other pituitary hormones besides gonadotropin. The classical view of the adenohypophysis asserts that each cell secretes a single hormone (Amar and Weiss, 2003). However, evidence accumulated during the past two decades suggests that there are subpopulations of multihormonal cells that may be involved in more than one neuroendocrine system (Mignot and Skinner, 2005). A number of papers indicate that GH secretion is related to LH surge in different species as the previous literature review, which revealed that the gonadotropes showed co-expressed GH mRNA. Furthermore, a small proportion of gonadotrophs of mouse and sheep can produce immunoreactive GH (Nunez et al., 2003; Mignot and Skinner, 2005). However, there are studies which revealed that such co-expression in gonadotrophs was specific not only to GH but also to prolactin. TSH and ACTH were detected within gonadotrophs, as well as LH was colocalized in thyrotrophs, lactotrophs, and corticotrophs in mouse and rat (Childs et al., 1994; Nunez et al., 2003). According to those previous findings, in this study the GH, TSH, and ACTH levels were elevated in both intact and gonadectomized rats after the GnRH stimulation test accompanied LH surge was induced. The distribution of pituitary hormone-producing cells of the adenohypophysis was mentioned in a literature preview. The gonadotroph distribution is scattered throughout the anterior pituitary rather than ganglion in special block like other hormone-producing cells (Heaney and Melmed, 2004). The activated gonadotrophs after GnRH administration whether exerted the paracrinicity throughout the adenohypophysis or not still remained vague. Although the potential paracrinicity of hormone-producing cells in adenohypophysis is too complicated to entirely comprehend, there are some studies dedicated to figure out (Denef, 2008).
In this study, the most interesting question is why the GH levels increased after the GnRH stimulation test accompanied by reduced IGF-I levels? As previously mentioned, obesity leads to the disruption of GH-IGF-I axis such that GH is generally reduced, while part of the disruption can be linked to the increased insulin that often accompanies obesity (Hosick et al., 2012). Insulin increases the number of cell surface GH receptors, thereby, increasing hepatic tissue sensitivity to GH. The result of increased hepatic GH sensitivity is a decreased amount of GH needed to stimulate IGF-I release (Leung et al., 2000). On the other hand, glucocorticoids are known to reduce insulin sensitivity and potential candidate mechanisms include reduced insulin binding to its receptor (Rizza et al., 1982). Although the mechanism is unclear, some observations reinforce the potential value of specific inhibitors of 11β-HSD1 to enhance insulin sensitivity (Walker et al., 1995).

In conclusion, gonadectomy in female animal model could lead to obesity. In addition, the basal IGF-I and ACTH levels positively correlated with body weights, probably implying that obesity after neutralization is accomplished with the elevation of IGF-I and ACTH. Meanwhile, GnRH administration facilitated other pituitary hormones such as GH, ACTH, and TSH secretion in both intact and gonadectomized rats.

References


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Figure 7 The correlation between body weights and basal GH levels. The body weights showed significantly negative correlation with basal GH concentrations ($r = -0.322, p < 0.05$).

Figure 8 The correlation of body weights with basal IGF-I and ACTH levels. (A) The body weights showed significantly positive correlation with basal IGF-I concentrations ($r = -0.346, P < 0.05$), and (B) basal ACTH ($r = 0.372, p < 0.05$).
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