

ORIGINAL**Neuropeptide Y mediates orexin A-mediated suppression of pulsatile gonadotropin-releasing hormone secretion in ovariectomized rats**

Machiko Kiyokawa, Toshiya Matsuzaki, Takeshi Iwasa, Rie Ogata,
Masahiro Murakami, Riyo Kinouchi, Shinobu Yoshida, Akira Kuwahara,
Toshiyuki Yasui, and Minoru Irahara

Department of Obstetrics and Gynecology, Institute of Health Biosciences, the University of Tokushima Graduate School, Tokushima, Japan

Abstract : Objectives : Reproductive functions are influenced by various feeding regulators. Orexin, which is one of orexinergic peptides, suppresses the pulsatile secretion of luteinizing hormone (LH) in bilaterally ovariectomized (OVX) rats. However, the mechanism of this effect is still not clear. To investigate whether neuropeptide Y (NPY) is involved in the orexin A-mediated suppression of pulsatile LH secretion, we evaluated the effects of NPY antibody on the suppressive effect of orexin A. **Methods :** Orexin A was administered intracerebroventricularly (icv) and NPY antibody (NPY-Ab) was injected before icv administration of orexin A in OVX rats. Pulsatile LH secretion was analyzed by measuring serum LH concentrations in the next 2 h in blood samples drawn at 6-min intervals by radioimmunoassay. **Results :** Administration of orexin A significantly reduced the mean LH concentration and LH pulse frequency. Co-administration of NPY antibody with orexin A significantly restored the suppressive effect of orexin A on the mean LH concentration and LH pulse frequency. **Conclusion :** NPY mediated the suppressive effect of intracerebroventricularly injected orexin A on pulsatile LH secretion, suggesting that hypothalamic orexin suppressed pulsatile gonadotropin-releasing hormone (GnRH) secretion via NPY in the hypothalamus of female rats. *J. Med. Invest.* 58 : 11-18, February, 2011

Keywords : orexin A, luteinizing hormone, neuropeptide Y, gonadotropin-releasing hormone.

INTRODUCTION

Starvation and weight loss are among the main causes of female reproductive disorders. Currently, a lot of women wish to become slim that often results in amenorrhea. However, the neurological

relation between feeding behavior and reproductive habits has not yet been completely clarified.

Among the known feeding regulators, some appear to influence gonadotropin secretion. Starvation stimulates the expression of orexin, which is one of the orexinergic peptides, in the lateral hypothalamic area (LHA) or the feeding center (1). Orexin neurons project their fibers to the arcuate nucleus (ARC), which contains neuropeptide Y (NPY) neurons, and to the paraventricular nucleus (PVN), which contains corticotrophin-releasing hormone (CRH) neurons (2, 3). NPY appeared to mediate the

Received for publication July 12, 2010 ; accepted September 10, 2010.

Address correspondence and reprint requests to Toshiya Matsuzaki, M.D., Ph.D., Department of Obstetrics and Gynecology, Institute of Health Biosciences, the University of Tokushima Graduate School, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan and Fax : +81-88-631-2630.

orexinergic effect of orexin, because this effect was blocked after co-administration with NPY Y1 receptor antagonist in rats (4). However, we found that orexin suppressed the hypothalamic pulsatile secretion of gonadotropin-releasing hormone (GnRH) in ovariectomized (OVX) rats (5). We used OVX rats, because it may be affected by ovarian factors such as estradiol, progesterone, inhibin and activin.

Orexin might directly regulate GnRH secretion because orexin neurons project their fibers into the preoptic area and into the group of septic nucleus that contain the GnRH cell bodies in rodents. In addition, orexin neurons project into ARC and PVN and indirectly suppress GnRH secretion via several neural pathways involving NPY, CRH, and proopiomelanocortin (POMC) (6); further, orexin suppresses the pulsatile secretion of luteinizing hormone (LH) via β -endorphin and CRH as we have reported in our previous studies (7, 8). However, the mechanism of orexin-mediated suppression of GnRH secretion remains to be completely clarified. In this study, we investigated whether NPY plays a role in the orexin-mediated suppression of GnRH secretion in OVX rats.

MATERIALS AND METHODS

Animals

Eight-week-old female Wistar rats (weight, 160–200 g) were purchased from Charles River Inc., Yokohama, Japan). The rats were housed in a room with controlled lighting (light period, 0800–2030) and temperature (24°C), and they were allowed ad libitum access to standard laboratory pellets of rat chow and tap water. All animal experiments were conducted in accordance with the ethical standards of Animal Care and Use Committee of the University of Tokushima. All rats were bilaterally ovariectomized under intraperitoneal anesthesia with pentobarbital sodium (40 mg/kg body weight) and used for the experiments 2 weeks later.

Implantation of a brain cannula and atrial cannulation

At 2 weeks after ovariectomy, a brain cannula was implanted under intraperitoneal pentobarbital anesthesia (50 mg/kg body weight). A 23-gauge stainless-steel tube guide cannula (length, 20 mm; outer diameter, 0.64 mm; inner diameter, 0.39 mm) was implanted into the third ventricle (3V) using the stereotaxic coordinates according to the atlas of

Paxinos and Watson (9). A sterile 29-gauge stainless-steel obturator with a polyethylene cap (length, 20 mm; outer diameter, 0.33 mm; inner diameter, 0.17 mm) was inserted into the guide cannula to ensure that the cannula remained patent. One week after brain cannulation, the rats were anesthetized intraperitoneally using a mixture of ketamine and xylazine (20 : 5 mg/kg body weight, ip), and a silastic tube (outer diameter, 1.0 mm; inner diameter, 0.5 mm; Kaneka Medics, Tokyo, Japan) was inserted into the external jugular vein and sewn into the skin after confirming its positioning in the right atrium (10). The tube was rinsed with heparinized saline (10,000 U/l saline) and threaded to an exit at the back of the neck. On the next day, the intratrial cannula was rinsed and connected to a long polyethylene tube containing heparinized saline. A steel pin was inserted into the open end of this tube, and the tube was led outside the cage to permit rapid blood sampling without handling the rats.

Feeding experiment

On the day after atrial cannulation, 3 nmol of orexin A (Peptide Institute, Inc., Osaka, Japan) dissolved in 5 μ l of distilled water or 5 μ l saline as control (Group C) were administered intracerebroventricularly (icv) during the dark period; 5 μ l of NPY antiserum (Group ON, Group N) (NPY-Ab; Peptide Institute, Inc., Osaka, Japan) or normal rabbit serum (Group O) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were administered icv 10 min before the injection of orexin A using a Hamilton microsyringe. After their rats were fed freely in individual cage in the darkness, we calculated the amount of food consumption in the next 2 h. The reduction in food weight was measured using a digital scale (HL-200; Tokyo, Japan) at 0 and 2 h after injection of saline or orexin A. In the feeding experiment, the rats were divided into the following 4 groups: Group C (saline, n=9), Group O (normal rabbit serum+orexin A, n=8), Group ON (NPY-Ab+orexin A, n=8) and Group N (NPY-Ab+saline, n=4).

Reproductive experiment

Three nmol of orexin A dissolved in 5 μ l of distilled water or 5 μ l of saline (Group C) was administered into the 3V; 5 μ l of NPY-Ab (Group ON) or normal rabbit serum (Group O) were administered 10 min before the injection of orexin A. In the reproductive experiment, the rats were divided into the following 3 groups: Group C (saline, n=9), Group O (normal rabbit serum+orexin A, n=8), and

Group ON (NPY-Ab+orexin A, n=8).

Blood samples were collected through the intratrial cannula just before icv administration and then at 6-min intervals over the next 2 h. After each sampling, an equal volume of heparinized saline was injected. Blood samples were centrifuged and the obtained plasma samples were stored at -40°C until the measurement of LH concentrations by radioimmunoassay (RIA).

RIA for the measurement of LH

Serum LH concentration was measured using a double-antibody rat RIA kit for LH (RPA552 ; GE Healthcare UK, Bucks, UK). The minimum detectable concentration was assayed at 0.09 ng/tube, and the intra- and interassay coefficients of variation (CVs) were 6.5% and 6.6%, respectively.

Statistical analysis

LH pulses were defined and identified using the established criteria, as described by Gallo (11) and DePaolo *et al.* (12). Further, CV was calculated using the LH concentrations in the ascending and descending phases of a suspected pulse. A pulse was assumed to have occurred if the CV was greater than twice the CV of the LH standard solution, which corresponded to the mean LH levels in the suspected pulse. The pulse amplitude indicated the difference between the peak and baseline values of LH concentrations. The mean LH concentration, pulse frequency (number of pulses during the 2-h period), and mean pulse amplitude were calculated for each animal. This method has been shown to reveal differences in the parameters of pulsatile hormone release of the groups, and these differences are similar to those obtained by cluster algorithm (13). The data were analyzed using one-way analysis of variance and subsequently by Fisher's protected least significant difference test. All statistical analyses were performed using StatView for Macintosh version 5.0 (SAS Institute, Cary, NC, USA). The differences were considered statistically

significant at the level of $p < 0.05$. All results are presented as mean \pm standard error of the mean, and determined using 8 or 9 samples for each group as indicated.

RESULTS

The profiles of rats in each group just before the icv administration are shown in Table 1. Those groups did not show significant differences in body weight or basal LH levels.

The results of statistical analysis for food intakes after icv administration are shown in Figure 1. The 2-h food intake in Group O after icv administration during the light period was significantly larger than that in Group C ($p < 0.05$). Food intake in Group ON was significantly lesser than that in Group O ($p < 0.05$). NPY-Ab did not affect food intake, but the orexin A-mediated stimulation of food intake was not observed after pretreatment with NPY-Ab.

The changes in serum LH concentration in three groups are shown in Figure 2 and Table 2. Group O

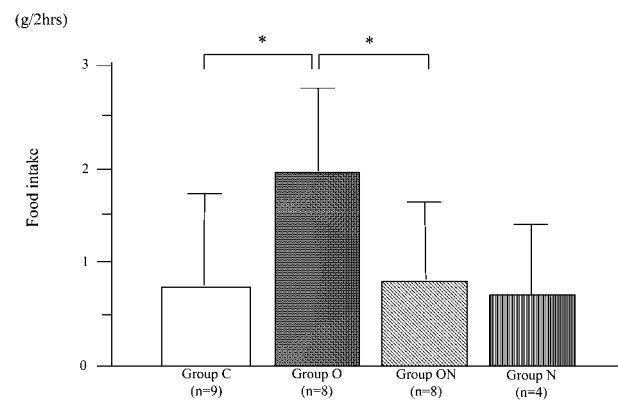


Figure 1 : Two-hour food intake after the intracerebroventricular (icv) administration in ovariectomized rats. The values are expressed as the mean \pm standard error of mean (SEM). Group C (saline : control), Group O (NRS+orexin A), Group ON (NPY antibody+orexin A), Group N (NPY antibody+saline). * : $p < 0.05$ compared with Group O. (one-way analysis of variance followed by Fisher's protected least significant difference test).

Table 1 Baseline Data of Rats

Group	Number of rats	Body weight (g)	Basal LH level ($\mu\text{g/l}$)*
Group C (saline)	9	286 \pm 4.61	8.46 \pm 2.61
Group O (NRS+orexin A)	8	277 \pm 4.96	8.24 \pm 2.40
Group O (NRS+orexin A)	8	277 \pm 4.96	8.24 \pm 2.40
Group N (NPY Ab+saline)	4	284 \pm 5.27	-

Note. Values are expressed as means \pm SEM.

* Basal LH level denotes the serum LH concentration just before icv administration of orexin A or saline.

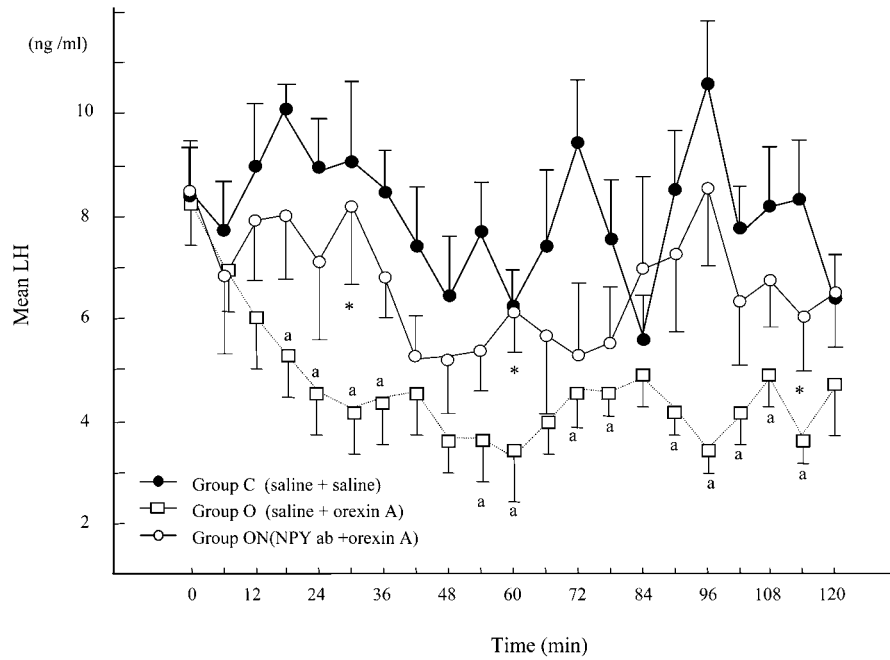


Figure 2 : Changes in the serum concentrations of LH in each group for 120 min after injection. Values are expressed as the mean \pm standard error of mean (SEM). a : $p < 0.05$ vs Group C, * : $p < 0.05$ vs Group O.

Table 2 The mean LH levels of 4 period after the icv administration

Group	LH level (ng/ml)			
	6-30 min.	36-60 min.	66-90 min.	96-120 min.
Group C	8.94 \pm 0.83	7.30 \pm 0.74	7.75 \pm 0.87	8.24 \pm 0.61
Group O	5.50 \pm 0.80*	4.47 \pm 1.00**	6.01 \pm 0.73	5.62 \pm 0.81**
Group ON	8.60 \pm 0.89 ^a	6.13 \pm 0.70	6.29 \pm 0.99	6.70 \pm 0.77

Note. Values are expressed as means \pm SEM.

* : $p < 0.01$ compared with group C, ** : $p < 0.05$ compared with group C. a : $p < 0.05$ compared with group O.

showed significantly lower serum LH levels than Group C at several time points after orexin A administration ; however, there was no significant difference between Group ON, in which NPY-Ab was co-administered with orexin A, and Group C.

The representative LH secretion profiles of each group are shown in Figure 3. In the Group C rat, the pulsatile secretion of LH was frequently observed for 2 h. In the Group O rat, which was injected with only orexin A, plasma LH level decreased rapidly after icv administration and the frequency of pulsatile LH secretion appeared to be

lower as compared to that of the Group C rat. In the Group ON rat, which was co-administered NPY-Ab and orexin A, pulsatile LH secretion appeared frequently, similar to that observed in the Group C rat.

The results of statistical analysis of pulsatile LH secretion are shown in Figure 4. Administration of orexin A (Group O) significantly reduced the mean LH concentration and the pulse frequency (Group O vs Group C, $p < 0.01$, respectively). Co-administration of NPY-Ab with orexin A (Group ON) significantly restored the suppression of mean LH concentration and pulse frequency in Group O (Group ON

vs Group O, $p < 0.05$, respectively) ; however, the pulse frequency were still significantly lower than in Group C ($p < 0.05$, respectively). No significant

differences were observed in the pulse amplitude of the 3 groups.

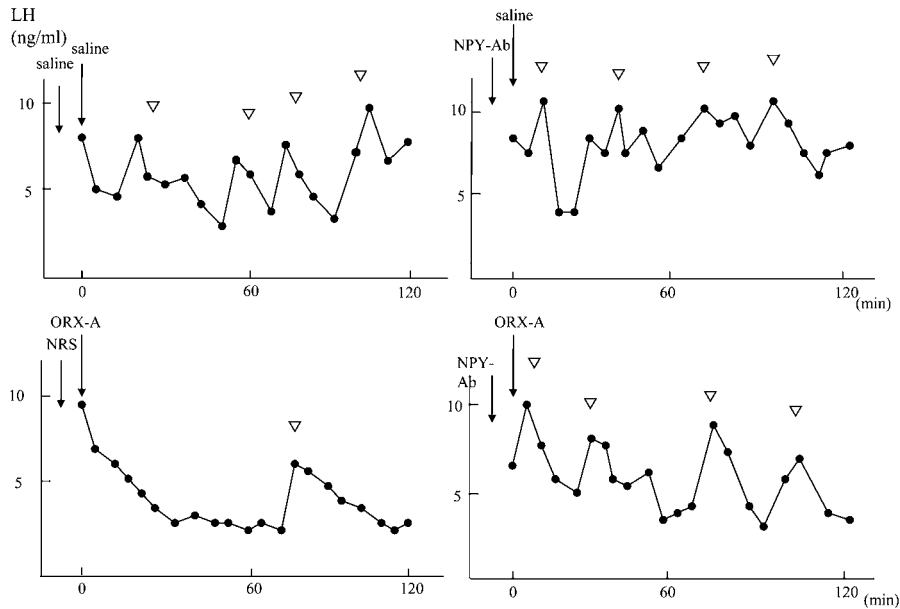


Figure 3 : Effect of the icv administration of NPY antibody before orexin A on the pulsatile LH secretion in ovariectomized rats. The representative profiles of 2 rats treated with orexin A or saline at 0 min and preinjection of NPY antibody or NRS at -10 min. Open reverse triangle (▽) denotes a pulse of LH secretion.

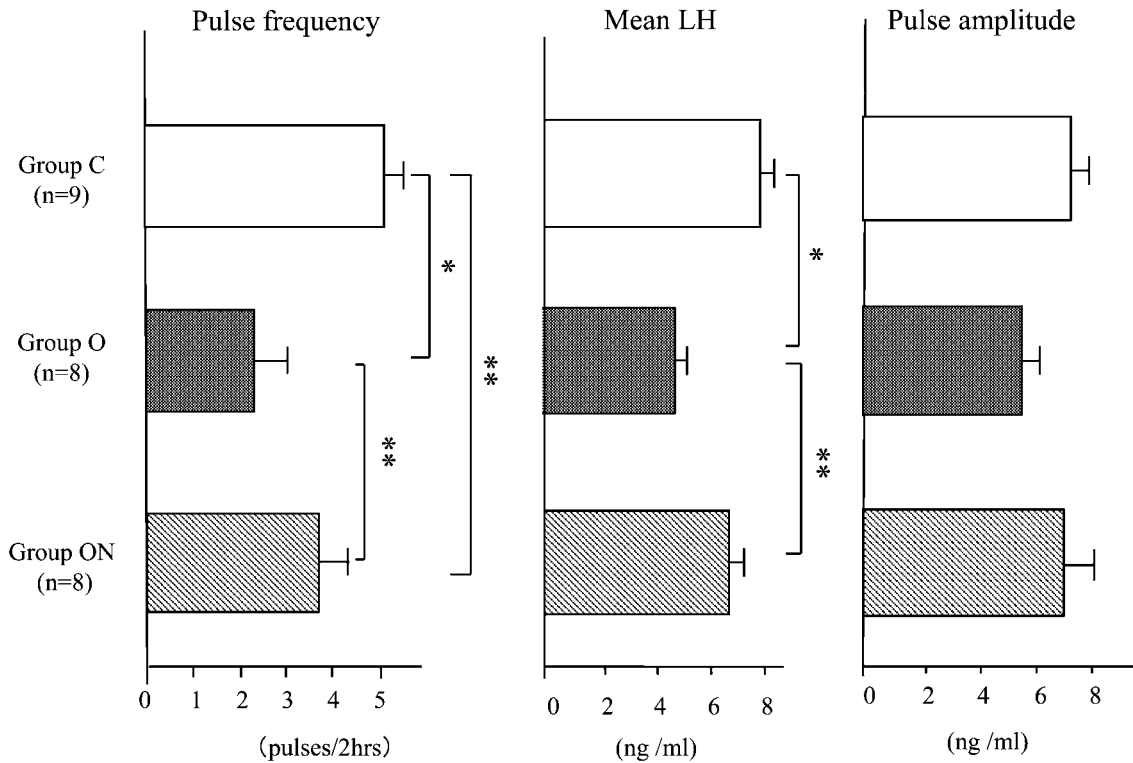


Figure 4 : Effect of the icv administration of NPY antibody before orexin A on the pulsatile LH secretion in ovariectomized rats. Values are expressed as the mean \pm standard error of mean (SEM). *: $p < 0.01$, **: $p < 0.05$ (one way analysis of variance followed by Fisher's protected lest significance difference test.)

DISCUSSION

The reproductive function is restrained if energy storage is insufficient for the maintenance of individual life and the conservation of species. In fasting condition, female animals show restrained estrous cycle and sexual behavior (14, 15). Therefore, the neurons that regulate appetite and nutritional state may influence GnRH secretion and control reproductive function; NPY would be the key peptide that serves as a link between orexinergic information from orexin and leptin and the signals (originating from the medulla oblongata) from the vagus nerve (16). Orexins, also known as hypocretins, were discovered and named based on their orexinergic effect. Thereafter, orexins have been reported to exert several effects, such as the stimulation of activities, control the sleep-wake cycle, influence the immune, autonomic, and neuroendocrine systems (17) and the suppression of gonadotropin secretion. With regard to the regulation of feeding activities, fasting induces the expression of prepro-orexin mRNA, which is a precursor of orexin in rat hypothalamus (3), and orexin increases food intake through NPY expression in the hypothalamic ARC (18). However, the mechanisms of the other functions of orexin, including the suppression of gonadotropin secretion, have not been completely clarified.

In the present study, the icv injection of orexin A stimulated food intake and suppressed gonadotropin secretion in OVX rats, which is in agreement with the results of previously published reports (5, 7). Pretreatment with NPY antibody completely neutralized the orexinergic effect of orexin A, which was consistent with the previously reported findings (19), indicating that the orexinergic effect of orexin A is mediated by NPY. Furthermore, pretreatment with NPY-Ab partially neutralized the suppressive effect of orexin A on LH secretion in OVX rats, indicating that this effect is also mediated by NPY, and recovery was observed in not only the serum LH level but also the frequency of pulsatile LH secretion. Because LH pulses are generated by GnRH pulses, the recovery of pulsatile LH frequency indicated that pulsatile GnRH release was restored by NPY-Ab. Therefore, the suppressive effect of orexin A on GnRH secretion appeared to be mediated by NPY in hypothalamus.

NPY plays an important role in energy homeostasis and regulation of food intake; the cell body of this neuron is located mainly in the ARC. The

fibers of the NPY neuron of ARC are projected into both the preoptic area (POA), which contains the GnRH neuron cell body, and into the medial basal hypothalamus (MBH) that regulates the release of GnRH, which contains GnRH neuron fibers [20]. NPY Y1 receptor is present in the GnRH nerve fibers in rats (20) and in GT-1 cells, which are the stocking cells of GnRH (21). In ovarian steroid-primed OVX rats, icv administration of NPY Y1 receptor antagonist suppressed the release of LH (22), which suggested that NPY may influence GnRH via the NPY Y1 receptor.

Action of NPY on the gonadotropin secretion differs depending on the presence or absence of estrogen. *In vivo* experiments suggested that NPY promoted LH secretion, in the case of pretreatment with estradiol, although NPY icv administration suppressed the pulsatile secretion of LH in OVX rats (23). The action of orexin on gonadotropin secretion is similar to that of NPY. Pulsatile LH secretion and serum LH levels are suppressed by icv injection of orexin in OVX rats (5, 24). However, serum LH level is elevated by icv injection of orexin in OVX rats supplemented with estradiol (23). GnRH release from the brain cells of adult female rats during pro-estrus are promoted by orexin (25). In this way, both NPY and orexin suppress the pulsatile secretion of GnRH and the basal secretion of gonadotropin and stimulate GnRH surge just before ovulation. This similarity in the effects of NPY and orexin suggests that the pathway of the action of orexin on GnRH is similar to that of NPY. NPY knockout (KO) mice do not show accelerated sexual development. However, unlike wild type mice, these mice do not show abnormal feeding behavior and body weight and become hyperphagic after food deprivation (26). Interestingly, although the activation of Y1 and Y5 receptors is involved in NPY-induced hyperphagia, the Y1-KO and Y5-KO mice develop late-onset obesity with increase in food intake and adiposity (27), whereas NPY-KO mice do not show any change in the feeding behavior or body weight. The compensation against the orexinergic NPY system, which would exist in these KO mice, has not been elucidated thus far.

Two mechanisms have been proposed for the suppression of reproductive function in the fasted state. One is the activation of the network of feeding regulators in the hypothalamus, which suppress the pulsatile secretion of GnRH. The other is the attenuation of the stimulatory effect of leptin on kisspeptin, which is a potent stimulator of GnRH secretion (28).

In the case of the former mechanism, NPY would be an important factor involved in the suppression of GnRH release by serving as a link in the activated network of the feeding regulators, including orexin. Further, we have clarified the pathway by which orexin A suppresses LH secretion through β -END (7), CRH, and urocortin 2 (8). By combining this result with the results of the present study, it can be suggested that orexin suppresses GnRH secretion in the hypothalamus via several pathways involving β -END, CRH, urocortin 2, and NPY.

In conclusion, NPY partially mediates the suppressive effect of orexin on pulsatile LH secretion and influences the orexinergic effect of orexin in OVX rats. The mechanism of this effect consists of a network of orexinergic neuropeptides that when activated would suppress reproduction in energy-deficient conditions

REFERENCES

1. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M : Orexins and orexin receptors : A family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92 : 573-585, 1998
2. Date Y, Ueta Y, Yamashita H, Yamaguchi H, Matsukura S, Kangawa K, Sakurai T, Yanagisawa M, Nakazato M : Orexins, orexinergic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. *Proc Natl Acad Sci USA* 96 : 748-753, 1999
3. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M : Orexins and orexin receptors : A family of orexin receptors : Implication in feeding behavior. *Regul Pept* 85 : 25-30, 1998
4. Russell SH, Small CJ, Dakin CL, Abbott CR, Morgan DG, Ghatei MA, Bloom SR : The central effects of orexin-A in the hypothalamic-pituitary-adrenal axis in vivo and in vitro in male rats. *J Neuroendocrinol* 13 : 561-566, 2001
5. Tamura T, Irahara M, Tezuka M, Kiyokawa M, Aono T : Orexins, orexinergic hypothalamic neuropeptides, suppress the pulsatile secretion of luteinizing hormone in ovariectomized female rats. *Biochem Biophys Res Commun* 264 : 759-762, 1999
6. Horvath TL, Diano S, van den Pol AN : Synaptic interaction between hypocretin (orexin) and neuropeptide Y cells in the rodent and primate hypothalamus : A novel circuit implicated in metabolic and endocrine regulations. *J Neurosci* 19 : 1072-1087, 1999
7. Irahara M, Tamura T, Matsuzaki T, Saito S, Yasui T, Yamano S, Kamada M, Aono T : Orexin-A suppresses the pulsatile secretion of luteinizing hormone via beta-endorphin. *Biochem Biophys Res Commun* 281 : 232-236, 2001
8. Iwasa T, Matsuzaki T, Kiyokawa M, Shimizu F, Minakuchi M, Kuwahara A, Maegawa M, Yasui T, Irahara M : The type 2 corticotrophin-releasing hormone receptor mediates orexin A-induced luteinising hormone suppression in ovariectomised rats. *J Neuroendocrinol* 19 : 732-738, 2007
9. Paxinos G, Watson C : The rat brain in stereotaxic coordinates. Academic Press, Australia, 1982
10. Harms PG, Ojeda SR : A rapid and simple procedure for chronic cannulation of the rat jugular vein. *J Appl Physiol* 36 : 391-392, 1974
11. Gallo RV : Pulsatile LH release during ovulatory LH surge on proestrus in the rat. *Biology of Reproduction* 24 : 100-104, 1981
12. De Paolo LV, King RA, Carrillo AJ : In vivo and in vitro examination of an autoregulatory mechanism for luteinizing hormone-releasing hormone. *Endocrinology* 120 : 272-279, 1987
13. Culler MD : Role of Leydig cells and endogenous inhibin in regulating pulsatile gonadotropin secretion in the adult male rat. *Endocrinology* 127 : 2540-2550, 1990
14. Tropp J, Markus EJ : Effects of mild food deprivation on the estrous cycle of rats. *Physiol Behav* 73 : 553-559, 2001
15. Temple JL, Millar RP, Rissman EF : An evolutionarily conserved form of gonadotropin-releasing hormone coordinates energy and reproductive behavior. *Endocrinol* 144 : 13-19, 2003
16. Schwartz MW, Woods SC, Porte D Jr, Seeley

- RJ, Baskin DG : Central nervous system control of food intake. *Nature* 404 : 661-671, 2000
17. Sakurai T : The neural circuit of orexin (hypocretin) : maintaining sleep and wakefulness. *Nat Rev Neurosci* 8 : 171-181, 2007
 18. Yamanaka A, Kunii K, Nambu T, Tsujino N, Sakai A, Matsuzaki I, Miwa Y, Goto K, Sakurai T : Orexin-induced food intake involves neuropeptide Y pathway. *Brain Research* 859 : 404-409, 2000
 19. Jászberényi M, Bujdosó E, Telegdy G : The role of neuropeptide Y in orexin-induced hypothalamic-pituitary-adrenal activation. *J Neuroendocrinol* 13 : 438-441, 2001
 20. Li C, Chen P, Smith MS : Morphological Evidence for Direct Interaction between Arcuate Nucleus Neuropeptide Y (NPY) Neurons and Gonadotropin-Releasing Hormone Neurons and the Possible Involvement of NPY Y1 Receptors. *Endocrinology* 140 : 5382-5390, 1999
 21. Besecke LM, Wolfe AM, Pierce ME, Takahashi JS, Levine JE : Neuropeptide Y stimulates luteinizing hormone-releasing hormone release from superfused hypothalamic GT1-7 cells. *Endocrinol* 135 : 1621-1627, 1994
 22. Jain MR, Pu S, Kalra PS, Kalra SP : Evidence that Stimulation of Two Modalities of Pituitary Luteinizing Hormone Release in Ovarian Steroid-Primed Ovariectomized Rats May Involve Neuropeptide Y Y1 and Y4 Receptors. *Endocrinol* 140 : 5171-5177, 1999
 23. McDonald JK, Lumpkin MD, DePaolo LV : Neuropeptide-Y suppresses pulsatile secretion of luteinizing hormone in ovariectomized rats : possible site of action. *Endocrinol* 125 : 186-191, 1989
 24. Pu S, Jain MR, Kalra PS, Kalra SP : Orexins, a novel family of hypothalamic neuropeptides, modulate pituitary luteinizing hormone secretion in an ovarian steroid-dependent manner. *Regul Pept* 78 : 133-136, 1998
 25. Russel SH, Small CJ, Kennedy AR, Stanley SA, Seth A, Murphy KG, Taheri S, Ghatei MA, Bloom SR : Orexin A interaction in the hypothalamo-pituitary-gonadal axis. *Endocrinol* 142 : 5294-5302, 2001
 26. Erickson JC, Clegg KE, Palmiter RD : Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature* 381 : 415-421, 1996
 27. Raposinho PD, Pedrazzini T, White RB, Palmiter RD, Aubert ML : Chronic neuropeptide Y infusion into the lateral ventricle induces sustained feeding and obesity in mice lacking either Npy1r or Npy5r expression. *Endocrinology* 145 : 304-310, 2004
 28. Smith JT, Acohido BV, Clifton DK, Steiner RA : KiSS-1 neurons are direct targets for leptin in the ob/ob mouse. *J Neuroendocrinol* 18 : 298-303, 2006