Estrogen and Leptin Have Differential Effects on FSH and LH Release in Female Rats[2] (44441)

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Abstract. **Prior experiments have shown that the adipocyte hormone leptin can advance puberty in mice. We hypothesized that it would also stimulate gonadotrophin secretion in adults. Since the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) is drastically affected by estrogen, we hypothesized that leptin might have different actions dependent on the dose of estrogen. Consequently in these experiments, we tested the effect of injection of leptin into the third cerebral ventricle of ovariectomized animals injected with either the oil diluent, 10 μg or 50 μg of estradiol benzoate 72 hr prior to the experiment. The animals were ovariectomized 3–4 weeks prior to implantation of a cannula into the third ventricle 1 week before the experiments. The day after implantation of an external jugular catheter, blood samples (0.3 ml) were collected just before and every 10 min for 2 hr after 3V injection of 5 μl of diluent or 10 μg of leptin. Both doses of estradiol benzoate equally decreased plasma LH concentrations and pulse amplitude, but there was a graded decrease in pulse frequency. In contrast, only the 50-μg dose of estradiol benzoate significantly decreased mean plasma FSH concentrations without significantly changing other parameters of FSH release. The number of LH pulses alone and pulses of both hormones together decreased as the dose of estrogen was increased, whereas the number of pulses of FSH alone significantly increased with the higher dose of estradiol benzoate, demonstrating differential control of LH and FSH secretion by estrogen, consistent with alterations in release of luteinizing hormone releasing hormone (LHRH) and the putative FSH-releasing factor (FSHRF), respectively.**

The effects of intraventricularly injected leptin were drastically altered by increasing doses of estradiol benzoate. There was no significant effect of intraventricular injection of leptin (10 μg) on the various parameters of either FSH or LH secretion in ovariectomized, oil-injected rats, whereas in those injected with 10 μg of estradiol benzoate there was an increase in the first hr in mean plasma concentration, area under the curve, pulse amplitude, and maximum increase of LH above the starting value (Δ_{max}) on comparison with the results in the diluent-injected animals in which there was no alteration of these parameters during the 2 hr following injection. The pattern of FSH release was opposite to that of LH and had a different time-course. In the diluent-injected animals, probably because of the stress of injection and frequent blood sampling, there was an initial significant decline in plasma FSH at 20 min after injection, followed by a progressive increase with a significant elevation above the control values at 110 and 120 min. In the leptin-injected animals, mean plasma FSH was nearly constant during the entire experiment, coupled with a significant decrease below values in diluent-injected rats, beginning at 30 min after injection and progres-

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sing to a maximal difference at 120 min. Area under the curve, pulse amplitude, and Δ_{max} of FSH was also decreased in the second hour compared to values in diluent-injected rats. In contrast to the stimulatory effects of intraventricular injection of leptin on pulsatile LH release manifest during the first hour after injection, there was a diametrically opposite, delayed significant decrease in pulsatile FSH release. This differential effect of leptin on FSH and LH release was consistent with differential effects of leptin on LHRH and FSHRF release. Finally, the higher dose of E_2 (50 μg) suppressed release of both FSH and LH, but there was little effect of leptin under these conditions, the only effect being a slight ($P < 0.04$) increase in pulse amplitude of LH in this group of rats. The results indicate that the central effects of leptin on gonadotropin release are strongly dependent on plasma estradiol levels. These effects are consistent with differential effects of estrogen on the release of LHRH and the putative FSHRF. [P.S.E.B.M. 1999, Vol 222]

The adipocyte hormone, leptin, also called ob protein, is a 167-amino acid product of the obese (*ob*) gene. This gene and its human homolog have been cloned and sequenced (1, 2). Peripheral administration of recombinant leptin to leptin-deficient *ob/ob* mice reduced their body weight and food intake, increased energy expenditure, and normalized blood glucose and insulin levels (3–6).

Leptin plays a role in reproductive functions, as well as in central metabolic regulation. Mice homozygous for mutation in the *ob* gene and therefore leptin-deficient (1) are infertile (7), and have decreased blood levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and gonadal steroids (7). Reproductive failure in female *ob/ob* mice is reversible by injecting exogenous gonadotropins and progesterone (7, 8), demonstrating their deficiency of reproductive hormones. Furthermore, a relationship between a critical amount of body fat and the onset of puberty has been demonstrated (9, 10). Chronic treatment of *ob/ob* female mice with exogenous leptin restored their fertility (11), increased plasma gonadotropin levels, and had a trophic effect on the reproductive organs (12). Moreover, the ability of leptin to accelerate the onset of puberty in normal female mice has been demonstrated (13).

Earlier *in vitro* and *in vivo* studies from our laboratory demonstrated an acute stimulatory effect of leptin on the hypothalamic-pituitary unit in adult rats (14). Leptin produced a dose-related increase in LH and FSH release from incubated hemi-anterior pituitaries and LH-releasing hormone (LHRH) secretion from median eminence–arcuate explants of male rats. Microinjection of leptin into the third cerebral ventricle significantly increased plasma LH concentrations during the first hr after its injection in ovariectomized, estrogen-primed rats. We hypothesized that the action of leptin to modify gonadotropin release may be estrogen-dependent; therefore, we investigated its role in the central effects of leptin on gonadotropin secretion. The results indicate that plasma FSH and LH are altered in response to central injection of leptin in an estrogen-dependent manner.

Materials and Methods

Animals. Adult female rats (initial weight 160–180 g) of the Holtzman strain (Harlan, Sprague-Dawley, Inc., USA) were used throughout the experiments. Upon arrival, the animals were allowed to acclimatize for 1 week and were housed two per cage in a room with controlled temperature (23°C–25°C) and lighting (lights on from 0700 and 1900 hr). A standard pellet diet and water were available *ad libitum.*

Experimental Procedure. In our experiments, we used a model that we have employed to evaluate the effects of peptides on gonadotropin release, the ovariectomized estrogen-primed rat (14). Ovariectomized rats are used because removal of negative feedback by ovarian steroids leads to increased concentrations of gonadotropins, readily measurable by radioimmunoassay (RIA) and eliminates estrous cycle–induced changes in gonadotropin concentrations. Ovariectomy was performed using isoflurane anesthesia (Ohmeda Caribe Inc., Bend, OR) 3–4 weeks prior to each experiment. Six to eight days before the experiment, a 23-gauge stainless steel guide cannula was implanted into the 3V using the technique of Antunes-Rodrigues and McCann (15) employing ketamine/acepromazine/xylazine anesthesia (90 ± 2 ± 6 mg/kg i.p., respectively). Three days (72 hr) before the experiments, the animals were injected subcutaneously with 0.1 ml of sesame oil (ovariectomized; $n = 8$), 10 μg of estradiol benzoate (ovariectomized 10 estradiol benzoate; $n = 6$), or 50 μg of estradiol benzoate (ovariectomized 50 estradiol benzoate; $n = 8$) in 0.1 ml of sesame oil.

One day before blood sampling, a Silastic catheter was introduced into the right external jugular vein, advanced to the right atrium according to the technique of Harms and Ojeda (16) using the same anesthetic as for the 3V cannula implantation. This catheter allowed for the removal of blood samples before and after microinjection of leptin.

Blood Sampling. The animals were left overnight in the experimental room for acclimatization. One hour before the experiment, polyethylene tubing filled with 0.9% NaCl containing 50 IU/ml of heparin was connected to the jugular catheter. Immediately after collection of the initial 0.3 ml blood sample (time 0), 10 μg of recombinant murine leptin from (Novartis, Basel, Switzerland; a gift to Dr. David York, Pennington Biomedical Research Center) dissolved in 5 μl of Krebs-Ringer bicarbonate buffer (KRB), or 5 μl of KRB alone was microinjected into the third ventricle

over a 30-sec period, and blood samples (0.3 ml) were collected every 10 min subsequently for 2 hr. Each time the volume of withdrawn blood was replaced by an equal volume of 0.15 *M* NaCl. Blood was centrifuged (2,500*g*), and plasma was stored at −20°C until RIA.

Hormone Assays. Plasma LH and FSH concentrations were determined using RIA kits supplied by the National Institute of Digestive Diabetes and Kidney Diseases (NIDDK) and expressed in terms of the NIDDKS RP-3 and RP-2 reference preparation, respectively.

Statistical Analysis. Mean hormone concentrations, pulse amplitude and frequency were identified by the PC-Pulsar program (18) version 3.0 (Gitzen JF, Ramirez VD, Neuroscience Program and Dept. of Physiology and Biophysics, University of Illinois, Urbana, IL) using default G (1–5) parameters (17) in each animal. The values of mean hormone concentrations, pulse amplitude, and the maximal plasma hormone increase (Δ_{max}) are expressed in nanograms of hormone per milliliter of plasma. The Δ_{max} is determined by taking the highest value during the experiment and subtracting from it the initial value for each rat. The area under the curve for LH and FSH was calculated by the trapezoid rule using a computer. All presented values are mean ± SEM. Multivariate analysis (MANOVA) was used to test for baseline homogeneity. The statistical differences of mean concentrations, pulse amplitude, and pulse frequency for LH and FSH between the three control groups were determined using PROC GLM, SAS program, version 6.2. All calculated parameters of LH and FSH release were analyzed by one-way analysis of variance, and statistical differences between two means were calculated by Student's *t* test.

Results

Effect of Estrogen on Pulsatile LH and FSH Release in Ovariectomized Rats. Both doses of estradiol benzoate (10 and 50 μg estradiol benzoate) injected s.c. 72 hr before the experiment profoundly depressed mean plasma LH concentrations ($P < 0.001$), and pulse amplitude ($P < 0.001$) approximately equally below the respective values in the ovariectomized control rats (Fig. 1). LH pulse frequency was progressively diminished as the dose of estradiol benzoate was increased (Fig. 1). The same doses of estradiol benzoate affected pulsatile FSH release differently (Fig. 2). The 10-μg dose of estradiol benzoate had no effect on the magnitude and pattern of FSH release, whereas the 50-μg dose of estradiol benzoate decreased mean plasma FSH concentrations ($P < 0.05$), but did not significantly change the other parameters of FSH release. FSH pulse amplitude was slightly, but not significantly, decreased, and frequency was not significantly increased. Since there were only 1.5 pulses over the 120-min period, and mean plasma FSH was significantly decreased, FSH release must have been reduced during the interpulse intervals.

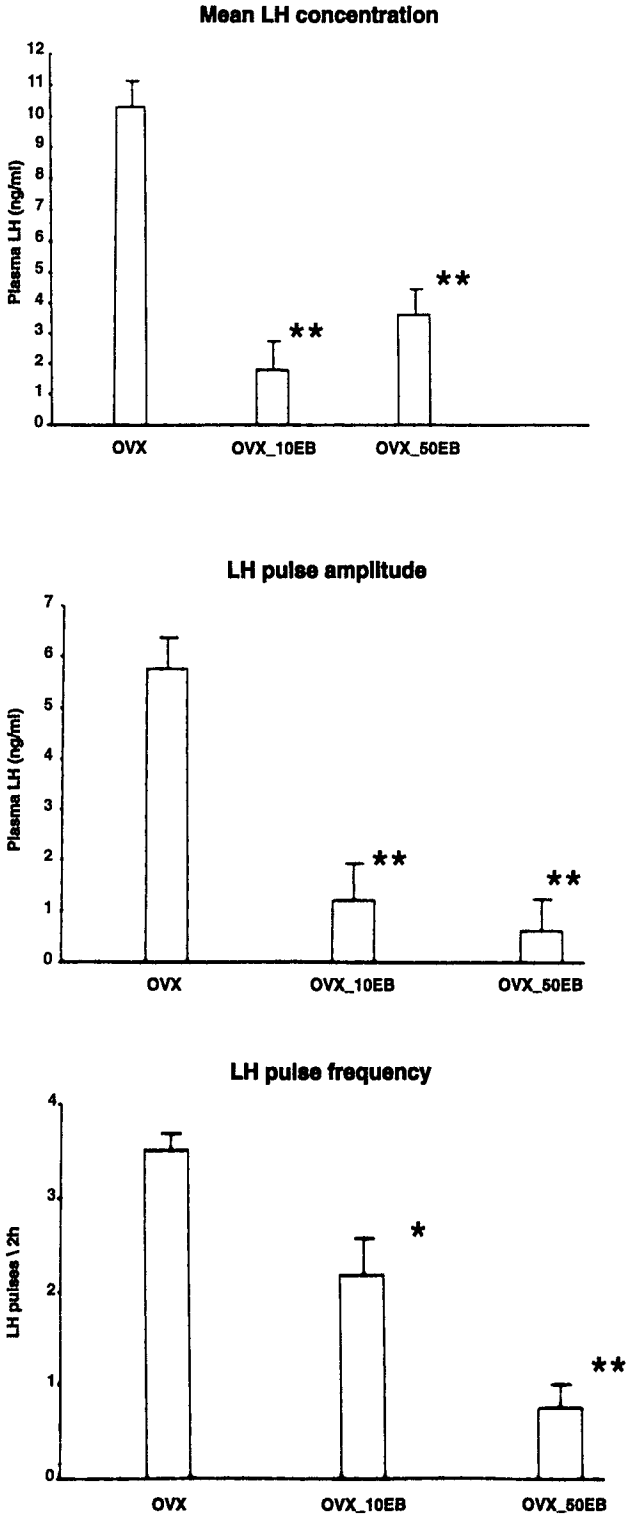

Figure 1. Parameters of LH secretion in ovariectomized animals injected with oil diluent, 10 or 50 μg of estradiol benzoate. No. of rats/group: ovariectomized, 8; 10 μg estradiol benzoate, 6; 50 μg estradiol benzoate, 8. In this and subsequent figures, the height of the column or point indicates the mean, the vertical line one standard error of the mean; $^{*}P < 0.05$ versus control, $^{**}P < 0.01$ versus control.

In ovariectomized rats, LH pulses occurred more frequently than FSH pulses (every 35 min vs 86 min). The 10-μg dose of estradiol benzoate reduced the LH pulse fre-

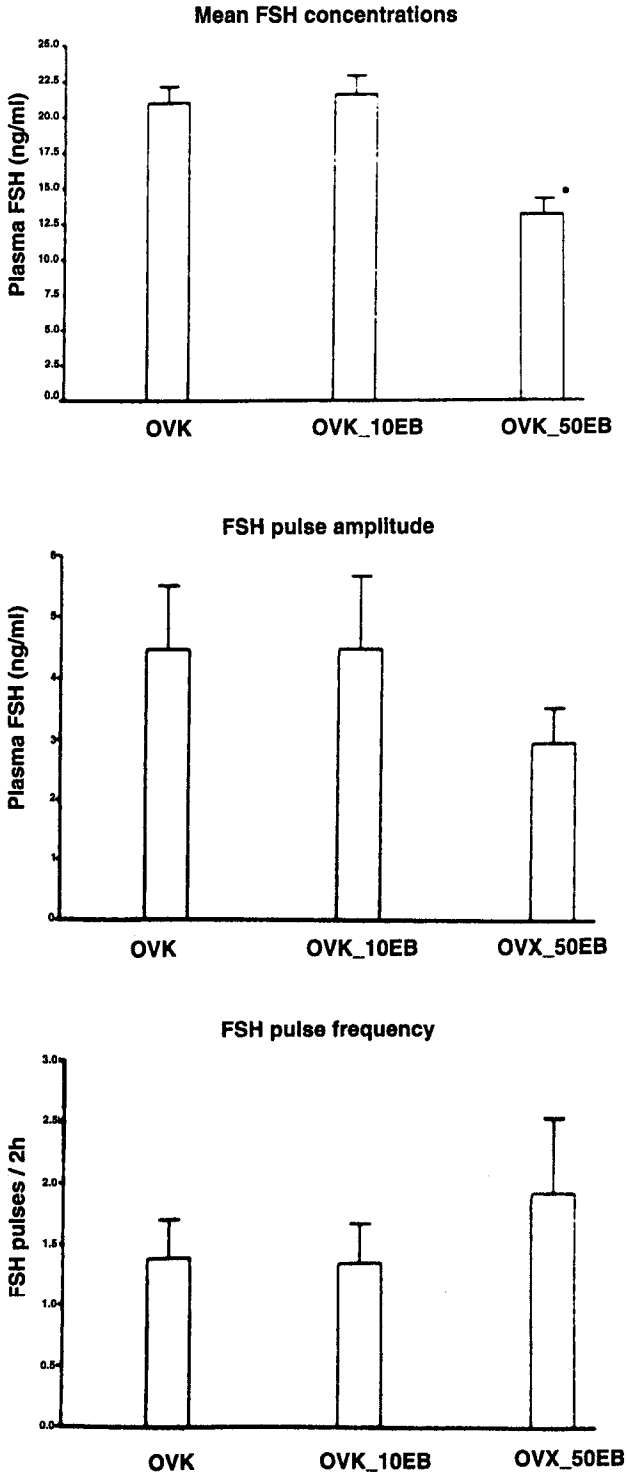

Figure 2. Parameters of FSH secretion in animals injected with oil, 10 or 50 μg estradiol benzoate.

quency to approximately one pulse per hr, and the 50-μg dose reduced the frequency further to a single pulse per 1.5 hr (Fig. 1). In contrast, FSH pulse frequency was not effected by either dose of estradiol (Fig. 2).

Effect of Estrogen on Simultaneous Pulses of FSH and LH and Pulses of Either Gonadotropin Alone. The number of pulses of LH and FSH alone and of

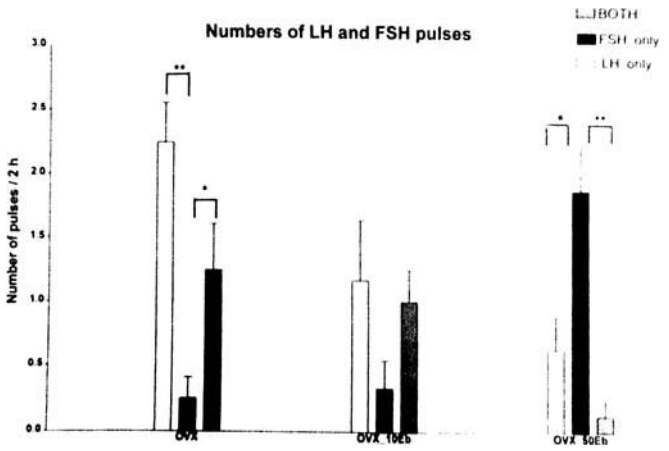

Figure 3. Differential effects of estradiol benzoate on the numbers of pulses of FSH or LH alone and the two hormones together; * and ** refer to $P < 0.05$ and $P < 0.01$ or better versus the indicated column.

both hormones simultaneously was compared. In ovariectomized animals, a large fraction (more than half) of the pulses were of LH only, and a very small number of FSH-alone pulses (0.2/2 hr) occurred. Roughly one-third of the pulses were of both hormones simultaneously (Fig. 3). This pattern changed as the estradiol benzoate dose was increased. With the 10-μg dose, the number of LH-alone pulses was reduced by half although this change was not statistically significant ($P < 0.1$). The number of FSH-alone pulses and pulses of both hormones together was not altered; however, when the dose of estradiol benzoate was increased to 50 μg, there was a highly significant ($P < 0.01$) reduction in the number of LH-alone pulses and a highly significant increase in the number of pulses of FSH alone, whereas the pulses of both hormones together were almost extinguished.

The time course of FSH and LH pulses was similar with a rapid upsweep to peak within 10 min and a slow decline from peak that reached trough values in 20 min. These profiles were not modified by estrogen.

Effect of Microinjection of Leptin into the Third Ventricle on Pulsatile LH and FSH Release. The injection of 10 μg of leptin into the third ventricle did not alter the patterns of pulsatile LH and FSH release significantly and the mean concentrations of both hormones as compared to those of diluent-injected rats in ovariectomized, oil-injected rats. Leptin was also ineffective in ovariectomized animals injected with 50 μg of estradiol benzoate except that it slightly increased the pulse amplitude of LH ($P < 0.04$). Leptin had no effect in these groups on the areas under the curves of plasma LH and FSH as well (Fig. 4).

In contrast, there were clear effects of leptin on plasma LH and FSH in the animals primed with 10 μg of estradiol benzoate 72 hr before the experiment. In the case of LH, the mean plasma concentrations in the leptin-injected animals increased at 10 min after injection and remained elevated until 70 min. They then returned to values similar to those

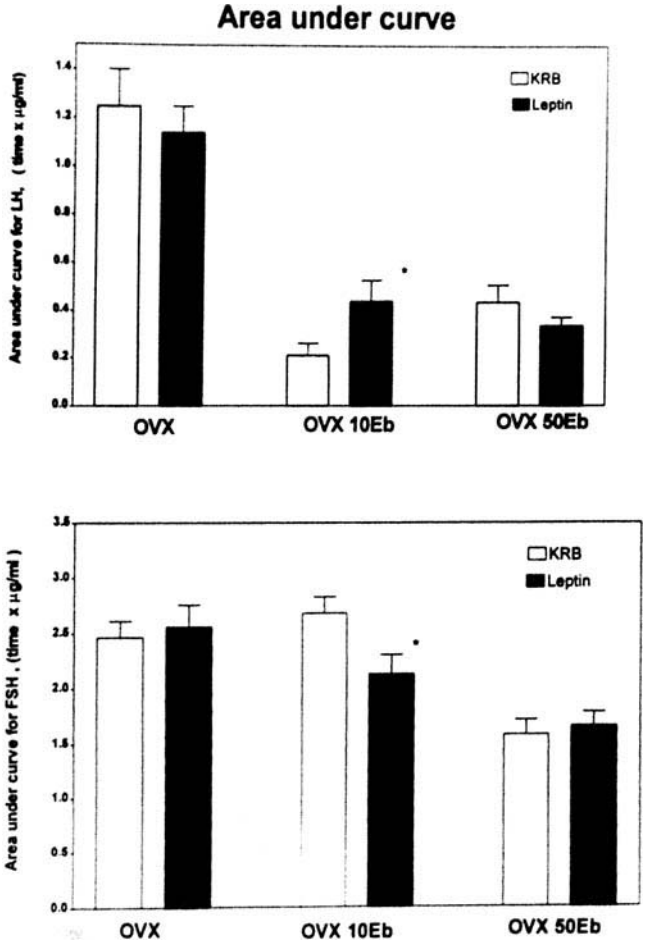

Figure 4. Areas under the curve of FSH and LH secretion in ovariectomized animals and those treated with either 10 or 50 µg of estradiol benzoate and injected with either diluent or 10 µg of leptin into the 3V. *$P < 0.05$ versus the diluent-injected controls.

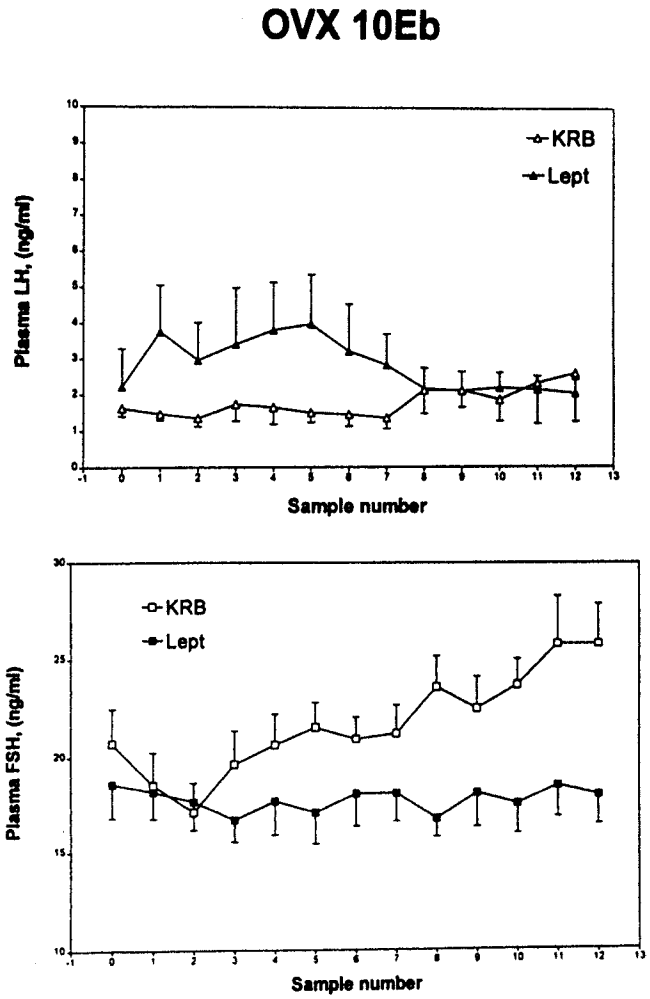

Figure 5. Patterns of FSH and LH secretion in animals injected with 10 µg estradiol benzoate and either the diluent or 10 µg leptin intraventricularly. Samples, numbered from 0 to 12, were taken every 10 min beginning just prior to the injection into the third ventricle. Plasma FSH concentrations were significantly lowered below initial levels at 20 min ($P < 0.01$) in control rats and became significantly increased above this nadir at 80–120 min and at these times were significantly higher ($P < 0.05$–0.01) than those of the leptin-injected rats. FSH values in leptin-injected rats were significantly elevated above 0 time at 110 and 120 min.

in the KRB-injected controls (Fig. 5). The area under the curve of LH was significantly increased by leptin during the 120-min duration of the experiment (Fig. 4), but this effect occurred during the first 70 min. Plasma LH levels were nearly identical between control and leptin-injected rats from 70–120 min (Fig. 5).

On the other hand, injection of KRB, although it failed to alter mean plasma LH concentrations during the 2-hr duration of blood sampling, produced a biphasic effect on plasma FSH concentrations with a significant decline ($P < 0.05$, paired t test) to a nadir at 20 min after the onset of sampling, followed by a gradual significant rise ($P < 0.05$) to a peak by the end of the experimental period (Fig. 5). On the other hand, the injection of leptin, although not affecting plasma concentrations of FSH during the first hour, completely prevented ($P < 0.01$) the rise in FSH that occurred during the second hr in the KRB-injected controls. The area under the curve of plasma LH was significantly increased by leptin over the 2-hr duration of the experiment whereas that of FSH was decreased during this period (Fig. 4).

This effect of leptin in the first hour with respect to LH and the second hour with respect to FSH, was also discernible by calculating the Δ_{max} and pulse amplitude for LH during the first hour, which were highly significantly elevated ($P < 0.01$), whereas in the second hour, they were unchanged (data not shown). Since these parameters were not altered in the second hour, when calculated over the 2-hr duration of the experiment, they were less significant ($P < 0.05$) (Fig. 6). Again, the pattern for FSH was different in that the Δ_{max} and pulse amplitude of FSH were significantly reduced both in the first and second hours with a greater effect ($P < 0.01$) in the second hour and an overall significant effect for the 2-hr period ($P < 0.05$) (Fig. 6). The 10-µg dose of estradiol benzoate failed to alter the pulse frequency of either FSH or LH (Fig. 6).

Discussion

In our previous paper (14), we reported that very low concentrations of leptin stimulated LHRH release from median eminence-arcuate nucleus explants and stimulated LH

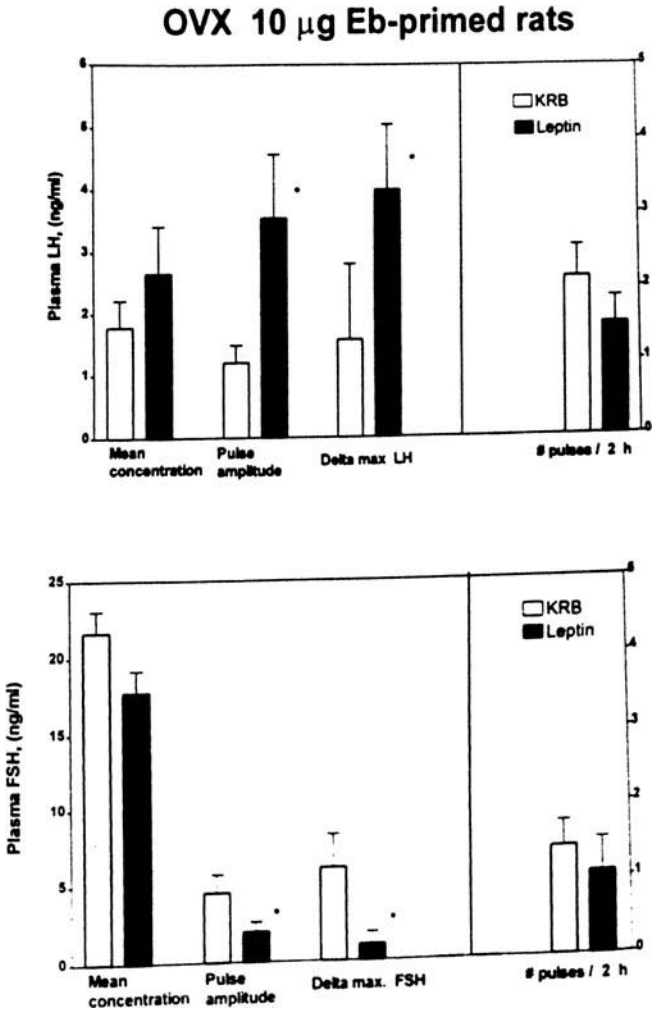

Figure 6. Parameters of FSH and LH secretion in animals primed with 10 μg of estradiol benzoate and then injected into the third ventricle with either diluent or leptin (10 μg) into the third ventricle. Values are calculated over the 2-hr sampling period and reflect significant increases ($P < 0.05$) in pulse amplitude and Δ_{max} LH and significant decreases of these parameters for FSH of leptin-injected rats over the 2-hr period. The effect on pulse amplitude and Δ_{max} for LH was maximal and highly significant ($P < 0.01$) in the first hour, whereas the effects on pulse amplitude and Δ_{max} FSH were maximal and highly significant in the second hour (data not shown).

and FSH release from incubated anterior pituitaries of intact male rats with a potency equal to that of LHRH itself. We also demonstrated in those experiments that the 10-μg dose of estradiol benzoate injected 72 hr prior to experiment in ovariectomized rats led to an increase in the Δ_{max} of plasma LH concentrations during the first hour in these animals. In this experiment, we further analyzed the results with that dose and studied the effects of oil alone and a higher dose of 50 μg of estradiol benzoate on the release of FSH and LH.

Both doses of estradiol benzoate produced an equal depression of plasma LH; there was little effect except of the high dose on plasma FSH concentrations. Furthermore, LH pulse frequency and the magnitude of pulses were dramatically inhibited in a dose-related manner by estrogen, whereas estrogen did not alter the frequency or magnitude of FSH pulses. A very interesting finding was the fact that in the ovariectomized animals, approximately 50% of the pulses were of LH only; an occasional pulse of FSH alone occurred with approximately 50% of the pulses of both hormones occurring simultaneously. This pattern changed as the dose of estrogen increased, so that with the higher dose of estrogen a remarkable suppression of LH-alone pulses, an increase of FSH-alone pulses, and a reduction in the number of pulses of both hormones released simultaneously occurred.

It is probable that part of the explanation for the different degree of depression in the pulsatile character of FSH and LH release is related to direct action of estradiol on the pituitary since the steroid was given 72 hr before the studies, and it is known that estrogen can alter responsiveness to LHRH in the rat. In general, it has been shown to increase responsiveness to this peptide (19, 20). No studies exist on the effect of estrogen alone on responsiveness to FSHRF although combined treatment of ovariectomized rats with 50 μg of estradiol benzoate and 25 μg of progesterone 72 hr prior to injection increases responsiveness to FSHRF (21); however, it is hard to see how these differences in pituitary sensitivity to the LHRH and FSHRF could account for the dramatic difference in the number of FSH-alone pulses and a decrease in LH-alone pulses that occur as the estrogen dose is increased. We hypothesize that the increase in FSH-alone and decrease in LH-alone pulses with increasing doses of estradiol is related to increased pulsatile FSHRF and decreased pulsatile LHRH release.

The differential effects of estrogen and leptin on FSH and LH release can be best explained by postulating the existence of an FSHRF. In recent experiments we have identified lamprey (l) GnRH-III as a potent FSHRF (21). l-GnRH is resident within the brain as determined by RIA of rat hypothalamic extracts fractionated by gel filtration on Sephadex G-25 (22). FSHRF is clearly separable from LHRH, and the FSH-releasing activity of purified FSHRF is abolished by a l-GnRH antiserum (22). l-GnRH cell bodies and axons are localized in areas that selectively control FSH (22). l-GnRH-III and FSHRF act to release FSH by activating nitric oxide synthase (NOS) (23; Yu WH *et al.*, unpublished data, 1998). The release of NO stimulates guanylyl cyclase to release cyclic guanosine monophosphate (cGMP) that induces FSH release. LHRH and leptin act similarly to stimulate LH and to a lesser extent FSH (23). Specificity of the effects is presumably conferred by specific receptors for each hormone on the gonadotropes. FSHRF receptors have not yet been identified, but in view of the structural differences between l-GnRH-III and LHRH, both decapeptides but with different amino acids in positions of 5–8 of the molecule, we believe that distinct FSHRF receptors will ultimately be identified.

On the basis of this research, we hypothesize that FSH-alone pulses are driven by the FSH-releasing factor (FSHRF) (21), whereas LH-alone pulses are driven by LHRH. Pulses of both hormones together could be due either to simultaneous pulses of both releasing hormones or to

large pulses of only LHRH since LHRH has intrinsic FSH-releasing activity. To determine which of these two possibilities is correct, it will be necessary to measure the levels of FSHRF and LHRH in portal blood. Similar findings with regard to separate pulses of FSH not accounted for by LHRH have recently been reported in castrate sheep (24).

The most intriguing results of our studies are that in rats pretreated with 10 μg of estradiol, leptin had opposite effects on the magnitude of LH and FSH release with a stimulatory effect on LH release in the first hour, and an inhibitory effect on FSH release manifest in the second hour. These effects were related to the dose of estrogen since little or no effect of leptin was found either in the oil-injected ovariectomized controls or in those animals that were given the higher dose of estrogen. The 10-μg dose of leptin clearly increased all parameters of LH release during the first 60–70 min after its injection with a variable time course in individual rats. In contrast, the microinjection of oil into the third ventricle plus the blood sampling procedure itself, although producing no change in plasma LH concentrations, produced an initial decline, followed by a delayed rise in FSH concentrations that was completely blocked by leptin. The inhibitory effect of leptin on FSH release developed and became maximal in the second hour in contrast to the more rapid effects in the opposite direction on LH release.

The minor stress of microinjection and repeated blood sampling had no effect on the pulsatile release of LH driven by LHRH in the animals given the 10-μg dose of estradiol benzoate; however, this mild stress caused an immediate inhibition of FSHRF release followed by a stimulation of the release of this peptide commencing immediately after the nadir was reached at 20 min that reached a maximum at the end of the experiment. Intraventricular injection of leptin produced quite a rapid effect to increase the pulsatile release of LHRH only during the first hour after its injection, whereas it produced a delayed inhibition of FSHRF release that became significant during the second hour of the experiment. Since the stress of the procedures blocked FSHRF release during the first 20 min, it is possible that the inhibitory action of leptin on FSHRF release could not be detected until this initial stress-induced inhibition had dissipated. Therefore, the onset of leptin-induced inhibition of FSHRF may have coincided with the onset of its stimulation of LHRH release, but the duration of the inhibition clearly outlasted the stimulation of LHRH release. The reason for the different time course of the effect on the release of these two peptides is not known.

Interestingly enough, in castrated male rats, we have shown that the stress of blood sampling produces a stimulation of FSH release at the same time that LH release is inhibited (25). Therefore, there is an apparent sex difference even in castrated animals in their response to the mild stress of connecting the catheter and drawing small blood samples. The mechanisms for this phenomenon remain unknown.

We hypothesize that the site of action of leptin in these experiments is in the region of the arcuate nucleus since incubation of median eminence–arcuate nuclear explants with leptin released LHRH (14, 23). Some of the leptin injected into the third ventricle in the current experiments undoubtedly reached the pituitary gland and even reached the peripheral circulation as well after passing through the pituitary gland (unpublished data, 1997); however, the *in vitro* sensitivity to leptin at the pituitary level is 100-fold less than at the hypothalamic level (14). Consequently, we believe that the results in the rats injected with 10 μg of estradiol benzoate are caused by its hypothalamic action to stimulate the release of LHRH and inhibit that of FSHRF. Furthermore, the effect on the anterior pituitary incubated *in vitro* was a stimulation of both FSH and LH release by leptin and not the differential effect seen here. That the effects were caused by this dose of estrogen is demonstrated by the lack of effects of leptin in the oil-injected rats and those injected with the higher (50-μg) dose of estradiol benzoate.

The evidence to date supports a role of leptin in induction of female puberty (5, 26–29). The current results suggest that this role may increase with increased estrogen secretion by the ovary as puberty nears and that leptin may play a crucial role in the onset of puberty in women (26). The reversion to a prepubertal state observed in patients with anorexia nervosa may be related to the decreased leptin release caused by the vanishing fat stores in these individuals (27). In collaborative research, we have shown recently that in women in the follicular phase of the menstrual cycle, blood leptin levels increase from a nadir in the morning to reach a peak at midnight–2 AM. The pattern of LH release is dramatically altered at that time. As the peak of leptin is reached, frequent small pulsations are replaced by large infrequent pulses that continue throughout the rest of the night (26). It is possible that this pattern in women is brought about by the increasing estrogen levels provided by the developing ovarian follicles.

It will be interesting to study the role of estrogen further in modifying the hypothalamic-pituitary response to leptin. For example, we do not whether the levels of estrogen and leptin that were achieved in the present study are achieved physiologically. In any event, the data presented are consistent with a role of estrogen in modifying the central responsiveness to leptin mediated by changes in pulsatile release of LHRH and FSHRF that probably cause the induction of puberty and control gonadotropin secretion during the estrous cycle in rats and menstrual cycle in women.

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