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Roles of Inhibin and Estradiol in the Regulation of Follicle-Stimulating Hormone and Luteinizing Hormone Secretion during the Estrous Cycle of the Rat[1]

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ABSTRACT

The relative contributions of inhibin and estradiol in the regulation of FSH and LH secretion were examined at various stages of the estrous cycle in the rat.

At 1100 h on metestrus, diestrus, or estrus or at 0500 h on proestrus, rats were ovariectomized or given an injection of normal goat serum, antiserum to inhibin (inhibin-AS), antiserum to estradiol (estradiol-AS), or both antisera to examine the role of gonadal hormones in the regulation of tonic gonadotropin secretion. Plasma samples were collected before and at 6, 12, and 24 h after the treatments. Further, to examine the effects of the treatments on preovulatory gonadotropin surges, the five treatments described were carried out at 0500 h on proestrus and blood samples were collected from 1100 h to 2000 h on the same day at 1.5-h intervals. There was a significant rise in the concentration of plasma FSH after injection of inhibin-AS as well as after ovariectomy on each day of the estrous cycle. These treatments, however, had less effect on estrous FSH secretion. The rise in FSH was greater with immunoneutralization against both inhibin and estradiol than with immunoneutralization against inhibin alone on diestrus and proestrus. Basal levels of LH were increased at all stages of the cycle through immunoneutralization against inhibin and were also increased through immunoneutralization against estradiol except at estrus. Especially on diestrus, a remarkable increase in LH secretion was induced at 6 h after immunoneutralization against both inhibin and estradiol (1449.3 ± 100.3% vs. control). The magnitude of the LH surge increased in inhibin-immunized rats, decreased in estradiol-immunized or ovariectomized rats, and remained at normal levels after injections of both antisera. The magnitude of the primary FSH surge increased very markedly in the inhibin-immunized group and decreased in the estradiol-immunized group. These results suggest that both estradiol and inhibin play a role in the regulation of LH secretion and that inhibin is a major regulator of FSH secretion during the estrous cycle of the rat. Furthermore, it is suggested that one or more extragonadal factors suppress estrous FSH secretion.

INTRODUCTION

In 4-day-cyclic rats, striking changes in gonadotropin secretion occur from the evening of proestrus to the early morning of estrus [1, 2]. Except during this periovulatory period, plasma concentrations of both FSH and LH remain at low basal levels. Hypothalamic GnRH, gonadal steroids, and inhibin play important roles in the regulation of FSH and LH secretion. Inhibin suppresses both FSH synthesis and release and plays a major role as a regulator of FSH secretion [3]. Passive immunization against inhibin causes an increase in the concentration of FSH in plasma [4, 5] and in the ovulation rate [6] in the female rat. Furthermore, large changes in inhibin secretion during the periovulatory period are involved in the secondary surge of FSH and result in the difference between the patterns of FSH and LH secretion during the periovulatory period [7–10]. On the other hand, gonadal steroids modulate gonadotropin secretion through GnRH in hypothalamus and gonadotropin gene expression at the level of the pituitary gland [11–18]. However, the relative contributions of estrogen and inhibin to regulation of gonadotropin secretion during the estrous cycle still have not been clarified.

The purpose of the present experiments was to examine these problems using cyclic rats through passive immunization against inhibin and/or estradiol as well as ovariectomy at various stages of the estrous cycle.

MATERIALS AND METHODS

Animals

Adult cyclic female rats of the Wistar strain weighing 270–330 g were used. They were kept under conditions of controlled temperature (25 ± 2°C) and lighting (lights-on from 0500 to 1900 h). Food and water were available ad libitum. Vaginal smears were checked daily, and only rats with at least two consecutive 4-day estrous cycles were used.

Inhibin Antiserum (Inhibin-AS)

[Tyr^{30}]-Porcine inhibin α(1–30) conjugated to rabbit serum albumin was used as the antigen. This conjugate was provided by Dr. N. Ling (Neurocrine Bioscience Inc., San Diego, CA). The conjugate (3.6 mg) was dissolved in 1 ml saline and mixed with an equal volume of Freund's complete adjuvant. A castrated goat was given 2 ml of the suspension (including 3.6 mg of the conjugate) s.c. at each immunization. First, second, and third immunizations were performed at 2-wk intervals and thereafter, monthly. Blood samples were obtained 2 wk after each injection. The sera were collected and examined for inhibin-AS titer, and titers of the antisera were checked in the following manner. The antisera were diluted with 0.05 M PBS (pH 7.4) containing 1% BSA, and the diluted samples were incubated with 5000 cpm of ^{125}I-labeled bovine 32-kDa inhibin (325 Ci/mmol) at 4°C for 24 h in a total volume of 200 μl. To separate bound radioligands, 100 μl of 1% bovine gamma globulin in PBS and 500 μl of 25% polyethylene glycol in PBS were added, and the mixture was agitated for 3 min. After centrifugation at 4°C and 1700 × *g*, radioactivity of the precipitate was counted in a gamma counter. The serum used in the present experiment had a titer of 1:1 000 000 as defined by final dilution of the antiserum required to bind 50% of added ^{125}I-labeled bovine 32-kDa inhibin. In vivo efficiency of the antiserum was ensured by an increase in plasma concentrations of FSH after an i.v. injection of six doses (6.25–200 μl) of the antiserum at 1100 h on metes-

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trus and diestrus. A dose-related increase in the basal secretion of FSH was observed at 24 h after the injection, and the maximal response was noted when 100 μl of the antiserum was injected. The capacity of the antiserum to neutralize the inhibin bioactivity of rat inhibin (ovarian homogenate) was also examined in vitro with use of a dispersed anterior pituitary cell bioassay system. The secretion of FSH from cultured rat anterior pituitary cells was suppressed in a dose-dependent manner by rat ovarian homogenate, and the maximal suppression of the ovarian homogenate could be reversed by the addition of increasing dosage of the antiserum. Human transforming growth factor β (TGFβ) and activin showed no cross-reaction with the inhibin-AS.

Estradiol Antiserum (Estradiol-AS)

The antigen used in the immunization was 1,3,5,(10)-estratrien-3,17β-diol-6-one 6-carboxymethyloxyme:BSA (6-keto-17β-estradiol 6-CMO:BSA; Steraloids, Inc., Wilton, NH). One milligram of the conjugate was dissolved in 1 ml saline and mixed with an equal volume of Freund's complete adjuvant. The 2-ml mixture (including 1 mg of the conjugate) was given to a castrated goat, and sera were collected and checked for estradiol-AS titer in the same protocol as described for the production of inhibin-AS. The serum used in the immunoneutralization experiments had a titer of 1:1 250 000 as defined by final dilution of the antiserum required to bind 50% of 5000 cpm of ^{125}I-labeled radioligand (estradiol-6-(*o*-carboxymethyl)oximino-2-[125-I]-iodohistamine), 2000 Ci/mmol (Amersham Life Science, Tokyo, Japan), in an RIA. The cross-reactivities of the antiserum with estrone and estriol were 23.0 and 0.4%, respectively; those with testosterone, progesterone, and pregnenolone were less than 0.1%. In vivo efficiency of the antiserum (25–200 μl) was ensured by an increase in plasma concentrations of LH at 6 h after an i.v. injection of the antiserum at 1100 h on diestrus, by delay of the next ovulation, and by suppression of the increase in uterine weight. The effect of the antiserum was maximal, at 100 μl/rat, on basal secretion of LH, and it was enough to delay ovulation by 1 day in 80% of the treated rats. The dosage was also enough to suppress the increase in uterine weight for 24 h.

Roles of Inhibin and Estradiol in the Regulation of Tonic Gonadotropin Secretion

To investigate the roles of inhibin and estradiol in the regulation of basal FSH and LH secretion during the estrous cycle, rats were given an injection of inhibin-AS and/or estradiol-AS. Other groups of animals were ovariectomized at various stages of the estrous cycle. Four points of the estrous cycle at which plasma concentrations of FSH and LH remained at low basal levels were selected (1100 h on metestrus, diestrus, and estrus and 0500 h on proestrus). At each point, rats were anesthetized with ether and the following treatments were carried out: 1) a single i.v. injection of 200 μl normal castrated goat serum (NGS) for controls; 2) simultaneous i.v. injections of 100 μl inhibin-AS and 100 μl NGS; 3) simultaneous i.v. injections of 100 μl estradiol-AS and 100 μl NGS; 4) simultaneous i.v. injections of 100 μl inhibin-AS and 100 μl estradiol-AS; and 5) ovariectomy and a single i.v. injection of 200 μl NGS. Just before and at 6, 12, and 24 h after the treatments, rats were anesthetized with ether, and 600-μl blood samples were collected with heparinized syringes from the jugular vein through the pectoralis major muscle. On proestrus, however, the bleeding at 12 h after treatment was omitted because this is the time of preovulatory gonadotropin surges. After each bleeding, 600 μl of saline was replaced. Blood samples were centrifuged immediately and plasma was separated and stored at −20°C until assayed for FSH and LH.

Effects of Immunoneutralization against Inhibin and Estradiol or of Ovariectomy on Preovulatory Gonadotropin Surges

To investigate the effect of inhibin and estrogen on the proestrous gonadotropin surge, experiments of immunoneutralization against inhibin and estradiol were undertaken. Rats were anesthetized with ether, and a right atrial cannula was implanted in the afternoon on diestrus; each rat was then placed in an individual cage. At 0500 h on proestrus, the five treatments described in the previous section were carried out. Sera were injected through the cannula. From 1100 h to 2000 h on proestrus (from 6 to 15 h after treatment), 600-μl blood samples were collected with heparinized syringes at 1.5-h intervals from the right atrial cannula of unanesthetized rats. Immediately after each bleeding, 600 μl heparinized saline (10 IU/ml) containing a 1:100 dilution of the corresponding serum in each experiment was injected into the rats through the cannula to maintain both the blood volume and the circulating antibody concentration. Plasma was separated as described above, and samples were stored at −20°C until assayed for FSH and LH.

RIA of FSH and LH

Concentrations of FSH and LH in the peripheral plasma were measured with use of NIDDK (Baltimore, MD) RIA kits for rat FSH and LH. Iodinated preparations were rat FSH-I-5 and LH-I-5. The antisera used were anti-rat FSH-S-11 and anti-rat LH-S-10. Results were expressed as rat FSH RP-2 and rat LH RP-2. The intra- and interassay coefficients of variation were 9.7% and 10.4% for FSH and 6.5% and 15.5% for LH, respectively.

Statistical Analysis

Values are presented as means ± SEM. Duncan's New Multiple Range test was used to compare the mean values of area under the FSH and LH curves between groups. A value of $p < 0.05$ was considered to be significant.

RESULTS

Effects on Basal Gonadotropin Secretion of Immunoneutralization against Inhibin and Estradiol or of Ovariectomy

Changes in the plasma concentrations of FSH and LH after the treatments conducted at various stages of the estrous cycle are shown in Figures 1 and 3, respectively. Areas under the FSH and the LH curves during 24 h for metestrus, diestrus, and estrus and during 6 h for proestrus after the treatments are illustrated in Figures 2 and 4, respectively.

Treatment on Metestrus (Figs. 1a, 2a, 3a, and 4a)

After passive immunization against inhibin or after ovariectomy, a marked increase in plasma concentrations of FSH was observed within 6 h. After treatment with estradiol-AS, on the other hand, FSH levels in the plasma showed no increase, as in the controls. The FSH rise in the

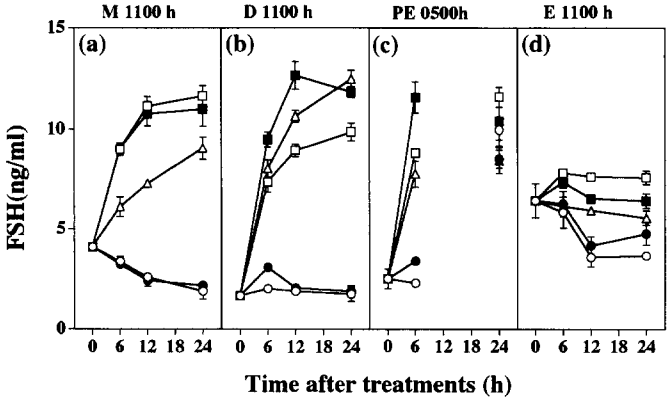

FIG. 1. Changes in plasma concentrations of FSH after ovariectomy (open triangle) or injection of inhibin-AS (open square), estradiol-AS (solid circle), or both antisera (solid square) at 1100 h on (**a**) metestrus, (**b**) diestrus, or (**d**) estrus or (**c**) at 0500 h on proestrus. Controls received an injection of NGS (open circle). Blood samples were collected from the jugular vein just before and at 6, 12, and 24 h after treatment. Values are mean ± SEM for five or six rats.

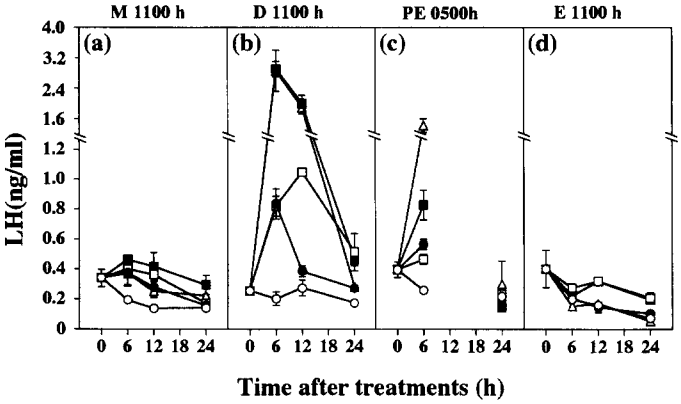

FIG. 3. Changes in plasma concentrations of LH after ovariectomy (open triangle) or injection of inhibin-AS (open square), estradiol-AS (solid circle), or both antisera (solid square) at 1100 h on (**a**) metestrus, (**b**) diestrus, or (**d**) estrus or (**c**) at 0500 h on proestrus. Controls received NGS (open circle). Blood samples were collected from the jugular vein just before and at 6, 12, and 24 h after the treatments. Values are mean ± SEM for five or six rats.

plasma after ovariectomy was gradual as compared with that in rats after treatment with inhibin-AS. Area under the FSH curve was significantly increased in the group treated with inhibin-AS alone, or with both inhibin-AS and estradiol-AS, as compared with that for control animals. Ovariectomy also increased area under the FSH curve; however, these increases were smaller than for rats treated with inhibin-AS alone or with both inhibin-AS and estradiol-AS.

In each experimental group, plasma concentrations of LH remained at slightly higher levels than for controls given NGS. Area under the LH curve was significantly increased in all experimental groups compared with controls. In the group treated with both inhibin-AS and estradiol-AS, a synergistic effect of the antisera was observed as compared with the effect in rats treated with estradiol-AS alone.

Treatment on Diestrus (Figs. 1b, 2b, 3b, and 4b)

Immunoneutralization against inhibin caused a marked increase in the plasma concentrations of FSH as shown in the metestrous rats. After treatment with estradiol-AS, a transient increase in plasma concentrations of FSH was observed at 6 h after the injection, in contrast to the results in metestrous rats, although no difference was noted in area under the FSH curve. Furthermore, a synergistic effect on area under the FSH curve was noted in the group treated with both inhibin-AS and estradiol-AS as compared to the group treated by immunoneutralization against inhibin alone. Area under the FSH curve in the ovariectomized rats was significantly larger than that for rats treated with inhibin-AS alone but was smaller than for the group given both inhibin-AS and estradiol-AS.

At 6 h after treatment, plasma concentrations of LH were increased in the group treated with estradiol-AS (416.7 ± 51.1% vs. control) and in the group given inhibin-AS (408.7 ± 33.1% vs. control). In the group treated with estradiol-AS, the rise in LH was temporal, while high concentrations of LH were sustained for more than 12 h in the inhibin-neutralized group. In the group given both inhibin-AS and estradiol-AS, a marked increase in plasma LH (1449.3 ± 100.3% vs. control) was observed at 6 h after treatment and decreased thereafter. These changes in LH in

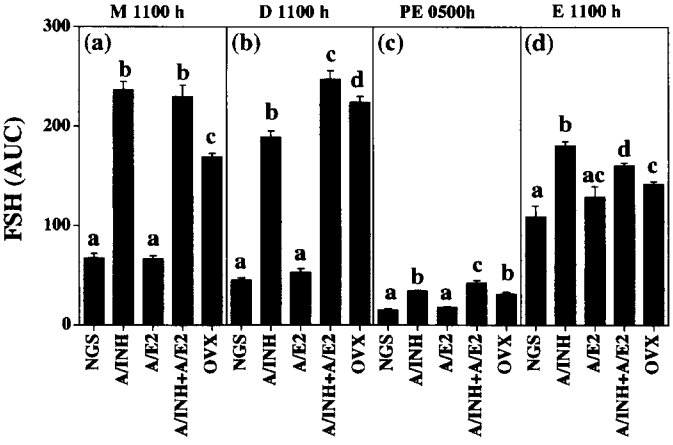

FIG. 2. Area under the FSH curve during 24 h after ovariectomy (OVX) or injection of inhibin-AS (A/INH), estradiol-AS (A/E2), or both antisera (A/INH+A/E2) at 1100 h on (**a**) metestrus, (**b**) diestrus, or (**d**) estrus or during 6 h after these treatments (**c**) given at 0500 h on proestrus. Controls were treated with NGS. Values are mean ± SEM for five or six rats. Bars with differing superscripts are significantly different ($p < 0.05$).

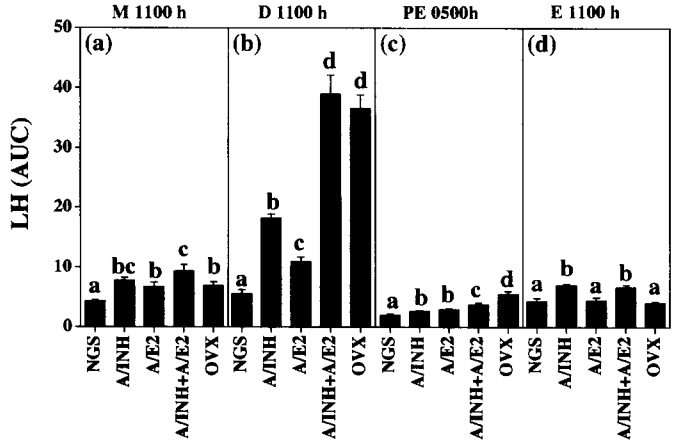

FIG. 4. Area under the LH curve during 24 h after ovariectomy (OVX) or injection of inhibin-AS (A/INH), estradiol-AS (A/E2), or both antisera (A/INH+A/E2) at 1100 h on (**a**) metestrus, (**b**) diestrus, or (**d**) estrus or during 6 h after these treatments (**c**) at 0500 h on proestrus. Controls received NGS. Values are mean ± SEM for five or six rats. Bars with differing superscripts are significantly different ($p < 0.05$).

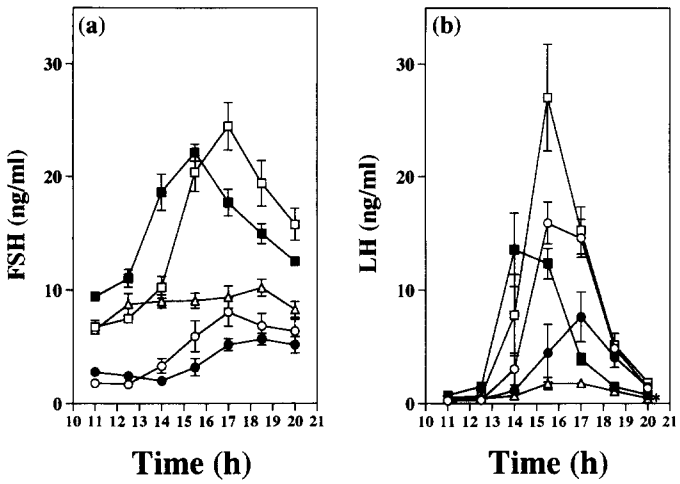

FIG. 5. Patterns of the preovulatory (**a**) FSH and (**b**) LH surge after ovariectomy (open triangle) or injection of inhibin-AS (open square), estradiol-AS (solid circle), or both antisera (solid square) at 0500 h on proestrus. Controls received NGS (open circle). Blood samples were collected from the jugular vein at 1.5-h intervals from 1100 h to 2000 h on the same day. Values are mean ± SEM for five or six rats.

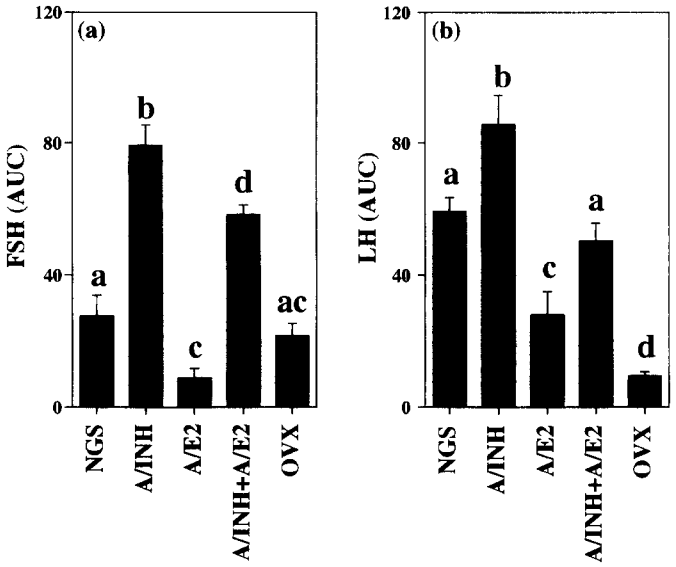

FIG. 6. Area under the (**a**) FSH and (**b**) LH curve from 1100 h to 2200 h on proestrus. Rats were ovariectomized (OVX) or given an injection of inhibin-AS, estradiol-AS, or both antisera at 0500 h on the same day. The area under the FSH curve was normalized for each rat by subtracting the values at 1100 h on proestrus. Values are mean ± SEM for five or six rats. Bars with differing superscripts are significantly different ($p < 0.05$).

the plasma showed a pattern similar to that for the ovariectomized animals. Area under the LH curve was significantly larger in the estradiol-AS-treated group than in controls; it was also significantly larger in animals treated with inhibin-AS than in controls or in estradiol-AS-treated animals. Further increases in area under the LH curve were noted in the group treated with both inhibin-AS and estradiol-AS as compared to rats treated with estradiol-AS alone or inhibin-AS alone; there was no difference in relation to ovariectomized rats.

Treatment on Proestrus (Figs. 1c, 2c, 3c, and 4c)

As in the preceding two phases, immunoneutralization against inhibin caused an increase in the FSH concentration within 6 h after treatment. Treatment with estradiol-AS had increased FSH levels by 6 h later as observed on diestrus, although area under the FSH curve during the 6 h after treatment was not different from that for controls. In the ovariectomized group, area under the FSH curve during the 6 h after treatment did not reach the value observed for the group immunoneutralized against both inhibin and estradiol, and was not different from the value for the group given inhibin-AS alone.

In each experimental group, area under the LH curve was significantly increased as compared to that for controls, and a synergistic effect of inhibin-AS and estradiol-AS was noted. The levels of area under the LH curve in ovariectomized rats were significantly higher than for the other groups.

Treatment on Estrus (Figs. 1d, 2d, 3d, and 4d)

In striking contrast to the results for the three other phases of the estrous cycle, a marked increase in plasma concentrations of FSH did not take place after immunoneutralization against inhibin or even after ovariectomy. Only small increases in plasma FSH were observed in animals treated with inhibin-AS or with both inhibin-AS and estradiol-AS, as well as in the ovariectomized group.

With respect to LH secretion, no effect of estradiol-AS was observed on area under the LH curve, while a small but significant increase was noted with administration of inhibin-AS as compared with control treatment.

Effects on Gonadotropin Surges of Immunoneutralization against Inhibin and Estradiol or of Ovariectomy

Changes in the plasma concentrations of FSH and LH, and area under the FSH and LH curves during the preovulatory gonadotropin surge, are shown in Figures 5 and 6, respectively.

Preovulatory FSH Surge (Figs. 5a and 6a)

Because of the elevation in plasma FSH levels that occurred in inhibin-AS-treated or ovariectomized rats, the area under the FSH curve was normalized for each rat by subtracting the value at 6 h after treatment (1100 h on proestrus).

In the control group, the primary surge of FSH was observed to be normal. The FSH surge in the inhibin-immunoneutralized group and in the group immunoneutralized against both inhibin and estradiol occurred earlier than in the other groups, and a marked increase in the magnitude of the FSH surge was noted. In the group given both inhibin-AS and estradiol-AS, plasma concentrations of FSH reached their peak values earlier than the time of the FSH peak in the other groups, and the magnitude was less than in the group treated with inhibin-AS alone. In the ovariectomized group, a marked rise of FSH in plasma in relation to levels at 1100 h on proestrus was not observed, and the size of the FSH surge was not different from that in controls. In the group given estradiol-AS, the magnitude of the FSH surge was less than in controls.

Preovulatory LH Surge (Figs. 5b and 6b)

In the controls, an LH surge was induced normally, and the maximal rise was noted during 1530–1700 h. As with the FSH surge, in the group given inhibin-AS and the group treated with both inhibin-AS and estradiol-AS, there was a

tendency for the LH surge to be initiated earlier than in the controls and for the time of the maximal rise to be advanced. Immunoneutralization against estradiol reduced the magnitude of the LH surge, while immunoneutralization against inhibin increased it. Interestingly, the size of the LH surge in the group given both inhibin-AS and estradiol-AS was not different from that of controls. In the ovariectomized group, a clear peak in the LH surge was not observed and plasma concentrations of LH remained at low levels, although these levels were higher than basal levels from metestrus to diestrus of the estrous cycle.

DISCUSSION

In the present experiment, a significant increase in FSH secretion was observed throughout the estrous cycle after immunoneutralization against inhibin. These results are consistent with the previous report of Rivier et al. [4] and suggest that inhibin is a major regulator of FSH secretion during the estrous cycle of the rat.

There were, however, some differences in patterns of the FSH increase among the stages of the estrous cycle when immunoneutralization against inhibin was undertaken. On the day of estrus, plasma concentrations of FSH after immunoneutralization against inhibin and even after ovariectomy were lower than those in the other three stages, although neither the level of GnRH in pituitary stalk plasma nor the sensitivity of FSH secretion to GnRH was lower than in metestrus and diestrus [19–21]. As plasma levels of inhibin on the day of estrus were lower than at other stages of the estrous cycle [22], the effect of immunoneutralization against inhibin at this stage seemed to be smaller than for the other stages. Further, these results suggest the possibility that the level of some stimulatory factor for FSH secretion other than GnRH may be low on estrus. It is postulated that activin, a stimulatory factor for FSH secretion, regulates FSH secretion by an autocrine or paracrine route, and that follistatin, an activin-binding protein, modulates the effect of activin on pituitary cells [23]. The expression of follistatin mRNA in pituitary cells is increased in proestrous evening [24], so it is not unreasonable to think that the level of follistatin protein in the pituitary gland may increase on estrus, resulting in inhibition of stimulating effects of activin on FSH secretion. Another possibility is that the pituitary releasable pool of FSH may be depleted after the primary and secondary FSH surges although the pituitary content of FSH has already recovered on estrous morning [22].

On the other days of the estrous cycle, the increase in FSH in plasma after ovariectomy was more gradual than in the group treated with both inhibin-AS and estradiol-AS. Plasma levels of the ovarian hormones decreased more quickly after treatment with corresponding antisera than after ovariectomy, resulting in an earlier response in the former group than in the latter. Further, the difference in the response in FSH release between these two groups may be derived from plasma levels of progesterone. In fact, the difference in area under the FSH curve between ovariectomized rats and rats treated with both estradiol-AS and inhibin-AS was greater on metestrus, when plasma levels of progesterone in the estrous cycle are high [22, 25], than in other stages. Progesterone enhances GnRH-stimulated FSH secretion in vitro [13], and administration of RU486, a progesterone antagonist, suppresses the expected increase in FSH secretion after immunoneutralization against inhibin or during the secondary surge of FSH [26]. In addition, progesterone response elements have been identified in the 5′-flanking region of ovine FSHβ gene [27]. With previous reports taken into consideration, the present data suggest that progesterone may facilitate FSH secretion during the estrous cycle at the level of the pituitary gland. Another possibility is that ovarian activin may stimulate FSH secretion in the physiological state and that ovariectomy terminates the positive stimulation from the ovary. Further, inhibin-AS may neutralize the inhibin produced in the pituitary gland, and this may physiologically suppress FSH secretion [23], resulting in the difference between the two groups.

On diestrus and proestrus, a synergistic effect of inhibin-AS and estradiol-AS on FSH secretion was observed, suggesting that estradiol is involved in the regulation of FSH secretion during these stages of the estrous cycle, though the effect of estradiol was much smaller than that of inhibin. Administration of estradiol-AS also induced a considerable increase in LH secretion on diestrus. Administration of estradiol or GnRH antagonist was found previously to suppress the increase in LH secretion after ovariectomy, but further suppression of LH secretion was not noted in ovariectomized rats treated with both GnRH antagonist and estradiol as compared to rats treated with GnRH antagonist alone [18]. Therefore, it is suggested that increased FSH and LH secretion after estradiol-AS administration is probably due to removal of suppressive effects of estradiol on GnRH secretion.

As with estradiol-AS, administration of inhibin-AS increased plasma levels of LH during the estrous cycle in the present experiments, in contrast to previous findings [4–6]. Effects of inhibin on LH secretion are controversial. However, it has been reported that inhibin suppresses GnRH-stimulated LH release [28, 29] and up-regulation of GnRH receptor by GnRH itself [30] in vitro. The present results showed a marked increase (408.7 ± 33.1%) in LH concentrations in plasma after administration of inhibin-AS, and a further increase (1449.3 ± 100.3%) was noted after simultaneous injections of inhibin-AS and estradiol-AS on diestrus. Inhibin as well as estradiol seems to be a physiological suppressive factor with respect to LH secretion during the estrous cycle in the rat.

On the other hand, a further increase in plasma levels of LH after ovariectomy was not observed, in contrast to the increase after immunoneutralization against both inhibin and estradiol except after treatment on proestrus. These results of ovariectomy suggest that progesterone is not of such great importance for the suppression of tonic LH secretion in an acute phase. We do not deny the importance of long-term inhibitory effects of progesterone on tonic LH secretion. Interestingly, administration of estradiol-AS or ovariectomy induced a transient increase in LH secretion as reported previously in ovariectomized rats [31]. GnRH secretion is temporally increased after ovariectomy [14], and therefore a transient increase in gonadotropin secretion after these treatments was probably induced by a transient increase in hypothalamic GnRH secretion. It is not clear why such a transient phenomenon is induced, but there is some possibility that an acute increase in GnRH secretion may deplete releasable GnRH from GnRH neurons or may suppress GnRH secretion itself by the ultrashort feedback system [32].

Preovulatory gonadotropin surges were also considerably affected by neutralization of inhibin and estradiol or by ovariectomy. An attenuated LH surge after immunoneutralization against estradiol on proestrous morning must be

due to the removal of positive effects of estradiol on gonadotropin surges. The amplifying effect of inhibin-AS on the LH surge may be induced by abolition of the suppressive effect of inhibin on the self-priming action of GnRH as demonstrated by Culler [33]. Removal of the suppressive effect of inhibin on the self-priming action of GnRH may also have induced an earlier initiation of the LH surge in the inhibin-immunoneutralized group. The possibility cannot be ruled out that the antiserum against inhibin used in these experiments cross-reacted with a gonadotropin surge-inhibiting/attenuating factor that is thought to be different from inhibin [34]. After immunoneutralization against both inhibin and estradiol, the magnitude of the LH surge was higher than in the group given estradiol-AS, and lower than after immunoneutralization against inhibin, probably due to the effects of a combination of a reduction of estradiol effects on preovulatory GnRH release and the removal of a suppressive effect of inhibin on the self-priming action. Earlier induction of preovulatory gonadotropin surges by immunoneutralization against both inhibin and estradiol may have been due both to an increase in tonic GnRH secretion and to removal of suppressive effects of inhibin on the self-priming action of GnRH. Increased tonic GnRH secretion may have primed pituitary gonadotrophs earlier than in the controls although preovulatory GnRH release was reduced. Delay of the LH surge by immunoneutralization against estradiol may be due to a reduction of preovulatory GnRH release under the suppressive effects of inhibin on the GnRH self-priming action. Ovariectomy remarkably suppressed the LH surge in spite of the reduction in inhibin, probably due to reduction in progesterone, which is known to facilitate the LH surge when administrated in estrogen-treated ovariectomized rats [35].

Inhibin is known to suppress both basal and GnRH-stimulated FSH release [3, 36]. In the present experiment, a remarkable increase in preovulatory FSH release was noted after administration of inhibin-AS; this is consistent with earlier findings [4]. It was due not only to an increase in basal levels but also to an increased sensitivity of FSH secretion to GnRH, because the normalized area under the FSH curve was much greater than for controls. As well as the LH surge, the preovulatory FSH surge was attenuated by administration of estradiol-AS, perhaps due to the reduction in GnRH release. In the ovariectomized group, basal levels of FSH were raised, probably due to reduction in inhibin, though only a slight increase was further observed at the time of the preovulatory gonadotropin surges, probably because of a decrease in preovulatory GnRH release as a result of lack of progesterone.

In summary, the present experiments demonstrate that inhibin is a major ovarian suppressive factor of FSH secretion during the estrous cycle and that estradiol is to a lesser degree involved with the regulation of FSH on diestrus and proestrus through GnRH secretion in the cyclic female rat. It is also suggested that progesterone may facilitate FSH secretion during the estrous cycle and that one or more extragonadal factors may suppress estrous FSH secretion. Furthermore, inhibin as well as estradiol is thought to suppress basal LH secretion; and progesterone, in an acute phase, seems not to be so important in the suppression of basal LH secretion. Concerning the preovulatory gonadotropin surge, inhibin decreases both FSH and LH, probably due to suppression of the self-priming action of GnRH.

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