FULL PAPER *Theriogenology*

Regulatory Role of Inhibin in Follicle-Stimulating Hormone Secretion and Folliculogenesis in the Guinea Pig[1]

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ABSTRACT. The effects of unilateral and bilateral ovariectomy and passive immunization against inhibin on follicle-stimulating hormone (FSH) secretions and follicular development in the guinea pig were investigated. Bilateral ovariectomy decreased plasma immunoreactive (ir-) inhibin rapidly and increased plasma FSH significantly. Unilateral ovariectomy decreased plasma ir-inhibin and increased plasma FSH temporarily, and doubled the number of ova released from the remaining ovary at the subsequent ovulation in guinea pigs. Injection of 1.0 m*l* inhibin antiserum significantly increased concentrations of plasma FSH at 6 hr onwards and the number of small follicles (100–200 μm in diameter) at 48 hr after the injection in guinea pigs bearing progesterone-containing implants. *In vitro* bioassay showed that inhibin antiserum could neutralize the suppression of ovarian homogenate on FSH secretion from cultured rat anterior pituitary cells. These results confirm the evidence that the ovary is the main source of inhibin secretion and both *in vitro* bioassay and passive immunization against inhibin show that the inhibin is a major regulator in the follicular development through FSH secretion in guinea pigs.

KEY WORDS: FSH, guinea pig, immunoneutralization, inhibin, unilateral ovariectomy.

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The guinea pig is a good biomedical and veterinary model for the study of reproductive biology because the luteal phase of its estrous cycle closely emulates that in large domestic animals and primates. The frequent attempts have been made to induce superovulation in guinea pigs since the number of offspring is limited from 2 to 4 in this species, but those practically ended in a failure.

Unilateral ovariectomy in guinea pigs [11], as in other animals [5, 7, 8, 12, 15], doubles the number of ova released from the remaining ovary at the subsequent estrus, thus the species-specific ovulation rate being maintained. It is reported that the increase in ovulation rate in the remaining ovary in hemiovariectomized animals is attributed to a decrease in incidence of atresia of the larger vesicular follicles [11].

Ovarian inhibin is a major regulator of FSH secretion in several species of animals [28, 31, 32]. Passive immunization against inhibin can increase the secretion of FSH in female guinea pigs [29] as well as in the other animals [14, 16, 17, 21, 26]. However, the mechanism that regulates the onset and completion of the compensatory response to hemiovariectomy remains obscure in guinea pigs. Thus, the role of inhibin in the regulation of FSH secretion and folliculogenesis needs to be further elucidated in guinea pigs.

A chronic subcutaneous progesterone implant prevents follicular growth and ovulation in the guinea pig, and removal of the implant results in rapid follicular growth and ovulation [20, 35]. These procedures make it possible to examine follicular function during the luteal phase and the rapid follicular growth. In the present study, effects of unilateral and bilateral ovariectomy and passive immunization against inhibin on tonic FSH secretion and follicular development in the guinea pig bearing the progesterone implant have been examined.

MATERIALS AND METHODS

Animals and implantation of progesterone-containing capsules: Adult female guinea pigs (*Cavia porcellus*) of the Hartley strain were used at 3–6 months of age. They were housed under controlled lighting (lights on 05:00–19:00 hr), and provided with commercial pellets and tap water *ad libitum*. All of experimental guinea pigs received a subcutaneous implant (Silastic tubing, 1.0 cm long, 0.4 cm in diameter. Dow Corning CO., Midland, MI, U.S.A.) filled with crystalline progesterone for more than 2 weeks, and received a cannula into the right atrium under ether anesthesia before being placed on experiment.

Preparation of inhibin antiserum: The antiserum to inhibin (inhibin-AS) was obtained from a castrated goat immunized against [Tyr30]-inhibin α(1–30) conjugated to rabbit serum albumin. This conjugate was kindly provided by Dr. N. Ling, Neuroendocrine Biosciences Inc., La Jolla, CA, U.S.A. The titer of the antiserum was determined as in our previous reports [2]. The serum used in the present experiment had a titer of 1:1,000,000 as defined by a final

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dilution of the antiserum required to bind 50% of added ^{125}I labeled bovine 32-kDa inhibin. The control serum was obtained from a castrated goat immunized against bovine serum albumin. *In vivo* efficiency of the antiserum was ensured by an increase in plasma concentration of FSH after an *i.v.* injection of the antiserum, and described previously [29].

Bioassay of inhibin: *In vitro* efficiency of the inhibin antiserum was measured by neutralization of the suppression of the ovarian homogenate on the secretion of FSH from cultured rat anterior pituitary cells. Bioactivity of inhibin was determined using an assay for suppression of FSH release from cultured cells of rat anterior pituitary as reported previously [33]. A partially purified bovine follicular fluid (bFF) inhibin preparation obtained after immunoaffinity chromatography (AF-BI) was used as a reference standard for bioassay [23]. Ovarian homogenates from guinea pigs were assayed using 5 doses, in quadruplicate, to generate FSH-inhibition curves.

Unilateral and bilateral ovariectomy: Fifteen guinea pigs in which the progesterone-containing tubes were removed were then ovariectomized unilaterally on the left side or bilaterally by the dorsal route under ether anesthesia. The control animals were sham-operated. Blood samples were collected through the inserted catheter at 0, 6, 12, 24 and 48 hr after operations. In the unilaterally ovariectomized animals, specific phases of the estrous cycle were determined by daily inspection of the vaginal membrane and vaginal cytology. On the day when an influx of leukocytes was observed to follow the smear that consisted predominantly of nucleated epithelial cells, animals were sacrificed by an overdose of ether (from 6 to 7 days after the surgery) and the remaining ovary was removed for counting the number of corpora lutea.

Passive immunization against inhibin: In the immunoneutralization experiment, ten guinea pigs carrying progesterone-containing tubes were randomly located to two groups (5 animals per each group), and injected through the cannula with 1.0 m*l* non-immune goat serum (NGS, control group) or 1.0 m*l* inhibin antiserum (immunized group). Blood samples were collected through the inserted catheter at 0, 6, 12, 24, 48 hr after injection. One ovary from each animal was randomly selected at 48 hr after injection for quantitative histological studies. Plasma samples were stored at –20°C until assayed for FSH.

Quantitative histology: Ovaries were immediately removed from animals and fixed in 4.0% paraformaldehyde solution at room temperature overnight. After fixation, the ovaries were sectioned as reported previously [29]. Serial sections of 10 μm thickness were prepared and stained with hematoxylin and eosin (HE).

Radioimmunoassays (RIA) for immunoreactive (ir-) inhibin and FSH: Plasma concentrations of ir-inhibin were measured in triplicate using a rabbit antiserum against bovine inhibin (TNDH-1) and ^{125}I-labeled 32 kDa bovine inhibin (bFF 32 kDa inhibin), as described previously [13]. Partially purified bFF inhibin was used for immunization in an adult castrated Japanese white rabbit. The inhibin antiserum (TNDH-1) showed no significant cross-reaction with LH, FSH and prolactin of rats, cattle and sheep, gonadotropin release hormone (GnRH), transforming growth factor or activin, whereas the antiserum cross reacts with bovine free inhibin α-subunit [13].

Concentrations of plasma FSH were measured by heterologous double-antibody RIA using a NIDDK RIA kit for rat FSH as described previously [29, 30]. The iodinated preparation was FSH-I-5, and the antiserum used was anti-rat FSH-S-11. Results were expressed in terms of NIDDK rat FSH-RP-2.

Statistics: All data were expressed as mean ± SEM. Plasma concentrations of FSH and ir-inhibin in Figures 1 and 4 were expressed as mean percent to the initial values of each group. Changes in plasma concentrations of FSH and inhibin after unilateral and bilateral ovariectomies and changes in plasma concentrations of FSH during passive immunization against inhibin were analyzed using a two-way ANOVA, with treatment and sampling time as the two factors, and followed by Duncan's Multiple Range test [30]. The differences between/among groups in ovulation rates after unilateral ovariectomy and the number of follicles after passive immunization against inhibin were analyzed using one-way ANOVA followed by Duncan's Multiple Range test [30]. A value of $P<0.05$ was considered to be statistically significant.

RESULTS

Effects of ovariectomy on the plasma concentrations of FSH and ir-inhibin, and ovulation: In sham-ovariectomized guinea pigs, removal of progesterone implant produced rapid follicular development accompanied by an increase in plasma ir-inhibin and elicited ovulation within 6 days. The plasma concentration of ir-inhibin in unilateral ovariectomized guinea pigs temporarily declined only at 6 hr ($P<0.05$, compared with the value of sham-ovariectomized guinea pigs) and thereafter remained comparable to those in control sham-ovariectomized animals (Fig. 1a). Plasma FSH levels were consistently higher in unilateral ovariectomized guinea pigs than in control animals, but significant differences in plasma FSH levels between these guinea pigs were observed only at 6 hr (Fig. 1b). Bilateral ovariectomy resulted in an abrupt decrease in plasma ir-inhibin (Fig. 1a) and a steadily increase in plasma FSH (Fig. b). The significant differences in plasma FSH were observed at 24 and 48 hr after the surgery.

The mean number of corpora lutea per ovary (±SEM) was 1.4 ± 0.1 (n=5) in control sham-ovariectomized guinea pigs and 3.0 ± 0.2 (n=5) in unilateral ovariectomized animals on 6 to 7 days after surgeries. A significant difference in the mean number of corpora lutea was evident between the two groups.

Bioassay of inhibin activity and in vitro efficiency of inhibin antiserum: Addition of serial dilutions of guinea pig ovarian homogenates gave a dose response suppression of

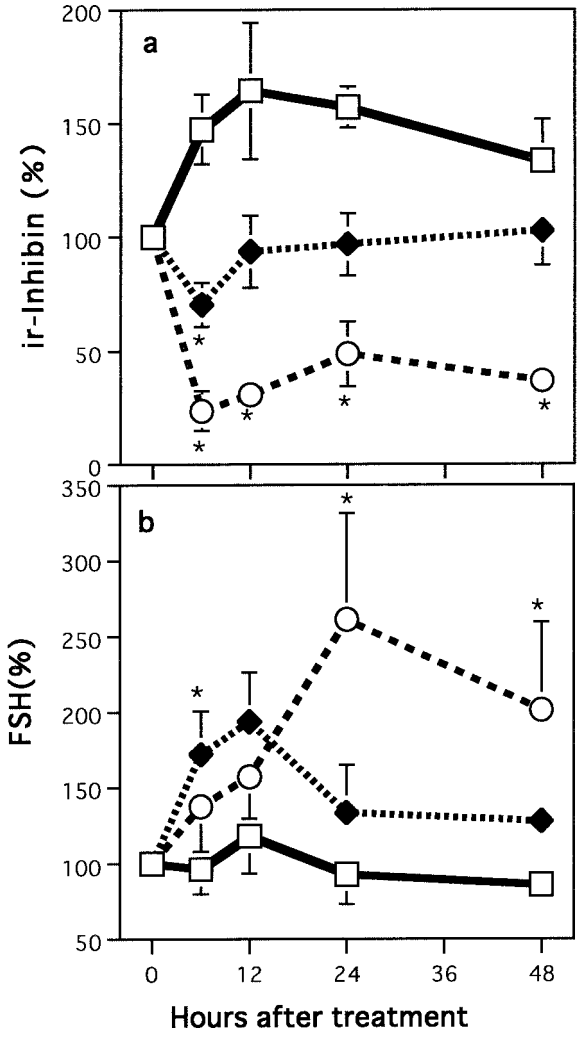

Fig. 1. Effects of unilateral (◆) on bilateral (○) ovariectomies and sham operation (□) on the plasma concentrations of immunoreactive (ir-) inhibin (a) and FSH (b). Each value represents the mean ± SEM percent to the initial values of five animals. * P<0.05 compared with the control value.

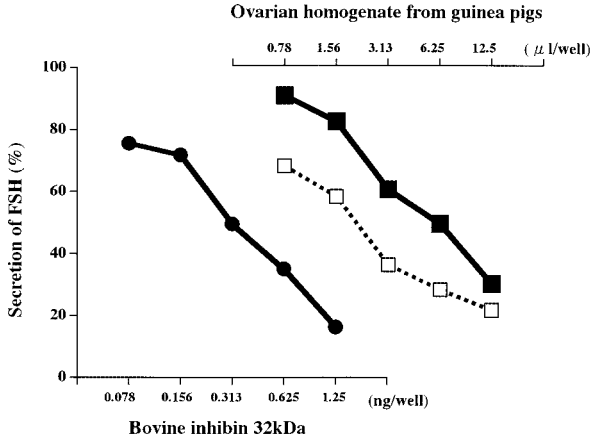

Fig. 2. Dose-response curves of partially purified bovine inhibin (●), ovarian homogenates from adult guinea pigs mixed with 50 μl normal rabbit serum (NGS) (□) or inhibin antisera (■) in the inhibin bioassay. Each value represents the mean of quadruplicate determinations.

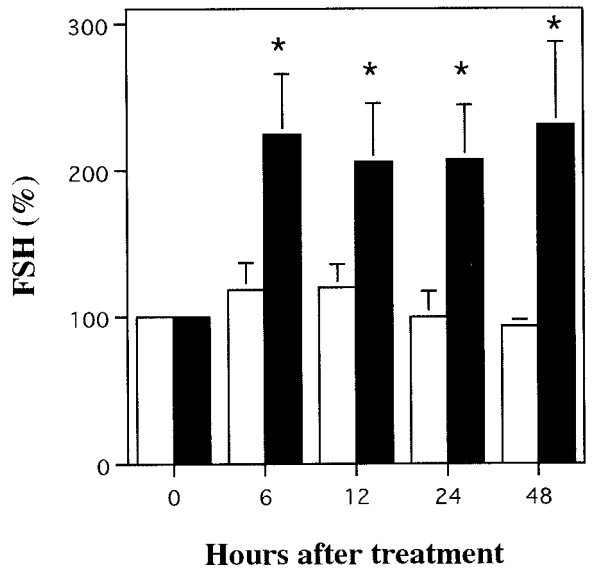

Fig. 3. Effects of a single injection of 1.0 m*l* control serum (□) or inhibin antiserum (■) on plasma concentrations of FSH in female guinea pigs bearing progesterone implant. Values represent mean percent to the initial values of each group ± SEM for 5 animals. * P<0.05 compared with the control value.

FSH secretion from cultured rat anterior pituitary cells and revealed parallel dose-response curves with the bovine inhibin standard (Fig. 2). The maximal suppression of the ovarian homogenate could be reversed by the addition of 50 μl of the inhibin antiserum (Fig. 2).

Effects of passive immunization against inhibin on the plasma concentrations of FSH and follicular development: There were no significant difference in plasma concentrations of FSH between control and passive immunized groups at 0 hr (control: 320.4 ± 76.9 vs passive immunized: 268.7 ± 21.2 *p*g/m*l*). Percentage of plasma concentrations of FSH to initial value in the passive immunized group increased significantly than that in the control group from 6 through 48 hr after a single injection of 1 m*l* inhibin antiserum (Fig. 3). The numbers of small follicles (100–200 μm in diameter) significantly increased at 48 hr after the injection (Fig. 4).

DISCUSSION

This study is the first to describe the effects of unilateral ovariectomy on plasma concentrations of ir-inhibin in guinea pigs. This study also provides the evidences that

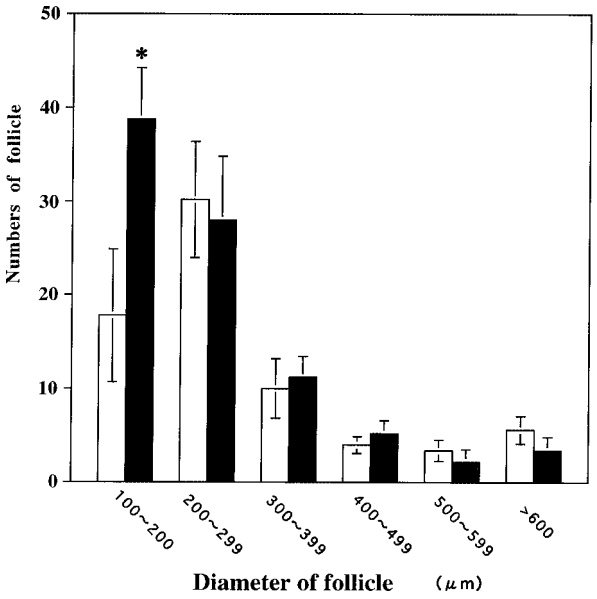

Fig. 4. The size distribution of follicles greater than 100 μm in diameter, in one ovary of five different guinea pigs at 48 hr after injection with 1 m*l* NGS (□) or 1 m*l* inhibin antisera (■). Each value represents the mean ± SEM of 5 animals. * P<0.05 compared with the control value.

inhibin is a major regulator of folliculogenesis through FSH secretion by using passive immunization against inhibin and *in vitro* bioassay in the female guinea pig. In the present study, unilateral ovariectomy induced a transient decline in plasma concentrations of ir-inhibin in guinea pigs. This partial removal of negative feedback at the pituitary causes an increase in circulating FSH. This increase in plasma FSH then results in the recruitment of a new set of follicles and induces expression of inhibin mRNA in the remaining ovary [6]; this presumably causes the subsequent increase in circulating ir-inhibin that inhibits FSH secretion and thereby limits the degree of follicular compensation. While bilateral ovariectomy decreased the plasma ir-inhibin throughout the experimental period and thereafter increased the plasma FSH significantly. These results confirm that the ovary is the main source of inhibin secretion in guinea pigs. Although, it is now beyond question that the gonad is likey to be a major source of inhibin in males and females, circulating ir-inhibin was measurable even after the gonadectomy [9]. Previous reports demonstrated that the adrenal gland is probably a source of ir-inhibin in mammals [4, 10] and avian species [27, 37], though amount of secretion of bioactive inhibin are much less in adrenal glands than gonads. In addition, placenta secrete a large amount of immunoreactive and bioactive inhibin during pregnancy in primates [1, 18, 25].

In the present study, passive immunization against inhibin resulted in an increase in the number of small follicles but not in large follicles 48 hr after the injection of anti-inhibin serum. Progesterone in the implants suppresses the development of large follicles [19, 20, 35] which are the main source of estradiol secretion [31, 32]; and passive immunization against inhibin increases FSH, which result in an increase in the numbers of small follicles. The inhibitory effects of exogenous progesterone on folliculogenesis are demonstrated in cyclic rats through the hypothalamic-pituitary axis, especially via lowering serum LH [34]. The present results concerning with FSH secretion compare favorably with the results of passive immunization against inhibin in other animals, such as rats [26], hamsters [16, 17], cows [14], mares [24] and sheep [21, 36], implying passive immunization against inhibin can induce superovulation in guinea pigs. Inhibin has the ability to suppress FSH secretion by directly inhibiting expression of the gene encoding the FSH β subunit in the pituitary gland [3, 22]. In the present study, it clearly showed that the FSH secretion from cultured cells of rat anterior pituitary could be suppressed by guinea pig ovarian homogenates and this suppression could be neutralized by the inhibin antiserum, this certainly meant that the inhibin was a suppressor in the regulation of FSH secretion in guinea pigs.

In conclusion, results of the present experiment suggest that in guinea pigs the ovary exerts a limited compensatory function and inhibin is an important factor in the regulation of folliculogenesis through the suppression of FSH secretion. The present results also suggest that immunoneutralization of inhibin may be a useful method to induce multiple ovulation in guinea pigs.

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