Dexamethasone or Triamcinolone Increases Follicular Development in Immature Female Rats

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ABSTRACT—We have previously reported that dexamethasone increased follicle-stimulating hormone (FSH) secretion via suppression of inhibin in immature female rats. In the present study, we investigated the effects of dexamethasone or triamcinolone on follicular development and ovarian functions (estradiol and inhibin secretion) in equine chorionic gonadotropin (eCG)-primed immature female rats. Dexamethasone significantly increased the number of ovulated oocytes in immature female rats treated with 5 i.u. eCG. Serum concentration of FSH in eCG-treated female rats was increased by administration of dexamethasone or triamcinolone, showing the peak value at 9 h after its administration, although the levels of inhibin markedly decreased at that time. Serum concentrations of inhibin and estradiol in eCG-treated female rats increased at 24 h after administration of dexamethasone or triamcinolone. These results demonstrate that dexamethasone or triamcinolone increases FSH secretion, and the excess amount of FSH strongly stimulates follicular development cooperating with exogenous eCG.

Keywords: Dexamethasone, Triamcinolone, Follicle-stimulating hormone, Follicular development, Immature female rat

In humans, the inhibition of sexual functions has often been observed during pathological circumstances accompanied by elevated levels of circulating corticosteroids (such as Cushing's syndrome) and usually disappears after a return to the normal adrenal function (1, 2). Administration of natural or synthetic glucocorticoids have been known to interrupt normal gonadotropin secretion and ovulation in experimental animals (3–6). It has also been reported that glucocorticoids inhibited luteinizing hormone (LH) secretion after female castration or administration of gonadotropin-releasing hormone (7–10). Ovulation may constitute a cyclic inflammatory-like process, and several reports suggest that glucocorticoids, potent steroidal anti-inflammatory drugs, may exert an antiovarulatory effect (11).

In contrast, many clinical reports in obstetrics and gynecology have shown that glucocorticoid therapy improved the menstrual rhythm and ovulatory activity in human polycystic ovarian syndrome (12, 13). Administration of glucocorticoids enhances follicle-stimulating hormone (FSH) release in vitro (14) and increases the pituitary content of FSH (15–17) by selectively increasing FSH β messenger RNA (mRNA). We have also reported that dexa-

methasone increased FSH secretion via suppression of inhibin in immature female rats (18), in addition to the direct effect of dexamethasone on FSH synthesis in gonadotrophs. The increase of FSH secretion by corticosteroids has been proposed to be a possible cause for the restoration of ovulation in polycystic ovarian syndrome (19).

It is clear that glucocorticoids enhance FSH secretion, but many reports show the inhibition of ovarian functions after administration of glucocorticoids (3–6). There are no data showing that the increase of FSH secretion caused by glucocorticoids affects follicular development and ovarian functions in female rats. In the present study, we investigated the follicular development and ovarian functions (estradiol and inhibin secretion) in response to the excess amount of FSH induced by dexamethasone or triamcinolone in equine chorionic gonadotropin (eCG)-primed immature female rats.

MATERIALS AND METHODS

Animals and treatments

Immature female Wistar-Imamichi strain rats were obtained from the Institute for Animal Reproduction (Ibaraki). Rats were housed under controlled temperature and lighting conditions and supplied with food and water

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ad libitum. Immature female rats were used on 26 days of age throughout this study. Dexamethasone (9 α -fluoro-16 α -methylprednisolone; Sigma, St. Louis, MO, USA) or triamcinolone acetonide (Sigma) was dissolved in 0.2 ml sesame oil containing 10% dimethyl sulfoxide (vehicle) and subcutaneously injected at 8:00 h. Blood samples (1.5 ml) were drawn via the abdominal aorta under ether anesthesia. In a preliminary study, we confirmed that administration of sesame oil containing 10% dimethyl sulfoxide did not affect serum concentrations of FSH, LH, estradiol and inhibin.

Effects of dexamethasone on ovulation

To examine the effects of dexamethasone on ovulation in immature female rats, 4 to 6 rats of each group were subcutaneously injected with eCG (5 or 10 i.u.) and dexamethasone (500 μ g/rat) or vehicle at 8:00 h on 26 days of age. Human chorionic gonadotropin (hCG; 10 i.u.; Sankyo Zoki Co., Tokyo) dissolved in 0.2 ml saline was injected intravenously under ether anesthesia at 17:00 h the next day. Animals were killed by decapitation at 11:00 h at 28 days of age, and the oviducts were examined for oocytes.

Effects of dexamethasone or triamcinolone on gonadotropin, inhibin and estradiol secretion

We have previously reported that dexamethasone increased FSH secretion via suppression of inhibin, and the levels of FSH reached a peak at 9 h after its administration (18). To examine the follicular development in response to the excess amount of FSH induced by dexamethasone, blood samples were drawn from 6 rats of each group under ether anesthesia at 9 and 24 h after injection of dexamethasone (500 μ g/rat) or vehicle. Serum was collected and stored frozen at -20°C until assay for FSH, LH, inhibin and estradiol.

Several reports suggest that endogenous glucocorticoids (cortisol and corticosterone) and dexamethasone increase FSH secretion, but there are few data showing an increase of FSH secretion induced by any other synthetic glucocorticoid such as triamcinolone. To examine the effects of triamcinolone, another synthetic glucocorticoid, on ovarian function, blood samples were drawn from 6 rats of each group under ether anesthesia at 24 h after injection of triamcinolone (2 mg/rat) or vehicle. Blood was centrifuged, and the supernatant was collected and stored frozen at -20°C until assay for inhibin and estradiol.

Effects of dexamethasone or triamcinolone on gonadotropin secretion

To examine the effects of dexamethasone and triamcinolone on gonadotropin secretion, six animals of each group were injected s.c. with dexamethasone (500 μ g/rat), triamcinolone (2 mg/rat) or vehicle. Blood samples were drawn

under ether anesthesia at 0, 3, 6, 9, 12 and 24 h after their administration. Blood was centrifuged and the supernatant was collected and stored frozen at -20°C until assay for LH and FSH.

Radioimmunoassay (RIA)

Concentrations of LH and FSH were measured using National Institute of Diabetes and Digestive and Kidney Disease (NIDDK) rat RIA kits for rat LH and FSH. Hormones for iodination were rat LH-I-7 and rat FSH-I-7. The antisera used were anti-rat LH-S-10 and anti-rat FSH-S-11. The results are expressed in terms of NIDDK rat LH-RP-2 and FSH-RP-2. The intra- and inter-assay coefficients of variation were 5.5% and 8.9% for LH and 4.3% and 10.3% for FSH, respectively.

Inhibin (20) and estradiol (21) were measured by double-antibody RIAs using ^{125}I -labeled radioligands as described previously. The intra- and inter-assay coefficients of variation were 3.7% and 6.4% for inhibin and 6.2% and 7.4% for estradiol, respectively.

Statistical analyses

The percentages of females showing ovulation were compared using the chi-square test and Fisher's exact probability test. All results except for frequency of ovulation are expressed as the mean $\pm$ S.E.M. and were analyzed using one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) test; a value of $P < 0.05$ was considered significant.

RESULTS

Effects of dexamethasone on ovulation

Dexamethasone (500 μ g/rat) induced ovulation in 5/6 rat (83.3%) treated without eCG, while 2/5 rats (40%) treated with vehicle and no eCG showed ovulation (Fig. 1a). There is no significant difference in the ratio of ovulation induced by hCG between the control and the dexamethasone-treated group. Ovulation was confirmed in all female rats treated with 5 or 10 i.u. eCG (Fig. 1a). The number of oocytes in the oviduct was increased by eCG (0, 5 or 10 i.u.) in a dose-related manner. Dexamethasone significantly increased the number of ovulated oocytes in rats treated with 5 i.u. eCG, although it did not affect the number of oocytes from rats treated with 0 or 10 i.u. eCG (Fig. 1b). Data shown in Fig. 1b indicate the average values calculated from only ovulated oocytes.

Effects of dexamethasone or triamcinolone on gonadotropin, inhibin and estradiol secretion

Dexamethasone decreased serum concentration of inhibin and increased serum concentration of FSH at 9 h after dexamethasone administration in rats treated with eCG

Fig. 1. Effects of dexamethasone (Dex) on the ratio of ovulation (a) and the number of oocytes (b) in eCG-primed immature female rats. Animals were treated with eCG (0, 5 or 10 i.u.) and dexamethasone (500 μ g/rat) at 8:00 h at 26 days of age. hCG (10 i.u.) was injected intravenously under ether anesthesia at 17:00 h on the day after dexamethasone treatment. Animals were killed by decapitation at 11:00 h at 28 days of age, and the oviducts were examined for oocytes. * $P < 0.05$, compared with the control, analyzed using one-way ANOVA followed by Fisher's PLSD test.

Fig. 2. Effects of dexamethasone (Dex) on serum concentrations of FSH (a), inhibin (b), LH (c) and estradiol (d) in eCG-primed immature female rats. The level of each hormone was measured at 9 and 24 h after dexamethasone (500 μ g/rat) administration. * $P < 0.05$, compared with the control, analyzed using one-way ANOVA followed by Fisher's PLSD test.

(Fig. 2: a and b). In contrast, serum concentration of inhibin increased at 24 h after its administration, although serum concentration of FSH did not change at the same time point in dexamethasone-treated rats (Fig. 2: a and b). Serum concentration of LH did not change as a result of dexamethasone-treatment (Fig. 2c). Serum concentration

of estradiol increased at 24 h after dexamethasone administration, although it did not change at 9 h after dexamethasone-treatment (Fig. 2d).

Serum concentrations of inhibin and estradiol significantly increased at 24 h after administration of 2 mg triamcinolone (Fig. 3: a and b).

Fig. 3. Effects of triamcinolone (TA) on serum concentrations of inhibin (a) and estradiol (b) in eCG-primed immature female rats. The level of each hormone was measured at 24 h after triamcinolone (2 mg/rat) administration. * $P < 0.05$, compared with the control, analyzed using one-way ANOVA followed by Fisher's PLSD test.

Fig. 4. Effects of dexamethasone (Dex) (a) or triamcinolone (TA) (b) on serum concentration of FSH in eCG-primed immature female rats. The levels of FSH were measured at 0, 3, 6, 9, 12 and 24 h after administration of dexamethasone (500 μ g/rat) or triamcinolone (2 mg/rat). * $P < 0.05$, compared with the control, analyzed using one-way ANOVA followed by Fisher's PLSD test.

Effects of dexamethasone or triamcinolone on gonadotropin secretion

Serum concentration of FSH markedly increased as a result of administration of dexamethasone or triamcinolone and showed the peak values at 9 h after glucocorticoid injection. The increased level of FSH was reduced to the control level by 24 h after administration of dexameth-

asone or triamcinolone (Fig. 4: a and b). Serum concentration of LH did not change at any time point after administration of dexamethasone or triamcinolone (data not shown).

DISCUSSION

Administration of glucocorticoids have been reported to enhance FSH release in vitro (14) and to increase the pituitary content of FSH (15–17) by selectively increasing FSH β messenger RNA (mRNA). We have already reported that, in addition to the direct effect of dexamethasone on FSH synthesis in gonadotrophs, dexamethasone increased FSH secretion via suppression of inhibin in immature female rats (18). In the present study, follicular development and ovarian functions in response to the increased level of FSH induced by dexamethasone were investigated using eCG-primed immature female rats. Dexamethasone slightly increased the ratio of ovulation in immature female rats treated without eCG (Fig. 1a), but there is no significant difference in the ratio of ovulation between the control (40%) and the dexamethasone-treated group (83.3%). Ovulation was confirmed in all female rats treated with 5 or 10 i.u. eCG (Fig. 1a), and dexamethasone caused superovulation in female rats treated with 5 i.u. eCG. These results suggest that dexamethasone stimulates follicular development in immature female rats.

Serum concentration of inhibin decreased and serum concentration of FSH increased in rats treated with eCG at 9 h after administration of dexamethasone (Fig. 2). These results are similar to our previous observations (18). This effect of dexamethasone is probably due to its direct action on ovarian functions because the presence of glucocorticoid receptor in the ovary has been demonstrated (22). On the other hand, serum concentrations of inhibin and estradiol increased in eCG-treated rats at 24 h after dexamethasone administration (Fig. 2). Triamcinolone also in-

creased the serum levels of inhibin and estradiol in eCG-treated rats at 24 h after its injection (Fig. 3). Serum concentration of FSH increased in eCG-treated rats after administration of dexamethasone or triamcinolone, and it showed the peak value at 9 h after the drug administration (Fig. 4). The increased levels of FSH were reduced to control level by 24 h after the drug injection. These results suggest that dexamethasone or triamcinolone increased the serum levels of FSH, and the excess amount of FSH stimulated follicular development by cooperating with exogenous eCG. As a result of the accelerated follicular development induced by glucocorticoid administration, superovulation and hypersecretion of inhibin and estradiol were observed. In our previous study, we demonstrated that dexamethasone inhibited ovarian functions, especially, inhibin secretion, and increased the serum levels of FSH until 9 h after glucocorticoid administration (18). In the present study, we have shown that glucocorticoids such as dexamethasone or triamcinolone have two different actions on ovarian functions depending on the time after its administration. In the early phase of glucocorticoid action, it increases FSH secretion mediated by suppression of inhibin, and this increased level of FSH stimulates inhibin and estradiol secretion from granulosa cells in the later phase of glucocorticoid actions.

It has been reported that administration of progesterone at 24 h after eCG-treatment in immature female rats advances the preovulatory gonadotropin surge and ovulation by 24 h (23). Brann et al. reported that deoxycorticosterone and triamcinolone, like progesterone, were able to advance the gonadotropin surge and ovulation in eCG-primed rats (19), although they did not demonstrate ovarian functions as a result of glucocorticoid injection. The animal model was different from our experiment, but this is the only report that mentioned the positive effects of glucocorticoids on ovulation in experiment animals.

In summary, the present study has demonstrated that glucocorticoids such as dexamethasone or triamcinolone have two different actions on ovarian functions depending on the time after its administration. In the early phase of the action, it increases FSH secretion mediated by suppression of inhibin, and the excess amount of FSH strongly stimulate follicular development, cooperating with exogenous eCG in the later phase of the action. As a result, dexamethasone or triamcinolone induces superovulation and hypersecretion of ovarian hormones, inhibin and estradiol. These results suggest that the beneficial effects of glucocorticoids in inducing ovulation in polycystic ovarian syndrome may be due in part to the stimulated follicular development in response to the excess amount of FSH induced by glucocorticoids.

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