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Dexamethasone increases follicle-stimulating hormone secretion via suppression of inhibin in rats

Atsushi Tohei, Hiroshi Kogo *

Department of Pharmacology, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

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Abstract

In the present study, the effects of dexamethasone on follicle-stimulating hormone (FSH) secretion in immature female rats were investigated. Dexamethasone increased the selective secretion of FSH and decreased plasma concentrations of inhibin in immature female rats. The effects of dexamethasone on FSH secretion were not confirmed in rats treated with ovariectomy or immunoneutralization against inhibin. In addition to the direct effect of dexamethasone on FSH synthesis in gonadotrophs, the present study has clearly demonstrated that the increased level of FSH in dexamethasone-treated rats is mediated by suppression of ovarian function, especially by the inhibition of inhibin secretion.

Keywords: Dexamethasone; FSH (follicle-stimulating hormone); Inhibin; Ovarian function; (Rat, female, immature)

1. Introduction

The ability of stress to interfere with reproductive functions in mammals has been long recognized (Selye, 1939; Rivier and Rivest, 1991). Stress-related hormones, for example, corticotropin-releasing hormone (CRH), pro-opiomelanocortin (POMC)-derived peptides and adrenal corticosteroids, can influence reproductive function by acting on the hypothalamic–pituitary–gonadal axis (Rivier and Rivest, 1991). It has been reported that β-endorphin, a major product of the maturation process of POMC, and CRH participate either directly or indirectly in the inhibition of luteinizing hormone-releasing hormone (LHRH) neuronal activity during stress (Rivier and Rivest, 1991; Rivest and Rivier, 1995). Glucocorticoids secreted from adrenal glands have also been known to interrupt normal gonadotropin secretion. Several reports have shown that glucocorticoids block the postorchidectomy rise in serum luteinizing hormone (LH) and inhibit LH release in response to LHRH (Baldwin, 1979; Ringstrom and Schwartz, 1984, 1985, 1987). In contrast, glucocorticoid treatment significantly enhances follicle-stimulating hormone (FSH) release in vitro (Baldwin et al., 1991) and increases the pituitary content of FSH (Ringstrom et al., 1991; McAndrews et al., 1995; Kilen et al., 1996) by selectively increasing FSHβ messenger RNA (mRNA). The increases in FSHβ mRNA and protein levels were confirmed in the presence of an LHRH antagonist (Ringstrom et al., 1992; McAndrews et al., 1994, 1995), suggesting that glucocorticoids directly affect the pituitary rather than the hypothalamus.

The secretion of FSH is controlled by, in addition to LHRH and gonadal steroids, the specific regulatory glycoprotein inhibin secreted from granulosa cells in females and from Sertoli cells in males (De Jong, 1988). The presence of glucocorticoid receptors in the ovary has been demonstrated (Schreiber et al., 1982), and glucocorticoids are known to inhibit the FSH-induced differentiation of granulosa cells (Schoonmaker and Erickson, 1983) and the secretion of estrogen (Hsueh and Erickson, 1978) and inhibin (Suzuki et al., 1987) in vitro. In addition to females, males also show a decrease in testosterone (Doerr and Pirke, 1976; Bambino and Hsueh, 1981; Tohei et al., 1997) and inhibin (Tohei et al., 1997) secretion after administration of glucocorticoids.

It is clear that glucocorticoids inhibit ovarian function in vitro and selectively increase FSH secretion, but there are no data showing that FSH secretion in vivo is mediated by inhibin after administration of glucocorticoids in female rats. In the present study, we investigated the effects of

* Corresponding author. Tel.: +81-426-76-4526; fax: +81-426-76-4526.

E-mail address: kogo@ps.toyaku.ac.jp (H. Kogo)

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dexamethasone on FSH secretion in vivo in immature female rats and whether or not the increased levels of plasma FSH are always mediated by the levels of inhibin after dexamethasone administration.

2. Materials and methods

2.1. Animals and treatments

Immature female Wistar–Imamichi strain rats were obtained from the Institute for Animal Reproduction (Ibaraki, Japan). Rats were housed under controlled temperature and lighting conditions and supplied with food and water ad libitum. Twenty-six-day-old rats were used throughout this study. This age was chosen because inhibin levels are high enough and endogenous inhibin plays a physiological role at this age (Rivier and Vale, 1987). Dexamethasone (9α-fluoro-16-methylprednisolone, Sigma, St. Louis, MO) was dissolved in 0.2 ml sesame oil containing 10% dimethyl sulfoxide and injected s.c. at 0800 h. In a preliminary study, we confirmed that administration of sesame oil containing 10% dimethyl sulfoxide does not affect plasma concentrations of FSH, LH, estradiol and inhibin.

2.2. Antiserum against inhibin

Inhibin antiserum obtained from a castrated goat immunized against [Tyr^{30}] inhibin α(1–30) conjugated to rabbit serum albumin was provided by Dr. Taya (Laboratory of Veterinary Physiology, Tokyo University of Agriculture and Technology). Biological activity testing of the inhibin antiserum showed that a single i.v. injection of six doses (6.25–200 μl) of the antiserum at 1100 h on the day of metestrus and diestrus in adult female rats increased plasma concentrations of FSH in a dose-related manner within 6 h after the injection. Human-transforming growth factor β and activin showed no cross-reaction with inhibin antiserum (Kishi et al., 1995; Arai et al., 1996b). The control serum was obtained from a castrated male goat immunized against bovine serum albumin.

2.3. Effects of dexamethasone on gonadotropin secretion

To examine the effects of dexamethasone on gonadotropin secretion in vivo, five animals of each group were anesthetized with ether, and blood samples (1.5 ml) were drawn via the portal vein at 0, 3, 6, 9, 12 and 24 h after administration of dexamethasone (50 or 500 μg/0.2 ml sesame oil). Then, animals were killed by decapitation, and anterior pituitary glands were separated from the neural lobe and weighed. The pituitary glands were homogenized in 1 ml 0.05 M phosphate-buffered saline (PBS) at 4°C. The supernatant and plasma were collected and stored frozen at −20°C until assay for LH and FSH.

2.4. Effects of dexamethasone on inhibin and estradiol secretion

To examine the effects of dexamethasone on ovarian function, blood samples (1.5 ml) were drawn from six animals of each group under ether anesthesia 6 and 9 h after injection of dexamethasone (500 μg) or vehicle (sesame oil containing 10% dimethyl sulfoxide). Then, animals were killed by decapitation, and ovaries were removed, weighed and homogenized in 2 ml 0.05 M PBS at 4°C. The supernatant and plasma were collected and stored frozen at −20°C until assayed for estradiol and inhibin.

2.5. Effects of dexamethasone on FSH secretion in ovariectomized rats

To examine the effects of dexamethasone on FSH secretion mediated by ovarian factors, 5 animals of each group were ovariectomized 3 days before the experiment. Blood samples (1.5 ml) were drawn from ovariectomized rats under ether anesthesia 9 h after injection of dexamethasone (500 μg) or vehicle (sesame oil containing 10% dimethyl sulfoxide). Blood was centrifuged and the supernatant was collected and stored frozen at −20°C until assay for FSH.

2.6. Effects of dexamethasone on FSH secretion in inhibin immunized rats

To examine the effects of dexamethasone on FSH secretion mediated by inhibin, six animals of each group were given an i.v. injection of 200 μl inhibin antiserum or normal goat serum 1 h before administration of dexamethasone (500 μg). Blood samples (1.5 ml) were drawn under ether anesthesia 9 h after injection of dexamethasone (500 μg) or vehicle (sesame oil containing 10% dimethyl sulfoxide). Blood was centrifuged and the supernatant was collected and stored frozen at −20°C until assay for FSH.

2.7. Radioimmunoassay (RIA)

Concentrations of LH and FSH were measured using National Institute of Diabetes and Digestive and Kidney Disease (NIDDK) rat radioimmunoassay (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 (Hamada et al., 1989) and estradiol (Taya et al., 1985) were measured by double-antibody RIAs using ^{125}I-labelled 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.

2.8. Statistical analyses

All results are expressed as the means ± 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.

3. Results

3.1. Effects of dexamethasone on gonadotropin secretion

Plasma concentrations of FSH were markedly increased as a result of administration of dexamethasone and showed peak values 9 h after injection of both doses (50 and 500 μg). The increased levels of FSH were restored to control levels by 24 h after injection of dexamethasone (Fig. 1a). The pituitary content of FSH was not changed by administration of dexamethasone, though it was significantly increased 9 h after injection of 50 μg dexamethasone (Fig. 1b). Plasma concentrations of LH were suppressed after administration of dexamethasone and the values were significantly lower at 3 and 9 h in the 500 μg dexamethasone-treated groups compared to the control group (Fig. 2a). The pituitary content of LH was not changed at any time point after administration of dexamethasone (Fig. 2b).

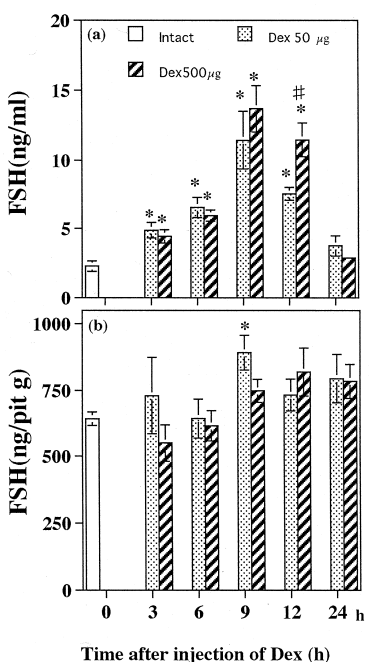

Fig. 1. Effects of dexamethasone (Dex) on plasma concentration (a) and pituitary content (b) of FSH in immature female rats. The levels of FSH were measured 3, 6, 9, 12 and 24 h after a single s.c. administration of 50 or 500 μg dexamethasone. * Indicates $P < 0.05$ compared with control and # indicates $P < 0.05$ compared with 50 μg dexamethasone-treated groups analyzed using one-way ANOVA followed by Fisher's PLSD test.

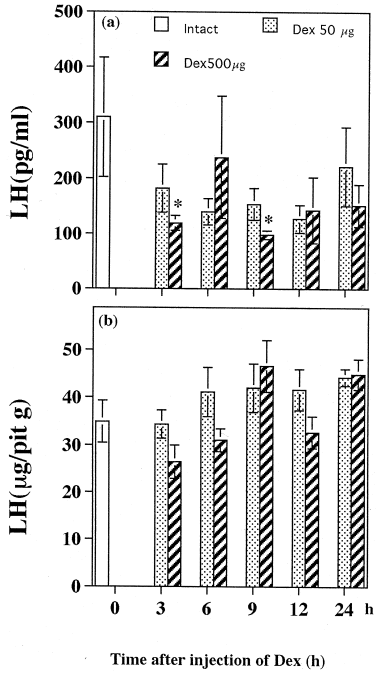

Fig. 2. Effects of dexamethasone (Dex) on plasma concentration (a) and pituitary content of LH (b) in immature female rats. The levels of LH were measured 3, 6, 9, 12 and 24 h after a single s.c. administration of 50 or 500 μg dexamethasone. * Indicates $P < 0.05$ compared with the control group analyzed using one-way ANOVA followed by Fisher's PLSD test.

3.2. Effects of dexamethasone on inhibin and estradiol secretion

Plasma concentrations of inhibin significantly decreased in dexamethasone-treated groups at both time points (6 and

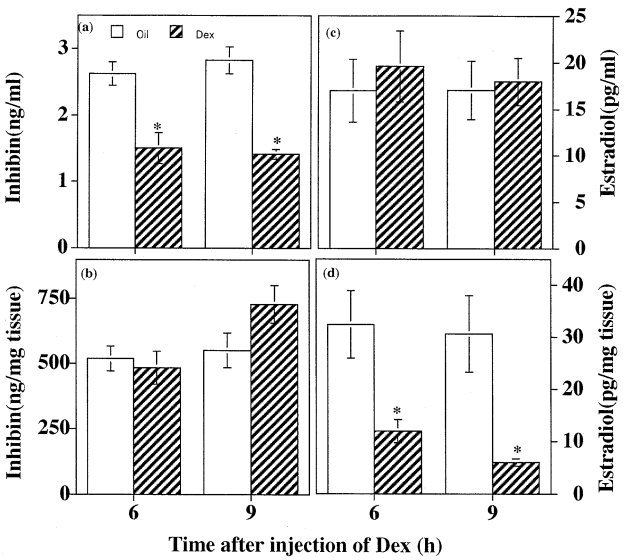

Fig. 3. Effects of dexamethasone (Dex) on the levels of inhibin in plasma (a) and ovary (b) and on the levels of estradiol in plasma (c) and ovary (d) in immature female rats. The levels of inhibin and estradiol were measured 6 and 9 h after a single s.c. administration of 500 μg dexamethasone or vehicle. * Indicates $P < 0.05$ compared with control analyzed using one-way ANOVA followed by Fisher's PLSD test.

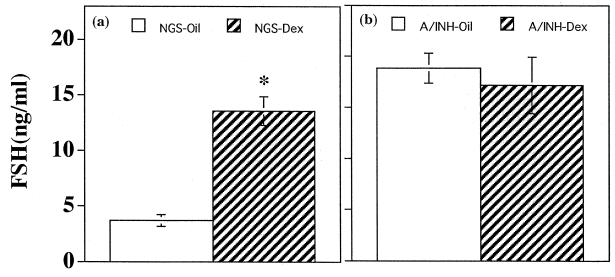

Fig. 4. Effects of dexamethasone (Dex) on plasma concentrations of FSH in immature female rats pretreated with normal goat serum (NGS) (a) or inhibin antiserum (A/INH) (b). Injection of normal goat serum or inhibin antiserum was carried out 1 h before dexamethasone treatment. The levels of plasma FSH were measured 9 h after a single s.c. administration of sesame oil or 500 μg dexamethasone. * Indicates $P < 0.05$ compared with respective control analyzed using one-way ANOVA followed by Fisher's PLSD test.

9 h after dexamethasone treatment) compared to the control groups (Fig. 3a). The ovarian content of inhibin did not change at 6 h after injection of dexamethasone (dexamethasone did not affect ovarian weight), but increased at 9 h after the treatment compared to the control, though not statistically significant (Fig. 3b). There was no significant effect of dexamethasone on plasma concentrations of estradiol (Fig. 3c). The ovarian content of estradiol was significantly suppressed in dexamethasone-treated groups at both time points compared to the control groups (Fig. 3d).

3.3. Effects of dexamethasone on FSH secretion in ovariectomized rats

Basal concentrations of FSH were markedly increased in ovariectomized rats (54.7 ± 1.83 ng/ml) compared to those of ovary-intact rats (Fig. 1a; 2.28 ± 3.68 ng). The increased levels of plasma FSH in ovariectomized rats were not further enhanced as a result of dexamethasone administration (50.46 ± 3.84 ng/ml).

3.4. Effects of dexamethasone on FSH secretion in inhibin immunized rats

Plasma concentrations of FSH increased as a result of dexamethasone administration in normal goat serum-pretreated immature female rats (Fig. 4a). Injection of inhibin antiserum to the intact rat increased plasma concentrations of FSH. However, the increased levels of plasma FSH induced by passive immunization against inhibin were not enhanced in the dexamethasone-treated rats pretreated with inhibin antiserum (Fig. 4b).

4. Discussion

In the present study, administration of dexamethasone increased FSH secretion in immature female rats. In contrast, the secretion of LH decreased as a result of dexamethasone treatment. Several reports have shown that cortisol (Suter and Schwartz, 1985) and corticosterone (Baldwin et al., 1991) enhance FSH release in vitro, and that corticosterone selectively increases FSHβ-subunit mRNA in vitro (McAndrews et al., 1994, 1995; Kilen et al., 1996). These results also support our in vivo results in the present study.

Plasma concentrations of inhibin significantly decreased in dexamethasone-treated rats, but in contrast to plasma concentrations, the ovarian content of inhibin did not change at 6 h and increased at 9 h after the injection, though not statistically significantly (Fig. 3). Plasma concentrations of estradiol were not changed, but the ovarian content of estradiol decreased as a result of dexamethasone injection. These results suggest that dexamethasone inhibits ovarian function in terms of inhibin secretion and estradiol synthesis, and the duration of the effect of dexamethasone on inhibin secretion is not long compared to that on estradiol synthesis (Fig. 3). Previous studies of the effects of dexamethasone on ovarian function have shown that it inhibits the FSH-induced differentiation of granulosa cells (Schoonmaker and Erickson, 1983) and the secretion of estrogen (Hsueh and Erickson, 1978) and inhibin (Suzuki et al., 1987) in vitro. The presence of glucocorticoid receptors in the ovary has also been demonstrated (Schreiber et al., 1982). However, few data have shown the effects of dexamethasone on ovarian function in vivo. In the present study, it was clearly demonstrated that ovarian function was inhibited as a result of administration of dexamethasone. The reason why the plasma concentrations of estradiol did not change after the administration of dexamethasone is probably that the plasma concentrations of estradiol in about 25- to 29-day-old female rats are very low by nature (Dohler and Wuttke, 1975) and that the decreased levels of ovarian estradiol hardly reflect its plasma concentrations.

To clarify the effects of dexamethasone on FSH secretion in vivo mediated by inhibin in immature female rats, ovariectomized and inhibin-immunized rats were used in the present study. Both ovariectomy and immunoneutralization against inhibin increased plasma concentrations of FSH, but the effects of dexamethasone on FSH secretion disappeared in ovariectomized and inhibin-immunized rats. These results suggest that dexamethasone increases FSH secretion mediated by inhibition of inhibin secretion. The secretion of FSH is controlled by the specific regulator inhibin secreted from granulosa cells in female rats (De Jong, 1988) and immunoneutralization against inhibin markedly increases plasma concentrations of FSH in adult female rats (Sander et al., 1991; Arai et al., 1996a). In immature rats, plasma concentrations of inhibin are very low before 5 days of age, and then progressively increase until day 17, when they show an abrupt rise (Rivier and Vale, 1987). On days 20 to 30, inhibin values are not significantly different from those of adult females, and the

immunoneutralization of endogenous inhibin markedly increases plasma inhibin levels, though its immunoneutralization has no effect on FSH secretion in 10 day old female rats (Rivier and Vale, 1987). In contrast to inhibin, the physiological role of estradiol in modulating FSH secretion is not important during the estrous cycle of rats (Arai et al., 1996a,b). It has been reported that plasma concentrations of FSH do not increase in adult female rats treated with immunoneutralization against estradiol at any stage of the cycle (Arai et al., 1996a). Dexamethasone treatment did not affect plasma concentrations of estradiol in the present study, suggesting that endogenous estradiol in 26-day-old female rats is not important in increasing plasma concentrations of FSH as a result of dexamethasone administration.

In summary, the present study has clearly demonstrated that dexamethasone selectively increases in vivo secretion of FSH in immature female rats. The increased levels of FSH in dexamethasone-treated rats are mediated by suppression of ovarian function, especially by the inhibition of inhibin secretion, in addition to the direct effect of dexamethasone on FSH synthesis in gonadotrophs.

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References

Arai, K., Watanabe, G., Taya, K., Sasamoto, S., 1996a. Effects of passive immunization against estradiol on LH and FSH in the diestrous female rat. J. Reprod. Dev. 42, 185–191.

Arai, K., Watanabe, K., Taya, K., Sasamoto, S., 1996b. Roles of inhibin and estradiol in the regulation of follicle-stimulating hormone and luteinizing hormone secretion during the estrous cycle of the rat. Biol. Reprod. 55, 127–133.

Baldwin, D.M., 1979. The effects of glucocorticoids on estrogen-dependent luteinizing hormone release in the ovariectomized rat and on gonadotropin secretion in the intact female rat. Endocrinology 105, 120–128.

Baldwin, D.M., Srivastava, P.S., Krummen, L.A., 1991. Differential actions of corticosterone on luteinizing hormone biosynthesis and release in cultured rat anterior pituitary cells: interactions with estradiol. Biol. Reprod. 44, 1040–1050.

Bambino, T.H., Hsueh, A.J.W., 1981. Direct inhibitory effect of glucocorticoids upon testicular luteinizing hormone receptor and steroidogenesis in vivo and in vitro. Endocrinology 108, 2142–2148.

De Jong, F.H., 1988. Inhibin. Physiol. Rev 68, 555–607.

Doerr, P., Pirke, K.M., 1976. Cortisol-induced suppression of plasma testosterone in normal adult males. J. Clin. Endocrinol. Metab. 43, 622–629.

Dohler, K.D., Wuttke, W., 1975. Changes with age in levels of serum gonadotropins, prolactin, and gonadal steroids in prepubertal male and female rats. Endocrinology 97, 898–907.

Hamada, T., Watanabe, G., Kokuho, T., Taya, K., Sasamoto, S., Hasegawa, Y., Miyamoto, K., Igarashi, M., 1989. Radioimmunoassay of inhibin in various mammals. J. Endocrinol. 122, 697–704.

Hsueh, A.J., Erickson, G.F., 1978. Glucocorticoid inhibition of FSH-induced estrogen production in cultured rat granulosa cells. Steroids 32, 639–648.

Kilen, S.M., Szabo, M., Strasser, G.A., McAndrews, J.M., Ringstrom, S.J., Schwartz, N.B., 1996. Corticosterone selectively increases follicle-stimulating hormone β-subunit messenger ribonucleic acid in primary anterior pituitary cell culture without affecting its half-life. Endocrinology 137, 3802–3807.

Kishi, H., Taya, K., Watanabe, G., Sasamoto, S., 1995. Follicular dynamics and secretion of inhibin and oestradiol-17β during the oestrous cycle of the hamster. J. Endocrinol. 146, 169–176.

McAndrews, J.M., Ringstrom, S.J., Dahl, K.D., Schwartz, N.B., 1994. Corticosterone in vivo increases pituitary follicle stimulating hormone (FSH)-β messenger ribonucleic acid content and serum FSH bioactivity selectively in female rats. Endocrinology 134, 158–163.

McAndrews, J.M., Ringstrom, S.J., Dahl, K.D., Schwartz, N.B., 1995. Effects of corticosterone and testosterone on pituitary gonadotropin content, secretion, bioactivity and messenger RNA levels in the presence or absence of GnRH in male rats. Endocrine 3, 13–20.

Ringstrom, S.J., Schwartz, N.B., 1984. Examination of prolactin and pituitary–adrenal axis components as intervening variables in the adrenalectomy-induced inhibition of gonadotropin response to castration. Endocrinology 144, 880–887.

Ringstrom, S.J., Schwartz, N.B., 1985. Cortisol suppresses the LH, but not the FSH, response to gonadotropin-releasing hormone after orchidectomy. Endocrinology 116, 472–474.

Ringstrom, S.J., Schwartz, N.B., 1987. Differential effect of glucocorticoids on synthesis and secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). J. Steroid Biochem. 27, 625–630.

Ringstrom, S.J., McAndrews, J.M., Rahal, J.O., Schwartz, N.B., 1991. Cortisol in vivo increases FSHβ mRNA selectively in pituitaries of male rats. Endocrinology 129, 2793–2795.

Ringstrom, S.J., Suter, D.E., Hostetler, J.M., Schwartz, N.B., 1992. Cortisol regulates secretion and pituitary content of the two gonadotropins differentially in female rats: effects of gonadotropin-releasing hormone antagonist. Endocrinology 130, 3122–3128.

Rivest, S., Rivier, C., 1995. The role of corticotropin-releasing factor and interleukin-1 in the regulation of neurons controlling reproductive functions. Endocr. Rev. 16, 177–199.

Rivier, C., Rivest, S., 1991. Effect of stress on the activity of the hypothalamic–pituitary–gonadal axis:peripheral and central mechanisms. Biol. Reprod. 45, 523–532.

Rivier, C., Vale, W., 1987. Inhibin: measurement and role in the immature female rat. Endocrinology 120, 1688–1690.

Sander, H.J., Kramer, P., van Leeuwen, E.C., van Cappellen, W.A., Meijs-Roelofs, H.M., De Jong, F.H., 1991. Ovulation rate, follicle population and FSH levels in cyclic rats after administration of an inhibin-neutralizing antiserum. J. Endocrinol. 130, 297–303.

Schoonmaker, J.N., Erickson, G.F., 1983. Glucocorticoid modulation of follicle-stimulating hormone mediated granulosa cell differentiation. Endocrinology 113, 1356–1363.

Schreiber, J.R., Nakamura, K., Erickson, G.F., 1982. Rat ovary glucocorticoid receptor: identification and characterization. Steroids 39, 569–584.

Selye, H., 1939. Effect of adaptation to various damaging agents on the female sex organs in the rats. Endocrinology 25, 615–624.

Suter, D.E., Schwartz, N.B., 1985. Effects of glucocorticoids on responsiveness of luteinizing hormone and follicle-stimulating hormone to

gonadotropin-releasing hormone by male rat pituitary cells in vitro. Endocrinology 117, 855–859.

Suzuki, T., Miyamoto, K., Hasegawa, Y., Abe, Y., Ui, M., Ibuki, Y., Igarashi, M., 1987. Regulation of inhibin production by rat granulosa cells. Mol. Cell. Endocrinol. 54, 185–195.

Taya, K., Watanabe, G., Sasamoto, S., 1985. Radioimmunoassay for progesterone, testosterone and estradiol-17β using ^{125}I-iodohistamine radioligands. Jpn. J. Anim. Reprod. 31, 186–197.

Tohei, A., Tomabechi, T., Mamada, M., Akai, M., Watanabe, G., Taya, K., 1997. Effects of repeated ether stress on the hypothalamic–pituitary–testes axis in adult male rats with special reference to inhibin secretion. J. Vet. Med. Sci. 59, 329–334.