Glucocorticoid counteracts the suppressive effect of tumor necrosis factor- α on the surge of luteinizing hormone secretion in rats

Takashi Matsuwaki, Masatoshi Suzuki, Keitaro Yamanouchi and Masugi Nishihara

Department of Veterinary Physiology, Veterinary Medical Science, The University of Tokyo, Tokyo 113-8657, Japan
(Requests for offprints should be addressed to M Nishihara; Email: amnishi@mail.ecc.u-tokyo.ac.jp)

Abstract

We have previously reported that tumor necrosis factor- α (TNF- α) suppressed pulsatile secretion of luteinizing hormone (LH) in adrenalectomized (ADX) rats, which was restored by replacement of glucocorticoid. In the present study, we examined the role of glucocorticoid in inducing the preovulatory LH surge under conditions of infectious stress. Intravenous injection of TNF- α (1 μ g) into the proestrous rats at 1300 h attenuated the LH surge and decreased the number of oocytes ovulated. The inhibitory effect of TNF- α on the LH surge was blocked by pretreatment with indomethacin, suggesting that the

effects of TNF- α were mediated by prostaglandins (PGs). On the other hand, ADX markedly enhanced the inhibitory effect of TNF- α on the LH surge and subsequent ovulation, which was almost completely restored by pretreatment with a subcutaneous injection of corticosterone (10 mg). These results suggest that glucocorticoid counteracts the inhibitory effect of the cytokines on the preovulatory LH surge by suppressing PG synthesis, and thereby helps to maintain reproductive function under infectious stress conditions.

Journal of Endocrinology (2004) **181**, 509–513

Introduction

Infectious stress exerts a broad influence on the neuroendocrine system, resulting in the activation of the hypothalamic–pituitary–adrenal (HPA) axis and the suppression of the hypothalamic–pituitary–gonadal axis. There are many reports suggesting that the former is the cause of the latter: for example, both corticotropin-releasing hormone (CRH) (Rivier & Vale 1984, Rivier *et al.* 1986, Williams *et al.* 1990) and glucocorticoid (Baldwin 1979, Briski & Sylvester 1994, McGivern & Redei 1994) have been shown to suppress pulsatile luteinizing hormone (LH) secretion in rats and monkeys. We have previously shown, however, that suppression of the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator activity by tumor necrosis factor- α (TNF- α), which is one of the major cytokines responsible for the coordination of host defence mechanisms (Michie *et al.* 1988, Sanna *et al.* 1995, Yoo *et al.* 1997b), was enhanced by α -helical CRH, a CRH receptor antagonist (Yoo *et al.* 1997a). In addition, we have also shown that adrenalectomy (ADX) enhanced the inhibitory effect of TNF- α on pulsatile LH secretion in ovariectomized rats, and that the secretion was completely restored by the replacement of corticosterone (Matsuwaki *et al.* 2003). From these results, we hypothesize that the activation of the HPA axis plays a role in maintaining LH pulses under infectious stress conditions.

In addition to the pulsatile secretion, LH is secreted in a surge fashion during the preovulatory period. These two methods of LH secretion appear to be governed by two intrinsically different neuronal systems – the GnRH pulse generator and the GnRH surge generator respectively (Nishihara *et al.* 1999). In fact, the effects of stress on the LH pulse differ from those on the LH surge, and are affected by sex steroids (Cagampang *et al.* 1991). For example, immobilization (Hashimoto *et al.* 1990, Kam *et al.* 2000) and injection of interleukin-1 (Rettori *et al.* 1991, MohanKumar *et al.* 2002) suppress both the LH pulse and the LH surge, while hypoglycemia inhibits only the LH pulse (Cagampang *et al.* 1990, Kawaguchi *et al.* 1998) in rats. In addition, there are reports demonstrating that glucocorticoid suppresses the LH pulse in rats (Baldwin 1979, Briski & Sylvester 1994) and monkeys (McGivern & Redei 1994), but does not affect the LH surge in rats (Sridaran & Blake 1980) and cows (Dobson & Smith 2000). These observations suggest that stress and/or glucocorticoid may differently affect GnRH pulse versus surge generators. It is therefore necessary to reveal the effects of glucocorticoid on the LH surge in gonad-intact rats under stress conditions in order to understand the physiological significance of glucocorticoid in maintaining reproductive function.

In the present study, to elucidate the role of glucocorticoid in inducing the LH surge under conditions of

infectious stress, we examined the LH surge and subsequent ovulation following TNF- α injection, and the effect of ADX and corticosterone replacement on them. In addition, since it has been shown that prostaglandins (PGs), at least partially, mediate the suppressive effect of TNF- α on LH pulses (Yoo *et al.* 1997a) and that glucocorticoid inhibits PG synthesis (Hirata *et al.* 1987), we also examined the possible involvement of PGs in the effects of TNF- α on the LH surge.

Materials and Methods

Animals

Female rats of the Wistar-Imamichi strain were obtained from the Imamichi Institute for Animal Reproduction (Tsuchiura, Japan). The animals were maintained under controlled lighting conditions (lights on 0500–1900 h) and given free access to food and water. A number of the animals were adrenalectomized (ADX) under ether anesthesia at 8 weeks of age (body weight 230–250 g). For ADX animals, 0.85% saline was given as drinking water. The rats were subjected to the following experiments after at least 1 week of recovery. All of the experiments were carried out at the age of 9 to 11 weeks. The experiments were conducted according to the Guidelines for the Care and Use of Laboratory Animals, Graduate School of Agriculture and Life Sciences, the University of Tokyo.

Experimental procedures

All of the intact ($n=15$) and ADX ($n=15$) rats used in the present study showed at least two successive 4-day estrous cycles, which were confirmed by their vaginal smears. The day before the experiments, a silastic cannula was inserted into the jugular vein under ether anesthesia. The distal end of the cannula was tunneled subcutaneously to the back of the neck. On the day of the experiment, i.e. the day of proestrus, the animals were moved to the experimental room and allowed at least 2 h for adaptation. A blood sample (200 μ l) was withdrawn through the indwelling jugular cannula from freely moving animals without anesthesia at 1-h intervals from 1200 h to 1900 h. After the withdrawal of each blood sample, an equal volume of heparinized saline (10 U/ml), in which erythrocytes were suspended, was replaced through the cannula. At 1200 h, five ADX animals were subcutaneously injected with corticosterone (Wako, Osaka, Japan; 10 mg/0.3 ml sesame oil). At 1300 h, ten ADX animals including those pretreated with corticosterone were administered TNF- α (R & D Systems, Minneapolis, MN, USA; 1 μ g/50 μ l saline) through the indwelling jugular cannula. Ten intact rats were also treated with TNF- α at 1300 h; five of them had been intravenously injected with indomethacin (Cayman Chemical Company, Ann Arbor, MI, USA;

1 mg/100 g body weight), a PG synthesis inhibitor, 10 min before the injection of TNF- α . The remaining five intact and five ADX animals received saline at 1300 h. The collected blood samples were allowed to clot for 1–2 h at room temperature and centrifuged at 5000 g for 15 min. The separated serum was stored at -80°C until assayed. Between 0800 h and 1000 h on the next day, i.e. the day of estrus, the number of oocytes in the ampulla of the oviduct of both sides was counted.

Hormone assay

Serum concentrations of LH and corticosterone were measured with a rat LH RIA kit and a rat corticosterone RIA kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) respectively. The intra- and interassay coefficients of variation for LH assays, which were calculated from five replicated determinations for a pool of rat serum containing 12.9 ng/ml LH, were 4.1% and 5.8% respectively. For corticosterone, the intra- and interassay coefficients of variation, which were calculated from five replicate determinations for a pool of rat serum containing 231.5 ng/ml corticosterone, were 7.4% and 11.8% respectively.

Statistical analysis

The data were statistically analyzed by one-way factorial ANOVA followed by post-hoc comparison of Scheffé's test between the two groups. Differences were considered significant at $P<0.05$.

Results

Changes in serum LH levels in the afternoon of proestrus are shown in Fig. 1. Table 1 shows the LH concentrations at 1200 h and at the peak of the surge, and the number of oocytes in both oviducts the next morning. Serum LH levels at 1200 h were not different among all the experimental groups. When TNF- α was injected into intact rats, the LH surge tended to be suppressed, although not significantly, and the number of oocytes ovulated was significantly decreased. This inhibitory effect of TNF- α on the LH surge was completely blocked by pretreatment with indomethacin, whereas no oocytes were detected in any of the animals pretreated with indomethacin. Although ADX did not affect the LH surge and the number of oocytes ovulated, injection of TNF- α into the ADX animals almost entirely suppressed the LH surge. In addition, four out of the five ADX animals injected with TNF- α had died by the next morning, and no oocytes were observed in the oviduct of the remaining animal. When corticosterone was injected 1 h before TNF- α injection in ADX animals, the LH surge was restored and the number of oocytes in the oviducts was not different

Figure 1 The effect of TNF- α on the spontaneous surge secretion of LH in the afternoon of proestrus in both intact and adrenalectomized (ADX) rats. TNF- α (1 μ g/50 μ l saline) or saline was injected i.v. at 1300 h. Intact rats were injected i.v. with indomethacin (IND, 1 mg/100 g body weight) 10 min before the injection of TNF- α , and ADX rats were injected s.c. with corticosterone (CORT, 10 mg/0.3 ml sesame oil) at 1200 h. Each point and vertical bar represent the mean $\pm$ S.E. ($n=5$).

from that of intact animals. As shown in Fig. 2, the serum corticosterone concentration reached a peak (510.9 ng/ml) 2 h after corticosterone injection in ADX rats; this value was not significantly different from the peak value (439.7 ng/ml) in intact rats following TNF- α injection.

Discussion

In the present study, injection of TNF- α at 1300 h reduced the peak levels of the LH surge, although not significantly, and significantly decreased the number of oocytes ovulated. Since it has been shown that the LH surge in rats is blocked by the injection of compounds that

disrupt neuronal activity during the critical period, which is roughly from 1300 h to 1600 h in the afternoon of proestrus (Everett 1989, Stoker *et al.* 2001), the present result suggests that TNF- α can also decrease the neuronal activity required for the induction of the LH surge. There seems to be a threshold amount of LH secretion needed to induce ovulation, which is about a fourfold increase above the basal LH concentration (Turgeon & Barraclough 1973). The ratio of the peak-to-basal LH concentrations in animals injected with TNF- α was 6.3, while it was 10.2 in control animals in this study. The LH concentration in the TNF- α -injected animals may not have reached the level necessary to induce normal ovulation, although it exceeded the threshold level.

Table 1 Serum LH concentrations in the afternoon of proestrus and the number of oocytes in the oviducts the next morning (estrus). Values are means $\pm$ S.E. ($n=5$)

	Intact	Intact+TNF- α	Intact+IND+TNF- α	ADX	ADX+TNF- α	ADX+CORT+TNF- α
Serum LH (ng/ml)						
At 1200 h	2.1 $\pm$ 0.2 ^a	1.9 $\pm$ 0.2 ^a	2.2 $\pm$ 0.2 ^a	1.8 $\pm$ 0.2 ^a	1.6 $\pm$ 0.2 ^a	2.0 $\pm$ 0.2 ^a
At the peak	19.0 $\pm$ 0.3 ^a	12.4 $\pm$ 2.0 ^b	18.4 $\pm$ 2.0 ^a	18.6 $\pm$ 2.8 ^{a,b}	2.0 $\pm$ 0.1 ^c	18.3 $\pm$ 2.7 ^a
Number of oocytes ovulated	16.2 $\pm$ 0.5 ^a	7.2 $\pm$ 2.1 ^b	0	15.4 $\pm$ 0.9 ^a	NA	14.4 $\pm$ 0.5 ^a

Values with different letters in each line are significantly different ($P<0.05$, ANOVA followed by Scheffe's test). IND, indomethacin; CORT, corticosterone; NA, not available.

Figure 2 Serum concentrations of corticosterone following i.v. injection of TNF- α (1 μ g/50 μ l saline) in intact rats at 1300 h, or s.c. injection of corticosterone (CORT, 10 mg/0.3 ml sesame oil) in ADX rats at 1200 h. Each point and vertical bar represents the mean $\pm$ S.E. ($n=4$).

In the present study, pretreatment with indomethacin almost completely blocked the inhibitory effect of TNF- α on the LH surge. We have previously reported that PGs mediate the suppressive effect of TNF- α on the electrical activity of the hypothalamic GnRH pulse generator (Yoo *et al.* 1997a). Taken together, it is suggested that PGs play a pivotal role in mediating the inhibitory action of TNF- α on the surge as well as on the pulsatile secretion of LH. In spite of the normal LH surge, oocytes could not be detected in either side of the oviducts in the indomethacin-pretreated animals. This is probably due to the direct action of indomethacin on the ovary as reported previously (Shirotta *et al.* 1998).

ADX did not affect the estrous cycle, the LH surge or subsequent ovulation, consistent with a previous report (Sridaran & Blake 1980). However, ADX animals seemed to be much more susceptible to TNF- α because the LH surge was not observed in any of the ADX animals injected with TNF- α , and most of them died by the next morning. Pretreatment with corticosterone almost completely blocked the effect of ADX; all of the animals injected with corticosterone survived with a normal LH surge and ovulation. The serum profile of corticosterone following its injection into ADX animals was similar to that following TNF- α injection into intact animals, indicating that glucocorticoid plays an important role in inducing the LH surge and subsequent ovulation, as well as the LH pulse in ovariectomized rats (Matsuwaki *et al.* 2003), under infectious stress conditions. Although the neuronal systems inducing the LH pulse and surge, respectively, are regarded as intrinsically different (Nishihara *et al.* 1999),

glucocorticoid could counteract the suppressive effects of infectious stress on both systems.

As mentioned above, PGs are involved in mediating the inhibitory effect of TNF- α on the LH surge. On the other hand, glucocorticoid is known to inhibit PG synthesis (Hirata *et al.* 1987). Taken together, the increase in glucocorticoid secretion probably counteracted the cytokine effects by suppressing PG synthesis in the hypothalamus, and thereby restored the LH surge. Contrary to the present results, there are many studies indicating inhibitory effects of glucocorticoid on reproductive processes in rats (Baldwin 1979, Briski & Sylvester 1994, McGivern & Redei 1994). Although further studies are needed to reconcile the contradiction, one of the possible explanations for the difference in the effects of glucocorticoid is the difference in the stressors the animals suffered. Glucocorticoid may preferably interfere with the suppressive effect of stress on reproductive function when the effect of stress is mainly mediated by PGs in the hypothalamus as in the case of infectious stress examined in the present study.

The present study demonstrates that an increase in glucocorticoid levels is a prerequisite for the maintenance of the preovulatory LH surge under infectious stress conditions. Although CRH has been regarded to be inhibitory towards reproductive function (Rivier & Vale 1984, Rivier *et al.* 1986, Williams *et al.* 1990), we have previously shown that intracerebroventricular injection of α -helical CRH enhanced the TNF- α -induced suppression of the GnRH pulse generator (Yoo *et al.* 1997a). In addition, we have also shown that glucocorticoid maintains pulsatile secretion of LH under infectious stress conditions (Matsuwaki *et al.* 2003). Taken together, we conclude that the activation of the HPA axis and the resultant increase in glucocorticoid levels are physiologically involved in the maintenance of reproductive function, at least under conditions of infectious stress.

Funding

This work was supported by a Grant-in-Aid for Scientific Research, the Japan Society for the Promotion of Science (13854007, to M N).

References

- Baldwin DM 1979 The effect 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.
- Briski KP & Sylvester PW 1994 Antagonism of type II, but not type I glucocorticoid receptors results in elevated basal luteinizing hormone release in male rats. *Neuroendocrinology* **60** 601–608.
- Cagampang FR, Maeda K, Yokoyama A & Ota K 1990 Effect of food deprivation on the pulsatile LH release in the cycling and ovariectomized female rat. *Hormone and Metabolic Research* **22** 269–272.

- Cagampang FR, Maeda KI, Tsukamura H, Ohkura S & Ota K 1991 Involvement of ovarian steroids and endogenous opioids in the fasting-induced suppression of pulsatile LH release in ovariectomized rats. *Journal of Endocrinology* **129** 321–328.
- Dobson H & Smith RF 2000 What is stress, and how does it affect reproduction? *Animal Reproduction Science* **6–61** 743–752.
- Everett JW 1989 Neurobiology of reproduction in the female rat. A fifty-year perspective. *Monographs on Endocrinology* **32** 1–133.
- Hashimoto R, Arita J & Kimura F 1990 LH and PRL secretion in ovariectomized spontaneously hypertensive rats. *Acta Endocrinologica* **122** 540–544.
- Hirata F, Stracke ML & Schiffmann E 1987 Regulation of prostaglandin formation by glucocorticoids and their second messenger, lipocortins. *Journal of Steroid Biochemistry* **27** 1053–1056.
- Kam K, Park Y, Cheon M, Son GH, Kim K & Ryu K 2000 Effects of immobilization stress on estrogen-induced surges of luteinizing hormone and prolactin in ovariectomized rats. *Endocrine* **12** 279–287.
- Kawaguchi M, Funabashi T & Kimura F 1998 Insulin-induced hypoglycemia does not impair the surge of luteinizing hormone secretion in the proestrous rat. *Neuroscience Letters* **256** 131–134.
- McGivern RF & Redei E 1994 Adrenalectomy reverses stress-induced suppression of luteinizing hormone secretion in long-term ovariectomized rats. *Physiology and Behavior* **55** 1147–1150.
- Matsuwaki T, Watanabe E, Suzuki M, Yamanouchi K & Nishihara M 2003 Glucocorticoid maintains pulsatile secretion of LH under infectious stress condition. *Endocrinology* **144** 3477–3482.
- Michie HR, Manogue KR, Spriggs DR, Revhaug A, O'Dwyer S, Dinarello CA, Cerami A, Wolff SM & Wilmore DW 1988 Detection of circulating tumor necrosis factor after endotoxin administration. *New England Journal of Medicine* **318** 1481–1486.
- MohanKumar SM & MohanKumar PS 2002 Effects of interleukin-1B on the steroid-induced luteinizing hormone surge: role of norepinephrine in the medial preoptic area. *Brain Research Bulletin* **58** 405–409.
- Nishihara M, Takeuchi Y, Tanaka T & Mori Y 1999 Electrophysiological correlates of pulsatile and surge gonadotrophin secretion. *Reviews of Reproduction* **4** 110–116.
- Rettori V, Gimeno MF, Karara A, Gonzalez MC & McCann SM 1991 Interleukin 1 α inhibits prostaglandin E2 release to suppress pulsatile release of luteinizing hormone but not follicle-stimulating hormone. *PNAS* **88** 2763–2767.
- Rivier C & Vale W 1984 Influence of corticotropin-releasing factor on reproductive functions in the rat. *Endocrinology* **114** 914–921.
- Rivier C, Rivier J & Vale W 1986 Stress-induced inhibition of reproductive functions: role of endogenous corticotropin-releasing factor. *Science* **231** 607–609.
- Sanna PP, Weiss F, Samson ME, Bloom FE & Pich EM 1995 Rapid induction of tumor necrosis factor α in the cerebrospinal fluid after intracerebroventricular injection of lipopolysaccharide revealed by a sensitive capture immuno-PCR assay. *PNAS* **92** 272–275.
- Shirota M, Watanabe G, Taya K & Sasamoto S 1998 Effects of indomethacin on the selective release of follicle-stimulating hormone during the period of ovulation in the rat. *Journal of Veterinary Medical Science* **60** 1059–1065.
- Sridaran R & Blake CA 1980 Effects of long-term adrenalectomy on periovulatory increases in serum gonadotrophins and ovulation in rats. *Journal of Endocrinology* **84** 75–82.
- Stoker TE, Goldman JM & Cooper RL 2001 Delayed ovulation and pregnancy outcome: effect of environmental toxicants on the neuroendocrine control of the ovary. *Environmental Toxicology and Pharmacology* **9** 117–129.
- Turgeon J & Barraclough CA 1973 Temporal patterns of LH release following graded preoptic electrochemical stimulation in proestrous rats. *Endocrinology* **92** 755–761.
- Williams CL, Nishihara M, Thalabard JC, Grosser PM, Hotchkiss J & Knobil E 1990 Corticotropin-releasing factor and gonadotropin-releasing hormone pulse generator activity in the rhesus monkey. Electrophysiological studies. *Neuroendocrinology* **52** 133–137.
- Yoo MJ, Nishihara M & Takahashi M 1997a Involvement of prostaglandins in suppression of gonadotropin-releasing hormone pulse generator activity by tumor necrosis factor- α . *Journal of Reproduction and Development* **43** 181–187.
- Yoo MJ, Nishihara M & Takahashi M 1997b Tumor necrosis factor- α mediates endotoxin induced suppression of gonadotropin-releasing hormone pulse generator activity in the rat. *Endocrine Journal* **44** 141–148.

Received 18 February 2004

Accepted 2 March 2004

Made available online as an

Accepted Preprint 12 March 2004