Adrenal Influence on the Persistence of Pseudopregnancy in Rats

MITSUMORI KAWAMINAMI, MAKOTO YOSHIDA, YUTAKA MORIAI, SHIRO KURUSU
AND INORU HASHIMOTO

Veterinary Physiology, School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada, Aomori 034–8628, Japan

Abstract. The effect of adrenalectomy on the duration of pseudopregnancy was investigated in rats. When adrenalectomy was performed three days before cervical stimulation or when it was done on the first day (day 1, day of estrus) or day 2 of pseudopregnancy, the duration of the pseudopregnancy was significantly prolonged (3.0, 2.5 and 1.8 days respectively). This effect of adrenalectomy was not seen when operation was delayed until day 4 of pseudopregnancy. Adrenalectomy on day 2 of pseudopregnancy significantly increased prolactin (PRL) release at the time of the nocturnal PRL surge (5:00) on day 7. When rats were ovariectomized simultaneously with adrenalectomy on day 1, the stimulating effect of adrenalectomy on PRL release was more evident. The effect of active immunization against corticosterone on the continuation of pseudopregnancy was also examined. Neutralization of plasma corticosterone extended the duration of pseudopregnancy and the binding activity of the antiserum positively correlated with the length of continuing diestrus ($P < 0.05$). These results indicate the negative effect of the adrenal glands, which is probably due to corticosterone, on PRL release in pseudopregnant rats and that the early relief of this inhibition extends the duration of pseudopregnancy.

Key words: Pseudopregnancy, Adrenal gland, Corticosterone, Prolactin surge

(*Endocrine Journal* 49: 357–362, 2002)

THE pro gravid phase of rats, pseudopregnancy, is accompanied by daily prolactin (PRL) surges which are induced by mechanical stimulation of uterine cervix in the evening of proestrus [1, 2]. Twice daily PRL surges are the diurnal surge, which appears at the onset of darkness, and the nocturnal surge, which is usually larger than the diurnal surge and emerges after midnight to early morning [3]. They facilitate luteal progesterone production and last throughout the functional life span of the corpus luteum [4, 5]. Progesterone, in turn, augments recurrent PRL surges [6–9]. The surges cease at the end of pseudo-

pregnancy along with the decline of plasma progesterone concentration [2, 6, 8, 9]. We reported previously that the physiological levels of progesterone induce PRL surges without cervical stimulation [10]. It is thus suggested that the steroid signals play a significant role not only in stimulating PRL release at the surges but also in their induction. The mechanism by which progesterone affects the PRL surge, however, is not well understood.

It is well known that the hypothalamus-pituitary-adrenal axis, a major endocrine system involved with stress response, influences pituitary PRL secretion. Corticosterone was shown to inhibit PRL release and synthesis [11, 12]. Dexamethasone, however, was demonstrated to induce pseudopregnancy, probably by increasing PRL secretion [13]. On the other hand, adrenalectomy increased PRL release during the latter half of the lactational period [14, 15]. Corticotropin releasing hormone (CRH) has been

Received: January 7, 2002

Accepted: March 26, 2002

Correspondence to: Mitsumori KAWAMINAMI, Ph.D.,
Veterinary Physiology, School of Veterinary Medicine and
Animal Sciences, Kitasato University, Towada, Aomori 034–
8628, Japan

shown to increase PRL release and the PRL cell population [16, 17]. Therefore it is of interest whether the adrenal gland is involved in the induction and maintenance of pseudopregnancy, and also whether glucocorticoid exerts a negative effect on the PRL surges of pseudopregnancy. In the present study, we examined the effect of adrenalectomy on the functional life span of the corpora lutea which is considered to reflect on the duration of pseudopregnancy and on the PRL surges which are an indispensable luteotropic factor in rats.

Materials and Methods

Adult female Wistar-Imamichi rats (250–300 g) bred in our animal facility were used for the present study. They were kept in light (5:00–19:00) and temperature ($23 \pm 3^\circ\text{C}$) controlled rooms. Vaginal smears were observed every day and only rats showing at least two regular four-day estrous cycles were selected for use. Cervical stimulation was given by tapping the uterine cervix with a glass rod about one hundred times on the evening of proestrus. The estrous day following the day of the stimulation was designated as day 1 of pseudopregnancy.

Adrenalectomy was performed under ether anesthesia on the morning of estrous day, three days before the day of cervical stimulation (day -3), or on days 1, 2, 4 or 6 of pseudopregnancy. Adrenalectomized animals were given saline (0.9% NaCl) instead of usual drinking water. The effect of adrenalectomy was also observed in ovariectomized rats, in which ovariectomy and adrenalectomy were performed simultaneously on the estrous day following the day of cervical stimulation. Sham-operated rats were prepared for each group as a control.

Indwelling jugular vein cannula (silastic tube, id 0.012 inch, od 0.025 inch, Dow Corning, Midland, MI) were inserted in rats under ether anesthesia two days before the day of serial blood sampling. The cannula were flushed and filled with heparinized saline (0.9% NaCl, 100 U/ml heparin, 2 mg/ml cephalosporin, 2 mg/ml streptomycin) once or twice a day. About 200 μl of blood was collected every two hours for 24 h (from 9:00) through the cannula from conscious rats with a heparinized syringe. Plasma samples were stored at -80°C until radioimmunoassay of PRL. Changes in the plasma PRL concentration

of adrenalectomized pseudopregnant rats were examined. Adrenalectomy was performed two days after the day of the cervical stimulation (day 2). The adrenalectomized and intact pseudopregnant rats were attached to an indwelling jugular vein cannula on day 4 and subjected to serial blood sampling starting at 9:00 of day 6. Sham-operation of adrenalectomy was not done for the intact pseudopregnant rats, which experienced the general stress of surgery by cannulation on day 4. To see the changes in the plasma PRL concentration of ovariectomized and adrenalectomized rats, cervical stimulation was given in the evening of proestrus, and ovariectomy and adrenalectomy were performed two days later (day 2). These ovariectomized rats and ovari-adrenalectomized rats had the indwelling jugular vein cannula inserted on day 4 and were subjected to serial blood sampling from 9:00 of day 6.

The effect of active immunization against corticosterone on the length of pseudopregnancy was examined. Corticosterone hemisuccinate bovine serum albumin (2.5 mg/ml, Steraloids Inc., Wilton, NH) was emulsified with an equal volume of Freund's complete adjuvant (Iatron Lab., Tokyo, Japan). A 0.4 ml dose of this mixture was injected intradermally in the backs of the rats every two weeks. Bovine serum albumin (Sigma Chemical Co., ST. Louis, MO) was used for the control immunization. Cervical stimulation was given at the first proestrus after the sixth immunization. To titrate the binding activity of antiserum, one ml of blood sample was collected under ether anesthesia from jugular vein two days before the last immunization. Plasma samples were diluted ($\times 50$, $\times 250$, $\times 1,250$, $\times 6,250$, $\times 31,250$) and the binding activity to corticosterone was determined as the dilution factor at which the plasma bound 50% of ^3H corticosterone (4 ng, 1,2,6,7- ^3H corticosterone, DuPont Co., Wilmington, DE). Separation of bound and free ^3H -corticosterone was done using a dextran-coated charcoal.

The plasma concentration of PRL was assayed using a double antibody radioimmunoassay with NIDDK rat PRL assay kit as in previous studies [10]. Rat PRL RP-3 was used as the reference preparation. Each sample was run in duplicate and the results were averaged.

Multiple comparisons of differences between means were analyzed by one-way ANOVA and the Bonferroni method. P values less than 0.05 were consi-

dered to be significant.

Results

The duration of pseudopregnancy, namely the length of diestrous period, was significantly prolonged by adrenalectomy (Table 1). When adrenalectomy was performed three days before cervical stimulation (day -3), pseudopregnancy lasted three days longer than that of the control group of sham-operated rats. This effect of adrenalectomy diminished proportional to delay of the day of operation and disappeared when rats were adrenalectomized after day 4 of pseudopregnancy.

Augmented PRL release was seen in the adrenalectomized pseudopregnant rats (Fig. 1). PRL release at the nocturnal surge (5:00) was significantly higher than that of the control group. The stimulating effect of adrenalectomy on PRL release was even more evident in the ovariectomized rats (Fig. 2). Nocturnal PRL surges of rats which had only the ovaries removed were very low compared to those with simultaneous adrenalectomies.

The binding activity of immunized rat serum to ^3H -corticosterone correlated positively with the duration of pseudopregnancy ($P < 0.05$, Fig. 3). Although the average duration was not different significantly from the control group, rats with a higher

binding activity of corticosterone had a pseudopregnancy of up to 17 days duration. The duration of pseudopregnancy in control rats which were im-

Fig. 1. Changes in the plasma PRL concentration of adrenalectomized pseudopregnant rats. Cervical stimulation was given on the evening of proestrus and adrenalectomy ($n=6$) performed two days later (day 2). An indwelling jugular vein cannula was inserted into the adrenalectomized rat and intact pseudopregnant rat ($n=8$) on day 4. Serial blood sampling began at 9:00 of day 6. Asterisk reveals the significant difference from the corresponding control value ($P < 0.05$).

Fig. 2. Changes in the plasma PRL concentration of ovariectomized and adrenalectomized rats. Cervical stimulation was given on the evening of proestrus and ovariectomy and adrenalectomy performed two days later (day 2). An indwelling jugular vein cannula was inserted into these ovariectomized rats (OVX, $n=4$) and ovari-adrenalectomized rats (OVADX, $n=5$) on day 4. Serial blood sampling began at 9:00 of day 6. Asterisks reveal the significant difference from the corresponding control values ($P < 0.05$).

Table 1. Effect of adrenalectomy on the length of pseudopregnancy

Day of operation ^{a)}	Operation ^{b)}	n	Duration of diestrus (days) ^{c)}
-3	SHAM	5	12.6 ± 0.2
	ADX	5	15.6 ± 0.8*
1	SHAM	6	12.2 ± 0.4
	ADX	6	14.7 ± 0.7*
2	SHAM	4	12.0 ± 0.7
	ADX	8	13.8 ± 0.3*
4	SHAM	5	12.8 ± 0.6
	ADX	5	12.6 ± 0.5
6	SHAM	5	13.2 ± 0.4
	ADX	6	13.3 ± 0.4

a) The estrous day after the day of cervical stimulation was designated as day 1 of pseudopregnancy. b) Type of operation. ADX, adrenalectomy. SHAM, sham operation. c) mean ± S.E.M. Asterisks indicate significant difference to corresponding control group ($P < 0.05$).

Fig. 3. Correlation between the length of continuing diestrus and the dilution factor of anti corticosterone of actively immunized rats. Corticosterone hemisuccinate bovine serum albumin was inoculated to cycling rats every two weeks. Bovine serum albumin was used for the control immunization. Cervical stimulation was given at the first proestrus after sixth immunization. Binding activity of antiserum was determined as a dilution factor at which the diluted plasma bound 50% of ^3H corticosterone (4 ng, 1,2,6,7- ^3H corticosterone). The correlation between the length of continuing diestrus and the dilution factor was significant ($P < 0.05$).

munized with bovine serum albumin was also prolonged to 14.8 ± 0.7 days.

Discussion

The present data clearly show that the adrenal glands affect the persistence of pseudopregnancy of rats. Adrenalectomy at the beginning of pseudopregnancy extended the duration of continuing diestrus. As the continuation of the diestrous period in pseudopregnancy is thought to depend on luteal activity [6, 9, 18], the prolongation may be the result of the enhancement of luteal progesterone secretion.

PRL, the major luteotropic hormone of rats, is released as twice daily surges during pseudopregnancy, which is induced by the mechanical stimulation to the uterine cervix [1–3]. When plasma PRL levels are increased by grafting extra pituitary glands under the kidney capsule, the duration of continuing diestrous day has been shown to increase [19]. In the present

study, adrenalectomy on day 2 of pseudopregnancy was shown to increase PRL release at the time of nocturnal PRL surge on day 7. Although we did not examine PRL levels in the other experimental conditions in which adrenalectomy prolonged pseudopregnancy, the augmentation of PRL secretion by adrenalectomy seems to cause the enhancement of progesterone production and, in turn, the prolongation of continuing diestrous period.

The effect of adrenalectomy on the PRL release was more evident when the rats were ovariectomized. Progesterone would thus augment PRL release and compete with the negative effect of adrenal glands on PRL secretion in intact pseudopregnant rats. Since the progesterone level of pseudopregnant rats is significantly high on day 4, this may be the reason why the adrenalectomy was ineffective when delayed until day 4 of pseudopregnancy [20, 21].

Although there was no significant difference between the actively-immunized and the control rats, the neutralization of plasma corticosterone seems to extend the duration of pseudopregnancy. The dilution factor of the antiserum positively correlates with the length of continuing diestrus ($P < 0.05$). Thus adrenal corticosterone seems to be the major factor which affects the continuation of pseudopregnancy. Withdrawal of corticosterone by adrenalectomy augments PRL release and this facilitates progesterone secretion.

Corticosterone exerts its effect by binding to the glucocorticoid receptor and by interacting with glucocorticoid response element (GRE) in a certain DNA sequence. Although progesterone and glucocorticoid sometimes show similar biological activity and their receptors bind the same DNA sequence [22, 23], the specificity of each steroid hormone is well regulated [24]. The present data show corticosterone competitively suppresses PRL secretion which is augmented by progesterone during pseudopregnancy. This effect was seen when adrenal glands were removed in early pseudopregnancy or when the ovarian steroids were eliminated by ovariectomy. Therefore, the increased release of progesterone in the pseudopregnant rats would suppress the inhibitory effect of corticosterone on PRL release. It is thus of interest how progestin and glucocorticoid function inversely at the level of the hypothalamus and/or anterior pituitary gland, directly or indirectly, on PRL secretion.

Adrenalectomy, as well as immuno-neutralization of corticosterone, would be expected to induce higher CRH in the hypothalamus. CRH increases in the median eminence of the adrenalectomized rats [25, 26]. As it is known that CRH increases PRL secretion and chronic exposure to CRH augments the number of lactotopes in the primary culture of pituitary cells [16, 17], this may be one of the mechanisms which works in favor of increasing PRL secretion after adrenalectomy. Since the prolongation of pseudopregnancy was only observed when rats were adrenalectomized at the beginning of pseudopregnancy, the inhibition of gonadotropin secretion by CRH and subsequent delay of follicular development may not

take place in the present experimental conditions [27].

In conclusion, we showed that the adrenal glands significantly affect the hypothalamus-pituitary-ovary axis in pseudopregnant rats, probably due to the inhibitory effect of corticosterone on PRL release.

Acknowledgments

The authors wish to thank the National Institute of Diabetes and Digestive and Kidney Diseases of U.S.A. for the gift of the PRL assay kit. They also thank Ms. Janet K Cook for editing this manuscript.

References

- Freeman ME, Neill JD (1972) The pattern of prolactin secretion during pseudopregnancy in the rat: a daily nocturnal surge. *Endocrinology* 90: 1292-1294.
- Freeman ME, Smith MS, Nazian SJ, Neill JD (1974) Ovarian and hypothalamic control of the daily surges of prolactin secretion during pseudopregnancy in the rat. *Endocrinology* 94: 875-882.
- Smith MS, Freeman ME, Neill JD (1975) The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. *Endocrinology* 96: 219-226.
- Gorospe WC, Freeman ME (1981) An ovarian role in prolonging and terminating the two surges of prolactin in pseudopregnant rats. *Endocrinology* 108: 1293-1298.
- Smith MS, McLean BK, Neill JD (1976) Prolactin: the initial luteotropic stimulus of pseudopregnancy in the rat. *Endocrinology* 98: 1370-1377.
- De Greef WJ, Zeilmaker GH (1976) Prolactin and delayed pseudopregnancy in the rat. *Endocrinology* 98: 305-310.
- Gilman DP, Lu JK, Whitmoyer DI, Judd HL, Sawyer CH (1981) Relationship between progesterone and prolactin surges in aged and young pseudopregnant rats. *Biol Reprod* 24: 839-845.
- Kawaminami M AK, Arakawa H, Hashimoto I (1986) Persistence of prolactin (PRL) surges induced by progesterone in ovariectomized rats. *Jpn J Anim Reprod* 32: 95-100.
- Murakami N, Takahashi M, Suzuki Y (1979) Indispensable role of peripheral progesterone level for the occurrence of prolactin surges in pseudopregnant rats. *Biol Reprod* 21: 263-268.
- Kawaminami M, Takahashi M (1987) Induction of daily PRL surges in rats by chronic treatment with progesterone. *Endocrinol Jpn* 34: 37-44.
- Fang VS, Shian LR (1981) Adrenal influence on pituitary secretion of thyrotropin and prolactin in rats. *Endocrinology* 108: 1545-1551.
- Stephanou A, Sarlis NJ, Knight RA, Lightman SL, Chowdrey HS (1992) Glucocorticoid-mediated responses of plasma ACTH and anterior pituitary pro-opiomelanocortin, growth hormone and prolactin mRNAs during adjuvant-induced arthritis in the rat. *J Mol Endocrinol* 9: 273-281.
- De Greef WJ, van der Schoot P (1987) Effects of dexamethasone on ovarian activity in rats. *Acta Endocrinol (Copenh)* 116: 350-356.
- Taya K, Sasamoto S (1990) Involvement of the adrenal gland in the suckling-induced decrease in LH and FSH secretion and the concomitant increase in prolactin secretion in the rat. *J Endocrinol* 125: 279-285.
- Van der Schoot P, de Greef WJ (1983) Effect of adrenalectomy on the regulation of the secretion of gonadotrophins and prolactin in the lactating rat. *J Endocrinol* 98: 227-232.
- Morel G, Enjalbert A, Proulx L, Pelletier G, Barden N, Grossard F, Dubois PM (1989) Effect of corticotropin-releasing factor on the release and synthesis of prolactin. *Neuroendocrinology* 49: 669-675.
- Hoeffler JP, Frawley LS (1987) Hypothalamic factors differentially affect the proportions of cells that secrete growth hormone or prolactin. *Endocrinology* 120: 791-795.
- De Greef WJ, Zeilmaker GH (1978) Regulation of prolactin secretion during the luteal phase in the rat.

- Endocrinology* 102: 1190–1198.
19. Naito H, Suzuki Y (1978) The rat estrous cycle associated with functional corpora lutea and the mechanism of its recurrence. *Endocrinol Jpn* 25: 367–376.
 20. Hashimoto I, Henricks DM, Anderson LL, Melampy RM (1968) Progesterone and pregn-4-en-20 alpha-ol-3-one in ovarian venous blood during various reproductive states in the rat. *Endocrinology* 82: 333–341.
 21. Pepe GJ, Rothchild I (1974) A comparative study of serum progesterone levels in pregnancy and in various types of pseudopregnancy in the rat. *Endocrinology* 95: 275–279.
 22. Cairns C, Gustafsson JA, Carlstedt-Duke J (1991) Identification of protein contact sites within the glucocorticoid/progestin response element. *Mol Endocrinol* 5: 598–604.
 23. Strahle U, Boshart M, Klock G, Stewart F, Schutz G (1989) Glucocorticoid- and progesterone-specific effects are determined by differential expression of the respective hormone receptors. *Nature* 339: 629–632.
 24. Le Ricousse S, Gouilleux F, Fortin D, Joulin V, Richard-Foy H (1996) Glucocorticoid and progestin receptors are differently involved in the cooperation with a structural element of the mouse mammary tumor virus promoter. *Proc Natl Acad Sci USA* 93: 5072–5077.
 25. Antoni FA, Palkovits M, Makara GB, Linton EA, Lowry PJ, Kiss JZ (1983) Immunoreactive corticotropin-releasing hormone in the hypothalamoinfundibular tract. *Neuroendocrinology* 36: 415–423.
 26. Hashimoto K, Yunoki S, Kageyama J, Ohno N, Takahara J, Ofuji T (1981) Vasopressin and CRF-ACTH in adrenalectomized and dexamethasone-treated rats. *Neuroendocrinology* 32: 87–91.
 27. Rivier C, Rivier J, Vale W (1986) Stress-induced inhibition of reproductive functions: role of endogenous corticotropin-releasing factor. *Science* 231: 607–609.