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## Hyperprolactinemia and estrogen-induced rhythms in LH and prolactin release in the ovariectomized rat

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*Summary.* Short-term (9 days) hyperprolactinemia induced by pituitary grafts reduced basal plasma LH levels in ovariectomized rats whereas long-term (31 days) grafts increased basal LH levels. Although long-term grafts inhibited estradiol-induced prolactin surges, hyperprolactinemia had no effect on the LH surge. It is concluded that the estrogen-treated ovariectomized rat is not suitable for studying the effects of hyperprolactinemia on LH release.
*Key words.* Hyperprolactinemia, LH and prolactin.

Interference with ovulatory cycles is a frequent clinical manifestation of hyperprolactinemia in women. The underlying mechanisms responsible for this disruption remain unclear. Both exaggerated[2] and impaired[3] release of LH in response to luteinizing hormone releasing hormone have been reported.

Various animal models have been utilized to investigate the possible mechanisms involved. In intact female rats, hyperprolactinemia produced by prolactin-secreting tumors[4,5] pituitary grafts or dopamine antagonists[6] has been shown to suppress basal plasma levels of LH and to cause acyclicity. However, it has been questioned whether the intact female rat is a suitable model for studying the suppression of LH release by elevated plasma prolactin levels since this may lead to pseudopregnancy associated with increased progesterone secretion[7]. In the chronically ovariectomized rat, prolonged hyperprolactinemia does not affect basal plasma LH levels[8,9]. Recent studies indicate however, that steroid-induced release of LH may be selectively reduced by hyperprolactinemia[9], suggesting that this may be a more relevant model. This idea is further supported by findings that estrogen-induced positive feedback of LH release is inhibited in hyperprolactinemic patients[10]. On the other hand, some recent reports argue against the validity of this model[11,12].

The major aim of the present study was to determine whether hyperprolactinemia induced by anterior pituitary grafts could suppress the circadian rhythm of LH or prolactin release following administration of estradiol to ovariectomized rats.

*Materials and methods. Animals.* Female Wistar AG rats were obtained from C.S.E.A.L.-C.N.R.S. (Orleans) at 10–12 weeks of age. Following bilateral ovariectomy, the animals were kept under standardized lighting conditions (lights on 05.00–17.00 h) and provided with food and water ad libitum.

*Surgical procedures.* Three to four weeks after ovariectomy, the animals were assigned to one of three groups which were treated as follows: Group 1 (control): rats (n = 22) received a sham graft (fat tissue) under kidney capsule on day 0 of the experiment. A Silastic tube (Dow Corning 601-321, 0.5 cm in length) filled with estradiol was implanted s.c. in the back[13] 4 or 26 days later. Three days after the implant, an indwelling cannula was placed in a jugular vein. All of the above procedures were carried out under ether anesthesia. Two days after cannulation, blood samples of 0.3–0.4 ml were taken via the cannula at 2-h intervals beween 09.00 and 17.00 h. The samples were centrifuged and the plasma stored at −20 °C until assayed for prolactin and LH concentrations.

Group 2 (short-term graft): rats (n = 8) received a transplant consisting of three anterior pituitaries from donor rats under the kidney capsule on day 0 of the experiment. An estradiol implant was made four days later as described above. The animals were then treated exactly as the rats in group 1.

Group 3 (long-term graft): rats (n = 16) received a transplant of three anterior pituitaries, and estradiol was implanted 26 days later. The animals were then treated exactly as the rats in group 1.

*Chemical assays.* Plasma levels of LH and prolactin were determined by radioimmunoassay according to the methods of Niswender et al.[14,15]. All results were expressed in terms of NIAMDD rat LH-RP-1 and NIAMDD rat PRL-RP-1, respectively.

*Statistical analysis.* The data were analyzed initially by 2-way analysis of variance (treatment, time of day) with subsequent analysis of each variable by 1-way analysis of variance and Student's t-test.

*Results.* The plasma levels of prolactin throughout day 5 after estradiol implantation for the various treatment groups are shown in figure 1. All of the experimental groups which had received pituitary grafts had significantly elevated basal levels of prolactin at 09.00 h when compared with the sham control group. Preliminary studies (not shown) indicated that implantation of estradiol had no effect on the graft-induced elevation

Effect of anterior pituitary grafts on basal plasma levels of LH in ovariectomized rats with estradiol implant

| Duration of graft | Plasma LH |
|---|---|
| 0 (sham) | 161 ± 16[a] (22) |
| 9 days | 105 ± 8[b] (8) |
| 31 days | 264 ± 21[b] (16) |

Anterior pituitary or sham grafts were made as described in Methods. Five days after implantation of estradiol, blood was collected at 09.00 h. Numbers in parentheses represent the number of animals. [a] ng/ml + 1 SE. [b] Significantly different from control ($p < 0.05$).

of basal prolactin levels. Thus, this effect was due primarily to the spontaneous release of prolactin from the grafts. Each treatment group exhibited a surge of prolactin release beginning around 11.00 h, reaching a peak between 13.00 and 17.00 h. During the 15.00–17.00-h time interval, animals with the short-term graft (9 days) had significantly higher prolactin levels than control animals. However, in those animals which had received pituitary grafts 31 days earlier, there was a significant reduction in the amplitude of the estrogen-induced surge. To correct for differences in the baseline values for plasma prolactin, the data for each rat was recalculated as the increase in concentration over the 09.00 h value. When the data are expressed in this fashion, only the long-term grafted animals showed a significant change (decrease) in the afternoon surge of prolactin release at each time point when compared with the control group (fig. 2).

As with prolactin, there were also significant differences in plasma LH levels beween treatment groups at 09.00 h (table). Due to the variability in basal levels at this time, the LH levels at succeeding time intervals were also expressed as an increase over the 09.00 h value (fig. 3). The control group exhibited a marked increase in LH levels with a peak occurring at 15.00 h. Although plasma levels of LH in animals with the short-term graft were decreased at 13.00 and 15.00 h, the levels did not differ significantly from those in the control group. Those animals which had received grafts 31 days previously showed a slight, but insignificant, increase in plasma LH levels at all time periods.

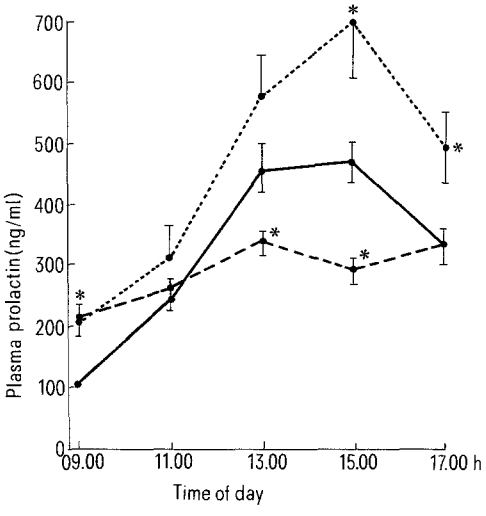


Figure 1. Plasma prolactin levels in chronic ovariectomized rats on day 5 following implantation of estradiol. Animals had previously received a sham graft (●—●), a short-term pituitary graft (●·····●) or a long-term pituitary graft (●————●). Each point represents the group mean ± SE (n = 7–22). *p < 0.05, compared with sham graft control.

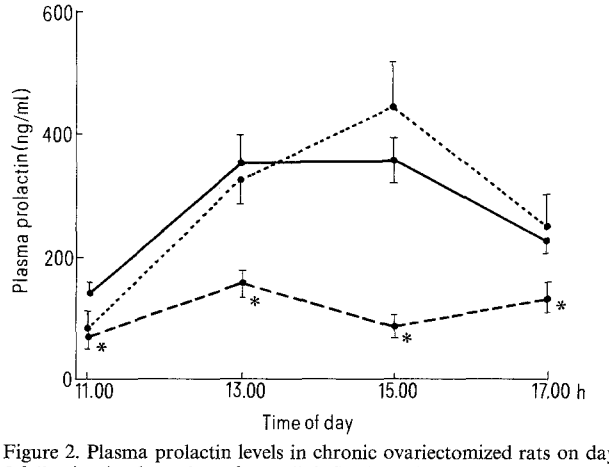


Figure 2. Plasma prolactin levels in chronic ovariectomized rats on day 5 following implantation of estradiol. See legend to figure 1 for details. The data at 11.00–17.00 h from figure 1 are expressed as the increase in concentration over the 09.00 h values. *p < 0.05, compared with sham graft control.

*Discussion.* The results of the present study indicate that chronically elevated levels of plasma prolactin can prevent the cyclic increase in prolactin, but not LH, release induced by estradiol. A number of studies have shown that prolactin secretion from the in situ pituitary gland during pseudopregnancy can be inhibited by renal pituitary grafts[16] or by hypothalamic prolactin implants[7]. Pituitary grafts have been reported to prevent the estradiol-induced increase in plasma prolactin levels four days after implantation of estradiol capsules in acutely ovariectomized rats[12] and two days after estradiol administration to prepubertal female rats[18]. The present study extends these findings in that prolactin-induced suppression of estradiol-induced prolactin surges can occur in chronic ovariectomized rats and that the duration of hyperprolactinemia appears to be an important factor. The long-term pituitary graft caused a marked suppression of prolactin release whereas the short-term graft had no effect. The mechanisms involved in this autofeedback action of elevated plasma prolactin levels are unclear. Hyperprolactinemia has been shown to increase dopamine turnover in the hypothalamus[19] and a recent study[20] suggests that the inhibitory feedback effects of prolactin on its own secretion involve a dopaminergic mechanism. It has also been suggested that endogenous opioids are involved in the negative feedback effect since naloxone enhanced the decrease in pituitary prolactin content caused by prolactin secreting tumors[5].

In the present study, short-term hyperprolactinemia (9 days) decreased basal plasma levels of LH, in agreement with Beck and Wuttke[8]. This appears to be a transient effect, however, as basal LH levels were actually increased after 31 days of hyperprolactinemia. Similar effects caused by long-term elevation of prolactin levels have been reported previously[9]. It does not seem likely, therefore, that changes in basal level of LH are involved in the inhibition of ovulation caused by hyperprolactinemia.

The positive feedback effect of estradiol on gonadotropin release is believed to be responsible for the ovulatory surges of

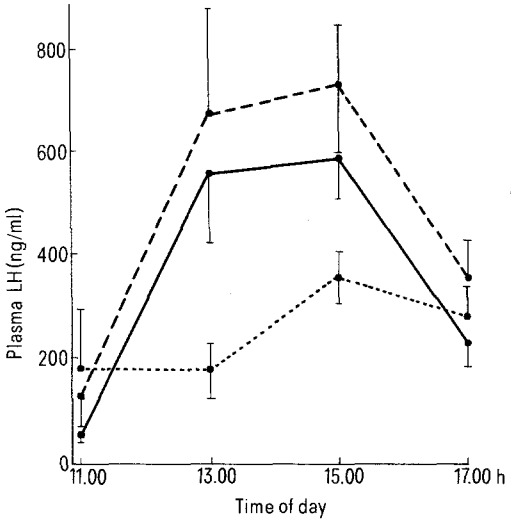


Figure 3. Plasma LH levels in chronic ovariectomized rats on day 5 following implantation of estradiol. See legend to figure 1 for details. The data at 11.00–17.00 h are expressed as the increase in concentration over the 09.00 h values.

LH seen in intact mature female rats[21]. It has been suggested that the anovulatory effect of hyperprolactinemia may be due to a reduction or blockade of this positive feedback action[22]. However, efforts to demonstrate or confirm this mechanism with experimental models of hyperprolactinemia have produced contradictory or inconclusive results. Studies in intact rats have shown that hyperprolactinemia can reduce, but not prevent, the positive feedback effect of estradiol[4,18]. It is not clear whether this reduction contributed to the acyclicity in these animals, however, due to the pseudopregnancy state. This complication can be avoided when ovariectomized animals are utilized. The only other known study employing chronic ovariectomized rats also failed to find any effect of elevated plasma prolactin levels on estrogen-induced release of LH[11], but the hyperprolactinemia was of rather short duration (10 days). The present study, which compared the effects of short term and long-term hyperprolactinemia, thus provides more definitive information on whether inhibition of steroid-induced release of LH can result from prolonged elevation of plasma prolactin levels. Taken together with the studies cited above, the present investigation strongly suggests that chronic hyperprolactinemia does not prevent estrogen-induced positive feedback in the female rat and thus this animal model is probably not adequate for studying mechanisms which underlie this effect observed in hyperprolactinemic patients.

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## Peptide-stimulated release of prolactin from the fowl anterior pituitary gland

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*Summary.* Anterior pituitary glands from broiler fowl were preincubated for 24 h in either medium 199 only or medium containing estradiol 17$\beta$, following which they were incubated in medium containing thyrotrophin releasing hormone (TRH), vasoactive intestinal polypeptide (VIP) or substance P (SP), alone or with the dopamine agonist, apomorphine. Estradiol priming stimulated release of prolactin and enhanced apomorphine-inhibition of prolactin release. TRH stimulated prolactin release, an effect reversed by apomorphine, and priming with estradiol potentiated both effects. VIP stimulated prolactin to a lesser degree and again this was inhibited by apomorphine and potentiated by estradiol. SP had little effect on the nonsteroid-primed pituitary, but stimulated release of prolactin after estradiol treatment, though less effectively than TRH or VIP.
*Key words.* Prolactin release, stimulation of; vasoactive intestinal polypeptide; substance P; thyrotrophin releasing hormone; estradiol 17$\beta$, effect on fowl pituitary responsiveness.

A number of peptides have been isolated from the mammalian hypothalamus that are able to affect the release of anterior pituitary gland hormones[1,2]. It is believed that these neuropeptides are released from nerve terminals into the hypophyseal portal system, where they are transported to the anterior pituitary cells[3,4]. Some of these peptides, including thyrotrophin releasing hormone (TRH), vasoactive intestinal polypeptide (VIP) and substance P (SP) stimulate the release of prolactin in vitro[2,5–7]. In birds, the effects of TRH on anterior pituitary function are well-documented[8–11], but little attention has been paid to the actions of other neuroactive peptides[12,13]. Recently, large quantities of VIP and SP have been detected, by radioimmunoassay and immunofluorescence techniques, in the central nervous system and particularly the hypothalamus and median eminence of domestic fowl. In addition, VIP stimulated the release of immunoassayable prolactin when administered to domestic fowl[14–16]. However, little is known concerning the interactions of prolactin stimulating and inhibiting agents in birds. The effects of the peptides (TRH, VIP and SP) on release of prolactin from fowl pituitary glands under different conditions in vitro were determined in the present study.

*Materials and methods.* Procedure. Anterior pituitary glands were dissected from the heads of freshly-killed 8- to 10-week-old broiler fowl and collected in ice-cold medium 199. The pituitaries were bisected and each half placed in a small plastic tube with 100 µl medium 199 or medium containing $10^{-8}$ M estradiol 17$\beta$. After through gassing with 95% $O_2$/5% $CO_2$ the tubes were sealed and placed in a water bath at 39 °C for 24 h. The medium was then aspirated and discarded. Fresh medium, containing the test substances as described in the results section, was then added. After gassing and sealing the tubes were incubated for a further 3 h, following which the medium was removed, diluted 10-fold and stored at −20 °C until assayed. Assay and data analysis. The homologous chicken prolactin