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# Thyroid Stimulating Hormone and Prolactin Secretion: Reduced Sensitivity to TRH-Stimulated Prolactin Release after Midpregnancy in Rats (41682)

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*Abstract.* Prolactin (PRL) and thyroid stimulating hormone (TSH) plasma concentrations were measured during the latter part of the dark period in early and mid–late pregnancy in the rat. On Days 4–5 and 7–8 of pregnancy, plasma PRL concentrations surged between 22:00 and 06:00 hr and TSH values increased between 22:00 and 02:00 hr. While the TSH pattern was maintained during the second-half of pregnancy, surges in PRL release ceased and PRL levels remained at <10 ng/ml. The effects of thyrotropin releasing hormone (TRH) administration on PRL and TSH secretion were then measured to determine whether the second-half of pregnancy is associated with a decrease in sensitivity to an agent that can stimulate PRL release. Injection (iv) of cannulated pregnant rats with a low dosage (20 ng) of TRH stimulated a twofold increase in plasma TSH during both early (Days 5–9) and later (Days 14–18) pregnancy but did not change plasma PRL levels. Treatment with a high dosage (2 μg) of TRH induced a sixfold rise in plasma TSH during both phases of gestation. The higher dose of TRH also stimulated elevations in plasma PRL during early and mid–late pregnancy; however, both the absolute increase in the amount of PRL in plasma and the percentage increase over baseline levels were greater from Days 5–9 than from Days 14–16 of gestation. These data indicate that the neuroendocrine sensitivity to factors that stimulate PRL secretion changes as pregnancy progresses, and suggest that nocturnal secretion of PRL and TSH during pregnancy may be regulated, in part, by a common trophic factor.

The patterns and effectiveness of prolactin secretion are altered during pregnancy in many mammals. In rats, cervical stimulation during mating induces large diurnal and nocturnal surges of prolactin in blood for the first 8 to 10 days of gestation (1). From Day 11 of pregnancy until 1–2 days prior to delivery, prolactin concentrations remain low and no surges are evident (1, 2). The disappearance of these prolactin (PRL) surges is associated with the establishment of an endocrine placenta and the secretion of rat placental lactogen (3–5). Recently, we have found that plasma thyroid stimulating hormone (TSH) concentrations also increase during the latter part of the dark phase during both early and late pregnancy in rats (6). Since it is established that thyrotropin releasing hormone (TRH) can cause the release of TSH and PRL in many mammals, including the rat (7–9), it seemed possible that the nocturnal increases in TSH and PRL might be regulated in part by a common trophic signal. However, an explanation is required for the dissociation of the release of those two hormones during the night after mid–pregnancy.

In the present study we examined the hypothesis that a differential shift in sensitivity to trophic stimulation of TSH and PRL release might occur between the early (prior to Day 9) and later (Days 14–18) phases of pregnancy. We proposed that the pituitary and/or neuroendocrine systems regulating PRL release might be more responsive to trophic stimulation during early pregnancy when PRL surges occurred than during later pregnancy when these surges were absent. Our experimental approach involved the measurement of plasma concentrations of endogenous nocturnal TSH and PRL during pregnancy in cannulated rats and the determination of the effects of different dosages of TRH on plasma PRL and TSH concentrations when the prolactin surges are evident (early pregnancy) and absent (mid–late pregnancy).

**Materials and Methods.** *Animals.* Sprague–Dawley female rats purchased from Simonsen Laboratories (Gilroy, Calif.) were maintained



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under controlled environmental conditions in our laboratory (lights on 05:00–19:00 hr; temperature 20–23°C). Food and water were supplied *ad libitum* throughout the study. Daily vaginal smears were taken from all animals beginning 2–3 weeks after their arrival in the laboratory. Females (215–250 g) exhibiting at least two consecutive 4-day estrous cycles were housed with males on the afternoon of the next proestrus. The day sperm were found in the vaginal lavage was designated Day 1 of pregnancy. Females were then fitted with intraatrial cannulas (10) on Days 2–3 of pregnancy. A total of 23 pregnant rats were used in the two experiments in this study.

*Bleeding schedule and TRH treatment.* In the first experiment a series of blood samples were collected at 4-hr intervals on each of three nights during pregnancy. Initial samples (0.7 ml/sample) were taken at 22:00 hr ± 10 min on Days 4, 7, and 14 of pregnancy. Subsequent samples were obtained 4 and 8 hr later at 02:00 and 06:00 hr on Days 5, 8, and 15 of gestation. These sampling points were selected to coincide with the reported time of the nocturnal surge of prolactin during early gestation in rats (1, 3, 4). Blood samples were centrifuged immediately after collection for 10 min at 2000 RPM and the plasma was refrigerated at −20°C until assayed for prolactin and TSH content. After centrifugation, red blood cells were suspended in 0.5 ml 0.9% saline and returned through the cannula to each rat.

In the second experiment thyrotropin releasing hormone (TRH), a hormone capable of stimulating release of both TSH and PRL, was administered to 12 pregnant rats via intraatrial cannulas. Each animal received 2 μg TRH (Beckman, lot No. 05706), 20 ng TRH, or saline vehicle (200 μl) at 09:30–10:30 hr on Day 5, 7, or 9 and again on Day 14, 16, or 18 of pregnancy. Each treatment was administered to each animal once during both early and later pregnancy. The sequence of treatments was randomized such that an approximately equal number of animals received each treatment on each bleeding day. Blood samples (0.3–0.4 ml/sample) were collected on ice prior to (0 min) and 5, 10, 30, and 60 min after treatment. Samples were processed as described in the first experiment except that the red blood cells from the five sample points for each animal were pooled in 1.0 ml 0.9% saline and returned via the cannula after all blood sampling had been completed for the day. Plasma samples were later assayed for prolactin and TSH content.

*Hormone assays.* Plasma prolactin and TSH concentrations were measured by radioimmunoassay with the materials supplied with the prolactin and TSH assay kits distributed by NIAMDDK (Dr. A. Parlow). Prolactin values represent the average of duplicate determinations at volumes of 10 or 20 μl plasma, while TSH values were assayed in duplicate at volumes of 20 or 50 μl plasma. Prolactin is expressed in terms of NIAMDD-rat-PRL-RP-1, which has a biological potency equivalent to 11 IU/mg. TSH is expressed in terms of NIAMDD-rat-TSH-RP-1, which has a biological potency equal to 0.22 USP TSH units/mg. The ranges of sensitivity of the prolactin and TSH assays were 0.1–0.2 and 4–8 ng/tube, respectively. The upper limit of readability on the standard curves were 6 ng for PRL and 300 ng for TSH. The intraassay and interassay coefficients of variation were 9 and 16% for prolactin samples and 7 and 13% for TSH samples.

*Statistical analysis.* Data were analyzed with ANOVA, multiple $t$ test comparisons, Scheffe's test, the Mann–Whitney $U$ test, and the Sign test.

**Results.** The patterns of prolactin and TSH concentrations in pregnant rats bled during late night and early morning phases of the light-controlled day (experiment 1) are shown in Fig. 1. On Days 4–5 and 7–8 of pregnancy, prolactin concentrations increased significantly between the 22:00 and 02:00 hr sampling points, and then declined between 02:00 and 06:00 hr. In contrast to the high levels and surges in PRL found during early pregnancy, plasma concentrations of PRL at all three time points on Day 14 were less than the detectable limits (10 ng/ml) of the prolactin assay. The patterns of plasma TSH concentration found during early pregnancy were similar to those of prolactin at this stage of pregnancy. Plasma TSH levels rose in females between 22:00 and 02:00 hr on Days 4–5 and 7–8 ($P < 0.05$) of pregnancy. TSH levels rose from about 250 to 400 ng/ml between 22:00 and 02:00 hr during these sampling periods.

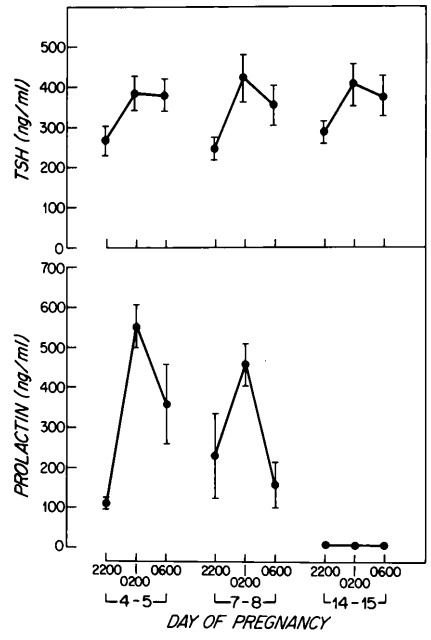


FIG. 1. Plasma concentrations of TSH and prolactin during pregnancy in rats. Blood samples were collected through indwelling atrial cannulas at 22:00, 02:00, and 06:00 hr on Days 4–5 and 14–15 of gestation. Significant increases in plasma TSH were found on all sampling days, whereas prolactin concentrations surged on Days 4–5 and 7–8 but not on Days 14–15 of pregnancy. The values are expressed as means ± SEM. The *N*s for the three sampling periods were 11 (Days 4–5), 10 (Days 7–8), and 8 (Days 14–15).

However, in contrast to the shift in the pattern and levels of PRL concentration between Days 7 and 8 and 14 and 15 of pregnancy, neither the levels nor the patterns of plasma TSH concentrations changed between Days 7 and 14 of pregnancy. TSH concentrations on Days 14–15 of gestation rose ($P < 0.05$) from 283 ng/ml at 22:00 hr on Day 14 to 403 ng/ml at 02:00 hr on Day 15 of pregnancy (see Fig. 1).

In the second experiment, baseline (0 min) plasma concentrations of PRL, but not TSH, changed during pregnancy (Table I). While TSH levels ranged from about 315–380 ng/ml at 09:30 hr (0 min) from days 5–18 of pregnancy, plasma PRL levels decreased significantly ($P < 0.01$) from 21 ng/ml on Day 5 to 15 ng/ml on Day 9, and then were about 10 ng/ml from days 14–18 of pregnancy. Mean baseline levels of plasma prolactin, therefore, were significantly higher ($P < 0.05$) during early pregnancy (Days 5–9) than during later pregnancy (Days 14–18).

Prolactin and TSH responses to TRH administration during pregnancy are shown in Figs. 2 and 3. Treatment of pregnant rats with the higher dosage of TRH (2 μg) stimulated significant increases in plasma PRL concentrations during both early ($P < 0.05$) and later ($P < 0.05$) pregnancy at both 5 and 10 min after TRH injection; PRL concentrations were highest at the 5-min sample point. The levels of PRL in plasma and the amount of increase in PRL concentration from baseline at 5 and 10 min after 2 μg TRH treatment, however, were higher during early pregnancy. Neither the lower dosage of TRH (20 ng) nor the vehicle alone affected plasma PRL concentrations during either early or mid–late gestation (Fig. 2).

The patterns and magnitudes of the TSH responses to both dosages of TRH are shown in Fig. 3. Plasma TSH levels increased significantly ($P < 0.01$–$0.001$) in response to both

TABLE I. BASAL PLASMA CONCENTRATIONS OF PROLACTIN AND TSH (0 min) IN PREGNANT RATS BLED BETWEEN 09:30 AND 10:30 hr[a]

| | Early pregnancy | | | Mid–late pregnancy | | |
|---|---|---|---|---|---|---|
| Hormone | Day 5 | Day 7 | Day 9 | Day 14 | Day 16 | Day 18 |
| Prolactin (ng/ml) | 20.8 ± 1.1* | 18.5 ± 2.5** | 14.7 ± 0.8 | 10.3 ± 0.6 | 9.8 ± 0.5 | 10.9 ± 2.1 |
| THS (ng/ml) | 330.5 ± 17.5 | 333.6 ± 23.5 | 357.9 ± 30.5 | 381.1 ± 32.3 | 342.1 ± 39.6 | 316.2 ± 17.3 |

[a] The values represent the means ± SEM for 8–11 animals bled on each day.
* $P < 0.01$ compared with Day 9, 14, 16, and 18 of pregnancy.
** $P < 0.01$ compared with Day 14, 16, and 18 of pregnancy.

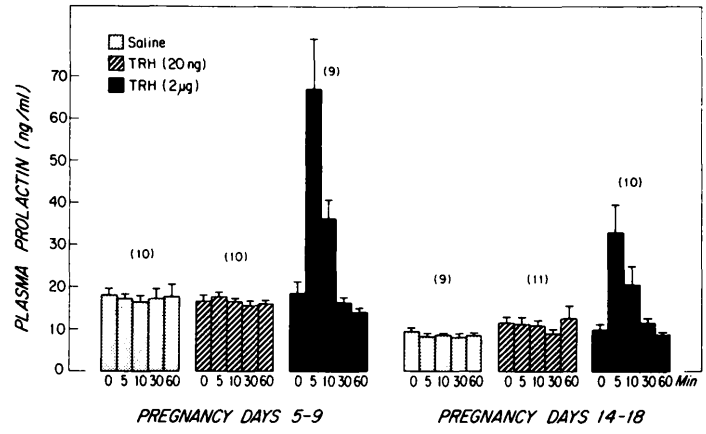


FIG. 2. The effects of TRH treatment on plasma prolactin concentrations during early and later pregnancy. Plasma prolactin content was unaffected by the 20-ng dose of TRH. The 2-μg dose of TRH resulted in significantly elevated prolactin values at 5 and 10 min ($P$ values < 0.05–0.01). The elevations in plasma prolactin at 5 and 10 min after 2 μg TRH were higher during early pregnancy ($P < 0.05$). Eleven samples were collected between 09:30 and 11:30 hr.

dosages of TRH. The magnitudes of the rises in TSH concentration after the 2 μg and 20 ng TRH treatment were about six- and two-fold, respectively, with peak concentrations found at 10 min. The TSH responsiveness to TRH did not differ between early and mid–late pregnancy.

**Discussion.** The present data indicate that the dissociation of nocturnal TSH and PRL release after midpregnancy in the rat may result in part from a shift in pituitary sensitivity to factors capable of stimulating PRL release. Although the mechanisms controlling the shift in pituitary sensitivity to PRL release are unknown, a number of possibilities exist. The larger PRL response to the 2-μg dose of TRH during early pregnancy when compared with later pregnancy may represent a diminished inhibition of pituitary prolactin release, an increased stimulation of pituitary PRL release

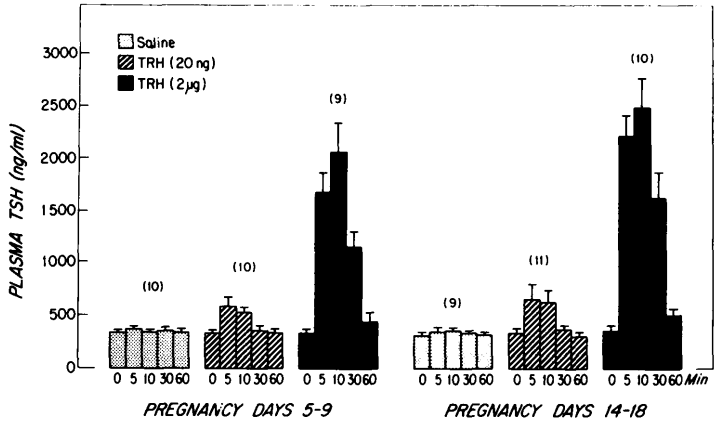


FIG. 3. The effects of TRH administration on plasma TSH concentrations during early and later pregnancy. A dose of 20 ng TRH stimulated a significant rise in plasma TSH at the 5- and 10-min sampling points ($P < 0.01$–$0.001$) during both early (Days 5–9) and later (days 14–18) pregnancy. The high dose of TRH (2 μg) stimulated large increases in TSH concentrations at 5, 10, and 30 min during both stages of gestation. Eleven samples were collected between 09:00 and 11:00 hr.

by a PRL-releasing factor, or both of these processes. Conversely, the suppression of PRL responsiveness during the second-half of pregnancy may result from increased inhibition or decreased stimulation of PRL release.

One attractive possibility that might account for this shift in PRL response involves a rise in dopaminergic activity during the course of pregnancy. Dopamine (DA) can inhibit PRL release (11) and may be a PIF under certain physiological conditions, such as during lactation in rats (12, 13). DeGreef and Neill (14) have found lower overall concentrations of DA in hypophysial stalk plasma in ovariectomized rats exhibiting nocturnal and diurnal surges. Recently, McKay *et al.* (15) found that dopamine concentrations in the anterior pituitary gland of pregnant rats sacrificed at 09:00 and 12:00 hr on Day 13 of pregnancy were about three times greater than the concentrations present during early pregnancy (Days 2 and 6). *In vitro* studies have demonstrated that pituitary responsiveness to TRH-induced PRL release is attenuated by DA treatment (16), whereas the effectiveness of TRH in releasing PRL in lactating rats is increased when hypophysial portal DA levels are lowered prior to TRH administration (12). Therefore, it is feasible that the increased PRL responsiveness to TRH as well as the higher basal PRL concentration during early pregnancy in the present study may have resulted from a decreased release of DA from the hypothalamus during the first 10 days of pregnancy, i.e., the attenuation of an inhibitory agent. After Day 10 of gestation in rats it has been proposed that both basal and rhythmic components of PRL release are actively suppressed by placental lactogen (3–5) and/or a uterine factor other than placental lactogen (17, 18). Whether the suppressive effects of placental lactogen and/or uterine factors on prolactin secretion after midpregnancy involve a possible direct shortloop feedback of these factors at the pituitary level or an action at the CNS, perhaps at the level of the tuberoinfundibular dopaminergic system, are the subjects of future inquiry.

A second mechanism that might account for the increased release of PRL during pregnancy is an enhanced secretion of prolactin releasing factor (PRF) into the hypophysial portal system during early gestation. This mechanism, however, would not likely account for the decrease in responsiveness to TRH during the latter half of pregnancy.

Our data demonstrate that during pregnancy in rats the threshold dosage of TRH needed to cause a significant increase in plasma TSH levels is lower than that required to release prolactin. These differential responses to the lower dose of TRH are consistent with earlier studies. Burnet and Wakerley (19) reported that 50 ng of TRH induced a rise in TSH, but not PRL, in lactating rats, whereas higher doses of TRH (1–25 $\mu$g) stimulated increases in both TSH and PRL (7, 8).

The findings of the present study can be compared with those in other species. In pregnant sheep as in pregnant rats, TRH-stimulated prolactin release is correlated with basal prolactin levels (20, 21). Fitzgerald *et al.* (20) found that the greatest release of TRH-induced PRL from the pituitary gland occurred when basal PRL levels were elevated. In humans, in contrast, there is no apparent change in prolactin responsiveness to TRH during pregnancy, although basal levels do change (22). With respect to TSH responsiveness to TRH during pregnancy, our findings and those in humans are similar. In neither rats nor humans have changes in sensitivity to TRH-induced TSH release been detected as a function of stage of pregnancy.

The increase in plasma TSH levels that occurs between 22:00 and 02:00 hr throughout pregnancy (6) may be due to one or more physiological events. Whereas this increase in plasma TSH concentrations at 02:00 hr may result from an increase in trophic stimulation of TSH release from the pituitary gland, it is also possible that a suppressive influence upon TSH secretion and/or an increased rate of TSH clearance exists around 22:00 hr, effects which abate by 02:00 hr and then return later in the morning. The mechanism(s) responsible for the increased TSH levels in plasma at 02:00 hr remain(s) to be determined.

Finally, it should be noted that although concurrent increases in plasma TSH and PRL occur at night during early pregnancy, and both can be released by TRH during early gestation in rats, we do not interpret our data to mean that the nocturnal surges in PRL and

TSH are necessarily stimulated by TRH itself. Rather, the data indicate that during the early light period and possibly at night in early pregnancy some factor which can stimulate TSH as well as PRL may be released into the hypophysial blood. Whereas this factor can stimulate PRL release during early pregnancy, it may be ineffective after Day 10 of gestation in overriding a postulated increase in PRL inhibitory activity.

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1. Smith MS, Neill JD. Termination at midpregnancy of the two daily surges of plasma prolactin initiated by mating in the rat. Endocrinology **98**:696–701, 1976.
2. Amenomori Y, Chen CL, Meites J. Serum prolactin levels in rats during different reproductive states. Endocrinology **86**:506–510, 1970.
3. Yogev L, Terkel J. The temporal relationship between implantation and termination of nocturnal prolactin surges in pregnant lactating rats. Endocrinology **102**:160–165, 1978.
4. Yogev L, Terkel J. Timing of termination of nocturnal prolactin surges in pregnancy rats as determined by the number of fetuses. J Endocrinol **84**:421–424, 1980.
5. Yogev L, Terkel J. Endogenous inhibition of prolactin secretion in pregnant lactating rats. Endocrinology **110**:158–162, 1982.
6. Bridges RS, Terkel J, Sawyer CH. Changes in plasma concentrations of thyroid-stimulating hormone during pregnancy in rats. Proc Soc Exp Biol Med **106**:374–377, 1979.
7. Deis RP, Alonso N. Prolactin release induced by synthetic thyrotrophin releasing factor in female rats. J Endocrinol **58**:673–674, 1973.
8. Mueller GP, Chen HJ, Meites J. *In vivo* stimulation of prolactin release in the rat by synthetic TRH. Proc Soc Exp Biol Med **144**:613–615, 1973.
9. Grosvenor CE, Mena F. Evidence that thyrotropin-releasing factor may function in the release of prolactin in the lactating rat. Endocrinology **107**:863–868, 1980.
10. Harms PG, Ojeda SR. A rapid and simple procedure for chronic cannulation of the rat jugular vein. J Appl Physiol **36**:391–392, 1974.
11. MacLeod RN. Regulation of prolactin secretion. In: Martini L, Ganong WF, eds. Frontiers in Neuroendocrinology. New York, Raven Press, Vol. 4: pp 169–194, 1976.
12. DeGreef WJ, Visser TJ. Evidence for the involvement of hypothalamic dopamine and thyrotropin-releasing hormone in suckling-induced release of prolactin. J Endocrinol **91**:213–223, 1981.
13. Plotsky PM, Neill JD. The decrease in hypothalamic dopamine secretion induced by suckling: Comparison of voltametric and radioisotopic methods of measurement. Endocrinology **110**:691–696, 1982.
14. DeGreef WJ, Neill JD. Dopamine levels in hypophysial stalk plasma of the rat during surges of prolactin secretion induced by cervical stimulation. Endocrinology **105**:1093–1099, 1979.
15. McKay DW, Pasieka CA, Moore KE, Riegle GD, Demarest KT. Semicircadian rhythm of tuberoinfundibular dopamine neuronal activity during early pregnancy and pseudopregnancy in the rat. Neuroendocrinology **34**:229–235, 1982.
16. Fagin KD, Neill JD. The effect of dopamine on thyrotropin-releasing hormone-induced prolactin secretion in vitro. Endocrinology **109**:1835–1840, 1981.
17. Freeman ME. A direct effect of the uterus on the surges of prolactin induced by cervical stimulation in the rat. Endocrinology **105**:387–391, 1979.
18. Gorospe WC, Mick C, Freeman ME. The obligatory role of the uterus for termination of prolactin surges initiated by cervical stimulation. Endocrinology **109**:1–4, 1981.
19. Burnet FR, Wakerley JB. Plasma concentrations of prolactin and thyrotropin during suckling in urethane-anaesthetized rats. J Endocrinol **70**:429–437, 1976.
20. Fitzgerald BP, Evins JD, Cunningham FJ. Effects of TRH on the secretion of prolactin in ewes during the breeding season and seasonal anoestrus. J Reprod Fertil **61**:149–155, 1981.
21. Wright PJ, Jenkin G, Heap RB. Prolactin and LH release in response to LH-RH and TRH in ewes during dioestrus, pregnancy and post partum. J Reprod Fertil **62**:447–453, 1981.
22. Ylikorkala O, Kivinen S, Reinila M. Serial prolactin and thyrotropin response to thyrotropin-releasing hormone throughout normal human pregnancy. J Clin Endocrinol Metab **48**:288–292, 1979.

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