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Changes in Plasma Concentrations of Thyroid-Stimulating Hormone During Pregnancy in Rats[1] (40453)

ROBERT S. BRIDGES,[2] JOSEPH TERKEL,[3] AND CHARLES H. SAWYER

Department of Anatomy and Brain Research Institute, UCLA School of Medicine, Los Angeles, California 90024

A diurnal pattern of changes in levels of thyroid stimulating hormone (TSH) has been reported in cycling female rats (1), in which plasma TSH concentrations are high during the early part of the light period and then decline prior to the onset of the dark period. Patterns of TSH secretion during other reproductive states, e.g. pregnancy, have not been reported. It is known that secretion of other pituitary hormones, most notably prolactin (PRL), is dramatically altered in the pregnant rat. Throughout the first half of gestation daily nocturnal surges of PRL occur (2–4); these surges are not present in cycling female rats (5, 6). In addition, the finding that thyrotropin releasing hormone (TRH) can stimulate release of both TSH and PRL (7) enhances the possibility that TSH secretion, like PRL, may be altered by the state of pregnancy. We, therefore, measured plasma TSH levels in pregnant rats in order to characterize the pattern of TSH secretion during pregnancy and to determine whether the state of pregnancy affects the rhythmic pattern of TSH secretion found in cycling rats.

Materials and methods. Sprague-Dawley female rats (200–225 g, $N = 39$) purchased from Simonsen Laboratories (Gilroy, Ca.) were mated 3–4 weeks after arrival in our laboratory. The day sperm were found in the vaginal lavage was designated Day 1 of pregnancy. Pregnant animals were then fitted with intra-atrial cannulas (8) on Days 1–2 ($N = 23$) or Days 14–17 ($N = 16$) of pregnancy.

In the first experiment, 0.7 ml of blood per sample was collected from 8–11 cannulated animals at both 0900 hr and 1500 hr (lights on 0500–1900 hr) on Days 2, 4, 7, 10, 13, 16, 18, 20 and 22 of gestation. Although not all of these animals were bled on each of the days listed, those bled at 0900 hr on a given day also were bled at 1500 hr. In a second experiment eleven females were bled (0.7 ml) at 2200, 0200 and 0600 hr on Days 4, 7, 14 and 21 of pregnancy. In both experiments the blood samples were centrifuged immediately and plasma refrigerated at −20° until assayed for hormone content. After centrifugation red blood cells were suspended in 0.5 ml 0.9% saline and returned through the cannulae to the experimental animals.

Plasma TSH was measured by radioimmunoassay with the materials and instructions supplied with the TSH assay kit distributed by NIAMDD (Dr. A. Parlow). TSH values represent the average of duplicate determinations at volumes of 50 or 100 μl plasma. TSH is expressed in terms of NIAMDD-rat TSH-RP-1, which has a biological potency equivalent to 0.22 USP TSH units/mg. Data were analyzed with ANOVA and multiple t test comparisons.

Results. Plasma TSH concentrations in females sampled at 0900 hr and 1500 hr throughout pregnancy are shown in Fig. 1. The morning and afternoon plasma TSH levels remained relatively constant during the first 10 days of gestation (range of $\bar{X}$'s = 224–284 ng/ml). However, from Day 13 through Day 22 of pregnancy plasma TSH concentrations at 0900 hr were significantly higher than those measured at 1500 hr. Comparisons of hormone levels at 0900 hr during pregnancy revealed that plasma TSH concentrations were considerably higher in samples taken during the second half of gestation. The differential in morning TSH values started between Days 10 and 13 of pregnancy and was statistically significant on Days 16, 18 and 20 (vs. Day 10 values; $P < 0.01$, $P < 0.001$ and $P < 0.05$, respectively). In contrast

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[2] Present address: Laboratory of Human Reproduction and Reproductive Biology, Department of Anatomy, Harvard Medical School, 45 Shattuck Street, Boston, Massachusetts 02115.

[3] Present address: Department of Zoology, Tel-Aviv University, Tel-Aviv, Israel.

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to the shift in TSH titers found at 0900 hr, plasma TSH levels at 1500 hr remained constant throughout pregnancy (range of $\bar{X}$'s = 228–274 ng/ml).

TSH levels in blood samples ($N = 9$) that were obtained on Days 22 and 23 post-coitum 2–4 hr after onset of parturition were significantly higher than in samples taken prior to parturition ($\bar{X} \pm SE = 682 \pm 116$ ng/ml, $P < 0.01$ vs. Day 22 at 0900 samples).

The second experiment investigated the possibility that the apparent lack of a daily rhythm of TSH during the first part of gestation might be the result of a shift in the rhythm's timing, i.e., advancement or delay. A statistically significant rhythm in plasma TSH was found during the four nights that blood was collected ($P < 0.02$, see Fig. 2). TSH concentrations rose significantly to peak values during the dark between 2200 and 0200 hr on Days 4, 7 and 14 of pregnancy (P values all < 0.05). TSH levels also appeared to rise between 2200 and 0200 hr on Day 21 of gestation; however, the change in hormone

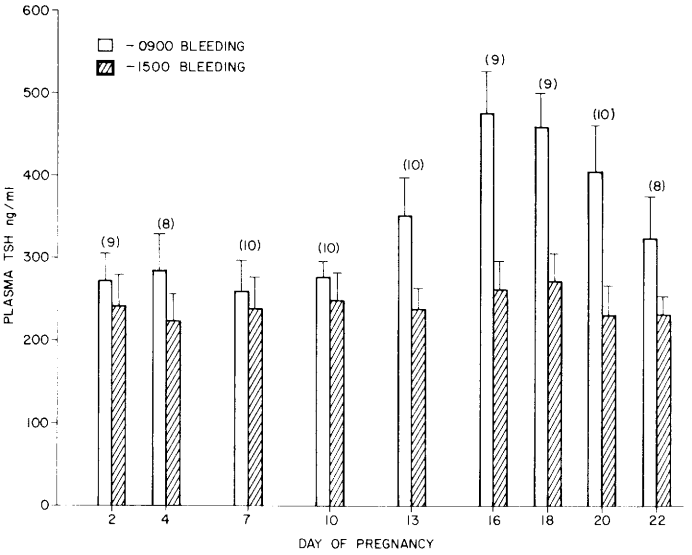

FIG. 1. Plasma TSH concentrations at 0900 hr and 1500 hr in rats during pregnancy. Values are expressed as $\bar{X} \pm$ SEM. Numbers in parentheses are the N for each bleeding time.

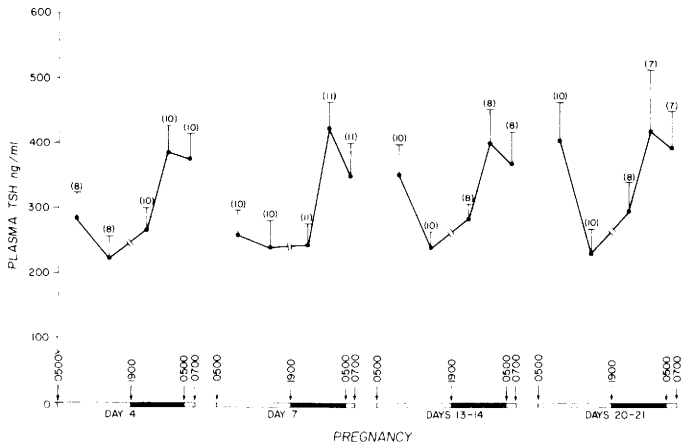

FIG. 2. Concentrations of plasma TSH in rats bled on days 4, 7, 13–14 and 20–21 of pregnancy. Values expressed as $\bar{X} \pm$ SEM. Numbers in parentheses are the N for each bleeding. The broken line separates the values obtained from two groups of animals, the first bled at 0900 hr and 1500 hr (see Fig. 1) and the second bled at 2200 hr, 0200 hr, and 0600 hr.

concentrations at this time was not statistically significant.

Discussion. A diurnal pattern of changes in plasma TSH concentrations was found throughout pregnancy in rats, a pattern that shifted between the first and second halves of gestation (Fig. 2). Although TSH levels were highest at 0200 and 0600 hr and lowest at 1500 hr throughout pregnancy, the daily timing for signalling the decline in TSH levels appeared to shift between the first and second halves of pregnancy. TSH levels at 0900 hr from Days 13 to 22 were as high as those found at 0600 hr throughout pregnancy, while at 0900 hr during the first 10 days of pregnancy, plasma TSH concentrations were only 60% of 0600 hr levels. This alteration in rhythmic secretion of TSH may reflect a shift in the periodicity of TSH secretion, the duration of hormone output, or a combination of both of these possibilities. It is interesting that similar differences are found in the secretion of another pituitary hormone, PRL, between the first and second halves of pregnancy in rats. PRL levels rise dramatically each night from Days 1 to 10 of pregnancy, but then remain low during the second half of gestation (4). This change in PRL secretion in the rat is interpreted as reflecting a shift from neural to placental control in the maintenance of pregnancy (9). It is possible that similar neurological changes during pregnancy may affect secretion of TSH.

In the present study we found that TSH levels were highest between 0200 and 0600 hr throughout pregnancy, precisely the time when nocturnal surges of PRL occur in rats during the first half of pregnancy (2–4). The temporal correlation between increased nocturnal secretion of TSH and nocturnal surges of PRL during the first half of pregnancy suggests that secretion of these two hormones may be regulated by common neuroendocrine factors. One such possible factor is TRH. Administration of large amounts of TRH stimulates release of both TSH and PRL under certain experimental conditions (7, 10, 11). A possible direct role for TRH in stimulation of the nocturnal rise of TSH (and perhaps PRL) awaits further investigation. If the nocturnal prolactin surge is stimulated by TRH there must be some mechanism for terminating the PRL surges at midpregnancy since the TSH "surges" are maintained. It has been suggested that the nocturnal PRL surges are inhibited at midpregnancy by factors secreted by the placenta (9). Perhaps these placental factors have no effect on TSH secretion.

In the present study plasma TSH levels were significantly elevated 2–4 hr after parturition. This elevation may have been the result of the changes in endocrine state induced by parturition and/or of pup-induced stimulation, i.e., suckling. It is unlikely that termination of pregnancy exerts a direct influence on the higher levels of TSH found 2–4 hr after birth, since plasma TSH concentrations in females whose pregnancies were terminated by a combination of ovariectomy and hysterectomy on Day 17 of gestation remained low or slightly suppressed from 6 to 72 hr after surgery (Bridges and Sawyer, unpublished data). It appears more likely that during the postpartum period suckling, a stimulus which is able to activate TSH secretion (11) and oxytocin release (12) in lactating rats, accounted for the elevated concentrations of plasma TSH. All newly parturient animals in our study were actively nursing their litters prior to or at the time of blood collection.

Finally, the diurnal pattern of the TSH rhythm in the pregnant rat differs from that reported in the cycling female (1). Whereas Fukuda *et al.* (1) found a morning–afternoon TSH rhythm with higher TSH levels in the early part of the light period in cycling rats, we found a morning–afternoon rhythm in TSH only during the second half of pregnancy. Furthermore, while Fukuda *et al.* reported basal levels of TSH throughout the dark period, we found that plasma TSH levels were highest during the last 3–4 hr of the dark period. Apparently, TSH secretion in the rat is dramatically modified by neuroendocrine influences during pregnancy which may also affect secretion of other pituitary hormones, including PRL.

Summary. A diurnal pattern of changes in plasma TSH concentrations was found throughout pregnancy in rats. TSH levels (~400 ng/ml) were highest at 0200 hr and 0600 hr and lowest (~240 ng/ml) at 1500 hr. The diurnal pattern in TSH levels changed between the first and second halves of gesta-

tion. Whereas TSH levels declined to basal levels by 0900 hr ($\bar{X}$ ~ 240 ng/ml) from Days 2 to 10, they remained high at 0900 hr ($\bar{X}$ ~ 400 ng/ml) from Days 13 to 22. The temporal correlation between the nocturnal secretion of TSH and PRL in the pregnant rat was discussed. Finally, a large increase in plasma TSH ($\bar{X}$ = 682 ng/ml) occurred 2–4 hr postpartum. This postpartum rise in hormone concentration appeared to result from pup-induced stimuli, i.e., suckling.

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