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Changes in Pituitary Prolactin Responsiveness to TRH during Pregnancy[1] (42308)

JAMES L. VOOGT

Department of Physiology, University of Kansas Medical Center, Kansas City, Kansas 66103

Abstract. Prolactin plasma concentration during pregnancy was determined in rats treated with thyrotropin-releasing hormone (TRH). Day 0 of pregnancy was defined as the day sperm were first found in the vagina. All blood samples were obtained in unanesthetized rats which had previously received a cannula in the right common carotid. On Day 8 of pregnancy, plasma prolactin concentrations reached a peak between 2400 and 0800 hr (lights on from 0600 to 1800 hr). Injection of TRH (1 μg/kg body wt) via the carotid artery increased plasma prolactin levels within 5 min. The largest increase occurred when TRH was given during the prolactin surge, whereas much smaller effects were found when TRH was given at the beginning or after the end of the surge period. Thus, the sensitivity of the prolactin cell to TRH appears to be the greatest when the secretory activity of the cell is high. It was then determined whether there was any change in the sensitivity of the prolactin cell to TRH after the prolactin surges had disappeared at midpregnancy. Injection of TRH between 1100 and 1200 hr increased prolactin less on Day 12 than on Day 8 of pregnancy. Since placental lactogen (PL) levels in the plasma are high on Day 12 compared to Day 8, and are inhibitory to prolactin secretion, it was reasoned that PL may be the factor which caused the reduced sensitivity to TRH. However, hysterectomy on Day 11 failed to increase the pituitary responsiveness to TRH the next day. In summary, these data indicate that the pituitary responsiveness to factors that stimulate prolactin, such as TRH, varies with relation to the time of pregnancy or presence of the nocturnal surge. What cellular mechanism is responsible for these sensitivity changes is not known. © 1986 Society for Experimental Biology and Medicine.

The two daily surges in prolactin that occur during pregnancy in the rat were first described by Butcher *et al.* (1). It was determined later that the diurnal surge terminated by Day 9 and the nocturnal surge by Day 11 of pregnancy (2). These prolactin surges are required for initiation of luteal function and continued progesterone secretion (3). The mechanisms controlling these surges have not been fully determined. Two neural factors which may play a role are dopamine (DA) and thyrotropin-releasing hormone (TRH). McKay *et al.* (4) found a biphasic variation in tuberoinfundibular DA neuronal activity during early pregnancy, which was subsequently lost in late pregnancy when the prolactin surges were no longer present. Although there is no direct evidence that TRH neurons are involved in these surges, TRH has been shown to have a direct stimulatory effect on the anterior pituitary gland and prolactin release (5, 6).

A second model in which serum prolactin levels increase rapidly followed by a decline is the suckled lactating rat. It was shown that TRH concentration in hypophysial portal blood increased slightly during suckling, correlated with prolactin release (7). In addition, the amount of DA the pituitary prolactin cells were exposed to altered the sensitivity of the cells to the effects of TRH (7, 8). It is possible that a change in the sensitivity of the pituitary to TRH during the first half of pregnancy partially accounts for the appearance of the twice daily prolactin surges.

After midpregnancy these prolactin surges are no longer present. A great deal of indirect evidence suggests that this is due to the inhibitory effects of rat placental lactogen (PL) on prolactin release (9–11). Stimuli that normally increase prolactin release such as suckling (12), or TRH (13), were ineffective when large amounts of PL are present during the second half of pregnancy.

The present study was designed to determine whether any change in prolactin responsiveness to TRH occurs at the time of the nocturnal surge, suggesting a role of TRH in controlling the surge. Secondly, it was determined whether the decreased responsiveness to TRH

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during the second half of pregnancy (13) is due to the presence of the uterine–placental unit.

Materials and Methods. *Animals.* Rats were purchased from Sasco (Omaha, Nebr.) of the Holzman strain and maintained on a 12:12 light dark cycle with lights on at 0600 hr. Males were placed with females and the presence of sperm in the vaginal lavage was designated as Day 0 of pregnancy. Blood samples were obtained from heparinized rats via a cannula in the right common carotid artery connected to a piece of extension tubing, such that the animals were not handled or distrubed just prior to or during each bleeding (14). Each animal remained in an individual cage at all times. Following each bleeding (0.3 ml blood), an equivalent amount of saline was injected via the cannula. In any one rat no more than a total of 2.1 ml of blood was removed, which represented about 10% of the total blood volume. When samples were taken during the dark period, a 25 W red light was used to illuminate the sampling area. All surgeries were done using either metaphan or ether as the anesthetic. Rats were cannulated 1 day prior to use in an experiment.

Experiments. In order to have an accurate description of the nocturnal prolactin surge, a group of rats was bled every 2 hr beginning at 2200 hr on Day 7 and continuing until 0800 hr on Day 8 of pregnancy. Further experiments were designed based on the timing of the prolactin surge found in this group.

The second set of experiments was done to compare prolactin responsiveness to TRH (1 μg/kg body wt) at different times on Day 8. Rats were injected via the cannula with either saline or TRH and bled at 0, 5, 15 and 25 min during the following time periods: 2400–0100, 0500–0600, and 1400–1500 hr. In general, rats were bled during only one time period, and received either saline or TRH, not both solutions.

In the third experiment, a comparison of the prolactin response to TRH was made between Day 8 and Day 12 pregnant rats, during a nonsurge time (1100–1200 hr). A blood sample was taken at zero time, followed immediately by an injection of 0.3 ml saline. Additional samples were taken at 5, 15, and 25 min. Just after the 25-min sample, an injection of TRH (1 μg/kg body wt) was given via the cannula and a second set of blood samples taken 5, 15, and 25 min later. In order to determine whether secretions from the uterine–placental unit contribute to the prolactin response to TRH, pregnant rats were hysterectomized on Day 11 and bled on Day 12, between 1100 and 1200 hr, following saline and TRH injections.

Hormone assays. All blood samples were centrifuged and frozen until assayed for PL and prolactin. To measure serum PL, the Nb2 lymphoma cell bioassay was used (15), with our modifications (16). Ovine prolactin (NIH-S-10) was used as the standard. To prevent the prolactin present in the plasma samples from stimulating the lymphoma cells to grow, antiserum to rat prolactin was added to each culture well. This antiserum (NIADDK—anti-rPL-ICF-1), generously supplied by Dr. Parlow, was used at a final concentration of 1:60,000. This will neutralize up to 25 ng of rat prolactin in the culture well, equivalent to 20,000 ng/ml plasma. Plasma samples (0.2 and 1.2 μl) were assayed in duplicate. Both the intra- and interassay coefficients of variation were 10%. The least concentrated standard used was 0.050 ng ovine prolactin.

Prolactin was assayed in triplicate by RIA. The assay materials were provided by the NIADDK Hormone Distribution Program, and the reference preparation used was NIADDK rat PRL-RP1.

Statistics. One-way analysis of variance was done to determine whether there was any significant prolactin response to TRH. Dunnett's *t* test was used to compare means with pretreatment values. Students *t* test was used to compare means between saline and TRH treatment groups.

Results. Rats cannulated on Day 7 of pregnancy demonstrated a significant elevation in prolactin between midnight on Day 7 and 0800 on Day 8 (Fig. 1). The prolactin surge did not begin until after 2400 hr, and was completed in most animals by 0800 hr. The large individual variation in prolactin values at 0200 and 0600 hr is primarily due to the lack of uniformity in the onset and termination of the surge among individual rats. At 0200 hr, two of six rats had not yet begun to show a prolactin surge, and at 0600 hr, two of six rats had completed the surge. At 0400, prolactin was elevated in all rats, although the magnitude of the surge varied considerably.

Based on the timing of the nocturnal surge

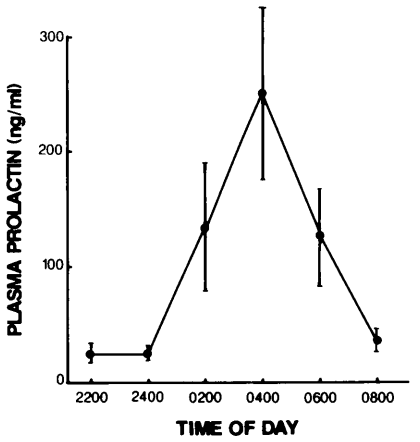

FIG. 1. Plasma concentration of prolactin at various times on Day 8 of pregnancy. Blood samples were collected through carotid cannulas inserted 1 day earlier. Significant increases occurred at 0200, 0400, and 0600 hr compared to 2200 hr. The data are expressed as means ± SEM. Six rats were used in this group.

found in the first experiment, several groups of rats were treated with a single intracarotid injection of TRH or saline at various times on Day 8. This was done to test whether there was any change in pituitary responsiveness to TRH dependent on the current state of prolactin secretion at the time of injection. The data are shown in Fig. 2. Injection of TRH at 2400 hr significantly increased prolactin compared to controls and compared to preinjection levels ($P < 0.05$). The greatest change in prolactin occurred after 5 min. Between 2400 and 0500 hr plasma prolactin had increased from 12 to 98 ng/ml, and injection of TRH significantly increased it an additional 112 ng in 5 min. This increment in prolactin at the 0500 hr time period was significantly greater than at the 2400 hr period. At 15 and 25 min prolactin returned to preinjection levels. Saline injection had no effect. At 1100 hr, plasma prolactin had become very low (3 ng/ml), indicating that the nocturnal surge was over. TRH injection at this time significantly increased prolactin, compared to preinjection or control levels, but the increase was much less than what occurred at 0500 hr. Similarly, at 1400 hr prolactin levels were quite low (16 ng/ml), and TRH treatment resulted in changes similar to that seen at 1100 hr.

The next experiment was done to test the pituitary prolactin response to TRH with day of pregnancy as the variable. In this experiment each rat served as its own control. To do this, saline was given immediately after the first blood sample was drawn. Three more samples were taken at 5, 15, and 25 min, at which time TRH was given via the carotid cannula. Three more samples were taken at 30, 40, and 50 min, or 5, 15, and 25 min after injection of the TRH. As seen in Fig. 3, saline injection had no effect on prolactin levels. Data from all three groups prior to TRH injection were combined because there was no difference among the groups, as expected. Injection of TRH (1 μg/kg body wt) significantly increased prolactin in all three groups after 5 min compared to preinjection levels. Prolactin quickly returned to baseline by 10 and 20 min. The Day 8 rats responded to TRH to a much greater extent than did the Day 12 rats. Surprisingly, Day 12 rats hysterectomized on Day 11 had the same prolactin response to TRH

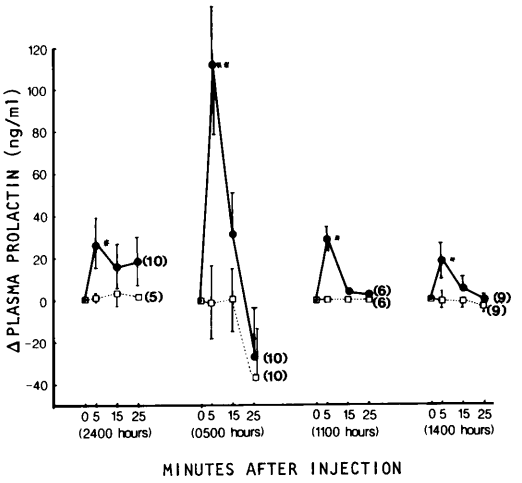

FIG. 2. Change in mean plasma concentration of prolactin at various times on Day 8 following injection of TRH (1 μg/kg body wt). TRH (●) or saline (□) was injected at time 0 immediately after a control blood sample was taken. This was followed by three more samples at 5, 15, and 25 min. The absolute change in plasma prolactin from time 0 was plotted at each time period because the actual plasma concentration of prolactin varied greatly from one time period to another, due to the prolactin surge. At each time period, the 5-min sample had significantly (*$P < 0.05$) more prolactin than at 0 or in saline-treated rats. The change in prolactin from 0 to 5 min during the 0500 hr time period was significantly greater (**$P < 0.05$) than at any other time period. Parentheses indicates number of rats/group.

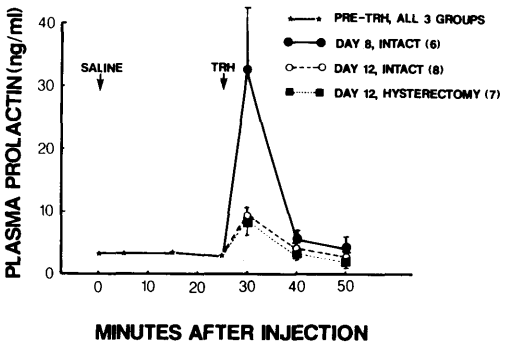

FIG. 3. Plasma concentration of prolactin after saline and TRH injection (1 μg/kg body wt) on Days 8 and 12 of pregnancy. Hysterectomy was done on Day 11 in one group. TRH significantly (*$P < 0.05$) increased prolactin at 5 min in all groups. The 5-min sample in Day 8 rats was significantly greater (**$P < 0.05$) than either group of Day 12 rats. Parentheses indicates number of rats/group.

as did the intact rats on Day 12. Plasma placental lactogen levels averaged about 3000 ng/ml in the intact Day 12 rats and were minimal or undetectable in the hysterectomized rats.

Discussion. The experiments of this study show that the response of pituitary prolactin cells to the presence of TRH depends on the current status of prolactin secretion. The largest change in prolactin secretion following TRH injection occurred during the prolactin surge (0500 hr), whereas much smaller changes occurred at the beginning and after the completion of the surge. This suggests that TRH does not initiate the nocturnal prolactin surge, but may have a physiological role in determining the magnitude or duration of the surge.

Most research regarding the importance of TRH in prolactin regulation has focused on the suckling-induced increase in prolactin. A brief suckling period has been reported to increase the sensitivity of lactotrophs to TRH (17). However, these results were not confirmed by others (18). Another approach has been taken to demonstrate an increased responsiveness of pituitary prolactin to TRH. A brief decrease in the level of dopamine reaching the pituitary significantly increased the prolactin response to an injection of TRH in lactating rats (7, 8), suggesting that internal factors do regulate lactotroph responsiveness to TRH during suckling.

The nocturnal prolactin surge is quite different from suckling-induced prolactin release. The nocturnal surge is initiated by a one time stimulation of the cervix, yet occurs daily for the first half of pregnancy. Thus each night the surge is initiated and sustained for several hours without any external stimuli, in contrast to what occurs during lactation. No measurements have been made of TRH secretion during the surge, nor have antibodies to TRH been injected to determine whether such treatment would terminate the surge. The present results suggest that initiation of the nocturnal prolactin surge is not a result of an increase in TRH reaching the pituitary. Injection of TRH at or near the beginning of the surge, presumably a time when the cellular mechanisms of the lactotroph for prolactin release are becoming activated, did not increase prolactin release any more than during the intersurge period (1100 or 1400 hr). Once the release mechanisms have been activated and prolactin secretion is occurring at a high rate, TRH is more potent in causing prolactin release as measured by the increase in the concentration of prolactin in the blood. This is similar to the results obtained in the suckling-TRH experiments referred to earlier (17). This does not prove that TRH participates in the nocturnal prolactin surge, but does suggest that any role it may have is limited to augmenting rather than initiating the release process. Insight into what neural mechanisms may be involved in this change in sensitivity to TRH during a 24 hr period can be found in several studies. McKay *et al.* (4) found that dopaminergic activity in the median eminence of pregnant rats on Day 8 was high at midnight, decreased until 0600 hr, and then increased again and remained high until 1800 hr. Others showed that a temporary decrease in dopamine reaching the pituitary enhanced pituitary prolactin release to an injection of TRH in lactating rats (7, 8). Our present experiments show that the greatest response to TRH occurred between 0500 and 0600 hr, which corresponds to the time of lowest dopamine activity.

The second part of this study concerns the mechanism for the termination of the nocturnal surges after Day 10 of pregnancy. One partial answer may be that the pituitary is not as sensitive to prolactin releasing factors at this time. Indeed, the data show (Fig. 3) that injection of TRH on Day 8 increased both the absolute amount of prolactin in plasma and the percentage increase over baseline com-

pared to a similar injection on Day 12. Similar results were reported by Bridges *et al.* (13) using a much larger dose of TRH (2 μg/rat).

The mechanism for this decreased sensitivity to TRH remains unclear. Numerous reports by our laboratory (10, 11) and that of Yogev and Terkel (9, 12) provide correlative evidence that placental lactogen is involved in terminating the prolactin surges at midpregnancy. Results of Demarest *et al.* (19) suggest that a uterine–placental factor, presumably placental lactogen, stimulates dopaminergic neurons in the median eminence and is responsible for terminating the nocturnal prolactin surges. We reasoned that this same factor may be involved in decreasing prolactin responsiveness to TRH after midpregnancy. However, the lack of any effect of hysterectomy on prolactin levels in response to TRH does not support this theory. Perhaps it is necessary to remove the uterus several days earlier, as Demarest *et al.* (19) did, in order to maintain lactotrophs which are highly sensitive to TRH.

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