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Tyrosine Hydroxylase Activity in the Median Eminence Area of the Pseudopregnant Rat during Surges of Prolactin Secretion (41058)[1]

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Abstract. Serum prolactin and LH levels were measured at several times during pseudopregnancy (PP) in the rat and compared to levels found in rats during diestrous Day 2. In the same rats, tyrosine hydroxylase (TH) activity and dopamine-β-hydroxylase (DBH) activity were measured in the median eminence area as indices of dopaminergic and noradrenergic neuronal activity, respectively. Serum LH and median eminence DBH were unchanged throughout the experiment. Serum prolactin was elevated at 1800 hr on Days 4 and 8 of PP and at 0100 hr on Days 5 and 9 compared to unstimulated controls. During the intersurge time (2200 hr) prolactin levels were low but slightly higher than controls. Tyrosine hydroxylase activity in the median eminence was low during the diurnal and nocturnal prolactin surges compared to unstimulated controls. It is concluded that the acute increases in prolactin levels in the blood that occur twice daily during PP may be related to the decrease in activity in dopamine containing neurons that occurs at that time.

A substantial amount of evidence indicates that dopamine is a physiological inhibitor of prolactin secretion, acting directly on anterior pituitary cells. It has been shown that the amount of dopamine found in stalk blood of rats is sufficient to partially account for suppression of prolactin release (1). Dopamine is capable of binding to cells in the pituitary (2) and has been shown to associate with the prolactin secretory granules (3, 4). However, there is evidence that dopamine cannot account for all of the inhibitory effects of the hypothalamus, and other hypothalamic factors may exist that are physiological regulators of prolactin release (5, 6).

The secretion pattern of prolactin during the life cycle of the female rat is characterized by abrupt fluctuations, such as the response to suckling, mating, and during proestrus. Only limited studies have been reported correlating these dynamic changes in prolactin release with changes in activity of dopaminergic neurons in the hypothalamus (7–9). In the present study the activities of tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) were determined as an index of dopaminergic and noradrenergic activity in the medial basal hypothalamus during the diurnal and nocturnal surges of prolactin in the pseudopregnant rat.

Materials and Methods. The animals used in this study were Sprague–Dawley female rats purchased from the Sasco Company (Omaha, Nb.) weighing 180–200 g. They were housed five per cage in a temperature-controlled room (21–24 c) with lights on from 0500 to 1900 hr. Food and water were available *ad libitum*. After two normal estrous cycles were recorded by obtaining vaginal smears daily, the cervix of each rat was stimulated mechanically for 1 min on the evening of proestrus using a glass rod. The first day of a leukocytic smear was designated as Day 1 of pseudopregnancy (PP). Rats were killed by rapid decapitation at 1800 hr (diurnal surge) or 2200 hr (between surges) on Days 4 and 8 of PP and at 0100 hr (nocturnal surge) on Days 5 and 9 of PP. Groups of nonstimulated rats were killed at 0100, 1800, and 2200 hr on diestrus Day 2.

Immediately after decapitation, the brain was removed and dissected on a cold aluminum block using a dissecting microscope. A piece of tissue weighing about 500

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μg representing the median eminence and some surrounding tissue was obtained. This method is described in an earlier report (10) and is similar to the one used by Chiocchio *et al.* (11). The remainder of the hypothalamus was also dissected and weighed. The dissected tissues were homogenized in 75 μl of cold Tris–Triton buffer (pH 6). Ten microliters was removed from each sample for protein determination (12) and stored at −80°. The remainder of the samples was then centrifuged at 5000*g* for 10 min and the supernatant was frozen and stored at −80° until assayed for TH and DBH activity.

Tyrosine hydroxylase activity was determined in 25 μl of tissue supernatant by slight alteration of the methods of Coyle (13) and Saavedra *et al.* (14), as described previously (10). Dopamine-β-hydroxylase activity was determined in 25 μl of tissue supernatant by the method of Molinoff *et al.* (15) as modified by Saavedra *et al.* (14).

Trunk blood was obtained following decapitation and the serum was separated from the cells and stored at −20° for subsequent radioimmunoassay (RIA). Each serum sample was assayed for LH and prolactin in duplicate or triplicate by the methods of Niswender (16, 17). The antisera, hormone for iodination, and reference preparation (NIAMDD-Rat Prolactin-RP-1) for the prolactin assay were provided by the NIAMDD Hormone Distribution Program. The antisera for the LH assay (GDN-15) was kindly provided by Dr. Gordon Niswender and the ovine LH for iodination (LER-1056-C2) was kindly provided by Dr. Leo E. Reichert. The reference preparation used was NIAMDD rat LH-RP-1. All hormone levels were calculated from logit-logdose standard curves generated on a calculator. Enzyme activity data and hormone data were analyzed by Student's *t* test. Two comparisons were made for each group of three means of data collected at a single time of day. Comparisons were made primarily between pseudopregnant and unstimulated diestrous rats. *P* values less than 0.05 were accepted as significant.

Results. The pseudopregnant rats showed the expected elevation in serum prolactin during the nocturnal (0100 hr) and diurnal (1800 hr) surge periods compared to a between surge time (2200 hr) or compared to rats killed at similar times during diestrus 2 (Fig. 1). The only time during the surge periods that prolactin levels in the pseudopregnant rats were not significantly higher statistically ($P < 0.10$) than in unstimulated rats was on Day 4 at 1800 hr. There was a large amount of variability in this pseudopregnant group so that the fivefold elevation in prolactin did not meet the $P < 0.05$ statistical criterion. Serum prolactin values in pseudopregnant rats bled between the surge times (2200 hr) were significantly higher ($P < 0.02$) than unstimulated rats when the data for PP_4 and PP_8 were combined. Serum LH levels did not vary significantly among any of the different groups of pseudopregnant rats nor was there any difference when pseudopregnant and diestrous rats were compared. The levels of LH were uniformly low (5–20 ng/ml serum).

Tyrosine hydroxylase activity in the median eminence in pseudopregnant and diestrous rats during the diurnal prolactin surge (1800 hr), the nocturnal prolactin surge (0100 hr), and the intersurge period (2200 hr) are shown in Fig. 2. Enzyme activity during the intersurge period was not different between pseudopregnant and diestrous rats. Significantly lower TH activity was found at 1800 hr on both Days 4 and 8 of PP compared to unstimulated controls. This is the inverse of serum prolactin levels at this time. During the nocturnal surge (0100 hr), TH activity was significantly lower on Day 5 of PP compared to controls. On Day 9 of PP, even though TH activity was only 31% of controls, variability in individual values made this nonsignificant. However, when the data of the 2 days of PP were combined, TH activity was significantly lower ($P < 0.01$) than found in unstimulated controls. As was found during the diurnal surge, TH activity in the median eminence varied inversely with prolactin levels during the nocturnal surge. Tyrosine hydroxylase in the remainder of the hypothalamus did not vary significantly between unstimulated and pseudopregnant rats.

Dopamine-β-hydroxylase activity in the median eminence or the remainder of the hypothalamus did not vary between pseudopregnant and control rats during

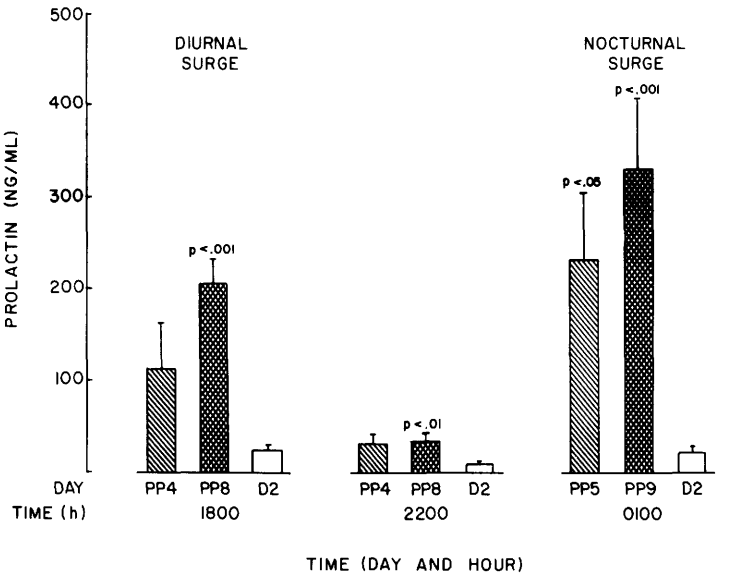

FIG. 1. Serum prolactin levels at various times during pseudopregnancy (PP) and diestrous Day 2 (D2). Each bar height represents the group mean ± 1 SE (n = 5 – 14). All p values represent comparisons between different days of PP and D2 at each time of day.

either of the surge periods or during the intersurge period (Table I). This suggests that median eminence norepinephrine involvement in prolactin secretion during PP may be minimal.

Discussion. The evidence that dopamine exerts a tonic inhibitory effect on prolactin secretion is substantial (18). In this study we sought an answer to the question whether the acute rise in prolactin that occurs twice daily during PP is related to changes in dopaminergic activity in neurons

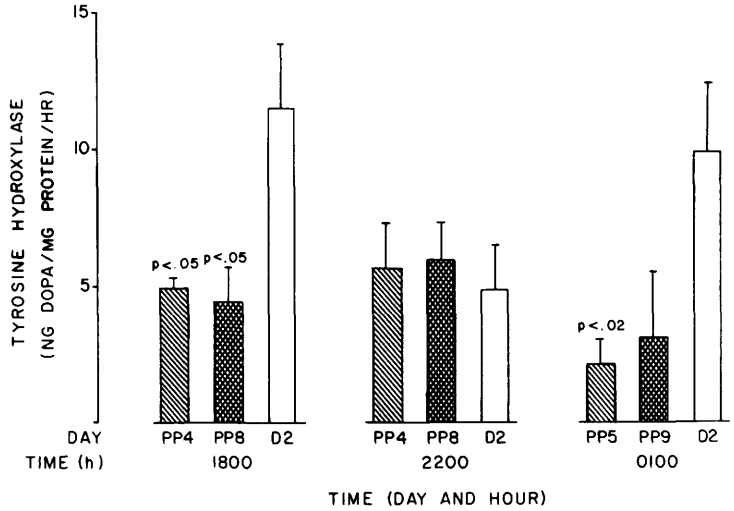

FIG. 2. Tyrosine hydroxylase activity in the median eminence area at various times during pseudopregnancy (PP) and diestrous Day 2 (D2). Each bar height represents the group mean ± 1 SE (n = 5 – 14). Tyrosine hydroxylase activity is measured in nanograms of dopa per milligram of protein per hour. All p values represent comparisons between days of PP and D2 at each time of day.

TABLE I. DOPAMINE-β-HYDROXYLASE[a] ACTIVITY DURING PSEUDOPREGNANCY

Time (hr)	Days		
	$PP_{4/5}$[b]	$PP_{8/9}$[b]	D_2
0100	3.55 ± 0.56[c]	4.30 ± 2.50	3.59 ± 0.26
1800	3.46 ± 0.41	4.29 ± 0.82	4.64 ± 0.63
2200	3.18 ± 0.97	2.75 ± 0.34	3.59 ± 0.74

[a] DBH units are expressed as nmoles *n*-methyloctopamine/mg protein/hr.

[b] $PP_{4/5}$ and $PP_{8/9}$. The rats were bled at 0100 hr on Days 5 and 9 of PP, and at 1800 and 2200 hr on Days 4 and 8 of PP.

[c] Mean ± SEM.

in the median eminence area of the hypothalamus. It has been shown that TH activity is closely linked to the rates of catecholamine utilization and release (19) and thus may serve as an index of neuronal activity (20). It has been shown that most of the TH in the median eminence is associated with dopaminergic neurons (21). Very recently Nicholson *et al.* (22) clearly showed that measurement of TH activity in the median eminence is a good index of dopaminergic neuronal activity. Thus the evidence is strong that measurement of TH activity during periods when hormonal levels in the blood are changing is a useful technique for studying the role of dopamine containing neurons in regulating the release of anterior pituitary hormones.

The prolactin surges occurred as expected at 0100 and 1800 hr during PP and were absent during the intersurge period (Fig. 1). This is in agreement with the findings of Freeman *et al.* (23). LH levels in the blood did not vary during the three time periods nor were they different from unstimulated diestrous controls. Thus the changes that occurred in TH activity are probably related to prolactin secretion and not LH secretion. The decline in TH activity during the prolactin surges compared to unstimulated control rats suggests that there is a decrease in dopaminergic neuronal activity during this time, resulting in the rise in prolactin. This also has been suggested by de Greef and Neill (24) in a study in which dopamine levels in hypophysial portal blood were measured and found to be lower in cervically stimulated rats than in controls. However, it cannot be concluded that there is a simple inverse relationship between dopamine secretion into the portal blood and prolactin secretion from the pituitary. DeGreef and Neill (24) found that a decrease in dopamine levels in the portal blood similar to magnitude to what occurred during the prolactin surges of PP, resulted in only a 1.5-fold rise in prolactin compared to a normal rise of 4- to 5-fold. Additionally, when all the TH activity data from rats killed during the prolactin surges (mean = 3.68 ± 0.67) were compared to the TH activity data during the intersurge period (mean = 5.67 ± 0.84), the level of significance between these two groups was not less than 5%. Thus it cannot be concluded that the rise in prolactin that occurs twice daily during PP is solely the result of a decrease in dopaminergic activity in the median eminence. There was a decrease in TH activity in diestrous rats at 2200 hr ($P < 0.10$) compared to 1800 hr, without any change in prolactin levels (Fig. 2). Thus, changes in dopaminergic activity in the median eminence may not always be reflected in changes in prolactin secretion. Other hypothalamic factors (5, 6) may also contribute to the acute changes that occur in prolactin secretion.

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