Increased Serum Prolactin Induced in Hypophysectomized Rats Bearing Ectopic Pituitaries[1] (37719)

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Surgical removal of the adenohypophysis (AP) from its normal site in vascular contact with the hypothalamus and transplantation to the renal capsule allows relatively autonomous prolactin secretion (1). Prior to radioimmunoassay methods for quantifying serum prolactin, many workers felt that prolactin release from such ectopic AP was greater than normal. However, Chen *et al.* (2) reported that transplantation of multiple ectopic AP into hypophysectomized (Hx) rats could not increase serum prolactin to the high levels noted in intact lactating rats. Nevertheless, prolactin release from ectopic AP is substantial and might serve as an *in vivo* assay for prolactin-inhibiting and prolactin-releasing factors (PIF and PRF). One potential problem in such an assay is the possible influence of the *in situ* hypothalamus on prolactin release from the ectopic AP. Studies involving exogenous L-dopa (3) and median eminence lesions (4) in Hx rats bearing ectopic AP suggest, but do not prove, that prolactin release may still be influenced by hypothalamic hypophysiotrophic hormones (HTH) reaching the ectopic AP via the systemic circulation. A second potential problem in the proposed assay system involves the function of the pars tuberalis after hypophysectomy. Recent studies (5, 6) revealed structural changes in this tissue after Hx, but there was no attempt to relate these changes to prolactin release. However, various authors (2, 3, 7) have reported detectable serum prolactin in Hx rats, and the possibility remains that prolactin is released in measurable amounts by the pars tuberalis after hypophysectomy.

The possibility that endogenous HTH could influence prolactin release either from ectopic AP or from pars tuberalis would greatly complicate interpretation of the results of our PIF–PRF assay sytem. For example, the injected test materials could be affecting endogenous HTH which, in turn, affect prolactin release. For this reason, the present experiments were designed to investigate the independence and stability of serum prolactin in Hx rats with and without ectopic AP.

Materials and Methods. Hypophysectomized female rats (Sprague–Dawley) weighing 180–200 g were purchased from a commercial supplier and received AP transplants as described in a previous paper (8). Two differences from the previous procedures were that 10–12 days elapsed between Hx and AP transplantation and that Hx rats without AP transplants were not subjected to sham surgery. All sampling of blood serum for prolactin assay occurred 7 or 11 days after transplantation. Blood samples (1.5 ml) were collected by heart puncture under anesthesia unless otherwise noted. Various anesthetics and sampling schedules were employed in different experiments.

Prolactin concentrations were quantified by a radioimmunoassay (RIA) system developed in our laboratory from reagents generously supplied by others. The antiserum to rat prolactin (Ab #10) was provided by Dr. J. D. Neill, and its validation was published by him (7). The rat prolactin used as standard in the assay (RP-1) was provided by the National Institute of Arthritis and Metabolic Diseases (NIAMD) and its potency was approximately 11 IU/mg. The purified rat prolactin for iodination (I-1) was also pro-

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vided by NIAMD, but two different vials of this preparation were used at different times. Even though both vials were labeled I-1, they will be designated in this paper according to the years in which they were received (*i.e.,* 1969-I-1 and 1972-I-1).

The RIA system developed using these reagents was of the equilibrium type with 3 days of equilibrium allowed at 4° among anti-prolactin, ^{131}I-prolactin, and the unlabeled prolactin in standards or unknowns in a diluent of 1% bovine serum albumin in 0.01 *M* phosphate-buffered saline. The tubes were incubated at 4° for 3 more days after addition of anti-rabbit gamma globulin to precipitate the antigen–antibody complex. After centrifugation and decanting of the supernate, the precipitate was counted in a crystal scintillation detector.

Rat prolactin (NIAMD-I-1) was radioiodinated using chloramine T, but the optimum conditions varied according to which vial was being used. For 1972-I-1, best results were obtained when 30 μg chloramine T was reacted with 2 μg rat prolactin and 1 mCi ^{131}I for 20 sec. Subsequent gel filtration chromatography (Sephadex G-75) resulted in a profile of radioactivity in 10-drop fractions which displayed a gradual increase in counts in the fractions preceding the one with maximal ^{131}I. Best assay results were obtained when ^{131}I-prolactin from the peak tubes was used. When the 1969-I-1 preparation was radioiodinated, a mixture of 45 μg chloramine T, 2 μg rat prolactin, and 2 mCi ^{131}I was reacted for 120 sec. In this case, the profile of radioactivity in the effluent fractions from the column showed a rapid increase to the fraction with maximal ^{131}I followed by a gradual decline. Best assay results were obtained with the third, fourth, and fifth 10-drop fractions following the peak.

Anti-prolactin serum was diluted to 1:16,000 with 1:400 normal rabbit serum, and 200 μl was added to each assay tube giving a final concentration of 1:64,000. When the specific binding of the ^{131}I-prolactin to the antibody was expressed as a percent of the total radioactivity, binding in the zero standard tubes (B_0) was approximately 30% for the 1969-I-1 preparation and 60% for the 1972-I-1 preparation. The binding in all other tubes was expressed as a percent of the B_0 for that particular assay. When testing for parallelism between increasing log dosages of standard or serum, the % B_0 was transformed to logit units. The minimum detectable serum concentration was 1–2 ng/ml, depending on whether 200 or 300 μl of serum was assayed. All serum samples for a given experiment, as denoted in *Results,* were assayed in a single RIA to eliminate assay to assay variation in the statistical evaluation of treatment effects using a simple *t* test.

Results and Discussion. Transplantation of AP equivalent to ⅛ of one gland in Hx rats caused a significant ($p < 0.01$) elevation of serum prolactin (Expt. 1, Table I). The detection of measurable prolactin in Hx rats without AP transplants confirms previous reports (2, 3, 7). Furthermore, we compared the log dosage response curves of increasing amounts of serum from Hx rats and Hx rats with ectopic AP. Both were parallel to the curve produced by increasing amounts of standard rat prolactin which agreed with a previous report in which NIAMD reagents were used (7).

In Expt. 2, the possible effect of urethane anesthesia on serum prolactin was investigated since several authors reported anesthetic effects on prolactin in intact rats (9–11). In this study, the first blood sample under urethane anesthesia was taken by heart puncture within 3–4 min after ip injection of a 1.2 g/kg dosage. In Hx-only rats and Hx rats with AP transplants, serum prolactin values were the same as those collected by rapid decapitation (Table I). A second blood sample was taken by heart puncture from the urethane-anesthetized rats 20 min after the first sample. The data in Table I clearly indicate that the prolactin concentration in this second sample was significantly elevated in Hx rats bearing transplants of either ¼ AP ($p < 0.05$) or ½ AP ($p < 0.01$). On the other hand, prolactin concentrations were significantly ($p < 0.01$) decreased in the Hx rats without ectopic AP. A comparison of the ¼ AP and ½ AP groups sampled under urethane reveals that the amount of the transplant affected serum prolactin only in the second sample.

The influence of pentobarbital (PB)

TABLE I. Influence of Amount of Ectopic AP and Method of Sample Collection on Serum Prolactin.

Expt. no. and method of blood collection	Serum prolactin (ng/ml) in rats with various amounts of ectopic AP			
	Hx only	⅛ AP	¼ AP	½ AP
Expt. 1				
Ether anesthesia	8.2 ± 0.6 (6)[a]	14.7 ± 0.6 (5)		
Expt. 2				
Decapitation without anesthesia	7.8 ± 0.6 (5)		30.8 ± 3.4 (6)	
Urethane anesthesia				
1st sample[b]	7.2 ± 0.7 (5)		26.2 ± 3.0 (9)	28.2 ± 1.6 (8)
2nd sample[c]	3.8 ± 0.2 (5)		34.5 ± 2.8 (8)	42.8 ± 2.7 (8)

[a] Means ± SE, and the number of observations in parentheses.
[b] Blood collected 3–4 min after urethane injection.
[c] Blood collected 20 min after the first sample.

anesthesia was investigated in Expts. 3–5 (Table II). All three studies were of the same experimental design, and the data were intended to be combined. However, they are presented separately because serum prolactin concentrations of comparable groups varied so much between experiments. Much of this variation was due to differences among the RIA used to quantitate serum prolactin from each experiment. Prolactin concentrations in Hx-only rats were much larger in Expt. 5, and serum prolactins in Hx rats with ½ AP transplants were also increased over those in Expts. 3 and 4. A comparison between Expts. 3 and 4 reveals that the ectopic AP elevated serum prolactin much more in Expt. 3. This difference may reflect the fact that the surgical transplantations in Expt. 4 were performed by someone less experienced in such procedures, possibly resulting in reduced viability of the AP transplants.

Separate examination of the results from each PB experiment (Table II) reveals that in no case did prolonged PB anesthesia (35 mg/kg) decrease serum prolactin in Hx-only rats as prolonged urethane anesthesia had done in Expt. 2. However, prolonged PB anesthesia in Hx rats with ectopic AP always tended to increase serum prolactin just as noted for urethane in Expt. 2. This increase in serum prolactin could sometimes be detected as early as 25 min after PB but in all cases serum prolactin was significantly increased by 45 min. The literature contains reports of PB administration to intact rats increasing prolactin release (10) as well as decreasing it (12). Wuttke *et al.* (13) investigated the possible mechanisms of action of PB on prolactin release. These authors concluded that PB acted directly on the pituitary to inhibit prolactin release, but that PB also acted on the hypothalamus to facilitate prolactin release by its inhibition of PIF. These dual mechanisms opposing each other may explain the divergent results reported by various authors using different experimental models. If the hypotheses of

TABLE II. Influence of Time After Pentobarbital (PB) Injection on Serum Prolactin.

	Minutes after PB injection[a]	Serum prolactin (ng/ml)	
		Hx only	Ectopic AP[b]
Expt. 3	5	3.6 ± 0.9 (2)[c]	31.7 ± 1.7 (5)
	25	3.7 ± 0 (2)	39.1 ± 2.4 (5)
Expt. 4	5	7.5 ± 0.8 (3)	18.1 ± 1.5 (3)
	25	6.7 ± 0.9 (3)	22.1 ± 2.9 (3)
	45	6.6 ± 1.2 (2)	26.9 ± 2.7 (3)
Expt. 5	5	19.3 ± 3.0 (3)	53.0 ± 5.7 (4)
	25	18.8 ± 3.8 (3)	62.5 ± 5.2 (4)
	45	14.0 ± 3.0 (2)	73.0 ± 4.2 (3)

[a] All rats sampled at the later time periods were also sampled at each of the previous periods.
[b] All ectopic AP transplants were equivalent to ½ AP.
[c] Means ± SE, and number of observations in parentheses.

Wuttke *et al.* (13) were correct, we would expect PB to act directly on the ectopic AP to decrease prolactin release. Since we observed an increase in serum prolactin, one possible explanation is that the PB effect on the hypothalamus predominated, and prolactin release was increased via HTH reaching the ectopic AP in the systemic circulation. This PB effect on circulating HTH could be either an increase in PRF or a decrease in PIF.

General Discussion. The effects of prolonged anesthesia with urethane and PB on serum prolactin in Hx-only rats and Hx rats with ectopic AP provided information concerning the potential usefulness of such rats in an assay for PRF and PIF. The present results demonstrated that serum prolactin was not stable in anesthetized Hx rats bearing ectopic AP. On the contrary, Lu and Meites (3), using similarliy prepared rats, observed comparable prolactin concentrations in first and second blood samples collected under brief ether anesthesia 30–60 min apart. The increased serum prolactin in the present study probably resulted from enhanced prolactin secretion, but decreased rates of peripheral metabolism for circulating prolactin might also have contributed. Furthermore, it was not possible to separate in these data the effect of prolonged anesthesia from the effect of multiple blood sampling. If, however, prolactin release from the ectopic AP was increased by these experimental conditions, the effect was probably mediated by circulating HTH. Lu and Meites (3) also concluded that exogenous L-dopa affected prolactin release by ectopic AP by altering circulating PIF. These conclusions point out the major weakness of the proposed assay system. Any injected test material that alters prolactin release could be working on either the ectopic AP or the hypothalamus.

The present results did not support the possibility of pars tuberalis or remnants of pars distalis tissue secreting prolactin under the influence of HTH. If this situation did exist, we would expect serum prolactin in the Hx-only rats to respond to the experimental conditions of anesthesia and blood sampling in a manner similar to that in Hx rats with AP transplants.

Summary. A radioimmunassay was developed for quantifying rat prolactin in blood serum, but the assay detected small amounts of immunoreactive prolactin in serum from hypophysectomized (Hx) rats. Prolonged anesthesia of these Hx rats with urethane significantly decreased their serum prolactin values, while prolonged anesthesia with pentobarbital (PB) had no significant effect.

Transplantation of pituitary tissue (AP) to the renal capsule of Hx rats elevated serum prolactin, and this effect was produced with as little as ⅛ AP. Prolonged PB or urethane anesthesia combined with multiple blood sampling of Hx rats bearing ectopic AP significantly increased serum prolactin. The absence of stable prolactin concentrations in these rats suggests that prolactin release by ectopic AP may still be influenced by hypothalamic hypophysiotrophic hormones in the systemic circulation.

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