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Hyperprolactinemia and Hyperadrenocorticism Accompanied by Normal Blood Pressure in Sprague–Dawley Rats[1] (41243)

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Abstract. Pituitary glands removed from adult female normotensive Sprague–Dawley (S–D) rats were implanted beneath the renal capsule of 100-day-old male S–D rats. Female neonatal S–D rats were given a single sc injection of 1.25 mg of testosterone proprionate (TP) suspended in sesame oil. Systolic blood pressure and blood samples were taken at various time intervals postimplantation and TP treatment prior to autopsy. Both the TP treatment and pituitary implants caused hyperprolactinemia, increased adrenal weight concomitant with thymus gland involution, hypersecretion of corticosterone, but no increase in systolic blood pressure. It is suggested that the failure of these hyperprolactinemic S–D rats to develop hypertension was due to the absence of a genetically mediated hypertensinogenic factor present in this normotensive strain which is activated by chronic hyperprolactinemia.

The ancient hormone prolactin has undergone a modern renaissance with the discovery that it is secreted by humans and that it has a broad spectrum of physiological effects, e.g., maternal behavior, adjustment to salinity, lipid and glucose mobilization, response to stress, and increased cardiovascular reactivity (1–3). Although the evidence to date is equivocal whether prolactin regulates aldosterone secretion directly, it is clear that prolactin and ACTH act synergistically causing increased adrenocortical steroidogenesis in response to stress and that prolactin per se will cause increased arterial activity (4–8). It has been suggested that prolactin may play a role in the pathogenesis of hypertension since it is involved in electrolyte metabolism in animals (9, 10) and in man (11, 12).

Interest in the role of prolactin in the pathogenesis of hypertension was heightened when Stumpe *et al.* (13) found increased prolactin production in patients with essential hypertension. These authors suggested that the increased production of prolactin in patients with hypertension was due to defective control of prolactin secretion by cerebral dopamine, i.e., purported prolactin-inhibiting factor (PIF). Sowers *et al.* (14) also found that the spontaneously hypertensive rat (SHR), a close animal prototype of essential hypertension in man, was hyperprolactinemic and suggested that a similar defect in central dopaminergic control of prolactin release may be operative in SHR. Although we have not found SHR to be hyperprolactinemic (15), we have found that SHR secrete extra quantities of prolactin in response to relatively innocuous stress (16, 17) and that their high blood pressure may be reduced effectively if they are given the prolactin-inhibiting drug, bromocryptine (18).

In order to gain more insight into the possible relationship of hyperprolactinemia to high blood pressure, we subjected normotensive, Sprague–Dawley rats to: (1) transplantation of pituitary glands to a site removed from hypothalamic control, i.e., beneath the kidney capsule, and (2) a single neonatal injection of testosterone proprionate. Both of these procedures are known to induce chronic hyperprolactinemia (19,

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20). We wished to determine whether prolonged hyperprolactinemia would induce hypertension in normotensive rats.

Materials and Methods. Adult, male and female, Sprague–Dawley rats were raised in our Animal Research Colony. The animals were housed in individual cages in temperature (26 ± 1°)- and light-controlled quarters (12 hr light/day), and provided food and water *ad libitum*.

Experiment 1. At 90–100 days of age, male Sprague–Dawley rats received renal capsular implants of four pituitary glands taken from 5-month-old, normal female Sprague–Dawley donors. An equal number of animals were subjected to sham operation. Blood pressure was determined at the time of operation using the Friedman:Freed microphonic manometer indirect tail cuff with light Seconal anesthesia and no restraint. Heparinized blood samples (1 to 2 ml) were taken by cardiac puncture 1 to 2 days prior to surgery between 0900 and 1100 hr. Blood pressure and blood samples were taken again at 1, 2, and 3 months post-transplantation. At the end of the experiment, rats were autopsied, and tissues removed and weighed. The kidneys were examined carefully for the presence or absence of vital pituitary tissue. Only those animals in which it was established that viable pituitary tissue was present were included in the data analyses.

Experiment 2. At 2–5 days of age, female pups were divided into two groups. One group (TP) was injected sc with 1.25 mg testosterone proprionate (Sigma) in 0.05 ml sesame oil. All animals were weaned at 24 days of age. At 30 days of age and at periodic intervals thereafter, blood pressure was taken again. One to two days later, heparinized blood samples were taken via cardiac puncture within 1 min after the rat had been removed from its cage. Samples were collected from the control group between 1600 and 1800 hr on the day of proestrus. (Prior studies in this laboratory have determined that serum prolactin levels are elevated at this time on the day of proestrus.) TP-treated females were also sampled at 1600–1800 hr. Blood was centrifuged, the plasma collected and frozen at −20° until assayed. Following collection of the final blood sample, rats were autopsied and appropriate tissues removed, trimmed, and weighed.

Daily vaginal smears were taken on both control and TP-treated rats starting with Day 50 and at 3-week intervals thereafter for the duration of the experiment. Only those females which exhibited regular 4-day cycles were included in the control group. Similarly, only those rats in the TP-treated group which exhibited constant estrus were included in this group.

Blood prolactin concentrations were determined by a double-antibody radioimmunoassay method (21) using the reagents in the rat prolactin NIH kit kindly provided by Dr. A. Parlow (NIAMDD). Samples were assayed in duplicate at two dilutions. Circulating corticosterone levels were measured by a modification of Murphy's method (22).

The data were analyzed using analysis of variance and a *t* test (23).

Results. *Experiment 1.* Transplantation of donor adult female pituitary glands beneath the renal capsule of 100-day-old male rats was successful with few rejections. The male recipients of female pituitary glands manifested significant ($P < 0.001$) enlargement of their adrenal glands concomitant with significant ($P < 0.001$) involution of their thymus glands (Table I). Kidneys and seminal vesicles were also significantly enlarged ($P < 0.01$ and 0.001, respectively, Table I). Circulating prolactin and corticosterone levels were significantly ($P < 0.001$) elevated in males bearing implants compared to sham-operated males (Table II). Despite the chronically elevated prolactin and corticosterone levels, the systolic blood pressure of rats bearing pituitary implants remained normal (Table II).

Experiment 2. Young, adult female Sprague–Dawley rats given a single neonatal injection of TP also displayed significant ($P < 0.01$ and 0.05) enlargement of their adrenal glands concomitant with thymus gland involution (Table III). Their hearts and kidneys were heavier than their sister controls (Table III). Despite the successful induction of chronic hyperprolac-

TABLE I. EFFECTS OF MULTIPLE RENAL IMPLANTS OF PITUITARY GLANDS FROM FEMALE DONORS (CHRONIC HYPERPROLACTINEMIA) ON THE BODY AND ORGAN WEIGHTS OF ADULT MALE RATS

	Sham operated	Pituitary recipient	*P* value
Body weight (g)	454 ± 17	462 ± 14	n.s.
Adrenals (mg)	25 ± 2	67 ± 4	<0.001
Thymus (mg)	300 ± 9	162 ± 11	<0.001
Heart (mg)	1384 ± 83	1320 ± 62	n.s.
Kidney (mg)	1311 ± 52	1573 ± 68	<0.01
Testis (mg)	2397 ± 67	2250 ± 91	n.s.
Seminal vesicles (mg)	276 ± 11 [14]	335 ± 10 [13]	<0.001

Note. Values represent means ± SE; [ ], number of animals; n.s., not significant.

tinemia, systolic blood pressure remained normal except at the outset of the experiment, i.e., 30 days of age (Fig. 1).

Discussion. Transplanted pituitary glands will remain functional although separated from their hypothalamic connection (19). Ectopic pituitary glands will secrete growth hormone, ACTH (24, 25), and extra prolactin (26, 27) when severed from hypothalamic control. The increased adrenal glandular weight, thymus gland involution, and increased corticosterone and prolactin levels attest to the effectiveness of the pituitary implants to cause augmented pituitary gland release of ACTH and prolactin in male recipients. We were compelled to use Sprague–Dawley rats as transplant recipients because WKy rats reject SHR pituitary glands (27). Female donors were used because of the strong likelihood that female pituitary glands would release more ACTH and prolactin than pituitary gland transplants taken from male donors. Similarly, neonatal injection of TP has been demonstrated to cause increased testicular and seminal vesicle weight and prolactin secretion (20).

Our finding of normal blood pressure despite chronic hyperprolactinemia in genetically normotensive Sprague–Dawley rats vs the finding of Sowers *et al.* of hyperprolactinemia in SHR (14) suggests that there may be a genetically mediated factor in SHR which is sensitive to the hypertension-inducing effects of prolactin (2, 3, 8–13). Alternatively, the Sprague–Dawley transplant recipients may have released antihypertensive compounds which could have counteracted the hypertensive effects of prolactin. We have found that genetically normotensive Sprague–Dawley pups nursed by SHR dams eventually developed high blood pressure (28) suggesting the transmission of some hypertensinogen through the mother's milk. Although investigators continue to report elevated vs nor-

TABLE II. PROLACTIN, CORTICOSTERONE, AND SYSTOLIC BLOOD PRESSURE LEVELS IN ADULT MALES BEARING RENAL IMPLANTS OF FEMALE PITUITARY GLANDS VS SHAM-OPERATED RATS

	Prolactin (ng/ml)		Corticosterone (μg/100 ml)		Blood pressure (mm Hg)	
Days postimplant	Sham	Pituitary implant	Sham	Pituitary implant	Sham	Pituitary implant
0	51 ± 8	49 ± 3	8 ± 1	7 ± 1	89 ± 2	92 ± 1
30	60 ± 11	271 ± 14*	9 ± 2	28 ± 4*	97 ± 3	94 ± 6
60	62 ± 17	291 ± 20*	11 ± 2	40 ± 7*	111 ± 6	114 ± 4
90	51 ± 7	276 ± 15*	11 ± 3	36 ± 11**	120 ± 5	126 ± 7

Note. Values represent means ± SE; sham operated, 14 animals; pituitary implants, 13 animals per group.
* $P = <0.001$.
** $P = <0.05$.

TABLE III. EFFECTS OF TESTOSTERONE-INDUCED CHRONIC HYPERPROLACTINEMIA ON BODY AND ORGAN WEIGHTS OF ADULT FEMALE RATS

	Control	Testosterone Treated	*P* value
Body weight (g)	267 ± 11	254 ± 4	n.s.
Adrenals (mg)	31 ± 1	47 ± 5	<0.01
Thymus (mg)	269 ± 28	171 ± 16	<0.05
Heart (mg)	782 ± 19	859 ± 32	<0.05
Kidney (mg)	782 ± 9	919 ± 19	<0.001
Ovary (mg)	28 ± 4	21 ± 2	n.s.
	[19]	[15]	

Note. Values represent mean ± SE; [ ], number of animals; n.s., not significant.

mal prolactin levels in animals (2, 3, 8–10, 14–16, 29) and in man (11–13, 30), it is intriguing that prolactin has been shown to cause increased sensitivity of the aortic wall to norepinephrine and angiotensin (2, 3, 8, 9). We would like to suggest that the role of prolactin in hypertension be investigated conjointly with adrenocortical function. Prolactin and ACTH are actively secreted and act synergistically to cause increased adrenocortical secretion during acute stress (4–7). Under strictly quiescent conditions, hypertensive SH rats secrete normal quantities of prolactin, corticosterone, and aldosterone during a 24-hr period (15, 31). However, under relatively innocuous conditions of acute stress, SHR respond by secreting extra prolactin, corticosterone, and aldosterone (16, 17). Extra responsiveness and extra production of corticomedullary hormones during stress is in harmony with the pathogenesis of hypertension. We suggest that the role of prolactin in experimental and essential hypertension be investigated under graded conditions of duress and in conjunction with adrenocortical hormones and sympathoamines.

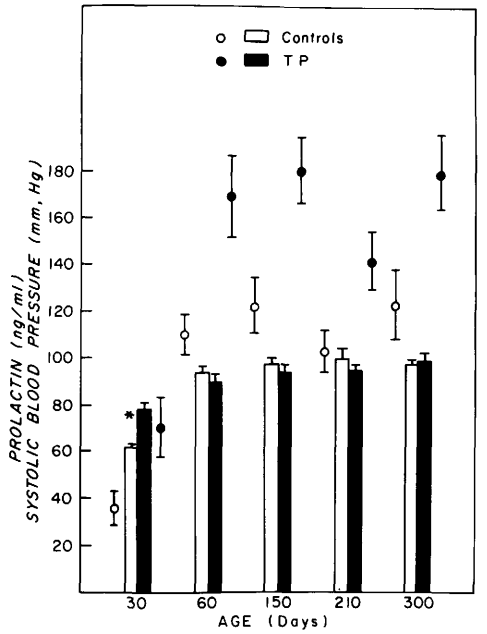

FIG. 1. Changes in systolic blood pressure (cf. barograms) with age in female, Sprague–Dawley rats made chronically hyperprolactinemic (cf. circles) by a single, neonatal injection of testosterone proprionate (TP). At 30 days of age, the blood pressure of TP-treated animals was significantly (*$P < 0.05$) above controls with no significant differences at 60 to 300 days of age. The prolactin levels of TP-treated females were significantly ($P < 0.05$) above controls ($P < 0.01$ at 60 and 150 days of age) throughout the experimental period. Mean ± SE, $n = 19$ for controls, $n = 15$ for TP-treated animals.

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