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Inhibition of Prolactin Secretion by Moderate and High Doses of Testosterone[1] (41817)

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Abstract. The effects of different doses of testosterone on basal and stimulated secretion of prolactin (PRL) were investigated. Intact female mice of the S/W strain were injected sc with 0, 1, 10, 20, 50, 250, 500, 1000, 2500, or 5000 μg of testosterone propionate (TP) once daily for 4 weeks. Serum testosterone concentrations of TP injected mice rose 2- to 5-fold above those of controls at 1- to 50-μg doses, and 25- to 600-fold over controls in mice given higher doses of the steroid. Administration of 1 μg of TP, the lowest dose tested, had no significant effects on basal serum PRL concentrations and only slightly inhibited the release of PRL induced by perphenazine, but the weight and PRL concentration of the pituitary gland were significantly depressed. However, at doses of 10, 20, and 50 μg TP, perphenazine-induced PRL release, pituitary PRL concentration, and pituitary gland weight were all reduced in a dose-related manner. The basal serum PRL concentrations decreased by the time the dose of TP reached 50 μg. In contrast, higher doses of TP (250 μg and above) reversed the suppression of these parameters: pituitary gland weight and basal serum PRL levels were restored to control levels, whereas pituitary PRL concentrations and perphenazine-induced PRL release were partially restored. These results suggest that administration of moderate and large doses of testosterone may suppress natural episodic and acute releases of PRL.

Androgens in high doses are sometimes used in the treatment of breast cancer (1–4). The antitumor effect of androgens is also seen in carcinogen-induced mammary tumors in rats (5, 6). Still unknown, though, is how androgens act in suppressing breast cancer or mammary tumor growth. One theory holds that androgens exert their antitumor effects by blocking the mammogenic effects of prolactin (PRL) at the level of the tumor cell (6). However, when Costlow *et al.* (7) measured PRL binding activity of mammary tumors after treatment with testosterone propionate (TP), they found that TP injections reduced PRL receptors, but not sufficiently to account for the entire regression. An alternative possibility, not yet explored, is that androgens suppress mammary tumor growth by interfering with the secretion of PRL. The objective of the present study was to determine the effect of low and high doses of a synthetic androgen, TP, on PRL secretion. We determined the effects of TP on the basal as well as stimulated secretion of PRL in mice and monitored the levels of testosterone achieved in blood by the different doses of TP administered.

Materials and Methods. *Animals.* Female mice of the outbred S/W strain, obtained from the Simonsen Laboratory (Gilroy, Calif.), were used. The mice were housed in plastic boxes (36 × 30 × 18 cm; 12 mice/box) and fed Wayne Lab Blox (6% fat, 24% protein, and 4.5% fiber) and tap water *ad libitum.* The animal room was maintained at 24 ± 1°C and was lighted from 0600–1800 hr.

Hormone injection. Androgen was administered in the form of TP (Sigma Chemical Co., St. Louis, Mo.). The steroid was dissolved in sesame oil and injected sc in a 0.05-ml volume. At approximately 100 days of age, the mice were divided into 10 groups, each consisting of 24 mice. One was the control group injected only with sesame oil, the vehicle used for dissolving TP. The other 9 groups were injected with one of the following doses of TP: 1, 10, 20, 50, 250, 500, 1000, 2500, or 5000 μg/mouse/day. The daily injections continued for 4 weeks. The mice were in all stages of the estrous cycle.

Provocative test for PRL release. Twenty-four hours after the last injection of TP or

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sesame oil, each group of mice was divided in half. One-half received a single ip injection of perphenazine (Trilafon, Schering Corp., Kenilworth, N.J.), a powerful PRL-releasing neuroleptic (8). The dose of perphenazine injected was 1 μg/g body weight and the volume of injection was 2.5 μl/g body weight. The other half of each group was injected with an appropriate volume of normal saline (0.85% NaCl). One hour after the perphenazine or saline injection, between 1000 and 1100 hr, all mice were decapitated, and their trunk blood and pituitary glands were collected. PRL release peaks 1 hr after perphenazine injection in intact as well as testosterone-treated mice (9).

Preparation of sera and pituitary extracts. Blood was collected individually from each mouse in conical glass centrifuge tubes and allowed to clot at 4°C for 2 hr. The serum was separated by centrifugation at 1000*g* for 30 min and stored at −20°C until assayed.

The pituitary gland from each mouse was removed immediately after sacrifice and weighed on a Mettler analytical balance. The tissue was homogenized with a motor-driven glass homogenizer in 0.05 *M* Na_2CO_3–$NaHCO_3$ buffer, pH 10 (10 mg/ml). A 25-μl aliquot of this homogenate was added to a centrifuge tube containing 2 ml of the same buffer, and the solution was centrifuged at 1000*g* for 30 min. A 25-μl aliquot of the supernatant was then added to 2 ml phosphosaline buffer (0.01 *M* phosphate, 0.15 *M* sodium chloride, and 0.01% merthiolate) containing 1% bovine serum albumin, pH 7.6, and stored at −20°C until assayed.

Radioimmunoassays (RIA). PRL in sera and pituitary extracts was analyzed by a homologous RIA for mouse PRL. The details of the assay procedure are described elsewhere (10); the only difference from the published method was that the tracer antigen was labeled using lactoperoxidase instead of chloramine-T. The biological potency of the mouse PRL standard used was 25 IU/mg.

Testosterone in samples of sera pooled from four mice each was measured by RIA as described previously (11). The anti-testosterone serum (S250) used in the RIA was provided by Dr. G. Niswender and was developed in sheep against testosterone conjugated at the C-11 position to bovine serum albumin. The specificity of this antiserum has been reported elsewhere (12).

The concentration of 17β-estradiol was also measured in the same samples of pooled sera by a previously described RIA (13). The antiserum to estradiol, produced in sheep against estradiol conjugated at C-6 to bovine serum albumin, was provided by Dr. L.-E. Edqvist.

Statistical analysis. The results were compared by analysis of variance and Duncan's new multiple range test.

Results. *Serum PRL concentrations.* The effect of TP on basal PRL concentrations (saline-injected subgroups of mice) and PRL concentrations after a provocative test (perphenazine-injected subgroups of mice) are shown in Fig. 1. Compared to some of the other strains (14), basal PRL concentrations were generally low in these mice, the control group (0 dose) having only 13 ± 2 ng/ml. Administration of 1, 10, and 20 μg of TP had no significant effect on this level ($P > 0.05$). However, 50-, 250-, and 500-μg doses of TP significantly decreased this basal concentration to 5–6 ng/ml ($P < 0.05$). At 1000-, 2500-, and 5000-μg doses, basal PRL increased to normal levels ($P > 0.05$).

Injection of perphenazine caused a 20-fold increase in serum PRL of control mice (0 dose), from 13 ± 2 to 247 ± 23 ng/ml. At the 1-μg dose of TP, PRL values averaged 216 ± 30 ng/ml and were not significantly different from controls ($P > 0.05$). However, 10-, 20-, 50-, and 250-μg doses of TP caused a marked dose-related inhibition of perphenazine-induced PRL release ($P < 0.01$), the concentration reaching a nadir at the 250-μg dose (19 ± 2 ng/ml). Perphenazine-induced PRL release then increased at the 500-, 1000-, 2500-, and 5000-μg doses of TP, the increment at the 5000-μg dose being significant ($P < 0.05$), just as the basal levels had increased, although PRL release was still significantly lower than normal ($P < 0.01$).

Pituitary PRL concentrations. The concentrations of PRL in pituitary glands of the various groups are illustrated in Fig. 2. Sesame oil-injected control mice had 6.4 ± 0.3 μg of PRL/mg of pituitary tissue. These concentrations decreased progressively at 1-, 10-, 20-, and 50-μg doses of TP ($P < 0.01$), the value at the 50-μg dose being only one-sixth of control. However, pituitary PRL concentrations

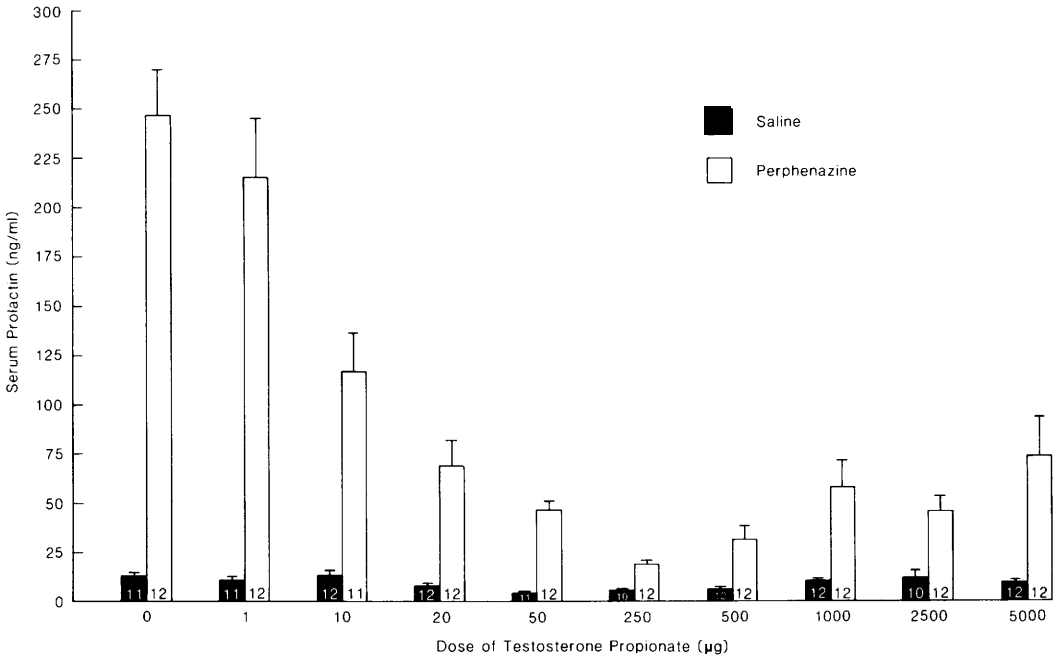

FIG. 1. Effect of increasing doses of TP on the release of PRL in response to challenge with a single injection of perphenazine (1 μg/g body weight, ip). The vertical lines at the top of the bars represent the SEM. The numbers at the base of the bars represent the number of animals in each group.

increased at all doses higher than 50 μg ($P < 0.01$), although they still averaged about one-half of control values ($P < 0.01$).

Pituitary gland weight. Changes in the weights of the pituitary glands, adjusted for body weight differences, are shown in Fig. 3. Pituitary gland weight was significantly decreased by 1, 10, 20, 50, 250, and 500 μg TP ($P < 0.01$), the decrease being dose-related up to 20 μg. However, at doses higher than 20 μg, the decrease in weight gradually diminished and the glands reached normal weight at a dose of 1000 μg and higher ($P > 0.05$).

Body weight. All groups of mice gained

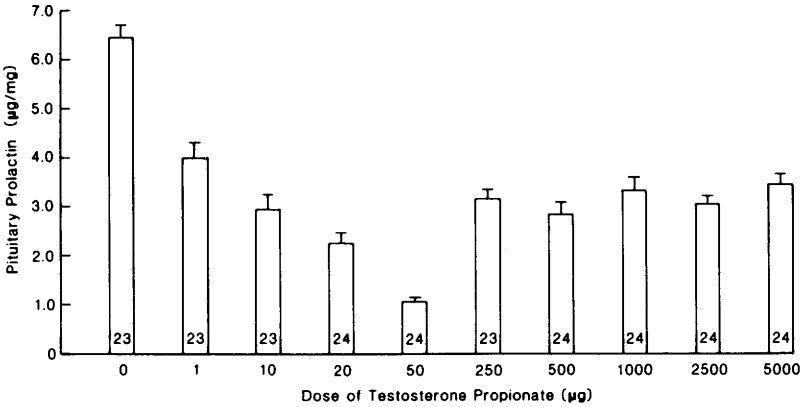

FIG. 2. Effect of increasing doses of TP on pituitary PRL concentration. The vertical lines at the top of the bars represent the SEM. The numbers at the base of the bars represent the number of animals in each group.

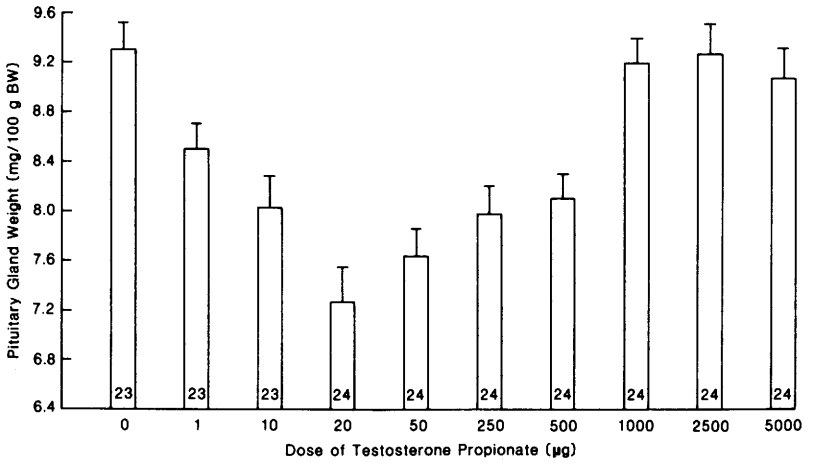

FIG. 3. Effect of increasing doses of TP on pituitary gland weight. The vertical lines at the top of the bars represent the SEM. The numbers at the base of the bars represent the number of animals in each group.

weight during the 4-week injection period ($P < 0.01$); however, the gain was somewhat greater ($P < 0.05$) in mice receiving 20 μg or more of TP than in those receiving lower doses (data not shown). No amount of TP had any deleterious effects on body weight gain.

Serum testosterone concentrations. The serum concentrations of testosterone at the time of sacrifice are given in Table I. Compared to controls, mice receiving 1, 10, and 20 μg TP showed no measurable increase in serum testosterone concentrations ($P > 0.05$). At the 50-μg dose, however, serum testosterone increased to values 5-fold greater than those of controls ($P < 0.05$). At doses higher than 50 μg, serum testosterone levels rose further ($P < 0.01$), as expected.

Serum estradiol concentrations. Serum estradiol concentrations averaged 29.9 ± 1.24 pg/ml in mice treated with sesame oil (0 dose) (Table I). This level decreased significantly at 1-, 10-, and 20-μg doses of TP ($P < 0.01$). However, serum estradiol returned to control levels at 50-, 250-, and 500-μg doses of TP ($P > 0.05$) and increased to higher than control levels at 1000, 2500, and 5000 μg ($P < 0.01$).

Discussion. The results of this study demonstrate that exogenous administration of an androgen influences PRL secretion. The effects produced, however, depend upon the dose administered. Furthermore, basal serum PRL levels do not reveal these effects as readily as do serum PRL concentrations after a provocative challenge or as do pituitary PRL concentrations.

Our data show that the androgen TP, even in very small doses, does not stimulate PRL secretion in mice. For example, at the lowest dose of TP tested, 1 μg/mouse/day for 4 weeks, both basal as well as perphenazine-induced serum PRL concentrations were unchanged. In contrast, the weight and PRL concentration of the pituitary gland were significantly re-

TABLE I. SERUM CONCENTRATIONS (MEAN ± SEM) OF TESTOSTERONE AND ESTRADIOL IN MICE TREATED WITH VARYING DOSES OF TESTOSTERONE PROPIONATE

Dose of testosterone propionate (μg)	Testosterone (ng/ml)	Estradiol (pg/ml)
0	1.1 ± 0.19 (6)	29.9 ± 1.24 (6)
1	2.0 ± 0.87 (3)	20.4 ± 1.48 (5)
10	2.4 ± 0.49 (6)	22.9 ± 1.18 (6)
20	2.4 ± 0.29 (5)	22.0 ± 1.86 (6)
50	5.6 ± 0.30 (6)	30.1 ± 0.83 (6)
250	25.9 ± 2.91 (6)	28.6 ± 1.31 (6)
500	54.0 ± 3.13 (6)	30.6 ± 2.54 (6)
1000	212.8 ± 40.8 (5)	41.6 ± 4.54 (5)
2500	258.2 ± 25.8 (6)	43.8 ± 4.07 (6)
5000	605.8 ± 130.1 (4)	55.4 ± 1.56 (5)

Note. Numbers in parentheses indicate the number of serum pools assayed for testosterone and estradiol.

duced by this dose. These results are consistent with our earlier observations that castration of male mice produced no changes in basal serum PRL but increased pituitary gland weight (9), and differ from those reported in rats (15–17). In moderate doses, on the other hand, the effect of TP on PRL secretion appeared to be markedly inhibitory. For example, TP in doses of 10, 20, and 50 μg, which increased serum testosterone concentrations only 2- to 5-fold above normal, drastically reduced perphenazine-induced PRL release, pituitary PRL concentrations, and pituitary gland weights. These reductions may have resulted from an estrogen deficiency caused by TP, since serum estradiol levels were lowered by 1, 10, and 20 μg of TP. On the other hand, TP could have produced these effects directly, since serum estradiol levels returned to normal at the 50-, 250-, and 500-μg doses but PRL levels remained low. In agreement with our results, Nolin (18) has reported that dihydrotestosterone, a nonaromatizable androgen, suppresses PRL secretion in female rats.

In large doses of TP, PRL secretion appeared to be subjected to the simultaneous influences of both testosterone and estradiol. At doses greater than 500 μg, serum estradiol levels increased significantly, in conjunction with increases in serum testosterone levels. The increase in serum estradiol was most likely due to the bioconversion of testosterone into estradiol (19). The net effect of the opposite influences of the two steroids was complete restoration of pituitary gland weight and basal serum PRL concentrations, but only partial restoration of pituitary PRL concentrations and perphenazine-induced PRL release. It was noteworthy that even a 2-fold increase in serum estradiol at the 1000-μg dose of TP was unable to completely reverse the inhibition of PRL release.

Thus, from these results, the major influence of moderate and large doses of TP on PRL secretion can only be characterized as inhibitory. This inhibition resembles the reduction in perphenazine-induced PRL secretion produced by large doses of estradiol benzoate (20), another sex steroid sometimes used in breast cancer therapy (1). Furthermore, we have observed that high doses of TP also suppress the physiologic release of PRL, such as that induced by pregnancy (11) or nursing (J. Kan, N. Lee, Y. N. Sinha, W. P. VanderLaan, and S. Ohno, unpublished). These results raise the question if the release of PRL is suppressed in patients receiving androgens for the treatment of breast cancer. At present, there is no convincing evidence that PRL is involved in human breast cancer. But if future work shows aberrations in daily circadian release (21), episodic bursts (22) or sleep-related nocturnal surges (23) of PRL to be important in the development and growth of breast cancer, conceivably androgens could produce tumor regression partly by suppressing these releases of PRL.

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1. Council on Drugs. Androgens and estrogens in the treatment of disseminated mammary carcinoma: Retrospective study of 944 patients. J Amer Med Assoc **172**:1271–1283, 1960.
2. Goldenberg I. Testosterone propionate therapy in breast cancer. J Amer Med Assoc **188**:1069–1072, 1964.
3. Goldenberg IS, Waters MN, Ravdin RS, Ansfield FJ, Segaloff A. Androgenic therapy for advanced breast cancer in women. J Amer Med Assoc **223**:1267–1268, 1973.
4. Volk H, Deupree RH, Goldenberg IS, Wilde RC, Carabasi RA, Escher GC. A dose-response evaluation of delta-1-testololactone in advanced breast cancer. Cancer **33**,9–12, 1974.
5. Young S, Baker RA, Helfenstein JE. The effects of androgens on induced mammary tumors in rats. Brit J Cancer **19**:155–159, 1965.
6. Quadri SK, Kledzik GS, Meites J. Counteraction by prolactin of androgen-induced inhibition of mammary tumor growth in rats. J Natl Cancer Inst **52**:875–878, 1974.
7. Costlow ME, Buschow RA, McGuire WL. Prolactin receptors and androgen-induced regression of 7,12-dimethylbenz(a)anthracene-induced mammary carcinoma. Cancer Res **36**:3324–3329, 1976.
8. Sulman FG. Hypothalamic Control of Lactation. New York, Springer-Verlag, 1st ed, pp88–102, 1970.
9. Sinha YN, Wickes MA, Salocks CB, VanderLaan WP. Gonadal regulation of prolactin and growth hormone secretion in the mouse. Biol Reprod **21**:473–481, 1979.
10. Sinha YN, Selby FW, Lewis UJ, VanderLaan WP. Studies of prolactin secretion in mice by a homologous radioimmunoassay. Endocrinology **91**:1045–1053, 1972.

11. Barkley MS. The temporal relationship between implantation, termination of prolactin surges, and increased testosterone secretion in the pregnant mouse. Endocrinology **110**:1529–1534, 1982.
12. Gay VL, Kerlan JT. Serum LH and FSH following passive immunization against circulating testosterone in the intact male rat and in orchidectomized rats bearing subcutaneous silastic implants of testosterone. Arch Androl **1**:257–266, 1978.
13. Lasley B, Hendrickx AG, Stabenfeldt GH. Estradiol levels near the time of ovulation in the bonnet monkey (*Macaca radiata*). Biol Reprod **11**:237–244, 1974.
14. Sinha YN, Vlahakis G, VanderLaan WP. Serum, pituitary and urine concentrations of prolactin and growth hormone in eight strains of mice with varying incidence of mammary tumors. Int J Cancer **24**:430–437, 1979.
15. Meites J, Clemens JA. Hypothalamic control of prolactin secretion. Vitam Horm **30**:165–221, 1972.
16. Kalra PS, Fawcett CP, Krulich L, McCann SM. The effect of gonadal steroids on plasma gonadotropins and prolactin in the rat. Endocrinology **92**:1256–1268, 1973.
17. Shin SH, Aiken RB, Roberts R, Howitt C. Effect of testosterone on serum prolactin in the castrated rat. J Endocrinol **63**:257–258, 1974.
18. Nolin JM, Campbell GT, Nansel DD, Bogdanove EM. Does androgen influence prolactin secretion? Endocr Res Commun **4**:61–70, 1977.
19. Naftolin F, Ryan KJ, Davies IJ, Reddy VV, Flores F, Petro Z, Kuhn M, White RJ, Yakaoka Y, Wolin L. The formation of estrogens by central neuroendocrine tissues. Rec Progr Horm Res **31**:295–319, 1975.
20. Sinha YN, Gilligan TA. Estrogen in high doses inhibits perphenazine-induced prolactin release. Endocrinology **110**:126–130, 1982.
21. Sinha YN, Salocks CB, Wickes MA, VanderLaan WP. Serum and pituitary concentrations of prolactin and growth hormone in mice during a twenty-four hour period. Endocrinology **100**:786–791, 1977.
22. Sassin JF, Frantz AG, Weitzman ED, Kapen S. Human prolactin: 24-hour pattern with increased release during sleep. Science **177**:1205–1207, 1972.
23. Parker DC, Rossman LG, VanderLaan EF. Sleep-related, nyctohemeral and briefly episodic variation in human plasma prolactin concentrations. J Clin Endocrinol Metab **36**:1119–1124, 1973.

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