Comparison of Prostatic Response to Pituitary Grafts in Castrate Rats Treated with Testosterone or Dihydrotestosterone

G. S. PRINS AND C. LEE

From the Department of Urology, Northwestern University, The Medical School, Chicago, Illinois

To understand further the effect of prolactin (PRL) on prostatic growth, the present study was undertaken to determine if the augmentation of testosterone (T)-stimulated growth by PRL is mediated before or after the formation of dihydrotestoterone (DHT). Mature male Sprague-Dawley rats were castrated and treated with subcutaneous Silastic implants containing either T or DHT. Serum PRL was elevated by grafting, under the kidney capsule, pituitaries from two female rats, while controls received grafts of muscle. Three weeks later, all animals were sacrificed. Animals bearing pituitary grafts had an average serum PRL level of 125 to 140 ng/ml while PRL levels for controls were 15 to 20 ng/ml. T treatment provided 1.37 ± 0.11 ng T/ml serum and the DHT capsules produced serum DHT levels of 1.16 ± 0.12 ng/ml. Under these conditions, the lateral prostate showed a specific growth response to PRL: wet weight, protein content, protein concentration, and DNA levels were all significantly greater in engrafted rats treated with either T or DHT than in their respective controls. On histologic examination, elevated PRL levels were associated with proliferative changes of the lateral lobe which included marked papillary infolding and acinar epithelial hyperplasia. This alteration was similar in both steroid-treated groups. These data show that PRL has the same effect on DHT-maintained lateral lobes as it does on the testosterone-maintained prostate and indicate that the PRL effect is not mediated prior to T conversion to DHT but rather at a site beyond DHT formation.

Key words: prostate, prolactin, testosterone, dihydrotestosterone.

Prolactin (PRL) acts synergistically with testosterone in promoting growth and function of the rat prostate gland (Grayhack et al, 1955; Grayhack and Lebowitz, 1967; Moger and Geschwind, 1972; Negro-Vilar, 1980). Recent work indicates that PRL exerts this effect through a permissive action on testosterone-mediated prostatic growth (Holland and Lee, 1980); however, the mechanism of interaction between these two hormones remains unclear. Testosterone is taken up by the prostate cell and converted irreversibly via 5 α-reductase to dihydrotestosterone (DHT), which serves as the active androgen in that tissue. Since PRL has been suggested as a regulator of 5 α-reductase activity in the rat liver (Lax et al, 1976), adrenal (Gustafsson and Stenberg, 1975; Witorsch and Kitay, 1972), and ovary (Polan and Behrman, 1981), it is reasonable to question whether PRL augments growth of the prostate by stimulating testosterone metabolism to DHT. Previous *in vitro* experiments that have addressed this issue have produced conflicting reports ranging from no alteration (Lasnitzki, 1972; Johansson, 1976a) to stimulation (Yamanaka, et al, 1975) and inhibition of DHT formation by PRL in the rat prostate (Manandhar and Thomas, 1976). Additionally, PRL was shown to inhibit DHT formation in the guinea-pig seminal vesicle (Mawhinney et al, 1975). It has been reported that PRL administration to castrate rats supplied with DHT increased the citric acid content of the lateral prostate (Walvoord et al, 1976), suggesting that the PRL synergism may be mediated at a site beyond DHT formation. In con-

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Reprint requests: G. S. Prins, Department of Urology, Northwestern University, The Medical School, Chicago, Illinois 60611.

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trast, others have demonstrated a PRL augmentation of prostatic androgen metabolism only when testosterone was administered to rats and not when DHT was supplied (Edwards and Thomas, 1977). It is presently unknown, therefore, whether the stimulatory effect of PRL on the prostate is at the site of testosterone conversion to DHT.

In light of these reports, the present study was undertaken to determine if the PRL growth effect on the androgen maintained prostate is mediated before or after the formation of DHT. Serum PRL was elevated with pituitary grafts in castrate rats provided with either testosterone or DHT implants. The effect of this treatment on prostatic weight, protein, and DNA content was analyzed three weeks later and compared with the two steroid-treated groups. In addition, the effect of this pituitary hormone was examined at the histologic level in order to determine which cells of the prostate were influenced by the treatments.

Materials and Methods

Animals and Experimental Protocol

Young adult male (250 to 275 g) and female (200 to 225 g) Sprague-Dawley rats (Charles River, Wilmington, Massachusetts) were maintained in a controlled environment (22 to 24 C; lights on 0500 to 1900 hours) throughout the course of the experiment. Tap water and Purina rat chow were provided *ad libitum*.

Animals were divided into two treatment groups; half received testosterone treatment and half were treated with DHT. All animals were castrated via the scrotal route under ether anesthesia. At the same time, anterior pituitaries from two females were transplanted under the right renal capsule of recipient male rats. Control animals received a sham graft which involved placement of a small piece of muscle under the kidney capsule. All rats were then given subcutaneous implants of Silastic tubing (inside diameter 1.575 mm; outside diameter, 3.175 mm; Dow Corning, Midland, Michigan) containing either crystalline testosterone or DHT. The lengths of the implants used (0.5 cm testosterone; 3.0 cm DHT) produced a serum androgen level which was submaintainance in relation with prostatic weight. These levels were desired since results of a previous study had demonstrated that PRL produces its greatest growth-promoting effect on the prostate when a submaintainance dose of circulating steroid is supplied (Holland and Lee, 1980).

Three weeks later, the animals were decapitated. Trunk blood was collected and the serum fraction stored in several aliquots at −20 C until assayed for hormone levels. The three prostate lobes (ventral, dorsal, and lateral) were dissected, weighed and either snap frozen in liquid nitrogen or fixed in 10% formalin. The adrenal glands were also dissected, cleaned, and weighed.

Tissue Analysis

Frozen tissue was thawed, homogenized in 2 ml distilled water at 4 C, and immediately used for quantitation of protein and DNA. The protein content of the individual lobes was determined according to the method of Lowry et al (1951), with bovine serum albumin as the standard. Tissue DNA levels were quantitated using the diphenylamine technique as described by Schneider (1957). Calf thymus DNA was used as the standard. Tissue fixed in formalin was processed through routine histologic procedures using hematoxylin and eosin (H and E) as stains.

RIA of Serum PRL and Testosterone

The levels of serum PRL were measured in duplicate samples (50 μl for control, 25 μl for pituitary grafts) by means of double-antibody RIA using the NIAMDD rat PRL kit, with results expressed in terms of NIAMDD rat PRL-RP-1 (Schwartz and Justo, 1977). Serum (50 μl aliquots) from castrate rats treated with testosterone implants was assayed for testosterone by RIA using Niswender antiserum #250 for testosterone as previously described (Schwartz and Justo, 1977).

RIA of Serum DHT

Serum from castrate rats treated with DHT implants was assayed for DHT by RIA following separation by thin-layer chromatography. Aliquots (0.25 ml) of serum were extracted twice with 3 ml diethyl ether after adding a tracer amount of ^{3}H-DHT (New England Nuclear Corp., Boston, Massachusetts) to correct for procedural losses. The pooled extract was evaporated to dryness under nitrogen, reconstituted in 100 μl acetone, and spotted in entirety on silica gel-60 plates (Merck; Darmstadt, West Germany), followed by a second wash of 50 μl acetone. A marker solution of ^{3}H-DHT was spotted on each side of the plate to serve as a reference. The plates were developed twice in chloroform ethyl acetate (8:2, v/v). The DHT band was located on the basis of a predetermined R_f value (0.64) and confirmed by analyzing the reference lanes for ^{3}H-DHT. The sample DHT band was scraped into ethanol-rinsed tubes and the steroid was eluted with three washes of 2 ml acetone. This fraction when dried was redissolved in 0.5 ml absolute ethanol; 100 μl was removed for determination of recovery and the remainder was used for assay, after evaporation under nitrogen.

The assay used for DHT quantitation was a modification of the testosterone RIA (Schwartz and Justo, 1977) using Niswender antisera #250 for testosterone. The cross-reactivity for DHT was measured at 58% and the final antiserum dilution used (1:20,000) allowed 50 to 60% binding of the total radioactive DHT. A standard curve ranging from 5 to 500 pg/tube was prepared with authentic DHT (Sigma Chemical Company, St. Louis, Missouri). The DHT content of the unknowns was determined from this curve after logit transformation. The lower limit of sensitivity ranged between 10 to 15 pg/tube. Distilled water blanks and an internal standard of pooled rat serum were run in triplicate through the en-

tire assay procedure. The interassay coefficient of variance was 13.3% and the intrassay coefficients of variance ranged from 4.1 to 7.9% for values between 20 and 300 pg on the standard curve. The accuracy of the method was determined in each assay by adding unlabeled steroid to 50 μl plasma pool. An example of recovery showed plasma, 0.60 ± 0.04 ng DHT/ml; plasma + 1 ng, 1.67 ± 0.07; plasma + 2 ng, 2.61 ± 0.14 (mean ± SE). In each assay, 50 μl serum from ovariectomized-adrenalectomized female rats were assayed in triplicate to determine the biological "blank". This value averaged 7.3 ± 0.7 pg for DHT and was always below the lower limit of sensitivity of the assay. This "blank" value was not subtracted from the unknown values in the present study.

Statistical Analysis

Pituitary-engrafted animals that did not show elevated serum PRL levels upon RIA were excluded from data analysis. All data are expressed as mean ± SE. One-way analysis of variance followed by Student's *t*-test were used for statistical analysis (Steel and Torrie, 1960).

Results

The serum hormone levels and adrenal gland weights observed in the rats after a three-week exposure to the experimental treatment are shown in Table 1. Serum PRL levels were significantly higher in the engrafted animals than in the controls, thus confirming the viability of the graft. The 0.5 cm testosterone implants gave an average circulating testosterone level of 1.37 ± 0.11 ng/ml, while the 3.0 cm DHT implants provided 1.16 ± 0.12 ng DHT/ml serum. The presence of a pituitary graft did not significantly alter these circulating androgen levels or the adrenal gland weights.

The effect of pituitary grafts on the prostate gland of the castrate rats treated with testosterone is summarized in Fig. 1. The weight, protein, and DNA content of the lateral lobe and the weight and protein content of the dorsal lobe were significantly greater in engrafted than in control animals. These parameters in the ventral lobe were not altered by PRL treatment. The differences observed in the dorsal lobe were not as marked as those in the lateral prostate: the increase in weight and protein content was 19 and 31% ($P < 0.05$) respectively in the dorsal lobe as compared with 99 and 140% ($P < 0.001$) in the lateral lobe. Only the lateral lobe of engrafted animals receiving DHT showed a significantly ($P < 0.001$) greater weight, protein, and DNA content than the control group receiving DHT alone (Fig. 2). The dorsal lobe exhibited a similar trend (13% increase in weight) but it was not found to be significant ($P > 0.05$). The protein concentration (expressed in terms of DNA) was calculated for the prostatic lobes in each animal. In both steroid treatment groups, the lateral lobe alone showed a marked protein increase ($P < 0.05$) in engrafted animals as compared with controls (Table 2).

Histologically, the lateral lobes of castrate rats maintained with implants of testosterone or DHT appeared normal (Jesik, et al, 1981). Areas of actively secreting acini, as judged by intense eosinic stained lumens, alternated with areas containing little or no secretions (Figs. 3, 4A). The acini population was a mixture of smooth, rounded acini and those with infolding of the epithelium to form papillae. The acinar epithelium was lined with cuboidal or low columnar cells possessing basally-located nuclei and a supranuclear clear zone (Figs. 3, 4B). Rats given ectopic pituitary grafts in addition to testosterone or DHT showed marked proliferative changes of the lateral lobe acinar epithelium as compared with their respec-

TABLE 1. Effect of Ectopic Pituitary Grafts and Androgen Implants on Serum PRL and Androgen Levels and on Adrenal Gland Weight

	Testosterone		DHT	
	Control	Graft	Control	Graft
Number of animals	12	12	13	13
Body weight (g)	332 ± 8	310 ± 9	349 ± 7	336 ± 7
Adrenal (mg)	57.6 ± 2.8	61.3 ± 4.5	52.1 ± 2.6	59.0 ± 2.5
Serum PRL (ng/ml)	16.3 ± 2.4	139.6 ± 10.8†	20.4 ± 3.1	123.6 ± 14.9†
Serum androgen* (ng/ml)	1.45 ± 0.20	1.30 ± 0.11	1.22 ± 0.18	1.12 ± 0.16

Values are mean ± SE.
* Testosterone values given for testosterone-treated animals, DHT values given for DHT-treated animals.
† $P < 0.001$; significantly different from control animals in each group.

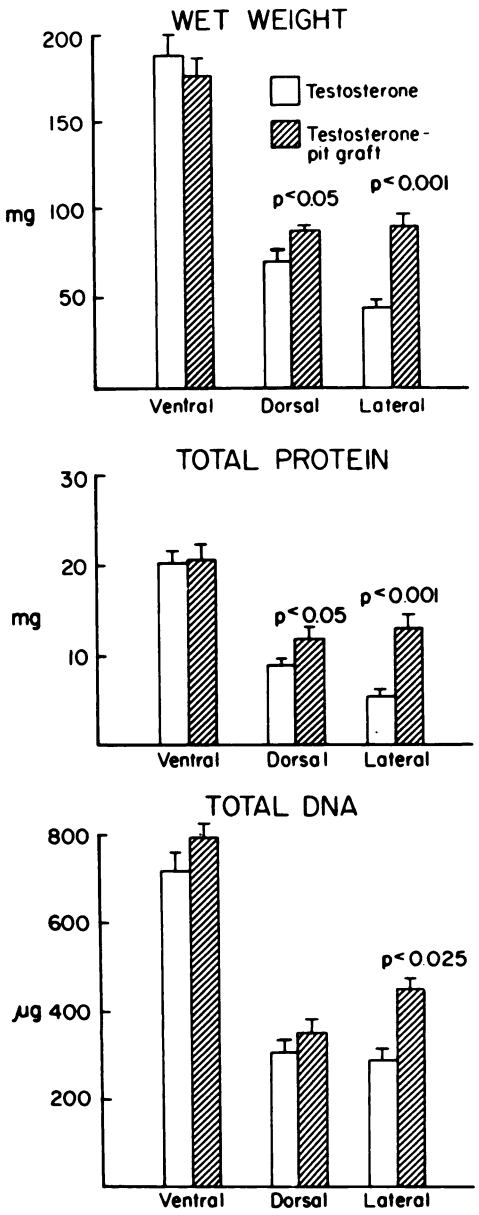

Fig. 1. Effect of ectopic pituitary grafts (two pituitaries per recipient) and testosterone implants (1.36 ng/ml serum testosterone) on wet weights, protein, and DNA content of the three prostate lobes in castrate rats. Each bar represents the mean of 12 rats for weight and eight rats for protein and DNA content, with SE above.

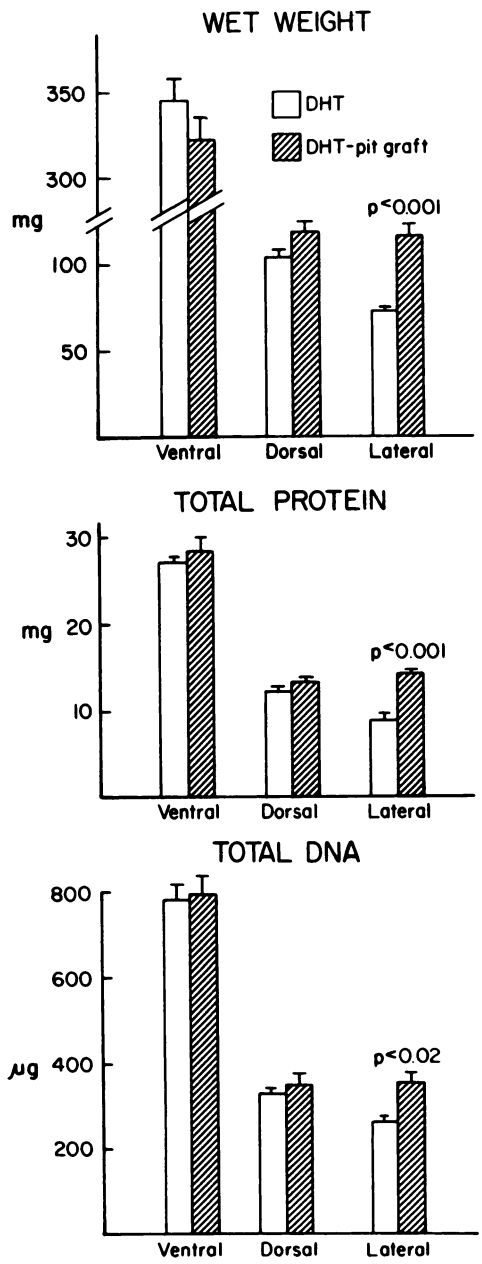

Fig. 2. Effect of ectopic pituitary grafts (two pituitaries per recipient) and DHT implants (1.14 ng/ml serum DHT) on wet weights, protein, and DNA content of the three prostate lobes in castrate rats. Each bar represents the mean of 13 rats for weight and nine rats for protein and DNA, with SE above.

tive controls. There was a large increase in the number of acini with papillary infolding and in the extent of epithelial infolding within each acini (Figs. 3, 4C). Piling up and crowding of cells was frequently observed, as was bridging of the epithelial layer across the lumen; both are indicators of hyperplastic growth (Figs. 3, 4D). Overall, the histologic alterations of the lateral lobe were comparable in engrafted animals maintained with either steroid. No significant changes were noted in the ventral or dorsal lobes.

TABLE 2. Effect of Ectopic Pituitary Grafts on Protein Concentration (mg Protein/mg DNA) in the Three Prostatic Lobes in Castrate Rats Maintained with Testosterone or DHT

	Testosterone		DHT	
	Control	Graft	Control	Graft
Number of Animals	12	12	13	13
Ventral	28.2 ± 3.3	26.0 ± 2.4	35.8 ± 1.8	36.5 ± 1.7
Dorsal	29.3 ± 1.9	33.2 ± 2.0	36.5 ± 1.7	38.3 ± 1.3
Lateral	18.2 ± 1.4	28.8 ± 1.9*	34.8 ± 2.0	41.0 ± 1.5*

Values are mean ± SE.
* $P < 0.05$; significantly different from control animals in each group.

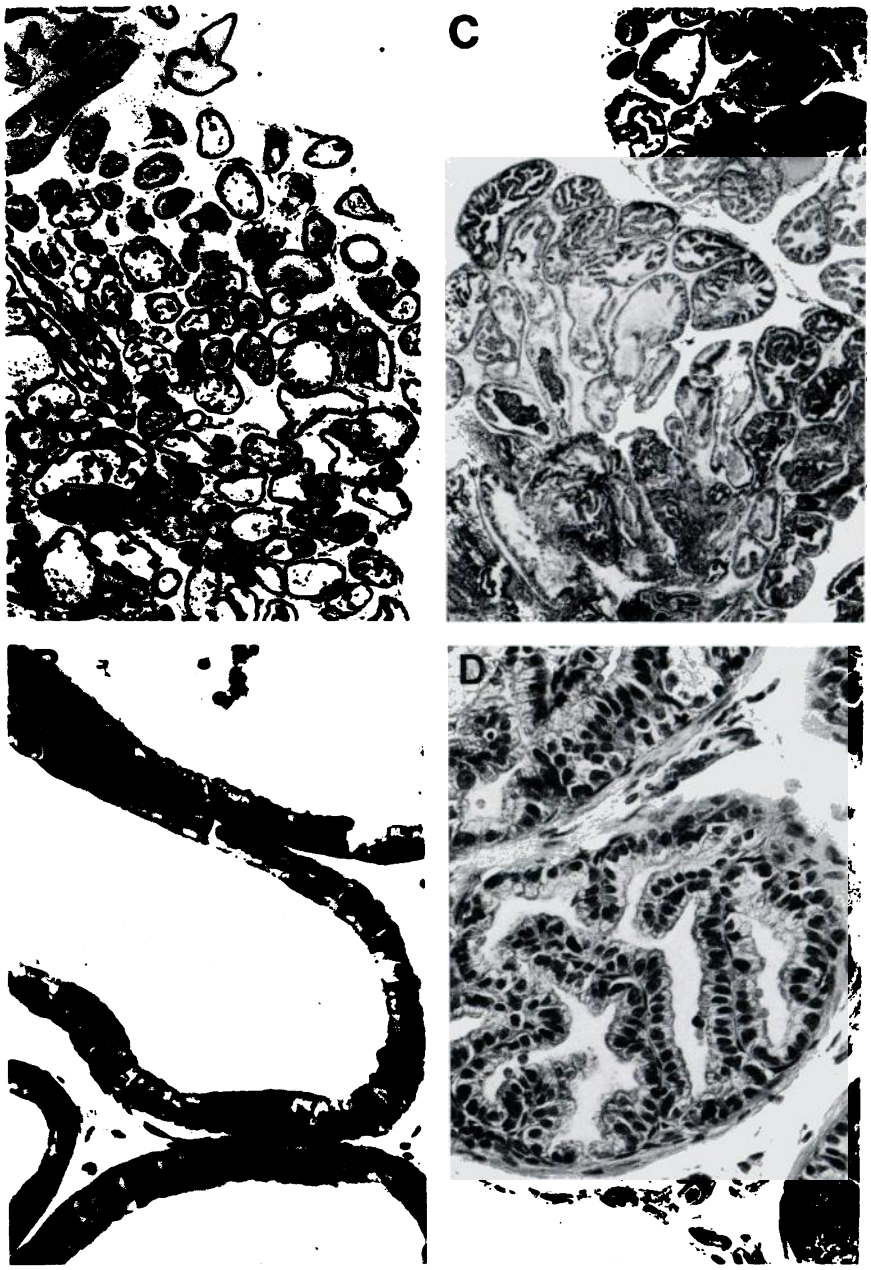

Fig. 3. Tissue sections from the lateral prostate of castrate rats treated with testosterone alone or testosterone plus pituitary grafts. A. Testosterone treatment alone; acini, loosely packed within the stromal matrix, are a mixture of those with a smooth epithelial lining and those whose epithelial layer is thrown into folds. Stained secretions are visible within the acini (×30). B. Higher power view of acini with testosterone treatment alone; cuboidal or low columnar cells show a basally-located nuclei and a supranuclear clear zone (×315). C. Testosterone plus pituitary grafts; most acini now show infolding of the epithelial lining and papillary hyperplasia. (×30). D. Higher power view of acini treated with testosterone plus pituitary grafts; note marked crowding and heaping up of cells, extensive epithelial infolding, and bridging of the epithelial layer across the lumen (×315).

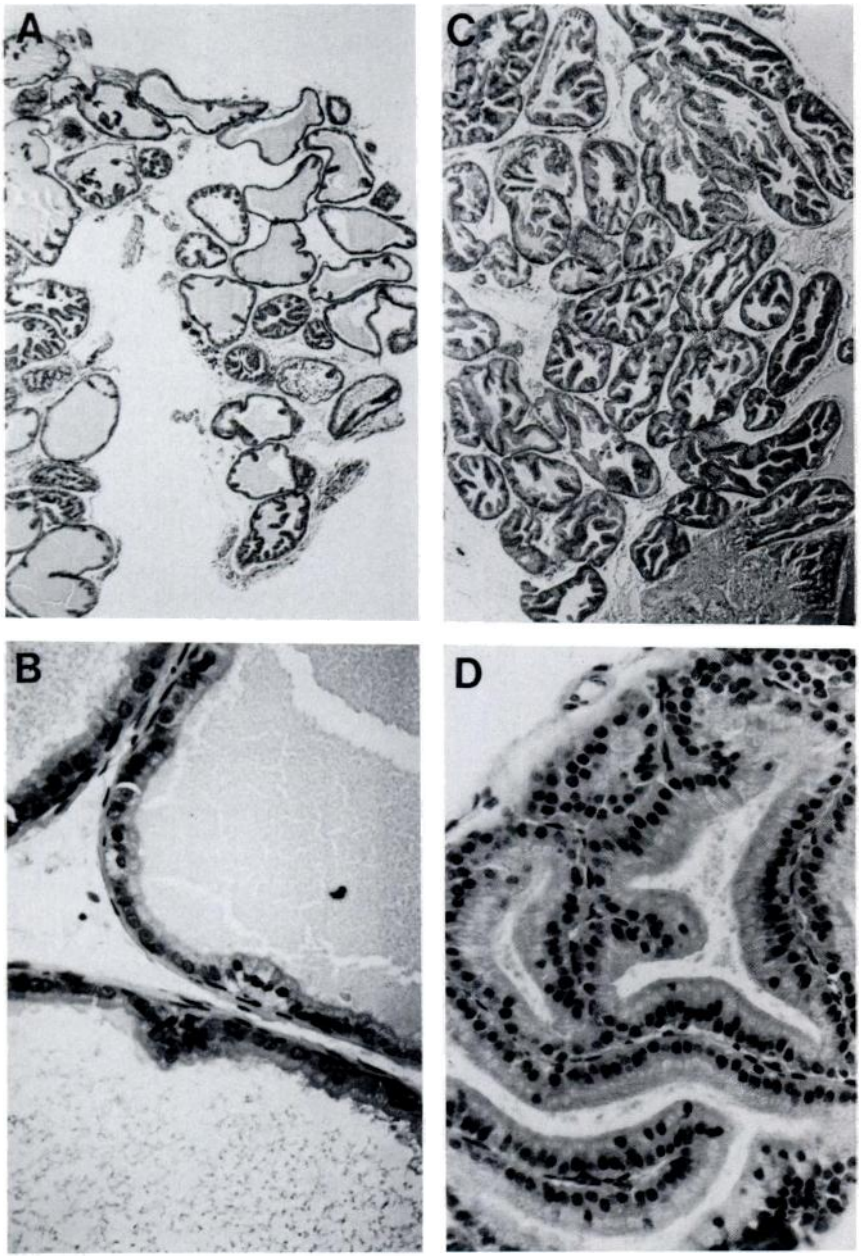

Fig. 4. Tissue sections from the lateral prostate of castrate rats treated with DHT alone or DHT plus pituitary grafts. A. DHT treatment alone; mixture of acini with a smooth epithelial layer and those with an infolded epithelium forming papillae. Eosinophilic secretions fill the lumen (×30). B. Higher power view of acini with DHT treatment alone; cuboidal cells line the acini in an orderly fashion. Basal nuclei and a supranuclear clear zone are apparent (×315). C. DHT plus pituitary grafts; papillary hyperplasia and thick epithelial lining are visible (×38). D. Higher power view of acini with DHT plus pituitary grafts; note cell crowding and piling up, increased cell height and extensive papillary hyperplasia (×380).

Discussion

The results of the present study clearly indicate that the effect of ectopic pituitary grafts on the DHT-maintained prostate of castrate rats is similar to the effect observed in the testosterone-maintained gland. The elevated serum PRL levels produced by the grafts in animals treated with either steroid were associated with a stimulation of the lateral prostatic lobe, involving an increase in cell number (DNA content), an increase in either cell size, glandular secretions or both (protein to DNA ratios), and epithelial hyperplasia as seen histologically. This finding correlates with earlier reports that the rat lateral prostate displays a specific response to prolactin (Grayhack and Lebowitz, 1967) and pituitary grafts (Holland and Lee, 1980) in the presence of testosterone which includes augmentation of secretion as well as proliferation. The present results confirm and extend

this observation to include proliferative synergism between PRL and DHT which resembles the synergism observed between PRL and testosterone. In addition, the histologic data provide visualization of the proliferative effects of PRL on the rat prostate and demonstrate a particular effect on the epithelial cell population.

Prolactin has been shown to enhance the activities of 5 α-reductase in the rat liver and ovary (Lax et al, 1976; Polan and Behrman, 1981). Furthermore, an elevated activity of 5 α-reductase has been associated with benign hyperplasia of the human prostate gland (Bruchovsky et al, 1980). It therefore seems reasonable to speculate that PRL may likewise stimulate the same enzyme in the rat prostate, thereby inducing a similar hyperplastic condition. However, this hypothesis was not substantiated by the present findings. Since PRL was capable of synergizing with DHT to promote prostatic growth in a manner that mimicked the known synergism with testosterone, the primary *in vivo* effect of PRL in the rat does not appear to be a result of an increased metabolism of testosterone to DHT. A similar conclusion was reached by Keenan et al (1981a,b) concerning the mouse seminal vesicle, where PRL was found to augment the proliferative actions of testosterone, DHT, and 5 α-androstane-3 α,17 β-diol. The present findings are also in agreement with our earlier observations (Prins and Lee, 1982) that the rate of conversion of pulse-injected ^{3}H-testosterone to ^{3}H-DHT in the three prostatic lobes was not found to be altered by the presence of pituitary grafts. These data support the alternative hypothesis that the synergistic effect of PRL and androgen on the rat prostate is primarily mediated at a site beyond DHT formation in the androgen mechanism of action.

That the dorsal lobe also exhibited a response on some parameters to the pituitary transplants in the present study is not surprising since several investigators have shown a PRL-testosterone synergism on growth and function of the dorsolateral prostate (Gunn et al, 1965; Moger and Geschwind, 1972; Slaunwhite and Sharma, 1977; Negro-Vilar et al, 1977). In the present report, animals bearing testosterone capsules exhibited a significant increase (19%, $P < 0.05$) in the weight of the dorsal lobes when pituitary grafts were present; whereas in the animals treated with DHT, the difference observed in the dorsal lobe weight with elevated PRL was not significant (13%, $P > 0.05$). The differences between these two steroid treatments in terms of the dorsal lobe response to pituitary grafts could indicate that the PRL-testosterone synergism is different from the PRL-DHT synergism. However, it should be pointed out that not only is the dorsal prostate far less sensitive to PRL than is the lateral lobe (Harper et al, 1976), but its response to PRL is also less consistent. In our previous studies, the dorsal prostate did not respond to the synergistic effect of PRL with testosterone as was found herein, while a consistent effect was always noted in the lateral lobe (Holland and Lee, 1980; Prins and Lee, 1982). Thus, if PRL could affect the dorsal prostate in a synergistic fashion involving testosterone and not DHT, it would be at best an inconsistent effect and one of small magnitude.

That the transplanted pituitary gland may influence the levels of other hormones in the circulation in addition to PRL deserves a comment. While it has been reported that anterior pituitary grafts are capable of secreting small amounts of LH and FSH in hypophysectomized rats (Lu et al, 1977), it has also been shown that ectopic grafts diminish LH and FSH production by the *in situ* pituitary gland in intact rats, thereby lowering the circulating level of gonadotropins (Bartke et al, 1977; McNeilly et al, 1978). Whether other adenohyphophyseal hormones are produced or altered by the pituitary grafts in the rat is not clear at the present time. It has been shown that plasma GH levels are not modified by grafts in the male hamster (Bartke et al, 1980). In the present study, the adrenal gland weight was not influenced by the presence of pituitary grafts for three weeks; therefore, it seems unlikely that the grafts secreted ACTH in any significant quantity. It should be noted that even if pituitary grafts secrete small amounts of other hormones, no consistent stimulatory effect on the prostate has been shown for the other pituitary hormones including GH, ACTH, FSH, and TSH (Grayhack et al, 1955; Chase et al, 1957; Gunn et al, 1965; Moshang and Bongiovanni, 1973; Johansson, 1976b). Thus, it is reasonable to assume that the effect of pituitary grafts on the prostate gland is the result of elevated serum PRL produced by the graft.

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References

Bartke A, Smith MS, Michael SD, Peron FG, Dalterio S. Effects of experimentally-induced chronic hyperprolactinemia on testosterone and gonadotropin levels in male rats and mice. Endocrinology 1977; 100:182–186.

Bartke A, Goldman BD, Bex FJ, Kelch RP, Smith MS, Dalterio S, Doherty PC. Effects of prolactin on testicular regression and recrudescence in golden hamster. Endocrinology 1980; 106:167–172.

Bruchovsky N, Rennie PS, Shnitka TK. Cellular factors contributing to the high concentration of dihydrotestosterone in hyperplastic and carcinomatous human prostates. In: Iacobelli S, ed. Hormones and cancer. New York: Raven Press, 1980; 457–470.

Chase MD, Geschwind II, Bern HA. Synergistic role of prolactin in response of male rat sex accessories to androgen. Proc Soc Exp Biol Med 1957; 94:680–683.

Edwards WD, Thomas JA. Effects of androgens and/or prolaction on ventral prostate transplants. Urol Int 1977; 32:303–311.

Grayhack JT, Bunce PL, Kearns JW, Scott WE. Influence of the pituitary on the prostatic response to androgen in the rat. Bull Johns Hopkins Hosp 1955; 96:154–163.

Grayhack JT, Lebowitz JM. Effect of prolactin on citric acid of lateral lobe of prostate of Sprague-Dawley rat. Invest Urol 1967; 5:87–94.

Gunn SA, Gould TC, Anderson WAD. The effect of growth hormone and prolactin preparations on the control by interstitial cell stimulating hormone of uptake of ^{65}Zn by the rat dorsolateral prostate. J Endocrinol 1965; 32:205–214.

Gustafsson JA, Stenberg A. Influence of prolactin on the metabolism of steroid hormones in rat liver and adrenals. Acta Endocrinol 1975; 78:545–553.

Harper ME, Danutra V, Chander JA, Griffiths K. The effect of 2-Bromo-ergocryptine (CB 154) administration on the hormone levels, organ weights, prostatic morphology and zinc concentrations in the male rat. Acta Endocrinol 1976; 83:211–224.

Holland JM, Lee C. Effects of pituitary grafts on testosterone stimulated growth of rat prostate. Biol Reprod 1980; 22:351–355.

Jesik CJ, Holland JM, Lee C. An anatomical and histological study of the rat prostate. Prostate 1981; 3:81–97.

Johansson R. Effect of some synandrogens and antiandrogens on the conversion of testosterone to dihydrotestosterone in the cultured rat ventral prostate. Acta Endocrinol 1976a; 81:398–408.

Johansson R. Effect of prolactin, growth hormone and insulin on the uptake and binding of dihydrotestosterone in the cultured rat ventral prostate. Acta Endocrinol 1976b; 81:854–864.

Keenan EJ, Klase PA, Thomas JA. Effects of prolactin on DNA synthesis and growth of the accessory sex organs in male mice. Endocrinology 1981a; 109:170–175.

Keenan EJ, Klase PA, Thomas JA. Interaction between prolactin and androgens in the accessory sex organs of male mice. J Endocrinol 1981b; 90:323–330.

Lasnitzki I. The effect of prolactin on rat prostate glands in organ culture. In: Boynes AR, Griffiths K, eds. Prolactin and carcinogenesis. Cardiff, Wales: Alpha Omega Alpha Publishing Co., 1972; 200–206.

Lax ER, Ghraf R, Schriefers H, Herrmann M, Petutschnight D. Regulation of the activities of the enzymes involved in the metabolism of steroid hormones in rat liver: the effect of administration of anterior hypophyseal hormones and gonadotrophin preparations to hypophysectomized rats. Acta Endocrinol 1976; 82:774–784.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-Phenol reagent. J Biol Chem 1951; 193:265–275.

Lu KH, Grandison L, Huang HH, Marshall LS, Meites J. Relation of gonadotropin secretion by pituitary grafts to spermatogenesis in hypophysectomized male rats. Endocrinology 1977; 100:380–386.

Manandhar MSP, Thomas JA. Effect of prolactin on the metabolism of androgens by the rat ventral prostate gland *in vitro*. Invest Urol 1976; 14:20–22.

Mawhinney MG, Belis JA, Thomas JA, Lloyd JW. Effect of prolactin on androgen metabolism by the guinea-pig sex accessory organs. J Pharmacol Exp Ther 1975; 192:243–249.

McNeilly AS, Sharpe RM, Davidson DW, Fraser HM. Inhibition of gonadotrophin secretion by induced hyperprolactinaemia in the male rat. J Endocrinol 1978; 79:59–68.

Moger WH, Geschwind II. The action of prolactin on the sex accessory gland of the male rat. Proc Soc Exp Biol Med 1972; 141:1017–1021.

Moshang T, Bongiovanni M. End-organ metabolism of testosterone. I. Effects of hypophysectomy and growth hormone. J Steroid Biochem 1973; 4:11–15.

Negro-Vilar A, Saad WA, McCann SM. Evidence for a role of prolactin in prostate and seminal vesicle growth in immature male rats. Endocrinology 1977; 100:729–737.

Negro-Vilar A. Prolactin and the growth of prostate and seminal vesicles. In: Spring-Mills E, Hafez ESE, eds. Male accessory sex glands. New York: Elsevier-No. Holland Bio. Med. Press, 1980; 223–233.

Polan M, Behrman H. Prolactin-stimulated ovarian androgen metabolism. Am J Obstet Gynecol 1981; 139:487–491.

Prins GS, Lee C. Influence of prolactin-producing pituitary grafts on the *in vivo* uptake, distribution and disappearance of ^{3}H-testosterone and ^{3}H-dihydrotestosterone by the rat prostate lobes. Endocrinology 1982; 110:920–925.

Schneider WC. Determination of nucleic acids in tissues by pentose analysis. In: Colowick SP, Kaplan NO, eds. Methods in enzymology. New York: Academic Press, 1957; 686–684.

Schwartz NB, Justo SN. Acute changes in serum gonadotropins and steroids following orchidectomy in the rat: role of the adrenal gland. Endocrinology 1977; 100:1550–1556.

Slaunwhite WR, Jr, Sharma M. Effects of hypophysectomy and prolactin replacement therapy on prostatic response to androgen in orchiectomized rats. Biol Reprod 1977; 17:489–492.

Steel RGD, Torrie JH. Principles and procedures of statistics. New York: McGraw-Hill, 1960.

Walvoord DJ, Resnick MI, Grayhack JT. Effect of testosterone, dihydrotestosterone, estradiol and prolactin on the weight and citric acid content of the lateral lobe of the rat prostate. Invest Urol 1976; 14:60–65.

Witorsch RJ, Kitay JJ. Pituitary hormones affecting adrenal 5 α-reductase activity: ACTH, growth hormone and prolactin. Endocrinology 1972; 91:764–769.

Yamanaka H, Kirdani RY, Saroff J, Murphy GP, Sandberg AA. Effect of testosterone and prolactin on rat prostatic weight, 5 α-reductase and arginase. Am J Physiol 1975; 229: 1102–1109.