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# Effects of Pituitary Grafts on Testosterone Stimulated Growth of Rat Prostate

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## ABSTRACT

The effect of prolactin and testosterone on the lateral prostatic growth was studied in Fischer 344 rats. Two pituitaries from adult female rats/animal were grafted under the renal capsule of 30-day-old castrated males. The control castrates received surgical exposure of the kidney but no grafts. Silastic tubing either empty or packed with crystalline testosterone for 0.5, 2.0 or 4.0 cm of its length was implanted s.c. into each rat immediately following the grafting or sham operation. Three weeks later, all animals were sacrificed. The average serum prolactin in pituitary-grafted animals was 430 ng/ml. In nongrafted controls, the average was 15–50 ng/ml. In rats bearing pituitary grafts and 0.5 cm of testosterone tubing, lateral prostate weight was 88% greater than in controls given 0.5 cm of testosterone tubing alone. In animals with grafts and 2.0 cm testosterone tubing, the increase in lateral prostate weight was only 27%. Furthermore, in those given 4.0 cm testosterone tubing, lateral prostate weights in grafted and control animals were not significantly different. Pituitary grafts did not increase the weight of ventral prostate, dorsal prostate, seminal vesicle or coagulating gland beyond that stimulated by the testosterone tubing. Results demonstrated an inverse relationship between circulating testosterone levels and the degree of synergism with prolactin on the weight of the lateral prostate in rats.

## INTRODUCTION

The augmentation by prolactin of the testosterone effect on growth of the lateral lobe of the prostate has been well documented (Grayhack, 1963; Grayhack and Lebowitz, 1967). However, the mechanism of this relationship remains unclear (Lloyd et al., 1973; Walvoord et al., 1976; Negro-Vilar et al., 1977). The presence of cytosol receptors for androgens (Bruchovsky and Wilson, 1968; Liao and Fang, 1969) and membrane receptors for prolactin (Aragona and Friesen, 1975) seems to indicate that the initial actions of these 2 hormones in the prostrate cells are different. However, the fact that prolactin alone in the absence of testosterone has no effect on prostatic growth (Grayhack et al., 1955) suggests that prolactin action may eventually be mediated through the action of testosterone.

From the above considerations, it seemed that the effect of prolactin would be more pronounced with a small amount of circulating testosterone than with a saturating level of testosterone. The present experiment was designed to test this hypothesis by administering different levels of testosterone in castrated male rats with and without a high level of endogenous prolactin. Testosterone was administered by implanting Silastic tubing containing various amounts of crystalline testosterone. Prolactin was provided by pituitary homografts under the renal capsule.

## MATERIALS AND METHODS

Inbred Fischer 344 male rats, 30 days old, were purchased from Charles River Breeding Laboratories, Wilmington, MA. They were kept in large plastic cages, 5 rats/cage, in an air conditioned room with lights on between 0500–1900 h. Commercial wood shavings were used as the bedding. Tap water and Purina rat chow were provided *ad libitum*.

All animals were castrated via the scrotal route under ether anesthesia. At the same time, anterior pituitaries from two 90-day-old female Fischer 344 rats were transplanted under the right renal capsule of recipient male rats. Each pituitary was cut longitudinally into 2 halves and the posterior pituitary removed before being grafted. Control animals received sham operation by surgical exposure of the kidney and incision into the kidney capsule without the pituitary grafting. Silastic tubing (i.d., 1.575 mm; o.d., 3.175 mm; Dow Corning, Midland, MI) containing crystalline testosterone (Sigma Chemical Co., St. Louis, MO) of either 0.5, 2.0 or 4.0 cm length was prepared according to the method of Legan et al. (1975). Empty capsules 2 cm in length were prepared as controls. Immediately following pituitary grafting or sham opera-

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tion, each animal received one of the silastic tubings implanted s.c.

Three weeks later, animals were killed by decapitation. Blood specimens were collected and the serum fraction from each animal was stored at −20° C for prolactin and testosterone determination by radioimmunoassay (Schwartz and Justo, 1977; Nequin et al., 1979). Kidneys bearing pituitary grafts were fixed in 10% formaldehyde solution for histological procedures. Three lobes of the prostate (ventral, dorsal and lateral), the seminal vesicles, the coagulating glands and the adrenal glands were dissected and weighed to the nearest mg on a Smith Roller torsion balance. DNA and RNA contents of the tissue were determined according to the method recommended by Munro and Fleck (1966) using calf thymus DNA and Torula yeast RNA, respectively, as the standards.

All data were expressed as mean ± SD. Unpaired Student's t test (Steel and Torrie, 1960) was employed to compare the organ weights and hormone levels between pituitary grafted animals and nongrafted controls at each testosterone level.

## RESULTS

Table 1 shows the fresh weights of sex accessory organs in castrated rats receiving different treatments. Comparisons were made between animals with and without pituitary grafts at each length of testosterone tubing. The only significant difference in organ weights between pituitary-grafted and nongrafted rats was in the lateral lobe of the prostate in animals receiving 0.5 or 2.0 cm of testosterone tubing. Furthermore, the increase in the weight of the lateral prostate by pituitary grafting was more pronounced in the group given 0.5 cm testosterone tubing than that given 2.0 cm tubing. With 4.0 cm testosterone tubing, the pituitary grafts were not associated with further lateral prostate growth beyond that stimulated by the testosterone tubing alone. Augmented prostatic growth was not observed in pituitary grafted-castrated rats receiving empty Silastic tubing.

Results of histological studies revealed that pituitary grafts under the renal capsule were viable (Fig. 1). The viability of the graft is further reflected by the elevated serum levels of prolactin as indicated in Table 2. Animals bearing the pituitary grafts had a 10-fold increase in serum prolactin levels compared with nongrafted animals. Table 2 also shows the serum levels of testosterone in different groups. Silastic testosterone tubing, at each length, maintained a relatively constant level of circulating testosterone. Furthermore, the presence of pituitary grafts did not significantly alter the serum testosterone level for each length of the tubing.

Contents of RNA and DNA were measured in the lateral lobes of the prostate in animals implanted with 0.5 and 2.0 cm of testosterone tubing. Figure 2 shows that pituitary grafting resulted in a significant increase in RNA and DNA content in the lateral prostate of rats receiving the testosterone but no graft. This difference disappeared when the tubing length was increased to 2.0 cm.

## DISCUSSION

The present study confirms earlier observations that the lateral lobe of the rat prostate responds uniquely to prolactin stimulation in the presence of testosterone (Grayhack, 1963; Grayhack and Lebowitz, 1967). The effect of prolactin has also been observed when dorsal and lateral lobes of the prostate are combined and weighed together (Moger and Geschwind,

TABLE 1. Effect of testosterone implants and pituitary homografts on weights of sex accessory organs in young male rats (mean ± SD).

| Treatments | | | Body weight (g) | | Prostate weight (mg) | | | Seminal vesicle (mg) | Coagulating gland (mg) |
|---|---|---|---|---|---|---|---|---|---|
| Testosterone tubing (cm) | Pituitary graft | n Rats | Initial | Final | Ventral | Dorsal | Lateral | | |
| Empty | − | 5 | 55 ± 6 | 150 ± 16 | 8 ± 1 | 2 ± 0.2 | 6 ± 1 | 8 ± 2 | 2 ± 0.5 |
| Empty | + | 5 | 50 ± 9 | 144 ± 5 | 10 ± 1 | 2 ± 0.5 | 6 ± 1 | 9 ± 2 | 2 ± 0.5 |
| 0.5 | − | 10 | 56 ± 4 | 152 ± 9 | 69 ± 14 | 34 ± 11 | 25 ± 11* | 67 ± 30 | 19 ± 9 |
| 0.5 | + | 10 | 58 ± 5 | 150 ± 7 | 69 ± 10 | 32 ± 7 | 47 ± 5 | 79 ± 23 | 22 ± 6 |
| 2.0 | − | 10 | 59 ± 4 | 160 ± 14 | 142 ± 16 | 55 ± 13 | 52 ± 7* | 179 ± 21 | 45 ± 6 |
| 2.0 | + | 10 | 57 ± 4 | 154 ± 12 | 132 ± 11 | 52 ± 8 | 66 ± 8 | 180 ± 11 | 38 ± 4 |
| 4.0 | − | 5 | 49 ± 4 | 148 ± 10 | 168 ± 28 | 72 ± 8 | 98 ± 17 | 318 ± 40 | 74 ± 18 |
| 4.0 | + | 5 | 54 ± 6 | 151 ± 11 | 178 ± 30 | 77 ± 5 | 91 ± 9 | 365 ± 80 | 79 ± 11 |

*Value is significantly different from that of the animals bearing pituitary grafts receiving the same length of testosterone tubing by t test (P<0.01).

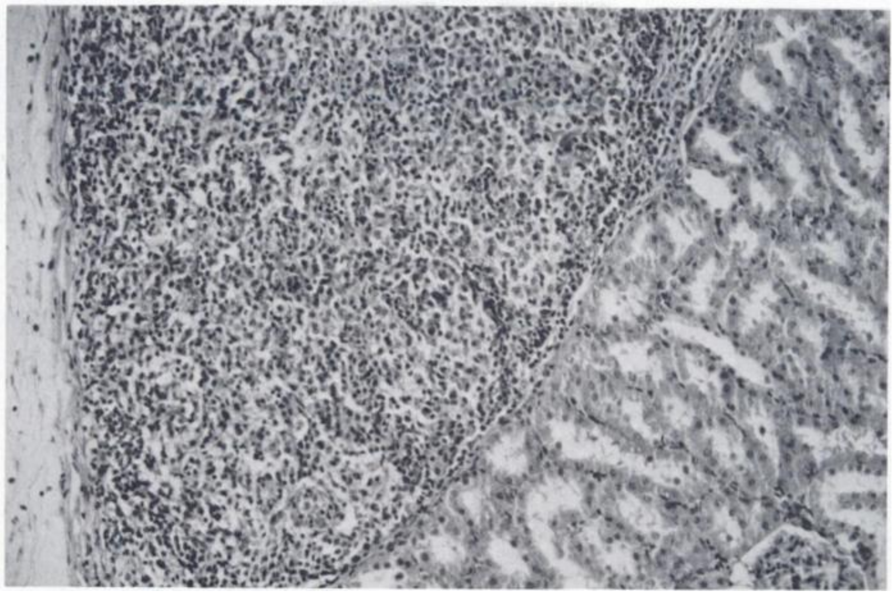

FIG. 1. Pituitary tissue grafted under the renal capsule for 3 weeks. Hematoxylin and eosin. ×40.

1972; Slaunwhite and Sharma, 1977; Negro-Vilar et al., 1977). These results are consistent with the concept that the effect of prolactin on the lateral prostate is mediated through testosterone. At 1.2 ng/ml circulating testosterone, prolactin is able to stimulate further growth of the lateral prostate to the point equivalent to the prostate weight in animals with 3.0 ng/ml circulating testosterone without the excess prolactin (Table 1, Fig. 2.). The present findings in castrated control rats also confirmed the observation of Grayhack et al. (1955) that prolactin alone had no effect on the growth of the lateral prostate of castrated rats.

Anterior pituitary grafts placed under the renal capsule, as in this experiment, are able to produce prolactin in large quantities (Chen et al., 1970). Although the production of LH and FSH diminishes under these conditions (Bartke et al., 1977), it is unclear if other pituitary hormones, such as growth hormone, ACTH and TSH, are produced in significant amounts. In the present study, the observation that grafting of pituitaries resulted in a heavier adrenal gland

TABLE 2. Effect of testosterone implants and pituitary homografts on serum prolactin and testosterone levels and on adrenal weights in young male rats (mean ± SD).

| Treatments | | | | | |
|---|---|---|---|---|---|
| Testosterone tubing (cm) | Pituitary grafts | n Rats | Serum prolactin (ng/ml) | Serum testosterone (ng/ml) | Adrenal gland (mg) |
| Empty | − | 5 | 16 ± 9* | 0.38 ± 0.15 | ND[a] |
| Empty | + | 5 | 357 ± 120 | 0.48 ± 0.18 | ND |
| 0.5 | − | 10 | 56 ± 46* | 1.19 ± 0.65 | 35 ± 6 |
| 0.5 | + | 10 | 504 ± 262 | 1.17 ± 0.54 | 39 ± 5 |
| 2.0 | − | 10 | 47 ± 40* | 2.93 ± 1.38 | 31 ± 4* |
| 2.0 | + | 10 | 414 ± 131 | 3.02 ± 0.90 | 40 ± 6 |
| 4.0 | − | 5 | 48 ± 17* | 3.73 ± 0.47 | ND |
| 4.0 | + | 5 | 379 ± 84 | 3.85 ± 0.72 | ND |

[a]ND, adrenal gland weights not determined.

*Value is significantly different from that of the animals bearing pituitary grafts by t test ($P<0.01$).

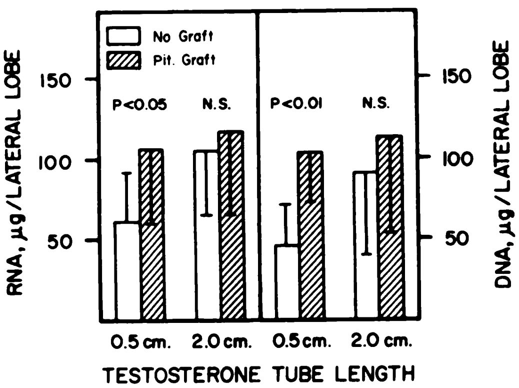


FIG. 2. Total content of RNA and DNA in the lateral prostate of castrated rats supplemented for 3 weeks with 0.5 or 2.0 cm Silastic tubing containing crystalline testosterone. Comparison was made between animals with or without the homogenetic pituitary grafts under the renal capsule. Vertical bars denote SD.

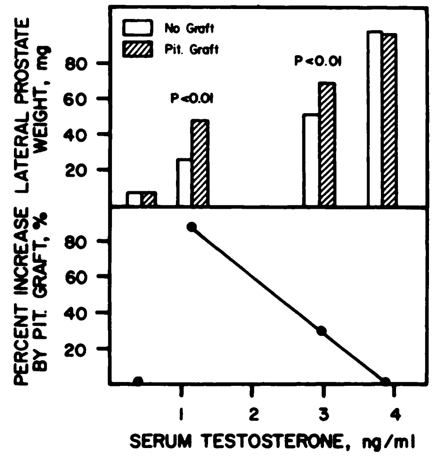


FIG. 3. Summary of the effect of pituitary homografts on the weight of the lateral prostate as plotted against average serum testosterone levels.

weight in animals receiving 2.0 cm testosterone tubing (Table 2) may indicate the possibility of ACTH production. However, it is unlikely that adrenal steroids have any significant effect on prostatic growth. Results from a separate study in this laboratory indicate that adrenalectomy did not cause any significant change in the lateral prostate weight in castrated rats bearing pituitary homografts (Kolbusz et al., 1979). These findings agree with those of Moore (1953) and VanderLaan (1960), although large doses of ACTH elicited some androgenic effect in castrated male rats (Lostroh and Li, 1957; Tullner, 1963).

The interaction between prolactin and testosterone on prostatic growth has been referred to as synergism in which the combined effect of 2 hormones on the prostate is greater than the additive effects of each individual hormone acting alone. Results of the present study seem to indicate that the occurrence of this synergistic relationship depends on the level of serum testosterone. As summarized in Fig. 3, using prostatic weight as the criterion, the synergistic effect diminished as the level of serum testosterone increased.

The present study, however, is unable to define the exact site of mechanism of interaction between prolactin and testosterone. The cascade of testosterone action on prostatic tissue includes: 1) conversion of testosterone to dihydrotestosterone; 2) binding of dihydrotestosterone to cytosol androgen receptor; 3) translocation of receptor-dihydrotestosterone complex to the nucleus; and 4) binding of this complex to chromosome acceptor sites (Liao et al., 1975). Any of the above sites or combinations of them would be possible sites for prolactin action. This mechanism of interaction between prolactin and testosterone within the lateral prostate warrants further investigation.

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