Sex Steroid-induced Changes in Collagen of the Prostate and Seminal Vesicle of Rats

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The effects of androgens alone or in combination with estrogen or prolactin on the collagen of the prostate and seminal vesicles were studied in prepubertal and adult rats. Castration decreased the collagen content of the male accessory sex organs of both prepubertal and adult rats. Androgens showed stimulatory effects in castrated rats irrespective of the age. However, in intact animals, the stimulatory effects of androgens were evident only before puberty. Only the seminal vesicle of prepubertal rats responded to the stimulatory effect of estrogen given along with androgens. Prolactin did not elicit any appreciable effect either in the prostate or the seminal vesicles when administered along with androgens.

Key words: testosterone propionate, dihydrotestosterone, estradiol, prolactin, collagen, seminal vesicle, ventral prostate.

J Androl 1986; 55-58.

The fibromuscular stroma provides the template for an orderly and finite growth of epithelial cells (Vracko, 1974). Collagen is considered to be one of the important components of the fibromuscular stroma (Miller, 1976). In prepubertal rats, the decrease in the collagen contents of the ventral prostate and seminal vesicles due to castration was restored after dihydrotestosterone (DHT) treatment (Mariotti et al, 1981). Estrogen and androgens have a synergistic effect on canine and human prostatic neoplasia (Walsh and Wilson, 1976; Deklerk et al, 1979; Vermeulen et al, 1979). Conflicting reports, however, are available on the effects of androgens and estrogens on the rat prostate (Grayhack, 1965; Shimazaki et al, 1969; Karr et al, 1974; Robaire et al, 1979). Apart from estrogen, prolactin also modulates the actions of androgens on male accessory sex organs (Thomas and Keenan, 1976). This paper reports the effects of the interaction of testosterone and DHT with estradiol or prolactin on collagen levels in the ventral prostate and seminal vesicles of prepubertal and adult rats.

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The work of Dr. N. Srinivasan was supported in part by the Council of Scientific and Industrial Research, New Delhi.

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Submitted for publication December 31, 1984; revised versions received May 20, 1985 and August 29, 1985; accepted for publication September 4, 1985.

Material and Methods

Animals

Healthy male albino rats of the Wistar strain were housed in a well-ventilated and humidity-controlled animal quarter at a temperature of 25 ± 2 C with a 14-hour light: 10-hour dark schedule. These rats were fed a standard balanced pellet diet (Gold Mohur, Hindustan Lever, India) and provided with drinking water *ad libitum*.

The animals were divided into prepubertal (25-days old) and adult (120-days old) age groups consisting of both intact and castrated animals. Castration was performed by the scrotal route under light ether anesthesia. Hormone treatment in the castrated animals was started seven days after surgery.

Depending upon the type of hormone treatment, the animals were divided into six subgroups:

Subgroup I: Animals received daily injections of testosterone propionate (TP) alone for 21 days. Dosage: 1 mg TP/prepubertal rat, 2 mg TP/adult rat.

Subgroup II: Animals received daily injections of TP plus E_2-17β (E_2) for 21 days. Dosage: 1 mg TP + 10 μg E_2/prepubertal rat, 2 mg TP + 20 μg E_2/adult rat.

Subgroup III: Animals received TP + ovine prolactin (PRL)

daily for 21 days. Dosage: 1 mg TP + 250 μg PRL/prepubertal rat, 2 mg TP + 500 μg PRL/adult rat.

Subgroup IV: Animals received DHT alone daily for 21 days. Dosage: 1 mg DHT/prepubertal rat, 2 mg DHT/adult rat.

Subgroup V: Animals of this group received daily injections of DHT + E_2 for 21 days. Dosage: 1 mg DHT + 10 μg E_2/prepubertal rat, 2 mg DHT + 20 μg E_2/adult rat.

Subgroup VI: Animals of this group received daily injections of DHT + PRL for 21 days. Dosage: 1 mg DHT + 250 μg PRL/prepubertal rat, 2 mg DHT + 500 μg PRL/adult rat.

Each subgroup consisted of 40 intact (20 prepubertals and 20 adults) and 40 castrated (20 prepubertal and 20 adults) rats. The specified hormone treatment was given to 20 animals (ten intact and ten castrates) from each age group, and an equal number of age-matched controls received the vehicle alone. Steroid hormones were dissolved in propane-1,2-diol, and PRL was dissolved in 0.02 M sodium hydroxide. Each hormone was injected i.p. separately at 0900 hours. Twenty-four hours after the last injection, the animals were killed by decapitation. Ventral prostate lobes were freed of external fascia. Seminal vesicles were freed from the anterior prostate, and the luminal fluid was expressed completely without destroying the epithelium. The tissues were washed in physiologic saline, weighed, and stored at −20 C until used.

Estimation of Collagen

Collagen was estimated by the method of Stegemann (1958). This method involves the oxidation of hydroxyproline to pyrrole-2-carboxylic acid by chloramine-T in a weak acid buffer (*p*H 6.0). Pyrrole-2-carboxylic acid is converted to pyrrole in the presence of perchloric acid, which destroys the chloramine-T and prepares the material for the formation of chromophore with p-dimethylamino benzaldehyde.

Chemicals

All chemicals and reagents used were analytic grade (Sigma Chemical Company, St. Louis, MO). The results were analyzed using Student's *t* test.

Results

Ventral Prostate

Castration reduced prostate tissue weights and collagen contents in both prepubertal and adult rats. Administration of TP/DHT markedly enhanced the organ weight in all groups of rats studied. The collagen content also was elevated by these androgens in all groups except the intact adults, which showed no significant change (Table 1).

E_2 given along with TP/DHT further increased prostate organ weights only in intact prepubertals. In intact adults, it decreased the organ weight. PRL combined with androgens further increased the organ weight of intact prepubertal and adult rats. The combination of either PRL or E_2 with androgen

TABLE 1. Influence of Sex Steroids and Prolactin on Ventral Prostate Weight and Collagen Content in Intact and Castrated Prepubertal and Adult Rats

	Intact Animals			Castrated Animals			
	Prepubertal		Adult*	Prepubertal		Adult	
Hormone Treatment	Organ Weight (mg)	Collagen Content (μg/Organ)	Organ Weight (mg)	Organ Weight (mg)	Collagen Content (μg/Organ)	Organ Weight (mg)	Collagen Content (μg/Organ)
Control	41 ± 3†	24.1 ± 3.0	162 ± 10	5 ± 0.5^{C1}	10.0 ± 2.1^{b1}	28 ± 3^{C1}	72.1 ± 8.2^{C1}
Testosterone propionate	53 ± 5^{b1}	59.3 ± 3.9^{C1}	331 ± 26^{C1}	48 ± 4^{C2}	44.2 ± 3.8^{C2}	267 ± 17^{C2}	162.3 ± 13.0^{C2}
Testosterone propionate + estradiol	101 ± 7^{C3}	63.4 ± 6.4	116 ± 9^{C3}	48 ± 4	49.7 ± 3.4	261 ± 16	166.4 ± 14.2
Testosterone propionate + prolactin	86 ± 7^{b3}	61.5 ± 4.2	411 ± 25	59 ± 5	45.2 ± 3.1	268 ± 17	163.6 ± 13.2
Dihydrotestosterone	74 ± 6^{C1}	67.1 ± 5.1^{C1}	324 ± 24^{C1}	59 ± 4^{C2}	50.1 ± 3.7	253 ± 15^{C2}	166.2 ± 14.8^{C2}
Dihydrotestosterone + estradiol	132 ± 9^{C4}	69.9 ± 6.2	118 ± 8^{C4}	62 ± 5	52.6 ± 3.9	273 ± 15	168.9 ± 13.9
Dihydrotestosterone + prolactin	100 ± 8^{a4}	68.7 ± 5.9	403 ± 24^{a4}	68 ± 5	50.9 ± 3.6	263 ± 19	161.1 ± 13.4

*Collagen content in intact adult control = 158 ± 14 (μg/organ). The data on collagen content in intact adult treatment groups are not presented, as there were no significant changes.

†Each value is Mean ± SEM of 10 animals/estimations. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{C}P < 0.001$.

1 = compared with intact control; 2 = compared to castrated control; 3 = compared with testosterone propionate; 4 = compared with dihydrotestosterone.

had no added effect on androgen-induced changes in prostate collagen contents (Table 1).

Seminal Vesicle

Castration diminished the organ weight and collagen content of the seminal vesicles of prepubertal and adult rats. TP/DHT, when given alone, increased the organ weights in all the groups studied. The seminal vesicle collagen content was also increased by androgens in all groups, except the intact adults, which showed no significant changes (Table 2).

E_2 given along with TP/DHT further enhanced seminal vesicle weights and collagen contents in intact and castrated prepubertal rats. In intact adults, E_2 combinations decreased organ weights but had no significant effect on seminal vesicle collagen levels. In castrated adults, this combination did not produce any changes in organ weight and collagen content that were significantly different from those of TP/DHT alone. Coadministration of PRL with TP/DHT further increased organ weights only in intact prepubertal and adult rats. This combination, however, had no significant effect on the collagen content of the seminal vesicles of any of the groups studied (Table 2).

The body weights of the rats were not altered by any of the hormone treatments (data not shown). An important finding is that none of the treatment protocols caused any significant changes in the collagen content of the ventral prostate and seminal vesicles of intact adult rats (data not shown).

Discussion

Androgen-induced increases in the prostate and seminal vesicle weights and collagen contents in prepubertal rats may be due to cell proliferation and stromal growth. The stroma provides the infrastructure for the developing epithelial cells (Vracko, 1974). The data also reflect the anabolic effects of androgens as reported by earlier workers (Mawhinney and Neubauer, 1979; Neubauer and Mawhinney, 1980). E_2 potentiated the action of androgens on collagen contents in the seminal vesicles but not in the ventral prostate of prepubertal rats. This differential response of the seminal vesicles and ventral prostate may be due to the varying sensitivity of these organs to E_2 (Fujii and Villee, 1968; Mawhinney and Neubauer, 1979). The presence of relatively more estrogen receptors in the seminal vesicles (Robinette et al, 1978) and the absence of collagen-rich smooth muscle in the rat ventral prostate (Deklerk and Coffey, 1978) may also contribute to this differential response.

Castration-induced reduction in organ weights

TABLE 2. Influence of Sex Steroids and Prolactin on Seminal Vesicle Weight and Collagen Content in Intact and Castrated Prepubertal and Adult Rats

	Intact Animals			Castrated Animals			
	Prepubertal		Adult*	Prepubertal		Adult	
Hormone Treatment	Organ Weight (mg)	Collagen Content (μg/Organ)	Organ Weight (mg)	Organ Weight (mg)	Collagen Content (μg/Organ)	Organ Weight (mg)	Collagen Content (μg/Organ)
Control	22 ± 3†	81.0 ± 6.0	147 ± 12	6 ± 0.7^{C1}	29.4 ± 3.1^{C1}	77 ± 8^{C1}	379.1 ± 21.9^{a1}
Testosterone propionate	41 ± 5^{b1}	138.1 ± 10.6^{C1}	239 ± 18^{C1}	57 ± 6^{C2}	103.0 ± 9.4^{C2}	280 ± 20^{C2}	462.4 ± 27.1^{a2}
Testosterone propionate + estadiol	102 ± 9^{C3}	189.2 ± 16.2^{b3}	101 ± 9^{b3}	89 ± 7^{b3}	138.2 ± 10.7^{a3}	258 ± 24	464.0 ± 26.6
Testosterone propionate + prolactin	93 ± 9^{C3}	139.4 ± 11.7	440 ± 36^{C3}	64 ± 7	104.3 ± 9.9	256 ± 24	452.8 ± 30.1
Dihydrotestosterone	89 ± 8^{C1}	146.7 ± 13.1^{C1}	309 ± 25^{C1}	61 ± 8^{C2}	109.9 ± 9.0^{C2}	256 ± 21^{C2}	467.2 ± 27.6^{a2}
Dihydrotestosterone + estadiol	131 ± 10^{b4}	227.3 ± 21.6^{b4}	112 ± 9^{C4}	97 ± 9^{b4}	139.4 ± 10.1^{a4}	264 ± 22	464.6 ± 28.9
Dihydrotestosterone + prolactin	117 ± 9^{a4}	154.5 ± 14.7	410 ± 34^{C4}	69 ± 8	106.1 ± 10.4	261 ± 23	459.8 ± 29.3

*Collagen content in intact adult control = 439 ± 29 (μg/organ). The data on collagen content in the intact adult treatment groups are not presented, as there were no significant changes.

†Each value is the Mean ± SEM of 10 animals/estimations. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

1 = compared with intact control; 2 = compared with castrated control; 3 = compared with testosterone propionate; 4 = compared with dihydrotestosterone.

and collagen levels of these accessory sex organs may be attributed to the degradation of basement membranes and interstitial tissues, and the absence of cell proliferation due to the depletion of androgens (Vracko, 1974). These effects are very pronounced in prepubertal rats since androgens are essential at this initial period of growth (Rajfer and Coffey, 1978). The present study suggests that sex hormone treatment does not result in benign prostatic hyperplasia nor in the development of abnormal growths in adult rats.

The antagonistic effect of E_2 given along with TP/DHT on the organ weights of intact adult rats may be attributed to an activated negative feedback effect of the pituitary-gonadal hormone axis in these animals (Verjans et al, 1974). Despite the active negative feedback effect, collagen levels were unaffected in these animals. E_2 given along with androgens probably has a direct effect on fibromuscular growth (Mawhinney and Neubauer, 1979). The lack of any significant influence of PRL on collagen levels in the ventral prostate or seminal vesicles may be due to the absence of prolactin receptors in the stromal region of these organs.

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