Effects of Androgen Treatment on the Male Rat Aorta

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ABSTRACT Androgen was given to male rats to determine if it exerted effects on the aortic wall distinct from those of estrogen deficit. Although a general anabolic effect was avoided, significant vascular effects were observed. The amounts of mural fibrous proteins, elastin and collagen, were significantly increased in treated animals; noncollagenous, alkali-soluble protein, thought to reflect the cellular component, was unchanged with treatment. These effects were not detectable on microscopic examination and measurement of the vessel wall despite attempts to duplicate closely in vivo wall dimensions before study. These findings of distinct and marked effects of androgen on vascular metabolism extend the growing evidence for an important role of sex hormones in vessel wall structure and function.

INTRODUCTION

Recent studies have shown that female sex hormones exert profound effects on vessel wall morphology and metabolism. Estrogens administered to gonadectomized female rats (1, 2) and guinea pigs (3) markedly reduce the net amount of collagen in the aortic wall and skin. Labeled precursor studies of vascular collagen in females suggest that the mechanism responsible is a combination of accelerated turnover of a relatively small pool of soluble precursors of collagen and interference with progression of these precursors into the insoluble highly cross-linked form of collagen (1, 3). When administered to male hypertensive rats, estrogens virtually eliminate the marked morphological changes and accelerated accumulation of mural fibrous proteins usually seen in response to elevated blood pressure (4). Since estrogen administration to males also causes inhibition of androgen secretion, results of that experiment could either reflect a simple effect of estrogen or a combined effect of androgen deficit and estrogen excess. The present experiment was undertaken to ascertain if androgens exert a distinct effect on the morphology and composition of the male rat aorta.

METHODS

Male Carworth rats (CF-N) weighing 170–220 g at the onset of the experiment were given rat laboratory chow and drinking water ad lib. Depo-Testosterone,[1] in a dose of 10 mg, was given subcutaneously at biweekly intervals for a total of four doses to treated animals; controls were given subcutaneous injections of sterile water in a similar volume (0.1 cc). Systolic blood pressures were measured at biweekly intervals in lightly anesthetized animals using a tail cuff and an amplifier system as previously described (5). The experiment was terminated after 8 wk; total heart weights were obtained after cutting the great vessels at the base of the heart and blotting the hearts briefly.

Morphological studies. Four animals from each group were used; thoracic aortas were removed and placed on a frame which permitted extension to in vivo length. Then each segment was distended with a gelatin-barium sulfate mixture (6) to a pressure corresponding to the average systolic pressure of that animal in life. After gelling of the mixture within the segment was completed by immersion of the frame in cold formalin, the distended segment could be removed from the pressure system for further formalin fixation and subsequent histological preparation. Diameters were measured on X-rays of the segments taken after 24 hr of fixation (6). Then histological cross sections of segments, 5 μ thick, were stained with hematoxylin-eosin and Weigert-van Gieson stains. Micrometric techniques were used to measure wall thickness and medical lamellar complement (6). Tangential wall tension per lamellar unit (6) and wall stress (7) were calculated as previously described (4). Medial area was calculated using the formula: Area $= \pi(r_o^2 - r_i^2)$, where r_o = outer wall radius and r_i = inner wall radius. All wall measurements were corrected for tissue shrinkage as previously described (6). Results in the two groups were compared using Student's t test; differences were considered to be significant at the 5% level.

Chemical studies. Six animals from each group were used; thoracic aortic segments exactly delimited by the left

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[1] Testosterone cypionate, 100 mg/ml, Upjohn Company, Kalamazoo, Mich.

subclavian and celiac arteries were removed and the intima and media isolated (8). After dehydration in ethanol-ether for 2 hr and ether for 1 hr as previously described (4), each aortic segment was brought to dry weight after 72 hr at 55-60°C in a vacuum oven. Alkali hydrolysis was carried out using a modification (5) of Lansing's method; soluble and residue fractions were obtained as previously described (5). Hydroxyproline determinations were carried out on portions of each soluble fraction (9) and total collagen in each aorta was estimated using the value of 92 residues hydroxyproline per 1000 residues of amino acids obtained for rat tail tendon and rat skin collagens (10). The nitrogen content of a portion of each soluble and residue fraction was determined using Kjeldahl digestion and was then corrected to total nitrogen in each sample. For the soluble fraction, nitrogen attributible to collagen (assuming an 18.9% nitrogen content of collagen) was subtracted from total nitrogen; the difference was attributed to noncollagenous alkali-soluble proteins (NCASP)[2] (a nitrogen content of 16% was assumed in calculating the amount of NCASP). The residue fraction consisted only of elastin, based on results of previous amino acid analyses (5); amounts of elastin calculated from nitrogen results (using an assumed content of 18.9% nitrogen) correlated well with results obtained from amino acid analysis (5). Amino acid analyses of the residue were carried out to determine lysine content. Total elastin, collagen, and NCASP were calculated for each aorta based on sample results. Tests for significance of results were the same as those used for morphological studies.

RESULTS

Characteristics of the groups are shown in Table I. Final body weights, mean systolic blood pressures, and total heart weights did not differ in the control and treated groups. That anabolic effects of the androgen were not reflected in the final body weight of the treated group was probably due to the rapid body growth rate experienced by all animals.

Morphological. The gross appearances of the aortas of both groups were not different. Histologically, no consistent difference in amount of connective tissue or cellular features was qualitatively apparent. Aortic diameters of the groups did not differ significantly (Table II) and micrometric measurements detected no differences between the groups for a variety of parameters (Table II).

Chemical. Total aortic dry weights were significantly greater in the treated group than in controls (Table III). Likewise, the total amounts of both elastin and collagen were larger in the treated vessels than in the untreated vessels (Table III); the increase in elastin appeared to be proportionately larger than that of collagen. The amounts of NCASP were no different in the two groups (Table III).

When calculated as per cent of dry weight (Table IV), elastin did not differ in the two groups. However, the disproportionate net increase in amount of elastin compared to that of collagen in the treated vessels resulted in a significant decrease in the per cent of collagen in these vessels. NCASP per cents did not differ significantly in the groups.

When lysine contents of aortic elastin were calculated from chromatograms after amino acid analysis, no difference was found between the mean values of treated (10.5±0.6 residues/1000 residues) and control (9.6±0.9 residues/1000 residues) groups ($t = 0.87$, $0.5 > P > 0.4$).

TABLE I
Characteristics of Animal Groups

	Control	Treated	
Final body weight, *g*	362±12*	346±12	$t = 0.94$, $0.4 > P > 0.3$
Systolic blood pressure, *mm Hg*	105±1	105±1	$t = 0$, $P > 0.9$
Heart weight, *g*	1.01±0.05	1.03±0.03	$t = 0.30$, $0.8 > P > 0.7$

* Values expressed as mean ±SEM; based on 10 animals per group.

DISCUSSION

The types of constituents of the vessel wall are rather limited; fibrous proteins, cellular elements (predominantly smooth muscle), and glycosamino-glycans constitute the major groups. Undoubtedly some heterogeneity exists in the NCASP fraction; for example total mucopolysaccharides which normally constitute up to approximately 2.0% of aortic dry weight (11, 12) contain a small protein moiety. However, these and other potential contributors to NCASP are minor when compared to the mass of cellular protein in the vessel wall. We have therefore considered it reasonable to consider changes in NCASP to primarily reflect medial smooth muscle changes. Though this permits detection of major changes in the cellular compartment no conclusions about cell number, size, or activity can be derived from this measurement.

Several noteworthy findings have resulted from this study. It has been possible by chemical means to detect significant differences in fibrous protein amounts which escaped notice morphologically despite efforts taken to simulate in vivo dimensions. The fibrous protein increments in the treated group and the total aortic weight differences amounted to approximately 20% above control levels. The failure to morphologically detect these connective tissue changes is probably explained by the fact that the bulk of the wall volume mainly reflects the cellular compartment (see Figures, Ref. 5) and this component, as reflected in NCASP, did not change in the treated group.

It is of interest that accumulation of the fibrous proteins was enhanced in the treated vessel wall while at the

[2] *Abbreviation used in this paper:* NCASP, noncollagenous alkali-soluble proteins.

TABLE II

Dimensions of and Calculated Stresses on Male Thoracic Aortas

	Control	Treated	
Diameter, *mm*	2.27±0.04*	2.28±0.13	$t = 0.05$, $P > 0.9$
Wall thickness, *mm*	0.103±0.004	0.100±0.006	$t = 0.42$, $0.7 > P > 0.6$
Tension/lamellar unit, *dynes/cm* × 10^3	1.99±0.02	2.02±0.06	$t = 0.55$, $0.7 > P > 0.6$
Wall stress, *dynes/cm²* × 10^6	1.56±0.05	1.60±0.10	$t = 0.38$, $0.8 > P > 0.7$
Medial area, mm^2	0.771±0.046	0.742±0.067	$t = 0.36$, $0.8 > P > 0.7$

* Values expressed as mean±SEM; based on four animals per group.

same time body growth rate was not. The animals used were in a period of rapid body growth rate; this was deliberately chosen in order to overwhelm any general anabolic effects of testosterone. In retrospect, it was hardly warranted to expect a change in vessel components under these same conditions, and with only several injections of hormone over a short period of time even though the doses used were probably pharmacologic. That significant changes were seen suggests that androgen treatment exercised important effects on mural fibrous protein metabolism, effects distinct from those of estrogen treatment. Androgen has been shown to be associated with increases in the concentration of fibrous proteins in gonadectomized chick aortas (13) and in the content of skin collagen in hirsute women (14) so that precedents for the effects described here exist.

Implications from these findings must be drawn cautiously since administration of a hormone may trigger other hormonal changes thereby complicating interpretation of its effects. Additionally, the study included only rapidly growing animals; effects of androgens on older animals remain to be shown.

This study gives further indication of the dissociation which can occur between the responses of fibrous components and cellular components of vessels. Fibrous proteins increased in the androgen-treated vessels; NCASP did not. In previous studies of untreated animals, using hypertension as a model, striking increases in aortic NCASP were shortly followed by sharp increases in the fibrous proteins. Reversal of hypertension led only to a fall in the cellular fraction, resulting in an elevated concentration of the fibrous components (5). When male hypertensives were treated with estrogen, aortic fibrous proteins remained at levels no different from those of normotensives; however, a slight but definite increase in NCASP was found (4). Those findings together with the ones reported here raise the strong possibility that the size of the cellular component, as reflected in NCASP, primarily reflects wall stress; when tension is elevated this compartment is increased; when it is not, no change in the cellular component can be detected. Conversely, it is possible to exert a predominant influence on mural accumulation of the fibrous proteins independently of stress: estrogens limit accumulation despite hypertension; androgens stimulate accumulation despite normal wall tensions. That levels of mural fibrous proteins are increased after chronic hypertension is not explained by this apparent capability of the vessel wall for hemodynamic-metabolic dissociation; perhaps another stimulus, for example, healing of cellular damage, is operative in addition to the easily detected elevated wall stress. In any case it can be concluded from the present study that the male sex hormone studied, like the female ones, significantly influences vascular fibrous protein metabolism.

Others have proposed that the degree of fibrous protein accumulation may influence permeability characteristics of vascular tissue (15) or otherwise predispose to atherosclerosis (16, 17). If the findings here are projected to extremes it is possible to foresee adverse consequences of prolonged androgen effect and potential benefits from sustained exposure to estrogens. It should be possible to test these projections experimentally.

TABLE III

Total Aortic Weights and Total Amounts of Wall Components

	Control	Treated	
Total aortic dry weight, *mg*	5.47±0.15*	6.72±0.11	$t = 6.79$, $P < 0.001$
Elastin, *mg*	2.15±0.09	2.72±0.07	$t = 4.85$, $P < 0.001$
Collagen, *mg*	1.36±0.04	1.57±0.03	$t = 4.13$, $P < 0.01$
NCASP, *mg*	0.85±0.08	0.93±0.07	$t = 0.71$, $0.5 > P > 0.4$

* Values expressed as mean±SEM; based on six animals per group.

TABLE IV

Per Cents of Aortic Wall Components

	Control	Treated	
Elastin, %	39.27±1.20*	40.52±1.01	$t = 0.80$, $0.5 > P > 0.4$
Collagen, %	24.96±0.54	23.43±0.37	$t = 2.34$, $P < 0.05$
NCASP, %	15.63±1.37	13.78±0.94	$t = 1.12$, $0.3 > P > 0.2$

* Values expressed as mean±SEM; based on six animals per group.

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