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ANDROGENS REGULATE CELL GROWTH, LYSOSOMAL HYDROLASES AND MITOCHONDRIAL CYTOCHROME *c* OXIDASE IN MOUSE AORTA

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Summary

The aorta in male mice shows higher activities of several lysosomal hydrolases and of cytochrome *c* oxidase, an inner mitochondrial membrane enzyme, than in female mice. Orchiectomy abolishes this sex difference, whereas testosterone administration induces an accretion of RNA and protein and elevated activities of lysosomal hydrolases and cytochrome *c* oxidase. However, the outer mitochondrial membrane enzyme monoamine oxidase is unaffected by sex, orchiectomy or testosterone. Thus, androgens regulate cell growth and enzymes associated with lysosomes and the inner mitochondrial membrane.

Introduction

Cardiovascular diseases exhibit a marked preference for men until the sixth and seventh decades when the incidence of these diseases increases sharply in women [1]. The basis for this sex difference remains an enigma. Estrogens have long been thought to protect premenopausal women from artherosclerosis. However, considerable doubt has been cast on this hypothesis by recent findings that estrogens, whether administered to women in oral contraceptives [2] or men for the treatment of prostatic carcinoma [3] and coronary heart disease [4], actually increased the incidence of myocardial infarction and cardiovascular mortality. On the other hand, androgens have attracted little attention as a potential risk factor in artherosclerosis, although testosterone administration has been shown to influence the fibroelastic structure [5] and glycosaminoglycan [6] and fibrous protein composition [7] of the aorta, and to enhance arterial thrombogenesis after intimal injury [8].

We recently observed a testosterone-mediated sex difference in the fine structure of mouse kidney proximal tubules affecting lysosomes and mitochondria and the tissue activities of several enzymes associated with these organelles [9,10]. These findings prompted a search for similar testosterone-mediated effects in the mouse aorta. We now present evidence showing that the mouse aorta exhibits a vigorous response to testosterone administration characterized by an accretion of RNA and protein and increased activities of cytochrome *c* oxidase and several lysosomal hydrolases. Further, endogenous androgens exert a substantial regulatory influence on the aorta which is manifested as a sex difference in tissue levels of lysosomal hydrolyses and cytochrome *c* oxidase.

Methods and Materials

Animal experiments

Adult A/J mice (Jackson Laboratory, Bar Harbor, ME, U.S.A.) of both sexes were used. Sex differences were examined in male and female mice of similar size and age. The effects of endogenous androgens were investigated by comparing intact and orchiectomized male mice. Orchiectomy was done through a scrotal incision under trichloroethylene anesthesia and mice were killed 8 or 63 days postoperatively. Female mice or orchiectomized male mice were given testosterone propionate (Sigma Co., St. Louis, MO, U.S.A.) (0.5 or 1.0 mg in 0.05 ml ethyl oleate) by subcutaneous injection every other day and decapitated 24 h after the fourth injection. Control mice were given ethyl oleate vehicle or were untreated. The entire aorta from its cardiac origin to its termination at the iliac bifurcation was excised and stored at −70°C.

Biochemical assays

The frozen aortas were minced, homogenized in 0.7 ml of cold 0.3 M sucrose, and assayed for protein [11], RNA [12], DNA [13], β-glucuronidase (EC 3.2.1.31), hexosaminidase (β-*N*-acetylhexosaminidase, EC 3.2.1.30), β-galactosidase (EC 3.2.1.23), arylsulphatase (EC 3.1.6.1) [14], cytochrome *c* oxidase [15], and monoamine oxidase [16]. Maximum enzyme activities were obtained under these assay conditions owing to the disruptive affects of freeze-thawing and the hypotonicity of the enzyme substrates on the lysosomal and mitochondrial membranes. All enzyme substrates came from Sigma Chemical Co. Data were analyzed using the Student's *t*-test.

Results

It can be seen in Table I that the specific activity of cytochrome *c* oxidase in the aorta was greater in male mice than in female mice, whereas the specific activity of monoamine oxidase was similar in the two sexes. Orchiectomy produced a decrease in cytochrome *c* oxidase activity that was well developed 8 days postoperatively. Conversely testosterone propionate administration in female and orchiectomized male mice induced a substantial increase in specific activity of cytochrome *c* oxidase in the aorta. In contrast, orchiectomy and testosterone propionate treatment had no effect on the monoamine oxidase. Testosterone propionate administration in female mice also increased the concen-

TABLE I

EFFECTS OF SEX, ORCHIECTOMY AND TESTOSTERONE ADMINISTRATION ON DNA, RNA, PROTEIN, CYTOCHROME *c* OXIDASE, AND MONOAMINE OXIDASE OF MOUSE AORTA

Expt. A. Male mice were unoperated (control) or orchiectomized 63 days before sacrifice. Female mice received testosterone propionate (TP) (1 mg/mouse in 0.05 ml ethyl oleate) by subcutaneous injection every other day and killed 24 h after the fourth injection. Five control females received ethyl vehicle and five received no vehicle. Expt. B. Female mice received TP (0.5 mg/mouse) or ethyl oleate vehicle only every other day and killed 24 h after the fourth injection. Expt. C. Male and female mice of similar age and weight were used without further treatment. Expt. D. Male mice were orchiectomized and 24 h later alternate day treatment with TP (0.5 mg/mouse) or ethyl oleate vehicle was begun. Animals were killed 24 h after the fourth injection along with intact untreated control males. Enzyme units: cytochrome *c* oxidase. ΔA_{550}/ml per h; monoamine oxidase, 1 μg 4-hydroxyquinolone produced per h. Data are means ± S.E. (number of animals). a, b, c: $P < 0.05, 0.01, 0.001$ (treated vs corresponding control). d, e, f: $P < 0.05, 0.01, 0.001$ (females, control vs males, control).

Constituent	Experiment	Males, control	Males, orchiectomized	Males, orchiectomized, TP	Females, control	Females, TP
DNA (μg/aorta)	B	—	—	—	9.01 ± 0.50(5)	8.37 ± 0.76(5)
RNA (μg/μg DNA)	B	—	—	—	1.82 ± 0.02(5)	2.23 ± 0.05(5) [c] (122.5%)
Protein (μg/μg DNA)	B	—	—	—	97 ± 4(5)	113 ± 5(5) [a] (116.5%)
Cytochrome *c* oxidase (U/mg protein	A	3.34 ± 0.28(7)	2.38 ± 0.30(7) [a] (71.3%)	—	2.54 ± 0.22(10) [d] (76.1%)	3.72 ± 0.35(5) [a] (146.5%)
	D	3.58 ± 0.31(5)	2.50 ± 0.032(2) [a] (69.8%)	5.05 ± 0.74(5) [b] (202%)	—	—
Monoamine Oxidase (U/mg protein)	C	82.6 ± 5.6(6)	—	—	73.2 ± 4.8(6)	—
	D	106 ± 8.5(5)	102 ± 7.2(2)	103 ± 9.8(5)	—	—

TABLE II

EFFECTS OF SEX, ORCHIECTOMY AND TESTOSTERONE ADMINISTRATION ON LYSOSOMAL HYDROLASES IN MOUSE AORTA

Experimental details are given in Table I. Data are means ± S.E. (number of animals). a, b, c: $P < 0.05, 0.01, 0.001$ (treated vs corresponding controls); d, e, f: $P < 0.05, 0.01, 0.001$ (females, control vs males, control).

Constituent	Experiment	Males, control	Males, orchiectomized	Males, orchiectomized, TP	Females, control	Females, TP
β-Glucuronidase (nmol/h/mg protein)	A	342 ± 14(7)	219 ± 60(7) [c] (64%)	—	228 ± 30(10) [e] (66.7%)	425 ± 80(5) [c] (186.4%)
	D	358 ± 35(4)	266 ± 25(5) [a] (74.3%)	505 ± 60(5) [b] (189.9%)	—	—
Hexosaminidase (nmol/h/mg protein)	A	698 ± 50(7)	465 ± 40(7) [b] (66.6%)	—	480 ± 60(10) [e] (68.8%)	1080 ± 70(5) [c] (225%)
	D	680 ± 70(4)	470 ± 70(5) [a] (69.1%)	840 ± 90(5) [b] (178.7%)	—	—
β-Galactosidase (nmol/h/mg protein)	A	83 ± 4(7)	64 ± 4(7) [b] (77.1%)	—	74 ± 4(10) [d] (89.2%)	142 ± 10(5) [c] (191.9%)
Arylsulphatase (nmol/h/mg protein)	A	189 ± 10(7)	154 ± 10(7) [a] (81.5%)	—	94 ± 15(10) [e] (49.7%)	147 ± 20(5) [b] (156.4%)

tration of RNA and protein in the aorta without affecting the total DNA.

Table II presents the results on the lysosomal hydrolases of the aorta. The specific activities of β-glucuronidase, hexosaminidase, β-galactosidase and arylsulphatase were substantially greater in the aorta of male mice than in the aorta of female mice. Orchiectomy caused an early decrease in the activities of the acid hydrolases, while testosterone propionate administration in female mice and orchiectomized male mice induced marked elevations in acid hydrolase activities in the aorta.

To assess the possibility that the testosterone propionate-induced increases in enzyme activities might be due to the removal of an inhibitor or the release of a stimulator, a mixing experiment was carried out. Cytochrome *c* oxidase, β-glucuronidase, hexosaminidase, β-galactosidase and arylsulphatase were assayed in total homogenates of aortas from orchiectomized controls (8 days postoperative) and testosterone propionate-treated (4 · 0.5 mg in 8 days) orchiectomized mice, and in mixtures of the two. The activities of cytochrome *c* oxidase and the four hydrolases were clearly additive (results not shown), indicating that the testosterone propionate-mediated increases in enzyme activities apparently reflect real differences in tissue enzyme content, and are not due to the effect of an enzyme stimulator or inhibitor.

Discussion

Our results demonstrate that testosterone exerts a trophic or anabolic effect on the mouse aorta, as evidenced by the testosterone propionate-induced accretion of RNA and protein. The total DNA of the aorta was essentially constant, indicating that the number of aortic cells was unchanged by testosterone propionate treatment. These findings suggest that androgen-mediated growth of the aorta in adult mice occurs by an accumulation of functional cytoplasm, i.e., by cellular hypertrophy, and not by hyperplasia.

In addition to stimulating the growth of the aorta, testosterone propionate induced a 42—103% increase in specific activity of cytochrome *c* oxidase, an inner mitochondrial membrane enzyme [17], without affecting monoamine oxidase, an outer mitochondrial membrane enzyme [17]. We have observed a similar testosterone propionate-induced increase in cytochrome *c* oxidase activity in mouse kidney [10], heart [18] and skeletal muscle [18,19]. Moreover, testosterone propionate administration induces striking changes in ultra structure of mitochondria in mouse kidney proximal tubules [10] and epithelial cells of rat ventral prostate [20]. It should be noted that several inner mitochondrial membrane proteins, including three of the seven subunits of cytochrome *c* oxidase, are synthesized on mitochondrial ribosomes under the direction of the mitochondrial genome, whereas synthesis of outer membranes components such as monoamine oxidase is carried out on cytoplasmic ribosomes programmed by the nuclear genome [21]. Therefore, androgens may regulate the rate of synthesis of cytochrome *c* oxidase, and possibly other proteins associated with the inner mitochondrial membrane, by a preferential action on the mitochondrial protein-synthesizing system in target cells in a manner similar to thyroid hormones [22]. An androgen-mediated increase in cytochrome *c* oxidase (and other inner membrane constituents?) would serve to augment the respiratory capacity of aortic mitochondria.

Testosterone treatment also induced large increases in specific activity of four hydrolases in the mouse aorta. Comparable increases in acid hydrolases have been observed in testosterone propionate-treated mouse kidney [9,10], heart [18] and skeletal muscle [18,19]. In several target tissues testosterone propionate decreases lysosomal enzyme latencies, destabilizes the lysosomal membrane [18,23], and induces ultrastructural changes in the lysosomal system, including an intensification of autophagy and an accumulation of hypertrophied lysosomes filled with layered, myelin-like membranes (myeloid bodies) [9,10,20]. It is likely, therefore, that the testosterone propionate-mediated increase in lysosomal enzyme activities in the aorta is associated with augmented activity of the lysosomal-vacuolar system in aortic cells.

The present study has disclosed a hitherto unrecognized sex difference in the specific activity of cytochrome *c* oxidase and several lysosomal hydrolases in the mouse aorta. Orchiectomy produced a decrease in cytochrome *c* oxidase and several lysosomal hydrolases in the mouse aorta, thereby abolishing this sex difference. These findings indicate that endogenous androgens exert a significant regulatory influence over the turnover of lysosomal enzymes and mitochondrial cytochrome c oxidase. In addition, endogenous androgens presumably regulate the turnover of RNA and protein in the aorta. Inasmuch as regulation of protein turnover may involve changes in either rates of protein synthesis or degradation or both, it will be important to assess the effect of testosterone on these metabolic parameters. An augmentation in lysosome-mediated protein degradation may prove to be a significant component of the androgenic response in the aorta, in view of the testosterone propionate-induced increase in activity of lysosomal hydrolases. Myocytes constitute the bulk of the cellular elements of the aorta and are likely to be the principal target cells of androgens, although endothelial cells and fibroblasts also may participate in the androgenic response. This inference is supported by the finding that myocytes of rat aorta possess specific androgen receptors detectible by radioautography (Sheridan, P., personal communication).

The androgen-mediated effects in the aorta reported in this communication may be of importance with respect to certain sex differences in arterial composition, metabolism, and function, such as collagen and elastin content [24], vasopressor response to norepinephrine [25,26], and vasodepressor response to arachidonic acid [26], as well as pathological processes that exhibit a male sex preference, e.g., coronary artery atherosclerosis in man [1], irreversible hypertension-induced medial hypertrophy of the aorta [27], spontaneous intimal lesions of the caudal artery [28], and arterial thrombogenesis in the rat [8].

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