Testosterone-Mediated Sexual Dimorphism of the Rodent Heart

Ventricular Lysosomes, Mitochondria, and Cell Growth are Modulated by Androgens

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SUMMARY. The ventricular myocardium was studied in A/J mice and in Sprague-Dawley rats. In male mice, the ventricles were slightly larger and the specific activities of the lysosomal hydrolases, β-glucuronidase, hexosaminidase, β-galactosidase, and arylsulphatase, and the inner mitochondrial enzyme cytochrome c oxidase were substantially higher than in female mice. Orchiectomy abolished this sex difference. Testosterone administration induced myocardial hypertrophy and accretion of RNA and protein without altering the DNA, and substantial increases in the activities of the lysosomal hydrolases and cytochrome c oxidase. However, the mitochondrial membrane enzyme monoamine oxidase was unaffected by sex, orchiectomy, and testosterone administration. Heart lysosomes from male mice showed a smaller structure-linked latency of the lysosomal enzymes and a greater fragility of the lysosomal membrane to osmotic and mechanical stress than those from female mice. This sex difference was also abolished by orchiectomy and restored by testosterone replacement. Similar sex differences were observed in the rat with respect to heart size, RNA and protein. In male mice, the activities of the lysosomal enzyme latency and membrane stability. These findings indicate that endogenous androgens regulate myocardial cell growth, the activity of enzymes associated with lysosomes and the inner mitochondrial membrane, and some physicochemical properties of lysosomes.

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HEART muscle cells possess specific androgen receptors and are potentially susceptible to the direct influence of circulating androgens (Krieg et al., 1978; McGill et al., 1980; McGill and Sheridan, 1981). However, this androgenic influence has not been characterized nor has its significance been assessed. We previously found a testosterone-mediated sexual dimorphism in ultrastructure of mouse kidney proximal tubules involving the lysosomes and mitochondria and the activities of several enzymes associated with these organelles in mouse kidney (Koenig et al., 1978; Koenig et al., 1980a), skeletal muscle (Koenig et al., 1980b), aorta (Goldstone et al., 1981), and brain (Koenig and Lu, 1980). We now report that the ventricular myocardium exhibits a vigorous response to testosterone administration which features hypertrophy, accretion of RNA and protein, and increased activities of mitochondrial cytochrome c oxidase and several lysosomal hydrolases. Furthermore, endogenous androgens exert a potent regulatory influence on the heart which is expressed as a sex difference in ventricle size and the activities of cytochrome c oxidase and lysosomal hydrolases. There is also a testosterone-mediated sex difference in heart lysosomes involving the latency of the hydrolytic enzymes and the stability of the lysosomal membrane. Some of these results have appeared in abstract form (Koenig and Goldstone, 1980).

Methods

Animal Experiments

Adult A/J mice (Jackson laboratory, Bar Harbor ME) and Sprague-Dawley rats (Holtzman, Madison WI) were used for these experiments. Male and female mice and rats of similar size and age were compared for sex differences. To study the effects of endogenous androgens, male mice and rats were orchiectomized through a scrotal incision under trichloroethylene anesthesia. For investigating the effects of exogenous testosterone, female mice or orchiectomized male mice were given testosterone propionate (TP) (Sigma Chemical Co.) (0.05-1.0 mg in 0.05 ml ethyl oleate) by subcutaneous injection every other day and killed by decapitation 24 hours after the fourth injection. Control animals were given ethyl oleate vehicle or no injection. Female rats were given TP (1 mg/100 g body weight in ethyl oleate) or vehicle according to the same treatment protocol. Hearts were quickly excised, the atria were removed and the ventricles were weighed and stored at −70°C.

Biochemical Assays

The frozen hearts were minced and homogenized in 9 volumes of 0.3 M sucrose. The homogenates were assayed for protein (Lowry et al., 1951), cytochrome c oxidase (EC 1.9.3.1) (Wharton and Tzagoloff, 1965), monoamine oxidase (EC 1.4.3.4) (Rosano and Jones, 1975), the lysosomal hydrolases 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) (Goldstone et al., 1973;
Patel and Koenig, 1976), RNA (Munro and Fleck, 1966), and DNA (Giles and Myers, 1965).

For lysosomal enzyme latency and membrane fragility determinations, mouse heart ventricles were rapidly excised and homogenized in 9 volumes of cold 0.3 M sucrose in a Potter-Elvehjem type glass homogenizer by one pass with a motor-driven Teflon pestle. Rat ventricles were cut into small pieces and homogenized in 9 volumes of cold 0.3 M sucrose by 4–5 passes against a rapidly rotating Teflon pestle. The homogenate was centrifuged at 1000 g for 10 minutes and the pellet was discarded. The supernatant was centrifuged at 16000 g for 20 minutes to deposit a mitochondrial-lysosomal (M-L) fraction, and the supernatant was centrifuged at 100,000 g for 60 minutes to give a soluble fraction. All procedures were done at 4°C. Aliquots of M-L fractions were resuspended in 0.3 M sucrose (for enzyme latency) and 0.05 M sucrose (for osmotic fragility) at 4°C. Free enzyme activities were assayed by incubating M-L fractions for 10 minutes at 37°C in the appropriate substrates. For hexosaminidase, the substrate was 3 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide, 80 mM sodium acetate buffer, pH 5.2, 300 mM sucrose (Goldstone et al., 1973). Total enzyme activity was assayed in the presence of 0.1% Triton X-100 to fully activate the latent enzymes. Free activity was expressed as percentage of total activity. Aliquots of soluble fractions and whole homogenates were assayed for 10 minutes at 37°C in the substrates with 0.1% Triton X-100. Soluble activities were expressed as percentage of the whole homogenate.

Enzyme substrates and Triton X-100 were supplied by Sigma Chemical Co. All other reagents were of the best analytical grade. Data were analyzed by Student’s t-test.

Results

The ventricles in male mice were slightly heavier than those in female mice and they revealed a greater specific activity of cytochrome c oxidase, an inner mitochondrial membrane enzyme. However, there was no sex difference in monoamine oxidase, an outer mitochondrial membrane enzyme (Table 1). Orchiectomy produced a decrease in ventricle weight, protein content, and cytochrome c oxidase activity that was already well developed 8 days postoperatively. Conversely, TP administration in female mice induced an increase in ventricle wet weight, protein content, and cytochrome c oxidase activity. TP also enhanced total ventricle RNA without affecting total DNA. TP administration in orchietomized mice induced a similar increase in RNA content.

### Table 1

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Experiment</th>
<th>Males, control</th>
<th>Males, orchietomized</th>
<th>Females, control</th>
<th>Females, TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (g)</td>
<td>A</td>
<td>23.5 ± 0.61 (7)</td>
<td>22.7 ± 0.33 (7)</td>
<td>18.19 ± 0.50 (10)</td>
<td>18.7 ± 0.7 (5)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>18.5 ± 1.02 (6)</td>
<td>21.2 ± 0.5 (8)</td>
<td>19.6 ± 0.9 (5)</td>
<td>20.7 ± 1.1 (5)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>22.8 ± 0.7 (8)</td>
<td>3.94 ± 0.04 (10)</td>
<td>22.7 ± 0.33 (7)</td>
<td>4.38 ± 0.06 (5)</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>4.20 ± 0.07 (7)</td>
<td>20.6 ± 1.3 (8)</td>
<td>3.72 ± 0.05 (6)</td>
<td>(93.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.97 ± 0.04 (8)</td>
<td>4.11 ± 0.07 (8)</td>
<td>4.11 ± 0.07 (8)</td>
<td>(110.5%)</td>
</tr>
<tr>
<td>Wet wt (mg/g body wt)</td>
<td>A</td>
<td>112.9 ± 2.4 (7)</td>
<td>110.6 ± 6.3 (7)</td>
<td>114.2 ± 5.1 (10)</td>
<td>115.8 ± 4.0 (5)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.92 ± 0.12 (5)</td>
<td>1.66 ± 0.09 (5)</td>
<td>1.92 ± 0.12 (5)</td>
<td>1.66 ± 0.09 (5)</td>
</tr>
<tr>
<td>RNA (µg/mg)</td>
<td>A</td>
<td>4.54 ± 0.32 (5)</td>
<td>5.50 ± 0.36 (5)</td>
<td>2.36 ± 0.06 (5)</td>
<td>3.27 ± 0.07 (5)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>3.26 ± 0.06 (5)</td>
<td>2.36 ± 0.06 (5)</td>
<td>2.36 ± 0.06 (5)</td>
<td>3.27 ± 0.07 (5)</td>
</tr>
<tr>
<td>Cytochrome c oxidase (units/mg)</td>
<td>A</td>
<td>1.55 ± 0.08 (5)</td>
<td>2.27 ± 0.22 (5)</td>
<td>2.27 ± 0.22 (5)</td>
<td>146.5%</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.81 ± 0.08 (6)</td>
<td>1.20 ± 0.09 (6)</td>
<td>1.81 ± 0.08 (6)</td>
<td>1.20 ± 0.09 (6)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.30 ± 0.08 (4)</td>
<td>1.04 ± 0.08 (4)</td>
<td>1.30 ± 0.08 (4)</td>
<td>1.04 ± 0.08 (4)</td>
</tr>
<tr>
<td>Monoamine oxidase (units/mg)</td>
<td>B</td>
<td>23.1 ± 2 (5)</td>
<td>24.1 ± 2.2 (5)</td>
<td>23.1 ± 2 (5)</td>
<td>24.1 ± 2.2 (5)</td>
</tr>
</tbody>
</table>

Data are means ± sem (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). Experiment A: Male mice were unoperated (control) or orchietomized 63 days before sacrifice. Female mice received four subcutaneous injections of testosterone propionate (TP) (1 mg/mouse in 0.05 ml ethyl oleate) by subcutaneous injection in 7 days and were killed 1 day later. Control females received ethyl oleate vehicle or no injection. Experiment B: Female mice received four injections of TP (0.5 mg/mouse in 0.05 ml ethyl oleate) or ethyl oleate vehicle in 7 days and were killed 1 day later. Experiment C: Male and female mice of similar age and weight were used without further treatment. Experiment D: Male mice were sham-operated (control) or orchietomized, and 24 hours later treatment was begun. Four doses of TP (100 µg/mouse in 0.05 ml ethyl oleate) or ethyl oleate vehicle were given by subcutaneous injection in 7 days and animals were killed on the 8th day. Intact male mice were also run. Enzyme units: cytochrome c oxidase, ΔA600/hour; monoamine oxidase, µg 4-hydroxyquinolone generated/hour. The numbers in parentheses are expressed as the percent of the corresponding controls, or of control males.
increase in ventricle weight and cytochrome c oxidase activity. Monoamine oxidase was unaffected by orchiectomy or TP.

The results for mouse ventricular lysosomal hydrolases are presented in Table 2. The specific activities of β-glucuronidase, hexosaminidase, β-galactosidase, and arylsulphatase were also significantly greater in ventricles from male mice than those from female mice. After orchiectomy, the activities of all four hydrolases decreased to the female levels, whereas TP administration in female mice or orchiectomized male mice produced the obverse effects. The decrease in acid hydrolase activities was also well developed 8 days post-orchiectomy.

In male rats the ventricle wet weight was somewhat greater than in female rats (Fig. 1). The specific activities of the lysosomal hydrolases β-glucuronidase, hexosaminidase, β-galactosidase, and arylsulphatase were all substantially greater in ventricles of male rats than in those of female rats (Fig. 2). Orchiectomy resulted in a significant decrease in ventricle wet weight and specific activities of three of four hydrolases. TP administration in female rats induced significant increases in ventricle weight and specific activities of all four lysosomal hydrolases.

To assess the possibility that the TP-induced increases in enzyme activities might be due to the removal of an inhibitor or the release of a stimulator, we performed a mixing experiment. Cytochrome c oxidase, β-glucuronidase, hexosaminidase, β-galactosidase, and arylsulphatase were all substantially greater in ventricles of male mice than those from female mice, heart lymphospectrometry of the ventricles from orchiectomized controls (8 days postoperative) and TP-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 TP-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.

Mice exhibit a significant sex difference in enzyme latency and membrane stability of heart lysosomes.

### Table 2: Effects of Sex, Orchiectomy, and Testosterone Administration on Lysosomal Hydrolases in Mouse Heart Ventricles

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Experiment</th>
<th>Males, control</th>
<th>Males, orchiectomized</th>
<th>Females, control</th>
<th>Females, TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Glucuronidase</td>
<td>A</td>
<td>74 ± 6 (7)</td>
<td>53 ± 6 (7)* (71.6%)</td>
<td>56.9 ± 4 (10)*</td>
<td>75.4 ± 7 (5)*</td>
</tr>
<tr>
<td>(nmol/hr per mg protein)</td>
<td>D</td>
<td>72 ± 6 (4)</td>
<td>55 ± 5 (4)* (76.4%)</td>
<td>(76.9%)</td>
<td>(132.5%)</td>
</tr>
<tr>
<td>Hexosaminidase</td>
<td>A</td>
<td>162 ± 7 (7)</td>
<td>117 ± 4 (7)* (72.2%)</td>
<td>136 ± 5 (10)*</td>
<td>199 ± 8 (5)*</td>
</tr>
<tr>
<td>(nmol/hr per mg protein)</td>
<td>D</td>
<td>207 ± 5 (4)</td>
<td>158 ± 9 (4)* (76.3%)</td>
<td>(84%)</td>
<td>(146.3%)</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>A</td>
<td>32 ± 1 (7)</td>
<td>23 ± 1 (7)* (71.9%)</td>
<td>26.5 ± 1 (10)*</td>
<td>38.2 ± 2 (5)*</td>
</tr>
<tr>
<td>(nmol/hr per mg protein)</td>
<td>D</td>
<td>164 ± 0.5 (7)</td>
<td>12.3 ± 0.4 (7)* (72.6%)</td>
<td>(72.6%)</td>
<td>(12.1%)</td>
</tr>
<tr>
<td>Arylsulphatase</td>
<td>A</td>
<td>164 ± 0.5 (7)</td>
<td>12.3 ± 0.4 (7)* (73%)</td>
<td>11.9 ± 0.5 (10)*</td>
<td>14.4 ± 0.7 (5)*</td>
</tr>
<tr>
<td>(nmol/hr per mg protein)</td>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SEM (number of animals): a, b, c: P < 0.05, 0.01, 0.001 (treated vs. corresponding controls). e, f: P < 0.05, 0.01, 0.001 (females, control vs. males, control). Experimental details are given in Table 1, and in the text.
changes in hexosaminidase latency and osmotic sensitivity of heart lysosomes. These data show that and testosterone replacement (50 μg in 0.05 ml ethyl oleate subcut.) or ethyl oleate vehicle (OR) every other day for four doses. The hearts were excised 24 hours after the last injection. The results are means ± SEM (n = 3).

Discussion

Our findings show that testosterone exerts a potent anabolic effect on the ventricular myocardium of the adult mouse. Thus, a short period of TP administration produced a moderate increment in ventricle wet weight and total protein and a larger increase in total RNA. The total ventricular DNA remained constant during this period, indicating that the number of myocytes was unchanged by androgen treatment. Therefore androgen-induced growth of the myocardium occurs by an accumulation of functional cytoplasm, i.e., by myocellular hypertrophy, and not by hyperplasia.

In addition to stimulating ventricle growth, testosterone increased the specific activity of cytochrome c oxidase, an inner mitochondrial membrane enzyme (Schnaitman and Grunwalt, 1968), and induced an even greater increase in the total ventricle cytochrome c oxidase activity. In contrast, monoamine oxidase, an outer mitochondrial membrane marker (Schnaitman and Grunwalt, 1968), was unaltered by hormone treat-
ment. TP treatment also decreased the equilibrium density of ventricular mitochondria on density gradient centrifugation, suggesting that androgens produce structural alterations in these organelles (unpublished observations). In mouse kidney, TP administration induces an increase in cytochrome c oxidase activity without affecting monoamine oxidase, reduces the equilibrium density of mitochondria, and induces striking changes in ultrastructure of mitochondria in the proximal tubules, notably, an increased mitochondrial volume, greater prominence of the inner membrane and decreased electron density of the matrix (Koenig et al., 1980a; and in preparation). It is pertinent to note in this context 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 membrane components such as monoamine oxidase is carried out on cytoplasmic ribosomes programmed by the nuclear genome (Tzagoloff et al., 1978). 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 (Bouhnik et al., 1979). An androgen-induced increase in cytochrome c oxidase, and possibly other inner membrane constituents, would serve to augment the respiratory capacity of heart mitochondria.

Finally, TP treatment induced substantial increases in specific and total activities of four lysosomal hydrolases in the ventricular myocardium. Testosterone administration also decreased the enzyme latency and stability of heart lysosomes and reduced their equilibrium densities, and these effects could be demonstrated within 1 hour after hormone administration (unpublished observations). Similar alterations occur in TP-treated mouse kidney (Koenig et al., 1980c; and in preparation) and are associated with ultrastructural changes in the lysosomal-vacuolar system of proximal tubules, including an increase in autophagy, an accumulation of enlarged lysosomes filled with myelin-like membranes (myeloid bodies), and augmented exocytosis of lysosomes into the tubule lumen resulting in a striking increase in lysosomal enzymuria and proteinuria (Koenig et al., 1978, 1980a).

Our studies have revealed a previously unrecognized sexual dimorphism involving the specific activities of cytochrome c oxidase and several acid hydrolases in mouse ventricular myocardium. Orchietomy produces a loss in ventricle bulk, total protein, cytochrome c oxidase, and lysosomal hydrolases, thereby abolishing this sexual dimorphism, whereas testosterone replacement attenuates or abolishes this loss. In addition, our data have disclosed a sex difference in mouse heart lysosomes with respect to the latency of the contained enzymes and the osmotic and mechanical stability of the lysosomal membrane, as well as the equilibrium density of the lysosomes (unpublished findings), and this sex difference is also abolished by orchietomy and restored by a replacement dose of TP. These findings establish that the sexual dimorphism of the mouse heart is mediated by endogenous androgens. The rat heart displays an analogous sexual dimorphism in ventricle size and acid hydrolase activities, and in the enzyme latency and membrane stability of isolated lysosomes, which also appears to be dependent on endogenous androgens. In mouse kidney, these indices of testosterone-induced lysosomal "destabilization" have been found to reflect the properties of a lysosomal population of low equilibrium density ($p \sim 1.125$) consisting largely of autolysosomes (autophagic vacuoles) and heterolysosomes (phagolysosomes resulting from merger of pinocytic vacuoles and lysosomes) that is expanded at the expense of the classical "dense body" lysosomes comprising the major high-density lysosomal fraction ($p \sim 1.18$) (unpublished findings). It seems likely, therefore, that testosterone enhances autophagy, and possibly pinocytosis to some extent, in ventricular myocytes.

It is clear from the present findings that endogenous androgens exert an important regulatory influence over the metabolism of the ventricular myocardium. Thus androgens would appear to merit inclusion among those hormones, e.g., growth hormone, thyroid hormone, insulin, which regulate protein turnover in the heart (Hjalmarson et al., 1975; Rannels et al., 1977). Since regulation can involve changes in either rates of protein synthesis or degradation, or both, it will be important to determine the effects of androgens on these metabolic parameters. In light of the androgen-induced increase in lysosomal hydrolases and the associated decrease in enzyme latency, membrane stability, and equilibrium density of heart lysosomes, an acceleration of lysosome-mediated protein degradation (catabolism) may prove to be a significant feature of the androgenic response in the ventricular myocardium.

The androgen-mediated effects in the ventricular myocardium described in this report may be important in relation to certain sex differences in cardiac function and metabolism, e.g., norepinephrine uptake by heart muscle (Salt, 1972), norepinephrine-induced vasopressor response (Bhargava et al., 1967; Baker et al., 1978), arachidonic acid-induced vasodepressor effect (Baker et al., 1978), as well as pathophysiological processes exhibiting a preference for the male sex, such as right ventricular hypertrophy produced by chronic hypoxia (Moore et al., 1978), isoproterenol-induced myocardial necrosis (Wexler and Greenberg, 1979), and myocardial infarction and sudden death associated with coronary artery atherosclerosis (McGill and Stern, 1979).

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