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Androgens regulate mitochondrial cytochrome *c* oxidase and lysosomal hydrolases in mouse skeletal muscle

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The gastrocnemius, a fast-twitch white muscle, and the soleus, a slow-twitch red muscle, were studied in A/J mice. The specific activities of the lysosomal hydrolases, β-D-glucuronidase, hexosaminidase, β-D-galactosidase and arylsulphatase, the inner-mitochondrial-membrane enzyme cytochrome *c* oxidase, and the outer-mitochondrial-membrane enzyme monoamine oxidase, were greater in the soleus than in the gastrocnemius. The specific activities of the lysosomal hydrolases and cytochrome *c* oxidase in the gastrocnemius and soleus were substantially higher in male mice than in female mice. Orchiectomy abolished this sex difference. Testosterone increased the activities of the lysosomal hydrolases and cytochrome *c* oxidase and coincidentally induced muscle hypertrophy and an accretion of protein and RNA, but total DNA remained constant. Monoamine oxidase was unaffected by sex, orchiectomy and testosterone. These findings indicate that endogenous androgens regulate the activity of enzymes associated with lysosomes and the inner mitochondrial membrane, as well as muscle fibre growth in mouse skeletal muscle.

Testosterone and related androgens have a well established anabolic or growth-promoting effect in skeletal muscle (Kochakian, 1959, 1975; Mayer & Rosen, 1977). This anabolic response presumably is mediated by specific androgen receptors present in skeletal muscle (Michel & Baulieu, 1974; Dubé *et al.*, 1976; Krieg, 1976), and involves an increase in the activity of ribosomes, RNA polymerase and template activity of chromatin DNA (Breuer & Florini, 1965, 1966). Androgen-induced muscle growth apparently reflects a simple hypertrophy of muscle fibres, as there is no major change in structure (Venable, 1966) or composition of skeletal muscle (Kochakian, 1959), and the pattern of synthesis of muscle proteins is qualitatively unaffected (Florini, 1970).

We noted a testosterone-dependent sex difference in ultrastructure of mouse kidney proximal tubules involving lysosomes and mitochondria and the tissue activities of several enzymes associated with these organelles (Koenig *et al.*, 1978, 1980*a*). These observations prompted a search for similar testosterone-mediated effects in mouse skeletal muscle. We now report that the anabolic response of the mouse gastrocnemius and soleus to testosterone is accompanied by a striking increase in the activity of mitochondrial cytochrome *c* oxidase and four lysosomal hydrolases. Furthermore endogenous androgens exert a regulatory influence over these muscles which is expressed as a sex difference in muscle size and the activity of lysosomal hydrolases and cytochrome *c* oxidase. A brief report of a portion of this work has appeared in abstract form (Koenig & Goldstone, 1980).

Experimental

Animal experiments

Young adult A/J mice (Jackson Laboratory, Bar Harbor, ME, U.S.A.) weighing 17–23 g were used. Male and female mice of similar size and age were compared for sex differences. To evaluate the effects of endogenous androgens, male mice were orchiectomized through a scrotal incision under trichloroethylene anaesthesia and killed 8 or 63 days later. For investigating the effects of exogenous testosterone, female or orchiectomized male mice were given testosterone propionate (Sigma Chemical Co., St. Louis, MO, U.S.A.) (0.5 or 1.0 mg in 0.05 ml of ethyl oleate) by subcutaneous injection every other day and killed by decapitation 24 h after the fourth injection. Control animals were given ethyl oleate vehicle or no injections. The gastrocnemius, a fast-twitch white muscle, and the soleus, a slow-

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twitch red muscle, were excised, weighed and stored at −70°C.

Biochemical assays

The frozen muscle tissues were minced and homogenized in cold 0.3 M-sucrose (1 ml for gastrocnemius, 0.6 ml for soleus). Protein was determined by the method of Lowry *et al.* (1951), RNA by u.v. spectrophotometry (Munro & Fleck, 1966) and DNA by the method of Giles & Myers (1965). β-D-Glucuronidase (EC 3.2.1.31) was assayed with phenolphthalein glucuronic acid and arylsulphatase (EC 3.1.6.1) with *p*-nitrocatechol sulphate as substrates, as described previously (Goldstone *et al.*, 1973). Hexosaminidase (β-*N*-acetylhexosaminidase, EC 3.2.1.30) was assayed with *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide, and β-D-galactosidase (EC 3.2.1.23) with *p*-nitrophenyl β-D-galactopyranoside as substrates as described by Patel & Koenig (1976). Enzyme units are μmol of substrate cleaved/h. Cytochrome *c* oxidase (EC 1.9.3.1) was assayed by the method of Wharton & Tzagaloff (1965) and monoamine oxidase (EC 1.4.3.4) as described by Rosano & Jones (1976).

Maximum enzyme activities were obtained under these assay conditions owing to the disruptive effects of freeze-thawing and the hypo-osmoticity of the enzyme substrates on the lysosomal and mitochondrial membranes. Enzyme units are A_{550}/min for cytochrome *c* oxidase and μg of 4-hydroxyquinolone cleaved/h for monoamine oxidase.

Results

It can be seen in Table 1 that the gastrocnemius in male mice was heavier, contained more protein and displayed a higher specific activity of cytochrome *c* oxidase than that of female mice. Long term orchiectomy (63 days postoperative, Expt. A) produced a decrease in wet weight and protein content, whereas testosterone propionate in female mice or acutely orchiectomized mice induced an

Table 1. *Effects of sex, orchiectomy and testosterone on wet weight, protein, DNA, RNA, cytochrome c oxidase and monoamine oxidase of mouse gastrocnemius*

Expt. A. Male mice were unoperated (control) or orchiectomized 63 days before being killed. Female mice received testosterone propionate (1 mg/mouse in 0.05 ml of ethyl oleate) by subcutaneous injection every other day and killed 24 h after the fourth injection. Control females received ethyl oleate vehicle. Expt. B. Female mice received testosterone propionate (0.5 mg/mouse) or ethyl oleate vehicle only every other day and were 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 testosterone propionate (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. Results are means ± S.E.M. (number of animals in parentheses). * $P<0.05$; ** $P<0.01$ (treated versus corresponding controls). † $P<0.05$; ‡ $P<0.01$ (females, control versus males, control). Significance was determined by Student's *t* test. TP, Testosterone propionate.

	Expt.	Males, control	Males, orchiectomized	Males, orchiectomized, TP	Females, control	Females, TP
Wet weight (mg/g body weight)	A	4.96 ± 0.15 (7)	4.28 ± 0.14 (7)** (86.3%)	—	4.32 ± 0.06 (10)‡ (87.1%)	4.85 ± 0.10 (5)** (112.3%)
	D	5.08 ± 0.19 (4)	4.82 ± 0.10 (5)	5.25 ± 0.1 (5)* (108.9%)	—	—
Protein (mg/muscle)	A	9.22 ± 0.50 (7)	8.86 ± 0.38 (7)	—	7.95 ± 0.27 (10)† (86.2%)	8.78 ± 0.44 (5)* (110.4%)
	D	8.21 ± 0.33 (4)	7.60 ± 0.42 (5)	8.82 ± 0.34 (5)* (116.1%)	—	—
DNA (μg/muscle)	B	—	—	—	12.8 ± 0.9 (5)	12.9 ± 1.3 (5)
RNA (μg/muscle)	B	—	—	—	27.9 ± 2.8 (5)	46.7 ± 7.1 (5)* (167.4%)
(μg/μg of DNA)	B	—	—	—	2.19 ± 0.13 (5)	3.63 ± 0.30 (5)** (165.8%)
Cytochrome *c* oxidase (units/mg of protein)	B	—	—	—	1.19 ± 0.19 (5)	1.76 ± 0.17 (5)* (147.9%)
	C	2.53 ± 0.13 (6)	—	—	1.95 ± 0.07 (6)‡ (77.1%)	—
	D	3.15 ± 0.36 (4)	2.99 ± 0.28 (5)	4.33 ± 0.45 (5)* (144.8%)	—	—
Monoamine oxidase (units/mg of protein)	C	12.6 ± 0.81 (6)	—	—	12.1 ± 0.55 (6)	—
	D	16.5 ± 0.88 (5)	16.7 ± 0.82 (5)	14.5 ± 0.61 (5)	—	—

increase in wet weight, protein and cytochrome *c* oxidase activity of the gastrocnemius. In contrast, monoamine oxidase was unaffected by sex, orchiectomy or testosterone propionate. Testosterone propionate also induced an increase in total RNA without altering total DNA in the gastrocnemius.

The specfic activity of cytochrome *c* oxidase in the soleus muscle was also greater in male mice than in female mice, although the specific activity of monoamine oxidase was similar in the two sexes (Table 2). Orchiectomy produced a decrease in cytochrome *c* oxidase activity, and testosterone propionate in female and orchiectomized male mice induced an increase in cytochrome *c* activity in the soleus. However, monoamine oxidase activity in the soleus was unaffected by these treatments. Monoamine oxidase, and to a lesser extent cytochrome *c* oxidase, were higher in the soleus than in the gastrocnemius. Testosterone propionate in female mice also increased the concentration of RNA in the soleus without affecting the total DNA.

Fig. 1 (*a*, *b*, *c*) presents the results on the lysosomal hydrolases of the gastrocnemius and soleus muscles. It is noteworthy that the specific activities of the acid hydrolases were 2- to 5-fold greater in the soleus than in the gastrocnemius. The specific activity of β-D-glucuronidase, hexosaminidase and β-D-glactosidase in both muscles was greater in male mice than in female mice, but no sex difference was observed for arylsulphatase (Fig. 1*a*). The activities of all four hydrolases in the gastrocnemius and soleus decreased after long term orchiectomy, and increased after testosterone propionate (Fig. 1*b*, *c*). The decrease in β-D-glucuronidase, hexosaminidase and arylsulphatase activity was already maximal 8 days after orchiectomy (results not shown).

A mixing experiment was performed to evaluate the possibility that the testosterone propionate-induced increases in enzyme activities might result from the removal of an inhibitor or the release of an activator. Cytochrome *c* oxidase, β-D-glucuronidase, hexosaminidase, β-D-galactosidase and arylsulphatase were assayed in homogenates of the gastrocnemius muscles from orchiectomized controls (8 days postoperative) and those given testosterone propionate (4 × 0.5 mg in 8 days), and in mixtures of the two. The activities of cytochrome *c* oxidase and of the four hydrolases were clearly additive (results not shown). These results suggest that testosterone-propionate-induced increases in enzyme activities reflect real differences in tissue enzyme content, and are not due to the effect of an enzyme inhibitor or activator.

Discussion

The present study indicates that mouse soleus, a red muscle, displays specific activities of lysosomal and mitochondrial enzymes higher than those obtained in the gastrocnemius, a white muscle. Similar observations have been made in rat (Wallace & Lewis, 1975; Koenig & Goldstone, 1980) and avian red and white skeletal muscle (Stauber & Schottelius, 1975). Our findings confirm that testosterone exerts a potent anabolic action on mouse skeletal muscle, as evidenced by the

Table 2. *Effects of sex, orchiectomy and testosterone on DNA, RNA, cytochrome c oxidase and monoamine oxidase of mouse soleus*

Experimental details are as given in Table 1. Results are means ± S.E.M. (number of animals in parentheses). $^{*}P<0.05$; $^{**}P<0.01$ (treated versus corresponding controls). †$P<0.05$ (females, control versus males, control). Significance was determined by Student's *t* test. TP, Testosterone propionate.

	Expt.	Male, control	Male, orchiectomized	Male, orchiectomized, TP	Female, control	Female, TP
DNA (μg/muscle)	B	—	—	—	3.30 ± 0.32 (5)	3.40 ± 0.48 (5)
RNA (μg/muscle)	B	—	—	—	7.01 ± 0.53 (5)	11.17 ± 1.13 (5)** (159.3%)
(μg/μg of DNA)	B	—	—	—	2.16 ± 0.16 (5)	3.11 ± 0.26 (5)** (144%)
Cytochrome *c* oxidase (units/mg of protein)	B	—	—	—	2.45 ± 0.11 (5)	3.17 ± 0.28 (5)* (129.4%)
	C	4.25 ± 0.16 (6)	—	—	3.51 ± 0.26 (6)† (82.6%)	—
	D	2.61 ± 0.1 (4)	2.25 ± 0.1 (5)* (86.2%)	3.13 ± 0.18 (5)* (139.1%)	—	—
Monoamine oxidase (units/mg of protein)	C	36 ± 0.9 (6)	—	—	37.8 ± 1.8 (6)	—
	D	41.8 ± 3.0 (5)	43.1 ± 2.4 (5)	44.0 ± 2.7 (5)	—	—

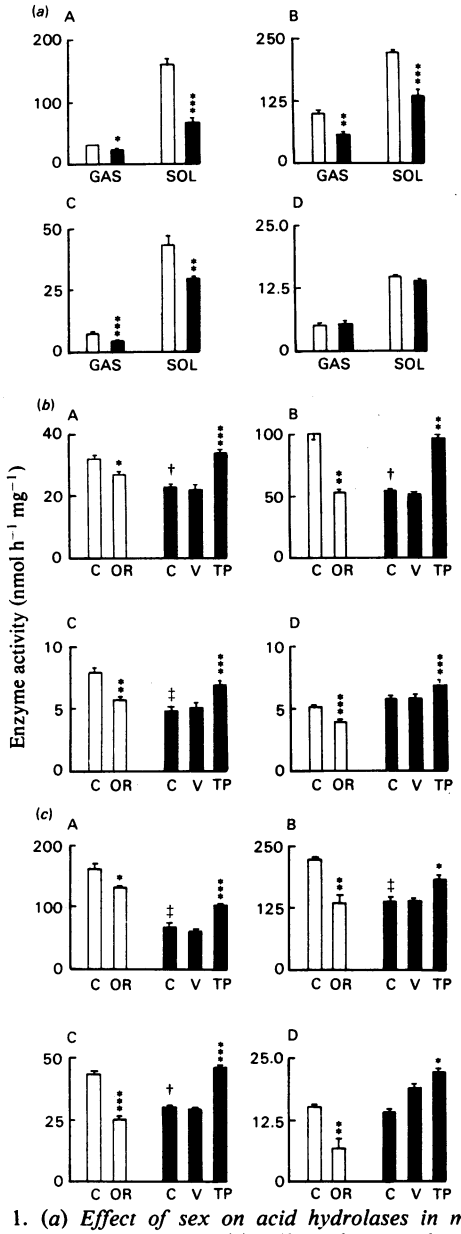

Fig. 1. (*a*) *Effect of sex on acid hydrolases in mouse gastrocnemius and soleus.* (*b*) *Effect of sex, orchiectomy and testosterone on acid hydrolases in mouse gastrocnemius.* (*c*) *Effect of sex, orchiectomy and testosterone on acid hydrolases in mouse soleus*

Experimental details are as given for Expt. A in legend for Table 1. Abbreviations used: GAS, gastrocnemius; SOL, soleus; C, untreated control; OR, orchiectomized (63 days postoperatively); V, ethyl oleate vehicle, control; TP, testosterone propionate. Males, (□); females, (■). Results are means ± S.E.M. ($n = 7$–10), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (versus corresponding control); †$P < 0.01$, ‡$P < 0.001$ (versus control males). A, β-D-Glucuronidase; B, hexosaminidase; C, β-D-galactosidase; D, arylsulphatase.

testosterone propionate-induced accretion of RNA, protein and wet weight. The total DNA of the muscle was constant, indicating that the number of muscle nuclei was unchanged by testosterone proprionate. These findings are consistent with the view that androgen-induced growth of skeletal muscle in adult mice occurs by an accumulation of cytoplasm, i.e. by myocellular hypertrophy, and not by hyperplasia.

Of particular interest is our observation that testosterone propionate induced a substantial increase in specific activity of cytochrome *c* oxidase, an inner-mitochondrial-membrane enzyme (Schnaitman & Greenwalt, 1968), in both the gastrocnemius and the soleus without affecting monoamine oxidase, an outer-mitochondrial-membrane enzyme (Schnaitman & Greenwalt, 1968). We have observed a similar testosterone propionate-mediated increment in cytochrome *c* oxidase activity in mouse kidney (Koenig *et al.*, 1980*a*), heart (Koenig & Goldstone, 1980; H. Koenig & A. Goldstone, unpublished work), and aorta (A. Goldstone, H. Koenig & C. Y. Lu, unpublished work). Further, testosterone propionate induces dramatic alterations in the fine structure of mitochondria in mouse kidney proximal tubules (Koenig *et al.*, 1980*a*) and epithelial cells of rat ventral prostate (Koenig *et al.*, 1979). It is noteworthy that several inner-mitochondrial-membrane proteins, including three of the seven subunits of cytochrome *c* oxidase, are synthesized on mitochondrial ribosomes directed by the mitochondrial DNA, whereas synthesis of outer membrane proteins such as monoamine oxidase is carried out on cytoplasmic ribosomes under the direction of the nuclear genome (Tzagoloff *et al.*, 1979). it seems reasonable to suggest that androgens may regulate the rate of synthesis of cytochrome *c* oxidase, and possibly other inner-membrane proteins, by a preferential action on the mitochondrial protein-synthesizing system in a manner similar to thyroid hormones (Bouhnik *et al.*, 1979). An androgen-mediated increase in cytochrome *c* oxidase, and possibly other inner-membrane constituents, would serve to enhance the respiratory capacity of muscle mitochondria.

Testosterone also elicited striking increments in the total specific activity of four lysosomal hydrolases in the mouse gastrocnemius and soleus. Free activities of the hydrolases were not determined in this study. We have observed similar increases in lysosomal hydrolases in kidney of mice given testosterone propionate (Koenig *et al.*, 1978, 1980*a*), heart (Koenig & Goldstone, 1980; H. Koenig & A. Goldstone, unpublished work), and aorta (A. Goldstone, H. Koenig & C. Y. Lu, unpublished work). In several target organs testosterone propionate reduces lysosomal enzyme

latencies, labilizes the lysosomal membrane (Koenig & Goldstone, 1980; Koenig *et al.*, 1979; 1980*b*), and induces ultrastructural alterations in the lysosomal-vacuolar system, including an increase in autophagy and an accumulation of enlarged lysosomes filled with myelin-like membrane arrays (Koenig *et al.*, 1978, 1979, 1980*a*). The testosterone propionate-induced elevation of lysosomal hydrolase activities in skeletal muscle is thus likely to be related to an enhanced activity of the lysosomal-vacuolar system of muscle fibres.

This study has revealed a previously unrecognized sex difference in specific activity of cytochrome *c* oxidase and three of the four lysosomal enzymes in mouse gastrocnemius and soleus muscles. Orchiectomy decreased these enzyme activities and largely abolished the sex difference, whereas testosterone restored these activities. We do not know why arylsulphatase showed no sex difference even though it responded to orchiectomy and testosterone treatment. These findings establish that endogenous androgens regulate the tissue levels of lysosomal enzymes and mitochondrial cytochrome *c* oxidase. The testosterone-induced increase in lysosomal enzyme activities suggests that an enhancement in lysosome-mediated protein degradation (catabolism) may be an important concomitant of the anabolic response of skeletal muscle to androgens.

The androgen-mediated actions in skeletal muscle described in this report may be of significance in relation to sex differences in muscle strength and metabolism and the mechanism of action of anabolic androgenic steroids on skeletal muscle. Androgenic effects also may be implicated in the abnormal exercise-induced increase in serum creatine kinase which occurs in healthy men, but not women (Shumate *et al.*, 1979), and in other pathophysiological processes exhibiting a male sex preference.

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