Effects of castration and androgen treatment on androgen-receptor levels in rat skeletal muscles

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Effects of castration and androgen treatment on androgen-receptor levels in rat skeletal muscles. J. Appl. Physiol. 87(6): 2016–2019, 1999.—The effects of castration and dihydrotestosterone (DHT) treatment on levels of skeletal muscle androgen receptor (AR) were examined in three groups of adult male rats: 1) intact normal rats, 2) rats castrated at 16 wk of age, and 3) rats castrated at 16 wk of age and given DHT for 1 wk starting at week 17. All animals were killed at 18 wk of age. Castration caused a decrease (P < 0.05) in the weights of the levator ani and bulbo cavernosus muscles. The administration of DHT to the castrated rats increased (P < 0.05) the weights of the levator ani and bulbo cavernosus muscles. Castration caused a significant downregulation of AR levels in the bulbo cavernosus (P < 0.05) but had no significant effect on AR levels in the levator ani muscle. DHT administration to the castrated group upregulated AR levels in the bulbo cavernosus and levator ani muscles. The plantaris muscle did not significantly (P > 0.05) change for any of the treatments. These findings suggest that the effects of castration and androgen replacement differentially affect skeletal muscle mass and AR levels.

IN ADDITION TO THEIR VIRILIZING effects in the reproductive tracts of males, androgens have anabolic effects in some skeletal muscles, but the mechanism of androgen control of muscle size is poorly understood (5, 23). In adult animals, castration produces atrophy of the rat levator ani and bulbo cavernosus muscles, frog flexor carpi radialis and flexor carpi centralis muscles, and several guinea pig muscles, such as the sternomastoid, spinotrapezius, and latissimus dorsi muscles, whereas the readministration of androgenic hormones to castrated animals results in hypertrophy of these muscles (5, 11, 16, 20). Electrical stimulation of rat gastrocnemius muscle results in an increase in the number of androgen receptors (ARs), whereas the administration of an androgen antagonist suppresses the hypertrophy seen in exercised gastrocnemius muscle (7, 8). As recently demonstrated in men, the administration of supraphysiological amounts of androgenic steroids (600 mg/wk testosterone enanthate) for 10 wk resulted in an increase in skeletal muscle mass and strength (3). In addition to animal data (5, 11, 16, 20), it is likely that the anabolic effects of androgens are directed by the same receptor that mediates androgen action in the urogenital tract. Furthermore, Kreig et al. (12) have also reported that the number of ARs varies among skeletal muscles, which may explain the regional differences in the physiological response to androgen administration.

The purpose of this study, therefore, was to assess the effects of castration and androgen replacement on AR levels in the rat bulbocavernosus, levator ani, and plantaris muscles. These findings have been reported in abstract form (1).

METHODS

Animals and hormonal manipulations. Eighteen 16-wk-old male Holtzman/Sprague-Dawley rats were obtained from Harlan Laboratories (Indianapolis, IN). The animals were housed in the animal facilities of the University of Texas Southwestern Medical Center under a 12:12-h light-dark cycle with free access to standard laboratory chow and water. Animal care and use protocols were approved by the institutional review board of the University of Texas Southwestern Medical Center and were in accordance with the guidelines of the National Institutes of Health.

Rats were divided into three groups of six animals each as follows: 1) intact normal (N), 2) castrated (C), and 3) castrated plus dihydrotestosterone (C+DHT). The rats in C and C+DHT groups were castrated on week 16. Rats in the C+DHT group were given injections subcutaneously (DHT: 50 mg·kg⁻¹·day⁻¹ for 7 days) commencing on week 17. All animals were killed at 18 wk of age.

Tissue extraction. Animals were killed with an overdose of ether. The prostate gland and three skeletal muscles (levator ani, bulbocavernosus, and plantaris) were immediately removed, weighed, and minced. Tissue fragments were suspended (0.025–0.1 g/0.9 ml) in buffer containing 110 mM SDS, 100 mM dithiothreitol, 80 mM Tris (pH 6.9), 0.002% bromphenol blue, and 10% glycerol and were homogenized on ice in an all-glass Dounce homogenizer (10 strokes of a loose pestle, followed by 20 strokes of a tight pestle). The homogenate was then sonicated (at setting 7; model W350, Branson) for 10 s, boiled for 5 min, and centrifuged at 200,000 g for 30 min. Aliquots of the supernatant were used immediately for Western blotting or were stored at −80°C and thawed only once.

Immunoblot assay. Extracts were boiled for 3 min immediately before loading on the gel. Aliquots (15–50 µg protein) of each sample were applied to 1.5-mm × 20-cm × 20-cm 8% polyacrylamide gels containing 3.5 mM SDS and 0.2% N,N'-methylene-bis-acrylamide (2).

Electrophoresis was performed at room temperature at 20 mA per gel until the bromphenol blue dye front reached the bottom of the running gel. Proteins were then transferred to nitrocellulose membrane filters by using a Hoefer Transphor at 25 V in a buffer containing 192 mM glycine, 25 mM Tris, and 20% (vol/vol) methanol at room temperature for ~12 h.
Differences in androgen-receptor concentrations were established by competition studies in which the affinity-purified U402 antibodies were preabsorbed with excess synthetic NH₂-terminal 21-amino-acid peptide. The amount of immunoreactive AR protein was visualized as previously described (24) by incubating the nitrocellulose filters with affinity-purified antibodies directed against the NH₂-terminal 21-amino-acid sequence of the human AR (rabbit U402), followed by incubation with ¹²⁵I-labeled goat anti-rabbit IgG, F(ab')₂. Immunooblots were exposed to Kodak XAR2 film at −80°C for 1–7 days. The specificity of the method was validated by competition studies in which the affinity-purified U402 antibodies were preabsorbed with excess synthetic NH₂-terminal 21-amino-acid peptide. The amount of AR present in each tissue extract was estimated by comparing the immunodetectable AR in each tissue to a standard curve developed from a prostate preparation of multiple concentrations that were run on the same gel. This standard preparation was prepared at the start of the study and was stable for at least 3 mo when it was stored in aliquots at −80°C and was shown to be linear over a 10-fold range (15–150 µg protein). The sizes of the visualized proteins were inferred from the position of radiolabeled molecular weight standards (Rainbow Markers, Amersham, Arlington Heights, IL) included on each gel. The apparent molecular ratio of the major AR band of 110,000 was consistent with a previous report (24). A computing densitometer (model 300A, Molecular Dynamics, Sunnyvale, CA) was used to measure the density of 110,000-molecular-ratio AR bands visualized by autoradiography. Levels of immunoreactive AR protein in the various muscles were expressed as density units per microgram protein and are shown as a percentage of the amount measured in the protein standard. Protein levels in tissue extracts were measured by the method of Lowry after precipitation with 10% (wt/vol) trichloroacetic acid in the presence of 0.8 mM sodium deoxycholate.

Statistics. Descriptive statistics include means ± SE. A one-way ANOVA was used to determine significance. If an F ratio was found to be significant, all pairwise multiple comparisons were made via the Student-Newman-Keuls method. P < 0.05 was selected to indicate statistical significance.

RESULTS

Body weight. There were no significant differences in body weight among groups after treatment (P > 0.05; N: 489.0 ± 10.0 (SE) g; C: 456.7 ± 13.4 g; C + DHT: 486.0 ± 13.2 g).

Muscle weights. Castration for 2 wk caused a significant decrease in the weight of the levator ani and bulbocavernosus muscles (and in the weight of the ventral prostate) but had no effect on the weight of the plantaris muscle (Table 1). Similarly, treatment of the C + DHT group for 1 wk with DHT resulted in growth of the bulbocavernosus and levator ani muscles back to the normal range but had no effect on the weight of the plantaris muscle (Table 1).

AR levels. In the N male animals, AR levels were approximately threefold higher in the bulbocavernosus and levator ani muscles than in the plantaris muscle (Fig. 1 and Table 2). Castration caused a significant (P < 0.05) decrease in the AR level in the bulbocavernosus muscles, an average 51% decline (P = 0.09) in the levator ani muscle, and a nonsignificant (P > 0.05) decrease in the plantaris muscle. However, the administration of DHT to the C + DHT group caused a significant rise in AR levels (compared with those in the C group) in both the levator ani and bulbocavernosus muscles and a nonsignificant (P > 0.05) increase in the AR level in the plantaris muscle (Table 2).

DISCUSSION

The response of skeletal muscles to androgen deprivation and/or administration is muscle and species specific. For example, the flexor carpi radialis muscle of the male frog Xenopus laevis atrophies in response to castration and returns to normal size within 7 days of testosterone administration (6), whereas these procedures have no discernible effect in the sartorius muscle of the same species. Similarly, in the guinea pig the temporalis muscle is highly sensitive to androgens (14). The temporalis muscle of the female guinea pig contains type IIa fibers predominantly, whereas in the male, testosterone causes hypertrophy of the muscle and an increase in type IIb fibers (9, 14). It is believed that the pectoral and shoulder girdle muscles are the most androgen-dependent muscles in humans (23). In the rat, the bulbocavernosus and levator ani muscles, which play a major role in copulation and/or ejaculation (4), are known to be particularly sensitive

Table 1. Prostate and skeletal muscle wet weights

<table>
<thead>
<tr>
<th>Muscle</th>
<th>N, mg</th>
<th>C, mg</th>
<th>% change from N</th>
<th>C + DHT, mg</th>
<th>% change from C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>128 ± 12</td>
<td>22 ± 3*</td>
<td>-83</td>
<td>101 ± 6†</td>
<td>+359</td>
</tr>
<tr>
<td>Levator ani muscle</td>
<td>61 ± 4</td>
<td>50 ± 4*</td>
<td>-51</td>
<td>58 ± 6†</td>
<td>+93</td>
</tr>
<tr>
<td>Bulbocavernosus muscle</td>
<td>201 ± 13</td>
<td>148 ± 8*</td>
<td>-26</td>
<td>214 ± 7†</td>
<td>+45</td>
</tr>
<tr>
<td>Plantaris muscle</td>
<td>91 ± 9</td>
<td>102 ± 3</td>
<td>+12</td>
<td>101 ± 4</td>
<td>-1.0</td>
</tr>
</tbody>
</table>

Data are means ± SE. N, normal intact rats; C, castrated rats; C + DHT, castrated plus dihydrotestosterone-treated rats. Significant differences (P < 0.05) * between C and N and † between C + DHT and C.

Table 2. Androgen-receptor concentrations in skeletal muscles

<table>
<thead>
<tr>
<th>Muscle</th>
<th>N, DU</th>
<th>C, DU</th>
<th>% change from N</th>
<th>C + DHT, DU</th>
<th>% change from C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levator ani</td>
<td>5.25 ± 1.20</td>
<td>2.54 ± 0.80</td>
<td>-51</td>
<td>7.79 ± 1.48*</td>
<td>+207</td>
</tr>
<tr>
<td>Bulbocavernosus</td>
<td>4.47 ± 0.78</td>
<td>2.00 ± 0.20</td>
<td>-55</td>
<td>10.70 ± 2.10*</td>
<td>+435</td>
</tr>
<tr>
<td>Plantaris</td>
<td>1.62 ± 0.31</td>
<td>0.97 ± 0.20</td>
<td>-40</td>
<td>2.17 ± 0.37</td>
<td>+124</td>
</tr>
</tbody>
</table>

Data are means ± SE. The units are density units (DUs) per µg protein relative to prostate (percent of prostate) with prostate standardized to 100. There were no significant differences between C and N. * Significant difference between C + DHT and C (P < 0.05).
to androgen levels (16). Atrophy of these muscles after castration is associated with a loss of contractile proteins that continues for ~45 days and is reversed by androgen administration (16, 22). In the present study, the weight of the bulbocavernousus and levator ani muscles dropped significantly within 2 wk of castration and was no different from the control weight within a few days of androgen treatment. In contrast, the plantaris muscle was unaffected by either castration or DHT treatment. A similar lack of androgen responsive

ess has been observed in the extensor digitorum longus and soleus muscles of male mice and the flexor digitorum brevis muscle of rats (13, 21).

The findings in the present study confirm and extend the conclusions of Kreig et al. (12). One could postulate that androgen responsiveness of muscles in rats correlates with the AR level and imply that the presence of some critical amount of AR separates major androgen target tissues from tissues with minor or no responsiveness. It is striking that, in the two muscle groups known to respond dramatically to androgen in the rat (i.e., bulbocavernousus and levator ani), the levels of AR are only ~5% as high as in the ventral prostate, which was used for the control for this experiment. The cause for variation in AR levels in different muscles and the minimal AR level needed to exert a major effect are unclear.

The findings in the present study do provide some insight into the mechanisms by which AR levels in muscles are regulated. That is, the administration of DHT caused a significant increase in the AR level compared with the C rats. This finding is in contrast to the report by Rance and Max (15) that castration caused an increase and that testicular testosterone caused a decrease in the level of AR in the levator ani muscle. The differences in these two studies are almost certainly due to the fact that it is difficult to measure AR levels in the presence of ligand. The effects of androgens on AR vary among tissues. Namely, androgen administration causes downregulation in AR levels in the rat penis (19), whereas, in the rat adrenal and prostate, androgens appear to upregulate AR levels (2). The nature of the upregulation by androgens is not entirely clear and may be the passive consequence of the fact that its ligand-occupied receptor is more stable and has a slower half-life (10). Regardless of the mechanism, it is intriguing to speculate that the upregulation of AR levels via the administration of pharmacological amounts of androgens might convert some muscles that normally have a minor or no response to muscles with enhanced androgen responsiveness. The present study was too short in duration to determine whether such changes do occur.

The anabolic potencies of different androgens vary. Although DHT is one of the most potent, naturally occurring anabolic hormones that has been identified (11), the synthetic androgens 7α-methyl-19-nortestosterone and 19-nortestosterone are even more potent anabolic steroids (18). Because athletes may self-administer synthetic androgens for many months or years at even higher doses than used in this study (17), it is possible that upregulation of AR levels might be an important part in the anabolic effects of androgen abuse.

In summary, these data are unique in that they demonstrate changes in skeletal muscle mass that may be associated with the observed changes in AR levels. Future work should examine the effects of different androgens and lengths of administration on the response of the AR in various skeletal muscles.

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