Development of sex differences in the rabbit masseter muscle is not restricted to a critical period

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First published October 19, 2001; 10.1152/japplphysiol.00953.2001.—The proportions of muscle fibers of different phenotype in the adult rabbit masseter differ greatly in different sexes. These sex differences are not apparent in young adults, but arise under the influence of testosterone in the males. We examined whether this switch occurred during a critical period of postnatal development. Testosterone was administered to young adults 1, 2, or 4 mo after castration, and also to adult females. Samples of masseter muscle were taken at four monthly intervals after the onset of treatment and examined for the expression of the different myosin heavy chain (MyHC) isoforms by using a panel of monoclonal antibodies. Despite the length of androgen deprivation, treatment with testosterone produced a marked MyHC isoform switch from α-slow/β to IIa. This male proportion of fibers of different phenotypes persisted well beyond the return of serum testosterone levels to pretreatment levels. Thus brief exposure to testosterone produces a permanent change in the proportions of masseter muscle fibers of different phenotypes, and the capacity for this change is not restricted to a critical period.

Masticatory muscles; sexual dimorphism; testosterone; myosin heavy chain

The muscles of mastication of several mammalian species are strongly sexually dimorphic. In mice (14), guinea pigs (4, 20, 27, 28), rabbits (13, 17), and rhesus monkeys (29), the proportions of muscle fibers of different phenotype differ markedly in males and females. In all of these species, these sex differences arise during postnatal maturation as changes in the males only. These changes are androgen mediated because they fail to occur in the absence of testosterone (15, 20) and hormone treatment produces the change (15, 20, 34).

In the rabbit masseter muscle, we described fibers of two general phenotypes (15). Some fibers contain the slow/β myosin heavy chain (MyHC) isoform, and all of these fibers also contain the cardiac α MyHC isoform. Within this general phenotype, we described four distinct phenotypes, called I₁ to I₄, depending on their immunoreactivity to four different monoclonal anti-slow/β antibodies (16). The rest of the fibers contain the IIa MyHC isoform. Very few fibers (<0.10%) normally contain all three of these isoforms or some other combinations of them. In adult females (>6 mo old), nearly equal proportions of these two phenotypes are present, and in young adults of both sexes, the same is true. In adult males, nearly 80% of fibers are of the IIa phenotype, indicating that during the period between 2 and 6 mo of postnatal age, nearly 30% of masseter fibers undergo a MyHC isoform switch from α-β/slow to IIa. In particular, only fibers of the I₁ phenotype are thought to undergo this change.

In castrated young adult rabbits, the MyHC isoform switch characteristic of normal males never occurs (15), and if these animals are treated with testosterone for as little as 3 wk, an isoform switch of comparable magnitude to that observed during normal development takes place (34). On the basis of these findings, we speculated that sex differences in rabbit masseter muscle arise under the influence of testosterone during a critical period of postnatal maturation (34).

The concept of a critical period was first popularized in studies of the development of the visual system, in which it was shown that visual experience during a limited time period is required for the development of normal vision (10, 22). Deprivation of sight in an eye for as little as a week during this period results in a complete loss of vision in that eye. In the neuromuscular system, it has been shown that the survival of both pelvic floor muscles and the motoneurons innervating them depends on the availability of testosterone during a limited period of postnatal development (7, 25). Two features are essential to this concept: developmental changes take place only during the critical period, not before or after it; and, once the changes have occurred, they are not reversible. Thus we hypothesized that androgens stimulate the MyHC-isoform switch, which defines sex differences in phenotype proportions in the rabbit masseter muscle, and that they are not only able to produce this switch during a restricted critical period of postnatal maturation, but also make the change so produced permanent. To test this hypothesis, we delivered testosterone for short periods (3 wk) either to male rabbits castrated as young adults and then al-

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allowed to survive for different periods before androgen treatment or to adult females. If the effects of the androgen were limited to a critical period, then hormone treatment after that critical period (or in adult females) would be predicted to have no effect. In contrast to our hypothesis, androgen treatment in all animals produced a similar α-slow/β to IIa MyHC isoform switch, indicating that a critical period is not present. In addition, the MyHC isoform composition of these muscles was retained in the male proportions well after the cessation of testosterone treatment, suggesting that the brief exposure to androgens resulted in a permanent change.

METHODS

Experiments were conducted on six male and two female New Zealand white rabbits obtained from commercial vendors. All experiments were conducted in accordance with National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Males were obtained at age 2 mo (2,000 g body weight). All were castrated under ketamine (35 mg/kg), xylazine (5 mg/kg), and acepromazine (5 mg/kg) anesthesia. Two animals each were then allowed to survive for 1, 2, or 4 mo before testosterone treatment began. Castrated males and both adult (>6 mo old, body weight = 4.35 kg) females were then treated with a sustained-release dosage form of testosterone, delivered as tablets implanted subcutaneously between the scapulae. All animals were anesthetized with ketamine (35 mg/kg) and xylazine (5 mg/kg) before tablet implantation, and each animal received two 200-mg tablets, as per the recommendation of the manufacturer (Innovative Research of America).

Before implantation, and at monthly intervals thereafter, serum was withdrawn for analysis of plasma testosterone levels. At these same time points, a surgical biopsy was performed from the anesthetized rabbits. A small piece of masseter muscle was removed and frozen rapidly in isopentane cooled just to its freezing point in liquid nitrogen. Tissue samples were then stored frozen at −50°C until processed for histology. Tissue samples were removed from left and right masseters and from anterior and posterior locations on alternate occasions. All samples were removed from the most superficial part of the superficial masseter (MSSs), as we have shown this region to be sexually dimorphic (17). At the end of the period of study, all but two of the animals (those in which the delay was 4 mo) were euthanized with Euthanasia 4 solution (iv). These latter animals were allowed to live for 7 mo after the onset of hormone treatment before euthanasia.

Serial 10-μm-thick histological sections were cut from the frozen muscle samples on a cryostat at −23°C. Adjacent sections were reacted with different monoclonal antibodies to different MyHC isoforms. The antibodies used, their source, and their isoform specificity are shown in Table 1. The specificity of each of these antibodies to rabbit MyHC isoforms is well established. The methods for immunohistochemical detection of MyHC isoforms are described in detail elsewhere (16). Briefly, sections mounted on slides were incubated in primary antibody solution for 18 h at 4°C. After washing, the sections were incubated for 1 h at room temperature (24°C) in a solution of peroxidase-conjugated goat, anti-mouse immunoglobulin (Jackson Immunobiologicals). Immunoreactivity was then demonstrated by a standard diaminobenzidine reaction that deposits a reddish-brown reaction product at the sites of antibody binding.

For analysis, images of sections stained with each of the antibodies were captured by using an image-processing computer, and these images were then combined to form low-power (×10) photomontages of the entire tissue section. Individual fibers were identified in each montage, and the staining with each of the different antibodies was noted for each fiber. Fibers with similar patterns of immunoreactivity for the different antibodies were assigned to phenotypes, and the proportions of each muscle fiber phenotype in each muscle sample were determined. A total of 16,606 fibers were studied in the eight rabbits. Proportions at different times after the onset of androgen treatment were compared with each other by using a multiway ANOVA. Significance of differences was evaluated by using post hoc (Scheffe) testing.

Serum collected at the time of each muscle biopsy was analyzed at the Radioimmunoassay Facility at the Yerkes Regional Primate Research Center of Emory University. In addition to these samples, serum was obtained from three other adult male (>6 mo old) rabbits and analyzed similarly. As reported previously (15, 20, 34), all serum testosterone data were studied as a proportion of the mean serum levels for these three animals.

RESULTS

Phenotypes in the rabbit masseter muscle. In previous papers, we have reported that the rabbit masseter muscle contains two basic phenotypes. Some fibers contain both the cardiac-α and slow/β MyHC isoforms, and these fibers could be subdivided into four groups (I1, I2, IIa, and IIx) based on their immunoreactivity to four different monoclonal anti-slow/β antibodies. The remainder of the fibers contain the type Ila MyHC isoform. Typical patterns of staining of tissue sections of rabbit masseter muscle fibers with these antibodies are shown in Fig. 1. In this series of images taken from consecutive serial cryostat sections stained with different antibodies, the two main phenotypes seen in the adult masseter are apparent. Fibers stained with antibodies BA-D5 and BA-G5 contain the slow/β and cardiac-α MyHC isoforms, respectively. Other fibers are immunoreactive with antibody SC-71, indicating that they contain the Ila MyHC isoform. Note that two different populations of these fibers are found: small intensely stained fibers (Ila dark) and larger fibers that react weakly with antibody SC-71 (Ila light; Fig. 1).

With the use of four different monoclonal antibodies to the slow/β MyHC isoform, we identified four differ-

Table 1. Antibodies used and their specificity

<table>
<thead>
<tr>
<th>Antibody</th>
<th>MyHC Isoform Specificity</th>
<th>Ref.</th>
<th>Source</th>
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<tbody>
<tr>
<td>BA-G5</td>
<td>Cardiac α</td>
<td>35</td>
<td>ATCC</td>
</tr>
<tr>
<td>BA-D5</td>
<td>Slow/β</td>
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<td>ATCC</td>
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<tr>
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<td>36</td>
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<td>23</td>
<td>DSHB</td>
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<td>A4.840</td>
<td>Slow/β</td>
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<td>DSHB</td>
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<tr>
<td>S-58</td>
<td>Slow/β</td>
<td>11</td>
<td>*</td>
</tr>
<tr>
<td>SC-71</td>
<td>Ila</td>
<td>36</td>
<td>ATCC</td>
</tr>
<tr>
<td>MY32</td>
<td>All type II and neonatal</td>
<td></td>
<td>Sigma Chemical</td>
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MyHC, myosin heavy chain; ATCC, American Type Culture Collection; DSHB, Developmental Studies Hybridoma Bank; *, generous gift of Dr. Frank Stockdale.
ent slow/β phenotypes in the adult rabbit masseter muscle (16). In Fig. 2A, similar results are presented from a section through the masseter muscle of an adult female rabbit. Images of identical muscle regions were obtained from adjacent serial cryostat sections, each of which was reacted with a different anti-slow/β anti-

![Diagram of muscle fibers and antibodies](image)

Fig. 2. The effects of androgens on slow/β and cardiac-α myosin heavy chain (MyHC) isoform expression in the rabbit masseter muscle. Consecutive serial sections were stained for the demonstration of the slow/β and cardiac-α MyHC isoforms by using different monoclonal antibodies. Images were obtained from identical regions of these sections and were overlaid to identify muscle fibers of different phenotype. These different phenotypes were then marked with different colors, as shown in the key. A: images were obtained from sections of the masseter of an intact adult female rabbit. Fibers of the four different slow/β phenotypes all contain the cardiac-α MyHC isoform. B: images were taken from serial sections through the masseter of a young adult male rabbit. Note that fibers of the I1 phenotype are not immunoreactive for antibody BA-G5 and thus do not contain the cardiac-α MyHC isoform (blue fibers). C: images were taken from sections of the masseter of the same animal but 4 wk after treatment with testosterone. Note the smaller proportion of fibers of the I1 phenotype (red). All fibers of the I4 phenotype are immunoreactive for antibody BA-G5 (gray). Scale bar = 100 μm.
body or with antibody BA-G5, which is specific for the cardiac-α MyHC isoform. The specificity of these antibodies to rabbit MyHC isoforms was demonstrated in a previous publication (16). The resulting images were aligned and superimposed, and fibers of different slow/β phenotype were assigned different colors. Fibers reacting only with antibodies BA-D5 and BA-G5 (I₁), for example, appear red, whereas fibers reacting to all five antibodies (I₄) appear gray. Fibers unstained by any of the five antibodies contain the I₁a MyHC isoform.

Testosterone treatment of castrated males. Young adult males were castrated and allowed to survive for 1, 2, or 4 mo before testosterone was administered. In each set of animals, testosterone was administered for 3 wk in a sustained-release dosage form. As noted previously (15, 20, 34), such treatment resulted in an elevation of serum levels to 3–4 times that of normal adult males (data not shown). Serum levels returned to pretreatment levels within a week of cessation of treatment (i.e., by 1 mo after onset of treatment). Muscle samples were obtained at the time of onset of androgen treatment and at 1-mo intervals thereafter. In tissues from both intact and castrated young adult males, fibers of different phenotypes were noted that are comparable to those noted in intact animals with one exception. Most, but not all of the fibers of the I₄ phenotype (fibers reacting positively to all four of the anti-slow/β antibodies used), do not react with antibody BA-G5. This means that these fibers contain the slow/β MyHC isoform but not the cardiac-α isoform (Fig. 2B, blue fibers). This staining pattern was not observed in normal adult males or any animals after androgen treatment (Fig. 2, A and C, gray fibers).

In all three sets of animals, testosterone treatment resulted in a change in masseter muscle fiber phenotype proportions. Three weeks of testosterone treatment resulted in a significant (P < 0.05) decrease in the proportions of fibers of the I₁ phenotype (Fig. 3, red bars), which was detectable as early as 4 wk after the onset of treatment. This decrease persisted for the remainder of the study period (4 mo). Slight increases in the proportion of I₁ fibers were found at the end of the study period in some animals, but these increases were not statistically significant. No changes in the proportions of the I₂ to I₄ phenotypes were noted.

![Fig. 3. Effects of delayed testosterone treatment on the proportions of muscle fibers of different phenotypes in the rabbit masseter muscle. Data from young adult males are shown in panels A–C, whereas D contains data from adult females. Young adult males were castrated at 2 mo of age and given testosterone for 3 wk beginning 1 (A), 2 (B), or 4 mo (C) later. Adult females were given the same doses of androgen and examined at the same time points (D). In each graph (A–C), the percentage of fibers of seven different phenotypes is shown at the onset of treatment and at monthly intervals thereafter. *Statistical significance (P < 0.05) of values compared with proportions at the onset of hormone treatment.](http://jap.physiology.org/
A significant \( P < 0.05 \) increase in the proportion of fibers immunoreactive to antibodies BA-D5, BA-G5, and SC-71 but not to any of the other anti-slow/\( \beta \) antibodies was found. Fibers with this pattern of immunoreactivity express both the I\(_1\) and I\( \alpha \)a phenotypes. In all animals, this increase was transient, appearing as early as 1 mo after the onset of hormone treatment but disappearing as early as 1 mo later (Fig. 3, green bars).

Accompanying the testosterone-induced decrease in the proportion of I\(_1\) fibers was an increase in the proportions of fibers reacting to antibody SC-71 (specific to the I\( \alpha \)a MyHC isoform) but not to any of the anti-slow/\( \beta \) antibodies. As noted above, rabbit masseter muscle fibers react with this antibody with two distinct staining intensities, termed I\( \alpha \)a dark and I\( \alpha \)a light. After testosterone treatment, a significant increase in the proportion of I\( \alpha \)a light fibers is noted in all animals (Fig. 3, light blue bars). In some animals, this increase in I\( \alpha \)a light fibers came at the expense of I\( \alpha \)a dark fibers. Only I\( \alpha \)a dark fibers in these muscles also contained the slow/\( \beta \) MyHC isoform (Fig. 4). In animals in which hormone treatment was delayed for either 1 or 2 mo after castration, the greatest increase in I\( \alpha \)a light fibers was found 2 mo after the onset of testosterone treatment. Yet, by the end of the 4-mo study period (3 + 1 mo after the end of hormone treatment), the proportions of these fibers had decreased to those found in the masseter muscles of untreated young adult rabbits. In animals in which testosterone treatment had been delayed by 4 mo, a similar increase in the proportions of I\( \alpha \)a light fibers was noted, except that it occurs later and lasts longer than the transient change observed in other groups.

The proportions of fibers of the I\( \alpha \)a dark phenotype (Fig. 3, dark blue bars) in the rabbit masseter decreased dramatically after hormone treatment only in some animals (1- and 4-mo delays), but by the end of the study period, it was the dominant phenotype in all animals. At this time, the proportions of fibers of the seven phenotypes studied were not significantly different from those reported for adult males (13, 17).

In two rabbits, masseter muscles were examined as long as 7 mo after the brief (3 wk) exposure to testosterone. Although no quantitative analyses of the histological sections from these muscles was performed, it was clear from simple examination that the proportion of fibers reacting positively to antibody BA-D5 (slow/\( \beta \) MyHC) remains reduced relative to that observed at the time of onset of testosterone treatment. By 2 mo after hormone treatment, this reduction was evident as an increase in the number of BA-D5\(^+\) fibers and the appearance of a substantial number of fibers that reacted weakly to this antibody (Fig. 5B) relative to that observed before treatment (Fig. 5A). By 4 mo after the onset of treatment, only a few BA-D5\(^+\) fibers were found (Fig. 5C), and after 7 mo, this same state was observed (Fig. 5D).

Testosterone treatment of adult females. In adult (>6 mo old) females, androgen treatment resulted in elevation of serum testosterone to values comparable to that noted above (data not shown). Treatment with testosterone for 3 wk, as performed on castrated males, produced generally similar changes in muscle fiber phenotype proportions. Results are shown in Fig. 3D. Androgen treatment resulted in a decrease in the proportion of fibers of the I\(_1\) phenotype that was significant by as early as 1 mo after treatment onset and persisted throughout the duration of the study. Accompanying this decrease was a transient increase in the proportion of fibers containing the \( \alpha \), slow/\( \beta \), and I\( \alpha \)a MyHC isoforms. A transient increase in the proportion of fibers of the I\( \alpha \)a light phenotype was noted. The proportion of fibers of the I\( \alpha \)a dark phenotype first

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**Fig. 4.** Transient expression of I\( \alpha \)a MyHC isoform in I\(_1\) fibers. The format is similar to that of Fig. 1. Images were obtained from cryostat sections from a biopsy of the masseter muscle of a male rabbit 6 mo after castration and 2 mo after the onset of testosterone treatment. Sections were reacted with antibodies A4.951 (A), BA-D5 (B), and SC-71 (C) (see Table 1). Three slow/\( \beta \) fibers, which are BA-D5\(^+\) and A4.951\(^-\) (I\(_1\) fibers) and also are immunoreactive for antibody SC-71 (specific for I\( \alpha \)a MyHC isoform), are indicated by dots. Note also that only I\( \alpha \)a dark fibers also contain the slow/\( \beta \) MyHC isoform. Scale bar = 100 \( \mu \)m.
decreased significantly and then increased to become the dominant phenotype. No significant changes in the proportions of fibers of the I₂ to I₄ phenotypes were found at any time. All fibers of the I₄ phenotype, both before and after testosterone treatment, were also immunoreactive to antibody BA-G5, indicating the presence of the cardiac-α MyHC isoform (data not shown).

DISCUSSION

The muscles of mastication of several mammalian species are sexually dimorphic with respect to the proportions of fibers of different phenotypes. In guinea pigs (4, 20, 27, 28), mice (14), macaques (29), and rabbits (13, 17), significant sex differences exist in the proportions of muscle fibers of different phenotypes. The phenotype proportions found in young animals of both sexes are similar, and sex differences are achieved by postnatal changes in males. In all of these species, testosterone is implicated in the change.

In a previous publication, we hypothesized that testosterone-mediated changes in phenotype proportions in the rabbit masseter muscle occur during a critical period of postnatal development (34). The hallmark of such a hypothesis is that the androgen effects will be restricted to the critical period and that they will be ineffective in promoting a change in muscle fiber phenotype proportions outside of the bounds of the critical period (7, 25). At 2 mo of age, only adult MyHC isoforms are present in the rabbit masseter muscle (6), and the proportions of muscle fibers of different phenotypes in young adult male rabbits are not significantly different from those of adult females (15). However, rabbits continue to grow until they are 6 mo old (38), and, at that time, muscles from males consist of fibers of different phenotypes in very different proportions from muscles of females (17). Thus, if a critical period exists during which testosterone stimulates a change in fiber phenotype proportions, it lies between ages 2 and 6 mo.

In the present study, we performed experiments to test the critical period hypothesis. Rabbits were castrated as young adults and allowed to survive for as long as 4 mo before they were treated with testosterone. Administration of testosterone after such a delay was designed to delineate the bounds of the critical period. The principal finding of this study is that a brief exposure to testosterone results in a change in phenotype proportions in the masseter muscle of these rabbits. Despite the length of delay before hormone treatment, androgen administration resulted in a change in phenotype proportions comparable to that noted during normal development. It is notable that, in the longest delay studied (4 mo), animals were fully grown (6 mo old) and testosterone was administered after the end of the hypothetical critical period.

We interpret these findings to mean that the effectiveness of testosterone in producing a change in rabbit masseter muscle fiber proportions is not limited to a critical period of postnatal maturation. Androgens are capable of producing such an effect past the bounds of the proposed critical period, a conclusion that is supported by the finding that testosterone administered to adult females results in changes in phenotype proportions in the masseter, which are remarkably similar to those noted after androgen administration to young adult males. Unlike the findings of others studying pelvic floor muscles and their motoneurons (9, 18, 19), androgen deprivation in young adult male or adult

Fig. 5. Long-term retention of testosterone-induced muscle fiber phenotypes. Images were obtained from histological sections of biopsies of the masseter muscles of a single male rabbit, which had been castrated at 2 mo of age and given testosterone for 3 wk beginning 4 mo later. All sections were reacted with antibody BA-D5, which is specific for the slow/β MyHC isoform. Biopsies were obtained before testosterone treatment (A), 2 mo after the onset of testosterone treatment (B), 4 mo later (C), and 7 mo later (D). Scale bar = 100 μm.
female rabbits has no effect on their ability to respond to testosterone administered later in life. However, our conclusion must be tempered slightly by comparison with results obtained in the developing visual system. Binocular visual deprivation is known to prolong the critical period in cats (12). It is possible that our androgen deprivations might result in a similar extension of a critical period in the rabbit masseter muscle. Until we can examine the effects of testosterone on older rabbits, this concern cannot be dismissed.

A second feature of the critical-period hypothesis is that the changes produced are irreversible. Despite the finding that no real critical period exists for androgen-induced changes in masseter phenotype proportions, the changes produced by testosterone could be permanent. Indeed, testosterone rescues the levator ani muscle of female rats permanently from involution if administered for a brief period during the first week of postnatal life (9). We view the androgen-induced change in phenotype proportions found during postnatal maturation of the rabbit masseter as evidence for two types of MyHC isoform switches. Some fibers that are of the I1 phenotype stop expressing the α and slow/β isoforms and begin expressing the IIa isoform only. Relatively shortly (4 wk) after the onset of testosterone treatment, in both castrated young adult males and adult females, a significant number of fibers were observed that contained the α, slow/β, and IIa MyHC isoforms. The anti-slow/β antibodies used, these fibers reacted exclusively with antibody BA-D5. At later times, this population was absent in all animals studied. We interpret such a transient expression as evidence of the proposed I1-to-IIa MyHC isoform switch. This isoform switch persists well beyond the duration of hormone treatment. Coupled with the more casual observation that the proportion of fibers containing the slow/β MyHC isoform remains reduced as long as 9 mo after the onset of testosterone treatment, we conclude that this isoform change is permanent. This finding is in contrast to the observations made in guinea pigs (20, 27) and mice (14), in which testosterone appears to be required to maintain the male proportions of different phenotypes. Whether other α-slow/β fiber phenotypes switch is not clear, but because no significant changes in their proportions were observed, either during normal development (17) or after androgen treatment, we think that the I2 to I4 populations do not respond to testosterone.

Among the population of rabbit masseter muscle fibers containing IIa MyHC isoform, two distinct groups were noted, IIa dark and IIa light, based on the intensity of immunoreactivity to antibody SC-71. An increase in the proportion of rabbit masseter muscle fibers of the IIa light phenotype occurred after testosterone treatment. In most animals, this increase was transient and had disappeared by the end of the study period. In most instances, this increase in the proportion of IIa light fibers was at the expense of the proportion of IIa dark fibers. We have speculated previously that these IIa light fibers might contain the IIx MyHC isoform in addition to IIa MyHC isoform (34).

The simplest explanation of the above observations is that androgens induce a decrease in the expression of IIa MyHC isoform and an increase in the expression of the IIx isoform in IIa dark fibers. Once androgens are removed, this isoform switch is reversed.

The effect of testosterone could be myogenic, neurogenic, or both. There is a strong opinion that muscle fiber phenotype is regulated by motoneurons (3, 31, 32). In particular, it is postulated that the pattern of activity of motoneurons forms an anterograde signal to muscle fibers and that postsynaptic changes in muscle fiber calcium concentrations form a second messenger, which transmits that neuronal signal to the muscle fiber (40). The calcium/calmodulin-regulated phosphatase, calcineurin, has been implicated by some as a key player in such a signaling pathway (Ref. 8 but cf. Ref. 37). Trigeminal motoneurons have been shown to contain abundant androgen receptors (30, 42), and, although an effect of androgens on the pattern of masseter motoneuron activity has yet to be demonstrated, testosterone-mediated changes in the content of choline acetyl transferase in both pelvic floor (33) and phrenic motoneurons (5), which is implied to promote enhanced motoneuron-to-muscle fiber signaling, have been described.

On the other hand, others, notably those studying the development of androgen-sensitive muscles, both in the pelvic floor (9, 18, 19) and in the guinea pig temporalis (27), have been strong advocates of a myogenic site of action of testosterone. In the motoneurons innervating pelvic floor muscles, androgen sensitivity develops only after the critical period for testosterone-mediated muscle survival (24–26). A strong case can be made that such motoneuron sensitivity is induced through developmentally earlier androgen signaling in muscle fibers (2, 39) and that retrograde signaling molecules, such as neurotrophins, play a necessary role in the process (1, 41). We have found androgen-receptor immunoreactive nuclei in the muscle fibers of the rabbit masseter of both sexes (Schwartz and English, unpublished observations), but we do not yet know whether testosterone binding to its receptor on these fibers is sufficient by itself to induce androgen sensitivity in masseter motoneurons, let alone a change in motoneuron firing patterns, which result in changes in muscle fiber phenotype proportions as observed in the present study.

A variation on this myogenic model is one in which androgens induce a change in the allototype of masticatory muscle fibers. Hoh first introduced the concept of a jaw muscle allototype (21). He based it on the finding that masticatory muscle fibers reinnervated by a limb muscle nerve, which normally innervates a predominantly fast-twitch muscle (extensor digitorum longus) did not express MyHC isoforms characteristic of limb muscle (IIa and IIb) but a masticatory muscle-specific MyHC isoform (IIm). Thus he viewed masticatory muscles as a distinct class of muscle fibers in which the properties could be altered by the signals imposed by motoneuron activity in a different manner than those of limb muscle fibers. We hypothesize that testoster-
one, by binding to muscle fiber receptors, induces a change in the allotype of masseter muscle fibers such that they respond differently to neuronal activity than they do before exposure to the androgen. Whether testosterone might also change the activity patterns of the trigeminal motoneurons, a change in allotype would result in a change in muscle fiber phenotype proportions.

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