Sexually dimorphic expression of myosin heavy chains in the adult mouse masseter

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Eason, Jane M., Gail A. Schwartz, Grace K. Pavlath, and Arthur W. English. Sexually dimorphic expression of myosin heavy chains in the adult mouse masseter. J Appl Physiol 89: 251–258, 2000.—Little is known regarding the role of androgenic hormones in the maintenance of myosin heavy chain (MHC) composition of rodent masticatory muscles. Because the masseter is the principal jaw closer in rodents, we felt it was important to characterize the influence of androgenic hormones on the MHC composition of the masseter. To determine the extent of sexual dimorphism in the phenotype of masseter muscle fibers of adult (10-mo-old) C57 mice, we stained tissue sections with antibodies specific to type IIA and IIB MHC isoforms. Females contain twice as many fibers containing the IIA MHC as males, and males contain twice as many fibers containing the IIB MHC as females. There is a modest amount of regionalization of MHC phenotypes in the mouse masseter. The rostral portions of the masseter are composed mostly of type IIA fibers, whereas the midsuperficial and caudal regions contain mostly type IIB fibers. Using immunoblots, we showed that castration results in an increase in the expression of type IIA MHC fibers in males. Ovariectomy has no effect on the fiber type composition in females. We conclude that testosterone plays a role in the maintenance of MHC expression in the adult male mouse masseter.

hormones; plasticity; masticatory muscle

BOTH ANDROGEN AND ESTROGEN receptors have been detected in skeletal muscles of several species of animals (7, 8, 28). These include both rat limb and perineal muscles (7, 8), as well as rabbit gastrocnemius and soleus muscles (28). Maintenance of the rat levator ani muscle is testosterone dependent (5, 16). In rats, the female levator ani muscle normally degenerates postnatally, but neonatal injection of testosterone prevents degeneration of the muscle (5). In adult males, castration results in a decrement in levator ani weight, but this is reversed by testosterone injections (16). Testosterone also plays a role in sex differences in the expression of different myosin heavy chain (MHC) isoforms in the muscles of mastication in adult guinea pigs (temporalis) (17, 22) and rabbits (masseter) (6, 12). In muscles of young guinea pigs (22) and rabbits (6), MHC composition is the same in both sexes. With the onset of puberty, MHC composition in the males of both species changes with respect to the females (6, 22).

Despite the potential for genetic manipulation as a tool to study hormonal control of masticatory muscles, very little literature exists describing sexual dimorphism of the adult mouse masseter muscle. Using ATPase histochemistry, Schiaffino (30) showed that adult mouse masseter uniformly contains “fast twitch” fibers with “moderate to high” levels of succinate dehydrogenase. However, a comparison of females to males to determine potential differences due to sex was not made. Sekino et al. (33) found that lactate dehydrogenase isozymes showed sexually dimorphic distribution patterns in the adult mouse masseter. Males who were castrated before puberty developed lactate dehydrogenase enzyme patterns similar to those of adult females (33). The proportion of fibers of the mouse masseter that contain different MHC isoforms is not known, but we hypothesized that only type II or fast-twitch isoforms would be present. Furthermore, we hypothesized that the composition would be different in adult males and females.

Androgens have been implicated in the development of such sexual dimorphism, but it is not clear whether they are required only for the development of sex differences or whether sexually dimorphic expression of different MHC isoforms continues to be androgen-dependent throughout life. Lyons and co-workers (22) found that castration of adult guinea pigs altered the composition of temporalis muscle fibers sufficiently to conclude that androgens are required for the maintenance of sexual dimorphism in that muscle. In contrast, Eason et al. (10) showed that castrating adult rabbits resulted in so little an effect on masseter muscle fiber composition that androgens do not play a role in maintaining sexual dimorphism in that muscle. We hypothesize that any sexual dimorphism found in the adult mouse masseter muscle is regulated by androgens, as is found in phylogenetically more closely related guinea pigs.

Even less is known about the possible role estrogen may play in control of masticatory muscle fiber phenotype. In general, estrogen does not affect MHC compo-
sition in rat limb skeletal muscle (20, 23, 34). However, these studies examined the effects of estrogen on skeletal muscles that are not overtly sexually dimorphic. The potential role estrogen may play in differential phenotype expression and maintenance of MHC composition in sexually dimorphic muscles cannot be overlooked. We hypothesize that any sexual dimorphism found in the mouse masseter muscle is not regulated in the adult by estrogens.

In this study, we conducted experiments aimed at testing the three hypotheses stated above. First, we evaluated whether a sex-based difference exists in MHC isoform composition of the adult mouse masseter. Second, we investigated whether androgen manipulation in adult mice would eliminate or greatly attenuate this sexual dimorphism. Finally, we determined whether estrogen deprivation in female mice would alter any sexual dimorphism in masseter muscle fiber phenotype. We did find a striking difference in the MHC expression of the masseter muscle between adult male and female mice. Castration of adult males altered the proportions of masseter fibers of different phenotypes significantly, but it did not completely eliminate sex differences. Depriving adult females of estrogen by ovariectomy had no significant effect on sexual dimorphism. Preliminary findings have been previously reported (9).

MATERIALS AND METHODS

Animals and tissue harvesting. A total of 12 adult (10-month-old) C57 mice were used for this study. We chose to use adult animals so that any developmental issues could be excluded. For the first experiment, three intact males and three intact females were used to determine sex-based differences in the MHC composition of the masseter using immunohistochemistry. Animals were anesthetized with pentobarbital sodium (70 mg/kg intraperitoneally), and the masseter muscles were harvested bilaterally. One masseter was harvested for biochemical analysis, and the remaining masseter was harvested and quick frozen for immunohistochemistry. For the second experiment, three adult males and three adult females were anesthetized with pentobarbital sodium (70 mg/kg intraperitoneally) and underwent gonadectomies. After a 6-wk survival period, animals were anesthetized, and tissue was harvested as described above. We chose a 6-wk survival period because previous studies have shown that changes in MHC composition occur 4–6 wk after induction of either hypo- or hyperthyroidism in the pharyngeal muscle of rats (26) and the rat soleus (18). Plasma assays of testosterone/estrogen were not performed because previous studies have indicated that removing the gonads was adequate to ensure gonadal status (6, 22, 24).

Immunohistochemistry and antibodies. Serial transverse sections (10 μm thickness) were cut in a plane roughly parallel to the zygomatic arch on a cryostat. Sampling was repeated at 100-μm intervals throughout the length of the muscle. Sections were reacted with specific primary antibodies. Previous observations from our laboratory utilizing Coomassie blue-stained gels showed that mouse masseter contains only three MHC isoforms: IIa, IIx, and IIb (Fig. 1). Therefore, we used the following antibodies for these experiments. Antibody SC-71 (American Type Tissue Culture) recognizes the epitope for MHC IIa (31), and antibody BF-F3 (American Type Tissue Culture) is specific for MHC IIb (31). The commercially available antibody MY32 (Sigma) was used to recognize all fast-twitch myosins (type IIa, IIb, IIx). Antibody 332, which detects both MHC IIa and IIb (31), was kindly provided by Professor W. A. Weijs. We determined the monospecificity of antibodies SC-71 and BF-F3 to the mouse masseter. Both SC-71 and BF-F3 recognize a single band that migrates to the relative positions of IIa and IIb, respectively (Fig. 2). With this series of antibodies, we were able to identify fibers of the three different phenotypes: IIa, IIx, and IIb. Because antibodies SC-71 and 332 both recognize IIa fibers, and 332 also recognizes IIb fibers coexpressing IIa and IIx could not be differentiated from fibers expressing IIa only. We attempted to use antibody RT-D9 (American Type Tissue Culture) to recognize fibers containing both type IIx and IIb MHC, because Schiaffino et al. (31) have shown that this antibody recognizes the epitope for both type IIx and IIb MHC. However, despite repeated attempts, we were unable to obtain staining in mouse tissue using this antibody.

Methods for immunohistochemistry have been previously described (12). Briefly, tissue sections were incubated in blocking solution composed of 0.1 M Tris-HCl and 2% normal goat serum containing 0.03% Triton X-100 (T-NGS) for 30 min, followed by incubation in primary antibodies at the following dilutions: 1:5 SC-71, 1:1 BF-F3, 1:400 MY32, and 1:10 332. Primary antibodies were diluted in T-NGS. After overnight incubation, sections were incubated in a peroxidase-conjugated goat antimouse immunoglobulin secondary antibody (Capel) at a dilution of 1:100. A standard diazonium-benzidine reaction was used to visualize antibody binding.
**Tissue analysis.** Images of stained sections were captured by utilizing a Macintosh-based processing system and NIH Image software. Two male and two female masseters were stained with antibodies SC-71, BF-F3, and MY32. Because no antibodies exist that recognize only type IIX fibers, MY32 was used in conjunction with both SC-71 and BF-F3 to determine fibers that contained only type IIX. One pair of three serial sections from each masseter of all animals was taken to represent the muscle. From these representative sections, an average of six randomly selected images of microscope fields were captured from each of four regions for each antibody. Fibers were classified by identification of fibers from the paired sections. Fibers that were not recognized by the paired sections were omitted. The images were printed on a laser printer, and the total number of cells in these images, as well as the number of SC-71- and BF-F3-stained cells were counted. Because MY32 stained all cells, the number of SC-71- and BF-F3-stained cells was subtracted from the total number of cells counted to determine the number of fibers containing type IIX MHC. The number of cells expressing each of the three phenotypes was then expressed as a percentage of the total number of cells counted. Near the completion of this study, we were able to obtain antibody 332 from Professor W. A. Weijs, and this antibody was substituted for MY32 in the immunohistochemical analysis of the remaining male and female mice in the first experiment. For the male and female mice whose masseters were stained with SC-71, BF-F3, and 332, images were obtained from three consecutive sections. From these representative sections, an average of six randomly selected images of microscope fields was captured from each of four regions for each of the three antibodies. Fibers stained with 332, but not SC-71, were counted as type IIX fibers. The number of cells expressing each of the three phenotypes was counted and expressed as a percentage of the total number of cells counted.

**Gel electrophoresis.** Gel electrophoresis was used to determine the MHC content of the mouse masseter. To compare MHC content of the masseter, samples of mouse soleus, diaphragm, and tibialis anterior were also subjected to electrophoresis. Briefly, extraction of MHC was accomplished based on a modification of the methods of Butler-Browne and Whalen (3) and Hughes et al. (19). The muscles were finely minced and homogenized in a high-salt buffer (0.3 M NaCl, 0.1 NaH2PO4, 0.05 M Na2HPO4, 1 mM MgCl2, 10 mM EDTA, 0.1% Tween 20 (TBST). Membranes were incubated overnight at 4°C in primary antibodies in the following dilutions: 1:3 SC-71 or 1:1 BF-F3. Primary antibodies were diluted in blocking buffer. Antibody solution was poured off, and the membranes were washed for 30 min in TBST. Membranes were then incubated in secondary antibody, peroxidase-conjugated goat anti-mouse (1:10,000 in TBST and 5% normal goat serum) at room temperature for 1 h. After a final wash, the membranes were reacted with an enhanced chemiluminescence reagent (Amersham) and exposed to Kodak Xomat film. Blots were then scanned into a Macintosh-based scanning system, and the bands were analyzed. The area and optical density of each sample were determined, and the mean optical density of each group was calculated. The mean optical density of the intact female group for both antibodies was arbitrarily chosen to be 100%. Thus the mean optical density for each of the three remaining groups for each antibody was expressed as a percentage of intact females.

Results were analyzed by a two-factor (hormonal status × MHC phenotype) ANOVA with appropriate post hoc testing. Significance level was established at \( P < 0.05 \).

**RESULTS**

**Immunohistochemistry.** Based on immunohistochemical staining, three different muscle fiber phenotypes were observed in the adult mouse masseter. Because of the antibodies chosen for this study (see METHODS), fibers with the phenotypes IIA/IIX and IIX/IIB could not be differentiated from fibers containing only IIA MHC or IIB MHC, respectively. Despite this limitation, females have more cells containing the IIA MHC, irrespective of the cell’s actual phenotype, than males, whereas males have more cells containing the IIB MHC, also irrespective of the cell’s actual phenotype, than females. Representative immunohistochemical serial sections from both male and female masseters reacted with SC-71, 332, and BF-F3 are shown in Fig. 3. Despite the small sample size, power analysis revealed a power of at least 0.80 to detect differences in muscle fiber phenotype in comparisons between and within all compartments. Therefore, we are comfortable that the data indicate that true differences exist between and within groups, and, in fact, the magnitude of this difference may have been greater with a larger sample size.

Sexual dimorphism exists in the mouse masseter. In the male mouse masseter muscle, a significantly
greater number of fibers containing the IIb MHC are found than in females (24.1 ± 7.4 vs. 9.8 ± 3.2%, means ± SE) (Fig. 4), and females contain significantly more fibers of the IIa phenotype than males (28.8 ± 4.6 vs. 15.8 ± 6.0%) (Fig. 4). Based on tendinous origins and insertions, the masseter was divided into four regions: rostral, caudal, midsuperficial, and middeep (Figs. 5 and 6).

To simplify the reporting of data, we only indicated significant differences between the most relevant comparisons of regional differences within a sex and sex differences within homologous regions for each of the MHC phenotypes. When MHC phenotype percentages were compared across both sex and region, several notable differences were found (Table 1). Both the female rostral and middeep regions contained significantly greater amounts of the IIa MHC isoform than the corresponding male regions (54.5 ± 5.9 vs. 13.6 ± 5.1% rostral and 29.0 ± 3.9 vs. 14.0 ± 2.1% middeep). Comparison of the remaining two regions in the females to the corresponding regions in the males showed no significant differences in type IIa MHC. Within the female group, the rostral region contained significantly greater amounts of type IIa MHC than any other region, whereas the middeep region contained significantly greater amounts of type IIa MHC than the midsuperficial region. There were no significant differences between any regions within the male group.

Significant differences in the proportion of type IIb MHC were noted between sexes. The proportion of type IIb fibers in the male midsuperficial, middeep, and caudal regions was significantly greater than that of the corresponding regions in the female (43.2 ± 7.1 vs. 24.1 ± 6.2%; 15.2 ± 5.3 vs. 1.4 ± 0.7%; and 37.9 ± 15.0 vs. 13.5 ± 5.6%, respectively). Within the male group, the midsuperficial and caudal regions contained significantly greater amounts of IIb fibers than both the rostral and middeep regions (43.2 ± 7.1 and 37.9 ± 15.0 vs. 10.1 ± 2.5 and 15.2 ± 5.3%, respectively). Within the female group, the midsuperficial region contained greater amounts of IIb fibers than both the rostral and middeep regions (24.1 ± 6.2 vs. 0.2 ± 0.1 and 1.4 ± 0.7%, respectively) (Table 1).

![Fig. 3](Image)

Fig. 3. Images taken from serial sections to show representative staining patterns obtained with antibodies SC-71, BF-F3, and 332. Antibody SC-71 labels type IIa-containing fibers, BF-F3 labels type IIb-containing fibers, and 332 labels both type IIa- and IIx-containing fibers. 1, Fibers containing type IIb MHC isoform; 2, fibers containing type IIx MHC isoform; 3, fibers containing type IIa MHC isoform; IM, intact male, IF, intact female. Scale bar = 100 μm.

![Fig. 4](Image)

Fig. 4. Proportions of fibers labeled IIa, IIx, and IIb in intact male and female.

![Fig. 5](Image)

Fig. 5. Representative, low-power cross sections of female mouse masseters stained with antibodies SC-71 (A) or BF-F3 (B). Dark lines indicate tendinous origins and insertions, used to divide the muscle into four compartments: rostral (R), midsuperficial (MS), middeep (MD), and caudal (C). Note regionalization of fibers containing the IIa (A) or IIb (B) MHC isoforms. Scale bar = 1.00 mm.
The cells in both sexes contained greater proportions of the IIx MHC. These fibers were located throughout the muscle in both sexes; however, some regions were comprised of more type IIx-containing fibers than others. In general, areas with a high content of either type IIa or IIb MHC contained smaller amounts of type IIx MHC. For example, the IIx isoform is the predominant phenotype in the female rostral region, and this region contained significantly fewer IIx-containing fibers than either the female middeep or midsuperficial regions (45.3 ± 5.9% vs. 69.6 ± 3.9% and 45.3 ± 5.9% vs. 70.2 ± 5.2%, respectively). Likewise, regions with a lower content of type IIa or IIb MHC contained larger amounts of the IIx phenotype. The male rostral region, which contains a small percentage of the IIb phenotype, contained significantly greater amounts of the IIx MHC compared with either the male midsuperficial or caudal regions (76.3 ± 8.6% vs. 46.3 ± 8.9% and 76.3 ± 8.6 vs. 36.9 ± 6.9%, respectively).

Immunoblotting indicated that the IIa MHC composition of intact females was significantly greater than that of intact males (100 vs. 11%) (Table 2; Fig. 7). Castration significantly increased the amount of IIa MHC in males compared with intact males (62 vs. 11%), but IIa MHC composition was still significantly less than in intact females (62 vs. 100%). This indicates that castration did not completely convert IIa MHC expression to the levels of intact females. There was no difference in the IIa MHC composition between intact females and ovariectomized females (100 vs. 120%). Immunohistochemical results show a two times greater amount of type IIa MHC in females compared with males, whereas immunoblots show a 10 times greater difference between the two sexes. There are several possibilities to account for this difference. In general, the sensitivity of chemiluminescence and immunoblotting in quantifying proteins is greater relative to immunohistochemistry. Second, histological analysis is based on fiber counts and does not take fiber size into consideration. Finally, it is possible that some of the IIa-labeled fibers in the male also contain IIx MHC. Because we were not able to identify fibers containing both IIa and IIx, it is possible that these IIa/IIx hybrid fibers were scored as IIa-containing fibers. This would artificially inflate the proportion of fibers in the male that were histochemically identified as IIa-containing fibers. Taken together, these three factors may explain the greater differences between the sexes in the amounts of type IIa MHC observed with immunoblotting compared with immunohistochemical methods.

Both intact and castrated males contained significantly greater amounts of type IIb MHC compared with intact females (486 and 510 vs. 100%, respectively). Ovariectomized females contained significantly less type IIb MHC than both intact males and castrated males (135 vs. 486 and 510%, respectively). There was no significant difference in the proportion of IIb MHC between intact and castrated males (486 vs. 510%). Both intact and ovariectomized females contained similar amounts of type IIb MHC (100 vs. 135%). Immunohistochemical techniques show an ~2.5 times greater amount of type IIb-containing fibers in the intact male compared with the intact female.

Table 1. Sex and regional fiber type percentages

<table>
<thead>
<tr>
<th></th>
<th>IIa</th>
<th>IIx</th>
<th>IIb</th>
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<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rostral</td>
<td>13.6±5.1</td>
<td>76.3±8.6</td>
<td>10.1±2.5</td>
</tr>
<tr>
<td>Middeep</td>
<td>14.0±2.1</td>
<td>70.8±5.7</td>
<td>15.2±5.3</td>
</tr>
<tr>
<td>Midsuperficial</td>
<td>10.5±3.3</td>
<td>63.6±8.9</td>
<td>43.2±7.2</td>
</tr>
<tr>
<td>Caudal</td>
<td>25.2±13.5</td>
<td>36.9±6.9</td>
<td>37.9±15.0</td>
</tr>
<tr>
<td>Average</td>
<td>15.8±6.0</td>
<td>58.5±7.5</td>
<td>24.1±7.4</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rostral</td>
<td>54.5±5.9</td>
<td>45.3±5.9</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>Middeep</td>
<td>29.0±3.9</td>
<td>69.6±3.9</td>
<td>14.0±7.4</td>
</tr>
<tr>
<td>Midsuperficial</td>
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<td>70.2±5.2</td>
<td>24.1±6.2</td>
</tr>
<tr>
<td>Caudal</td>
<td>25.9±6.8</td>
<td>60.6±9.8</td>
<td>13.5±5.6</td>
</tr>
<tr>
<td>Average</td>
<td>28.8±4.6</td>
<td>61.4±6.2</td>
<td>9.8±3.2</td>
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</table>

Values are expressed as mean percentages ± SE. *Significantly different from female rostral region; †significantly different from female midsuperficial region; ‡significantly different from female middeep region; §significantly different from female caudal region; ¶significantly different from male rostral region; ††significantly different from male middeep region (P < 0.05 for all values); *Significantly different by sex.

Table 2. Western blot myosin heavy chain percentages

<table>
<thead>
<tr>
<th></th>
<th>IIa</th>
<th>IIb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact female</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Intact male</td>
<td>11*‡</td>
<td>486*‡</td>
</tr>
<tr>
<td>Castrated male</td>
<td>62*‡</td>
<td>510*‡</td>
</tr>
<tr>
<td>Ovariectomized female</td>
<td>120</td>
<td>135</td>
</tr>
</tbody>
</table>

Values are percentages. *Significantly different from intact female; †significantly different from ovariectomized female; ‡significantly different from intact male; P < 0.05 for all values.
male, whereas immunoblotting results show a 20 times difference between the two sexes. Again, these differences could be due to the greater sensitivity of immunoblotting compared with immunohistochemistry, as well as the fact that immunohistochemistry does not take fiber size into account. Finally, because we were not able to discern differences between fibers containing only IIb MHC and those containing both IIx and IIb, it is possible that these hybrid IIx/IIb fibers were scored as IIb-containing fibers. This would artificially inflate the proportion of fibers in the male that were histochemically identified as IIb-containing fibers.

**DISCUSSION**

The results of this study are the first to show that the adult mouse masseter is a sexually dimorphic muscle and that maintenance of the adult male fiber type proportions is dependent on testosterone. Males have greater amounts of cells that contain type IIb MHC, and females have greater amounts of cells that contain type IIa MHC. In what is a novel finding, this maintenance of sexual dimorphism is at least partly testosterone dependent because castration results in an increase of the type IIa phenotype in the adult male mouse masseter. After 6 wk, type IIa MHC content in castrated males was still only 54% that of intact females. Complete transition to female proportions of IIa MHC may not have occurred due to length of survival time after castration. However, Lyons et al. (22) castrated male guinea pigs, allowed them to survive for 110 days, and still did not see a complete conversion to the peptide-mapping characteristic of adult intact females. Thus we believe our results are not time dependent.

Altering sex hormone levels appeared to have no effect on the proportions of type IIb MHC content in either sex. Neither castration nor ovariectomy resulted in a change in the content of type IIb MHC. These results indicate that manipulating either testosterone or estrogen levels in the adult mouse may not affect masseter muscle fibers in a manner that results in a change of the proportion of fibers containing the type IIb MHC isoform. These results are different from studies examining the response of the temporalis muscle to castration in adult male guinea pigs (22). Using peptide mapping, Lyons et al. (22) found that the peptide maps of castrated adult guinea pigs showed protein banding patterns consistent with an increase in type IIa and a decrease in type IIb MHC. However, it is possible that IIb fibers in the adult male guinea pig temporalis respond differently to changes in testosterone levels compared with IIb fibers in the adult male masseter.

With regard to the effect of sex hormones on MHC composition in the female, comparisons to previous studies are difficult. There are very few studies examining the response of muscle fiber phenotype to ovariectomy, and these investigations show no shift in MHC composition of rodent limb muscles (20, 23, 34). However, these studies examined the effects of estrogen on skeletal muscles that are not overtly sexually dimor-
phic. The results of our study suggest that the presence of estrogen is not necessary to maintain the MHC composition of the adult female mouse masseter.

Myogenic vs. neurogenic effect of sex hormones. The changes in MHC expression we observed could be explained by a direct effect of testosterone on adult masseter muscle fibers that resulted in transformation of IIx-containing fibers, in the male, to IIa-containing fibers. MHC phenotype transformation appears to follow an obligatory pathway of I ↔ IIa ↔ IIx ↔ Iib (31). Changes in factors affecting muscle MHCs can push phenotype transformation either to the left or the right in this pathway. We propose that, in the male, testosterone acts directly on the muscle fiber by binding to androgen receptors. Androgens interact with androgen receptors within the cytoplasm, and this receptor/ligand complex is transported into the nucleus to stimulate tissue-specific transcription factors (25). The ligand-receptor complex binds to specific DNA sequences called hormone response elements and regulates transcription in either a negative or positive way (25). In our proposal, this binding would repress transcription of the IIa MHC gene and result in a decrease in the expression of IIa MHC. With castration, these inhibitory transcriptional factors would be removed, and susceptible fibers would begin expressing the IIa MHC at the expense of the IIx MHC. As a result, the fibers would undergo phenotype transformation by moving toward the left in the obligatory pathway.

Alternatively, this androgenic hormone may also have an indirect effect on MHC composition via the motoneurons innervating the masseter. It is widely held that MHC isoform expression in muscle fibers is regulated by the pattern of activity in their innervating motoneurons (27). Removal of androgens in castrated male mice may result in a change in the activity of the trigeminal motoneurons innervating the masseter muscle. A change in motoneuron excitability would be expected to result in a change in activation patterns of muscle fibers by altering the probability of firing of the motoneuron. However, there is no evidence to support the notion that testosterone levels affect motoneuron excitability and thus muscle fiber phenotype.

Evidence from the literature does suggest that the changes we observed were probably due to a myogenic, rather than a neurogenic, effect. Carlson et al. (4) grafted the levator ani muscle, which is testosterone sensitive, into the bed of the testosterone-insensitive extensor digitorum longus muscle. The levator ani muscle maintained its testosterone sensitivity, as demonstrated by cross-sectional area of fibers and wet weight of the muscle, even after reinnervation. It was then concluded that testosterone exerts its control over MHC phenotype via myogenic mechanisms. Thus it is likely that the results we observed in this study were due to the myogenic influences of testosterone.

It is unknown, at this time, why fiber type composition of the masseter, but not limb muscle, is susceptible to sex differences in rodents. We believe that this fiber type difference is due to testosterone for several reasons. The castrated males in this study increased the content of the IIa phenotype; this is similar to the results observed in adult, castrated guinea pigs (22). Furthermore, increases in type Iib MHC content occurred (22) in adult female guinea pigs that were administered testosterone. Although we did not administer testosterone to adult females in this study, we hypothesize that administration of testosterone to adult female mice would have resulted in a decrease in type IIa-containing fibers.

Potential functional implications. The sexual dimorphism in MHC composition may be responsible for any potential functional differences in contractile characteristics between sexes. The MHC composition of a fiber plays a role in determining the speed of shortening, with type Iib-containing fibers contracting the fastest and type IIX-, IIA-, and I-containing fibers contracting progressively slower (28). Power output, or the ability to move a load, is also correlated with MHC isoform composition (2). Compartments with a higher percentage of type Iib fibers would contract faster and with greater power output than those containing primarily type IIa or IIX fibers. The exact functional differences between sexes resulting from differences in MHC composition of the masseter are unknown at this time.

The regionalization observed in the distribution of muscle fiber phenotypes in this muscle is intriguing and indicates that the mouse masseter may be partitioned in the same fashion as both cat and rat triceps surae, as well as the rabbit masseter (13, 15, 37). These neuromuscular compartments are believed to function as mechanically distinct output elements in the neural control of movement. In the rabbit masseter, English et al. (11) showed that different compartments produce torques around the mandible with different trajectories. A previous investigation by Blanksma et al. (1) showed that the anterior portion of the human temporalis muscle is used more extensively than the posterior portion. Furthermore, Korfage and Van Eijden (21) showed that the anterior portion of the human temporalis muscle contained significantly greater portions of type I fibers compared with the posterior portion of the muscle. They concluded that these differences in fiber distribution might be related to differences in muscle function between the anterior and posterior portions of the muscle (21). In light of the regionalization of fiber types we observed in this study, it is tempting to speculate that the mouse masseter is also divided into distinct neuromuscular compartments with different functions. Each compartment would then be able to produce different mechanical actions on the mandible to provide for smooth, controlled movements. This hypothesis awaits further testing.

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