Histochemical Characteristics of the Masseter and Temporalis Muscles of the Rhesus Monkey (Macaca mulatta)

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ABSTRACT The histochemical characteristics, cross-sectional area and capillary of the skeletal muscle fibers of the anterior and posterior regions of the superficial masseter and the temporalis muscles are described for juvenile and adult rhesus monkeys of both sexes. Slow twitch fatigue resistant (S), fast twitch fatigue resistant (FR) and fast twitch fatigable (FF) fibers were found in varying proportions throughout the muscles; however some fibers with an intermediate myofibrillar ATPase activity were observed in the anterior masseter. No significant differences for any of the variables were found between male and female juveniles for a specific muscle sample site. However, considerable variation was found between juvenile and adult and between adult male and female monkeys in the percentages of different fiber types and the cross-sectional area of fibers in specific regions of the superficial masseter and temporalis muscles. We conclude from these observations that significant differences in function exist both within and between the different masticatory muscles of rhesus monkeys. Functional differences may result from the pronounced sexual dimorphism evident in the dentofacial complex of the rhesus monkey.

Mammalian skeletal muscles are composed of three distinct fiber types which can be identified histochemically. Physiological and histochemical characteristics have been correlated for the three fiber types of cat gastrocnemius muscle (Burke et al., '71). Although most skeletal muscles tend to be heterogeneous with respect to fiber type, the proportion and cross-sectional area of each type often varies widely both within and between muscles. Variation in the proportion of fiber types has been found between young and adult animals (Maxwell et al., '73), and for skeletal muscles both within and between species (Maxwell et al., '77).

Most research on the histochemical characteristics of muscle fibers has focused on the muscles of the appendicular skeleton; relatively little is known about the muscles of mastication. Recent studies of the masticatory muscles in laboratory animals (Taylor et al., '73; Schiaffino, '74), livestock (Suzuki, '77) and man (Ringqvist, '73, '74) demonstrate that, like most limb muscles, these muscles are generally heterogeneous with respect to fiber type. More importantly, however, these studies demonstrate that there are major differences between species in the proportion of the three fiber types comprising the muscles of mastication. Since muscular and skeletal components of the masticatory complex differ greatly amongst species of mammals (Turnbull, '70), considerable variation is expected in histochemical characteristics between the muscles of mastication of these species.

There is no previously reported study on the histochemical characteristics of the muscles of mastication of non-human primates. This is

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unfortunate since one species of monkey in particular, *Macaca mulatta* (rhesus monkey), is used extensively as an animal model for normal craniofacial growth and function in man, and as an experimental model for the investigation of alteration of craniofacial growth and form. The purpose of this study is to describe the histochemical characteristics, cross-sectional areas and capillary of fibers from the superficial masseter and the temporalis muscles in rhesus monkeys. Variability within the muscles is evaluated by reporting data from both anterior and posterior regions. Comparisons are also made between juvenile and adult monkeys of both sexes.

**METHODS**

Masticatory muscle of 12 juvenile, seven adult female and six adult male rhesus monkeys (*Macaca mulatta*) were studied. The monkeys were preanesthetized with ketamine (10 mg/kg im) followed by an intravenous injection of sodium pentobarbital to induce surgical anesthesia. The right superficial masseter and temporalis muscles were exposed and samples were excised from standardized sampling sites near the anterior and posterior borders of superficial lamina of these muscles (fig. 1). Samples were then quick frozen in isopentane cooled with dry ice for histochemical preparation.

Cross-sections 14 μm thick were cut from the frozen blocks in a cryostat at −20°C. Sections were incubated for succinic acid dehydrogenase (SDH) (Nachlas et al., '57), for myofibrillar ATPase (Chayen et al., '73) with or without preincubation for 15 minutes at pH 10.2, and for capillary membrane phosphatase (Maxwell et al., '77) activities (figs. 2-4). Without the preincubation at pH 10.2 fast twitch and slow twitch muscle fibers of the masseter could be differentiated with myofibrillar ATPase, but slow twitch fibers had a greater intensity of reaction than normally observed for slow twitch fibers (fig. 5). This is in contrast to the excellent differentiation observed without preincubation in temporalis muscle (figs. 11, 12). Preincubation at pH 10.2 inhibited the demonstration of activity of slow twitch fibers such that they were more easily distinguished from fast twitch fibers (figs. 7-10, 13-16). The myofibrillar ATPase activity
demonstrated by each type of muscle fiber was completely inhibited by fixation of sections in calcium formol and by inclusion of parahydroxymercuribenzoate (PHMB) in the incubation medium (fig. 4). A section of each muscle sample incubated for myofibrillar ATPase activity was projected at 1,000 × magnification and the outlines of individual fibers in each of three or four 25 cm × 40 cm sample areas were traced. At least 100 fibers were traced from each muscle. Fibers completely within the sample area and fibers which crossed the top and right boundaries and the top corners of the sample area were included in the sample. Fibers crossing the other boundaries and corners of the sample area were excluded. Each fiber was classified as fast twitch or slow twitch based on myofibrillar ATPase activity (Burke et al., '71; Peter et al., '72; Maxwell et al., '73, '77).

A serial section incubated for SDH activity was then projected, the same sample fibers were superimposed on the tracing, and these fibers were further classified as fatigue resistant or fatigable (Burke et al., '71; Maxwell et al., '77). Fibers classified as fatigue resistant had distinct SDH activity and subsarcolemmal aggregates of diformazan, especially near capillaries. Fibers which were classified as fatigable had very little SDH activity in subsarcolemmal regions of the fibers. Both localization and intensity of activity were used in determining fiber classification. Based on the characteristics of individual fibers in these two serial sections, slow twitch fatigue resistant (S), fast twitch fatigable (FF) and fast twitch fatigable (FF) fibers were identified. These fiber classifications correspond respectively to the slow twitch oxidative (SO), fast twitch oxidative glycolytic (FOG) and fast twitch glycolytic (FG) fibers described by Peter et al. (’72).

A third serial section incubated for capillary membrane phosphatase activity was projected and the locations of capillaries relative to the same sample fibers were drawn on the tracings. The number of capillaries within the sample area as well as the number of capillaries adjacent to each fiber in the sample were counted.

Fiber area was determined by planimetry. Mean fiber area, the percentage of each type of fiber in the sample (percentage composition), fibers per mm², capillaries per mm² and capillary to fiber ratio (capillaries per mm² ÷ fibers per mm²) were calculated. Data from the muscles within each group were pooled and statistical measures calculated. Differences between the means were tested by t-test and significance accepted at the P < 0.05 level.

RESULTS

**Masseter muscle**

Photomicrographs of representative anterior and posterior samples of masseter muscle are in figures 5-10. The percentage of each histochemical fiber type in the anterior and posterior samples of the superficial masseter muscles varied according to age and sex of the animal (table 1). In juvenile animals no significant differences were observed between males and females, so data from both sexes were pooled for analysis. In sections from the anterior region of the superficial masseter incubated for myofibrillar ATPase activity, a small (usually less than 4%) proportion of fibers displayed an intensity of reaction slightly greater than normally observed for slow twitch fibers, but markedly less than the intensity displayed by fast twitch fibers (see example in fig. 7). Since these fibers were more similar to slow twitch than fast twitch fibers in histochemically demonstrated characteristics, they were included with the slow twitch fibers for statistical analysis. However, there are no studies of contractile properties of these muscle fibers on which to confirm this classification. No intermediate ATPase fibers were observed in posterior masseter.

In samples from the anterior region of the superficial masseter muscles, adult females (fig. 7) had more S and less FF fibers than adult males (fig. 9). Samples from the posterior aspect of the superficial masseter muscle of adult males did not differ in percentage composition from those of juvenile animals, but adult females (fig. 8) had significantly more S fibers than juveniles (fig. 6). Differences in percentage composition of posterior samples of superficial masseter between adult males and adult females were not statistically significant. In each group, the posterior (figs. 8, 10) compared to anterior (figs. 5, 7, 9) samples of superficial masseter had significantly more fast twitch fibers and fewer slow twitch fibers.

Except for larger FF fibers in anterior masseter of adult females than juveniles, mean fiber area in anterior or posterior regions of superficial masseter muscle was not significantly different between adult females.
and juvenile animals (table 1). However, the mean area of both FR and FF fibers was significantly greater in either region of the muscle of adult males (figs. 9, 10) compared to adult females (figs. 7, 8) or juvenile (figs. 5, 6) animals. Further, there was a different inter-relationship between the relative size of the different fiber types in adult males as compared to the juvenile animals or adult females. Whereas FF fibers were significantly larger than S fibers in the adult males, FF fibers in muscles of adult females and of males were similar in size or smaller than S fibers. For the same fiber types in posterior compared to anterior sites of masseter muscle only for adult females.

**Temporalis muscle**

Representative photomicrographs of anterior and posterior temporalis muscle are in figures 11-16. In temporalis muscle, no fibers of intermediate ATPase activity were observed. As observed for masseter muscle, sex related differences in percentage composition and mean fiber area (table 1) were observed in the anterior region of temporalis muscle. Compared to juveniles, the muscles of adult males had significantly more FR fibers. There were significantly more S and less FF fibers in the posterior temporalis of adult females than of adult males. In the posterior region of temporalis muscles, there were no significant differences in percentage composition between juvenile and adult animals of either sex. Although the muscle fiber areas for muscles of adult females did not differ from those for juveniles, the fast twitch fibers in both regions of the temporalis muscles of adult males (figs. 15, 16) were several fold larger than in the muscles of either adult females (fig. 13,
HISTOCHEMICAL CHARACTERISTICS OF MASTICATORY MUSCLE FIBERS 393

TABLE 2
Capillary of masseter and temporalis muscles of juvenile and adult monkeys. (Data are mean ± standard error of the mean).

<table>
<thead>
<tr>
<th></th>
<th>Capillaries</th>
<th>Capillary to fiber ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of adjacent capillaries</td>
<td>S</td>
<td>FR</td>
</tr>
<tr>
<td>Anterior masseter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juveniles</td>
<td>4.49 ± 0.29</td>
<td>3.17 ± 0.30</td>
</tr>
<tr>
<td>Adult males</td>
<td>5.69 ± 0.39</td>
<td>4.35 ± 1.11</td>
</tr>
<tr>
<td>Adult females</td>
<td>5.58 ± 0.38</td>
<td>3.89 ± 0.75</td>
</tr>
<tr>
<td>Posterior masseter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juveniles</td>
<td>4.59 ± 0.39</td>
<td>3.85 ± 0.22</td>
</tr>
<tr>
<td>Adult males</td>
<td>4.79 ± 0.55</td>
<td>5.35 ± 0.19</td>
</tr>
<tr>
<td>Adult females</td>
<td>4.60 ± 0.47</td>
<td>3.60 ± 0.49</td>
</tr>
<tr>
<td>Anterior temporalis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juveniles</td>
<td>4.47 ± 0.27</td>
<td>3.17 ± 0.31</td>
</tr>
<tr>
<td>Adult males</td>
<td>4.43 ± 0.45</td>
<td>5.03 ± 0.51</td>
</tr>
<tr>
<td>Adult females</td>
<td>6.52 ± 0.80</td>
<td>4.29 ± 1.03</td>
</tr>
<tr>
<td>Posterior temporalis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juveniles</td>
<td>3.94 ± 0.35</td>
<td>3.63 ± 0.29</td>
</tr>
<tr>
<td>Adult males</td>
<td>3.79 ± 0.64</td>
<td>4.86 ± 0.41</td>
</tr>
<tr>
<td>Adult females</td>
<td>4.06 ± 1.03</td>
<td>3.99 ± 0.68</td>
</tr>
</tbody>
</table>

1 Adult monkeys significantly different from juvenile monkeys (P < 0.05).
2 Adult females significantly different from adult males (P < 0.05).
3 Posterior significantly different from anterior (P < 0.05).

14) or juveniles (figs. 11, 12). Within each group, there were more S fibers in anterior (figs. 11, 13, 15) than posterior (figs. 12, 14, 16) temporalis muscle samples. Compared to posterior samples, the anterior samples of temporalis muscle of juveniles and of adult females had significantly fewer FF fibers, but no significant difference in percentage of FR or FF fibers was observed between anterior and posterior temporalis of adult males. Mean fiber areas of anterior temporalis were not significantly different from those of posterior temporalis muscles.

There was a greater number of capillaries adjacent to S fibers of anterior temporalis of adult females than adult males. More capillaries were observed adjacent to FR and FF fibers in anterior temporalis and adjacent to FF fibers in posterior temporalis of adult males than in temporal muscles of juveniles (table 2). No significant differences were observed in capillary to muscle fiber ratio between sexes, between juveniles and adults or between anterior and posterior temporalis muscle samples (table 2). Slight differences in capillaries per mm² resulted from the differences in mean fiber area.

DISCUSSION

Individual fibers of the masticatory muscles of juvenile and adult rhesus monkeys exhibit histochemical characteristics which are consistent with the classification of S, FR and FF fibers used for other muscles and other species. Considerable intramuscular variation in the percentage of different fiber types occurs between the anterior and posterior regions of the superficial masseter and temporalis muscles of each age and sex group. Although the posterior region of each muscle is relatively similar in composition between groups, the anterior region of each muscle exhibits sex related variation. The anterior superficial masseter is comprised predominantly of slow twitch fibers, and the anterior regions of both masseter and temporalis have more S fibers than posterior regions of these muscles in monkeys of each age and sex category. These data are in contrast to the apparent lack of intramuscular variation in fiber type proportions reported for the masseter muscle in cattle, sheep, swine, dogs, guinea pigs and rats (Suzuki, '77) and in the masseter and temporalis muscle in the cat (Taylor et al., '73). However, in many previous studies the possibility of intramuscular variation in the proportion of fiber types has not been adequately considered. It is erroneous to characterize muscles as complex as the superficial masseter or the temporalis of the rhesus monkey on the basis of samples from a single anatomical region.

Differences in percentage composition in the anterior region of the superficial masseter
are evident between sexes and between age groups. There are more slow twitch fibers in anterior masseter and anterior temporalis muscles of adult females compared to juveniles. There is a significantly higher proportion of S fibers and lower proportion of FF fibers in the anterior portion of either muscle of females relative to males. Changes in overall histochemical composition of rat (Kugelberg, '76) and guinea pig (Maxwell et al., '73) soleus muscles have been observed with increasing age, and of rat soleus muscles in adaptation to alterations of hind limb function (Booth et al., '73). Thus, the differences we observed between juvenile and adult female monkeys may result from the conversion of some fast twitch fibers to slow twitch fibers during growth.

Masseter muscles of the rhesus monkey differ from limb muscles of monkeys and other species in the presence of some fibers with an intermediate level of myofibrillar ATPase activity. Fibers with intermediate intensity of myofibrillar ATPase activity have been previously reported for adult human masseter muscle (Ringqvist, '74) and have been observed in soleus muscles of growing rats (Kugelberg et al., '76). The physiological significance of intermediate myofibrillar ATPase activity in these fibers is not known, but could represent a transition of fibers from fast twitch to slow twitch or vice versa (Kugelberg et al., '76). Transitional fibers could occur in monkey masseter as a result of normal differences in jaw function associated with age and/or growth, or as a result of changes in intermaxillary relations or occlusal states. This possibility is consistent with the views of Ringqvist ('73; Ringqvist et al., '77) who found fibers with intermediate ATPase activity in the masseter muscle of adult but not fetal human subjects, and a greater percentage of intermediate ATPase fibers in adult humans with mandibular prognathism than in subjects with normal jaw relations. This interpretation differs from that of Taylor ('76), who would classify intermediate ATPase fibers described by Ringqvist as fast twitch fatigue resistant (FR).

Although the body weight of male and female juveniles is not significantly different, adult males weigh 50% more than adult females. This difference in overall body weight is reflected in differences between sexes in muscle fiber cross-sectional area. As male monkeys grow from juveniles to adults, muscle fibers hypertrophy several fold. Growth is not nearly so pronounced for the muscle fibers of female monkeys. Most of the difference in mean fiber area between the adult male and female animals occurs in the fast twitch fiber population. Whereas FR and FF fibers are approximately the same size in the muscle samples from juvenile as from adult female monkeys, these fast twitch fibers are 2- to 3-fold larger in the muscles of adult males than of juveniles. These differences in the growth of fibers result in easily observed differences in the histochemical appearance of muscle samples from adult males and females. While fast twitch fibers become larger than slow twitch fibers in the muscles of adult males, slow twitch fibers are as large or larger than fast twitch fibers in adult female muscles. Slow twitch fibers are also larger than fast twitch fibers in samples of human masseter muscles reported by Ringqvist ('74), who speculated that the reduced requirement for forceful chewing with modern foods impaired hypertrophy of fast twitch fibers. Such an explanation would not seem to explain completely the differences between masseter muscles of male and female monkeys, since in captivity both sexes receive the same diet.

A strong correlation has been reported for human masseter muscle between the size of fast twitch fibers and maximum bite force (Ringqvist, '74). Thus, it is reasonable to predict greater bite force for male than for female monkeys. Similarly, differences in mean area of some types of fibers in posterior compared to anterior samples of masseter muscles likely indicate differences in functional tension development by muscle fibers of these two sites. Only in posterior samples of superficial masseter muscles are there significant differences in capillarity between male and female monkeys. Data from numerous other muscles of many species indicate a remarkable similarity of capillarity between muscles of greatly differing functional abilities and maximum blood flow (Maxwell et al., '77; Plyley and Groom, '75). Thus, the small difference between sexes in capillarity for masticatory muscles in monkeys may not have great physiological significance.

The results of this study demonstrate the existence of intramuscular, age and sex variation in the composition of the masseter and temporalis muscles in the rhesus monkey. Differences in histochemical characteristics and
cross-sectional areas of specific fiber types indicate that functional and behavioral differences may exist both between the anterior and posterior regions of these muscles, and between the muscles of male and female monkeys. The masseter and the temporalis muscle of females develop relatively more slow fibers during growth at the expense of the proportion of both fast twitch fiber types. The muscles of adult male monkeys are characterized by the retention of a larger proportion of both fast twitch fiber types and by pronounced hypertrophy of the fast twitch fibers. The influence of motor neurons upon the characteristics of muscle fibers is well established (Henneman and Olsen, '56), and adaptation of muscle fiber characteristics is task specific (Gordon, '67; Kowalski et al., '69). Thus, a sex related difference in percentage composition of the anterior regions of the muscles and site dependent differences between anterior and posterior regions of the muscles may be indicative of important differences in the recruitment and function of these regions. The adaptive significance of these sex differences remains unclear, however, the masticatory muscles of the adult male monkey appear to be considerably more powerful than those of the adult female.

Extensive sexual dimorphism is evident in the craniofacial skeleton and in the dentition of rhesus monkeys. Adult female rhesus monkeys remain much more similar to juveniles in terms of craniofacial morphology than do adult males. At the time of adolescence, the craniofacial complex in male monkeys begins to manifest distinct secondary sex characteristics, including proportionally larger jaws and markedly larger canines (McNamara and Graber, ’75; McNamara et al., ’76). Such differences in skeletal and dental morphology coincide with differences we observe in muscle fiber characteristics, and may indicate variations in the biomechanics of jaw movement during mastication.

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LITERATURE CITED


Abbreviations

S, Slow twitch fatigue resistant    FF, Fast twitch fatigable
FR, Fast twitch fatigue resistant  PHMB, Parahydroxymercuribenzoate

PLATE I
EXPLANATION OF FIGURES

2 Photomicrograph of a cross section of a sample of anterior masseter muscle from an adult female rhesus monkey, incubated for succinic acid dehydrogenase activity. Example fibers are identified. Magnification is × 310.

3 Photomicrograph of a serial cross section from the same muscle, incubated for myofibrillar adenosine triphosphatase activity. Comparison of identified fibers with the same fibers in figure 2 will illustrate classification nomenclature. Magnification is × 310.

4 Photomicrograph of a serial section of the same muscle, incubated for capillary membrane phosphatase activity. Fixation of sections in Ca-formol and inclusion of PHMB in the incubation medium inhibit expression of activity by muscle fibers, but permit display of phosphatase activity in capillary endothelium. Magnification is × 310.
PLATE 2
EXPLANATION OF FIGURES

All figures in plates 2 and 3 are photomicrographs of muscle cross sections incubated for myofibrillar adenosine triphosphatase activity either with (figs. 7-10, 13-16) or without (figs. 5, 6, 11, 12) preincubation at pH 10.2, and are at the same magnification of × 120.

5 Anterior masseter from a juvenile monkey. Note few small, dark, fast twitch fibers.

6 Posterior masseter from a juvenile monkey, indicating higher proportion of fast twitch fibers than anterior masseter.

7 Anterior masseter from an adult female monkey. Fast twitch fibers are smaller than slow twitch fibers. A fiber with an intermediate activity is indicated (arrow).

8 Posterior masseter from an adult female monkey. Fast twitch and slow twitch fibers are similar in size.

9 Anterior masseter from an adult male monkey showing relatively large fast twitch fibers.

10 Posterior masseter from an adult male monkey. Note large fast twitch fibers.
PLATE 3
EXPLANATION OF FIGURES

11 Anterior temporalis from a juvenile monkey, showing higher proportion of fast twitch fibers than anterior masseter.

12 Posterior temporalis from a juvenile monkey.

13 Anterior temporalis from an adult female monkey.

14 Posterior temporalis from an adult female monkey.

15 Anterior temporalis from an adult male monkey. This section illustrates the very large fast twitch fibers observed in males and the higher proportion of fast twitch fibers in males than females.

16 Posterior temporalis from an adult male monkey.