Masticatory myosin unveiled: first determination of contractile parameters of muscle fibers from carnivore jaw muscles

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Toniolo L, Cancellara P, Maccatrozzo L, Patruno M, Mascarello F, Reggiani C. Masticatory myosin unveiled: first determination of contractile parameters of muscle fibers from carnivore jaw muscles. Am J Physiol Cell Physiol 295: C1535–C1542, 2008. First published October 8, 2008; doi:10.1152/ajpcell.00093.2008.—Masticatory myosin heavy chain (M MyHC) is a myosin subunit isoform with expression restricted to muscles derived from the first branchial arch, such as jaw-closer muscles, with pronounced interspecies variability. Only sparse information is available on the contractile properties of muscle fibers expressing M MyHC (M fibers). In this study, we characterized M fibers isolated from the jaw-closer muscles (temporalis and masseter) of two species of domestic carnivores, the cat and the dog, compared with fibers expressing slow or fast (2A, 2X, and 2B) isoforms. In each fiber, during maximally calcium-activated contractions at 12°C, we determined isometric-specific tension (P0), unloaded shortening velocity (v0), and the rate constant of tension redevelopment (KTR) after a fast shortening-relengthening cycle. At the end of the mechanical experiment, we identified MyHC isoform composition of each fiber with gel electrophoresis. Electrophoretic migration rate of M MyHC was similar in both species. We found that in both species the kinetic parameters v0 and KTR of M fibers were similar to those of 2A fibers, whereas P0 values were significantly greater than in any other fiber types. The similarity between 2A and M fibers and the greater tension development of M fibers were confirmed also in mechanical experiments performed at 24°C. Myosin concentration was determined in single fibers and found not different in M fibers compared with slow and fast fibers, suggesting that the higher tension developed by M fibers does not find an explanation in a greater number of force generators. The specific mechanical characteristics of M fibers might be attributed to a diversity in cross-bridge kinetics.

force; shortening velocity

MYOSIN, THE MOTOR OF MUSCLE CONTRACTION, is a hexamer composed of two heavy subunits (MyHC) and four light subunits (MyLC). MyHCs are responsible for ATP hydrolysis and interaction with actin, and the regulated expression of MyHC isoforms with distinct enzyme and motor kinetics is a major mechanism of modulation of muscle fiber contractile performance.

In mammals, at least nine distinct genes coding for MyHC isoforms are expressed in skeletal muscles (1). They are grouped in clusters located in different chromosomes and forming three distinct subfamilies. 1) The subfamily of fast isoforms comprises genes coding for three isoforms (2A, 2X, and 2B) expressed in adult fast fibers of limb and trunk muscles and genes coding for extraocular, embryonic, and neonatal isoforms. 2) The subfamily of cardiac isoforms is composed of two genes, coding for β/slow MyHC, expressed in cardiac muscle and in slow or type 1 fibers of skeletal muscles, and for α-MyHC, expressed in cardiomyocytes and in specialized skeletal muscles (masticatory, extraocular, and laryngeal), respectively. 3) Only the gene coding for the masticatory or M MyHC belongs to the third subfamily. This isoform represents a subfamily by itself not only because of the distinct chromosomal localization but also because sequence analysis carried out in cat, dog, and human shows a large diversity compared with all other MyHC isoforms (12, 15, 19); also phylogenetic comparisons point to its close link with MyHC isoforms present in distant animals as shark or crocodile and with nonmuscle isoforms (12, 19). Masticatory MyHC has been first identified in cat temporalis muscle, more precisely in posterior temporalis bundle where it is expressed in all fibers (23) and has been defined as “superfast” because contraction time parameters of the motor units of the cat temporalis muscle were faster than those recorded in fast motor units of the limb muscles (29). A well-developed sarcoplasmic reticulum and a high (2–3 times higher than fast fibers in limb muscles) ATPase activity have been also observed in temporalis fibers (23). The most accepted name “M" derives from the exclusive localization of the expression in the masticatory muscles (12, 22). M MyHC is expressed in muscles derived from the first branchial arch, i.e., jaw-closer muscles as masseter, temporalis, pterygoideus medialis and lateralis, and also tensor veli palatini and tensor tympani, although with pronounced interspecies differences (12, 22). It is not expressed in jaw-depressor muscles despite the common embryological origin. In carnivores, jaw-closer muscles are virtually composed only by M fibers (i.e., fibers expressing M MyHC) possibly associated with few slow fibers, whereas, in primates, slow fibers and 2A fast fibers are also present, and, in marsupials, M fibers coexist with fibers expressing α- and β-MyHC (11).

No comprehensive studies on structure and function of fibers expressing M MyHC are presently available. There are indications that M MyHC combines with specific masticatory myosin light subunits (MyLC) (18) and integrates in the sarcomeric structure together with other specific masticatory isoforms as masticatory α-tropomyosin (22) and masticatory myosin-binding protein C (10, 31, 33). Although ATPase activity has been measured and found high both in M fibers (24) and in M myosin preparations (23), more limited information is available on the mechanical properties. Preliminary
results based on the determination of \( f_{\text{min}} \) (the frequency at which the dynamic stiffness of the active fiber reaches a minimum) and reported by Hoh in his review (12) suggest that cat fibers expressing M MyHC are not faster than fibers expressing 2A or 2X MyHC. In a recent presentation at the Biophysical Society Annual Meeting, Reiser and Bicer (21) have confirmed that the raccoon M fibers are not faster but develop a significantly higher force compared with slow and fast 2A fibers.

In view of the uncertainty still existing about the physiology of the fibers expressing M MyHC, we sought to extend our work on the contractile properties of dog muscle fibers (30) to include fibers expressing M MyHC. In addition, we decided to repeat a similar analysis also in cat muscle fibers, with the aim to confirm or disprove the conclusion reached in dog using a second species of common domestic carnivore. MyHC isoforms of the cat have been electrophoretically separated (14, 28), and three isoforms, i.e., one slow and two fast, 2A and 2X, respectively, have been identified in trunk and limb muscle fibers, whereas no information is available on force and shortening velocity of the corresponding fiber types.

**MATERIALS AND METHODS**

**Sampling of dog and cat muscles.** Muscle samples were collected from adult dogs and cats euthanized in accordance with institutionally approved protocols at the Veterinary Clinics of the University of Padova. The care and use of all of the animals in this project were in accordance with institutionally approved protocols. The muscle fibers described in this study originated from the eight dogs mentioned in our recent paper (30) and from four cats.

The following muscles were dissected to obtain samples with different composition in myosin isoforms: 1) *longissimus dorsi, diaphragm, soleus* (only in cats), *tibialis anterior* (only in dogs), and *semimembranosus* taken as examples of trunk and limb muscles, 2) *temporalis* and *masseter* sampled as specific sources of M fibers.

Dissected samples were divided in small bundles immersed in ice-cold skinning solution (see *Single fiber mechanics*) with 50% glycerol and used for muscle fiber mechanics or frozen in isopentane cooled with liquid nitrogen for protein electrophoresis.

**Gel electrophoresis.** Muscle samples for protein electrophoresis were solubilized in Laemmli solution (Tris 62.5 mM pH 6.8, glycerol 10%, SDS 2.3%, β-mercaptoethanol 5%, with E-64 0.1% and leupeptin 0.1% as antiproteolytic factors). After being heated for 5 min at 80°C, appropriate amounts of the protein suspension were loaded onto polyacrylamide gels (about 1 μg of total protein/lane). For isofrom identification in single fibers, segments 1–2 mm long were solubilized in 20 μl of Laemmli solution, and 2–3 μl were loaded onto gels. MyHC isoforms were separated on 8% polyacrylamide slab gels according to the protocol described previously (30).

**MyHC concentration determination.** In a subset of fibers, MyHC concentration was measured with a method based on electrophoretic separation and densitometry, derived from those described by Geiger et al. (8) and by D’Antona et al. (6). Briefly, the MyHC concentration in individual fiber segment was obtained as the ratio between the protein content and the volume. The volume was determined as the product of the segment length by the cross-sectional area (CSA), which, to increase the precision of the measurement for this particular set of fibers, was calculated assuming an elliptical shape. Both length and CSA were measured while the fiber segment was mounted by means of small aluminum clips in the experimental set up, as described below. The two diameters of the ellipsoid were measured on a horizontal plane and on a vertical plane, respectively. The diameters on the horizontal plane were calculated as described below for CSA determination for mechanical studies, i.e., from video images taken at 400× magnification. The vertical diameter or depth was measured by carefully focusing on the top of the fiber and then on the bottom and measuring the distance between the two focus planes as proposed by Larsson and Moss (13). When the fiber segments were removed from the set up, care was taken to cut them along the edges of the clips and immerse them in precisely controlled volumes of Laemmli solution (10 μl) enriched with antiproteolytic factors (see above). After solubilization (5 min at 80°C), a known fraction of such volume (2–3 μl) was run on 10–20% polyacrylamide gradient gels together with known amounts of purified myosin (M-3889; Sigma, St. Louis, MO). Gels were then silver stained (Silver stain plus; Bio-Rad, Hercules, CA), and the brightness-area product (BAP, i.e., the product of the area of the band by the average brightness, subtracted local background after black-white inversion) of the MyHC band was determined with the accuracy of 1,200 dpi. The choice of the gradient gels was aimed to visualize all MyHC as a sharp single band, whereas the choice of silver staining was aimed to optimize the sensitivity of the method. The loading of known amounts of purified myosin provided the data to obtain, for each gel, a calibration curve that allowed the determination of the amount of myosin present in the fiber segment under investigation by interpolation. An example of calibration curve is depicted in Figure 6, A and B. As can be seen, the calibration curve showed a nonlinear trend over the whole range, likely due to saturation of the silver staining. Importantly, the amounts of MyHC from individual fiber segments fell in all cases in the initial steep part of the curve, where the sensitivity is higher. MyHC isoform composition of each fiber used for MyHC quantification was determined by 8% polyacrylamide gel electrophoresis on a second fraction of the sample.
Characterization of the contractile properties of single muscle fibers was carried out as recently described (30). Muscle fiber bundles were kept at 4°C in skinning solution without ATP mixed with 50% glycerol for no longer than 2 wk after sampling and, on the day of the experiment, were washed with ice-cold skinning solution containing ATP. Single fibers were manually dissected under a stereomicroscope (10–60× magnification) and bathed for 30 min in skinning solution containing 1% Triton X-100 to ensure membrane solubilization; fiber segments of 1–2 mm length were then cut, and light aluminum clips were applied at both ends.

Skinning, relaxing, preactivating, and activating solutions employed in single fiber experiments were prepared as previously described (3). The pH of all solutions was adjusted to 7.0 at the temperature at which solutions were used (12°C or 24°C). Protease inhibitors (E64 10 μM and leupeptin 40 μM) were added to all solutions.

The fiber segments were transferred to the experimental set up and, with the help of the aluminum clips, mounted between the force transducer (AME-801 SensorOne, Sausalito, CA) and the electromagnetic puller (SI, Heidelberg, Germany) equipped with a displacement transducer. Each fiber segment was mounted in relaxing solution at slack length and then stretched by ~20% to reach a sarcomere length close to 2.8 μm and at the same time avoid any increase in resting tension that might alter the results of the slack test protocol. A sarcomere length of 2.8 μm ensures that, during maximal isometric contraction, tension is developed at the upper edge of the plateau of the tension-length relation, taking into account that series compliance is extended by ~5% (see Ref. 30), and that shortening during slack test takes place over the plateau. Video images were collected at high (400×) magnification, and on such images sarcomere length and three diameters on the horizontal plane equally spaced along the segment were measured. CSA was calculated from the average of the three diameters assuming a circular shape without corrections for fiber swelling. As recently shown (5), the assumption of a circular shape leads to an overestimation of CSA by about 20%, without differences among fiber types. From relaxing solution, each segment was transferred into the preactivating solution for at least 2 min and then maximally activated by immersion in the activating solution (pCa 4.6). Relaxation was induced by returning the segment to relaxing solution, and the cycle activation-relaxation was repeated five or six times. During activation after maximal tension was achieved, a quick release (amplitude 7–15% of segment length) was performed to make the fiber slack and measure the time to tension redevelopment. From data of displacement and time to tension redevelopment, unloaded shortening velocity (v₀) and series elasticity were calculated according Edman’s procedure [slack test protocol (7)]. In a separate maximal activation, the quick release was followed within 15 ms by a relengthening to initial length, and the rate constant of the subsequent tension redevelopment (K_TR) was determined as described by Brenner and Eisenberg (4). For K_TR calculation, the curve of tension redevelopment was fitted with a single exponential. Isometric tension (P₀, i.e., isometric force normalized to CSA) was calculated from the drop of force, which followed a quick release of amplitude sufficient to make the fiber slack. At the end of the mechanical experiment, all fibers were stored in Laemmli solution for electrophoretic identification of MyHC isoforms.

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**Table 1. List of single fibers dissected and successfully characterized from dog and cat skeletal muscles**

<table>
<thead>
<tr>
<th></th>
<th>M</th>
<th>1-Slow</th>
<th>2A</th>
<th>2X</th>
<th>Hybrid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dog</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temporalis</td>
<td>34</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
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<td>9</td>
<td>11</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Longissimus</td>
<td>0</td>
<td>10</td>
<td>12</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Semimembranosus</td>
<td>0</td>
<td>4</td>
<td>35</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Tibialis Anterior</td>
<td>0</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td><strong>Cat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temporalis</td>
<td>53</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>15</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
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<td>6</td>
<td>45</td>
<td>5</td>
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<td>11</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

Fibers are classified on the basis of their MyHC isomorph composition and of their muscle of origin. Hybrid fibers were discarded and not used for mechanical analysis or for myosin quantification.

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**Fig. 2.** Cross-sectional area (CSA) of the cat (A) and dog (B) fibers classified according to muscle of origin and MyHC isoform composition. Values are means ± SE.
**Statistical analysis.** Data were expressed as means and standard errors. One-way ANOVA, followed by Newman-Keuls test, was used for comparison among fiber types. Statistical significance was set at \( P < 0.05 \). The Prism software (GraphPad, San Diego, CA) was utilized for linear and nonlinear fitting and statistical analysis.

**RESULTS**

A total of 345 fibers were dissected from cat and dog muscles and successfully characterized. Gel electrophoresis was employed to identify after the mechanical experiment which isoform was present in each muscle fiber segment. Figure 1 shows examples of MyHC isoform separation and identification in dog and cat muscles. The identification of canine MyHC isoforms was based on previously published evidence (2, 30) that shows that M MyHC migrates just above slow MyHC. Canine masseter and temporalis muscles express minor amounts of slow MyHC in addition to the predominant M MyHC (see Figure 1). For the cat, the three main isoforms were identified in agreement with previous studies (14, 28).

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The distribution of the fibers classified on the basis of their MyHC isoform composition is reported in Table 1. Only M fibers were dissected from cat masseter and temporalis, whereas, in dog temporalis, two hybrid fibers containing slow and M MyHC were found. In trunk and limb muscles, the most abundant fiber type was 2A in the dog (68% of the fibers analyzed) and 2X in the cat (52%). Both in longissimus and semimembranosus 2X fibers were predominant in the cat, whereas 2A were predominant in the dog. A few 2X fibers were found in feline diaphragm but none in canine diaphragm.

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**MyHC isoform expression**

Fig. 3. Kinetic parameters of single muscle fibers from cat and dog muscles classified on the basis of their MyHC isoform composition. Histograms on the left (A, C) show dog fibers, and histograms on the right (B, D) show cat fibers. Note that values from 2B fibers are shown for the dog, where pure 2B fibers were found in laryngeal muscles and mechanically characterized, whereas no 2B fibers were found in cat muscles. The upper histograms show the average (with SE) values of unloaded shortening velocity (\( v_o \)), and the lower histograms show average values of rate constant of tension development (\( K_{TR} \)). *Significantly different from type 1 fibers. &Significantly different from type 2X fibers. $Significantly different from type 2A fibers. Numbers of fibers for each group for dog were 21 slow fibers, 62 2A fibers, 11 2X fibers, 4 2B fibers, and 34 M fibers. Numbers of fibers for each group for cat were 17 slow fibers, 12 2A fibers, 29 2X fibers, and 50 M fibers.
Figure 2 shows the average values of CSA of the fibers studied in mechanical experiments classified on the basis of their MyHC isoform composition and muscle of origin. As can be seen, the thickness of M fibers was comparable to that of slow fibers and lower than that of fast fibers, particularly of 2X fibers.

Figure 3 shows the values of the kinetics parameters, which characterize various fiber types in dog and cat muscles at 12°C. For dog also the average values of a small group of 2B fibers dissected from laryngeal muscles are reported for the sake of comparison (see Ref. 30). No evidence of the presence of 2B fibers in cat muscles is hitherto available. In both animal species, slow fibers showed significantly lower $v_0$ and lower $K_{TR}$ than any fast fiber. In addition, a significant difference in $v_0$ and $K_{TR}$ values was found between 2A and 2X fibers in the cat but not in the dog. Importantly, in both animal species, the kinetic parameters $v_0$ and $K_{TR}$ of M fibers were virtually identical to those of 2A fibers.

The values of isometric tension, i.e., force normalized to CSA, $P_0$, are reported in Fig. 4. In both species, the isometric tension generated by M fibers was significantly greater than that of any other fiber type.

To explore the possibility that a differential sensitivity to temperature was responsible for the higher isometric tension developed by M fibers, a separate set of cat fibers was studied at 24°C. The results are shown in Fig. 5. As can be seen, M fibers developed significantly higher tension than slow and 2A fibers also at 24°C, but the difference with 2X fibers was below the statistical significance. The ratio between the isometric tension developed at 24°C and that developed at 12°C ranged between 1.67 for M fibers, 2.10 for 2X fibers, 1.82 for 2A fibers, and 1.32 for slow fibers. A pronounced diversity in the kinetic parameter $K_{TR}$ was present at 24°C among the four fiber types, 2X fibers showing the highest value, slow fibers the lowest, and 2A and M fibers similar intermediate values. The ratio between $K_{TR}$ values at 24°C and those measured at 12°C ranged between 1.94 and 3.33.

In the fibers studied in mechanical experiments at 24°C, myosin concentration was determined to ascertain whether the diversity in $P_0$ values among fiber types could be ascribed to different myosin concentrations. The results are reported in Fig. 6C and show that no significant difference was present among the four groups of fibers studied.

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**Figure 5.** Isometric tension ($P_0$) (B) and rate of tension redevelopment ($K_{TR}$) (A) measured in cat single fibers at 24°C. Values are means ± SE. *Significantly different from type 1 fibers. &Significantly different from type M fibers. M fibers, $n = 23$; 2X fibers, $n = 23$; 2A fibers, $n = 9$; slow or 1 fibers, $n = 8$.

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**Figure 4.** Isometric tension ($P_0$) developed by single muscle fibers from cat and dog muscles classified on the basis of their MyHC isoform composition. A: dog fibers. B: cat fibers. Note that values from 2B fibers are shown for the dog, where pure 2B fibers were found in laryngeal muscles and mechanically characterized, whereas no 2B fibers were found in cat muscles. Values are means ± SE. *Significantly different from type 1 fibers. &Significantly different from type M fibers. Number of fibers for each group is the same as in Fig. 3.
was also similar in M fibers and 2A fibers. In contrast, the values of isometric tension, $P_o$, of M fibers were in both species higher than those of any other fiber type.

A second element of novelty in this study is given by the data on the contractile parameters of the fibers of cat trunk and limb muscles. Interesting comparisons can be made with muscle fibers of other species and in particular of the dog. In feline fibers, $v_o$ increases regularly from slow to fast 2A to fast 2X as expected from previous observations in several species (17, 30). Only minor diversity in $v_o$ values exists between corresponding fibers of the dog and the cat. According to the scaling principle (17, 30), similar values of kinetic parameters should imply an approximately similar body size. Actually, whereas the body mass of adult cats is rather consistent in all breeds, a large diversity in body mass is present among various dog breeds [see discussion in Toniolo et al. 2007 (30)]. It is thus uncertain which body size of the dog must be considered for interspecies comparison. Furthermore, although present results are far to be conclusive in this respect, cat muscles are expected to be faster because they are more rich in 2X fibers, whereas 2A fibers are predominant in dog muscles.

Muscles and muscle fibers are classified as fast or slow in relation to a number of distinct functional parameters: rate of tension rise and decline during isometric twitch, speed of shortening at low or zero load, and rate of ATP hydrolysis, just to mention a few. Jaw-closer muscles of carnivores have been defined fast or superfast muscles on the basis of the isometric twitch kinetics (29). They have also been considered fast in relation to the rate at which ATP is hydrolyzed by myosin alone (23) or by myosin integrated in skinned fibers (24). The parameters that characterize the contraction kinetics in maximally activated skinned fibers ($v_o$ and $K_{tr}$) determined in the present study show, in substantial agreement with the available preliminary evidence on raccoon fibers (21) and on cat fibers (12), that M fibers are as fast as 2A fibers. A peculiar feature of M fibers is their ability to generate high specific tension (i.e., force normalized to CSA, $P_o$). The present results show that M fibers can achieve $P_o$ values higher than any other fiber type at 12°C and also at 24°C. Skinned fibers become unreliable if activated at higher temperatures unless specific methods as temperature jumps after activation at low temperature are applied (20). It will be of interest to assess whether the difference in tension development is still present at physiological temperature.

The possibility that the difference in specific tension was due to a different density of the force generators, i.e., the myosin heads, was explored by determining myosin concentration in feline muscle fibers. The values obtained of myosin concentration in M fibers are around 50 &/l, corresponding to a head concentration of 200 $\mu$M in good agreement with previously published data (8, 32). The lack of any significant difference in myosin concentration among the four groups of fiber examined gives support to an explanation on the basis of kinetic diversity. Such an explanation leaves open the question whether the difference in specific tension still exists at physiological temperature as the kinetic parameters of the cross-bridge cycle are characterized by distinct temperature sensitivity (16).

The explanation of the higher tension developed by M fibers in terms of cross-bridge cycle can be based on the assumptions that unitary force exerted by an attached cross bridge is constant and that isometric tension is determined by the frac-
tion of strongly bound cross bridges. Huxley’s 1957 cross-bridge model predicts that the fraction of cross bridges attached is given by $f/(f + g)$, whereas $K_{TR}$ is given by $(f + g)$. The direct comparison between M fibers and 2A fibers shows that in jaw muscles the specific isometric force is 40% greater in M than that in 2A fibers while the $K_{TR}$ is unchanged. To fit with Huxley’s model, the elevated isometric force must result from an increase in cross-bridge attachment rate ($f$) and an equivalent diminution in the rate of cross-bridge detachment ($g$) so that $f/(f + g)$ rises, but $K_{TR}$ does not change. It is worth underlining that difference in $g$ values might be restricted to the detachment rate in isometric conditions since $v_0$, which is proportional to the detachment rate constant during filament sliding at zero load, displays similar values in M fibers and in 2A fibers.

An important caveat must be considered before entering in any further discussion on the explanation of the specific features of M fibers: in M fibers, not only MyHC but also other proteins are present with specific masticatory isoforms, among them MyLC, tropomyosin, and C protein (10, 18, 22). Available evidence confirms, however, that MyHC isoforms dictate both $v_0$ and ATP consumption rate with only a minor modulatory effect of MyLC isoforms (see Refs. 17 and 25). It is, therefore, tempting to find the basis of the specific contractile parameters of M fibers in the amino acid sequence of M MyHC. In a recently published study (15), we have compared the primary structure of M MyHC with other sarcomeric MyHC isoforms in the dog, paying particular attention to those regions where the interisoform diversity is greater. Loop 1 has been shown to modulate filament sliding velocity and ADP release rate (27) and is very short in M MyHC with only 8 residues compared with 12 in slow MyHC and 14 in 2B MyHC (15). The origin of the low detachment rate constant suggested by the above discussion on the actomyosin interactions might be found in the short length of loop 1, which controls ADP release. On the actin-binding surface, loop 2 has been proposed as determinant not only for the diversity of ATPase rate between isoforms but also for the specific sensitivity of ATPase to alkali or acid preincubation (15). M MyHC exhibits a very short loop 2 (17 residues instead of 23 present in most fast MyHC), whereas loop 3 is 1 residue longer in M MyHC than in other sarcomeric MyHC isoforms.

In his review article, Hoh (12) suggests, “Early during mammalian radiation, some taxa (carnivores, chiropterans, primates, most marsupial orders) retained masticatory myosin expression where high force and power in jaw closers remained functionally advantageous to their life style. Others (rodents, ungulates, rabbits) replaced masticatory myosin with functionally more appropriate isoforms normally expressed in limb muscles or the heart.” It is, however, possible that the explanation is more complex since, as shown by the present results, the combination of high force and moderate velocity values should generate a peak power not much different in M fiber compared with other fast fibers. It is possible that presence or the absence of M MyHC expression depends on the combination of the evolutionary pressure related with feeding habits, the presence of mutations in the coding or regulatory part of the gene, and the specific programs of gene expression based on embryological origin (first branchial arch). In this respect, the observation that M MyHC is expressed also in muscles without any masticatory function (tensor veli palatini) (22) and in satellite cells of masticatory muscles transplanted in limb muscles (9) plays in favor of a major role of the embryological origin. The mutations in human M MyHC gene documented by Stedman (26) also bring support to the view of a complex interaction of several factors, and it will be of interest to study the mechanisms that, in species as the rodents, have led the complete disappearance of M MyHC expression.

REFERENCES