Histoenzymology and Morphometry of the Masticatory Muscles of Tufted Capuchin Monkey (Cebus apella Linnaeus, 1758)

By

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- Received for Publication, October 27, 1999 -

Key Words: Histoenzymology, Tufted capuchin monkey, Masseter, Temporal muscle, Digastric muscle, Masticatory muscles, Fiber types

Summary: Samples of the anterior and posterior regions of the masseter and temporal muscles and of the anterior belly of the digastic muscle of 4 adult male tufted capuchin monkeys (Cebus apella) were removed and stained with HE and submitted to the m-ATPase reaction (with alkaline and acid preincubation) and to the NADH-TR and SDH reactions. The results of the histoenzymologic reactions were similar, except for acid reversal which did not occur in fibers of the fast glycolytic (FG) type in the mandibular locomotor muscles. FG fibers had a larger area and were more frequent in all regions studied. No significant differences in frequency or area of each fiber type were detected, considering the anterior and posterior regions of the masseter and temporal muscles. The frequency of fibers of the fast oxidative glycolytic (FOG) and slow oxidative (SO) types and of FOG area differed significantly between the anterior belly of the digastic muscle and the mandibular locomotor muscle. The predominance of fast twitch (FG and FOG) fibers and the multipenniform and bipenniform internal architecture of the masseter and temporal muscles, respectively, are characteristics that permit the powerful bite typical of tufted capuchin monkeys.

The skeletal muscle system represents the major organic system of the human body and each muscle is specialized for the generation of force and movement in a specific manner and direction (Stal, 1994). The orofacial region is of great physiological and psychosocial importance for human beings. The face is the major structure responsible for the expression of emotions and communication and the mouth is the first route of satisfaction for newborn infants and young children. Its physiological importance is demonstrated by the rich sensory innervation of face and mouth and by the great cortical somatotopic representation. Thus, the orofacial region is characterized by a complex organization of muscles which are involved in a variety of complex movements such as mastication, speech, deglutition, respiration and facial expression (Stal, 1994). Despite the importance of this region, available histoenzymologic data about skeletal muscles mainly concern the trunk and limbs (Ringqvist, 1971; Maxwell et al., 1979; Mao et al., 1992; Stal, 1994).

Data about the histoenzymology of limb and trunk muscles should not be extrapolated to masticatory muscles in view of the various differences between these muscle types (Mao et al., 1992; Tuxen et al., 1992). As an example, mandibular muscles have been shown to contain whole bundles or large groups of densely packed fibers of the same histochemical type. A similar finding in limb muscles suggests denervation followed by reinnervation (Dubowitz and Brooke, 1973). In human mandibular muscles, type II fibers have considerably smaller diameters than type I fibers (Ringqvist, 1974a, b; Taylor, 1975; Eriksson et al., 1981, 1982). Furthermore, all fiber types present smaller areas in masticatory muscles than do the corresponding types in muscles of the lower limbs (Ringqvist, 1971, 1974a; Eriksson and Thornell, 1983). The mandibular muscles of some species contain a

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larger proportion of type IIC fibers (Eriksson et al., 1981, 1982; Eriksson and Thornell, 1983), which are scarce in limb muscles (Brooke and Kaiser, 1970; Dubowitz and Brooke, 1973). Different specific changes between mandibular and limb muscles occur during aging, probably owing to genetic differences, functional performance and hormonal influence (Monemi, 1998). The mandibular locomotor muscles are phenotypically much different from the somatic muscles of trunk and limbs (Hoh, 1992), the mandibular locomotor muscles of the cat present fibers that contract isometrically twice as fast as fast-twitch fibers of limb muscles (Hoh, 1991), and the mandibular muscles can rapidly adapt to the functional demand by altering the composition of their fibers (Sfondrini, 1996).

Non-human primates have been used in biomedical research since the times of Aristotle (Moulias and Berat-Muller, 1968). The phylogenetic proximity of these animals to man (Lapin, 1972) and their marked anatomical, biochemical and behavioral similarities (Zamecnik, 1976) have caused simians to be important animals for research that benefits human beings. However, up to 1979 there were no reports on the histoenzymologic characteristics of the masticatory muscles of non-human primates (Maxwell et al., 1979).

Some monkeys are omnivorous like man and a basic similarity exists between the masticatory muscles of man and of Rhesus monkeys in terms of electromyographic pattern (McNamara, 1974).

Old World primates were the first to be utilized in biomedical research but New World monkeys, among them tufted capuchin monkeys (Cebus apella), later became of high interest to researchers, especially during the last three decades (Szabuniewicz, 1971).

Although the masticatory musculature of tufted capuchin monkey has been described in terms of morphology (Oliveira et al., 1977; Madeira and Oliveira, 1979), there are no reports of its histochemical characteristics.

Thus, considering that histoenzymology is an indispensable method for the evaluation of muscle tissue, that the musculature of the orofacial region is of great importance for human beings, that the information about the histoenzymology of masticatory muscles is scarce, and that non-human primate are greatly similar to human beings, we undertook the present study in order to describe the histological and histoenzymologic characteristics, as well as the frequency and area of fiber types in the masticatory muscles of tufted capuchin monkey.

Material and Methods

Four tufted capuchin monkeys (Cebus apella Linnaeus, 1758) were anesthetized with sodium thiopental (30 mg/kg body weight) (Abbott Laboratorios do Brasil Ltda.) and samples were removed from the anterior and posterior regions of the masseter and temporal muscles and from the anterior belly of the digastic muscles. The animals were identified as adult males according to the criteria proposed by Hoppenheimer (1981). After surgery, the animals were identified, held in captivity until they had fully recovered, and then returned to the habitat from which they had been removed.

The muscle samples were kept at room temperature for 15 minutes according to the method of Khan (1977) and then covered with neutral teflon and frozen in liquid nitrogen by the method of Werneck (1981). The frozen samples were kept at 

20°C in a cryostat chamber for one hour as indicated by Pullen (1977) and cut into 10 μm thick sections. The sections were stained with HE (Behmer et al., 1976) and submitted to the m-ATPase reaction with alkaline and acid preincubation by the method of Padykula and Herman (1955), to the nicotinamide adenine dinucleotide tetrazolium reductase reaction (NADH-TR) by the method of Pearse (1968) modified by Dubowitz and Brooke (1973) and to the succinate dehydrogenase (SDH) reaction by the method of Nachalas et al. (1957) modified by Wegman and Tordet-Coidroit (1960).

The slides were photographed with a Nikon photomicroscope at 60× magnification. On the basis of the photographs, the fibers were classified as fast-twitch glycolytic (FG), fast twitch oxidative glycolytic (FOG) and as slow twitch oxidative (SO) fibers as proposed by Peter et al. (1972).

The area of at least 100 muscle fibers from each region was measured with the aid of a Digigraf digital board, model Renoir, coupled to a computer. The area of each fiber was measured three times and the mean and standard deviation of the three measurements was calculated. The mean was considered only when the standard deviation was less than 5%.

The data about the frequency and area of each fiber type were submitted to analysis of variance for fully randomized experiments and for a fully randomized block. The differences were considered to be significant at the 5% level of significance when the calculated F value was higher than the tabulated value. The significance of variance was analyzed by the F test of Snedecor and Cochran (1980). When significant differences were detected, the Tukey test was used to determine differences in frequency and area within fiber types, between
fiber types, and within and between the muscle regions studied. Differences were considered to be significant at the 5% level.

**Results**

The muscle fibers of the samples from different regions of the masseter, temporal and digastric muscles showed similar histological characteristics, i.e., they were polygonal in most cases, with pe-

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**Fig. 1.** Reactions performed on the anterior belly of the digastric muscle of tufted capuchin monkey. 1a – alkaline m-ATPase; 1b – acid mATPase; 1c – NADH-TR; 1d – SDH; 1e – HE. Mf = muscle fiber, n = nucleus, e = endomysium, p = perimysium, FG = fast-twitch glycolytic fiber, FOG = fast-twitch oxidative and glycolytic fiber, and SO = slow oxidative fiber.
ripheraly located nuclei and with a small amount of connective tissue. However, some fibers were spherical and others were elliptical, with varying sizes. With few exceptions, we observed cells in which the nucleus was dislocated to the central region, as shown in Figs. 1A and 2A.

The histoenzymologic characteristics of the fibers of the anterior belly of the digastric muscle are presented in Table 1 and in Figs. 1B, C, D, E and F. The samples from the anterior and posterior re-

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**Fig. 2.** Reactions performed on the mandibular locomotor muscles (masseter and temporal muscles) of tufted capuchin monkey. 2a – alkaline m-ATPase; 2b – acid mATPase; 2c – NADH-TR; 2d – SDH; 2e – HE. Mf = muscle fiber, n = nucleus, e = endomysium, p = perimysium, A = FG – fast-twitch glycolytic fiber, B = FOG – fast-twitch oxidative and glycolytic fiber, and C = SO – slow oxidative fiber.
gions of the masseter and temporal muscles presented similar results to those obtained for samples from the anterior belly of the digastric muscles in all reactions, as shown in Figs. 2B, C, D, E and F. However, no acid reversal occurred in FG fibers in the mandibular locomotor muscles.

The fibers of the masticatory muscles were classified into FG, FOG and SO according to the criteria of Peter et al. (1972). Table 2 shows the frequency of the different fiber types in the masticatory muscles.

Analysis of variance showed a significant difference in the frequency of the different fiber types and the Tukey test showed a significant difference between the FG and SO and FOG and SO fiber types.

The results obtained for the mandibular locomotor muscle were similar both for the anterior and posterior regions and for the muscle as a whole. Analysis of variance showed a significant difference in the frequency of FG and FOG fibers between the belly of the digastric muscle and the mandibular locomotor muscle of the animals. In contrast, no difference in frequency of SO fibers was detected between the analyzed muscles.

The calculation of the mean area of the different fiber types in the anterior belly of the digastric muscle and in the mandibular locomotor muscles are presented in Table 3. Analysis of variance showed a difference in the mean areas of the different fiber types and the Tukey test showed that this difference was between FG and SO fibers and between FOG and SO fibers.

In the masseter regions and in the muscle as a whole, analysis of variance showed a difference in the mean areas of the different fiber types, but the Tukey test did not permit the identification of which fiber types differed from one another.

In the temporal muscle region and in the muscle as a whole, analysis of variance showed a difference in the mean areas of the various fiber types and the Tukey test showed that this difference was between

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<th>Table 1. Histoenzymologic characteristics of the fibers of the anterior belly of the digastric muscle of tufted capuchin monkey</th>
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<th>Table 2. Frequency (N) and percentage (%) of FG, FOG and SO fibers in the masticatory muscles of tufted capuchin monkey</th>
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<th>Table 3. Mean areas (±SD) in μm² of fibers of the FO, FOG and SO types in the masticatory muscles of tufted capuchin monkey</th>
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FG and FOG fibers and between FG and SO fibers. In the anterior belly of the digastic muscle and in the mandibular locomotor muscles, no significant difference in mean area was detected between FG and SO fibers. However, a significant difference in FOG fibers was detected between the anterior belly of the digastic muscle and the mandibular locomotor muscles.

On the basis of the results obtained, we may state that the mandibular locomotor muscles of tufted capuchin monkeys are similar in terms of histological and histoenzymologic characteristics. However, they differ from the anterior belly of the digastic muscle in some aspects, especially in frequency and mean area of fiber types.

Discussion

The results obtained in histoenzymologic studies of the masticatory muscles are difficult to compare due to the use of different histochemical reactions such as succinate dehydrogenase (SDH) (Tsukamoto and Mori, 1966), Sudan black (Hiiemae, 1971), m-ATPase plus succinate dehydrogenase (Bennet et al., 1977; Roxk et al., 1984); m-ATPase plus NADH-TR (Clarck and Luschei, 1981; Eriksson et al., 1982), and due to the various criteria for fiber type classification (I, IIA and B; FG, FOG and SO; A, B and C; FF, FR and S). However, the classification of Peter et al. (1972) was used here since the fibers of the mandibular locomotor muscles and of the digastic muscle of capuchin monkeys have histoenzymologic characteristics more compatible with this classification.

The anterior belly of the digastic muscle of tufted capuchin monkeys presented a mosaic pattern in terms of fiber type distribution, similar to that observed in other animal species (Tsukamoto and Mori, 1966; Roxk et al., 1984; Maxwell et al., 1981). The fiber type most frequently detected was FG, in agreement with data reported for rabbits and cats (Tsukamoto and Mori, 1966) and for man by Eriksson et al. (1982). However, this was in contrast to data reported for Macaca fascicularis (Clarck and Luschei, 1981) and for Macaca mulatta (Maxwell et al., 1981), in which SO type fibers were more frequent. Considering that in tufted capuchin monkeys the amount of type II fibers (FG and FOG) is elevated (88%), we may state that these fibers are associated with rapid mouth-opening movements (Matthews and Smith, 1972; Taylor and Cody, 1973). These data, however, are presumably imprecise. The anatomical constitution of the temporomandibular joint of the tufted capuchin monkey permits little or absent lateral movement of the mandible, possibly justifying the lower presence of SO fibers, which are involved in postural and movement-orienting activities.

According to Rowerson et al. (1983), in primates the digastic muscle also has the task of elevating the hyoid bone and consequently acts in deglutition. Oliveira et al. (1981) detected an active participation of this muscle during the second phase of mouth closing in tufted capuchin monkeys (co-contraction) for the stabilization and more suave execution of the end of this movement. Considering that the anterior belly of the digastic muscle of tufted capuchin monkeys also contains a high percentage of fibers with oxidative metabolism (FOG and SO) (60.45%), which are more resistant to fatigue, this fact is in agreement with data reported by others about the participation of this muscle in other activities in addition to mouth opening. The areas of FG fibers were larger than the areas of FOG fibers, which in turn were larger than the areas of SO fibers.

In the mandibular locomotor muscle of tufted capuchin monkeys, FG fibers did not present acid reversal. A similar fact was reported by Ringqvist (1974a) and by Ringqvist et al. (1977) for the human masseter, i.e., type II fibers were alkaline and acidstable. This characteristic was also observed in the masticatory muscles of other animals such as rabbits (Schiaffino, 1974), Rhesus monkeys (Maxwell et al., 1980) and pigs (Suzuki and Cassen, 1980).

The data about the percentage of different fiber types in the masseter muscle are variable. Type I fibers have been variously detected as the majority (Eriksson, 1982; Eriksson and Thornell, 1983), or present in the same amounts as type II fibers (Serratrice et al., 1976; Boyd et al., 1984), or in smaller amounts (Ringqvist, 1971, 1974c, 1982). This lack of uniform results may be explained by the wide variety of the material studied and of the sites of sample removal (Tuxen et al., 1992), since the masseter has shown regional variations (Serratrice et al., 1976; Eriksson, 1982; Eriksson et al., 1983) in addition to variation in functional demand according to the feeding habits of the animals.

FG fibers were present in larger amounts in the anterior and posterior regions of the masseter of the tufted capuchin monkey, in agreement with previous studies on this muscle (Ringqvist, 1971, 1973, 1974a; c; Serratrice et al., 1976) and on the mandibular muscle of carnivores (Mao et al., 1992). However, discordance exists among primates since in the masseter of Macaca mulatta type I fibers were found to correspond to 60% of the total (Maxwell et al., 1979).

According to Mao et al. (1992), type I fibers are
postural, are organized into smaller motor units and are responsible for more precise movements performed with low force. According to these investigators, there is a perceptible predominance of type I fibers in the anterior region of the human masseter close to the first molar. These fibers serve to provide precision of the bite since they are closer to the point of force application.

The fibers located in the posterior region of the masseter are closer to the temporomandibular joint and therefore elevate the mandible more rapidly than anterior fibers. In the posterior region of this muscle there is a predominance of type II fibers which contract rapidly and produce explosive force, thus providing a greater velocity of mandible elevation, although with a lower precision of the movement (Mao et al., 1992). This regional difference was not observed in the superficial portion of the masseter of tufted capuchin monkeys, a finding possibly justified by the morphological characteristics of the temporomandibular joint of this primate.

With respect to the area, FG fibers had a wider diameter than FOG fibers, which were larger than SO fibers, i.e., there was a predominance of type II fibers over type I fibers, as is the case for carnivores (Mao et al., 1992) and for Macaca mulatta (Maxwell et al., 1979). The inverse occurs in man, with a predominance of type I fibers over type II fibers. This may possibly be explained by the fact that man is increasingly eating soft foods requiring a greater participation of type I fibers and inducing atrophy of type II fibers (Mao et al., 1992).

The morphological characteristics of the human temporomandibular joint permit wide lateral movements of the jaw. Type I fibers, in addition to having a postural activity, must participate in the orientation of the mandible during the first phase of elevation, thus permitting type I fibers, responsible for force, to act in a more effective manner. The more elevated cusps of human teeth may be responsible for a process of adjustment at the end of the elevation movement, orienting in a more precise manner the positioning of the mandible by occlusal overlapping.

The temporal muscle of capuchin monkeys is the bipenniform type (Madeira and Oliveira, 1979) and therefore presents characteristics of velocity and power. FG fibers predominate both in terms of percentage and area both in the anterior and the posterior region, followed by FOG fibers and finally by SO fibers. Thus there is a wide predominance of type II fibers which are active in fast contractions. These two factors, a bipenniform muscle and a predominance of type II fibers (FG and FOG), are necessary characteristics for the potent and rapid bite of capuchin monkeys.

Differences in the percentage and area of the different fiber types between the anterior and posterior regions of the temporal muscle have been reported in the literature (Hiemae, 1971; Maxwell et al., 1979; Eriksson and Thorne, 1983; Gorniak, 1986). A predominance of type II fibers in the temporal muscle of capuchin monkeys agrees with data reported in previous studies (Ringqvist, 1971; Mascarello et al., 1979; Vignon et al., 1980; Gorniak, 1986). Information about the masseter muscle of herbivores is conflicting, with some authors stating that the amount of white and red fibers is identical (Baker and Laskin, 1986) and others reporting a prevalence of slow-twitch fibers (Mascarello et al., 1979).

A relationship is believed to exist between the fiber type predominating in masticatory muscles and the diet of the animal, or the way the animal obtains its food (Baker and Laskin, 1969; Suzuki, 1977; Rowlerson et al., 1982, 1983).

In its natural habitat, the tufted capuchin monkey uses its maxillaries to extract food from tree branches and from fibrous fruits, as well as to defend itself. Thus it needs a powerful bite, which justifies the greater presence of type II fibers in its mandibular locomotor muscles.

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