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Expression of alpha-cardiac myosin heavy chain in mammalian skeletal muscle

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Abstract. We have investigated the reactivity of different human, rat and cat muscles to a monoclonal antibody directed against human α-cardiac myosin heavy chain. We have found that special fiber subpopulations of human masseter and extraocular muscles, as well as the bag fibers of human, rat and cat muscle spindles, were reactive to this antibody, indicating that these fibers expressed α-cardiac myosin heavy chain or a closely related isoform. This isomyosin was present in the spindle bag fibers at early fetal stages, whereas its expression in masseter and extraocular muscle fibers was not detected during the first 22 weeks of gestation. Our results add to the list of muscle proteins which are expressed in locations or at developmental stages other than those initially described, suggesting that a revision of the present nomenclature of the subgroups of myosin heavy chains might be considered in the future.
Key words. Masseter; extraocular muscles; muscle spindle; bag fibers; immunocytochemistry; human.

The myosin heavy chain (MHC) is the main component of the sarcomeric thick filament, and different MHC isoforms have been identified[1]. These MHCs are encoded by a large multigene family and their expression is tissue-specific and developmentally regulated[2]. Recent data suggest that some MHCs are expressed in tissues or at developmental stages other than those in which they were initially characterized: the adult human masseter has been shown to express fetal MHC[3], and an α-cardiac like MHC is present in nuclear bag fibers of rat muscle spindles[4]. Histochemical[5,6] and immunocytochemical[3,7] data suggest that the human masseter muscle has a complex myosin composition. The extraocular muscles also have a special MHC composition, including the expression of slow tonic MHC[8–11] and a tissue-specific fast MHC[1,11,12]. The possible expression of hitherto unknown MHC isoforms has not been excluded. We have therefore investigated the reactivity of a variety of muscles to a monoclonal antibody raised against α-cardiac MHC[13,14]. In this study we have demonstrated the expression of α-cardiac MHC in a subpopulation of extrafusal fibers, both in human masseter and extraocular muscles, and in the bag fibers of human, rat and cat muscle spindles.

Material and methods

Muscle samples from the extraocular muscles of two adults and from the masseter and the biceps muscles of four adults, three 7- to 9-week-old, one 3-month-old and four 4- to 7-year-old healthy human subjects were collected one to three days after death[15]. Masseter, extraocular and limb muscles were collected from human fetuses, obtained from legal abortions at 14-, 18-, 20- and 22 weeks of gestation. The investigation was approved by the Medical Ethical Committee of the University of Umeå. The diaphragm and the masseter muscles of two adult Sprague-Dawley rats and the masseter of an adult cat were collected under sodium pentobarbital anesthesia.

Muscles were rapidly frozen in propane chilled in liquid nitrogen. Serial frozen cross-sections were cut in a Reichert-Jung (Nussloch, Germany) cryostat at −20 °C. The sections for demonstration of myofibrillar ATPase activity after preincubation at pH 10.3, 4.6 and 4.3[16] were 8 μm thick, whereas sections for immunocytochemistry were 4–6 μm thick. The monoclonal antibody F 88.12F8 (Sera-lab, Sussex, England) was raised against human α-cardiac MHC and has been shown to bind specifically to this MHC in both rat and hu-

man[13, 14]. In addition, the following previously characterized monoclonal antibodies (mAb) were used: mAb ALD 19[17] directed against slow tonic MHC[18]; mAb 9812[19] which recognizes slow twitch MHC, and mAb 37 EH 11[20] directed against desmin. The polyclonal antibody NN 5 directed against fetal MHC[21] was also used. The mAb A 6 against M-protein Mr 165,000[22] was used as a negative marker for spindle bag_1 fibers (Thornell and Pedrosa-Domellöf, unpublished observations).

A standard peroxidase-antiperoxidase (PAP) technique[24] was employed. PAP products were purchased from Dakopatts, Glostrup, Denmark. Enzyme histochemical fiber typing was done according to previously published criteria[6, 16]. Muscle spindle fibers were classified as bag_1, bag_2 and chain according to morphological, enzyme- and immunohistochemical criteria[23]. The sections were photographed in an Olympus Vanox (Tokyo, Japan) microscope.

Results

In the *adult human masseter* a few extrafusal fibers were moderately to lightly stained with the antibody against α-MHC (fig. 1 a). These fibers were unevenly distributed within the fascicles and were absent from large areas of the muscle. The fibers reactive to anti-α MHC were a subset of the type 2 C and the ATPase intermediate fiber populations (fig. 1 b) and were more frequent in the superficial than in the deep portion of the muscle. Reactivity to anti-α MHC was never found in type 1 fibers. Heterogeneity in fiber size and type, as well as variability in reactivity with the antibodies against slow twitch (fig. 1 c) and fetal (fig. 1 d) MHC, was as described previously[5, 7]. The nuclear bag fibers in muscle spindles also displayed a positive reactivity to the antibody against α-MHC (fig. 2). Nuclear bag_2 fibers were strongly stained whereas the nuclear bag_1 fibers were moderately to weakly stained.

In the *fetal masseter* between 14 and 22 weeks of gestation (14–22 wG), reactivity to anti-α MHC was only seen in the spindle bag fibers (data not shown). From 7 weeks after birth, a subpopulation of the extrafusal fast fibers was also stained with the antibody against α-MHC. The pattern of staining was similar to that described in the adult (see above).

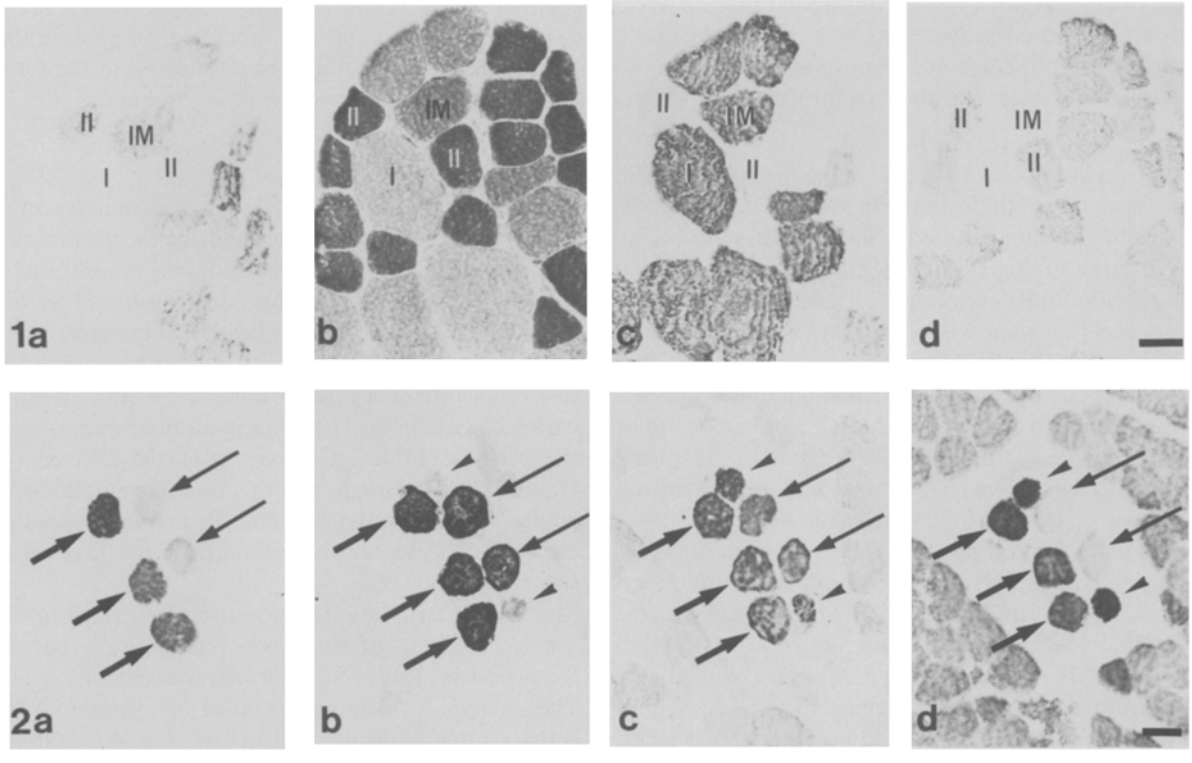

Figure 1. Adult human superficial masseter stained to demonstrate α-MHC (*a*), mATPase activity at pH 10.3 (*b*), slow twitch MHC (*c*) and fetal MHC (*d*). Type 1 (I) fibers lack α-MHC whereas some type 2 (II) and ATPase intermediate (IM) fibers express this MHC isoform. Notice the staining heterogeneity with the mAbs against slow twitch and fetal MHC. × 320, bar 20 μm.

Figure 2. Muscle spindle from a 4-year-old masseter stained with anti-α (*a*), anti-slow tonic (*b*) and anti-fetal (*c*) MHC and with anti-M protein (*d*). The nuclear bag_2 fibers (thick arrows) are strongly stained with anti-α MHC, whereas the nuclear bag_1 (thin arrows) fibers are weakly stained and the chain fibers (arrowheads) are unstained. × 300, bar 20 μm

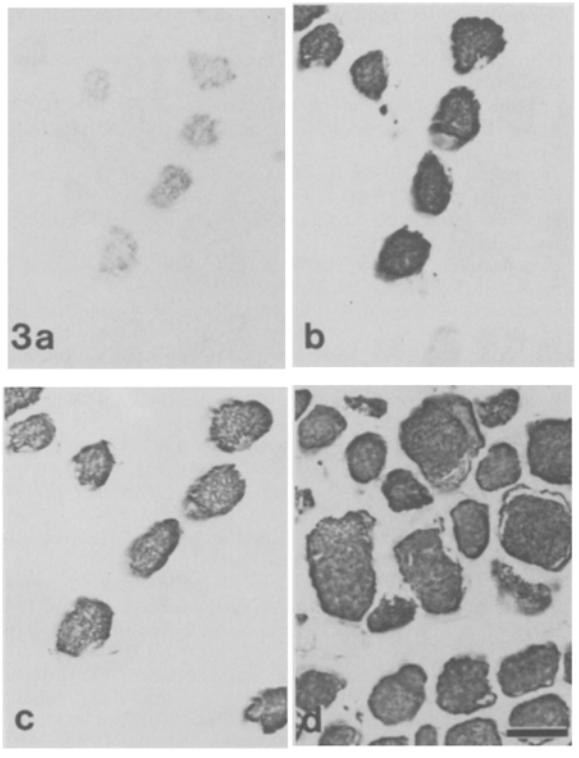

Figure 3. Adult human extraocular muscle stained with anti-α (*a*), anti-slow tonic (*b*) and anti-slow twitch MHC (*c*), and anti-desmin mAbs (*d*). Notice that not all slow tonic fibers (*b*) are stained with the mAb against α-cardiac MHC. ×450, bar 20 μm.

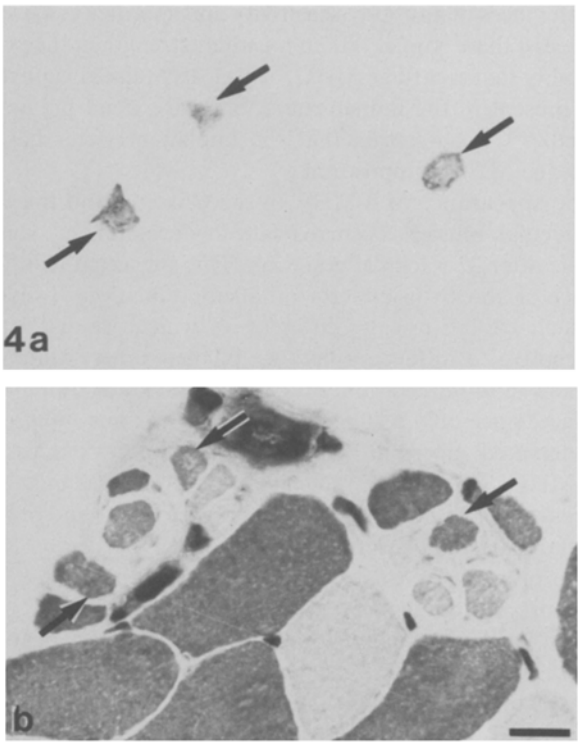

Figure 4. Adult cat masseter muscle spindles stained with anti-α MHC (*a*) and treated to demonstrate mATPase activity at pH 10.3 (*b*). Three bag fibers (arrows) are stained with the α-MHC mAb. ×450, bar 20 μm.

In the *adult human extraocular muscles* (fig. 3), a few fibers were strongly to moderately stained with anti-α MHC (fig. 3a). These fibers were a subpopulation of the fibers which reacted with the slow tonic MHC (fig. 3b) and the slow twitch MHC (fig. 3c) antibodies. The *extraocular muscles of fetuses* aged 18 wG and 22 wG were unstained with anti-α MHC (not shown). In *fetal and postnatal limb muscles*, reactivity to anti-α MHC was only seen in the intrafusal bag fibers.

Staining with anti-αMHC was also observed in the spindle bag fibers of *adult cat* (fig. 4) and *rat* (not shown) *masseter muscles*, but the extrafusal fibers were unstained. In the *adult rat diaphragm* muscle no staining was observed with α-MHC antibody (not shown).

Discussion

Our data show that an MHC isoform identical with or closely related to α-cardiac MHC is expressed in a subpopulation of extrafusal fibers in adult human masseter and extraocular muscles, as well as in nuclear bag fibers of human, cat and rat muscle spindles. The mAb used in this study was raised against human α-cardiac MHC and it has been shown by Western blots of denatured and native myosins, as well as in immunofluorescence studies, to bind specifically to this MHC[13,14]. So far, it has not been possible to show unequivocally, by immunoblotting, that the MHC in the masseter and the extraocular muscles is identical to the α-cardiac isoform. However, we have previously shown, with immunocytochemistry, that the mAb against α-cardiac MHC does not cross-react with slow tonic MHC[4], and the present study shows that it does not cross-react with slow twitch, embryonic or fetal MHC, or any of the isoforms of fast twitch MHC, including 2X or 2D[25,26] and superfast[27] MHC. Our proof of this is: i) the staining patterns obtained with anti-α MHC did not coincide with those of the other antibodies tested; ii) anti-α MHC did not stain the extrafusal fibers of either adult or developing fast or slow limb muscles; and iii) anti-α MHC did not stain the rat diaphragm, which is known to contain a significant proportion of type 2X fibers[25], nor did it stain the cat masseter, which contains fibers that express superfast MHC[27]. Interestingly, the reactivity to the mAb against α-cardiac MHC was found in type 2C and ATPase intermediate fibers in the masseter, whereas in the extraocular muscles it was detected in slow fibers, raising the question whether the MHCs detected by the anti-α MHC antibody in the masseter and the extraocular muscles are identical.

With the mAb used in the present study we only observed a few reactive fibers, whereas in a recent investigation[28], using a different antibody against α-cardiac MHC, 20–45% of adult human masseter fibers were reported to be stained. This discrepancy in results may be attributed to

differences in antibody sensitivity and specificity. Nevertheless, these studies, taken together, strengthen the possibility that α-cardiac MHC, or a closely related isoform, is present in the human masseter, and extend previous studies [5-7] suggesting that the human masseter has a unique MHC composition.

The appearance of α-MHC in the masseter and the extraocular muscles occurred late in development, some time after 22 weeks of gestation. This suggests the influence of an extrinsic factor on a subpopulation of fibers which are intrinsically different, or which are distinctly receptive to influences because of their innervation. In addition to innervation, other factors must be of importance, since only a subgroup of the slow tonic, multiply innervated, fibers in the extraocular muscles expressed α-MHC. Similarly, in the masseter α-MHC was only expressed in subsets of the different type 2 fibers, and of the intermediate ATPase fibers.

The presence of α-cardiac MHC in these muscles is an important finding since the expression of this MHC isoform has been thought to be exclusive to the myocardium [2]. The present nomenclature of MHC isoforms is based upon the original location of identification (e.g., α-cardiac MHC) or the period of development (e.g., neonatal MHC) at which the protein was first characterized. However, a growing amount of evidence shows that the functional specialization and adaptive capacity of different muscles [1] involve the whole repertoire of muscle genes. Obviously, some MHC isoforms can be expressed at developmental stages [3] and/or, as shown here, in muscles, distinct from those initially reported and used to name the isoforms. Therefore, a revision of the present nomenclature of the MHCs may be considered in the future.

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