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Histochemical and immunohistochemical study on muscle fibers in human extraocular muscle spindles

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Abstract

Human extraocular muscles are unique in several ways including their endowment with proprioceptive organs. Aim of this study was to establish a classification of intrafusal muscle fibers of human extraocular muscles based on their histochemical and immunohistochemical properties and to determine their relationship to extrafusal extraocular muscle fiber types in this respect. Using light microscopy, intrafusal muscle fibers were followed on consecutive cross-sections and classified according to the localization of their myonuclei and to their enzyme- and myosin-immunohistochemical characteristics. Sixteen muscle spindles in human extraocular muscles counted as 'true' spindles revealed 27% nuclear chain fibers [40.1 μm ± 10.4; perimeter ± SD] and 73% anomalous fibers [44.1 μm ± 12]. Seven 'false' muscle spindles showed only anomalous fibers [43.8 μm ± 11.1] and entirely lacked nuclear chain fibers. Six fiber types were distinguished according to their histochemical and myosin heavy chain immunohistochemical properties. Fiber type 1 [46.3 μm ± 13.3] was made up of fast-twitch myosin heavy chain isoform. Fiber type 2 [39.5 μm ± 10] additionally expressed a developmental myosin heavy chain isoform. Fiber type 3 [42.8 μm ± 10.4] consisted of pure slow-twitch positive muscle fibers. Slow-twitch MHC and fast-twitch myosin heavy chain isoform were found in fiber type 4 [43.3 μm ± 9]. Fiber types 5 and 6 showed different myosin heavy chain patterns than fiber types 1–4. The vast majority of nuclear chain fibers displayed fiber type 2 features, but 12% of nuclear chain fibers were found to be of fiber type 1. Among anomalous fibers in true spindles the frequency of fiber type 1 was much higher than in false spindles. On the other hand, fiber type 4 was found more often in false than in true spindles. With regard to their histochemical and immunohistochemical properties intrafusal muscle fibers in human extraocular muscles differ both from intrafusal muscle fibers in other skeletal muscles and from extrafusal muscle fibers in extraocular eye muscles. These conspicuous differences to skeletal muscle spindles relate to their morphology and myosin heavy chain characteristics. In particular, the occurrence of anomalous fibers might reflect dynamic neuronal processes and might be necessary for modulating and adapting processes in advancing age, as well as maintaining proprioceptive input during the whole life.

Keywords: human extraocular muscles; histochemistry; immunohistochemistry; myosin heavy chain isoforms; muscle spindles

1. Introduction

The interaction of extraocular muscles (EOMs) provides positioning of the eyes within the orbits and therefore is crucial for binocular vision. In human EOMs muscle spindles and palisade endings were thought to be responsible for proprioception (Büttner-Ennever et al., 2005). As recent studies in human (Lukas et al., 2000) and non-human EOMs (Blumer et al., 2001; Konakci et al., 2005a,b) demonstrated effector functions of palisade endings, human EOM muscle spindles remain the only structures with proprioceptive potency.

EOMs are amongst the fastest and most fatigue-resistant skeletal muscles (Porter et al., 1995). Because of their unique genetic repertoire they were introduced as an own allotype

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(Hoh et al., 1989). Extrafusal muscle fibers of adult EOMs express developmental myosin heavy chain (MHC) (Kjellgren et al., 2003; Wasicky et al., 2000), slow-tonic MHC (Fujii et al., 1990) and cardiac MHC isoforms (Rushbrook et al., 1994), MHC isoforms that normally occur only during muscle fiber formation and in adult intrafusal muscle fibers.

Human EOMs are richly supplied with muscle spindles (Lukas et al., 1994) that differ morphologically from common skeletal muscle spindles in several respects (Blumer et al., 1999; Bruenech and Ruskell, 2001; Lukas et al., 1994; Ruskell, 1989) indicating a function different from that of spindles in limb muscles. In human EOMs, ultrastructural analysis revealed two sets of encapsulated spindle-like structures (Lukas et al., 1994; Ruskell, 1989): so-called 'true' muscle spindles, expressing sensory neuronal contacts on their intrafusal fibers, and so-called 'false' spindles fiber lacking these contacts. In 'true' muscle spindles two types of intrafusal muscle fibers were found: (1) nuclear chain fibers (NCFs) presenting a row of centrally located myonuclei, and (2) anomalous fibers (AMFs) resembling extrafusal muscle (Blumer et al., 1999; Bruenech and Ruskell, 2001; Lukas et al., 1994; Ruskell, 1989).

In 'false' muscle spindles only anomalous fibers were found (Blumer et al., 1999; Bruenech and Ruskell, 2001; Lukas et al., 1994; Ruskell, 1989). The occurrence of these anomalous fibers and other particularities led to speculations about degenerative alterations within human EOM muscle spindles (Ruskell, 1989). But the discovery of similar changes in infant EOMs (Blumer et al., 1999; Bruenech and Ruskell, 2001) threw new light on the possible functions of human EOM muscle spindles.

In skeletal muscles neither 'false' muscle spindles nor anomalous fibers were described. On the other hand, nuclear bag fibers were found very sparsely in human EOM muscle spindles (Lukas et al., 1994). In skeletal muscles two types of nuclear bag fibers and nuclear chain fibers are regular constituents of muscle spindles (Boyd, 1980, 1981). Each fiber type was shown to own a specific MHC pattern (Barker and Banks, 1994; Maier et al., 1988; Pedrosa et al., 1990), reflecting its special role within muscles' movement and stretch reflex (Boyd, 1980, 1981; Taylor et al., 1998, 1999; Walro and Kucera, 1999).

Former studies on muscle spindles of human EOMs (Blumer et al., 1999; Bruenech and Ruskell, 2001; Lukas et al., 1994; Ruskell, 1989) were based on material fixed for light microscopy and/or ultrastructural analysis, which did not allow the investigation of the MHC pattern of intrafusal muscle fibers. The present study was done to characterize for the first time human EOM intrafusal muscle fibers according to their MHC profile in combination with enzyme-histochemistry. The MHC pattern may provide valuable and additional clues concerning EOM muscle spindle function and the role of the muscle fibers, since the fiber type reflects functional demands (Pette, 2002). Our present study is the first one to investigate histochemical and immunohistochemical characteristics of intrafusal muscle fibers of human EOMs without prior fixation procedure.

2. Material and methods

2.1. Sample collection

Two human medial rectus muscles (from a 52-year-old female and from a 68-year-old male, respectively, who did not suffer from neuromuscular disease) were harvested post-mortally according to the Austrian federal law of transplantation. Methods for securing human tissue were humane and complied with the tenets of the Declaration of Helsinki.

Immediately after excision from the orbit, the muscles were transferred into a 20% sucrose solution at pH = 7.4 for up to 20 min followed by freezing in liquid nitrogen. Slides were kept at −80 °C till they were used. Consecutive transversal sections (5–10 μm) were cut on a Leitz Cryocut 3000 cryostat microtome.

2.2. Histochemistry

Sections were stained with haematoxylin and eosin (HE) and for nicotine amid tetrazolium reductase (NADH-TR) (Dubowitz and Brooke, 1973).

Staining for myofibrillar actomyosin adenosine triphosphatase (mATPase) was performed following alkaline (pH = 10.4) and acid (pH = 4.3) preincubation according to Guth and Samaha (1969).

2.3. Immunohistochemistry

For immunohistochemical detection of MHC isoforms unfixed sections were incubated with primary monoclonal antibodies (ab) against fast myosin heavy chain (NCl-MHCf/Novocastra Laboratories Ltd./Newcastle UK) and/or A 4.74 (Developmental Studies Hybridoma Bank/University of Iowa), slow myosin heavy chain (NCl-MHCs/Novocastra Laboratories Ltd./Newcastle UK), and/or A 4.840 (Developmental Studies Hybridoma Bank/University of Iowa). NCl-MHCf shows specificity for all type 2A and 2B muscle fibers, NCl-MHCs for type 1 muscle fibers and NCl-MHCn stained fibers containing neonatal MHC (Ecob-Prince et al., 1989). A 4.74 showed reactivity for all fast fibers and A 4.840 for slow-twitch fibers (Webster et al., 1988).

For detection of developmental MHC isoforms anti-developmental MHC ab and anti-neonatal MHC ab (NCl-MHCd; NCl-MHCn/Novocastra Laboratories Ltd./Newcastle UK) and the anti-embryonic ab F 1.652 (Cho et al., 1993) (Developmental Studies Hybridoma Bank/University of Iowa) were used. NCl-MHCd was reported to show identical results as BF-G6 (Brueckner et al., 1996), an anti-embryonic myosin ab used and raised by Schiaffino et al. (1988), therefore we interpret NCl-MHCd as embryonic.

The sections were incubated with the primary antibodies for 1–6 h at room temperature or at 37 °C. After washing the sections in phosphate buffered saline three times for 10 min (PBS, pH = 7.4), they were incubated with the secondary antibody (goat–anti-mouse peroxidase conjugated immunoglobulin, NCl G-AMP, polyclonal/Novocastra Laboratories