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Research article

Myosin heavy chain composition of the human sternocleidomastoid muscle

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SUMMARY

The sternocleidomastoid (SCM) muscle is one of the neck muscles responsible for head posture and control of head movement. It functions in rotation, inclination, protraction, extension and flexion of the head, whilst chewing and in exerting increased respiratory efforts. This study is the first one describing the myosin heavy chain (MyHC) isoform composition of the SCM muscle of presumably healthy young males for the purpose of better understanding the contractile properties of the muscle as well as to help in evaluation of pathologically altered structure of the muscle. Autopsy samples were processed immunohistochemically to reveal the MyHC isoform composition. The muscle fibres expressed MyHC-1 (31.5%), -2a (29.7%) and -2x (4.3%) or co-expressed MyHC-2a with MyHC-2x (26.8%), MyHC-1 with MyHC-2a (4.1%) and/or MyHC-1, -2a with -2x (1.1%).

In addition to the MyHC isoforms, characteristic of adult limb muscles, a very low percentage of muscle fibres (0.2–2.7%) expressed MyHC-neo, which is normally not found in adult limb muscles. Only two samples exhibited MyHC-neo at a rather higher percentage (6.3% and 7.5%) of muscle fibres. The high share of hybrid fibres and the presence of MyHC-neo in the SCM muscle differ from that of adult limb muscles where hybrid fibres are rare and the expression of immature MyHC isoforms occurs only in pathological or experimental conditions. Since the SCM muscle shares the same embryogenic potential as limb muscles, its distinct MyHC expression appears to be associated with twin innervation and with the intrinsic specialisation to perform multiple functions.

1. Introduction

The muscles of the neck are responsible for the stabilisation of head posture and the control of head movement. More than 20 pairs of muscles act on the cervical vertebrae and head to generate multidirectional force and movement. One member of this complex is the sternocleidomastoid (SCM) muscle. It extends from the sternal manubrium and the medial end of the clavicle to the mastoid process of the temporal bone.

Electromyographic analysis has shown that the SCM muscle is active during heterolateral rotation and homolateral counter-resistance inclination movements, protraction, extension and flexion (Costa et al., 1990), the coactivation of the SCM muscle during chewing (Chandu et al., 2005) and its progressive recruitment during increased respiratory efforts (Costa et al., 1994). The impairment of the SCM muscle has been found to be associated with neck pain and cervical osteoarthritis (Falla et al., 2003). The SCM is one of the muscles involved in spasmodic torticollis and cervical dystonia (Lin and Chou, 1997). Long lasting exposure of shoulder/neck to low level repetitive, monotonous or static work is today widespread in modern society; work related neck and shoulder disorders are a significant problem worldwide (Zaza, 1998). In order to understand the pathologically altered structure of the muscle, it is an obvious prerequisite to obtain the best possible normal ranges of MyHC assembly in healthy individuals.

Based on myosin ATPase histochemistry, Johnson et al. (1973) reported that 35% and 65% of the SCM of young males contained type 1 and type 2 fibres respectively. The only study dealing with muscle fibre types and subtypes in human SCM revealed fibre type transformation in patients who underwent spondylodesis for cervical dysfunction of different ethiologies (Uhlig et al., 1995). However, the former study lacks comparison with control muscles, therefore it could not reliably be concluded whether the transformation resulted from muscle dysfunction, or whether hybrid fibres were characteristic of unaffected SCM muscle from control subjects.

Since muscle fibre contractile properties correlate with MyHC composition (Schiaffino and Reggiani, 1996) head and neck muscles with complex functional demands should have a complex pattern of MyHC isoform expression (Hoh, 2005). Apart from the unique functional requirements, the intrinsic specialisation of cranial muscles appears to be associated with their embryologic origin as well as with their innervation pattern (Butler-Browne et al., 1988; Jung et al., 1999). Multiple functions performed by the SCM muscle and its twin innervation by the eleventh cranial nerve and branches

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from the cervical plexus (Caliot et al., 1984) predict that the MyHC composition, which has not yet been investigated in humans, is rather complex.

This study was designed to determine the MyHC isoform(s) composition of the SCM muscle for the purpose of better understanding the physiological and pathological behaviour of the muscle. Our previous studies (Snoj-Cvetko et al., 1996a,b) have confirmed that area density, i.e. the area fraction of fibres expressing a particular MyHC isoform, and measured stereologically, corresponds strongly with the relative amount of the same MyHC isoform in the muscle homogenate, estimated by the SDS electrophoresis. In this study, we analysed the expression of MyHC isoforms in muscle fibres and their cross-sectional area in the SCM muscle in young males using immunohistochemistry. The main findings are as follows: (i) the SCM muscle expresses MyHC isoforms characteristic for adult limb muscles (MyHC-1, -2a and -2x) as well as MyHC-neo, normally not found in adult limb muscles; and (ii) more than 30% of all fibres are hybrid fibres, most of which co-express MyHC-2a with MyHC-2x, while approximately one third of all fibres contained MyHC-1 and one third MyHC-2a.

2. Materials and methods

2.1. Muscle specimens

Muscle samples were obtained by autopsy from presumably healthy subjects within 24 h post mortem. The specimens were excised from the superficial part of the middle portion (between origin and insertion) of the SCM muscle of 15 young males (aged 18–40 years, $\bar{x} \pm \mathrm{SD} = 30.3 \pm 6.3$ years) who had suffered a sudden accident. None of the subjects exhibited signs of functional disorder of the craniovertebral system. On the basis of the histological examination, none of the muscle specimens exhibited signs of muscle disease. The muscle sampling was approved by the National Medical Ethics Committee of the Republic of Slovenia.

2.2. Immunohistochemistry

The muscle specimens were rapidly frozen in liquid nitrogen and stored at −80 °C until they were cut into 10 μm thick transverse sections which were processed to demonstrate the expression of MyHC isoforms. The following monoclonal antibodies specific to MyHC isoforms were applied: BA-D5 (MyHC-1), BF-35 (against all MyHC isoforms except MyHC-2x) (Schiaffino et al., 1989), A4-74 (Alexis Biochemicals) (MyHC-2a and 2x), 6H1, specific to MyHC-2x (Lucas et al., 2000). Two antibodies specific to developmental (MyHC-emb) and neonatal (MyHC-neo) (Novocastra Laboratories, Newcastle upon Tyne, UK) and antibody specific to α-cardiac MyHC (anti-MyH6, Sigma, St. Luis, MO, USA) were also applied.

Serial muscle cryosections were preincubated for 30 min in phosphate buffered saline containing 0.5% bovine serum albumin (PBS/BSA) and rabbit serum (1:40). The muscle sections were then incubated with a primary antibody in a humidified box overnight at 4 °C. All the antibodies were diluted with PBS/BSA (1:100 for BA-D5, 1:30 for A4-74, non-diluted for BF-35, 1:10 for MyHC-neo, 1:20 for MyHC-emb, 1:3000 for 6H1, and 1:200 for α-cardiac MyHC). The reactivity of the monoclonal antibodies was revealed with the HRP conjugated secondary antibody (P260; Dako), diluted 1:100 in PBS/BSA with rabbit serum (1:40) using the indirect immunoperoxidase technique (Gorza, 1990). The control sections, where the primary antibody was replaced with PBS/BSA, were completely unstained. In order to confirm the specificity of the antibody against MyHC-neo and MyHC-emb, the sections from the human foetal limb muscle and the adult masseter muscle served as a positive control and the adult vastus lateralis as a negative control.

2.3. Image acquisition

The images of muscle sections from differently stained serial sections were captured with a Nikon digital camera DXM1200F connected to a Nikon Eclipse E800 microscope using Lucia GF software (version 4.82) (Laboratory Imaging, Prague, Czech Republic).

2.4. Stereology and software for muscle fibre type analysis

The expression of MyHC isoforms was estimated in two ways: (i) by the percentage of the area of muscle fibres containing particular MyHC isoforms; and (ii) by the numerical and area percentage of fibre types that were defined for each fibre through a successive series of sections, stained for different MyHC isoforms.

(i) The area percentage of muscle fibres expressing particular MyHC isoforms was estimated for each MyHC isoform from three sample areas per muscle, encompassing 500–600 muscle fibres, and applying the point grid plug-in of the Ellipse programme (ViDiTo, Košice, Slovakia).
(ii) We identified the fibre types from the series of variously stained sections using our own software (Karen et al., 2009). On average, 220 fibres were analysed per muscle (3300 in total). The fibres were classified into pure fibres (types 1, 2a and 2x) expressing MyHC 1, 2a or 2x, and hybrid fibres (types 2a/2x, 1/2a, 1/2x and 1/2a/2x) co-expressing two or three different adult MyHC isoforms.

Any fibres that were not recognised in each of the four sections (<4%) were omitted from the study. The numerical and area percentage and fibre diameter for each fibre type were estimated.

2.5. Statistics

The Systat Version 5 statistical package was used to calculate the mean values and standard error of the mean for the diameter, numerical and area proportion of each fibre type within a muscle sample.

3. Results

In the SCM muscle of young adult males, slow and fast fibre types were distributed in a mosaic pattern, without any evident compartmentalisation (Fig. 1).

3.1. Area fraction of muscle fibres expressing particular MyHC isoforms

The largest portion of the SCM muscle cross-sectional area co-expressed MyHC-2a and 2x (63.5%), 38.4% of the area fraction contained MyHC-1 and 34.5% MyHC-2x (Fig. 2). The portion of fibres expressing MyHC-neo was small and highly variable throughout the specimens; however, their area fraction has not been analysed.

3.2. Expression of adult MyHC isoforms in individual muscle fibres

Based on the MyHC expression, five major fibre types were identified: 1, 2a, 2x, 2a/2x and 1/2a (Fig. 3). Overall, the adult SCM muscle is a fast muscle characterised by a relatively high proportion of type 2 (fast) fibres (60.6% of the total fibre population). A total of 65.5% of fibres expressed only one MyHC isoform (pure fibres) and 34.5% expressed two or more MyHC isoforms (hybrid fibres). Most pure fibres were of type 1 (31.5%) and type 2a fibres (29.7%), whereas 2x fibres were rare (4.3%). Most of the hybrid fibres