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Regional differences in fibre type composition in the human temporalis muscle

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ABSTRACT

Anatomical and electromyographic studies point to regional differences in function in the human temporalis muscle. During chewing and biting the anterior portions of the muscle are in general more intensively activated and they are capable of producing larger forces than the posterior portions. It was hypothetised that this heterogeneity in function is reflected in the fibre type composition of the muscle. The composition and surface area of different fibre types in various anteroposterior portions of the temporalis muscle were investigated in 7 cadavers employing immunohistochemistry with a panel of monoclonal antibodies against different isoforms of myosin heavy chain. Pure slow muscle fibres, type I, differed strongly in number across the muscle. In the most posterior portion of the muscle there were 24% type I fibres, in the intermediate portion 57%, and in the most anterior portion 46%. The mean fibre cross-sectional area (m-fcsa) of type I fibres was 1849 μm^2, which did not differ significantly across the muscle. The proportion of pure fast muscle fibres, type IIA and IIX, remained more or less constant throughout the muscle at 13% and 11% respectively; their m-fcsa was 1309 μm^2 and 1206 μm^2, respectively, which did not differ significantly throughout the muscle. Pure type IIB fibres were not found. The relative proportion of hybrid fibres was 31% and did not differ significantly among the muscle portions. Fibre types I+IIA and cardiac α+I+IIA were the most abundant hybrid fibre types. In addition, 5% of the type I fibres had an additional myosin isoform which has only recently been described by means of electrophoresis and was named Ia. In the present study they were denoted as hybrid type I+Ia muscle fibres. It is concluded that intramuscular differences in type I fibre distribution are in accordance with regional differences in muscle function.

Key words: Masticatory muscles, myosin, temporalis muscle.

INTRODUCTION

Muscle fibres are traditionally classified into 3 groups depending on their physiological behaviour. Type I fibres are slow contracting, fatigue resistant and generate small forces, type IIA fibres are fast contracting, fatigue resistant and generate larger forces, and type IIB fibres are fast contracting, fatigue resistant and generate the largest forces. The speed of muscle fibre contraction is largely determined by the heavy chain of the myosin molecule (MHC). Histochemical ATPase studies (Ringqvist, 1974; Eriksson & Thornell, 1983) showed that human masticatory muscles generally contain a much higher proportion of 'hybrid' fibres dubbed IM and IIC than is usual in most limb and trunk muscles.

Immunohistochemical techniques, which reveal different isoforms of MHC, show that in human limb and trunk muscles at least 4 isoforms of MHC are expressed, namely types I, IIA, IIB and IIX (Schiaffino et al. 1989, 1994), in contrast to human jaw closing muscles which express also MHC-fetal, even at adult age (Butler-Brown et al. 1988), and MHC-cardiac α (Bredman et al. 1991), an MHC isoform normally expressed in the myofibrils of the atrium of the heart only. The latter 2 MHCs cannot be identified by ATPase histochemistry. Type IIB fibres in man defined by ATPase histochemistry have been shown to contain an MHC that is the homologue of MHC IIX in rodents (Schiaffino et al. 1989; Smerdu et al. 1994). The MHC content of motor units and their physiological properties are correlated (Schiaffino et al.

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Table 1. *Monoclonal antibodies and their specific binding*

Myosin heavy chain isoforms							
MHC antibody	MHC-1	MHC-IIA	MHC-IIB	MHC-IIX	MHC-cardiac-α	MHC-fetal	MHC-Ia
219-1D1	+	−	−	−	−	−	−
249-5A4	−	−	−	−	+	−	−
333-7H1	−	+	−	−	−	−	−
340-3B5	−	+	+	+	−	−	−
332-3D4	−	+	−	+	−	−	+
Antifetal	−	−	−	−	−	+	−

+, positive reaction between MHC and antibody; −, negative reaction between MHC and antibody.

1988; Kwa et al. 1995). Reiser et al. (1985) showed in their study that fibres with a slow velocity of shortening contained slow MHC, while fibres with a higher velocity of shortening contained both fast and slow MHCs. The velocity of shortening increased as the proportion of fast-type MHC increased.

A heterogeneous distribution of fibre types across the muscle has been reported for a number of muscles (Eriksson & Thornell, 1983; Gillott et al. 1994; Lexell et al. 1994; De Ruiter et al. 1996).

The human temporalis muscle is architecturally complex. Within the muscle regional differences exist in length, spatial orientation and position of muscle fibres, and in cross-sectional area (Van Eijden et al. 1996, 1997). Hence, during jaw movements fibre and sarcomere excursions are not the same for various muscle portions, and as a consequence the maximum force and excursion range of the muscle portions differ. This suggests that different portions are specialised for certain functions and that the muscle can actually exert different mechanical actions. In addition, electromyographic studies in which fine wire electrodes have been inserted into various muscle portions have demonstrated a differential activation depending on the motor task that was executed (Wood, 1986; Blanksma & Van Eijden, 1990, 1995; McMillan, 1993; Blanksma et al. 1997). These studies indicated that the anterior regions of the temporal muscle are in general more intensively used than the posterior regions. The question can be raised whether the heterogeneous distribution of different muscle fibre types in the temporalis muscle reflect the differences observed in anterior/posterior muscle activity. Indeed, ATPase enzyme histochemistry has pointed to a heterogeneous distribution of type I and type IIB fibres (Eriksson & Thornell, 1983). However, ATPase enzyme histochemistry does not give a complete picture as it is unable to discriminate between all MHC isoforms.

The aim of the present study was to determine the distribution of different fibre types in the human temporalis muscle by using immunohistochemistry and to examine the possible existence of regional differences within the muscle.

MATERIALS AND METHODS

In this study we used the right temporalis muscle of 7 Caucasian cadavers (4 males, 3 females; mean age ± S.D., 71.6 ± 15.0 y). Five cadavers had upper and lower dental prostheses, 2 were partially dentate. The muscles were obtained within 12–36 h after death. After the muscle was cut from its attachment sites, it was split into anterior and posterior halves by a vertical section running through the tip of the coronoid process. In addition, from 5 of the cadavers a sample was taken from the medial gastrocnemius for comparison of fibre types and fibre type cross-sectional areas. The muscles were rapidly frozen in liquid nitrogen cooled isopentane and stored at −80 °C until required for further processing.

Immunohistochemistry

Serial transverse sections (10 μm) of the whole anterior and posterior muscle halves were cut in a cryomicrotome (Model HM 500 M, Adamas Instruments BV, Leersum, the Netherlands). The sections were taken halfway through the belly of the muscle, just above the muscle's tendon plate; they were cut perpendicularly to the main direction of each muscle half. The sections were mounted on microscope slides coated with AAS (3-aminopropyltriethoxysilane; Henderson, 1989). Consecutive sections were fixed overnight in a mixture of methanol : acetone : acetic acid : water (35 : 35 : 5 : 25) at −20 °C (Wessels et al. 1988) and incubated with the monoclonal antibodies (Table 1) raised against purified myosin in our laboratory (Bredman et al. 1991). Anti-fetal MHC was

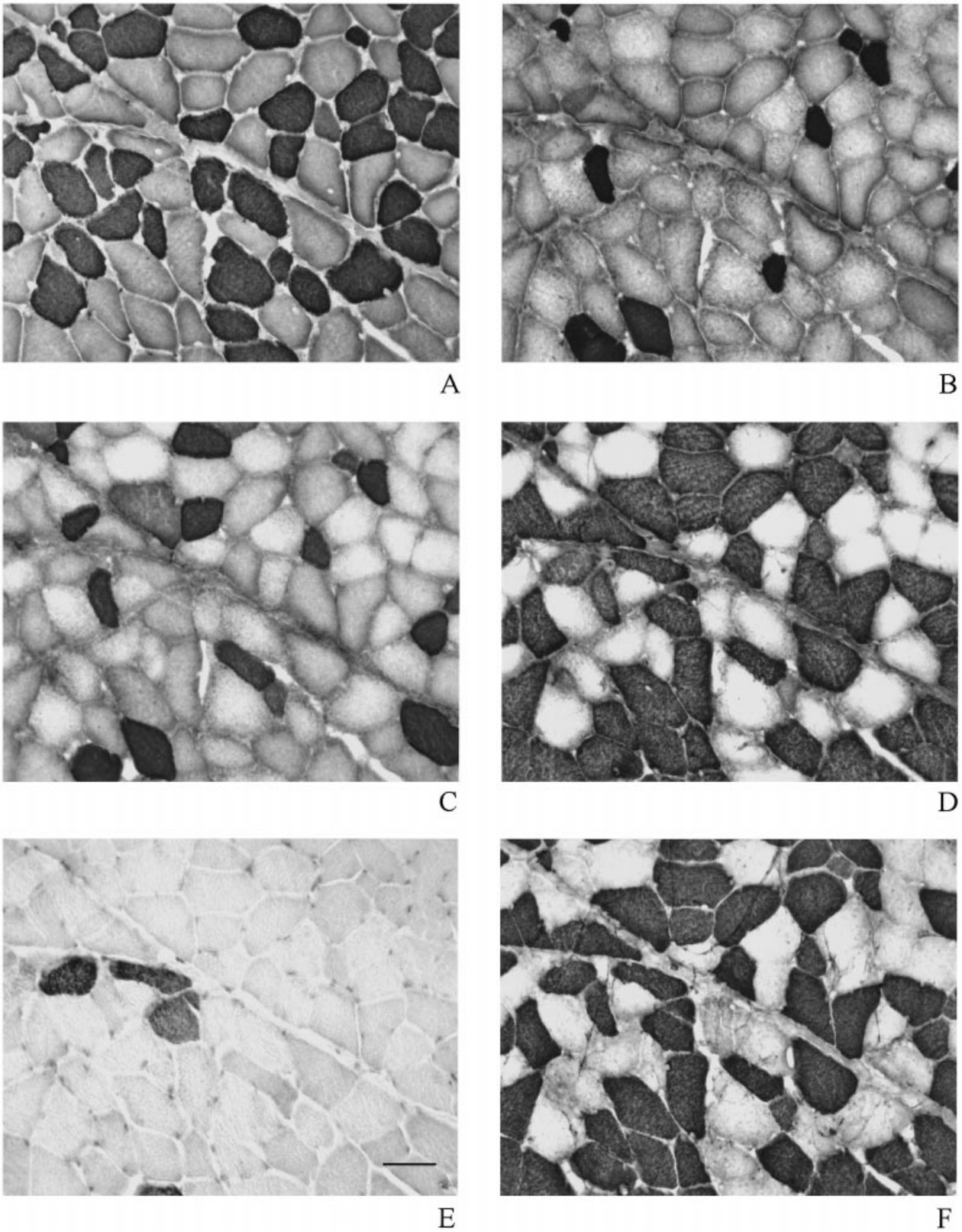

Fig. 1. Light micrographs of 6 consecutive sections of the temporalis muscle incubated with monoclonal antibodies against myosin heavy chains. *A*, anti-I MHC; *B*, anti-cardiac-α MHC; *C*, anti-IIA MHC; *D*, anti-fast MHC; *E*, anti-fetal MHC; *F*, anti-IIA/IIX/Ia MHC. Bar, 50 μm.

purchased (Novocastra Laboratories Ltd, UK). The indirect unconjugated immunoperoxidase technique (PAP-technique) was applied to detect the specific binding of the different antibodies and nickel-DAB was used to visualise the staining (Hancock, 1982) (Fig. 1).

Sampling method and fibre cross-sectional area measurements

Samples were taken from 7 sites in the muscle, equidistant in an anteroposterior direction, 4 from the posterior muscle half and 3 from the anterior half. In each sample area (about 0.6–0.4 mm^2), 100–300 fibres (average 165) were drawn, by means of a projection microscope (Carl Zeiss, Oberkochen, Germany) and a mirror table, onto a transparent sheet. Each fibre was classified by means of a series of 6 consecutive incubated sections. Fibres that were not recognised in each of the 6 sections were omitted.

The cross-sectional area of the fibres was measured by reading the drawn sheets, together with a grade mark for correction of enlargement, via a flat-bed scanner (Hewlett-Packard, ScanJet 4c) into a personal computer. A custom made program, that converts the number of pixels into μm^2, was then used to determine the cross-sectional area of each muscle fibre in μm^2. In total more than 8000 fibres were analysed in the 7 temporalis muscles.

Statistical analysis

For each muscle the distribution and mean cross-sectional area (m-fcsa) of different fibre types were determined. Mean and standard deviation values (S.D.) were calculated for the 7 temporalis muscles. One-way analysis of variance (ANOVA) was used to assess differences in fibre type distribution and in fibre cross-sectional area. The level of significance was set at $P < 0.05$.

Reproducibility

To ascertain the reproducibility of this method a second series, directly succeeding the first series, of 6 consecutive sections of the anterior portion of 6 muscles were incubated in the same way as the first series. The same fibres classified in one area of the first series were followed and classified in the second. The results of both series were then compared.

RESULTS

From the test for reproducibility it appeared that of the 622 fibres compared, 41 fibres (6.6%) gave conflicting results. In half of these fibres MHC cardiac-α was not reproducible.

Table 2. *Fibre type composition* (*mean* ± S.D.) *in the temporalis muscle* ($n = 7$)

Fibre type	Mean (%)	S.D. (%)
I	45.0	8.2
IIA	13.5	8.2
IIX	11.0	10.7
Hybrid	30.6	9.9

Table 3. *Distribution of hybrid fibre types* (*mean* ± S.D.) *in the temporalis muscle*

Fibre type	Mean (%)	S.D. (%)	n
I + IIA	4.7	3.2	7
I + IIB	0.3	0.6	3
I + IIX	0.9	1.2	6
I + Ia	4.5	7.5	3
Fetal	0.2	0.3	4
Fetal + I	3.1	4.8	6
Fetal + IIA	0.8	0.5	7
Fetal + IIB	0.1	0.0	1
Fetal + IIX	3.3	3.9	7
Fetal + I + IIA	0.3	0.6	3
Fetal + I + IIB	0.3	0.3	5
Fetal + I + Ia	0.1	0.1	1
Alpha	0.1	0.1	3
Alpha + I	2.0	3.3	6
Alpha + IIA	2.6	0.9	7
Alpha + IIX	0.6	0.7	6
Alpha + I + IIA	3.6	2.5	7
Alpha + I + IIB	0.1	0.2	1
Alpha + I + IIX	0.2	0.2	5
Alpha + I + Ia	0.1	0.1	1
Fetal + alpha	0.1	0.1	4
Fetal + alpha + I	0.5	0.7	6
Fetal + alpha + IIA	0.3	0.3	5
Fetal + alpha + IIX	0.6	0.7	6
Fetal + alpha + I + IIA	0.6	0.7	6
Fetal + alpha + I + IIB	0.1	0.1	1
Fetal + alpha + I + IIX	0.1	0.2	2
Fetal + alpha + I + Ia	0.5	0.8	3

n = number of muscles where the specific fibre was found.

Fibre-type distribution

Table 2 lists the grand means and S.D. values for the various muscle fibre types; the S.D. values are a measure for interindividual variability.

Type I was the predominant fibre type (45%); in every subject the frequency did not vary much among the subjects (note relatively small S.D. values). Types IIA and IIX were the second (14%) and third (11%) most predominant pure fibre types, but their frequency varied considerably between the subjects (note relatively large S.D. values). The remainder (31%) were hybrid fibres which consisted of 2 or more MHC isoforms. The composition of these hybrid fibres and their occurrence are listed in Table 3. We observed

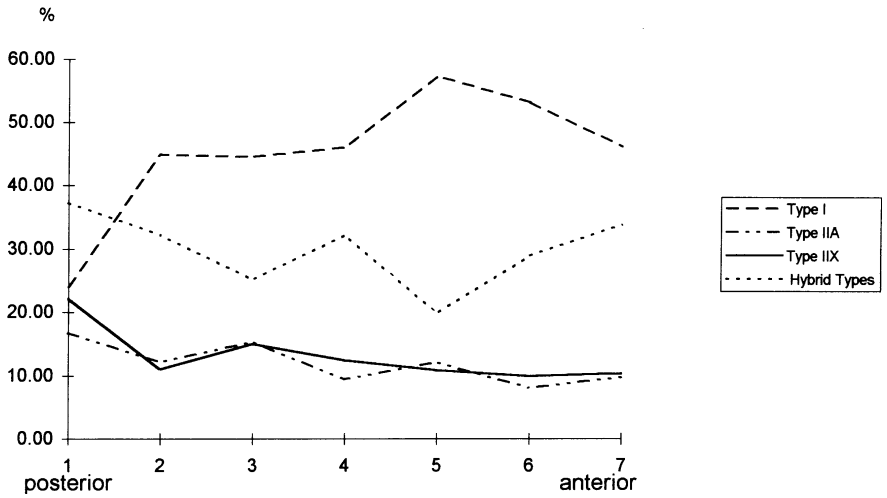

Fig. 2. Distribution of fibre types in different anteroposterior portions (1 ... 7) of the human temporalis muscle in mean percentages.

that some type I fibres also reacted positively with antibody 332-3D4, which normally only detects MHC-IIA and MHC-IIX. There was, however, no reaction in these fibres with antibody 340-3B5, which detects all fast MHCs. Electrophoretic studies showed that some pure slow fibres contained in addition to the MHC-I isoform a novel MHC isoform (Galler et al. 1997), which was named Ia. We think that antibody 332-3D4 is also able to detect this novel MHC. Therefore, those fibres were termed type I+Ia. Note that not all hybrid fibre types were found in every temporalis muscle, and that the frequency of any single hybrid fibre type was smaller than 5%. Types I+IIA, I+Ia and cardiac α+I+IIA were the most abundant hybrid fibre types, followed by types fetal+IIX, fetal+I, cardiac-α+IIA, and cardiac-α+I. The total number of hybrid fibres containing a combination of MHC-cardiac α with another MHC was almost the same as for hybrid fibres containing a combination of MHC-fetal with another MHC (12 ± 5% and 11 ± 8% respectively (mean$\pm$S.D.)). Few hybrid fibres contained both MHC-cardiac α and MHC-fetal in combination with other MHCs (3 ± 2%).

Figure 2 depicts the distribution of muscle fibres within the temporalis muscle. Pure type I fibres were heterogeneously distributed within the muscle. Their percentage varied between 24 ± 15% in the most posterior portion to 57 ± 11% in the intermediate portion, and to 46 ± 14% in the most anterior portion. The difference between the muscle portions was significant. The relative number of types IIA and IIX did not differ significantly between the muscle portions. Although the relative number of all hybrid fibres combined together was the highest in the posteriormost and anteriormost portions (respectively 37 ± 20% and 34 ± 16%) and the lowest in the intermediate portion (20 ± 17%), this difference was not significant.

Table 4. *Fibre type cross-sectional area (mean$\pm$S.D.) and coefficient of variation (mean$\pm$S.D.) in the temporalis muscle*

	Cross-sectional area (μm^2)		Coefficient of variation (%)	
Fibre type	Mean	S.D.	Mean	S.D.
I	1848.7	392.8	25.2	7.2
IIA	1309.4	746.6	40.5	17.2
IIX	1206.2	587.1	49.6	11.2
Hybrid	1287.2	580.6	40.7	18.6

Fibre cross-sectional area

Table 4 lists mean and S.D. values of fibre cross-sectional area in the temporalis muscle; the S.D. values are a measure for the interindividual variability, the coefficients of variation express the intra-individual variability. Type I fibres had the largest cross-sectional area. Fibre types IIA, IIX, and hybrid fibres had about 30% smaller areas than type I fibres, but the difference was not significant. Of all types, type I cross-sectional area showed the smallest inter-individual and intra-individual variability. No significant intramuscular differences in cross-sectional area were found.

DISCUSSION

The fan-shaped human temporalis muscle is architectually and mechanically heterogenous. This heterogeneity can only be effective if the different muscle portions are selectively controlled by the central nervous system. That this is indeed the case has been shown by electromyographic studies

(Møller, 1974; Wood, 1986; Van Eijden et al. 1990; Blanksma & Van Eijden, 1990). During biting, chewing and closing movements, the anterior muscle portions are more intensively used than the posterior portions. The results of the present study suggest that the muscle fibres in the muscle portions are adapted to this differential use. The more intensively used anterior muscle portions appeared to have a relatively large number of type I muscle fibres. Some studies have shown that chronic low-frequency electrical stimulation induces a fast-to-slow transition (Pette & Vrbová, 1985; Pette 1990). Thus type II fibres can be altered to type I fibres if they are chronically stimulated by the neurons. This could explain why there are more type I fibres in areas that are more intensively activated. Since type I fibres are activated first (Henneman et al. 1965), and their innervation ratio is small, it is likely that the anterior muscle portions are better equipped to regulate the magnitude of the produced chewing or biting force.

It should be realised that bite force and physiological cross-sectional areas of muscles decrease with age. A recent study (Monemi et al. 1998) showed that in the human masseter the proportion of type I fibres decreased during ageing from 63% to 33% and showed an increase in type IM and II fibres. In another study (Monemi et al. 1996) they noticed an increase of MHC-fetal in old human masseter muscles. Since we used muscles of older edentate subjects, the proportion of type I fibres in the temporal muscle could also be decreased while other fibre types could be increased.

Using ATPase histochemistry Eriksson & Thornell (1983) already showed that there is also a heterogenous distribution of fibre types across the muscle. The percentages of type I fibres found in the present study are not very different from the numbers found in young, dentate subjects by these investigators. However, a percentage of 81 type I fibres in the deep posterior muscle portion was not found in the present study. Eriksson & Thornell (1983) found in human temporalis and masseter muscles, next to a predominance of type I fibres, large numbers of IIB fibres, almost no IIA fibres, and many fibres with intermediate staining on ATPase histochemistry. We could scarcely find any IIB fibres. Sciote et al. (1994) were also unable to detect histochemically defined type IIB fibres in most of the masseter samples examined although they were found in control (nonmasticatory) muscles. In some temporalis muscles type IIA MHC was the predominant fast MHC type, in others type IIX was predominant. One explanation might be that standard ATPase enzyme histochemistry at pH 4.6 allows no differentiation between IIB fibres and IIX fibres (Aigner et al. 1983; Schiaffino et al. 1989). MHC-IIX was first demonstrated by means of immunohistochemistry and electrophoresis. Therefore, IIX fibres could mistakenly be called IIB fibres by investigators who only had ATPase enzyme histochemistry at their disposal. Another explanation might be that in our sample of relatively old muscles the expression of MHC-IIB was downregulated in favour of MHC-IIX or even MHC-IIA. This was observed in rat skeletal muscles (Sugiura et al. 1992; Larsson et al. 1993) where an age-related alteration from a fast fibre type profile into a more slow fibre type profile was demonstrated.

Next to the pure muscle fibres, which expressed only one MHC isoform, we were able to detect hybrid muscle fibres which expressed more than one MHC isoform. The contractile speed of those hybrid fibres lies between the contractile speed of the MHC isoforms they contain (Pette & Staron, 1990). The existence of different MHCs in a fibre allows a smooth transition of for instance force regulation needed in movements of the jaw muscles. Hybrid fibres are generally considered to arise when there is an alteration from one type into another type of muscle fibre. A suggestion was made of the following transition pathway of MHC isoforms: $I \leftrightarrow I/IIA \leftrightarrow IIA \leftrightarrow IIA/IIX \leftrightarrow IIX \leftrightarrow IIX/IIB \leftrightarrow IIB$ (Gorza, 1990; DeNardi et al. 1993). However, with our antibody panel it was not possible to distinguish IIA/IIX and IIX/IIB hybrid fibres. It is thus possible that a number of fibres which we designated as pure IIA fibres were actually hybrid fibres that express MHCs IIA and IIX, and a number of fibres which we designated as pure IIX fibres were actually hybrid IIX/IIB fibres. Single fibre electrophoresis could reveal if there is indeed an alteration from IIB to IIX fibres, and from IIX to IIA fibres.

In jaw closing muscles 2 distinct MHCs are expressed, namely MHC-fetal and MHC-cardiac α, which are said not to be expressed in skeletal muscle. Both MHCs were always expressed in combination with an other MHC. Hybrid fibres expressing MHC-cardiac α or MHC-fetal were found in all the temporal muscles investigated. The present study shows a higher percentage of hybrid fibres, namely 31% instead of the 7.1% found in the study by Eriksson & Thornell (1983). The fact that we were able to detect MHC-fetal and MHC-cardiac α could explain this difference.

Depending on different staining intensities only 2 hybrid fibres, named IM and IIC, can be demonstrated in masticatory muscles by ATPase histochemistry.

The precise MHC content of these fibre types is not yet fully clear.

Hybrid fibres containing MHC-fetal or MHC-cardiac α are normally not found in human limb or trunk muscles (Bredman et al. 1991; Monemi et al. 1996). It is not known why the temporalis muscle has so many fibres expressing MHC-fetal or MHC-cardiac α. MHC fetal expression was proposed to be typical for a developing muscle (Butler-Brown et al. 1988; Soussi-Yanicositas et al. 1990). MHC-fetal is also expressed in muscle fibres during regeneration (Satore et al. 1982; Schiaffino & Reggiani, 1996). Not known is during what specific conditions MHC- cardiac α is expressed in skeletal muscle fibres.

In the temporalis muscle all types of muscle fibres were significantly smaller than fibres of the same type in studies of human leg muscles (Edgerton et al. 1975; Green et al. 1981). We found that in the temporalis muscle type II fibres were smaller than type I fibres. This is in accordance with other studies of the temporalis muscle (Polgar et al. 1983) and other jaw closing muscles (Mao et al. 1992). The fact that type IIA fibres were smaller can therefore be considered as a normal phenomenon in the temporalis muscle. Some investigators (Clarkson et al. 1981; Lexell et al. 1988; Lexell, 1993) observed in skeletal muscles of older subjects that fast fibres were also smaller than slow fibres in contrast to what is normally found in skeletal muscles. A reduction in fibre size of type IIA fibres in skeletal muscles is said to be associated with denervation and also with inactivity. Table 4 shows that both the intra- and interindividual variability of the cross-sectional area of type I fibres was relatively small, while the variability of the cross-sectional areas of type II and hybrid fibres was relatively large. This suggests that type II and hybrid fibres types are more prone to changes than type I fibres.

The question why jaw closing muscles fibres are significantly smaller compared with limb and trunk muscles still needs to be answered.

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