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Muscle fibre types in the suprahyoid muscles of the rat

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ABSTRACT

Five muscle fibre types (I, IIc, IIa, IIx and IIb) were found in the suprahyoid muscles (mylohyoid, geniohyoid, and the anterior and posterior bellies of the digastric) of the rat using immuno and enzyme histochemical techniques. More than 90% of fibres in the muscles examined were fast contracting fibres (types IIa, IIx and IIb). The geniohyoid and the anterior belly of the digastric had the greatest number of IIb fibres, whilst the mylohyoid was almost exclusively formed by aerobic fibres. The posterior belly of the digastric contained a greater percentage of aerobic fibres (83.4%) than the anterior belly (67.8%). With the exception of the geniohyoid, the percentage of type I and IIc fibres, which have slow myosin heavy chain (MHCβ), was relatively high and greater than has been previously reported in the jaw-closing muscles of the rat, such as the superficial masseter. The geniohyoid and mylohyoid exhibited a mosaic fibre type distribution, without any apparent regionalisation, although in the later MHCβ-containing fibres (types I and IIc) were primarily located in the rostral 2/3 region. In contrast, the anterior and posterior bellies of the digastric revealed a clear regionalisation. In the anterior belly of the digastric 2 regions were observed: both a central region, which was almost exclusively formed by aerobic fibres and where all of the type I and IIc fibres were located, and a peripheral region, where type IIb fibres predominated. The posterior belly of the digastric showed a deep aerobic region which was greater in size and where type I and IIc fibres were confined, and a superficial region, where primarily type IIx and IIb fibres were observed.

Key words: Skeletal muscle; muscle histochemistry.

INTRODUCTION

The morphological, functional and metabolic characteristics of the skeletal muscles in vertebrates are mainly related to the cellular expression of different myosin heavy chain (MHC) isoforms. Of the 9 isoforms that have been described in different species of mammals, only a slow (MHCI/β) and 3 fast (MHCIIa, MHCIIb, and MHCIId/x) predominate in adult skeletal muscles (Schiaffino & Reggiani, 1996). Each muscle fibre has a specific composition of myosin heavy chains: thus type I, IIa, IIx and IIb fibres contain MHCβ, MHCIIa, MCHIIx, and MHCIIb isoforms respectively (Bär & Pette, 1988; Rowlerson, 1994: Schiaffino & Reggiani, 1996). In the rat, these muscle fibre types can be identified by combining immuno and enzyme histochemical techniques (Gorza, 1990; Lind & Kernell, 1991; Hämäläinen & Pette, 1993).

Of all the muscles in the cephalic region, the jaw muscles exhibit the greatest diversity of muscle fibre types with special MHC isoforms such as MHCIIm (Rowlerson et al. 1981, 1983; Kang et al. 1994; Sciote et al. 1995; Kirkeby, 1996) or cardiac MHCα (Bredman et al. 1991; d'Albis et al. 1991; Sciote et al. 1994; Stal, 1994). Most histochemical (Tamari et al. 1973; Taylor et al. 1973; Schiaffino, 1974; Ringqvist et al. 1977; Suzuki, 1977; Hiraiwa, 1978; Maxwell et al. 1979; Clark & Luschei, 1981; Rowlerson et al. 1981; Eriksson & Thornell, 1983; Rokx et al. 1984; Lindman et al. 1986; Bredman et al. 1990) and immunohistochemical (Bosley & Rowlerson, 1980; Rowlerson et al. 1983; Bredman et al. 1991, 1992; Weijs et al. 1993; Sciote et al. 1994, 1995; Stal, 1994; Kirkeby, 1996) research has focused on jaw-closing muscles, whereas the literature concerning jaw-opening and/or suprahyoid muscles is scarce (Maier, 1979; Rowlerson et al. 1983; Rokx et al. 1984) and often limited to the digastric muscle (Hiraiwa, 1978; Clark & Luschei, 1981; Eriksson et al. 1982; Andreo et al.

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1994; Bubb & Sims, 1986; Lev-Tov & Tal, 1987; Miller & Farias, 1988; Bredman et al. 1991).

In the rat, with the exception of Sfondrini et al. (1996) in the digastric, the limited number of histochemical studies of the suprahyoid muscles (Maier, 1979; Rokx et al. 1984; Kiliaridis et al. 1988; Van Lunteren et al. 1995, 1996) neither make reference to the possible presence of certain types of fibres such as IIx, a high percentage of which are found in other skeletal muscles, nor has any evaluation of the expression of different MHC isoforms been undertaken. Consequently, the aim of the present study is to evaluate the proportion and distribution of fibre types in the suprahyoid muscles that are involved to a greater or lesser extent in the masticatory cycle.

MATERIAL AND METHODS

Thirteen female Sprague-Dawley rats weighing 250–350 g each were killed with an overdose of chloral hydrate. Digastric (anterior belly and posterior belly), mylohyoid and geniohyoid muscles were immediately dissected from each animal. Small central cross-section segments (0.5 cm thick) were taken from each muscle, placed on metallic specimen holders with OCT compound (Tissue-Tek II), quickly frozen in isopentane cooled with liquid nitrogen (-160 °C) and stored at -80 °C. Transverse sections (10–12 μm) were cut in a cryostat at -25 °C, mounted on Vectabond-coated (Vector) glass slides, air-dried for a minimum of 30–60 min and processed histochemically or immunohistochemically.

Histochemical methods

Serial sections were stained for succinate dehydrogenase (SDH) and myosin adenosine triphosphatase (ATPase) after preincubation at pH 4.4, 4.7, 7.2 and 10.4.

SDH activity was revealed using a slight modification of the procedure of Kiernan (1981). Briefly, unfixed sections were incubated at 37 °C for 5–10 min in a fresh solution of 0.06 M phosphate buffer (pH 7.0) containing 0.04% nitro-blue tetrazolium (Sigma), 1.36% disodium succinate (hexahydrate) and 0.00075% Meldola blue oxacine (Sigma). The sections were then fixed for 7–10 min in 4% formaldehyde in 0.1 M phosphate buffer of pH 7.0, washed in distilled water, dehydrated in ethanol, cleared in xylene and embedded in Entellan (Merck).

For the demonstration of acid ATPase activity, unfixed sections were preincubated for 130 s (pH 4.7) or 5–7 min (pH 4.4) at 23–25 °C in a solution of 100 mM KCl or 100 mM KBr in 100 mM citrate buffer (Matoba & Gollnick, 1984) adjusted to pH 4.7 or 4.4. Thereafter, the following consecutive steps were carried out (Müller, 1974; Lind & Kernell, 1991): (1) sections rinsed for 30 s at room temperature in a 20 mM glycine buffer (pH 9.4) containing 20 mM $CaCl_2$; (2) sections incubated for 30 min (KCl) or 45 min (KBr) at 23–25 °C in a solution of 20 mM $CaCl_2$ and 2.5 mM ATP disodium salt in 40 mM glycine buffer (pH 9.4); (3) sections washed in 1% $CaCl_2$ (3×30 s); (4) sections immersed in 2% $CoCl_2$ for 3 min; (5) sections washed in distilled water (3×30 s); (6) sections revealed in 1.5% yellow ammonium sulphide for 3 min; (7) sections washed in distilled water and mounted in Vectashield (Vector).

Myofibrillar ATPase activity at pH 7.2 was demonstrated using a procedure similar to the modification of Hämäläinen & Pette (1993) of the Hughes method (1986), except for 2 differences: (1) in the incubation step, the temperature was increased to 39–40 °C and the time reduced to 45–50 min; (2) the sections were mounted in Vectashield.

For the demonstration of alkaline myofibrillar ATPase activity at pH 10.4, the sections were fixed for 5–7 min at 2–4 °C in 4% paraformaldehyde in 0.1 M cacodylate buffer of pH 7.6 containing 1% of $CaCl_2$ and 11.5% of sucrose. The sections were developed following the procedure of Guth & Samaha (1970) and either embedded in Entellan or Vectashield.

Table 1. *Specificities of the monoclonal antibodies used*

	Clone	Source	Working solution
Antibody to:			
MHCI/β (slow skeletal muscle)	NOQ7.5.4D	Sigma	1:4,000
MHCI/β (adult slow skeletal muscle)	A4.951	Alexis	1:10
Cardiac MHCα	F88 12F8, 1	Biocytex	1:2
MHCII (fast skeletal muscle)	MY-32	Sigma	1:1,600

Immunohistochemical methods

Cryosections of muscles were fixed for 5–10 min at 2–4 °C in 4% formaldehyde in 0.1 M phosphate-buffered saline (PBS) of pH 7.4 and washed in PBS (3×5 min). After preincubation for 30 min at room temperature in BSA diluent (1% bovine serum albumin, 0.6 v/v% Triton X-100 in 0.1 M PBS of pH 7.4), to reduce nonspecific binding, the sections were washed in PBS (3×5 min) and incubated for 60–90 min at room temperature with several monoclonal antibodies diluted in BSA (Table 1).

Table 2. *Immunohistochemical and histochemical characteristics of fibre types in the suprahyoid muscles of the rat**

	I	IIc	IIa	IIx	IIb
Slow MHCI/β	++	+/++	−	−	−
Fast MHCII	−	+/++	++	++	++
Cardiac MHCα	−	−	−	−	−
SDH	++	+++	+++	++/+++	+
ATPase pH 10.4	−	++	+++	++	+
ATPase pH 7.2	+	+++	+++	++	−
ATPase pH 4.7	++	−/+/++	−	+	+
ATPase pH 4.4	++	−/+/++	−	−	−

* −, negative; +, weak; ++, heavy; +++, heaviest.

Immunofluorescent staining. The sections were rinsed with PBS (3 × 5 min) and incubated for 1 h at room temperature in a fluorescent isothiocyanate (FITC) fluorochrome-conjugated horse secondary antibody to mouse IgGs (Vector, 1:200 in BSA diluent), washed in PBS and mounted in Vectashield.

Peroxidase staining. After incubation with the secondary antibody, some sections were washed in PBS (3 × 5 min) and treated for 1 h at room temperature with a HRP-conjugated anti-FITC rabbit tertiary antibody (Dako, 1:200 in BSA diluent). Once more, the sections were rinsed in PBS (3 × 5 min) and were developed with 0.05 diaminobenzidine in 0.1 M Tris buffer (pH 7.4) containing 0.0105 v/v% H_2O_2 for 10–20 min at room temperature. The slides were then washed, dehydrated, cleared and coverslipped with Entellan.

Quantitative and morphometric analysis

For the determination of muscle fibre composition, all fibres contained in each cross-section were typed to determine the percentage of each fibre type. Each muscle section was subdivided to assess the regionalisation. In addition, the percentage of fibre types in the central and peripheral regions of the anterior belly of the digastric were quantified separately, as were the deep and superficial regions of the posterior belly.

Photomicrographs of representative areas incubated for alkaline ATPase activity were calibrated with a micrometer and projected onto a rigid screen. Only areas free of artefact, with muscle fibres that had been cut perpendicular to their long axes and possessing well defined cell boundaries, were considered for analysis. The fibre size was measured using a Kontron Videoplan semiautomatic image analyser which derives the mean diameter (defined as the diameter of a circle of equal area to that of the muscle fibre section).

RESULTS

Fibre type composition

The combination of immuno and enzyme histochemical techniques enabled the distinction of type I, IIc, IIa, IIx and IIb fibres in the suprahyoid muscles of the rat (Table 2). No fibres containing MHCα (in our laboratory the antibody F8812F8,1 proved to be most effective in revealing this isoform in the jaw muscles of the rabbit) were detected.

Type I fibres were positive with the 2 antibodies used for detecting slow MHCI/β (clones NOQ7.5.4D and A4.951), but no reactivity was observed with the antibody used for detecting fast fibres (clone MY-32). The histochemical characteristics of these fibres are shown in Table 2 and do not appear to differ from those mentioned by other authors in other skeletal muscles in the rat.

Type IIc fibres were labelled using both antibodies for detecting slow and fast MHCs (Figs 1, 3*e*, *f*; Table 2). With slow MHCI/β antibodies, type IIc fibres varied in intensity, ranging from slightly positive to a degree similar to that observed in type I fibres. Histochemically, all the IIc fibres were heavily stained for myofibrillar ATPase after preincubation at pH 7.2 and 10.4. Nevertheless, acid ATPase activity of the type IIc fibres was heterogeneous: the weakly labelled-MHCβ fibres, the most abundant, were not stained after preincubation at pH 4.4, and had a light to intermediate intensity after preincubation at pH 4.7 (these fibres were only detected using immunohistochemical techniques); the other IIc fibres, which reacted heavily with the slow MHC antibodies, were also identified histochemically since these fibres were heavily stained after preincubation at pH 4.4 (though they were not as dark as type I fibres) and intermediate or dark after preincubation at pH 4.7.

Type IIa, IIx and IIb fibres reacted with MHCII antibody (Fig. 1*a*, *b*) and have been identified histochemically using various methods for mATPase

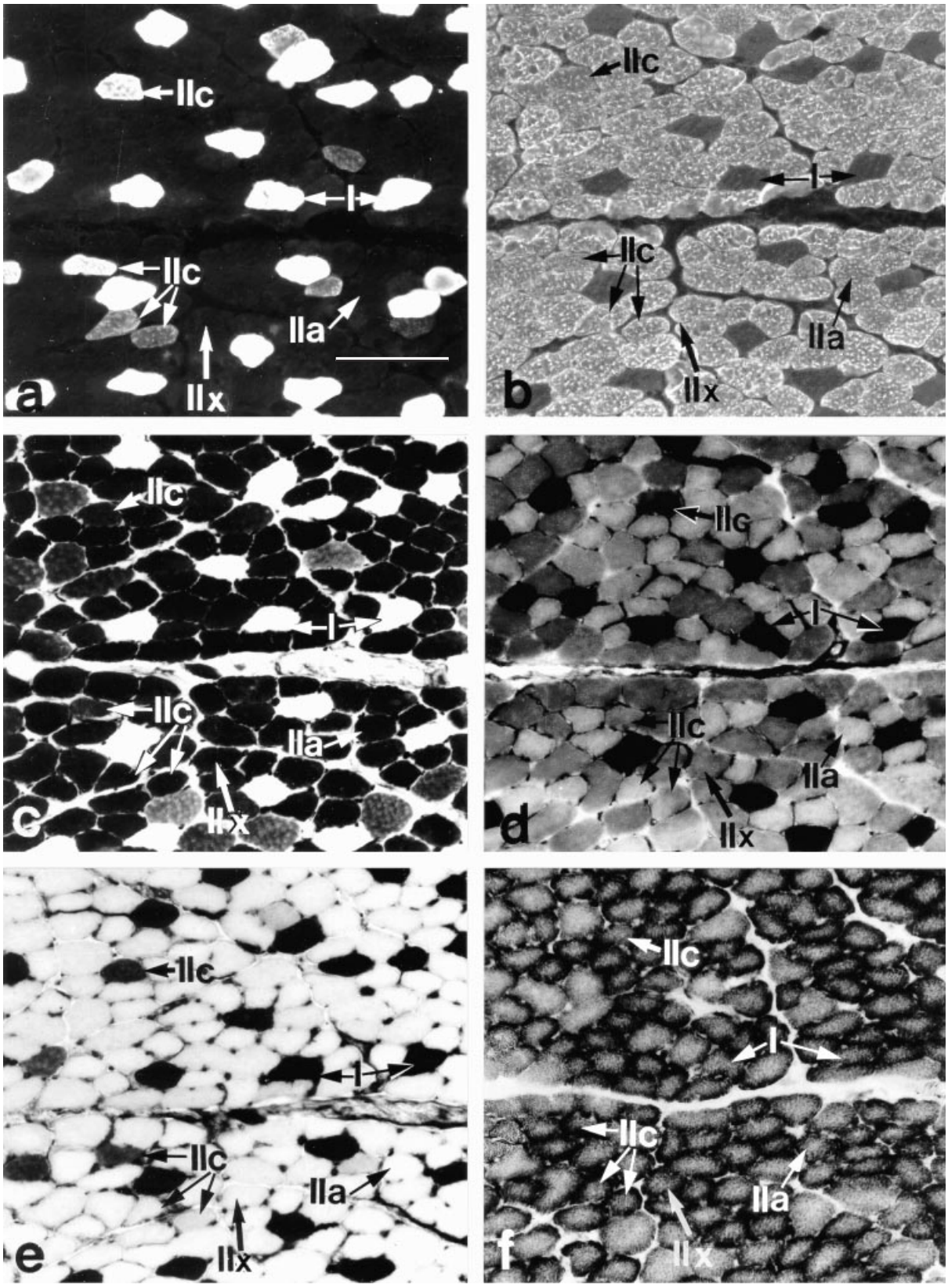

Fig. 1. Consecutive transverse sections of the central area of the anterior belly of the digastric muscle, reacted with Sigma MHCβ (*a*) and MHCII (*b*) antibodies, and stained for succinate dehydrogenase (*f*) and for myosin ATPase after preincubations at pH 10.4 (*c*), at pH 4.7 (*d*) and at pH 4.4 8 (*e*), showing the histochemical patterns of the type I, IIc, IIa, and IIx fibres. Bar, 100 μm.

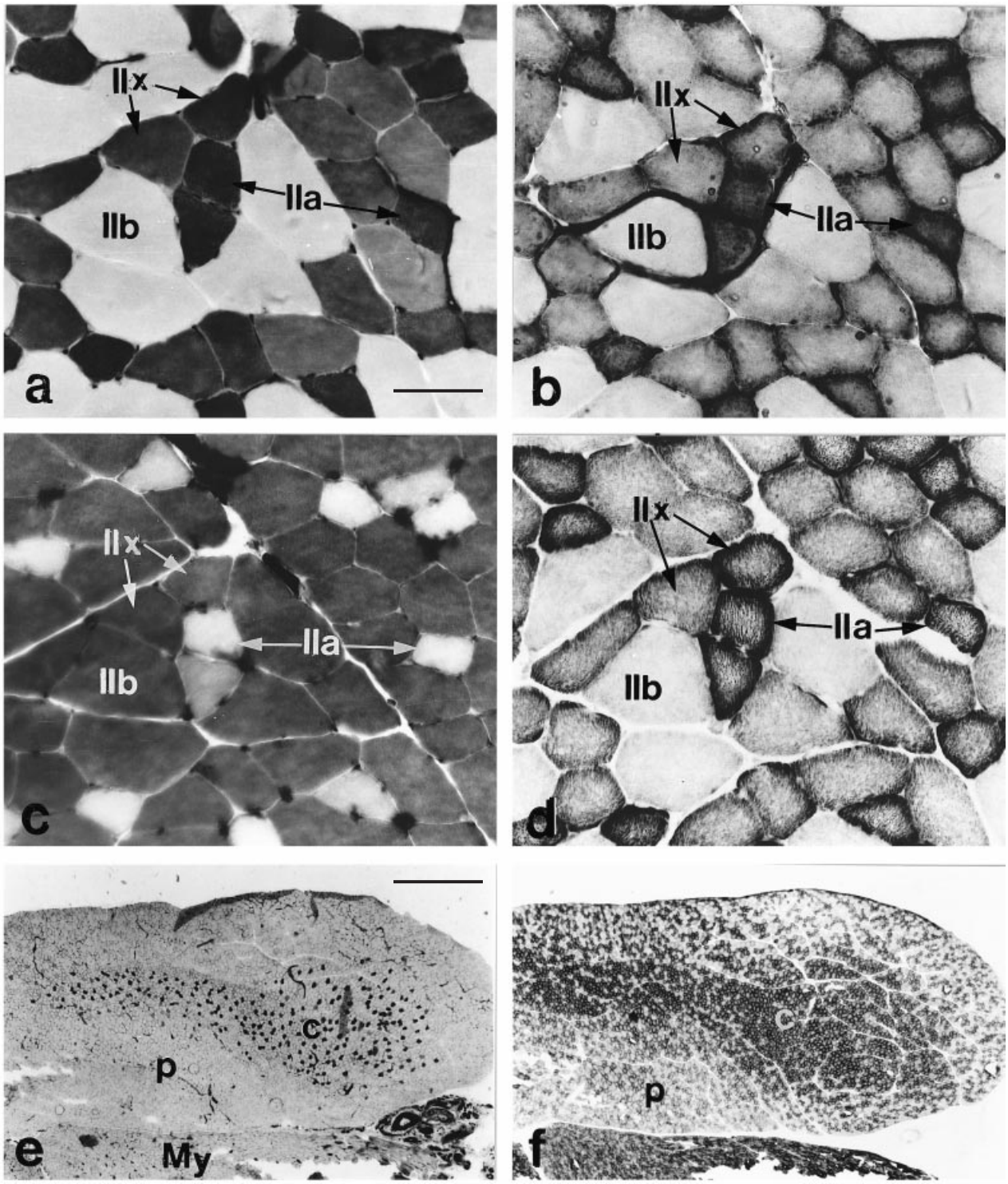

Fig. 2. (*a–d*) Serial transverse sections of the peripheral area of the anterior belly of the digastric muscle stained for succinate dehydrogenase (*d*) and for myosin ATPase after preincubations at pH 10.4 (*a*), at pH 7.2 (*b*) and at pH 4.7 (*c*) showing the histochemical patterns of the type IIa, IIx and IIb fibres. Bar, 50 μm. (*e*, *f*) Panoramic view of the anterior belly of the digastric muscle stained for acid myosin ATPase at pH 4.4 (*e*) and reacted with succinate dehydrogenase (*f*); the central oxidative area (c) containing type I fibres is clearly distinguished from the peripheral area (p). My, mylohyoid muscle. Bar, 500 μm.

staining (Hämäläinen & Pette, 1993). After preincubation at pH 4.4, type IIa, IIx and IIb fibres were not stained. It was possible to separate type IIa fibres from types IIx and IIb because the former appeared pale after acid preincubation at pH 4.7 (Figs 1*d*, 2*c*, 3*c*), and they were the darkest after preincubation at pH 7.2 and pH 10.4 (Figs 2*a*, *b*, 3*a*, *b*). Type IIb fibres were separated from type IIx and IIa fibres because they had a lighter appearance after alkaline preincubation and totally negative at pH 7.2, as well as

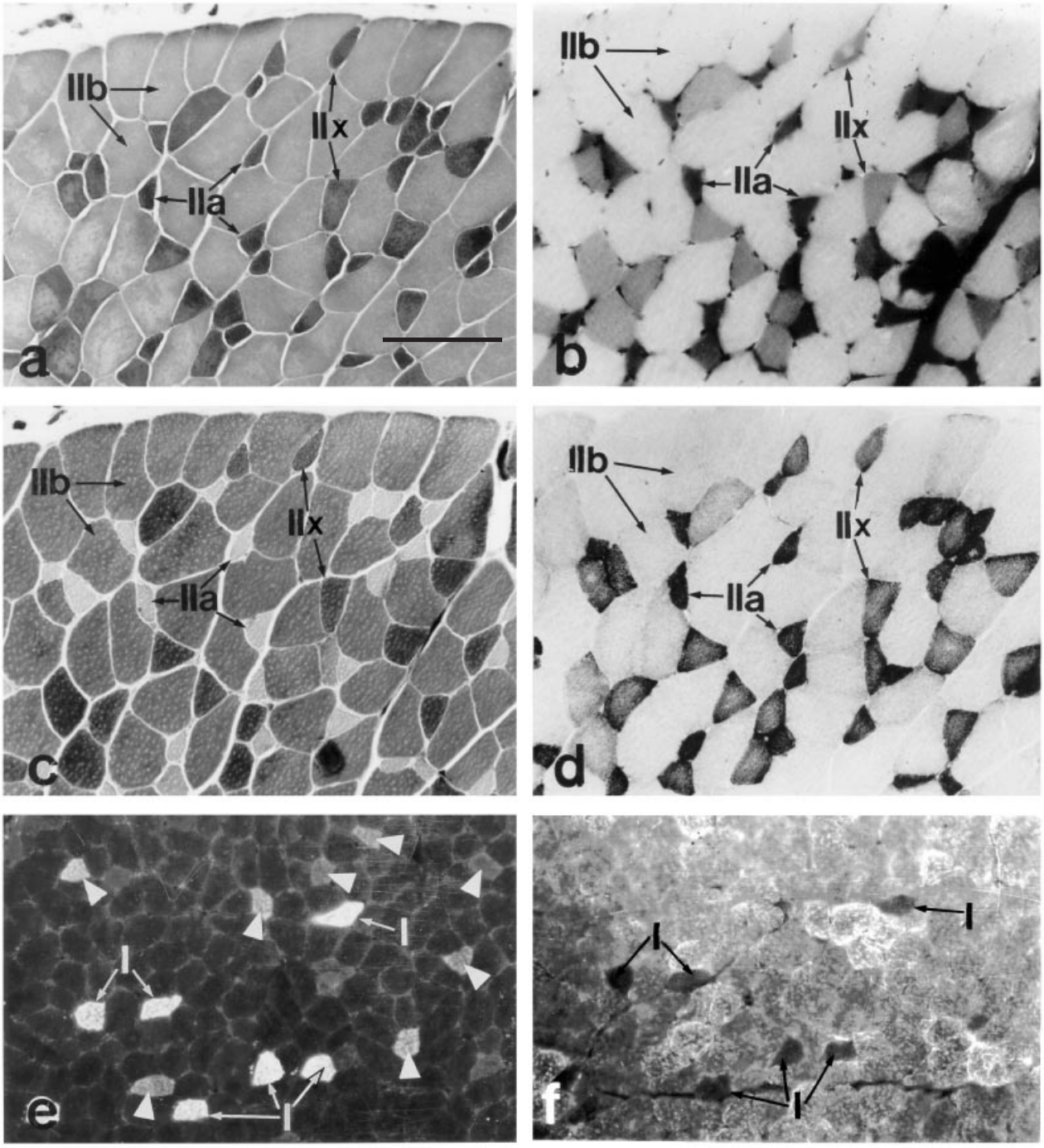

Fig. 3. (*a–d*) Successive transverse sections of the geniohyoid muscle stained for succinate dehydrogenase (*d*) and for myosin ATPase after preincubations at pH 10.4 (*a*), at pH 7.2 (*b*) and at pH 4.7 (*c*), demonstrating the histochemical patterns of the type IIa, IIx and IIb fibres. (*e*, *f*) Consecutive cross-sections of the mylohyoid muscle reacted with Sigma MHCβ (*e*) and MHCII (*f*) antibodies to show the immunohistochemical patterns of the type I (I) and IIc (arrowheads) fibres. Bar, 100 μm.

exhibiting a moderate mATPase activity at pH 4.7 and lower levels of SDH activity. Type IIx fibres exhibited intermediate properties in relation to types IIa and IIb. In comparison with type IIa, the IIx fibres separated because they were moderately positive for mATPase at pH 4.7, and they were distinguishable from type IIb fibres because they were darker at pH 7.2 and pH 10.4. It should be noted that with these classification criteria, type IIa and IIb fibres contain basically the isoforms MHCIIa and MHCIIb respectively, whereas type IIx fibres included both those that exclusively express the isoform MHCIIx and the hybrid forms that contain a mixture of MHCIIx/MHCIIa or MHCIIx/MHCIIb isoforms. These hy-

Table 3. *Mean value ($\mu m \pm$ S.D.) of muscle fibre diameter in the suprahyoid muscles of the rat*

	I	IIc	IIa	IIx	IIb
Digastric					
anterior belly	35.3±4.3	34.1±6.3	34.8±5.1	41.7±4.4	52.2±7.9
posterior belly	35.4±6.0	34.1±4.1	40.5±4.7	43.4±6.5	51.0±6.9
Mylohyoid	21.9±3.3	23.8±2.7	28.4±4.6	35.1±3.0	—
Geniohyoid	22.1±2.9	22.3±4.0	22.7±3.3	39.9±7.3	54.5±7.8

Table 4. *Percentage fibre type composition (± S.D.) of the suprahyoid muscles of the rat. Fibre type percentages of the central and peripheral regions of the anterior belly of digastric and the deep and superficial regions of the posterior belly of digastric are also given*

	I	IIc	IIa	IIx	IIb
Digastric (anterior belly)	4.6±1.0	2.8±2.7	27.5±5.7	32.9±2.8	32.2±3.2
Central	10.1±3.5	6.0±4.9	36.6±7.0	34.4±3.6	12.9±2.1
Peripheral	—	—	19.7±7.5	31.7±5.1	48.6±2.5
Digastric (posterior belly)	8.1±1.9	1.0±1.0	36.2±3.8	41.1±2.4	13.6±0.4
Deep	12.6±2.8	1.6±1.0	47.7±7.4	35.6±5.3	2.5±1.7
Superficial	—	—	15.0±11.3	51.1±10.7	33.9±4.8
Mylohyoid	1.2±1.0	3.4±1.7	27.3±3.6	68.2±2.1	0.1±0.1
Geniohyoid	0.5±1.2	0.9±0.5	30.8±2.0	27.2±2.7	40.6±1.2
*	0.0±0.0	0.0±0.0	32.1±3.1	28.2±2.5	39.7±4.3
**	0.0±0.0	1.8±1.1	28.1±2.0	28.7±1.9	41.3±1.1
***	1.4±0.9	0.6±0.4	32.6±2.4	25.2±2.9	40.2±1.0

*, Geniohyoid group without type I and IIc fibres (3 animals); **, Geniohyoid group with type IIc fibres (5 animals); ***, Geniohyoid group with type I and IIc fibres (5 animals).

brid forms can only be quantified with specific antibodies.

As for SDH activity, which is directly related to the aerobic capacity, it diminished in the order (IIa, IIc) > (I, IIx) > IIb. Moreover, type IIb fibres were the largest, type IIx the intermediate, and types I, IIa and IIc the smallest in all the muscles examined (Table 3). In terms of fibre size, the mylohyoid had the smallest fibres, and the geniohyoid exhibited the greatest variation in mean diameter ranging from the smallest (IIa, I and IIc) to the largest (IIb).

Fibre type percentage and distribution (Table 4)

The anterior and posterior belly of the digastric muscle showed notable differences in the proportion of fibre types. The posterior belly had a greater aerobic capacity than the anterior belly, since it had a greater percentage of fast aerobic fibres (types IIa and IIx) and slow contracting fibres (types I and IIc). In comparison with the mylohyoid and the geniohyoid, the 2 bellies of the digastric showed a clear regional differences in the distribution of fibre types. The regionalisation of the anterior belly consisted of a central and a peripheral area, (Fig. 2*e*, *f*), whereas in the posterior belly a deep area, extending towards the temporal bone, and a superficial area were observed. The central region of the anterior belly and the deep region of the posterior belly were highly aerobic, where type IIb fibres only represented 12.9% and 2.5% respectively. Furthermore, the fibres that contained slow myosin (I and IIc) were only found in this aerobic area. One of the reasons why the posterior belly had a greater aerobic capacity than the anterior belly lies in the fact the deep region encompassed 55.5±4% of the total muscle section, whereas in the anterior belly the central region only encompassed 35±2%. Moreover, a significantly higher percentage of type IIb fibres (48.6%) was found in the peripheral region of the anterior belly than in the superficial region of the posterior belly (33.9%), which highlights that the superficial region of posterior belly is more aerobic than the peripheral area of the anterior belly.

The mylohyoid was almost entirely made up of aerobic fibres, and most of the fast fibres were types IIx (68.2%) and IIa (27.3%), which were homogeneously distributed in the entire muscle. It is worth mentioning that fibres with slow myosin (I, IIc) were detected in all animals, and fewer type I fibres (1.2±1.0%) were observed than type IIc fibres

(3.4±1.7%). Moreover, the percentage of type IIc fibres was the highest of all the suprahyoid muscles studied. Although no clear regionalisation was observed in the mylohyoid muscle type I and IIc fibres were mainly located in the rostral 2/3.

Of the suprahyoid muscles examined, the geniohyoid had the lowest aerobic capacity given that it had the greatest percentage of type IIb fibres (40.6±1.2%). Moreover, this muscle had the greatest percentage (98–100%) of fast fibres (IIb, IIx and IIa), that were homogeneously distributed throughout the entire muscle in a mosaic arrangement. The expression of MHCβ in the geniohyoid was observed to vary significantly in the rat (Table 4): in some animals no MHCβ was detected, in others MHCβ was only detected as type IIc fibres and in a third group MHCβ was detected both in type I and IIc fibres. Nonetheless, the percentage of fibres containing slow MHCβ was never greater than 2%.

DISCUSSION

Fibre types

In this study, 5 types of muscle fibres (I, IIc, IIa, IIx, and IIb) were distinguished in the suprahyoid muscles of the rat. Although recently small quantities of cardiac MHCα have been reported in the extrafusal fibres of skeletal muscles in the hindlimbs of Sprague-Dawley rats (Dunn & Michel, 1997), no positive reaction was observed in the suprahyoid muscles. Thus our results agree with those described by other authors (Pedrosa et al. 1990; McWhorter et al. 1995) who restrict the presence of cardiac MHCα to the intrafusal fibres in the rat.

A small percentage of type IIc fibres, considered as a transitional form between types I and IIa (Rowlerson et al. 1988; Rowlerson, 1994), have been observed in the rat using immunohistochemical techniques in masseter (Rowlerson et al. 1988) and some hindlimb (Pierobon-Bormioli et al. 1981; Staron & Pette, 1993; Bottinelli et al. 1994) muscles. In contrast, our findings revealed a relatively higher percentage in all the suprahyoid muscles examined, particularly, in the central region of the anterior belly of the digastric (6.0±4.9%) and in the mylohyoid (3.4±1.7%). In the latter muscle, they accounted for the greatest number of the fibrillar population in comparison with type I fibres. Initially, type IIc fibres were defined as skeletal muscle fibres that show acid-stable and alkali-stable ATPase activity (Brooke & Kaiser, 1970). Recently, they have been defined as hybrid fibres that coexpress slow MHCβ and fast MHCII (Billeter et al. 1980; Pierobon-Bormioli et al. 1981; Rowlerson, 1994). As for the aerobic capacity, type IIc fibres have an intermediate activity in comparison with type I and IIa fibres (Hintz et al. 1984). It should be noted that in the jaw muscles of man (Eriksson et al. 1982; Eriksson & Thornell, 1983) and rabbit (Bredman et al. 1990) the vast majority of fibres that according to histochemical criteria have been classified as type IIc are not homologous with those in the rat, particularly if we consider recent findings that show that they express cardiac MHCα (Bredman et al. 1991). Histochemically, type IIc was observed to vary considerably in the suprahyoid muscles depending on the level of slow and fast MHCs. Type IIc fibres containing high levels of MHCβ were acid-stable and alkali-stable ATPase, whereas type IIc fibres with lower levels of MHCβ appeared pale after preincubation at pH 4.7, thus erroneously they may be classified as type IIa fibres. As this was the most frequently observed histochemical pattern in the mylohyoid and geniohyoid of the rat, this implies that without the use of immunohistochemical techniques the percentage of slow myosin fibres obtained would be considerably less than is really the case. It may be that in the rat the IIc fibres fulfil a function which is similar to fibres with cardiac MHCα in the jaw muscles of man and rabbit, given that the 2 type fibres have a contraction speed intermediate to types I and IIa (Kugelberg, 1976; Kwa et al. 1995; Sciote & Kentish, 1996).

The percentages of type I fibres in the digastric were 4.6% and 8.1% for the anterior and posterior bellies respectively, which is similar to the findings of Hiraiwa (1978) but much greater than the number described by other authors (Kiliaridis et al. 1988; Sfondrini et al. 1996). In larger mammals, the percentage of type I fibres in the digastric is greater than that found in the rat; thus they range from 10–25% in the rabbit and dog (Hiraiwa, 1978; Bub & Sims, 1986) to 1/3 in man (Eriksson et al. 1982), whereas in nonhuman primates (Clark & Luschei, 1981; Miller & Farias, 1988) and in cows (Hiraiwa, 1978) they account for 50%. Our findings revealed a smaller percentage of type I fibres in the mylohyoid (1.2%), which is in the range reported by Rokx et al. (1984), although is considerably less than the 40% detected in man (Vignon et al. 1980). In the geniohyoid only a small percentage of type I fibres was found in some rats (1.4%). Previous histochemical (Maier, 1979; Rokx et al. 1984) and immunohistochemical studies (Petrof et al. 1992) have also reported very few type I fibres in the geniohyoid of the rat, whereas in the cat they represent 16% (Dick & Van Lunteren, 1990). These findings

suggest that smaller animals have fewer slow contracting fibres than larger animals (Suzuki, 1977; Hiraiwa, 1978; Gauthier, 1986; Bredman et al. 1990).

In the suprahyoid muscles of the rat, a relatively high number of slow MHCβ-containing fibres (types I and IIc) were observed in the posterior (9.1%) and anterior (7.4%) bellies of the digastric as well as the in the mylohyoid (4.6%), in comparison with the mean value obtained in the geniohyoid (0.9%). These values are much higher than those reported by Rowlerson et al. (1988) in masseter (0.3%), and by Staron & Pette (1993) in adductor magnus (0.7%) and in tibialis anterior (2%). The expression of MHCβ in the mylohyoid in our Sprague-Dawley rats differed from the results obtained by Bär & Pette (1988), who did not find this isoform in the mylohyoid of the Wistar rat. This seems to indicate that besides sex, age and other factors (Rowlerson, 1994), the composition of MHCs in the muscles may also depend on the breed of rat. Moreover, the expression of slow MHCβ observed in the geniohyoid is also indicative of individual variations in fibre type patterns.

More than 90% of the fibres in the suprahyoid muscles of the rat are composed of fast contracting or type II fibres. With the exception of a few muscles such as soleus and the diaphragm, this is the pattern observed in the skeletal muscles of the rat (Bär & Pette, 1988; Petrof et al. 1992; Hämäläinen & Pette, 1993). The 3 common type II fibres which have so far been identified in the skeletal muscles of the rat (IIa, IId/x and IIb) were found in the 4 muscles under study. Type IIx fibres predominated in the mylohyoid and in the posterior belly of the digastric and account for a significant number of the fibrillar population in the anterior belly of the digastric and in the geniohyoid. The sum total of the percentages of aerobic fibres (I, IIc, IIa, and IIx) agrees with the findings of other authors (Hiraiwa, 1978; Maier, 1979), who suggested that the digastric and mylohyoid of the rat are fundamentally composed of aerobic (oxidative) fibres. Without doubt, of all the jaw muscles in the rat the mylohyoid is the one that contains the highest number of aerobic fibres; this view is also supported by Rokx et al. (1984). Although Kiliaridis et al. (1988) estimated that the anterior belly of the digastric contains 43.8–61.1% of type IIb fibres, our results (32.2%) come closer to the data (28%) obtained by Sfondrini et al. (1996). Whereas the percentage of anaerobic fibres in the geniohyoid was the highest of all the suprahyoids (40.4%), the sum total of all the aerobic fibres was approximately 60%, and this does not agree with the results obtained in the rat by Maier (1979) and in the cat by Dick & Van Lunteren (1990), who stated that in both cases the percentages are almost the same.

Regional variation of fibre types

Of the suprahyoid muscles examined only the anterior and posterior bellies of the digastric revealed a clear regionalisation, whereas in the mylohyoid and in the geniohyoid the fibre types were distributed in a mosaic pattern. Although slow contracting fibres have been reported in central areas of the mylohyoid of the rat (Maier, 1979), in our study type I and IIc fibres were homogeneously located among type IIx and IIa fibres. The almost exclusive location of these fibres with slow MHCβ in the rostral 2/3 of the mylohyoid may indicate that these fibres serve to stabilise the mandibular symphysis.

Like other skeletal muscles in the rat (Pullen, 1977; Lind & Kernell, 1991; Fuentes et al. 1998), the 2 bellies of the digastric exhibit 2 well defined regions: (1) a more aerobic region, central in the anterior belly and deep in the posterior belly, where the slow MHCβ-containing fibres (I and IIc) are located; (2) a less aerobic region, peripheral in the anterior belly and superficial in the posterior belly, where type IIb and IIx fibres predominate. The confinement of MHCβ-containing fibres to one area has already been reported in the masseter of the rat (Rowlerson et al. 1988). The pattern of regionalisation observed in the anterior belly of the digastric agrees with the observations of other authors (Maier, 1979; Rokx et al. 1984; Kiliaridis et al. 1988) who refered to a central oxidative region with type I fibres. Nevertheless, the regionalisation of the posterior digastric in deep and superficial areas does not coincide with the results obtained by Maier (1979) and Rokx et al. (1984), who described a pattern similar to that observed in the anterior digastric.

The regionalisation of the digastric into 2 areas has also been described in the guinea pig (Lev-Tov & Tal, 1987), but in this case both bellies exhibited the same distribution pattern and a similar percentage of fibres types. In contrast, the fibre type distribution in man (Eriksson et al. 1982) and nonhuman primates (Andreo et al. 1994) exhibits a mosaic pattern. Nonetheless, the digastric in the rat and man exhibit a certain similarity in as much that in both species the posterior belly has more aerobic fibres than the anterior belly. A possible explanation may lie in that both humans and rats have a fascial sling that attaches the tendon of the digastric to the hyoid bone, in such a way that both bellies have distinct functions during chewing and swallowing. In contrast, in the

guinea pig no union exists between the tendon of the digastric and the hyoid bone; thus the 2 bellies of the digastric function almost synchronously as if they were a single muscle (Lev-Tov & Tal, 1987).

Functional implications

The large number of aerobic muscle fibres and the relatively numerous slow MHCβ-containing fibres found in the suprahyoid muscles suggest that the function of these muscles in the chewing cycle is not relegated to a jaw-opening action. In fact, electromyographic studies have confirmed the involvement of the digastric (Weijs & Dantuma, 1975; Thomas & Peyton, 1983) and the other suprahyoid muscles (Weijs & Dantuma, 1975) in the grinding phase of the chewing cycle.

Of the suprahyoid muscles, the mylohyoid muscle is the most active during grinding, as well as being highly active during the jaw-opening phase (Weijs & Dantuma, 1975). In all likelihood, its main function in the chewing cycle is to stabilise the jaw in cooperation with tranversus mandibularis. Moreover, the mylohyoid also functions to pull the hyoid bone rostrally and elevate the floor of the oral cavity. These functions explain the highly aerobic nature of this muscle, as well as the relatively high number of slow MHC fibres.

The larger number of aerobic and slow fibres in the posterior digastric in comparison with the anterior belly, may be due to the fact that the former generates greater force in stabilising and closing the jaw while chewing, as well as having an important role in fixing the hyoid bone. In contrast, the distribution pattern of the fibres in the anterior belly (the peripheral region of which is mainly composed of type IIb fibres such as the geniohyoid) suggests that it has some functions independent to the posterior belly. Although the anterior belly transmits the force of the posterior disgastric during the jaw-opening phase, it is aligned with the anterior suprahyoid muscles to move the hyoid bone rostrally when swallowing.

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