Unloaded shortening velocities of rabbit masseter muscle fibres expressing skeletal or α-cardiac myosin heavy chains

James J. Sciote* and Jonathan C. Kentish

Department of Pharmacology, United Medical and Dental Schools, St Thomas's Campus, London SE1 7EH, UK

1. Some rabbit masseter fibres express the α-cardiac myosin heavy chain (MHC). To compare the biochemical and physiological properties of these fibres with other skeletal fibre types, we examined the histochemical and immunohistochemical staining characteristics, maximum velocity of shortening (V_0) and MHC isoform content of fibres from rabbit masseter and soleus muscles.

2. The fibre-type composition of muscle sections was determined with MHC antibodies and myofibrillar ATPase histochemistry. Fibres we designated 'type α-cardiac' were different from type I and type II fibres in that they stained positively with the α-cardiac MHC antibody and they maintained ATPase reactivity after acid and alkali pre-incubations. Samples of superficial masseter contained a few type I fibres, with the majority of fibres classified as either type II A or type α-cardiac. Soleus samples contained type I, II A and II C fibres.

3. The V_0 of chemically skinned fibres was determined by the slack-test method. Each fibre was subsequently characterized as type I, II A, II C or α-cardiac from MHC identification using gel electrophoresis (SDS–PAGE). In masseter fibres the V_0 values were (in muscle lengths s^{-1}): type I, 0·54 ± 0·05 (mean ± S.D., $n = 3$); type II A, 1·23 ± 0·34 ($n = 27$); type α-cardiac, 0·78 ± 0·08 ($n = 9$). In soleus fibres V_0 values were: type I, 0·55 ± 0·06 ($n = 14$); type II A, 0·89 ± 0·04 ($n = 8$); type II C, 0·73 ($n = 2$).

4. We conclude that the rabbit masseter muscle contains an 'α-cardiac' fibre type that is distinct from other skeletal fibres. This fibre type expresses only the α-cardiac MHC, has unusual myofibrillar ATPase reactivity and has a V_0 intermediate between type I and type II fibres.

Mammalian jaw-closing muscles contain fibre types specialized for masticatory function. In most carnivores and primates these muscles contain, in addition to type I and II C fibres, a type II masticatory fibre with a unique myosin heavy chain (type II M MHC) and a very fast twitch contraction speed (Rowlerson, Pope, Murray, Whalen & Weeds, 1981). Human jaw-closing muscles lack the II M fibre type but contain other fibres of unusual morphology and MHC composition (Sciote, Rowlerson, Hopper & Hunt, 1994). These fibres may express type I, II A, II B (and possibly II X) MHC homogeneously, or heterogeneously in various combinations with neonatal, embryonic and α-cardiac myosin heavy chains (Bredman, Wessels, Weijs, Korfage, Soffi & Moorman, 1991). In small mammals, the jaw-closing muscles contain type I, II A, II X (and rarely II B) fibres (Rowlerson, 1990). The rabbit is apparently unique for small mammals in that, like the human, some fibres also express the α-cardiac MHC (Bredman *et al.* 1991; d'Albis, Janmot, Mira & Couteaux, 1991), an isoform which was thought to be found almost exclusively in heart muscle. Although in skeletal muscle it is generally true that type I fibres, containing the type I MHC, have a slower maximum velocity of shortening than type II fibres, containing type II MHC (e.g. Reiser, Kasper, Greaser & Moss, 1988), the physiological properties of skeletal fibres containing α-cardiac MHC are unknown. The present study was designed to examine the properties of fibres expressing the α-cardiac MHC in the rabbit masseter muscle. Although the α-cardiac MHC has been found to be expressed heterogeneously with other MHC isoforms, we identified an area of rabbit masseter with a relatively large population of fibres that were homogeneous for α-cardiac MHC. We then determined the histochemical and immunohistochemical staining properties, MHC isoform content

* Present address to which correspondence should be sent: New York University, College of Dentistry, Basic Science Division, 345 East 24th Street, New York, NY 10010-4086, USA.

and velocity of unloaded shortening (V_0) of these α-cardiac fibres and compared them with the type I and II fibres in rabbit masseter muscle and with the fibres in a limb skeletal muscle, namely the soleus.

A preliminary report of some of this work has been presented (Sciote & Kentish, 1995).

METHODS

Muscle selection and processing

Young adult male New Zealand White rabbits (~2·5 kg) were killed by overdose of sodium pentobarbitone (80 mg kg^{-1} I.V.). In initial experiments, muscle samples of ~0·5 cm^3 were taken from various areas of soleus, superficial masseter and, for comparison, from lateral psoas and adductor magnus. On the basis of MHC content and distribution in these samples, determined as described below, we selected specific areas of the masseter and soleus for further investigation: in the masseter this was the middle area of the superficial fascicle located close to the inferior border of the mandible; in the soleus we used a lateral area which was paler than the rest of the muscle. For the experiments reported here, these selected areas of masseter and soleus were obtained from six rabbits. Each sample area (approximately 5 mm × 5 mm × 2 cm) was divided into three parts designated for histochemistry/immunohistochemistry, physiological experiments or electrophoresis, as described below. In addition, samples were taken from the atria and were processed for electrophoresis only.

Histochemistry and immunohistochemistry

The muscle samples were frozen in isopentane cooled by liquid nitrogen to −70 °C and were sectioned serially at 10 μm on a cryostat. Sections were stained for reactivity to MHC-specific antibodies or myofibrillar ATPase activity to determine fibre types and their distribution. Adjacent samples containing the same fibres were prepared for sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) to determine MHC composition. Myofibrillar ATPase reactivity was determined by the staining method described by Snow, Billeter, Mascarello, Carpene, Rowlerson & Jenny (1982, method A). This technique involves staining after pre-incubation in buffer at pH 10·4, 10·2, 4·6, 4·3, or 4·2 to distinguish the fibre types. Type II fibres were separated as follows: II D fibres (probably the same as II X fibres) and II A fibres were reactive after pre-incubation in buffer at pH 10·4 and type II B were unreactive; II B and II D fibres were reactive after pre-incubation in buffer at pH 4·6 and II A unreactive. (All type II fibres were reactive after pre-incubation in buffer at pH 10·2 and unreactive after pre-incubation in buffer at pH 4·3.) Type I fibres were unreactive after pre-incubation in buffer at pH 10·4 and 10·2, and reactive after buffer at pH 4·6 and 4·3.

Immunohistochemical staining was by the indirect immunoperoxidase method after incubation in the following MHC antibodies: type I polyclonal, specific for type I MHC with minor reactivity for slow skeletal myosin light chains (MLCs); type II A polyclonal, specific for II A MHC; type fast monoclonal (clone MY-32, Sigma), selective for all type II MHCs; type α-cardiac monoclonal (clone MAS 366, Sera-lab, Crawley Down, Sussex, UK), specific for only the α-cardiac MHC (see below); type neonatal polyclonal, specific for neonatal MHC; and type II M polyclonal, specific for II M MHC. Anti-mouse IgG secondary antibody (A-9917, Sigma) was used with the primary monoclonal antibodies and anti-rabbit IgG secondary antibody (A-0545, Sigma) was used with the primary polyclonal antibodies. The specificity of the α-cardiac monoclonal antibody has previously been described by Leger, Bouvagnet, Pau, Ronccoci & Leger (1985). With our immunohistochemical staining method, this cardiac antibody (clone MAS 366) stains mammalian cardiac tissue and only skeletal fibres that contain the α-cardiac MHC, including fibre subpopulations in: rat extraocular muscle (DelGaudio, Sciote, Carroll & Esclamado, 1995), human masseter muscle (Sciote *et al.* 1994) and rabbit masseter muscle (present results). In these species, our consistent finding was that all skeletal fibres staining positively for MAS 366 also had strong myofibrillar ATPase activity after pre-incubation in either acid or alkali buffer. MAS 366 never stained skeletal fibres that could be histochemically or immunohistochemically classified as type I, II A, II B or II D in limb, masticatory, laryngeal or extraocular muscle. Specificities for the other antibodies are described by Sciote *et al.* (1994).

Slack-test determination of unloaded shortening velocity (V_0)

The glycerination technique and the experimental apparatus have been described previously (Palmer & Kentish, 1994). In brief, the muscle samples were dissected into small bundles of fibres, tied to capillary tubes and glycerinated for >2 days at −20 °C. Individual fibres (mean length, 2·8 mm) were then dissected from a bundle and glued with cellulose acetate in acetone to carbon-fibre rods attached to a high-speed servomotor (300S, Cambridge Technology Inc., Watertown, MA, USA) and to an isometric force transducer (AE801, SensoNor, Horten, Norway). The fibre could be moved between a series of different wells. Force and muscle length were recorded on a chart recorder and on an AT-compatible computer using a 12-bit D/A board (Digidata 1200, Axon Instruments) and pCLAMP software (Axon Instruments). pCLAMP was also used to generate the series of length changes for velocity measurements. A He–Ne laser beam was directed onto the fibre and the resulting diffraction pattern was focused onto a photodiode array in order to continuously monitor sarcomere length. All experiments were carried out at 15 °C.

After the mounting of the fibre, it was immersed in relaxing solution, which contained (mM): 55 potassium propionate, 6·24 $MgCl_2$ ($[Mg^{2+}] = 1$), 10 sodium phosphocreatine, 100 *N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid (Bes), 6·21 Na_2ATP ($[MgATP^{2-}] = 5$), 2·5 P_i, 1 dithiothreitol, 10 K_2EGTA, pCa ($-\log_{10}[Ca^{2+}]$) ~9·0, pH 7·1 at 15 °C, ionic strength 0·20 M. The sarcomere length was set to 2·5–2·7 μm and its uniformity along the fibre was checked. Fibres with non-homogeneous sarcomere length were discarded. The fibre was then moved into maximal activating solution (i.e. relaxing solution with 10 mM CaK_2EGTA; pCa 4·5) and was given ramp releases and quick re-stretches to enhance the stability of sarcomere length (Brenner, 1983). Once force was steady, V_0 was determined using the slack-test method (Edman, 1979), as illustrated in Fig. 1. A series of six shortening steps, each lasting 0·14 s, was imposed upon the muscle. After each step the muscle was returned to its original length for 3·86 s, during which force and sarcomere length recovered completely (note the constancy of the initial force in Fig. 1*B*). The muscle was returned to the relaxing solution and then placed in sample buffer and processed for electrophoresis, as described below. Data were excluded if, during the activation, the sarcomere length was unstable or steady force declined by >10%. For each length change (ΔL), the time it took for the fibre to shorten and just

redevelop force was measured (Δt; arrows, Fig. 1*B*). The graph of ΔL *vs.* Δt was fitted with a straight line by least squares regression (Fig. 1*C*) and V_0 was taken as the slope of this line. Data with $r < 0{\cdot}98$ were rejected. After calculating V_0, this value for each fibre was standardized to a sarcomere length of 2·4 μm (by dividing V_0 by 2·4 and then multiplying by the actual sarcomere length during activation) and then converted into muscle lengths per second.

Creatine phosphokinase was not included in the solutions since in pilot experiments its addition (1 mg ml^{-1}) did not alter V_0, as found previously with rabbit psoas fibres by Julian, Moss & Waller (1981). However, we did include P_i (2·5 mM) because this not only simulated the *in vivo* [P_i] but also helped to stabilize sarcomere length during activation. P_i decreased maximal force but did not affect V_0, as reported by Cooke, Franks, Luciani & Pate (1988). Finally, to check that the [Ca^{2+}] was optimal for V_0 we varied [Ca^{2+}] between pCa values of 5·9 and 4·5; this did not change V_0.

Electrophoresis

The samples of whole muscle were minced, homogenized in relaxing buffer (0·1 M KCl, 5 mM EDTA, 5 mM EGTA and 1 mM dithiothreitol, pH 7·0), and the contractile proteins were sedimented by centrifugation (Sciote *et al.* 1994). The final sediment was resuspended in electrophoresis sample buffer (0·15 M Tris–HCl, 4% SDS and 10% β-mercaptoethanol, pH 6·8). These protein samples were boiled for 5 min and then frozen at −40 °C until the electrophoresis was carried out. Individual segments of single fibres that were used for V_0 measurements were placed in sample buffer, ultrasonicated for 15 min, then heated and frozen as described.

Discontinuous SDS–PAGE was conducted on 0·75 mm thick separating gels of 6% (w/v) acrylamide cross-linked with bis-acrylamide (48 : 1·5, acrylamide : bis-acrylamide) and 37·5% glycerol, buffered with Tris at pH 8·8. The stacking gel contained 3% acrylamide with Tris buffer at pH 6·8. Running buffer consisted of 0·3% Tris, 1·44% glycine and 0·1% SDS. Proteins were electrophoresed with a constant current of 2·5 mA per gel at about 8 °C and were subsequently stained with silver.

RESULTS

Histochemistry and immunohistochemistry

Sections from adductor magnus and psoas were stained for myofibrillar ATPase reactivity only. Because the fibre types in masseter muscle are unusual, we first established that our histochemical fibre-type description of adductor magnus and psoas muscles agreed with previous studies, then used the staining of these muscles to help type those of the masseter. Adductor magnus contained type II B and II D (or II X) fibres and a few type II A or I fibres (results

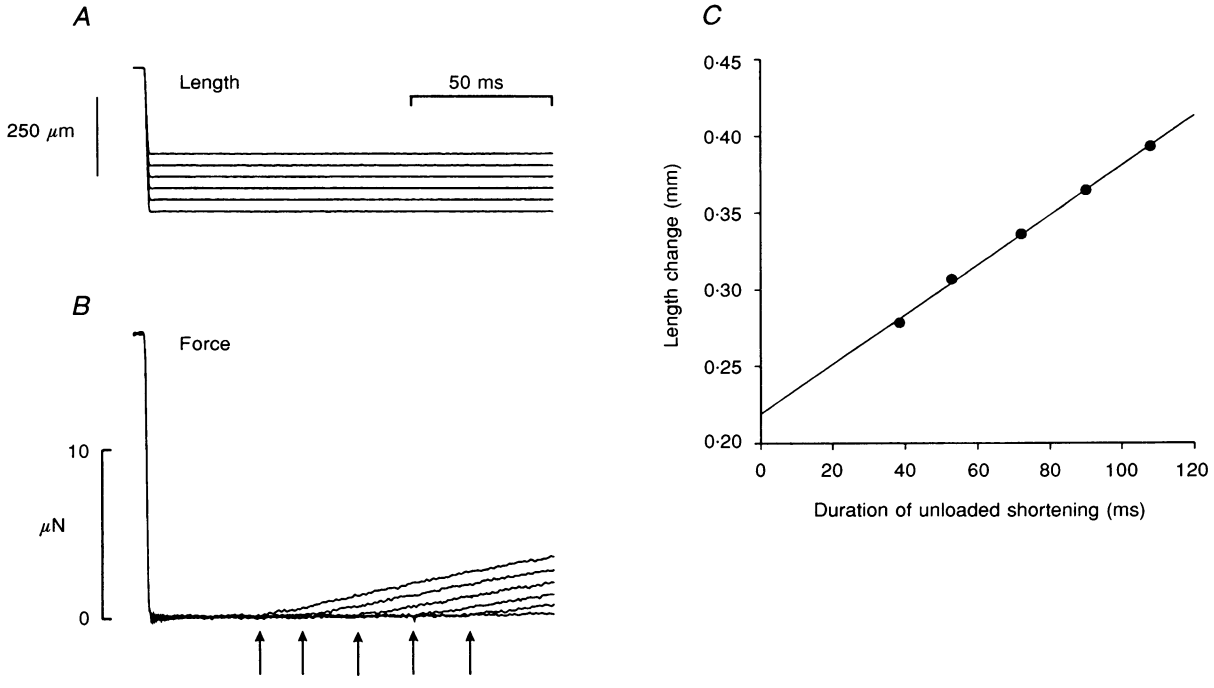

Figure 1. Determination of maximum velocity of shortening, V_0, of a skinned masseter α-cardiac fibre using the 'slack-test' method

A, during maximal activation (pCa 4·5) the muscle fibre was subjected to a series of step reductions in length (superimposed). *B*, the times for the fibre to shorten so as to take up the slack and redevelop force were measured (arrows). *C*, the slope of the relationship between length change and the time for force redevelopment equals V_0. In this fibre V_0 was 1·62 mm s^{-1} ($r = 0{\cdot}99$) at a sarcomere length of 2·7 μm, corresponding to a V_0 of 0·80 muscle lengths s^{-1} at a sarcomere length of 2·4 μm. Fibre length, 2·3 mm. Temperature, 15 °C.

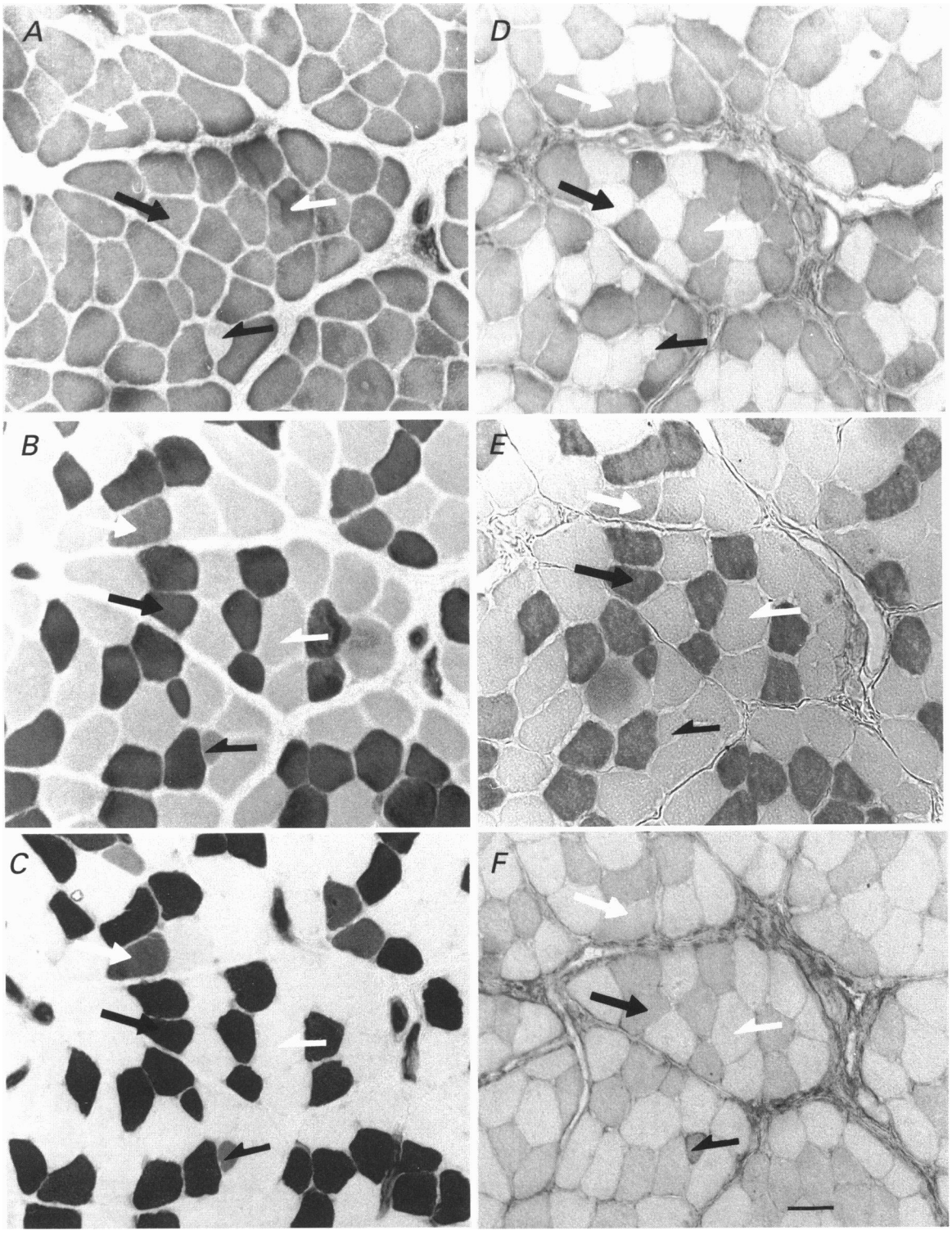

Figure 2. Serial sections from rabbit anterior superficial masseter muscle

A–C, sections stained for myofibrillar ATPase reactivity (dark staining) after pre-incubation in buffer of pH: 10·2 (*A*), 4·6 (*B*) and 4·3 (*C*). *D–F*, sections stained for reactivity to MHC antibodies: anti-type II A (*D*), anti-α-cardiac (*E*) and anti-type I (*F*). Type α-cardiac fibre (black arrow); fibre with MHC co-expression (white arrow); type II A fibre (white half-arrow); type I fibre (black half-arrow). Scale bar, 80 μm.

not shown), similar to the composition reported by Aigner *et al.* (1993). Psoas fibres contained up to 5% type I fibres and a mixture of type II fibres. This percentage composition differs from that found by Aigner *et al.* (1993), who reported no type I fibres, but this is probably due to the area sampled, since Weeds, Hall & Spurway (1975) also found type I fibres in the lateral area of the psoas muscle.

Samples from the soleus muscle contained mostly type I fibres, but some areas contained up to 10% type II A fibres (results not shown). A few type II C fibres could be found in these areas. The distribution of fibre types was variable between individuals, but selecting a relatively pale area of the soleus tended to increase the number of type II A fibres. Type I and II A fibres of the soleus muscle stained for ATPase reactivity as described for the psoas and adductor magnus muscles, and type II C fibres stained intermediately for myofibrillar ATPase after all buffer pre-incubations. Type I fibres stained positively for only the type I MHC antibody, type II A fibres for only the II A and fast MHC antibodies, and IIC fibres, to variable degrees, for the II A, fast and type I antibodies.

Results from the samples of masseter muscle are shown in Fig. 2. In the masseter, type II A fibres were common (white half-arrow, Fig. 2*D*) and type I fibres were relatively rare (black half-arrow, Fig. 2*F*). We identified an additional major fibre type which we designated 'type α-cardiac'; this fibre type stained intensely for myofibrillar ATPase after all buffer pre-incubations (black arrow, Fig. 2*A–C*). Type α-cardiac fibres stained positively for the α-cardiac MHC antibody (black arrow, Fig. 2*E*) and had moderate reactivity for the type I MHC antibody (black arrow, Fig. 2*F*). This type I polyclonal antibody is known (Rowlerson *et al.* 1981) to have moderate reactivity for slow MLCs, which suggests that these α-cardiac fibres contain slow MLCs. This confirms the finding of d'Albis *et al.* (1991), using protein electrophoresis, that when α-cardiac MHC is present in rabbit masseter it is associated with slow MLC isoforms. In the area of masseter sampled, a small subpopulation of fibres could not be classified into a specific type because of intermediate reaction for ATPase and antibody staining (white arrow, Fig. 2). None of the fibres in masseter or soleus stained positively for neonatal or type II M MHC antibodies (results not shown). In this area of masseter there were approximately 60% type II A fibres, 35% type α-cardiac, and the remainder either type I fibres or fibres with heterogeneous myosin expression. Since type II B or II D antibodies were not used in this study, we could not exclude the possibility that some of the rare heterogeneous fibres also contained either II B or II D MHC.

Electrophoresis

SDS–PAGE on whole-muscle samples separated the MHC isoforms into type I, α-cardiac and type II (Fig. 3*A*). The type I isoform was the fastest migrating and type II the slowest. Type II A, II B and II D isoforms, however, could not be separated from each other, and were represented by one band. The isoform content from SDS–PAGE (Fig. 3) matched the fibre type and composition of the muscle samples described by tissue staining. Thus, the adductor

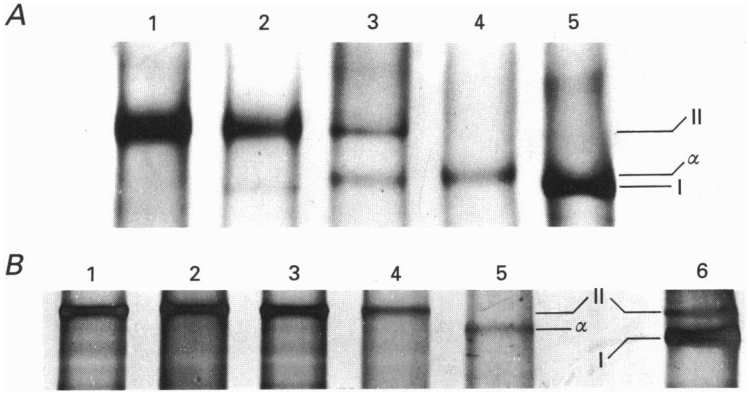

Figure 3. SDS–PAGE of rabbit whole-muscle homogenates (*A*) and skinned single fibres of masseter muscle (*B*) after slack-test measurements

A, lane 1, adductor magnus (containing type II MHC); lane 2, lateral psoas (containing type II and a little type I MHC); lane 3, masseter (containing types II and α-cardiac MHC); lane 4, atrium (containing α-cardiac MHC); and lane 5, soleus (containing type I MHC from a muscle sample with only type I fibres). *B*, lanes 1–5, single masseter fibres after slack-test (lanes 1–4, fibres containing type II MHC; lane 5, fibre containing α-cardiac MHC); lane 6, whole-muscle homogenate of soleus containing type I and II A MHC (taken from a muscle sample with both type I and II A fibres). Gels *A* and *B* were run separately, each with 6% acrylamide–37% glycerol (see text for details).

magnus had type II, the psoas had type II and a little type I, and the masseter had mainly types II and α-cardiac (Fig. 3*A*). In the soleus either type I alone or types I and II isoforms were present, depending upon the animal sampled (Fig. 3*A* and *B*). In addition, the MHC content of atrial muscle was determined, without tissue staining. Atrial muscle samples contained only the α-cardiac MHC, which migrated to the same position as the α-cardiac band in the masseter muscle samples.

With the SDS–PAGE performed on the single masseter and soleus fibres after the V_0 determinations (Fig. 3*B*) there were MHC bands identified as type I, α or II, as in the whole-muscle samples. A few fibres in the soleus were heterogeneous for type I and II MHC (and therefore were probably type II C).

V_0 and fibre type

We were able to determine both V_0 (using the slack-test method) and MHC isoform content (using SDS–PAGE) in twenty-two soleus fibres and thirty-nine masseter fibres. Although we could not distinguish between type II A, II B and II D fibres with electrophoresis, the results of tissue staining demonstrated that almost all type II fibres in masseter and soleus were II A. Figure 3*B*, which represents a typical gel for determination of MHC content after V_0 measurements, shows that there were approximately three type II A fibres for every one type α-cardiac fibre present in dissected masseter fibres. V_0 and MHC composition are plotted for each fibre in Fig. 4. The masseter fibres showed a wide range of V_0 values, from 0·49 to 2·12 muscle lengths s^{-1}. The V_0 values for the different fibre types in masseter were (in muscle lengths s^{-1}): type I, 0·54 ± 0·05 (mean ± S.D., $n = 3$); type II A, 1·23 ± 0·34 ($n = 27$); type α-cardiac, 0·78 ± 0·08 ($n = 9$). Thus, the type I fibres were slower than type II A, as in other muscles, and the α-cardiac fibres had intermediate V_0 values. In soleus fibres the range of V_0 values was smaller (Fig. 4). Mean V_0 values were (in muscle lengths s^{-1}): type I, 0·55 ± 0·06 ($n = 14$); type II A, 0·89 ± 0·04 ($n = 8$); type II C, 0·73 ($n = 2$). Thus, in soleus muscle type II A fibres were faster than type I fibres, with type II C fibres being intermediate in shortening speed. The range of V_0 for the type II A fibres in the masseter was far greater than those in soleus. Within each muscle type, mean V_0 values were significantly different between fibre types ($P < 0·001$ by Kruskal–Wallis one-way analysis of variance by ranks).

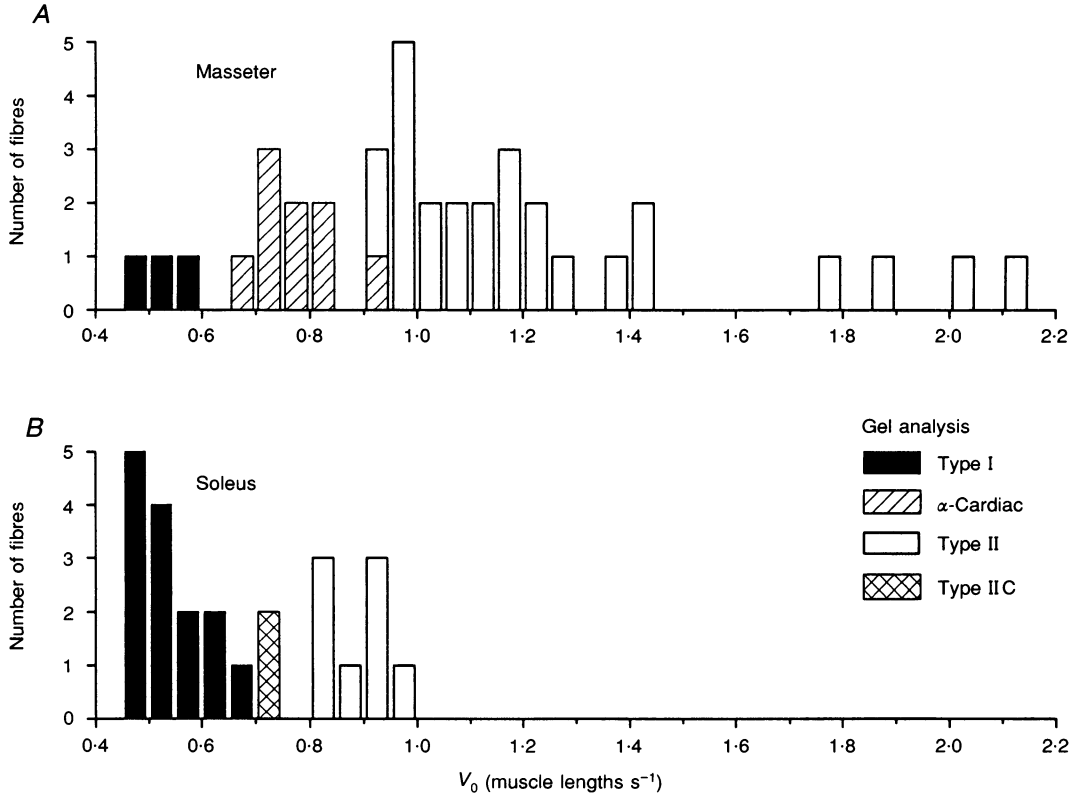

Figure 4. Summary of V_0 measurements from single skinned fibres in relation to their MHC composition

V_0 was determined by the slack-test method and MHC composition was determined by SDS–PAGE. The V_0 values were collected into bins of width 0·05 muscle lengths s^{-1}.

DISCUSSION

In this study we have described an unusual fibre-type population in the rabbit masseter that contains only one isoform of MHC, the α-cardiac isoform, and have correlated the presence of this isoform to a distinct V_0 compared with previously described skeletal fibre types. In both masseter and soleus muscles, type I fibres had the slowest V_0 and type II A fibres the fastest. The masseter α-cardiac fibres had a V_0 that was intermediate between type I and II A fibres and was similar to the type II C fibres of the soleus muscle. To our knowledge this is the first examination of the physiological properties of individual skeletal fibres expressing the α-cardiac MHC.

Fibre types in rabbit muscle

Skeletal muscle of rabbit is composed of four main fibre types (I, II A, II B and II D), with each fibre type containing a single MHC isoform. In addition, these MHC isoforms may exist in various combinations within individual fibres (Masanobu, Hämäläinen & Pette, 1995). For example, II C fibres contain both type I and II A MHC isoforms and both slow and fast MLC isoforms (Staron & Pette, 1986). With regard to the light chain composition, type I fibres have been shown electrophoretically to contain only the slow MLC isoforms $LC1s_a$, $LC1s_b$ and LC2s (Wagner & Weeds, 1977). Type II fibres contain the fast MLC isoforms LC1f, LC2f and LC3f (Termin & Pette, 1991), but some type II A fibres have also been shown to contain slow MLC isoforms (Staron & Pette, 1987).

The masseter muscle contains type I and II fibres, but the description of the type II fibres has varied. Previously, type II A and II B fibres have been described by histochemical and immunohistochemical staining (Bredman *et al.* 1991), yet a recent report has identified type II A and II D fibres and questions whether II B fibres are present to any appreciable extent (Kwa, Weijs & Juch, 1995). In masseter fibres α-cardiac MHC may be expressed singly or in combination with type I or II A MHC (Kwa *et al.* 1995). Because of these combinations, fibres with α-cardiac MHC have been described histochemically as very similar to type II C fibres, which have intermediate ATPase activity after either acid or alkali buffer pre-incubation (Bredman *et al.* 1991). Given our findings that in a specific area of masseter a major fibre population exists that homogeneously expresses the α-cardiac MHC, and that these fibres have a distinctive V_0, we have described this population as a separate fibre type termed 'α-cardiac.' In the present study we found only a limited number of fibres co-expressing MHCs, but we restricted our study to a superficial area of the masseter composed principally of α-cardiac and II A fibres.

V_0 and MHC composition

We found that, in both masseter and soleus muscle, fibres with type I MHC had a slow V_0, and fibres with type II MHC had a fast V_0. Soleus II C fibres, containing both type I and type II A MHC, had an intermediate V_0, as previously reported (Sweeney, Kushmerick, Mabuchi, Sreter & Gergely, 1988). Since our gel technique did not separate type II MHC into specific isoforms, further characterization of the fast fibres by SDS–PAGE was not possible. However, histochemical and immunohistochemical staining of other portions of the muscle samples demonstrated that soleus type II fibres and almost all masseter type II fibres were of type II A (Fig. 2). In addition, the mean V_0 of our type II fibres agrees with the V_0 of II A fibres found in other studies. Rabbit II A fibres described by Sweeney *et al.* (1988) had a mean V_0 of 0·98 muscle lengths s^{-1} in the vastus intermedius muscle and 0·86 muscle lengths s^{-1} in the tibialis anterior muscle. The V_0 for our type II A fibres was 0·89 muscle lengths s^{-1} in soleus and 1·23 muscle lengths s^{-1} in masseter. Masseter type II A fibres had a greater range of V_0 than found in soleus, and seemed to show a bimodal distribution (Fig. 4). If these V_0 values for masseter II A fibres do indeed represent two subpopulations of fibres (and this is by no means certain), this might be due to differing ratios of fast MLC isoforms, since there is evidence from rat that the type of fast MLC present in type II fibres can influence V_0 (e.g. Sweeney *et al.* 1988; Bottinelli, Betto, Schiaffino & Reggiani, 1994). On the other hand, in human fibres a similar, relatively large variation in the V_0 of a specific fibre type was not attributable to differing MLC isoforms (Larsson & Moss, 1993). Another possibility is that the type II A population in masseter may have included a few fibres containing type II B or II D MHC, corresponding to the rare unclassified fibre types identified by tissue staining (Fig. 2). Nevertheless, it may be that different MLC isoforms (slow MLC in soleus, fast MLC in masseter) were responsible for the variations in V_0 between the type II fibres from the different muscles, since the slowest contracting type II fibre of masseter was faster than the mean V_0 for soleus type II fibres (Fig. 4), even though their MHC profile should be the same.

Type I, α-cardiac and type II MHC isoforms (as a group) could be separated from each other electrophoretically so that fibres heterogeneous for these three isoform categories could be detected after slack-test measurements. The only heterogeneous fibres we found with single-fibre electrophoresis were two soleus type II C fibres co-expressing type I and II A MHC. We found that fibres containing the α-cardiac MHC alone had a distinct V_0 (mean, $0·78 \pm 0·08$ muscle lengths s^{-1}) that was intermediate between type I and type II A fibres. This supports, and offers an explanation for, the recent report by Kwa *et al.* (1995) that the twitch contraction time of rabbit masseter motor units homogeneous for α-cardiac fibres was intermediate in speed between motor units homogeneous for type I and type II A MHC. However, it should be noted that our technique gives a much more direct measure of myofibrillar contractile properties than does twitch contraction speed. It is interesting that the V_0 values of the masseter α-cardiac fibres in our experiments were always

faster than the type I fibres from the same muscle. Since both α-cardiac and type I fibres have slow MLC isoforms (d'Albis *et al.* 1991) the V_0 difference between these two fibres types must be due to differences in the MHC.

α-Cardiac MHC in cardiac *vs.* skeletal muscle

The finding that V_0 of α-cardiac fibres is about 1·4 times faster than that of type I fibres in masseter is consistent with the data from cardiac muscle, in which the α-isoform is considered to be fast and the β- (or type I) isoform, slow. For example, Pope, Hoh & Weeds (1980) showed that rat cardiac V_1 myosin (MHC composition αα) has an actin-activated ATPase activity that is 1·5–2 times greater than that of V_3 (composition ββ). Nevertheless, it is somewhat surprising that in the masseter the 'fast' α-cardiac fibres were slower than the type IIA fibres, and seemed to be slower than in previous studies that used cardiac muscle (most of which have used rat myocardium). For example, Sweitzer & Moss (1993) found that mean loaded shortening velocity for rat ventricular myocytes at 15 °C was 2·8 muscle lengths s^{-1}, and even faster speeds were found in individual cells with a higher proportion of α-cardiac MHC (cf. 0·78 muscle lengths s^{-1} in our α-cardiac fibres). It is unlikely that this discrepancy represents a difference between atrial and masseter α-cardiac MHCs, since d'Albis, Anger & Lompre (1993) demonstrated that the mRNA corresponding to most of the S2-subfragment of atrial α-cardiac MHC is present in rabbit masseter muscle. Therefore, there may be a real difference between α-cardiac fibres in masseter and heart muscle. Unfortunately, we are not aware of any data for the V_0 of rabbit cardiac muscle containing just the α-MHC against which we could compare our masseter data. (The atrial trabeculae in our rabbits were too short to be suitable for V_0 determinations.) If, in the same species, the masseter α-cardiac fibres are truly slower than cardiac fibres containing α-cardiac MHC, there are several possible explanations. One relates to the fact that cardiac atrial MLC isoforms in the rabbit are different from the slow MLC isoforms (LC1s and LC2s) found in masseter α-cardiac fibres (d'Albis *et al.* 1991), since the type of MLC present may influence V_0, at least in skeletal fibres (e.g. Sweeney *et al.* 1988; Bottinelli *et al.* 1994). Another possibility is that differences in other myofibrillar proteins, such as C-protein, contribute to the differences in V_0.

The unique functional requirements that would necessitate this additional α-cardiac fibre type in masticatory muscle are unclear. The α-cardiac MHC may also be found in mammalian extraocular muscles (Sartore *et al.* 1987) and transiently expressed in the rabbit diaphragm (d'Albis *et al.* 1993), but the functional properties of fibres containing α-cardiac MHC in these muscles have yet to be investigated. It is known that motor units of the rabbit masseter muscle are anatomically different from motor units of limb muscle, since they are typically restricted to relatively small anatomical compartments and have high fibre density (Weijs, Juch, Kwa & Korfage, 1993). Perhaps motor units are needed in specific fascicles for tasks unsuited to the type I and type II fibres. One possibility is that α-cardiac fibres, with their intermediate shortening speeds, allow a more precise control of jaw movements than is possible with a combination of type I and II fibres alone. Alternatively, the presence of α-cardiac MHC in skeletal muscle may be the result of development, since its expression is regulated in part by tissue-specific interaction of hormones upon distinct muscle cell populations. In some branchiomeric muscles (those muscles associated with branchial arch skeletal components), expression of the isoform is influenced by androgenic hormones, while in the heart, expression is influenced by thyroid hormone (d'Albis, Couteaux, Janmot & Mira, 1993). It is also possible that the α-cardiac fibres in these specialized skeletal muscles confer some other physiological or biochemical benefit that was not tested in the present study.

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