Fibre type composition of rabbit tibialis anterior and extensor digitorum longus muscles

J. LEXELL, J. C. JARVIS, J. CURRIE, D. Y. DOWNHAM AND S. SALMONS

Department of Neurology, University of Umeå, Umeå, Sweden; Departments of Human Anatomy and Cell Biology and Statistics and Computational Mathematics, University of Liverpool, Liverpool, UK

(Accepted 18 January 1994)

ABSTRACT

Rabbit tibialis anterior (TA) and extensor digitorum longus (EDL) muscles are used extensively in studies of stimulation-induced fibre type transformation, but the proportions and sizes of the 2 main fibre types, and the way in which they are distributed within the muscles, have never been described in any detail. In this study, transverse sections were processed by enzyme histochemical and immunohistochemical techniques and assessed morphometrically. The data were analysed by multivariate methods. In both TA and EDL muscles, the proportion of type 1 fibres varied significantly, and to a similar extent, within a cross-section, from lateral to medial and from superficial to deep parts. The fibre density, an indirect estimate of the mean muscle fibre area, also varied significantly, but not systematically, within a cross-section. For the EDL muscle, the proportion of type 1 fibres was consistently higher in the distal than in the proximal part of the muscle. The proportion of type 1 fibres was also significantly higher in the EDL than in the TA muscle for each of the 6 rabbits. There was no systematic variation between muscles from left and right limbs. The type proportions and fibre densities for both TA and EDL muscles differed significantly between individual rabbits, but not between sexes. The study provides a database that has hitherto been lacking on normal fibre type composition and its variation within and between these experimentally important muscles.

Key words: Muscle histochemistry; immunocytochemistry.

INTRODUCTION

Much of the available information on adaptive phenomena in mammalian muscle has come from experiments in which fast-twitch muscles in the anterior compartment of the lower hind limb of the rabbit were subjected to chronic stimulation (Salmons & Henriksson, 1981; Pette & Vrbová, 1992). There were several reasons behind the original choice of this experimental model (Salmons & Vrbová, 1969). First, the muscles in question – tibialis anterior (TA) and extensor digitorum longus (EDL) – were known to contain a predominance of fast-twitch fibres, against which background any acquisition of slow-twitch, oxidative characteristics could easily be detected. Secondly, the common peroneal nerve that supplies both muscles is readily accessible and electrodes can be placed close to the nerve. Thirdly, the sensory component of the common peroneal nerve appears to be less important than in, for example, the tibial nerve, and supramaximal stimulation can be applied without discomfort to the animal.

The TA and EDL muscles seem to respond similarly to stimulation, and in many studies the 2 have been used interchangeably. At first glance, this seems reasonable. The muscles are anatomically and physiologically similar: the muscles and their nerve and blood supplies are very closely associated, and both muscles are dorsiflexors of the ankle and fast-contracting. However, the muscles differ somewhat in their action and fibre architecture: the TA acts across one joint (the ankle) and is essentially parallel-fibred; the EDL acts across several joints (ankle, knee and phalanges) and is pennate. When the same measurements were carried out on both muscles, differences between the responses emerged. Changes in enzymes

Correspondence to Dr Jan Lexell, Department of Neurology, University of Umeå, S-901 85 Umeå, Sweden.
of several metabolic pathways had a more rapid time course in EDL than in TA (Brown et al. 1989). The 2 muscles differed in the time course of changes in the mRNAs encoding fast and slow myosin heavy chains (Brownson et al. 1992). The EDL is much more susceptible to stimulation-induced damage than the TA (Lexell et al. 1992, 1993). Outside the context of stimulation, the muscles differ qualitatively in their response to treatment with anabolic steroids (Salmons, 1992).

In view of their importance as an experimental model for the effects of stimulation, and the differences between their responses, there is a need for a more adequate database on the properties of the 2 muscles that would serve as a baseline for the effects of experimental intervention. In this study, we have analysed the rabbit TA and EDL muscles in terms of their fibre type composition: the extent to which the 2 muscles differ, their symmetry between left and right sides, and the way in which the composition varies with position in the cross-section, along the length of the muscle, and between individual animals and sexes.

**Materials and Methods**

TA and EDL muscles of the left and right hind limbs were removed from 3 male and 3 female New Zealand White rabbits with body weights in the range 2.5–3.1 kg. All animal procedures were performed in accordance with the Animals (Scientific Procedures) Act 1986.

**Preparative procedure**

Each of the 4 muscles was cut into 10–12 transverse slices by a specially made cutter incorporating equally spaced razor blades. Slices taken 5 and 15 mm proximal and 5 and 15 mm distal to the midbelly of the muscle were placed on cork discs, frozen in isopentane chilled with liquid nitrogen, and stored below −70 °C pending use.

Serial cryosections of 5–10 μm thickness were cut from each slice. To visualise type 1 and type 2 fibres, sections were stained for myofibrillar adenosine triphosphatase (mATPase) after alkaline (pH 9.4) preincubation (Guth & Samaha, 1970). In addition, 1 section from each muscle, taken 5 mm proximal to the midbelly, was also reacted with a monoclonal antibody (WB-MCHs) raised by Dr W. E. Brown against the rabbit slow myosin heavy chain isoform (anti-slow). Binding of the primary antibody was detected by immunofluorescence of a 2nd antibody, rabbit anti-mouse immunoglobulin conjugated with fluorescein isothiocyanate (FITC; Sigma Chemical Co. Ltd) and the sections were viewed with a Leitz Diaplan microscope fitted for epifluorescent illumination.

**Type 1 fibre identification**

From each section reacted with the anti-slow antibody, 2 areas of 4 mm² were selected at random. Images of these areas were produced and all type 1 fibres, recognised by their positive reaction with the antibody, were identified and localised. Images of the same areas from sections stained for mATPase were produced, and the results of the 2 staining techniques were compared.

**Sampling procedure**

All quantification was done on sections stained by mATPase. The sections were numbered 1–4 in sequence from the proximal to the distal end of the muscle. A square 1 × 1 mm grid was placed on the mounted section and a graticule with a square subdivided into 100 (10 × 10) smaller squares was placed in the eyepiece of the microscope. With the ×16 objective selected, a square of size 1×1 mm on the specimen grid corresponded approximately to the whole square in the eyepiece. Every 4th mm² throughout a muscle cross-section, referred to here as a ‘sample area’, was selected for measurement. Sample areas were given a coordinate, starting with A1 in the medial and superficial part of the muscle.

For each section, between 5 and 17 sample areas were used, depending on the size of the whole muscle cross-section. The numbers of type 1 and type 2 fibres in each selected sample area were counted and used to compute (1) the mean proportion of type 1 fibres in the muscle cross-section, and (2) the fibre density (the mean number of fibres per mm² in the muscle cross-section); the latter is an accurate indirect estimate of the mean fibre area (Lexell & Taylor, 1991).

**Data and analyses**

Each of the 928 sample areas was characterised by 9 variables: \( t_1 \), number of type 1 fibres; \( t_2 \), number of type 2 fibres; \( x \), the abscissa of the area of the section; \( y \), the ordinate of the area of the section; \( s \), the section (1, 2, 3, 4); \( l \), the limb (left, right); \( m \), the muscle (TA, EDL); \( i \), the individual rabbit (1, 2, 3, 4, 5, 6; male, female).

The 928 sample areas from the 6 rabbits were analysed by multivariate methods. The proportion of
Fibre type composition of rabbit muscles

RESULTS

Type 1 fibre identification

Every fibre that stained lightly for mATPase at pH 9.4, reacted positively with the anti-slow antibody; every fibre with the opposite mATPase staining reaction, stained negatively with the anti-slow antibody (Fig. 1a, b).

Results of multivariate analyses

Variability within cross-sections. Data on the fibre type composition of the left and the right TA and EDL muscles from the 6 rabbits are summarised in the Table. Means are calculated from all sample areas; standard deviations are calculated by pooling the variances from individual sections. Distributions

Fig. 1. Serial transverse cryostat sections of rabbit tibialis anterior muscle. (a) Myofibrillar ATPase after alkaline (pH 9.4) preincubation. (b) Reaction with a monoclonal antibody against rabbit slow myosin heavy chain isoform. × 300.

type 1 fibres in a sample area was analysed with the logistic model and expressed in terms of the 6 explanatory (independent) variables: $x$, $y$, $s$, $l$, $m$, and $i$. The fibre density was expressed in terms of the same 6 explanatory variables with the use of multivariate analyses of variance (MANOVA); the significance of the F statistics was used to assess the effects of these variables on the fibre density. The statistical package SAS (SAS Institute Inc., USA) was used throughout. The Logistic procedure was used to analyse the fibre type proportions. Analysis of the fibre density was performed with procedure GLM, which carries out the various tests and forms the necessary residual plots.
Table. Summary data on fibre type composition of right and left tibialis anterior (TA) and extensor digitorum longus (EDL) muscles from 3 male and 3 female rabbits

<table>
<thead>
<tr>
<th></th>
<th>Left</th>
<th>Right</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
<td>Mean*</td>
</tr>
<tr>
<td>Proportion of</td>
<td>TA</td>
<td>4.0 3.0</td>
</tr>
<tr>
<td>type 1 fibres (%)</td>
<td>EDL</td>
<td>6.8 2.5</td>
</tr>
<tr>
<td>Fibre density</td>
<td>TA</td>
<td>176 7</td>
</tr>
<tr>
<td>(no fibres mm⁻²)</td>
<td>EDL</td>
<td>172 7</td>
</tr>
</tbody>
</table>

* Obtained directly from the data for the sample areas; ** obtained by pooling the variances from each section.

Fig. 2. Variability in fibre type composition within cross-sections of rabbit tibialis anterior and extensor digitorum longus muscles. (a) Proportion of type 1 fibres (%). (b) Fibre density (mean number of fibres per mm²). Schematic drawing of section 3 from rabbit 5.

Both fibre type proportion and fibre density varied significantly within a muscle cross-section, and to a similar extent in TA and EDL and the left and right

of the proportion of type 1 fibres were positively skewed, whereas those for the fibre density were symmetric.
limbs (Fig. 2a, b). The proportion of type 1 fibres varied systematically from the medial to the lateral part of the cross-section; without exception, the highest proportion was found in the medial part of the muscle, which is closest to the tibia, whereas the lateral part was always composed exclusively of type 2 fibres (Fig. 2a). The proportion of type 1 fibres also increased gradually with depth (Fig. 2a). There was no such systematic variability in fibre density (Fig. 2b).

**Variability within muscles.** Figure 3a, b addresses the variability in fibre type composition along the length of TA and EDL. Each point is based on the 5–17 sample areas per muscle cross-section for the 6 rabbits. The proportion of type 1 fibres (Fig. 3a) varied significantly (P < 0.001) between cross-sections. The main contribution to this variability came from the EDL; the proportion of type 1 fibres was consistently higher in the distal than in the proximal part of this muscle (Fig. 3a). In terms of fibre density there was a significant (P < 0.001) but nonsystematic difference between cross-sections from both TA and EDL (Fig. 3b); the difference in means between sections never exceeded 4 fibres per mm².

**Variability between muscles, limbs and rabbits.** Figure 4a, b addresses the variability in fibre type composition between TA and the EDL and between the left and the right limbs for each of the 6 rabbits. Each point is based on all the sample areas from a muscle. Neither the proportion of type 1 fibres (Fig. 4a) nor the fibre density (Fig. 4b) differed significantly between the left and the right TA and EDL. There was a significant difference (P < 0.001) between TA and EDL in each rabbit for fibre type proportion but not
for fibre density; the proportion of type 1 fibres was consistently larger in EDL than in TA (Fig. 4a).

There was a significant difference ($P < 0.001$) between the rabbits, but not between the sexes, for fibre type proportion and fibre density, for both TA and EDL.

DISCUSSION

Quantitative assessment of the proportion and the size of the different fibre types has influenced our understanding of the structure and function of mammalian skeletal muscles: analyses of cross-sections of whole muscles from previously physically healthy males of different age-groups have revealed a complex distribution of the different fibre types and considerable changes throughout the whole fibre population with increasing age (Lexell, 1993). The 2 muscles analysed in this study, TA and EDL in the rabbit, have been used extensively in studies of the short and long-term effects of electrical stimulation but their fibre type compositions have not been described previously in this detail.

Immunohistochemistry confirmed the type 1 fibre identification made by staining for mATPase, and showed that in this species the latter technique leads to reliable separation of the fibre population into 2 major types: type 1 and type 2. No attempt was made to subclassify the type 2 fibre population, because of the recent description of diversity of fast myosin heavy chain (Aigner et al. 1993) and histochemical fibre types (Hämäläinen & Pette, 1993) in rabbit skeletal muscles. Furthermore, the available methods would have been impracticable when applied to a study of this scope.

Like many other muscles and species, the rabbit TA and EDL are heterogeneous in their fibre type composition. In terms of the variability within each section there was little difference between the 2 muscles: some areas had as much as 20% type 1 fibres whereas others had exclusively type 2 fibres. The standard deviation for both muscles was small, which indicates that few areas have a high proportion of type 1 fibres.

The 2 fibre types were distributed systematically within the cross-section of both TA and EDL, as found previously in animals and humans (Lexell et al. 1988; Newsholme et al. 1988). There was a significantly higher proportion of type 1 fibres in the medial and deep parts than in the lateral, superficial part of the muscles. The shift in fibre type proportion with depth is obvious to inspection in the rabbit TA and has attracted comment many times before. However, the increase in proportion of type 1 fibres towards the medial part of the muscle is a new finding, and it suggests that proximity to the bone, rather than depth per se, may be the determinant of these gradients. During embryological development, the muscle blocks of the limb form and divide in relation to skeletal elements, and it is during the primary wave of myogenesis that fibres are produced which express type 1 myosin. If subsequent waves of myogenesis tend to populate the more lateral and superficial parts of the muscle, this gradient may be retained, despite subsequent restriction of type 1 myosin expression (Narusawa et al. 1987). Part of the blood supply to TA enters the muscle on its medial aspect, so the vascular supply is most developed in this part of the muscle. This may be a factor in supporting a more oxidative metabolism in this part of the muscle of an adult animal.

The proportion of type 1 fibres differed significantly between the 2 muscles. In EDL the proportion of type 1 fibres was, on average, 3% higher than in the corresponding TA muscle. This may be related to their different functional properties; both muscles dorsiflex the ankle, but EDL also extends the knee and spreads the toes, and could therefore carry a greater burden of physiological activity. It could also explain the more rapid time course of the metabolic and molecular genetic responses of EDL to stimulation, for at the time of onset the fibres would have received a more significant history of use.

In EDL, there was a small but significant increase in the proportion of type 1 fibres in the proximal-to-distal direction. In our study of stimulation-induced damage we found that damage was less common in distal than in proximal portions of the muscle (Lexell et al. 1992, 1993). The difference in damage was approximately 3 volumes percent, similar to the difference in fibre type proportion. This would be consistent with a greater vulnerability to damage of type 2 fibres, as suggested by Maier & Pette (1987). On the other hand, EDL consistently showed much more damage than TA (Lexell et al. 1992, 1993), even though it has a significantly higher proportion of type 1 fibres. Obviously, stimulation-induced damage is a complex phenomenon that cannot be explained simply by a difference in fibre type proportion.

Fibre density can be used as a fast and accurate indirect measurement of mean fibre area (Lexell & Taylor, 1991). Fibre density varied significantly both within cross-sections and along the length of the muscle, but the variation was not systematic and the differences in actual values were small. Evidently, functional differences between different parts of the
Fibre type composition of rabbit muscles

muscle are not so pronounced as to cause systematic variability in this variable.

The findings presented here have practical implications. Because of the systematic variation in fibre type composition, care should be taken when single samples are analysed from either muscle; to reduce the influence of sampling error, the sample site should be clearly defined. The fact that no variability was found between left and right limbs, nor between male and female rabbits, suggests that neither asymmetry nor sex needs to be taken into account. Since variability in fibre area within and between muscles was very small, actual numbers of fibres can be expressed in terms of either the total number of fibres per unit area or the area itself.

ACKNOWLEDGEMENTS

This study was carried out while Jan Lexell was working at the Muscle Research Centre, University of Liverpool, England, supported by grants and scholarships from the Swedish Work Environment Fund, the Swedish Institute, the Swedish Society of Medicine, the Research Council of the Swedish Sports Federation, the Tore Nilsson Foundation and the Hans and Loo Osterman Foundation. The authors would also like to acknowledge the support given to this research by the British Heart Foundation.

REFERENCES


