Muscle fibre types and their distribution in the biceps and triceps brachii of the rat and rabbit

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ABSTRACT

Muscle fibre type composition and distribution in the biceps brachii (long head) and triceps brachii (long head) of the rat and rabbit were investigated using the following histochemical techniques: myosin ATPase, with preincubation at pH 10.4 and 4.35; succinate dehydrogenase (SDH) and glycogen phosphorylase. The muscle fibres were classified into slow-twitch (SO), fast-twitch glycolytic (FG), fast-twitch oxidative glycolytic (FOG and FOg) and fast-twitch oxidative fibres (FO). Significant differences in the regional distribution of muscle fibre types have been observed between the rat and the rabbit. In the rat, SO fibres were restricted to the deep regions of both biceps and triceps brachii, whereas FG fibres were located in the intermediate and superficial regions (the superficial regions contained the highest percentages of FG fibres). In the rabbit, SO and FG fibres were spread over the entire muscle, although SO and FG fibres were most abundant in the deep and superficial regions respectively. These findings indicate that the biceps and triceps brachii are more regionalised in the rat than in the rabbit.

Key words: Skeletal muscle; muscle histochemistry.

INTRODUCTION

Three (Peter et al. 1972; Pullen, 1977; Acosta & Roy, 1987) or 4 (Maltin et al. 1989; Lind & Kernell, 1991; Latorre et al. 1993) histochemically defined fibre types have been identified in skeletal muscles in mammals. According to their myosin ATPase activity, skeletal muscle fibres have been classified as I, IIA and IIB (Brooke & Kaiser, 1970), which appear to correspond quite closely to the metabolic classification described by Peter et al. (1972) in slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG) and fast-twitch glycolytic (FG) respectively (Spurway, 1981; Suzuki, 1990). Fibre types in limb muscles are frequently arranged in such a way that the percentage of slow-twitch and fast-twitch high oxidative fibres increases towards the deeper regions of the muscle near to the bone, whereas the percentage of fast-twitch low oxidative fibres increases towards the superficial areas of the muscle (Yellin, 1969; Pullen, 1977; Gunn, 1978; Armstrong et al. 1982; McIntosh et al. 1985; Acosta & Roy, 1987). The proportion and regional distribution of fibre types within the muscle appear to be related to the degree of functional complexity (Gonyea et al. 1981).

Although the muscles of the hindlimbs have been widely investigated in mammals (Maxwell et al. 1977; Dum & Kennedy, 1980; Alnaqeeb & Goldspink, 1986; Acosta & Roy, 1987; Suzuki & Tamate, 1988; Maltin et al. 1989; Chanaud et al. 1991; Williams & Dhoot, 1992; Lexell et al. 1994; Spurway et al. 1996), less is known of the histochemical properties of muscles in the forelimbs (Collatos et al. 1977; Gonyea & Bonde-Petersen, 1977; Armstrong et al. 1982; McIntosh et al. 1985; Maltin et al. 1989; Hermanson & Hurley, 1990; Hermanson et al. 1991; Lind & Kernell, 1991). The biceps and triceps brachii are 2 antagonistic muscles that play a key role for maintaining the stability and posture of the elbow joint and are involved in the complexity of movements of the forelimbs. However, little attention has been paid to their histochemistry (Collatos et al. 1977; Hermanson...
& Hurley, 1990) and even less to the regional distribution of fibre types (Armstrong et al. 1982; Lind & Kernell, 1991). Thus, the aim of the present study was to undertake a comparative study of biceps and triceps brachii in the rat and rabbit to determine their fibre type composition and regional distribution. The 2 species exhibit obvious differences in the function of their forelimbs: in the rabbit this function is limited to walking and jumping, whereas in the rat it extends to holding food, which may imply a greater degree of histochemical complexity.

**Materials and Methods**

Ten New Zealand–California rabbits weighing 1.65–1.75 kg each and 9 Sprague–Dawley rats weighing 250–400 g each were used. The animals were killed using an overdose of sodium pentobarbitone and immediately afterwards biceps brachii (long head) and triceps brachii (long head) were rapidly removed. Small central cross-section segments (0.5 cm thick) were taken from each muscle and placed on metallic specimen holders with OCT compound (Tissue-Tek II) and quickly frozen by immersion for 30 s in isopentane cooled to −160 °C by liquid nitrogen. After a minimum 1–2 h period at a temperature of −20 °C, transverse sections (10–12 µm) were cut in a cryostat at −20 °C, and allowed to dry at room temperature for 30–60 min.

**Histochemical methods**

Serial sections were selected for each of the histochemical techniques. The sections were incubated for succinate dehydrogenase (SDH), and glycogen phosphorylase activity, alkaline myosin adenosine triphosphatase (ATPase) (preincubation at pH 10.4) and acid ATPase (preincubation at pH 4.35). The technique of Guth & Samaha (1970) was used for the demonstration of acid and alkaline ATPase activity. Preincubation times were 15 and 60 min for the demonstration of alkali-stable ATPase and acid-stable ATPase respectively, and the incubation time was 25 min for both.

To determine the oxidative capacity of the muscles, SDH activity was demonstrated using the procedure described by Kiernan (1981), who added Meldola blue oxazine to the incubation medium as an intermediary electron acceptor (Kugler & Wrobel, 1978). This considerably accelerates the reaction and avoids spontaneous deposition due to a nonenzymatic reduction of formazan. Incubation was carried out for 5–20 min at 37 °C. Sections were then fixed for 7–10 min in 4% formaldehyde in 0.1 m phosphate buffer (pH 7.0).

Glycogen phosphorylase activity was demonstrated using Meijer’s (1968) incubation medium the subsequent phases following the procedures of Eränko & Palkama (1961) as described by Troyer (1980). The sections were incubated for 20 min at 37 °C, air dried and washed in 40% alcohol for 2 min. They were then air dried again, submerged in saccharine 0.32 m for a few seconds and stained with Gram iodine for 3 min. Finally, they were fixed as recommended by Sawyer (Pearse, 1972) and mounted in glycerine.

**Morphometric analysis**

Prior to morphometric analysis, representative areas from each muscle (deep, intermediate and superficial, which were reacted with the 4 histochemical techniques mentioned above) were selected and photographed at the same magnification. The photomicrographic fields of each area, which comprised 400–500 muscle fibres, were projected from slides and fibre type and frequency were determined. 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 transversely and possessing well defined cell boundaries, were considered for analysis. The fibres of each area were traced and classified before measuring their size, 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**

**Histochemical fibre type composition and fibre size**

According to the nomenclature of Peter et al. (1972), subsequently modified by Spurway (1981) and Maltin et al. (1989), 4 fibre types were observed in the biceps and triceps of the rat (Fig. 1a–c), i.e. slow-twitch (SO), fast-twitch glycolytic (FG), fast-twitch oxidative glycolytic (FOG) and fast-twitch oxidative (FO). The SO fibres exhibited alkali-labile and acid-stable ATPase activity, moderate to high SDH activity and the lowest glycogen phosphorylase activity. The FG fibres showed moderate to high alkaline ATPase activity, weak SDH activity and the highest glycogen
phosphorylase activity. The FOG fibres exhibited SDH and glycogen phosphorylase activity that ranged from moderate to high. The FO fibres showed the highest levels of alkali-stable ATPase and SDH activities as well as a low glycogen phosphorylase activity.
In the rabbit (Fig. 2a–c), similar fibre types were found apart from 2 differences: (1) all FG fibres showed high alkaline ATPase activity; (2) the fibres that showed the highest SDH activity (FO in the rat) exhibited moderate glycogen phosphorylase activity; for this reason they have been classed as FOg.

The morphometric analysis of the muscle fibres indicated the fibre type and fibre size were closely correlated (Table 1). In addition, the same morphometric pattern was observed in all muscles. FG fibres were found to have the largest diameter, SO and FOG fibres were intermediate in size and FO and FOg fibres were the smallest; except that for biceps in the rabbit the values for the mean diameter of FOg fibres were similar to those of SO fibres.

Both in the rat and rabbit (Table 2), the greatest percentage of fibres comprised fast contracting fibres (FG, FOG and FO/FOg). In the rabbit, the percentage of SO fibres was 10.2 ± 2.0% in biceps brachii and 14.7 ± 1.2% in triceps brachii whereas in the rat a considerably smaller number (6.9 ± 1.1% in biceps brachii, 3.7 ± 1.4% in triceps brachii) was observed. Moreover, in the rat, biceps brachii contained more SO fibres than triceps brachii but, in the rabbit, an inverse relationship was observed. In the rat, the percentage of oxidative fibres (SO, FOG, FO) was smaller (55.5% in biceps and 49.9% in triceps) than in the rabbit, where the percentage of oxidative fibres (SO, FOG, FOg) was 60.7% in biceps and 63.8% in triceps. This is due to the higher percentage of SO and FOg fibres in the rabbit.

**Distribution of fibre types (Table 2)**

In the rat, a panoramic view of the muscles revealed a histochemical regionalisation (Fig. 1d, e) consisting of 3 areas in each muscle: deep, intermediate and superficial (Fig. 3). The deep region, closest to the humerus, was the most oxidative and possessed all the SO fibres, whereas the superficial region was the most glycolytic and had the greatest percentage of FG fibres. The deep region of biceps and triceps brachii...
Table 2. Percentage fibre type composition (% ± s.d.) in the deep, intermediate and superficial regions and in the entire muscle (total) of biceps and triceps brachii*

<table>
<thead>
<tr>
<th></th>
<th>SO</th>
<th>FG</th>
<th>FOG</th>
<th>FO</th>
<th>FOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biceps brachii</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Deep</td>
<td>27.6±5.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Intermediate</td>
<td>—</td>
<td>51.1±6.1</td>
<td>37.6±7.2</td>
<td>11.3±2.1</td>
<td>—</td>
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<tr>
<td>Superficial</td>
<td>—</td>
<td>66.2±11.4</td>
<td>24.4±4.7</td>
<td>9.4±1.7</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>6.9±1.1</td>
<td>44.5±10.8</td>
<td>40.9±4.1</td>
<td>7.7±2.6</td>
<td>—</td>
</tr>
<tr>
<td>Triceps brachii</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Deep</td>
<td>18.4±4.0</td>
<td>—</td>
<td>81.6±2.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Intermediate</td>
<td>—</td>
<td>42.4±5.7</td>
<td>34.3±4.6</td>
<td>23.3±1.0</td>
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<tr>
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<td>—</td>
<td>75.3±0.5</td>
<td>23.0±2.1</td>
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<tr>
<td>Total</td>
<td>3.7±1.4</td>
<td>50.1±9.6</td>
<td>38.1±5.2</td>
<td>8.1±3.0</td>
<td>—</td>
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<tr>
<td>(b) Rabbit</td>
<td></td>
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</tr>
<tr>
<td>Biceps brachii</td>
<td></td>
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</tr>
<tr>
<td>Deep</td>
<td>14.2±2.3</td>
<td>32.9±4.5</td>
<td>30.7±1.8</td>
<td>—</td>
<td>22.2±2.2</td>
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<tr>
<td>Intermediate</td>
<td>8.8±1.7</td>
<td>36.3±5.1</td>
<td>27.9±5.9</td>
<td>—</td>
<td>27.0±5.2</td>
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<tr>
<td>Superficial</td>
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<td>48.5±9.3</td>
<td>18.0±7.3</td>
<td>—</td>
<td>25.9±3.7</td>
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<tr>
<td>Total</td>
<td>10.2±2.0</td>
<td>39.3±10.0</td>
<td>25.5±1.7</td>
<td>—</td>
<td>25.0±1.5</td>
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<td>Triceps brachii</td>
<td></td>
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<tr>
<td>Deep</td>
<td>27.3±2.4</td>
<td>18.4±4.3</td>
<td>35.9±4.5</td>
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<td>Intermediate</td>
<td>13.1±1.2</td>
<td>37.9±4.4</td>
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<td>17.8±2.9</td>
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<tr>
<td>Superficial</td>
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<td>22.6±1.3</td>
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<td>21.4±1.9</td>
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<tr>
<td>Total</td>
<td>14.7±1.2</td>
<td>36.2±2.9</td>
<td>29.9±2.2</td>
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<td>19.2±1.7</td>
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</table>

* The percentage of each fibre type in the entire muscle was calculated by counting the total number of fibres and the total number of each fibre type in each muscle.

Fig. 3. Cross-sections of the upper forelimb of rat and rabbit showing the location of the long heads of biceps and triceps brachii and their distribution into deep (dp), intermediate (in) and superficial (sp) regions. 1, triceps brachii (long head); 2, triceps brachii (lateral head); 3, triceps brachii (medial head); 4, dorso-epitrochlearis brachii; 5, humerus; 6, brachialis; 7, biceps brachii (long head); 8, coracobrachialis; 9, biceps brachii (short head); 10, deltoid.

was exclusively composed of fibres with moderate to high oxidative metabolism, i.e. SO and FOG fibres, the latter being the most abundant in this region both in biceps (72.4±4.4%) and triceps brachii (81.6±2.9%). The intermediate region contained FG, FOG and FO fibres and, although the greatest percentage of fibres were FG (51.1±6.1% in biceps and 42.4±5.7% in triceps), this area was more oxidative than the superficial region. In the intermediate region a greater number of oxidative fibres were observed in triceps than in biceps. In contrast to the other regions, the superficial regions of both biceps and triceps brachii were mainly composed of FG fibres (66.2±11.4% and 75.3±0.5% respectively). In biceps brachii, the FO fibres were quite evenly distributed between the intermediate and superficial regions (11.3±2.1% and 9.4±1.7% respectively), whilst in triceps brachii they were mostly located in the intermediate region (23.3±1.0%). Biceps and triceps brachii of the rabbit did not exhibit such a marked regionalisation of fibre types as in the rat. All fibre types were present in the deep, intermediate and superficial portions of each muscle (Table 2). Nevertheless, there was a gradient in the type of fibres, ranging from more oxidative in the deep region to more glycolytic in the superficial region, this gradient being more pronounced in triceps. The same gradient was also observed for SO fibres, which were more abundant in the deep than in the superficial region of both muscles. In the deep region of triceps, FOG fibres (35.9±4.5%) and SO fibres (27.3±2.4%) were the most numerous, whereas the most abundant fibre types in biceps were FG (32.9±4.5%) and FOG...
(30.7 ± 1.8%). In spite of the presence of FG fibres, the deep region appears to be an oxidative region since the percentage of fibres exhibiting moderate to very high oxidative activity (SO, FOG, FOg) was 81.6% in triceps and 67.1% in biceps. In the superficial region of both biceps and triceps, the percentage of FOG fibres decreased, while FOg fibres had no clear distribution pattern.

DISCUSSION

As for other limb muscles in mammals, biceps and triceps brachii both in the rat and rabbit are composed of type I (SO) and type II (FG, FOG, FO/FOg) fibres. It is widely accepted that the fibre type composition varies according to the type of animal and muscle in question, and that this is related to the function performed by each muscle. Considerably fewer slow contracting fibres are found in triceps brachii of the rat (3.7%) in comparison with the rabbit (14.7%), and the number in both animals is much smaller than in triceps brachii of other larger mammals such as the cat (26%, Collatos et al. 1977) and dog (35%, Armstrong et al. 1982). Moreover, a smaller percentage of type I fibres was observed in biceps brachii in the rat (6.9%) in contrast to the rabbit (10.2%), and the number in both animals was notably less than that described for biceps brachii of larger mammals such as the cat (20%, Collatos et al. 1977), dog (48%, Armstrong et al. 1982), horse (32–66%, Hermanson et al. 1991) and man (52–53%, Nygaard & Sánchez, 1982; 48%, Shorey & Cleland, 1988). In comparison with the rat and rabbit, the number of type I fibres was greater in other forelimb muscles of the cat (Collatos et al. 1977; Gonyea & Bonde-Petersen, 1977), dog (Armstrong et al. 1982) and primates (McIntosh et al. 1985). Given that type I fibre is designed for slow body movements and they are significantly present in postural muscles, the musculature of the forelimbs in larger animals appears to be more involved with maintaining a standing position than in smaller animals, such as the rat and rabbit. Moreover, this observation suggests a relationship between body weight and the number of slow contracting fibres in these forelimb muscles. Thus a possible explanation for the greater number of type I fibres found in the forelimbs of the rabbit than in the rat may lie in the larger size of the former.

It is difficult to compare our results with those reported by other authors in the hindlimbs of the rat and rabbit given that the literature regarding these animals has, with the exception of the study by Maltin et al. (1989) on the tensor fascia lata, focused on leg muscles (tibial anterior, gastrocnemius, extensor digitorum longus, soleus, plantaris). Bearing this in mind, and with the exception of soleus, the findings in the hindlimb of the rat (Maltin et al. 1989; Alnaqeeb & Goldspink, 1986; Williams & Dhoot, 1992) and rabbit (Spurway, 1980; Lexell et al. 1994) show low percentages of slow-contracting fibres which coincides with our results in biceps and triceps brachii. Similar results have been obtained in the thigh and leg muscles of the cat, except for gastrocnemius (Maxwell et al. 1977) and the central region of semitendinosus (Chanaud et al. 1991). In contrast, greater percentages of slow-contracting fibres have been observed in the hindlimb muscles of the dog (Maxwell et al. 1977) and primates (Acosta & Roy, 1987).

The FO fibres were considerably smaller in diameter than other fibre types found in biceps and triceps of the rat. These results lend support to the view that the size of muscle fibre types is related to their histochimical characteristics, i.e. the greater the oxidative activity the smaller the size of the muscle fibre type (Wachstein & Meisel, 1955). In rodents FO fibres are included in subtype IIA and showed low levels of parvalbumin (Füchtbauer et al. 1991). In the rabbit, however, the inverse relationship between fibre size and the level of SDH was not as marked, given that in biceps the FOg fibres were similar in size to those that exhibited lower SDH activity, i.e. FOG and SO fibres.

Our findings reveal considerable differences between the rat and rabbit in terms of the distribution of fibre types. In biceps and triceps brachii of the rat, the slow contracting fibres were only observed in the deep region, the most oxidative part of the muscle, whereas the fast contracting glycolytic fibres were only observed in the intermediate and, most abundantly, in the superficial regions. Other authors have reported that type I fibres are only found in the deep region of limb muscles in the rat (Pullen, 1977; Lind & Kernell, 1991) and mouse (Suzuki, 1990). In contrast, in the rabbit, although type I fibres were more numerous in the deep region, they were also observed throughout the entire muscle. A similar distribution pattern to that of the rabbit has been observed in the limb muscles of other mammals (McIntosh et al. 1985; Acosta & Roy, 1987; Suzuki & Tamate, 1988; Chanaud et al. 1991).

The distribution of fibre types in the rabbit suggests that the entire transverse section of biceps and triceps brachii may be involved in maintaining body posture. Nevertheless, as occurs in the limbs of the monkey (Acosta & Roy, 1987) and cat (Chanaud et al. 1991), there may be a functional compartmentalisation that permits the sequential activation of different regions.
of the muscle. This allows the deep regions (with a high oxidative capacity and rich in slow contracting fibres) to be active during all limb movements, whereas the superficial regions (rich in fast glycolytic fibres) are gradually incorporated as the movement gains intensity. In the rat, the regional segregation of the slow contracting fibres (deep region) and the fast glycolytic fibres (intermediate and superficial regions) may be due to functional specialisation designed to fulfil different motility needs as if they were 2 different muscles with different fibre type compositions all incorporated into a single muscle. Furthermore, the compartmentalised organisation of biceps and triceps brachii in the rat may be indicative of a greater specialisation than in the rabbit, and is probably associated with differences in posture or prehensile functions. As already noted, unlike the rabbit the rat uses its forelimbs to hold its food.

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