MUSCLE FIBRE TYPES AND FIBRE MORPHOMETRY IN THE TIBIALIS POSTERIOR AND ANTERIOR OF THE RAT: A COMPARATIVE STUDY

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Summary. Muscle fibre-type composition, fibre cross-sectional area and volume density were investigated in the rat tibialis posterior (TP) and compared to those in the tibialis anterior (TA), using histochemical and stereological methods. The muscle fibres were defined according to their myosin-ATPase (with preincubation at pH 10.4; 9.4; 4.5 and 4.3) activity. Succinate dehydrogenase and α-glycerophosphate dehydrogenase activities were also determined in the fibres. In whole muscle cross-section twenty fields were chosen for measurements (from superficial to deep and lateral to medial regions). The deep regions of TP muscle contained a higher proportion of type IIA and I fibres, the latter being restricted in distribution similar to those previously reported for the TA. The overall fibre-type proportions in the TP were 51.5%, 42.6% and 5.8% types IIA, IIB and I, respectively. The proportion of type I fibres was significantly higher than that in the TA. The estimated volume density values of fibre types were in the order IIB, IIA and I, for both muscles, indicating that the former constituted the main mass volume. This parameter is related to the size (area) and number of a given fibre type. The volume density of type I fibres was significantly higher in TP, while this parameter for type IIB fibres was significantly higher in TA. Differences in estimated parameters were noted also within muscles from various animals. These findings indicate that the rat TP is also regionalised muscle and suggest that sample sizes should be taken into consideration for the validity of quantitative results.

Key words: Muscle, fibres, rats, enzymology, morphometry

Introduction

Locomotory muscles of the rat hindlimb, such as tibialis anterior, are composed of three major fibre types designated as type I or slow-twitch oxidative (SO), IIA or fast-twitch oxidative glycolytic (FOG) and IIB or fast-twitch glycolytic (FG) fibres. The proportions of each fibre type vary between individual muscles within an animal and between homologous muscles of different species (1-7). The proportion of fibre types in a given muscle varies in people, as well. The samples of similar muscles from different subjects may show considerable variation in fibre-type composition, a difference that probably reflects the inherent histochemical mutability of muscle fibres and their capacity to adapt their properties to the range of functional loads (8). However, many investigators tend to believe that differences in the fibre-type composition, which are marked between sprinters and long-distance runners, are apparently genetically determined and that fibre-type composition does not change significantly during the training (9,10). Muscle fibre types defined by myosin-ATPase and oxidative enzyme histochemistry, such as succinate dehydrogenase, are related to two distinct physiological parameters, speed of contraction and resistance to fatigue respectively (11). Hence, the contractile properties of a whole muscle are dependent upon its fibre-type composition (11,12). For example, the slow soleus muscle in the adult rats is composed of approximately 80% slow-twitch (type I) fibres and 20% fast-twitch (type IIA and small number of undifferentiated fibres – type IIC) fibres, whereas the fast tibialis anterior muscle of the rat possess about 95% fast-twitch (types IIA and IIB) fibres and only 4% to 5% slow-twitch (type I) fibres (2-6).

It is known that certain human neuromuscular disorders selectively affect various muscles or are associated with pathologic variation in the proportion of fibre types (9, 13). It is also known that, for example, cross-innervation experiments have demonstrated changes in the histochemical and other properties of fibre types in a given muscle under the influence of foreign innervation (8). Therefore, the first pre-requisite for quantitative assessment of the extent of these changes is access to appropriate data on normal muscle fibre size and fibre-type proportions in the given muscles. These parameters as well as the regional distribution of muscle fibre types in the rat tibialis anterior have been investigated in numerous studies (2,5,6,14,15). In contrast, little attention has been paid to the rat tibialis posterior muscle, as well as to volume density of fibre types in both of these muscles. Therefore, the purpose of the present study was to determine proportions, sizes (areas) and volume densities of histochemically characterized fibre types in the adult rat tibialis posterior (TP) and to compare them
with the same parameters in the tibialis anterior (TA), using stereological methods.

**Material and Methods**

The tibialis posterior and anterior muscles were excised from seven adult male rats of Wistar strain under ether anaesthesia and prepared for freezing in isopentane cooled by liquid nitrogen. Serial cross-sections 10µm thick were cut in a cryostat at -20°C and stained for demonstration the activity of following enzymes: succinate dehydrogenase (SDH), NADH-tetrazolium reductase (NADH-TR), α-glycerophosphate dehydrogenase (α-GPDH), and myosin-ATPase (mATPase) with preincubation at pH 10.4; 9.4; 4.5 and 4.3 (13,16). The remaining sections were stained with hematoxylin-eosin (HE), van Gieson, periodic acid-Schiff (PAS) for glycogen and Sudan black B (for lipids) techniques.

Preliminary studies showed a variation in the regional fibre-type composition in the TP muscle, similar to those in the TA (5). According to this observation, in the present study, morphometric parameters were estimated from analysis of 20 fields of whole both muscle cross-section stained for mATPase after preincubation at pH 4.5 (from superficial to deep and lateral to medial regions), using stereological methodology, with test system M42 and objective ×40 (17). The obtained data of proportions of three major fibre types, their cross-sectional areas and volume densities, were used to calculate both standard deviations and standard errors. Statistical comparisons were made with Student’s t test. The level of significance was set at p<0.05.

**Results**

Histochemical techniques for demonstration of SDH, NADH-TR, α-GPDH and mATPase (pH 10.4; 9.4; 4.5 and 4.3) activities showed that the TP possesses three basic fibre types (type I, IIA and IIB), with considerable variation in the regional fibre-type composition. The deep regions of the muscle contained a higher proportion of type IIA and type I fibres, whereas the superficial regions contained a higher proportion of type IIB than type IIA fibres (type I fibres were absent) (Fig. 1). The three fibre types observed in the TP were indistinguishable from those previously seen in the TA. Type IIA (FOG) fibres possessed the high activity of SDH (and NADH-TR), with prominent subsarcolemmal accumulations of reaction product (Fig. 2). This fibre type exhibited a high mATPase activity (Fig. 3) and it was inactivated by acid preincubation at pH 4.5 (Fig. 3). Type I (SO) fibres generally contained a moderately high oxidative enzyme activity (SDH, NADH-TR) and low activity of mATPase (pH 10.4; 9.4) being acid stable, so it was activated by pretreatment with acid (pH 4.5 and 4.3) (Fig. 2 and 3). Type IIB (FG) fibres were characterized by low activities of SDH and NADH-TR, high activity of mATPase and by complete inhibition of their mATPase after preincubation at pH 4.3. Both IIA and IIB fibre types stained darkly for α-GPDH, which is indicative of a high concentration of enzymes for glycolysis, whereas type I fibres were stained weakly (Fig. 4).

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**Fig. 1a.** Cross-section of the superficial regions of the rat tibialis posterior muscle stained for NADH-tetrazolium reductase (NADH-TR, obj.×20).

**Fig. 1b.** Cross-section of the superficial regions of the rat tibialis posterior muscle stained for myosin-ATPase after preincubation at pH 4.3; mATPase darkly stained fibres of type I are absent (mATPase, obj.×20).

**Fig. 2a.** Cross-section of the deep regions of the rat tibialis posterior muscle stained for succinate dehydrogenase (SDH, obj.×20).
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Fig. 2b. Three major fibre types (I, IIA and IIB) are seen at higher magnification (SDH, obj.×60).

Fig. 3. Serial cross-sections of the deep regions of the rat tibialis posterior muscle stained for myosin-ATPase. (a) Myosin-ATPase reaction after preincubation at pH 9.4 (obj.×20). (b) Modified mATPase reaction following preincubation at pH 4.5 (obj.×20). (c) Modified mATPase reaction following preincubation at pH 4.3 (obj.×20).

The results of morphometric analysis showed that the overall fibre-type proportions in the TP were: 51.55% for type IIA, 42.62% for type IIB and only 5.83% for type I fibres (Table 1). The proportion of type I fibres in the TA was 4.44% (Table 1), indicating the significant preponderance of fast-twitch types of fibres in both analyzed muscles.

Table 1. The proportions of three major fibre types in the rat tibialis posterior and anterior muscles

<table>
<thead>
<tr>
<th>Fibre type</th>
<th>Tibialis posterior</th>
<th>Tibialis anterior</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.0583 ± 0.0118 *</td>
<td>0.0048 ± 0.0097</td>
</tr>
<tr>
<td>IIA</td>
<td>0.5155 ± 0.0320</td>
<td>0.5001 ± 0.0299</td>
</tr>
<tr>
<td>IIB</td>
<td>0.4262 ± 0.0340</td>
<td>0.4555 ± 0.0280</td>
</tr>
</tbody>
</table>

Values of fibre-type proportions from the superficial to deep and lateral to medial regions of both muscles are pooled for the overall proportions of fibre types.

Significantly higher from tibialis anterior: * p < 0.05

The predominating fibres of type IIA, with high activity of mATPase and oxidative enzymes, showed the lowest cross-sectional areas of all fibre types measured (Table 2). The proportions of type IIA fibres as well as the values for their cross-sectional area were larger in the TP than in TA, but differences were not statistically significant. However, the volume density (Vv) values of these fibres were significantly (p<0.05) higher in the former.

Table 2. Mean fibre cross-sectional areas for three major fibre types in the rat tibialis posterior and anterior muscles

<table>
<thead>
<tr>
<th>N</th>
<th>Fibre type</th>
<th>Tibialis posterior</th>
<th>Tibialis anterior</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean area (µm²)</td>
<td>Mean area (µm²)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. D.</td>
<td>S. E.</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>1156.62 ± 358.50</td>
<td>1197.54 ± 325.05</td>
</tr>
<tr>
<td></td>
<td>IIA</td>
<td>1107.03 ± 303.00</td>
<td>1046.34 ± 288.91</td>
</tr>
<tr>
<td></td>
<td>IIB</td>
<td>2305.64 ± 727.40</td>
<td>2400.25 ± 689.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.E.=30.30</td>
<td>S.E.=27.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.E.=25.61</td>
<td>S.E.=24.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.E.=61.48</td>
<td>S.E.=58.24</td>
</tr>
</tbody>
</table>

Values are quoted together with ± standard deviation (S.D.) and standard error (S.E.) of means

The type IIB fibres, the next most frequent type of fibres, were found to have the highest value for the cross-sectional area (Table 2). These fibres also had the highest value of volume density (Table 3) – this parameter strongly depends on the size and number of given fibre type. There was difference, although not
significant, in the mean area of type IIB fibres between the TP and TA, being larger in the latter. This is also the case with relative proportion of these fibres. The volume density values for type IIB fibres were, however, significantly (p<0.05) higher in the TA in comparison with those for their counterpart in the TP.

Table 3. Morphometric estimates of volume density (Vv) of three major fibre types in the rat tibialis posterior and anterior muscles

<table>
<thead>
<tr>
<th>Fibre type</th>
<th>Tibialis posterior</th>
<th>Tibialis anterior</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S. D.</td>
</tr>
<tr>
<td>I</td>
<td>0.0398 ± 0.0091</td>
<td>0.00371</td>
</tr>
<tr>
<td>IIA</td>
<td>0.3294 ± 0.0330</td>
<td>0.01347</td>
</tr>
<tr>
<td>IIB</td>
<td>0.5663 ± 0.0510</td>
<td>0.02082</td>
</tr>
<tr>
<td>Intertubulin</td>
<td>0.0645 ± 0.0120</td>
<td>0.00490</td>
</tr>
</tbody>
</table>

Vv in mm³

Values are expressed as mean ± standard deviation (S.D.) and standard error (S.E.) of means

Significantly higher from tibialis anterior: * p < 0.05
Significantly lower from tibialis anterior: ** p < 0.05

The type I fibres, which constitute a minor population of fibres, had a relatively low values for their cross-sectional areas (Table 2). The volume density values of these fibres were the lowest in comparison with those of types IIA and IIB (Table 3), which is due to a small number of them as well as their low sizes (areas). The obtained data of relative proportion and of volume density for these fibres in the TP were significantly (p<0.05) higher than those in the TA.

Discussion

The muscle fibres can be categorized as slow- or fast-twitch based upon several physiological and molecular criteria, including contractile protein isoforms and metabolic enzyme activity. Mammalian skeletal muscle fibres have been routinely categorized into three major types (SO or type I, FOG or type IIA and FG or type IIB) on the basis of their histochemical staining properties. The fibre types, identified using myofibrillar actomyosin ATPase histochemistry, correlate with the distribution of myosin isoforms in the fibres (18, 19). The limb and trunk muscles of adult rats express four myosin heavy chain (MCH) isoforms: one slow (MHC1a) and three fast (MHC1la, MHC1ld or IIX, and MHC1lb) (19,20). Thus, for example, type I fibres express MHC1 and fibre types IIA, IID or IIX, and IIB express MHC1la, MHC1ld or IIX, and MHC1lb, respectively. Histochemically, type IIX fibres resemble to IIB in their acid-mATPase activity, but have much higher oxidative (SDH) activity (18). The fibres which contain two MHC isoforms, a population of “hybrid” fibres, also exist in rat skeletal muscle (19).

The tibialis posterior muscle examined was found to contain approximately 94% histochemically fast-twitch fibres and about 6% slow-twitch fibres, indicating the significant preponderance of the fast-twitch types. Our data regarding the muscle fibre-type composition in the TP (Table 1) are similar to those in study of Ariano et al (1), in which FOG fibres constituted 51%, FG 44% and SO fibres only 5% of the whole population of them.

The regional variation of fibre types that slow-twitch fibres usually lie in deeply and fast-twitch fibres superficially, is a common observation for limb muscles. However, the degree of regionalisation varies for homologous muscles of different species (7,15). The regional variation in fibre-type composition, in which type I or SO fibres were restricted to the deep regions of muscle, has been demonstrated for numerous muscles of rat including sternomastoideus, tibialis anterior, extensor digitorum longus, biceps brachii (long head), triceps brachii (long head), gastrocnemius medialis, peroneus longus, flexor digitorum and hallucis longus (2,3,5,6,7, 14,15). In this study it was found that the tibialis posterior of the rat is also regionalised muscle.

The size of muscle fibres, their contractile and other properties can be influenced by a variety of factors such as changes in motor neuron activity, alterations in hormone levels or changes in physical activity, what was documented in numerous experimental studies (21-26). Although the molecular pathways by which environmental factors affect gene expression remain largely unknown, experimental data suggest that myogenin, as well as the other basic/helix-loop-helix muscle regulatory factors (MRFs), referred to as MyoD, Myf-5, and MRF4, may be involved in both the initial establishment as well as maintenance of fibre-type diversity in the developing organism (23). Recent evidence have suggested that MRFs and MEF2 (myocyte enhancer factor-2) act within a regulatory network that established the differentiated phenotype of skeletal muscle (27).

The determination of size and distribution of fibre types in normal muscles, as well as the other morphometric parameters (i.e. muscle fibre volume density), is pre-requisite in detection of their alterations in various animal experimental models and human diseases. Hence, morphometric analysis of normal muscles provide a quantitative basis for comparison and evaluation of lesions severity induced by the same factors (i.e. myotoxin, denervation) in different muscles; it can also be helpful in establishing the pathogenetic mechanisms of arised lesions.

Among the parameters proposed as a descriptor of muscle fibre size, fibre cross-sectional area appears to offer the greatest accuracy in that this measurement is the least susceptible to the effects of oblique sectioning or kinking of muscle fibres (9). In the present study, in both fast muscles examined, the three major fibre types had mean area values in the order: IIA, I and IIB, the latter being the largest. The type IIB fibres had the highest values for the cross-sectional area and volume density, indicating that they were most prominent in mass, although not in number, in both tibialis posterior and anterior muscles. There were significant differences between the volume densities of these fibres in the TP and TA; differences between the mean areas and proportions were, however, not statistically significant.
The predominating fibres of type IIA possessed the lowest cross-sectional areas in both rat muscles investigated. The mean value of diameter for type IIA fibres in the TA was also the smallest of all the fibre types measured (5). The volume density of these fibres, however, was relatively high and was found to be significantly higher in the TP than in the TA muscle. The type IIA fibres constituted 51.55% in TP and 50.01% in TA of the whole population of muscle fibres.

The values of cross-sectional areas for type I fibres were larger than for type IIA, but much smaller than those for type IIB fibres. These fibres, which were in minority and had the lowest value of volume density, constituted the minor mass volume in both rat muscles. Significant differences in the volume density and proportion of these fibres have been observed among the TP and TA muscles.

The estimated data regarding volume density in this study showed that the relative volume of muscle fibre types was 93.55%, whereas the relative volume of interstitium was 6.45% in TP, and 93.76% and 6.24% in TA, respectively. Interstitium represents the interstitial space and component tissue, including blood vessels and nerve bundles. In the extensive study of Kilarski and Sjöström (28), in which they analyzed rat and rabbit diaphragm, among other morphometric parameters, was estimated the volume density of muscle fibre types, but not volume density of interstitium. In an other study (29), the areal density for both muscle fibre types and interstitium in the rat extensor digitorum longus (EDL) was examined. The obtained value for areal density of interstitium was low, only 2.1% (29).

The results of Pullen (2) regarding the mean cross-sectional area for type IIB fibres in the TA are higher than those found in the present study. In this study the mean cross-sectional area of type IIB fibres was 3391.88µm², which is approximately three times higher than value for type IIA fibres. The mean cross-sectional area of type IIB fibres in this study was 2400.25 µm², and was approximately two times higher that one for type IIA. Considerable variation in the proportions and sizes of muscle fibre types between the reported estimates (1,2,5,6,14) may be the consequence of the rats strain differences, age and sex, the level of sections of muscle investigated and also of differences between the methods of quantitative analysis used by the various authors. Regarding the sizes of fast-twitch fibre types one has to take into consideration the conditions of limited movement of experimental animals, which may, and certainly are different, in various laboratories.

### Conclusion

In conclusion, based upon histochemically categorized muscle fibres of the tibialis posterior muscle, the considerable variation in this regional fibre-type composition, was found. Type I fibres were confined to the deep regions of muscle similar to those previously reported for the tibialis anterior. These finding indicates that the rat tibialis posterior is also regionalised muscle. The tibialis posterior have shown to contain overall 51.55% type IIA, 42.62% type IIB and 5.83% type I fibres. The proportion and volume density values of type I fibres, being in minority, were significantly higher in comparison to those of their counterpart in the tibialis anterior.

The estimated values for the mean cross-sectional area were in the order: types IIB, I and IIA fibres, while the values of volume density were in the order: types IIB, IIA and I fibres, indicating that type IIB fibres constituted the main mass volume in both rat analyzed muscles. The volume density of type IIB fibres was significantly higher in the tibialis anterior, while this parameter for type IIA fibres was significantly higher in the tibialis posterior. Differences in estimated parameters were noted also within muscles from various animals.

### References

14. Torrella JR, Whitmore JM, Casas M, Fouces V, Viscor G. Capillarity, fibre types and fibre morphometry in different sam-
Desanka Tasić, Dragan Dimov, Jasmina Gligorijević, Ljubinka Veličković, Katarina Katić, Miljan Krstić, Irena Dimov

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Kratak sadržaj: Ispitivana je kompozicija tipova mišićnih vlakana, njihova area (površina poprečno presećenih vlakana) i volumenska gustina u m. tibialis posterioru pacova i komparirana sa parametrima u m. tibialis anterioru, primenom histiohemijskih i stereoloških metoda. Mišićna vlakna su tipizirana na bazi aktivnosti njihove miozinske ATPaze sa preinkubacijom pri pH 10,4; 9,4; 4,5; i 4,3. U vlaknama su takođe određivane aktivnosti sukcinat dehidrogenaze (SDH) i α-glicerofosfat dehidrogenaze (α-GPDH). Merenja su vršena na dvadeset polja poprečne sekcije transversale oba mišioca. Ovaj metod je u direktnoj korelaciji sa veličinom (areom) i brojem datog tipa vlakana. Utvrđen je da vrlo duboke regije u m. tibialis posterioru sadrže veću proporciju vlakana tipa IIA i I, pri čemu je crvena serija bila ograničena u distribuciji na ove regije, slično nalažima koji su prethodno saopštěni za m. tibialis anterior pacova. Nađeno je da ukupna proporcija tipova vlakana u m. tibialis posterioru iznosi 51,5% za vlakna tipa IIA, 42,6% tipa IIB i 5,8% za vlakna I tipa. Ovaj parametar je u direktnoj korelaciji sa veličinom (areom) i brojem datog tipa vlakana. Volumenska gustina vlakana I tipa bila je značajno veća u m. tibialis posterioru, dok je volumenska gustina vlakana tipa IIB bila značajno veća u m. tibialis anterioru. Razlike u ispitivanim parametrima su takođe zapažene unutar mišića kod potiču od različitih životinja. Zaključeno je da je m. tibialis posterior pacova takođe regionalizovani mišić i sugerišano da pri proceni validnosti rezultata kvantitativne analize treba uzeti u obzir veličinu uzorka.