MUSCLE FIBER TYPES AND FIBER MORPHOMETRY IN THE SOLEUS MUSCLE OF THE RAT

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Summary. Muscle fiber type composition, distribution and size of fibers were investigated in the soleus muscle of young adult male and female rats, using histochemical and morphometric methods, to determine whether these parameters vary according to the sex of animals. Combined myofibrillar ATPase (mATPase) and metabolic enzyme histochemistry were performed on serial cross-sections from the belly region of muscle. Three fiber types (I, IIA, and IIC) were identified on the basis of mATPase activity after preincubation at pH values of 9.4, 4.5 and 4.3. The type IIC fibers, present in a small number (about 4%), were included within the type IIA fiber class for purposes of quantitative analysis. The fiber type composition of the slow soleus muscle showed type I dominance (78%). There has been observed a variation in the regional distribution of muscle fiber types. A relative number of type IIA fibers increased towards the lateral part of the muscle, and so did type IIC, but the general organization was not as regular as those previously found in the fast tibialis anterior and tibialis posterior muscles. The size of predominating fibers of type I was larger than of IIA type fibers in the soleus muscles from both groups. No significant difference was detected between male and female rats in respect to the morphometric parameters examined.

Key words: Fiber types, skeletal muscle, myofibrillar ATPase, morphometry, rats

Introduction

The phenotypic differences among skeletal muscle cells, termed fiber types, their potential for adaptation and underlying mechanisms have been a topic of study for several decades. Most mammalian skeletal muscles are composed of varying proportions of the three major types (I, IIA and IIB) of fibers, while certain muscles such as the rat soleus consist predominantly of type I fibers (1-4) determined by myofibrillar ATPase (mATPase) histochemistry. These fiber types show differences in morphological, metabolic and contractile properties. The functional characteristics of skeletal muscle are likely related to its fiber type composition. Thus, the rat soleus, slow-twitch muscle involved in maintaining posture, contains a high percentage of type I fibers, while the extensor digitorum longus of the rat, fast-twitch muscle, contains a high percentage of type IIA and IIB fibers (2-6).

The relative proportions of each fiber type vary between individual muscles within an animal, and between homologous muscles of different species, and even in different regions of the same muscle (3, 5-10). In addition, it has been established that fiber type composition in the slow soleus muscle of the rat undergoes continuous changes during the whole period of growth (11-13). The fiber type composition in this muscle has been extensively studied (4-7, 11, 12). However, reported data vary and could result from the use of different age and strains of rats or different methods of quantitative analysis used by various investigators.

The purpose of the present study was to investigate the fiber type composition, distribution and size of fibers in the rat soleus muscle and determine whether these morphometric parameters vary according to the sex of animals.

Materials and Methods

Ten young adult (5 male and 5 female) Wistar rats, weighing 200-250 g, were used in this study. The soleus (SOL) muscle was removed under ether anesthesia. The muscle samples were rapidly frozen in isopentane cooled by liquid nitrogen. Serial cross-sections 10 µm thick were prepared and stained using mATPase histochemistry after preincubation at pH 9.4, 4.5 and 4.3 for fiber typing and also processed for succinate dehydrogenase (SDH) and NADH-tetrazolium reductase (NADH-TR) to obtain data about their oxidative metabolism (2,3). The remaining sections were stained with hematoxylin-eosin, Van Gieson, Sudan black B (for lipids) and with periodic acid-Schiff (PAS) procedure for glycogen.

For the morphometric analysis, the image of the SOL muscle (in cross-section from the belly region) was projected on to the screen of a demonstration microscope and magnified to a constant size. The relative proportions
of fiber types were determined from direct counts of fiber numbers in squares along its deep to superficial and medial to lateral axes. 1000 fibers were counted, on ten square areas of a defined size (showing 90-120 fibers), in sections stained for mATPase after preincubations at pH 9.4 and 4.3. The type IIC fibers, present in a small number, were included within the type IIA fiber class for purposes of quantitative analysis.

The size of the muscle fiber types was determined by measuring the "lesser fiber diameter", since this measurement is the least affected by obliquity of the section or kinking of the muscle fiber (3). The measurement was performed on 100 fibers of type I and 100 of type IIA on the demonstration screen of the microscope from sections stained for mATPase after preincubations at pH 9.4 and 4.3. The pooled measurements obtained for each fiber type were used to calculate the mean values. Group values of morphometric parameters are expressed as means ± SD. The differences between the mean values for male and female groups were analyzed by Student's t test. Differences were considered statistically significant at p<0.05.

Results

In the rat SOL muscle, three fiber types (I, IIA and IIC) were observed on the basis of their intensity of coloration on mATPase after alkaline (pH 9.4) and acid (pH 4.5 and 4.3) preincubations (Fig. 1a-b). The type I fibers with low mATPase activity following preincubation at pH 9.4 exhibited a moderately high and diffuse activity of oxidative enzymes (Fig. 2). On serial sections reacted for mATPase after preincubation at both pH 4.5 and 4.3, these fibers showed enhanced activity. The type IIA fibers identified by high mATPase activity (Fig. 1a), that as acid-sensitive being inhibited after preincubation at pH 4.5, were found to contain a high level of SDH and NADH-TR activities with pronounced subsarcolemmal accumulations of reaction product. Namely, type IIA fibers possessed high mATPase activity and high oxidative potential. The type IIC fibers were indistinguishable from type IIA fibers on the standard mATPase reaction (pH 9.4) as both were dark (Fig. 1a). However, the mATPase activity in these fibers was not fully inhibited by preincubation at pH 4.3, having an intermediate coloration (Fig. 1b). Their SDH (and NADH-TR) reaction rather corresponded to type IIA than to type I fibers. However, the subsarcolemmal increase of oxidative enzyme activity was not so prominent (Fig. 2).

The fiber types in the SOL muscle on preliminary examination were fairly randomly distributed on both mATPase and oxidative enzyme reactions. However, a more detailed examination showed that the relative number of type IIA fibers increased towards the lateral part of the muscle. The type IIC fibers were estimated to constitute about 4% of the total fiber population, although the content of these fibers was variable among individual rats of both sexes.
Table 1. Muscle fiber type percentages in the rat soleus muscle. Values given are means ± SD.

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>Type I</th>
<th>Type IIA</th>
<th>Ratio I/IIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean I/IIA</td>
</tr>
<tr>
<td>M</td>
<td>5</td>
<td>76.86</td>
<td>4.40</td>
<td>23.14</td>
</tr>
<tr>
<td>F</td>
<td>5</td>
<td>79.36</td>
<td>6.30</td>
<td>20.64</td>
</tr>
</tbody>
</table>

Table 2. Ranges of size and diameters of fiber types in the rat soleus muscle. Values given are means ± SD and CV.

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>Type I Diameter (μm)</th>
<th>SD</th>
<th>CV</th>
<th>Type IIA Diameter (μm)</th>
<th>SD</th>
<th>CV</th>
<th>Ratio I/IIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>5</td>
<td>44.08 (43.30-45.16)</td>
<td>6.46</td>
<td>14.65</td>
<td>37.20 (36.78-37.60)</td>
<td>4.66</td>
<td>12.53</td>
<td>1.18</td>
</tr>
<tr>
<td>F</td>
<td>5</td>
<td>44.34 (39.88-49.08)</td>
<td>6.81</td>
<td>15.36</td>
<td>38.60 (36.10-40.06)</td>
<td>5.96</td>
<td>15.44</td>
<td>1.15</td>
</tr>
</tbody>
</table>

Discussion

The mATPase histochemistry after alkaline and acid preincubation is widely used for the identification of three major fiber types in the skeletal muscles: slow-twitch type I and fast-twitch types IIA and IIB according to the classification of Brooke and Kaiser (1). Two types of these fibers (I and IIA) were found in the slow SOL muscle of the rat, mixed with a variable number of type IIC fibers (2-4). The fiber types identified on the basis mATPase histochemistry correlate with the expression of myosin heavy chain isoforms (MHCs) (14). Namely, muscle fibers contain different MHCs responsible for their different ATPase activities and speed of contraction (14, 15). The MHCs are encoded by a multigene family, and four isoforms (i.e., I, IIA, IIX/d, and IIB) have been identified in adult skeletal muscles of small mammals including the rat, as well as in muscles of some large mammals such as the pig (14-16).

The different fiber type proportion among muscles is likely related to their function. Indeed, the SOL of the rat is a slow-twitch muscle involved in maintaining posture and weight bearing, so that type IIA fibers constitute a significantly smaller population of fibers in...
relation to type I, whereas type IIB fibers are absent in this postural muscle. The adult male and female SOL muscles examined were found to contain 78% type I fibers and 22% type IIA fibers (including about 4% fibers of type IIC). This is in accordance with previous results showing that type I fibers constitute about 80% of the total fiber population in this slow muscle. The present results regarding the fiber type composition of the SOL muscle from both groups are similar to the data previously published by Pullen (6). Pullen (6) reported the fiber type composition of the SOL muscle in rats of both sexes to be 76.3% type I and 23.7% type IIA, classified on the basis of their mATPase activity. This author found no difference between males and females in respect to fiber type composition of the SOL muscle, and the present findings agree with this. To our knowledge, there are no other data which indicate that sex differences exist in the fiber type composition of the SOL muscle.

The fiber type proportions are known to alter with age (11-13) and can alter under various kinds of physical exercise or following certain experimental procedures (13, 18-24). In the slow SOL muscle of the rat, the increase of slow-twitch type I fibers and decrease of fast-twitch type II fibers was found during the whole period of growth (11). During this adaptive process, the transformation from fast-twitch to slow-twitch fiber units occurred over the intermediate state (11). Similar changes in proportion of fiber types in this slow muscle were observed in other studies (12, 13), and intermediate stained fibers were termed "transitional" fibers (12). The intermediate /transitional fibers mainly corresponded to the type IIC, although various "types" of transitional fibers with different acid- and/or alkali-stable mATPase activity were found (11, 12), and they were also observed in this study (not shown).

It has been shown that in SOL muscle of the rat, between 5 and 34 weeks of age, the proportion of type II fiber units decreased from 33 to 10% and type I fiber units increased from 67 to 90%, which was paralleled by approximately the same relative changes in number of type II and type I fibers (11). Transformation from type II to type I, phenotypically expressed by variations in intensity of mATPase reaction in fibers under transformation (mATPase intermediate fibers), was complemented at a slower rate by a decrease in SDH activity (11). Kugelberg (11) noted a loss of intermediate fibers between one to six months after birth with a few intermediate fibers found at 34 weeks. Functional overload the SOL muscle induced by ablation of the gastrocnemius muscle was shown to accelerate the natural conversion of fiber types (19). Interestingly, the total number of fibers in the rat SOL muscle remained fairly constant during growth (11) and ageing (25). The variation between the reported estimates of fiber proportions in the rat SOL muscle (4-7, 11, 12, 14) could be explained by the different age and strain of the animals studied by the various authors, and by the use of different methods of quantitative analysis or histochemical methods (e.g. mATPase, oxidative enzyme or combination) used for muscle fiber typing. This last point is relevant even if the adult animals were studied because of the presence of intermediately stained fibers, some of which may be misclassified by enzyme histochemistry.

The transitional fibers showing variations in mATPase activity appear to contain two MHC isofoms within a single fiber. The fibers expressing more than one MHC isofom termed as "hybrid" fibers were found to exist in normal rat hindlimb muscles making an important population of fibers that should not be ignored (14). The hybrid fibers have also been found in other species and in human muscles (15, 26). Based on the use refined histochemical techniques for mATPase, Staron et al. (14) delineated the entire range of histochemically defined pure and hybrid fiber types in the hindlimb muscles of the adult rat. In the slow SOL muscle four fiber types were identified: I, IC, IIC and IIA, of which types IC and IIC represent hybrid fibers making 1.8% and 7.0%, respectively, of the total number of fibers (14). However, these data are different from those reported by Desaphy et al. (24) showing by immunocytochemistry that the 2-week unloaded SOL muscle of adult rats recovered a control proportion of slow/fast fibers (85% /15%) after 3 weeks of reloading, with a few remaining hybrid fibers co-expressing fast and slow MHCs. Namely, after 3 weeks of reloading, the proportion of pure slow and fast fibers were returned to control values with the exception of a small (<5%) population of MHCIIa fibers (24). The hybrid fibers could be detected by immunocytochemistry and in situ hybridization. However, because protein is longer-lived than the mRNA and/or because the protein may diffuse, a mismatch between the presence of the protein and its corresponding mRNA can occur (15). Hence, co-expression of MHCs was found to be more common at the protein than the mRNA level (15).

The fiber types in the slow SOL muscle were more evenly distributed throughout the cross-section than those previously found in the fast tibialis anterior and tibialis posterior muscles of the rat (10), although a distribution gradient was generally apparent. The relative number of type IIA fibers increased towards the lateral part of the muscle (as did type IIC). Thus, the difference in fiber type proportions was observed between the medial and lateral parts. The pattern of distribution observed in this study is similar to previously reported (4, 6, 11, 27), but the reason for such distribution of fast-twitch fibers is not fully known.

Although it is difficult to compare fiber size data between studies based on the use of different strains, age and sex of rats, the size of type I fibers was found to be larger than the type IIA in the adult rats of either sex and various strain, even if the different parameters of fiber size (cross-sectional area or diameter) were used (6, 11, 14, 18, 25). In this study, the predominating fibers of type I were found to be also larger than type IIA in both male and female rat SOL muscle (Table 2). Our data on the size of both fiber types are similar to those
found by Jaweed et al. (18) for control animals (young adult female rats). In their study the mean values for type I and IIA fibers were 42.9 μm and 37.9 μm, respectively. The mean values for both fiber types obtained in the present study are smaller than those found by Alnaqeeb and Goldspink (25) studied Sprague-Dawley rats, while Wistar albino rats were used by Jaweed et al (18) and in the present study. Wistar rats were also studied by Pullen (6), but the presented results of fiber type sizes are based on pooled data from both sexes.

The size of muscle fibers is influenced by a number of factors such as activity and innervation, growth, hormones and nutrition (3, 11, 13, 25, 28, 29). The muscles of the adult male in most mammalian species tend to be heavier, owing to larger fiber sizes, than those in the adult female. In adult male subjects, type II fibers are usually larger than type I fibers, in contrast to females where type I fibers tend to be larger. These differences are explained as due to greater physical activity and anabolic effects of androgens. In man testosterone appears to have selective effect on muscle fiber growth: type II fibers being larger in men than in women (28). Anabolic effects of androgens are demonstrated in animal models by either male castration or injection of androgens into female animals. Despite their observable effects, the response of skeletal muscle to androgens is less than in male accessory sex tissues because of low concentrations of hormone receptors and the absence of 5α-reductase which converts testosterone to its more potent congener, dihydrotestosterone (29). Accordingly, androgens can act within a muscle to support growth by increasing insulin-like growth factor-1 expression and in the hypothalamic-pituitary axis, where the synthesis and release of growth hormone is controlled (29).

In this study no significant difference in the size of both fiber types was found between males and females; the fast-twitch type IIA fibers being slightly larger in females. The ranges of the size and proportion of this fiber type in female rats were also larger than those in male rats, indicating that the group of males was more homogeneous than the group of females. The influence of other factors, such as the conditions under which the rats are maintained must be considered. One of the possible explanations is that the SOL muscle might be skeletal muscles which are less sensitive to androgens. In the male rat, as in man, testosterone appears to have a selective effect: type II fibers are larger in males than in females (28). In the study of hypogonadal mice it was found that type IIB fibers are the most dependent upon sex hormones for appropriate development (29), but fibers of type IIB are absent in the SOL, a postural muscle. In addition, the sizes of fiber types may also reflect functional requirements of the muscle to which belong. Thus, the slow SOL muscle to its involvement in maintaining posture and its weight bearing function will be expected to show a larger size of predominating slow-twitch type I fibers in both, males and females. Accordingly, the size of type I fibers was found to be larger than type IIA in the adult rats until they aged 34 weeks (11) or later (25).

Using the hypogonadal mouse model to examine the effects of diminished androgen and oestrogen hormones upon the muscle size and fiber type composition in murine gastrocnemius and SOL muscles, it was shown that muscle development in males generally retards, but has no apparent influence in females (29). The data of Sciote et al. (29) regarding the differences in the size of type I and IIA fibers of the SOL muscle between normal males and normal females 8 weeks of age (mice of this age represent pubescence and young adulthood) are similar to those found in this study, although we used young adult rats. It is clear that further comparative studies of male and female rat SOL muscles during growth are needed. They should be done according to rigorous criteria with respect to age and muscle mass of animals, using methods for successful detection age- and sex-related changes.

Changes in the size of muscle fibers and other properties (i.e. metabolic, functional) have been well documented in various experimental activity and inactivity models (18, 20-24, 30), suggesting the plasticity of muscle fibers. However, the molecular mechanisms, underlying these adaptive phenomena, are incompletely understood. Since MHC is the most abundant protein in muscle and because its polymorphism has influence on contractile, metabolic, and size properties of muscle fibers (15), a study of MHCs and expression corresponding mRNAs in muscle fibers during growth as well as during adaptive processes under the influence of the entire milieu and neural activity patterns may further our understanding of mechanisms underlying muscle fiber plasticity, and age- and sex-related differences.

**Conclusion**

In conclusion, the results obtained by the histochemical and morphometric analysis of the rat SOL, a postural muscle, in this study confirm the relationship between fiber type proportions and muscle function. The SOL muscle of both male and female rats examined was found to contain 78% of type I fibers and 22% of type IIA fibers (including about 4% fibers of type IIC).

A variation in the regional distribution of fiber types has been observed. The relative number of type IIA fibers increased towards the lateral part of the muscle, as did type IIC, but the general organization was not as regular as those previously found in the fast tibialis anterior and tibialis posterior muscles. The size of predominating fibers of type I was larger than of type IIA fibers in the soleus muscles from both groups. No significant difference was detected between males and females in respect to the morphometric parameters examined.
References

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Kratak sadržaj: Ispitivana je kompozicija tipova mišićnih vlakana, njihova distribucija i veličina u m. soleusu mladih odraslih mužjaka i ženki pacova, korišćenjem histoхemijskih i morфometrijskih metoda, da se utvrdi da li ispitivani parametri variraju prema polu životinje. Na serijskim poprečnim presecima mišića primenjena je kombinacija histoхemijskih metoda: miofibrilarna ATPaza (mATPaza) i enzimi metabolizma. Identifikovana su tri tipa vlakana (I, IIA i IIC) na osnovu aktivnosti mATPaze nakon preinkubacije pri pH 9,4; 4,5 i 4,3. Vlakna tipa IIC, koja su zastupljena u malom broju (oko 4%), uključena su u grupu vlakana tipa IIA za potrebe kvantitativne analize. Ispitivanje kompozicije tipova vlakana pokazalo je da u sporom m. soleusu dominira tip I (78%). Zapažena je varijacija u regionalnoj distribuciji tipova mišićnih vlakana. Idući prema lateralnom polu mišića povećavao se relativni broj vlakana tipa IIA, kao i tipa IIC, ali generalna organizacija nije bila tako regularna kako je prethodno utvrđeno za brze mišiće, tibialis anterior i tibialis posterior. Veličina predominirajućih vlakana tipa I bila je veća od vlakana tipa IIA u mišićima obe ispitivane grupe. Nije utvrđena značajna razlika između mužjaka i ženki pacova u ispitivanim morфometrijskim parametrima.

Ključne reči: tipovi vlakana, skeletni mišić, miofibrilarna ATPaza, morфometrija, pacovi