Slow motor units in female rat soleus are slower and weaker than their male counterparts

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Slow motor units in female rat soleus are slower and weaker than their male counterparts

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Abstract The aim of the study was to investigate sex-related differences in contractile properties, parameters of action potentials, and mechanisms of force regulation of motor units in the rat soleus muscle, which is a frequent experimental model in animal research. It was revealed that the mean mass of the muscle in males was bigger than in females, by approximately 80 %. However, the relation of the muscle mass to the body mass was not significantly different. These results correspond to approximately twice as much tetanic force per motor unit in male rats, and higher maximal contractile output, reflected by the force–time area per stimulus pulse. On the other hand, no differences were observed with respect to twitch forces of motor units. Thus the twitch-to-tetanus ratio was significantly higher in females. Additionally, the contraction and the half-relaxation times were significantly longer in female motor units, which might be due to differences in muscle architecture. The force–frequency curve in males was shifted rightwards with respect to females, indicating that the same relative level of tetanic force could be achieved at considerably lower stimulation frequency in females. The analysis of motor unit action potentials revealed about four times higher amplitudes in male rats, whereas the time parameters of action potentials were similar. The motor units in male and female rat soleus are considerably different and these observations should be taken in the consideration in various experiments on the muscle.

Keywords Sex differences · Soleus muscle · Motor unit · Rat

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Introduction

Sex differences in morphology and function of tissues and organs are meaningful with respect to basic knowledge, but it is especially important to realize that these differences exist when comparisons are made between results of similar experiments performed on male or female specimens. Studies on rat muscle, which is a frequent experimental model, show striking differences between male and female animals with respect to contractile and metabolic properties of motor units (MUs) and muscle fibers. For example, previous studies of the rat medial gastrocnemius MUs have revealed sex related differences of its MUs for male and female muscles (Celichowski and Drzymała 2006; Celichowski and Drzymała-Celichowska 2007). The male medial gastrocnemius muscle contains about 10 % more MUs, with higher participation of the fast fatigable and lower of the slow type in relation to females. Male MUs in this muscle are also stronger and have longer contraction time.

The soleus muscle has different physiological characteristics, most of all it is a typical slow-twitch muscle, composed predominantly of slow MUs (Kugelberg 1973; Burke et al. 1974; Chamberlain and Lewis 1989), however, it may contain two myosin heavy-chain (MHC) isoforms, type I and type IIa (Pette and Staron 2000; Drzymała-Celichowska et al. 2012). The soleus muscle has been widely investigated, however, so far the sex differences in MU proportions and their contractile properties have not been documented for this muscle. In the earlier studies (Mierzejewska-Krzyżowska et al. 2011, 2012) we have demonstrated for sex related differences in morphometric properties of soleus muscle fibers (number, diameter and cross-section area), which suggest that also functional characteristics of this muscle are different between sexes. In male rats, the mean number of muscle fibers visible on a cross-section was higher than in female rats, approximately by 11 %, whereas their diameter, and the cross-section area where higher by 8 and 19 %, respectively (Mierzejewska-Krzyżowska et al. 2012). Moreover, the myosin content appears to be sex-related. Male soleus contains about 13 % of the MHC IIa isoform, whereas the female muscle only 2 % (Drzymała-Celichowska et al. 2012). For human soleus muscle, Chow et al. (2000) have also presented distinct sex-based differences in its architecture. Female soleus muscles have longer average muscle fiber length, but male counterparts have a greater angle of pennation and a greater thickness in all examined parts of the muscle.

The aim of the present paper was to answer the question, whether the sex-related differences in myosin content and in the muscle architecture are equally reflected in distribution of MU types as well as in their contractile properties, and parameters of action potentials. This is the first electrophysiological study undertaken to test sex differences for the functionally isolated MUs of a slow muscle (soleus). We hypothesize that MUs of the female soleus would have longer twitch contractions and would develop lower contractile forces in comparison to MUs of the male counterpart. We also intended to reveal whether regulation of MU force through the firing rate is different between male and female soleus, and whether sex-related differences concern the MU contractile output, reflected by the force–time area per pulse, and in stimulation frequency for the optimal tetanus, characterized by the maximal force output.

Materials and methods

The present MU experiments were performed on 6-months old *Wistar* rats: five females (the mean body mass 257.7 ± 20.1 g), and five males (the mean body mass 474 ± 34.7 g). Additionally, the whole muscle properties were examined in five males, and five females (the mean body mass 458 ± 21.6 and 255.2 ± 15.6 g, respectively). All rats were born, housed and kept under the same conditions at one and the same place, so there was no additional influence of environment on the studied sex differences.

All the procedures were approved by the Local Ethics Committee and followed the European Union guidelines on animal care as well as the Polish Law on the Protection of Animals. Rats were anesthetized with sodium pentobarbital (initial dose of 60 mg kg^{-1} i.p., supplemented after 2 h with additional doses of 10 mg kg^{-1} h^{-1} i.p.), and adequacy of the anaesthesia was verified by the lack of withdrawal and pinna reflexes. At the end of the experiment the animals were killed by an overdose of sodium pentobarbital (180 mg kg^{-1} i.p.).

Experimental procedures were identical for male and female animals. The distal part of the soleus muscle was partly isolated from surrounding tissues, the innervation and blood supply to the examined muscle were left intact, whereas other hind limb muscles were denervated by cutting remaining branches of the sciatic nerve, and their distal tendons were cut. The laminectomy was performed over L2-S1 segments. Dorsal and ventral roots of the spinal nerves were cut proximally to the spinal cord. The animals were immobilized with steel clamps on the tibia, the sacral bone, and the L1 vertebra. The isolated spinal cord, ventral and dorsal roots of spinal nerves were covered with warm paraffin oil in a small pool formed by skin around the laminectomy. The studied muscle was immersed in a metal pool filled with the warm paraffin oil. The oil and animal core temperature were kept at a constant level (37 ± 1 °C) by an automatic heating system.

The studied muscle was connected to the inductive force transducer (the natural resonant frequency 300 Hz) through the soleus tendon. The isometric force of contractions was recorded in a muscle stretched up to the passive tension of 40 mN. These conditions were determined in a series of pilot experiments, which revealed that MUs of the soleus muscle developed the highest twitch force when muscle was stretched up to 40 mN. To achieve the evoked activity of single MUs, L5 or L4 ventral roots were split into very fine bundles of axons that were placed on a silver wire electrode and electrically stimulated (0.1 ms rectangular pulses of amplitude up to 0.5 V). Motor unit action potentials (MUAPs) were recorded with two silver wire electrodes (not insulated, 150 μm in diameter) inserted throughout the middle point of the muscle, perpendicular to its long axis. The distance between the two electrodes was about 5 mm. The force output and electromyographic signals were recorded with 12-bit analog-to-digital converter, with the sampling rate of 1 kHz (for force records) and 10 kHz (for action potentials). The "all or none" character of evoked twitch contractions and action potentials were used as criteria for a single MU isolation.

All investigated MUs were stimulated according to the following protocol:

(1) Five stimuli at 1 Hz (five single twitches and action potentials were recorded and then averaged).
(2) Series of stimuli at 10, 20, 30, 40, 50, 60, 75, 100 and 150 Hz frequencies and 500 ms duration to determine the force–frequency relationship.
(3) Five stimuli at 1 Hz (five single twitches were recorded and then the averaged twitch was obtained).
(4) The fatigue test (tetanic contractions evoked by trains of 14 stimuli at 40 Hz frequency, repeated every second for 4 min) (Burke et al. 1973).

10-s time intervals were applied between consecutive steps of the protocol. Examples of the experimental recordings of MU contractions are given in Fig. 1.

The whole-muscle contractions were evoked by electrical stimulation of the sciatic nerve. However, the muscle connected to the force transducer was stretched up to the optimal passive tension of 200 and 50 mN for males and females, respectively—in order to develop the highest twitch force of the muscle.

The studied MUs were classified as fast-twitch according to presence of "sag" in a 40 Hz unfused tetanic contraction, whereas "non-sagging" units were classified as slow-twitch (Burke et al. 1973; Grottel and Celichowski 1990). Additionally, the division was confirmed by an alternative method of classification of MUs as fast and slow on a basis of a profile of 20 Hz tetanic contraction, described by the 20 Hz index—below 2.0 for fast MUs or above 2.0 for slow ones (Krutki et al. 2008). Finally, the fatigue index was calculated on a basis of the standard fatigue test. For slow units the fatigue index was a ratio of force generated after 2 min of the test to the initial force, whereas for fast units it was a ratio of force reached by a MU 2 min after its force potentiated to the maximum at the beginning of the test to this maximal initial force (Kernell et al. 1975). Only a few fast units were present within the whole sample of functionally isolated MUs, they were not found in every soleus muscle investigated, and all of them had the fatigue index in a range 0.5–1.0, so were classified as fast resistant to fatigue (FR) (Kernell et al. 1983; Grottel and Celichowski 1990).

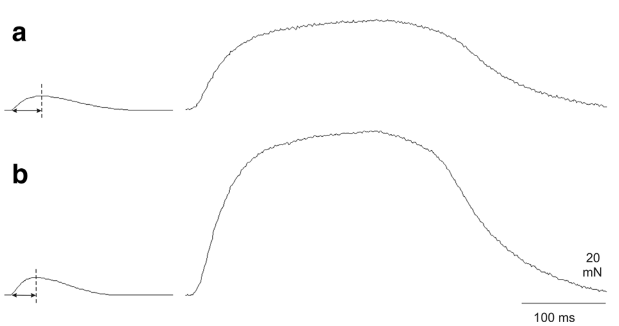

Fig. 1 Records of twitch contractions (*left*) and maximum tetani evoked at 150 Hz stimulation (*right*) for MUs of the female (**a**) and male soleus (**b**). Note similar twitch forces (female—9.8 mN, male—10.4 mN), contrasting to about twice lower tetanus force for the female MU (48.0 vs. 95.6 mN). The twitch-to-tetanus ratios for these units were 0.204 and 0.108 for female and male soleus MUs, respectively. The double arrows under the twitch records indicate the contraction times, 33.0 ms for the female and 28.5 ms for the male MU

For the twitch recordings, the contraction time (from the beginning of a twitch to the peak of the force record), the half-relaxation time (from the peak to the moment when force decreased to a half of the peak value) and the twitch force (the peak force amplitude) were calculated from the averaged twitch record (Celichowski and Bichler 2000). The maximum tetanus force was measured from the contraction evoked at 150 Hz, and the twitch-to-tetanus ratio was calculated.

Measurements of the force–time area per one pulse were performed for tetani evoked at all studied stimulation frequencies (10–150 Hz) at the plateau phase of the tetanic force, for the next to last contraction, i.e., for unfused tetani from the force minimum prior to the second last stimulus to the force minimum prior to the final stimulus or for fused tetani as the area between the two last stimuli (Celichowski et al. 2000).

The force–frequency curves were drawn using the peak force amplitudes obtained at different stimulation frequencies (from 1 to 150 Hz), expressed in percentage of the maximum force. The slope of the steepest part of each force–frequency curve was calculated as the relative increase (%) of the maximum tetanus force per 1 Hz increase in stimulation frequency around 50 % of the maximum force. Moreover, for each MU, the frequency of stimulation corresponding to 50 % of the maximum force was calculated (Kernell 1979).

For each MUAP recorded during the initial twitch, the following parameters were measured: the amplitude (from the minimum to the maximum of MUAP recording), the total duration (from the beginning to the end of electrical activity), the peak-to-peak time (between the minimum and maximum deflections of MUAP recording), and the number of turns, i.e., points of change in direction of the potential with at least 50 μV difference (Stålberg et al. 1986).

Statistical analysis was performed using the Statistica Stat Soft, 10.0 software. All data were expressed as mean values ± standard deviation (SD), and the minimum and maximum values were given. Normality of distribution of interval scale data was tested with the Shapiro–Wilk test. For statistical evaluation of significances of differences between the properties of male and female slow MUs and for comparisons of muscle masses, muscle length and twitch-to-tetanus ratio of the whole soleus muscle the Mann–Whitney *U* test was used. Comparisons of body masses and forces of the whole soleus muscle were made with the Student's *t* test. $P < 0.05$ value was the level for accepting statistical significance. Due to a low number of FR MUs, statistical comparison was performed for slow MUs only.

Results

The comparison of the overall body mass, mass of the soleus muscle and its length reflected commonly observed sex differences in mammals, as all these parameters were considerably higher for male rats in comparison to female animals (Fig. 2a–c). Although the body and muscle masses were higher for males, the relation of the muscle mass to the body mass was not statistically different between sexes (Fig. 2d), contrary to the medial gastrocnemius muscle, which in females was also considerably smaller, but constituted higher percentage of the body mass than in males (Celichowski and Drzymała 2006).

Substantial sex differences in the whole-muscle forces were found. The respective mean tetanus forces for the male and female soleus muscle were 1794 ± 96 and 924 ± 88 mN ($p < 0.01$). On the other hand, much smaller differences concerned twitch forces of the whole soleus muscle and the mean values amounted to 317 ± 42 and 239 ± 58 mN ($p < 0.05$), for male and female rats, respectively. As an effect, the twitch-to-tetanus ratio of whole soleus muscle were 0.18 ± 0.03 ($P < 0.05$) for male rats and 0.25 ± 0.05 ($P < 0.05$) for female rats.

Mechanical properties of 44 MUs and 49 MUs were measured in males and females, respectively. The vast majority of these MUs (41 in males and 47 in females) were classified as slow (S). Only in three MUs in males and two MUs in females was sag observed in tetanic contractions at 40 Hz (and additionally the 20 Hz tetanus index was below 2.0 in all these cases). Their fatigue indices were in a range 0.78–0.95, so they were classified as FR MUs (Table 1). It is worth noticing that only one FR MU per one rat was encountered during experiments, so for two

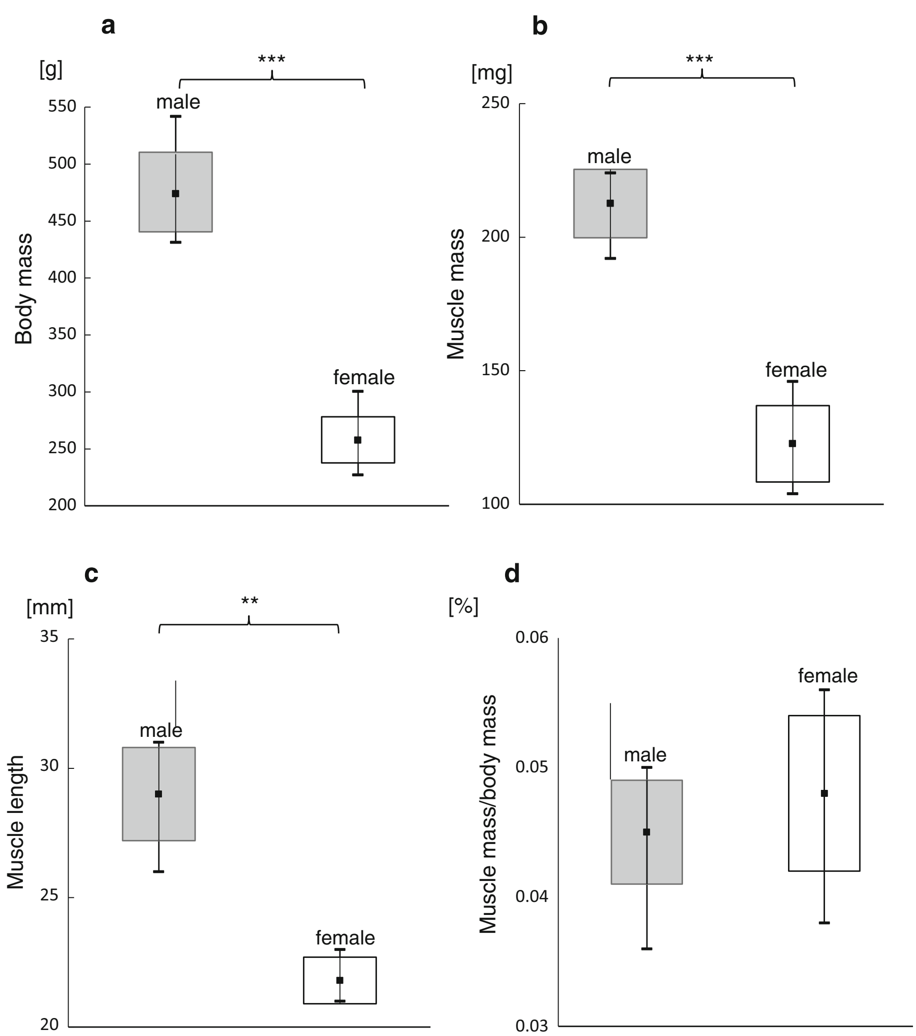

Fig. 2 Basic body and muscle morphometric properties. The mean values (*black points*), variability ranges (*bars*) and standard deviations (*rectangles*) of the rat body mass (**a**), the soleus muscle mass (**b**), the soleus muscle length (**c**) and the ratio of muscle to body mass (**d**) of male and female animals. ***—difference significant at $P < 0.001$; **—difference significant at $P < 0.01$ (the Student *t* test)

Table 1 The contractile properties of MUs in soleus

	CT (ms)	HRT (ms)	TwF (mN)	TetF (mN)	Tw/Tet	FatI
S						
Male	28.5 ± 6.4	44.5 ± 8.6	10.4 ± 3.3	95.6 ± 32.1	0.11 ± 0.03	0.98 ± 0.02
n = 41	19.0–48.0	27.0–65.0	4.9–19.8	44.2–238.0	0.05–0.21	0.92–1.00
S						
Female	33.2 ± 6.5	52.1 ± 9.86	9.9 ± 4.1	48.8 ± 16.9	0.19 ± 0.03	0.98 ± 0.03
n = 47	20.0–50.0	26.0–73.0	1.7–21.7	10.0–83.0	0.12–0.29	0.86–1.00
	***	***	N.S.	***	***	N.S.
FR						
Male	21.3 ± 4.0	25.0 ± 3.5	26.6 ± 4.0	106.7 ± 24.8	0.25 ± 0.03	0.85 ± 0.1
n = 3	19.0–26.0	23.0–29.0	22.9–31.0	80.0–129.0	0.23–0.29	0.78–0.90
FR						
Female	18.0 ± 2.8	23.5 ± 7.8	12.3 ± 2.6	53.3 ± 8.1	0.23 ± 0.08	0.91 ± 0.06
n = 2	16.0–20.0	18.0–29.0	10.5–14.2	47.6–59.0	0.17–0.29	0.86–0.95

The mean values, standard deviations and variability ranges of basic contractile properties of slow (S) and fast (FR) soleus MUs in male and female rats

CT the contraction time, *HRT* the half-relaxation time, *TwF* the twitch force, *TetF* the maximum tetanus force, *Tw/Tet* the twitch-to-tetanus ratio, *FatI* the fatigue index

***—difference significant at $P < 0.001$; N.S.—difference non-significant (the Mann–Whitney *U* test)

out of five male and in three out of five female rats no FR units were found.

For S MUs, the twitch time parameters revealed significant sex differences (contraction time and half relaxation time were shorter in males), but there was no difference with respect to the twitch force (Fig. 3; Table 1). On the other hand, the maximum tetanus force was substantially higher for male rats, what was reflected in differences of the twitch-to-tetanus ratio, which was significantly higher for females. No sex differences were observed in fatigability of soleus MUs.

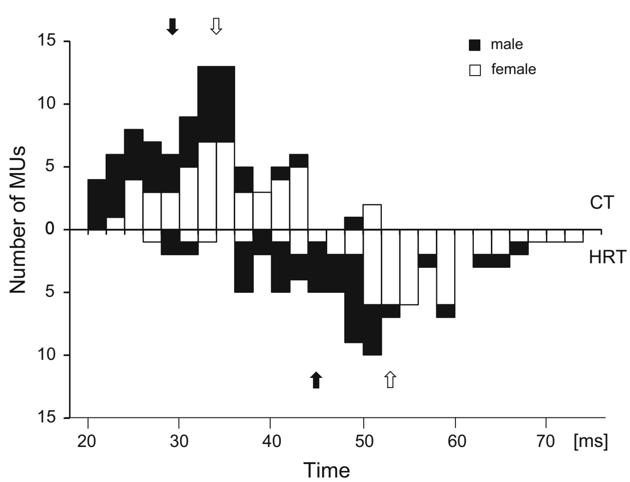

Fig. 3 The distribution of the contraction time (CT, *upper plot*) and the half-relaxation time (HRT, *lower plot*) for studied slow motor units of soleus muscles. Data for males on histograms are black bars, whereas for females—*white bars*. *Arrows* (males—*black*, females—*white*) indicate the mean CT or HRT values for each sex

Differences in the contraction time and twitch-to-tetanus ratio were well reflected in the force–frequency relationships of male and female MUs. The steep part of this curve (attributed to unfused tetanic contractions) was shifted rightwards in male rats with respect to the curve plotted for females, and the 50 % of the maximal force was achieved at a significantly higher mean frequency of stimulation in males (Fig. 4a; Table 2). There was also higher slope of the curve between 20 and 30 Hz for male soleus MUs, what indicated ability for a higher force increase in response to 1 Hz increase in the stimulation frequency (Table 2).

The maximum force–time area per one pulse was significantly higher for males, by about 27 % (Table 3). The tetanic contraction with the highest force–time area per one pulse was achieved at a higher frequency of stimulation for MUs in male rats, in relation to females (Table 3).

The analysis of the MUAPs revealed significant sex differences in amplitudes, which were about four times higher for MUs in males than in females (Fig. 5). The mean values of remaining MUAP parameters measured were not different.

Due to the small number of FR MUs in both male and female soleus, statistical comparison of contractile parameters of these units was not done. For each of the three cases of FR MUs in males twitch and tetanic forces were considerably higher in comparison to forces recorded for two fast MUs in females (Table 1), but mean values of the twitch time parameters or of the twitch-to-tetanus ratios were similar, and the force–frequency curves superimposed each other (Fig. 4b).

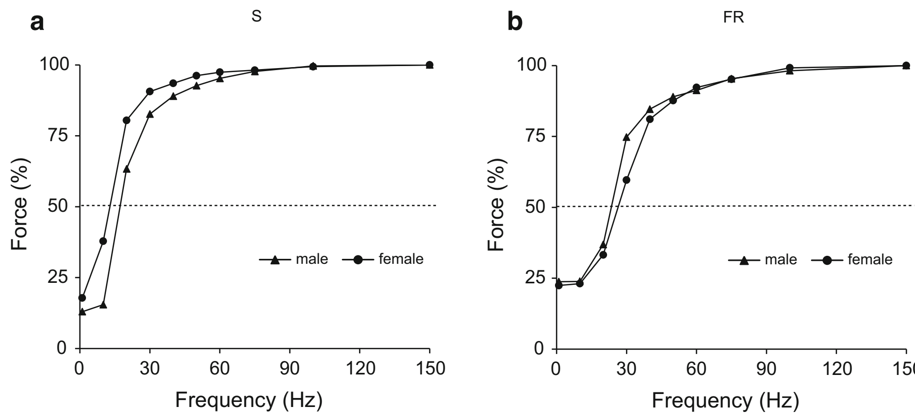

Fig. 4 The averaged force–frequency relationships for all studied motor units in soleus muscle. The contractile force is presented as a function of the stimulation frequency for the slow—S (**a**) and fast resistant to fatigue—FR (**b**) motor units of male and female soleus. The *dotted horizontal lines* indicate the 50 % of the maximum tetanus force. See Table 2

Table 2 Properties of the force–frequency relationships for slow MUs in soleus muscle

	Slope (%/ 1 Hz)	Frequency 50 % (Hz)
Male	4.86 ± 1.09	18.12 ± 4.9
n = 41	(1.60–6.30)	(12.0–43.0)
Female	4.36 ± 0.74	12.68 ± 2.6
n = 47	(2.90–6.00)*	(6.8–20.5)***

The mean values, standard deviations and variability ranges are presented for the slope of the curve between 20 and 30 Hz, and the frequency necessary to achieve 50 % of the maximum force (Frequency 50 %)

*** difference significant at $P < 0.001$; * difference significant at $P < 0.05$ (the Mann–Whitney *U* test)

Table 3 Parameters describing the tetanus with the highest force–time area (FTA) per one pulse and the stimulation frequency of this contraction for slow MUs of rat soleus

	FTA per pulse (ms·mN)	Frequency (Hz)
Male	1377.85 ± 332.63	22.19 ± 4.19
n = 41	(695–1967)	(20–30)
Female	971.31 ± 324.50	19.78 ± 2.54
n = 47	(310–1758)***	(10–30)*

The mean values, standard deviations and variability ranges are presented

*** difference significant at $P < 0.001$; * difference significant at $P < 0.05$ (the Mann–Whitney *U* test.)

Discussion

It was confirmed in this study that rat soleus muscle is composed mainly of S MUs (Chamberlain and Lewis 1989), but it is possible to find a small proportion of fast units as well (Leterme and Falempin 1996). However, no valid conclusion about sex differences could be drawn with respect to this subject, as identification of fast units was sparse for both sexes (five FR MUs collected from 10 muscles). Despite this, the proportion of fast MUs observed in our study (7 vs. 3 % of in males and females, respectively) was in line with the above mentioned higher content of MHC IIa isoform in males (Betto et al. 1986; Chamberlain and Lewis 1989; English and Schwartz 2002; Drzymała-Celichowska et al. 2012).

Substantial differences were found between the tetanic forces of MUs which were two times higher in males, whereas twitch forces were comparable, so the twitch-to-tetanus ratio was considerably higher in females. Kanda and Hashizume (1992) have studied innervation ratios in the rat medial gastrocnemius muscle and have concluded that the force of a MU contraction very strictly depends on a number of muscle fibers constituting the MU. Mierzejewska-Krzyżowska et al. (2011) have demonstrated that stronger male MUs in the rat gastrocnemius are composed of higher number of muscle fibers in comparison to females. It is likely that stronger MUs in the male soleus are also composed of higher number of muscle fibers in relation to females.

The previously mentioned sex-related differences in the twitch-to-tetanus ratios, approximately twice higher in females, were rather unexpected, and they probably indicate more efficient summation of individual twitches into tetanic contractions in soleus MUs of male rats due to differences in the geometry of the soleus MUs (spatial distribution of muscle fibers, length and pennation angle) in males and females. These differences may impact passive force transmission to the tendon (Zuurbier and Huijing 1992), and therefore influence effectiveness of summation of twitches into tetanic contractions. Indeed, studies in humans have indicated that males have bigger pennation angles than females (17.8 ± 6.1° and 15.1 ± 4.0° in the anterior soleus; 22.2 ± 7.1 and 17.6 ± 6.5 in the posterior soleus, for male and female subjects, respectively) and that

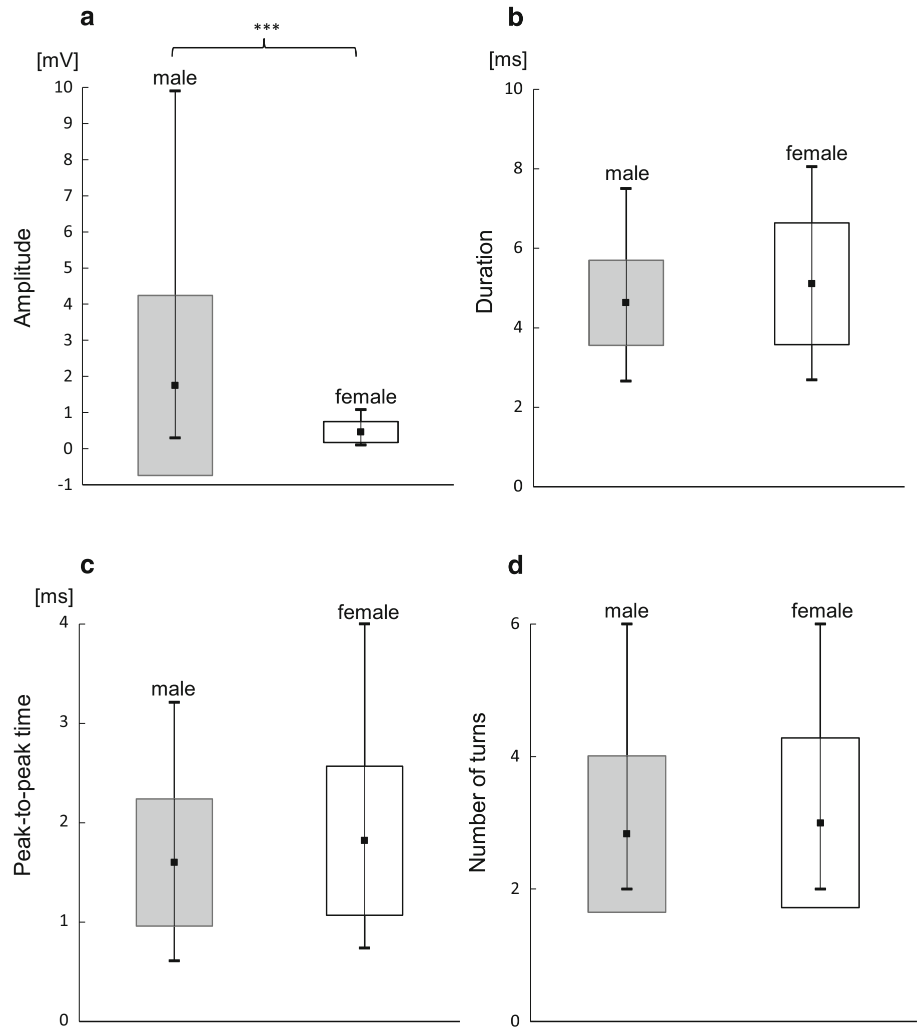

Fig. 5 The basic MUAP properties of male and female soleus. The *charts* present mean values (*black points*), standard deviations (*squares*) and variability ranges (*bars*) of MUAP parameters for slow soleus MUs: the amplitude (**a**), the duration (**b**), the peak-to-peak time (**c**) and the number of turns (**d**). ***—difference significant at $P < 0.001$ (the Mann–Whitney *U* test)

males have shorter muscle fibers than females (27.7 ± 9.7 and 30.0 ± 9.7 mm in the anterior soleus; 32.3 ± 7.7 and 37.2 ± 13.2 in the posterior soleus, in males and females, respectively) (Chow et al. 2000). However, for human soleus very low fiber length/muscle length ratio (0.063) has been reported (Lieber 2002), while the structure of the rat soleus muscle appeared to be different. In male rats, the unipennate soleus muscle has the pennation angle of 3.9 ± 2.4° and muscle fibers of 19.7 ± 1.9 mm in length were reported (Eng et al. 2008), but there have been no respective data for females so far.

Significant differences between males and females concerned also the twitch time parameters. The contraction time and half relaxation time are functionally important because they influence summation of successive twitches into tetanic contractions, and these processes are reflected in the course of the force–frequency relationship (Kernell et al. 1975, 1983; Mrówczyński et al. 2011). Therefore, the difference in twitch duration, which was shorter for male MUs, appears to be one of the major findings of this study. This difference may be due to several reasons. First, this may be an effect of differences in muscle architecture discussed above, and other morphometric parameters of muscles: e.g., the mean diameter of muscle fibers in the soleus muscle was by 8 % higher for males than for females (the mean of 59.50 ± 8.1 vs. 55.26 ± 3.04 µm, respectively; Mierzejewska-Krzyżowska et al. 2012). Second, this observation may be due to different intracellular rates of calcium release and uptake. However, this suggestion is highly speculative, as there is no relevant literature data available.

The discussed sex-related variations in the twitch time parameters in soleus MUs are opposite to those observed for S MUs in the rat medial gastrocnemius muscle. In that study the mean values of contraction and half-relaxation times have been significantly longer in males than in females (Celichowski and Drzymała 2006). However, it should be stressed that the time and force parameters of a twitch in S MUs of soleus muscle were definitely higher than the respective values measured in the medial gastrocnemius muscle. Reasons of the above discrepancies in contractile properties of S MUs between two muscles might be related to differences in architecture of these muscles and in metabolic enzyme activities between S MUs of two different muscles (Spamer and Pette 1977). The observed differences of MU properties between fast and slow muscles may explain some differences between upper and lower limb muscles. Namely, Senefeld et al. (2013) have found that the decrease of maximum voluntary isometric contraction of elbow muscles is similar for men and women but for the knee extensor is greater for men.

Shorter contraction time of MUs in male soleus suggests higher firing rates of their motoneurons. This suggestion is supported by a rightward position of the steep part of force–frequency curve for male MUs in relation to the curve for female MUs, which corresponds to higher stimulation frequencies of MUs in male rats. Thus, to achieve the same relative force a higher rate of motoneuronal firing is required in males than females. It is known that the mean firing rates of active motoneurons correspond to the frequencies indicated by the steep part of the force–frequency relationship (Kernell 1979; Hennig and Lømo 1987). However, so far there have been no studies indicating sex-related differences in motoneuronal firing properties.

The presented results reveal that the maximum force–time area per pulse is higher for male slow MUs than for female ones. In classical mechanics force–time area per pulse equals to an "impulse", and reflects an output of a MU contraction during short periods of force development, and maintenance of isometric force (Zajac and Young 1980; Celichowski et al. 2000). On the other hand, we have observed that the frequency of stimulation giving the maximal force–time area per one pulse was higher for MUs in male rats. Thus, motoneurons of the male soleus likely generate higher stimulation frequencies to obtain the same relative MU force as in female muscle, but the absolute force output of the male muscle is considerably greater. The analysis of MUAPs revealed strong sex-related differences which are in line with variations in MU forces. MUAP amplitudes were about four times higher in male rats. This is most probably related to previously discussed higher innervation ratios, higher muscle fiber diameters (Mierzejewska-Krzyżowska et al. 2012) and/or bigger muscle fiber density within MU territories in the male soleus, as the major factors determining MUAP amplitude (Kernell et al. 1983; Howard et al. 1988; Eijden and Turkawski 2002). On the other hand, there were no sex differences in MUAP time parameters (total duration and peak-to-peak times), which are strongly associated with length and diameter of muscle fibers (Eijden and Turkawski 2002; Dumitru et al. 1999; Gath and Stalberg 1975), probably because muscle fibers in rat soleus muscle are very short.

In conclusion, the rat soleus muscle is sexually dimorphic. Numerous contractile properties as well as electromyographic properties of its MUs are different for males and females. These results imply several variations in motor control processes and motoneuronal firing rate between male and female rats. Moreover, there is a small contribution of fast MUs in the soleus muscle, and their proportion may be higher for males.

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