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THE NUMBER OF MOTOR UNITS IN THE MEDIAL GASTROCNEMIUS MUSCLE OF MALE AND FEMALE RATS

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The number of motor units in the hindlimb locomotor muscle - the medial gastrocnemius - was estimated in male and female Wistar rats by comparison of the whole muscle tetanic force to the mean tetanic force of its motor units. Functional isolation of motor units was achieved by electrical stimulation of single axons from ventral roots of L4 - L5 spinal nerves, while the whole muscle force was measured during stimulation of the sciatic nerve. The medial gastrocnemius muscle is approximately 1.5 times bigger by mass, and is innervated by 10% more motoneurones in males compared to females. Estimation of the force that can be generated by the three types of motor unit in the studied muscle revealed that in both sexes fast fatigable units are responsible for over 60% of the total force output whereas the percentage of the force output contributed by slow units in females is approximately twice that in males. Concluding, the motor innervation of hindlimb skeletal muscles is sexually dimorphic and in the rat male medial gastrocnemius muscle contains about 10% more motor units than in females.

Key words: *skeletal muscle, gastrocnemius, motor unit, dimorphism, rat*

INTRODUCTION

Sexual differences in body size and morphology are widespread in the animal kingdom (1). However, there are limited data concerning the dimorphism of skeletal muscles. One sexually dimorphic small muscle - the levator ani - has been described by Tobin and Joubert (2). This muscle has sexual functions and cytochemical and ultrastructural techniques have revealed that the number of fibers and their cross-sectional area differ between sexes. Male muscles contain 10 times more fibers than female, and the average cross-section area of muscle fibers in the male is 7 times greater (2). The composition of non-sexual muscles in males and females of small mammals is also different (3, 4). The foot muscle - flexor digitorum brevis - shows significant differences in the number and diameter of muscle fibers between the sexes in rats, with higher values for these parameters in males (4). English *et al*. (5) studied the character of muscle fibers in the masseter muscle of male and female rabbits and found that the muscles of males contain a higher proportion of fast fibers. In our previous study (6) differences in the motor unit contractile properties of male and female rat medial gastrocnemius were examined. The mass of the medial gastrocnemius of male rats is approximately 1.5 times larger than in females. We found that the contraction times of male motor units in this muscle are significantly longer, whereas the forces are higher, although this difference was not significant. Moreover, the composition of motor units is different, with more fast fatigable and fewer slow units in male muscles. The twitch force of the three types of motor unit is higher in males although the difference is significant only for fast resistant to fatigue motor units. However, the ratio of the mean motor unit tetanus force to the muscle tetanus force is almost 2-fold smaller in male rats compared to females (6). These results indirectly suggest that the abundance of motor units is higher in male muscles. To resolve this issue, the number of motor units in male and female muscles needs to be estimated in a series of experiments performed under identical conditions for both sexes. This could be achieved by a variety of methods. The neuroanatomical method based on direct counting of motoneurons labeled with horseradish peroxidase injected into a muscle nerve has been applied in studies by Hashizume *et al*. (7) and Peyronnard *et al*. (8). However, the number of motoneurons may be overestimated by this technique due to the possible inclusion of fragments of the same motoneurons in serial sections. The electrophysiological method based on comparison of muscle force to the mean force produced by its motor units has been used in studies by Einsiedel and Luff (9) and Gardiner and Olha (10). This method appears to be the most suitable for the purpose of the present study, *i.e*. comparison of the number of motor units in the medial gastrocnemius in males and females. The confirmation of differences in the number of motor units in skeletal muscles between the sexes, together with previously described differences in motor unit contractile properties would point to separate strategies for motor control by the central nervous system.

MATERIAL AND METHODS

This study was performed using thirty-six 6-months old adult Wistar rats, under pentobarbital anesthesia (Morbital, initial dose of 60 mg/kg, *i.p*., supplemented during the experiments with doses of approximately 10 mg/kg as required). The depth of anesthesia was monitored by assessing withdrawal reflexes. The mean weight of male and female animals was 496.6 ± 60.0 g and 258.3 ± 21.0 g, respectively. The principles of laboratory animal care, as approved by the European Union and Polish Law on Animal Protection were followed. After the experiments, the animals were euthanized with an overdose of pentobarbital (180 mg/kg).

Two types of experiment were performed on the medial gastrocnemius muscle: the first involved the functional isolation and characterization of as many motor units as possible, while the second was recording the force of contraction of the whole muscle evoked by stimulation of the sciatic nerve.

The surgical procedures used have been described previously (11). Briefly, the medial gastrocnemius muscle was partly isolated from surrounding tissues. The innervation and blood supply to the examined muscle were left intact, whereas other muscles of the hindlimb were denervated by cutting the remaining branches of the sciatic nerve. Laminectomy was performed from the L2-S1 vertebra segments. Ventral and dorsal roots were cut proximally to the spinal cord. The animals were immobilized with special steel clamps on the tibia, sacral bone and the L1 vertebra. The isolated spinal cord, and 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 bath filled with the same oil. The temperature of the oil and the animal core were kept constant at 37 ± 1°C by an automatic heating system. The studied muscle was connected to an inductive force transducer *via* the Achilles tendon. The isometric force of evoked contractions was recorded in the muscle stretched to a passive tension of 100 mN. Under these conditions, the majority of motor units develop their highest twitch force (12). Muscle fiber action potentials were recorded with a silver-wire bipolar electrode inserted into the middle part of the muscle, perpendicular to the long axis. L5 and L4 ventral roots were split into very fine bundles of axons that were placed on the silver electrode and electrically stimulated (0.1 ms rectangular pulses with amplitude up to 0.5 V). "All-or-none" twitch contractions and muscle action potentials at increasing stimulation intensity were used as criteria for single motor unit isolation.

Contractions of the whole muscle were evoked by electrical stimulation of the sciatic nerve with 0.1 ms rectangular pulses (amplitude up to 1 V). The surgery of the hindlimb and procedure for immobilization during recordings were the same as for the motor unit isolation experiments. However, the muscle was stretched up to the optimal tension of 400 mN to record the highest whole muscle twitch force (13).

Data were stored on a computer using an AD converter (RTI Utilities, sampling rate of 1 kHz and 10 kHz for force and action potentials, respectively).

In all experiments the following stimulation protocol was applied: 1) 5 stimuli at frequency 1 Hz (5 single twitches were recorded and then the averaged twitch was calculated), 2) series of stimuli at 40 and 150 Hz of duration 500 ms (the unfused and fused tetani were recorded), 3) the fatigue test (tetani evoked by trains of 14 stimuli at 40 Hz frequency, repeated every second over 4 min) (14). Time intervals of 10 s separated the individual elements of the above protocol.

The isolated motor units exhibiting a "sag" in the 40 Hz unfused tetanus were classified as fast-twitch, whereas "non-sagging" units were classified as slow-twitch (S) (14, 15). The further division of fast motor units was based on variable fatigue resistance. The fatigue index (FI) was calculated as the ratio of the tetanic force reached by a motor unit two minutes after its force potentiated to the maximum at the start of the fatigue test, to this maximal initial force (16). Fast units with FI >0.5 were classified as fast resistant (FR), while those with FI <0.5 were fast fatigable (FF) (15, 17). For each motor unit, the contraction time (CT, from the beginning of a twitch to the peak of this force record), the half-relaxation time (HRT, from the peak to the moment when the force had decreased to half of the peak value) and the twitch force (TwF, the peak force amplitude) were calculated from the averaged twitch record (18). The maximum tetanus force (TetF) was measured at 150 Hz stimulation in both the motor unit characterization and whole muscle contractile properties experiments.

In total, 304 medial gastrocnemius motor units were investigated, 155 in male rats and 149 in females. The whole muscle properties were examined in 5 males and 6 females.

RESULTS

Table 1 presents mean values, standard deviations and variability ranges for the body mass, muscle mass and contractile properties of the medial gastrocnemius muscle in male and female rats. Both the muscle mass and the muscle force were significantly higher, and the twitch time parameters were longer in males.

The mean values, standard deviations and variability ranges for the contractile properties of isolated motor units are summarized in *Table 2.* The table presents data for each type of motor units and the total studied population, separately. These data are consistent with previously published results concerning the variability of the analyzed parameters of the three motor unit types in the two sexes (6).

The proportions of the three types of motor unit within the studied muscle were different in males and females. In males, the sample was composed of 46% of FF, 40% of FR and 14% of S motor units. In females, 40% of FF, 37% of FR, and 23% of S motor units were found (*Fig. 1A*). These proportions were statistically different in male and female muscles (the independence test χ_2, $p<0.05$), due to significantly different proportions of FF and S motor units (test for two factors of the structure, $p<0.05$, $p>0.05$, $p<0.01$ for FF, FR and S motor units, respectively).

The mean tetanus force for male and female motor units (*Table 2*) also differed significantly, with values of 144.3 mN and 100.8 mN, respectively. The mean whole muscle tetanus forces were 8260 mN and 5250 mN for male and female muscle, respectively. These data for motor units and the whole muscle allowed us to estimate the number of motor units in the medial gastrocnemius muscle of both sexes:

Males: 8260 mN / 144.3 mN ≈ 57 units
Females: 5250 mN / 100.8 mN ≈ 52 units

On the basis of the different proportions of the three motor unit types in the studied muscle, it was possible to estimate that the medial gastrocnemius muscle would be composed of approximately 26 FF, 23 FR and 8 S units in males,

Table 1. Mean values, ranges and standard deviations for body mass, muscle mass and basic contractile properties of the medial gastrocnemius muscle in male and female rats. CT - the contraction time; HRT - the half-relaxation time; TwF - the twitch force; TetF - the maximum tetanus force; ** - difference significant at P<0.01; * - difference significant at P<0.05; N.S. - difference non significant, P>0.05 (Mann-Whitney U-test).

	CT (ms)	HRT (ms)	TWF (N)	TETF (N)	Body mass [g]	Muscle mass [g]
♂ n=5	24.6±4.0 (21.0-29.0)	17.3±1.5 (16.0-19.0)	2.43±0.37 (2.00-2.70)	8.26±0.75 (7.50-9.00)	496.6±45.0 (450.0-540.0)	1.03±0.17 (0.83-1.15)
♀ n=6	23.0±2.0 (21.0-25.0)	13.0±1.0 (12.0-14.0)	1.65±0.13 (1.60-1.80)	5.25±0.27 (4.94-5.43)	258.3±24.8 (220.0-290.0)	0.75±0.21 (0.51-0.93)
	N.S.	*	*	*	**	**

Table 2. Mean values (± SD) and variability ranges of the basic contractile properties of motor units (MUs) in the male and female medial gastrocnemius muscle. FF - fast fatigable; FR - fast resistant; S - slow motor units. CT - the contraction time; HRT - the half-relaxation time; TwF - the twitch force; TetF - the maximum tetanus force; FI - the fatigue index; *** - difference significant at P<0.001; ** - difference significant at P<0.01; * - difference significant at P<0.05; N.S. - difference non significant, P>0.05 (Student's t-test).

MU type	CT (ms)	HRT (ms)	TwF (mN)	TetF (mN)	FI
FF♂ n=70	13.5±2.5 (9.0-20.0)	14.6±4.2 (8.0-26.0)	58.6±38.5 (4.0-157.0)	206.1±112.7 (15.8-452.0)	0.22±0.11 (0.01-0.47)
FR♂ n=63	15.1±2.4 (11.0-20.0)	18.9±6.2 (12.0-49.0)	23.9±18.12 (1.4-82.4)	109.0±59.1 (14.6-287.3)	0.77±0.11 (0.53-0.98)
S♂ n=22	24.2±4.8 (20.0-42.0)	39.1±5.7 (30.0-53.0)	8.1±3.7 (3.3-16.0)	48.1±11.5 (26.6-66.2)	0.97±0.03 (0.92-1.00)
FF+FR+S♂ n=155	15.7±4.6 (9.0-42.0)	19.7±9.6 (8.0-53.0)	37.3±34.6 (1.4±157.0)	144.3±103.9 (14.6-452)	0.55±0.32 (0.01-1.00)
FF♀ n=60	12.2±1.7 (8.0-16.0) ***	12.0±3.0 (8.4-22.4) ***	38.6±29.6 (2.0-135.2) ***	158.1±79.8 (9.1-350.0) **	0.24±0.12 (0.01-0.49) N.S.
FR♀ n=55	14.0±1.7 (11.2-20.0) **	16.5±4.4 (10.1-29.0) **	13.3±5.9 (1.90-27.05) ***	73.5±29.2 (13.3-171.0) ***	0.80±0.10 (0.55-0.99) *
S♀ n=34	22.4±2.7 (16.0-28.0) N.S.	35.7±5.4 (22.0-47.0) *	5.3±2.5 (1.7-13.8) **	43.0±13.7 (15.3-85.2) N.S.	0.97±0.03 (0.89-1.00) N.S.
FF+FR+S♀ n=149	15.2±4.5 (8.0-28.0) N.S.	19.1±10.1 (8.4-47.0) N.S.	21.7±23.8 (1.3-135.2) ***	100.8±72.8 (9.1-350.0) ***	0.61±0.33 (0.01-1.00) N.S.

whereas in females this muscle would contain 21 FF, 19 FR and 12 S motor units. Finally, the mean values of the motor unit forces presented in *Table 2*, permitted estimation of the degree to which the three types of motor units contribute to the total force generated by the muscle (*Fig. 1B*). The calculation was based on the number of motor units of the three types and the mean forces generated by each type. In males, the forces generated by the three types of unit within the muscle would be:

FF - 26 * 206.1 mN = 5358.8 mN (65% of the total force)
FR - 23 * 109.0 mN = 2507.0 mN (30% of the total force)
S - 8 * 48.1 mN = 381.8 mN (5% of the total force)

In females, forces generated by the three types of unit within the muscle would be:

FF - 20 * 158.1 mN = 3162.0 mN (63% of the total force)
FR - 19 * 73.5 mN = 1396.5 mN (27% of the total force)
S - 12 * 43.0 mN = 516.0 mN (10% of the total force)

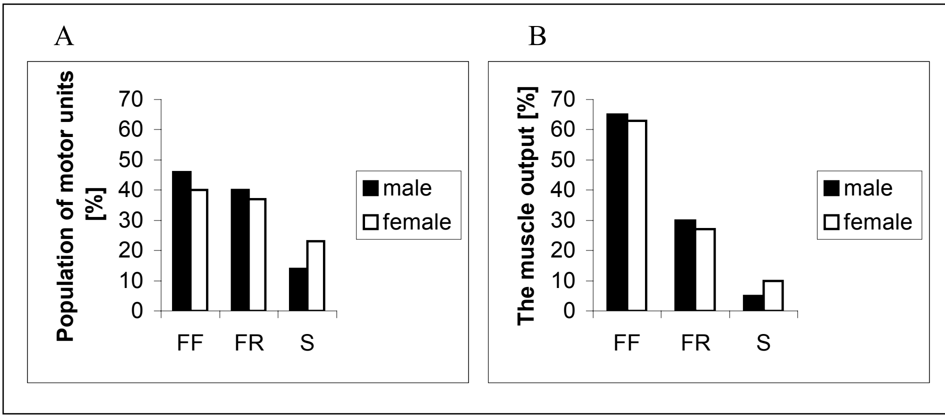

Fig. 1 A. Proportions of the three types of motor units (FF, FR and S) in male and female medial gastrocnemius muscles. B. Contribution of particular types of units to the whole muscle force output in the two sexes. FF - fast fatigable; FR - fast resistant; S - slow motor units.

The participation of particular types of motor units in the output of the medial gastrocnemius muscle for the two sexes is illustrated in *Fig. 1B*.

DISCUSSION

The main finding of the present study is that the male gastrocnemius muscle in rats is innervated by approximately 10% more motoneurones in males than in females. A similar sex-related difference might also be expected for other hindlimb skeletal muscles. The physiological significance of this observation should be considered in relation to the motor control processes. The central nervous systems of males and females have a different number of motor units that can be recruited into contraction. The higher number of units in male muscles suggests a more significant role for recruitment in the mechanism of force regulation. On the other hand, because of the lower number of motor units in female muscles it seems probable that changes in motoneuronal firing rate will be more important in force regulation. Furthermore, due to the shorter contraction times of female motor units found here and in a previous study (6) it might be expected that female motoneurones generate higher firing rates. Finally, the studied muscle of the two sexes contains different proportions of the three types of motor unit that are available for recruitment.

Analysis of the relative number of the three types of motor unit demonstrated that in both male and female rats the muscle is composed predominantly of FF units although the proportions are statistically different: 46% of the whole population in males compared with 40% in females. The proportions of S units are also different: 14 and 23%, for males and females, respectively. English and

Widmer (19) studied the proportions of muscle fibers in the masseter muscle of rabbits and they found that in males the muscle contains more fast fibers and fewer slow fibers than in females.

For all three types of motor units the twitch and tetanus forces were higher for males than females (*Table 2*). These differences are not due to the greater mass of male muscles but are likely to be related to the different innervation ratio values. Kanda and Hashizume (20) studied innervation ratios in the medial gastrocnemius muscle of the rat and they concluded that the force of motor unit contraction is strictly dependent on the number of muscle fibers constituting the motor unit. Therefore, it is expected that the stronger male motor units are composed of a higher number of muscle fibers. On the other hand the differences in the force parameters are likely to be due to the larger diameters of male muscle fibers (5), because the force produced by a motor unit is also proportional to the total cross-sectional area of its muscle fibers (21). Larger diameters have been reported for fast muscle fibers of male muscles (4). These indirect observations suggest higher innervation ratios in male muscles compared to those of females, although these differences are probably smaller than the differences in the force of motor units.

The body and muscle masses of male rats are evidently greater than those of female animals. In the present study the mass of the male gastrocnemius muscle was larger than the female. As discussed above, the innervation ratios of motor units are higher in the male gastrocnemius muscle compared to the female: the male muscles contain more motor units and therefore the number of muscle fibers in male muscles should also be higher.

In conclusion, we have found that the motor innervation of hindlimb skeletal muscles is sexually dimorphic in the rat. In males animals the medial gastrocnemius muscle is innervated by 10% more motoneurones than in females.

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