Decomposition of motor unit tetanic contractions of rat soleus muscle: Differences between males and females

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ABSTRACT

Mathematical decomposition of tetanic contractions of slow motor units (MUs) of the rat heterogeneous medial gastrocnemius muscle revealed immense variability of twitch-shape responses to successive pulses, contrary to results obtained for fast MUs. The aim of this study in rat soleus muscle, almost exclusively composed of slow MUs, was to reveal whether such variability of twitch-shape decomposed components was a common property of slow MUs in the two studied muscles, and whether ranges of the force amplitude or time parameters of these decomposed twitches showed sex differences. Unfused tetanic contractions evoked by stimulation at variable interpulse intervals were analyzed for 10 MUs of males and 10 MUs of females. Significantly higher variability between parameters of the decomposed responses was observed for male soleus MUs, as the mean ratio of forces of the strongest decomposed twitch and the first (the weakest) decomposed twitch amounted to 3.8 for males and 2.8 for females. The ratios of the contraction times of the longest decomposed to the first twitch were much more similar between male and female MUs, 2.6 and 2.9, respectively. Consequently, the mean ratio of the force–time area for the strongest decomposed to the first twitch was much bigger in male than female MUs (7.35 vs. 5.07, respectively). Our observations indicate that high variability of responses to successive stimuli is a general property of slow MUs in different rat muscles, but the mechanisms of summation of individual twitches into tetanic contractions of MUs are not identical for male and female rats.

1. Introduction

During voluntary activity, motoneurons generate trains of pulses at variable intervals, which substantially influence the development and profiles of tetanic forces of motor units (MUs). The discharge patterns of MUs have been most frequently studied by the decomposition of electromyograms into trains of MU action potentials (Boe et al., 2005; Moritz et al., 2005). On the other hand, the mathematical decomposition of unfused tetanic contractions of MUs into twitch-shape responses to successive pulses can provide information concerning contractile effects induced by individual action potentials. This method, applied to tetani evoked by stimulation of the medial gastrocnemius MUs at variable interpulse intervals (IPIs), resembling motoneuronal firing patterns observed during voluntary contractions, has revealed substantial variability of individual responses to successive pulses (Raikova et al., 2008). The decomposed twitches of fast MUs have displayed up to 2.2 times higher forces, and up to 2.5 times longer contraction times than the single twitch. For slow MUs, the forces of decomposed twitches have reached even 7 times higher values, while the twitch contraction and relaxation parameters have been up to 3.5 times longer than those of the single twitch (Celichowski et al., 2014).

The above observations have only been made for MUs of one muscle so far, and it is unknown whether similar variability in amplitudes and duration of responses summating into a tetanic contraction could be obtained for MUs of another muscle, anatomically and physiologically different. Such an example is the soleus, a homogeneous, slow-twitch muscle, composed predominantly of slow MUs (Kugelberg, 1973; Burke et al., 1974). Although the soleus and medial gastrocnemius muscles contain slow MUs, it has been shown that basic contractile properties of slow MUs of these two muscles are considerably different, as they are active in different motor tasks (Burke and Tsairis, 1973; Burke et al., 1974; Kanda and Hashizume, 1992; Drzymała-Celichowska and Krutki, 2015). Moreover, previous studies of rat soleus MUs

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have revealed that this muscle is also sexually dimorphic, and properties of its MUs are different for males and females (Drzymała-Celichowska and Krutki, 2015). MUs of the male soleus have shorter contraction times and generate higher tetanic forces, whereas the twitch forces are similar for animals of both sexes; consequently, the twitch-to-tetanus force ratios are considerably (about twice) higher for females.

It is expected that results of a decomposition of unfused tetani of the slow soleus MUs will not be identical to those previously observed for the medial gastrocnemius muscle. Moreover, significant sex differences will be found, in line with previous observations of sexual dimorphism in MU properties. The study is important for understanding processes of development of MU tetanic forces, and the specific role of slow MUs in the activity of skeletal muscles. Therefore, the principal purpose of this study was to mathematically decompose several unfused tetanic contractions of the soleus MUs, evoked by trains of stimuli at variable IPIs. We aimed at analyzing the contraction and relaxation times and the force and force–time area of all decomposed twitch-like responses to successive stimuli, separately for MUs of male and female soleus muscles, which had not been done so far. We compare and discuss the results in relation to muscle structure and function, in comparison with previous observations concerning slow MUs of the medial gastrocnemius and especially with respect to sex differences.

2. Materials and methods

2.1. Electrophysiological experiments, MU isolation, and recordings

The experiments were performed on adult Wistar rats (three males and three females). The body weight was 470–520 g for male and 260–300 g for female animals. Rats were anesthetized with pentobarbital sodium (Morbital, Biowet Puławy, 60 mg kg^{-1}, i.p., supplemented after 2 h with additional doses of 10 mg kg^{-1} h^{-1}), and the depth of anesthesia was controlled by monitoring pinna and limb withdrawal reflexes. After the experiments, the animals were killed by a lethal dose of the anesthetic (180 mg kg^{-1} i.p.). All experimental procedures were approved by the Local Ethics Committee and followed the European Union guidelines of animal care as well as principles of the Polish Law on The Protection of Animals.

Experimental procedures were identical for male and female animals. During surgery, 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 muscles were denervated by cutting remaining branches of the sciatic nerve. The laminectomy was performed over L2–S1 segments. Dorsal and ventral roots 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 operated hind limb, isolated spinal cord, and ventral and dorsal roots of spinal nerves were covered with warm paraffin oil. The oil and animal core temperature were kept at a constant level (37 ± 1 °C) by an automatic heating system.

The functional isolation of a single MU was achieved by splitting the L5 ventral roots into thin filaments, which were electrically stimulated with rectangular electrical pulses (duration of 0.1 ms, amplitude of up to 0.5 V), generated by the dual-channel square pulse stimulator (Grass Instrument Company, model S88). The isolation of an MU was confirmed when the action potential in a muscle and the twitch force were of the "all-or-none" type and did not change in shape and size with an increase in the stimulus strength. The MU action potentials were recorded with two non-insulated silver wire electrodes inserted into the muscle belly, perpendicularly to its long axis. The force was measured under isometric conditions by a custom made force transducer (deflection sensitivity of 100 µm per 100 mN). The muscle was stretched and kept at a passive force of 40 mN, at which the majority of its MUs had maximum twitch amplitudes.

The following experimental protocol was performed:

1. 5 single twitches were evoked (5 stimuli at 1 Hz) and the averaged twitch was estimated.
2. An unfused tetanus was evoked by a 500 ms train of stimuli at 40 Hz.
3. The maximum tetanus was evoked by a 200 ms train of stimuli at 150 Hz.
4. Tetanic contractions were evoked by trains of 41 electrical pulses at three frequencies, first at constant and then at random IPIs (prepared with the random number generator in MATLAB). This means that for each of the three applied

stimulation frequencies, two tetanic contractions were recorded. The following IPIs were applied: 70, 80, and 100 ms (i.e., the mean frequencies were 14.2, 12.5, and 10 Hz, respectively). For each of the random stimulation patterns, IPIs had a normal distribution within a variability range of 50–150% of the mean IPI, i.e., the respective ranges of IPIs were 35–105 ms, 40–120 ms and 50–150 ms.

5. After completing the above procedure, a standard fatigue test was performed: stimulation with trains of 14 pulses at 40 Hz, repeated each second within 3 min (Burke and Tsairis, 1973).

Ten-second intervals were applied between all successive steps of the above protocol and between all tetanic contractions evoked within point 4 of the protocol.

For each studied MU, the following parameters were calculated for its single twitch recordings: the contraction time (from the beginning of twitch to the force peak); the half-relaxation time (from the force peak to the moment when the force decreased to half of the peak value); and the peak twitch force. For the 150 Hz tetanus recordings, the maximum MU force was determined. For each unfused tetanus recorded at constant IPIs, the fusion index was calculated, as a ratio of the minimum force when the response to the last stimulus began to the peak force of the last response (Bakels and Kernell, 1995; Celichowski and Grottel, 1995); the relative force level of such a tetanus was determined as a percentage of the maximum tetanus force.

Among 67 MUs recorded during the experiments, 10 units of male and 10 of female soleus muscle were chosen for the mathematical decomposition. The selection criteria were a high signal-to-noise ratio and a lack of artifacts in force recordings (sporadically observed, due to respiratory movements or artifacts from the non denervated tail and hip muscles). The contractile properties of the selected MUs were within ranges of contraction force and time parameters obtained earlier for a large population of slow MUs in the soleus muscle of males and females. Moreover, for reliable comparison of results of decomposition, it was important to obtain similar relative force levels and similar fusion degrees of tetanic contractions for all studied MUs. Therefore, among four tetani recorded for each MU at different frequencies at constant IPIs, we chose those with force levels ranging from 46% to 70% of the maximum force, and with fusion indices in the range 0.78–0.94. Subsequently, the tetanus evoked at the respective mean frequency but in a random stimulation pattern was taken for the decomposition.

2.2. Decomposition of the tetanic contractions

The mathematical method for decomposition of an unfused MU force curve into successive twitch-shape contractions, which are mechanical responses to a train of electrical pulses, was described in detail previously (Raikova et al., 2007). To model the single twitch as well as the decomposed twitch-shape responses, a six-parameter analytical function was applied. These six parameters were: the lead time (T_{lead}); the half-contraction time (T_{hc}); the contraction time (T_c); the half-relaxation time (T_{hr}); the duration of the twitch (T_{tw}); and the force amplitude (F_{max}) – see the equations in Raikova et al. (2008). In this study, the algorithm and the computer program were ameliorated as described below:

1. From the five individual twitches recorded during the first step of the experiments, the most noiseless was chosen and the six parameters for this MU twitch were calculated so that the error between the experimental force and the model was minimal. This automatic process was followed by a visual inspection and manual adjustment of the parameters if necessary.
2. This model was compared with the visible part of the first contraction into the tetanus and, if necessary, some of the parameters were manually adjusted (Fig. 1a) to model the curve between the first and second pulses as precisely as possible.

3. The model of the first contraction was mathematically subtracted from the tetanic curve, and the contraction between the second and third pulses, i.e., the twitch response to the second pulse, was visible (green line in Fig. 1b). The basic twitch parameters for this second contraction (the amplitude, half-contraction time, and contraction time) were automatically calculated. To obtain the remaining three parameters, their values from the first modeled contraction were assigned initially and then were manually corrected to match the visible part between the second and third pulses, and to adjust the second modeled contraction (blue line in Fig. 1b). The force curve, obtained by subtraction of the first and the second models from the recorded curve (the red line in Fig. 1b), was visible. Then, the twitch parameters could be modified for the next, third contraction (i.e., the red line after the third pulse). This process was repeated till the last, 41st pulse (Fig. 1c).

2.3. Statistical analysis

All data are expressed as means ± standard deviations (SDs), and the minimum and maximum values are given. The normality of distribution of interval scale data