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Effect of aging on properties of motor unit action potentials in the rat medial gastrocnemius muscle

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ABSTRACT

The purpose of this study was to investigate whether age-related changes in motor unit (MU) contractile properties are reflected in parameters of motor unit action potentials (MUAPs). MUs of the medial gastrocnemius muscle were functionally isolated in anaesthetized Wistar rats. A control group of young animals (5–10 mo) was compared to two groups of old rats (24–25 mo and 28–30 mo). The basic contractile properties of MUs as well as the amplitude, total duration, peak-to-peak time, and number of turns within MUAPs were measured. Effects of aging were mainly observed for fast fatigable MUs (a prolongation of MUAPs and increased number of turns). The MUAP amplitude did not change significantly with aging in either MU type, but it correlated to the twitch or tetanic forces, which tended to increase with age, especially for slow MUs. We concluded that the prolongation of MUAPs and the greater incidence of signal turns was probably a result of a decrease in muscle fiber conduction velocity and/or an increase in their dispersion, and enlargement of MU territories – presumably caused by axonal sprouting of surviving motoneurons. The latter might also be responsible for the observed age-related tendency for a increase in MUAP amplitudes in slow MUs.

1. Introduction

Age-related changes in morphology and functional properties of muscle fibers, motor units (MUs) or whole muscles have been widely described in human subjects and numerous animal species. It has frequently been reported that denervated muscle fibers, due to motoneuronal death, are reinnervated by the terminal axonal collaterals of survived motoneurons, and transformation from fast to slow motor units usually takes place (Brown et al., 1981; Campbell et al., 1973). Moreover, considerable changes in contractile properties of MUs have been observed in old animals, e.g., a prolonged twitch contraction time, an increase in the maximum force of slow MUs and its decrease in fast MUs (Kadhiresan et al., 1996; Kanda and Hashizume, 1989; Larsson and Ansved, 1995; Sugiura and Kanda, 2004). In our previous paper (Łochyński et al., 2008) we have demonstrated that mechanical properties of MUs and mechanisms of force regulation are proceed with diverse intensity in different MU types. In fast fatigable (FF) MUs the twitch and tetanus forces transiently increase, in slow (S) MUs they increase almost twofold in advanced senescence, and the mean force of fast resistant to fatigue (FR) units does not change significantly across the life span.

Apart from force parameters, electromyographic signals from muscle units provide important information on muscle fiber function – not only from the physiological, but also from clinical perspectives. Recordings of motor unit action potentials (MUAPs) are especially important since they reflect electrical activity of single MUs – the principal neuromuscular components responsible for force production during muscle voluntary activity. However, due to technical difficulties in isolating single MUs, and invasive character of such experiments, the majority of studies on MU contractile properties and MUAP parameters have been performed on animals (for references see Krutki et al., 2008), and only exceptionally in humans – for example in small muscles of the hand, in which single MU force could be voluntary controlled and MUAPs detected (Bertram et al., 1995; Thomas et al., 2006). One should also notice that several studies on age-related changes in MUAP parameters in man have brought uneven results. A considerable increase in the mean MUAP amplitude, a significant prolongation of its duration, and an increase in the number of turns in old subjects have been commonly noted, e.g. for the brachial biceps, brachial triceps and anterior tibial muscles (Hayward, 1977; Howard et al., 1988; McGill et al., 1991). Surprisingly, the reverse observations (a decrease in the number of turns in MUAPs) in the same muscles have also been made by McGill et al. (1991).

The authors of the above cited papers have not been able to relate MUAP parameters to force properties of MUs since these units

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have not been type-indentified with respect to differences in contractile parameters (e.g., contraction time, force and fatigability). Furthermore, the majority of studies on age-related changes in MU properties are usually limited to two age groups, i.e., the young and the old. We considered the mentioned gaps in current knowledge and conclusions of our recent paper on aging in rat MUs (Łochyński et al., 2008) to design this research study. The goal of the present paper was to investigate the changes in MUAP parameters in three groups of animals: the control group of young adult Wistar rats (5–10 mo), and two groups of old rats (24–25 mo and 28–30 mo). The acute experiments were performed on functionally isolated and type-indentified MUs in the heterogeneous medial gastrocnemius muscle. We measured and compared basic MUAP parameters, i.e., the amplitude, the total duration, the peak-to-peak time and the number of turns. Results were referred to the time and force parameters of contractions simultaneously recorded from MUs, and the discussion was directed towards possible morphological and physiological mechanisms underlying differences between young and old subjects.

2. Methods

The study was performed on 19 male Wistar rats. The control group of young adult rats consisted of 5–10 mo animals ($n = 9$, body mass 380–580 g), while aged rats were divided into two experimental groups: 24–25 mo ($n = 6$, body mass 480–600 g) and 28–30 mo ($n = 4$, body mass 460–630 g). First signs of skeletal tissue degeneration are commonly observed at the age of 20 months, the half-mortality age of Wistar rats is 24 months, and the length of the maximal life span is 36 months (Larsson and Ansved, 1995).

The animals were anaesthetized with sodium pentobarbital (60 mg/kg, i.p., supplemented with doses of 10 mg/kg every 1 h). The depth of anaesthesia was controlled by pinna and limb withdrawal reflexes, and by monitoring the respiratory rate (90–120/min), and the heart rate (240–300/min). After the experiments animals were killed with an overdose of sodium pentobarbital. All procedures followed the Polish Law on Animal Care and European Union guidelines, and were accepted by the Local Ethics Committee.

During surgery, the medial head of the gastrocnemius muscle was partly isolated from the surrounding tissues, while the nerve branches as well as the blood vessels to the muscle were left intact. All other muscles innervated by the sciatic nerve were denervated. The laminectomy was performed over L2-S1 segments of the spinal cord, and dorsal as well as ventral roots were cut at the L4–L5 level. The whole exposed area was covered with a warm paraffin oil in a pool formed by skin around the laminectomy. The Achilles tendon was cut, attached to the isometric force transducer (sensitivity of 100 μm per 100 mN) by a low-elastic surgical thread, and stretched up to the passive force of 100 mN which ensured the highest contractile force of the majority of contracting MUs (Celichowski and Grottel, 1992). The studied muscle was immersed in a special pool filled with the paraffin oil, kept at 37 ± 1 °C by an automatic heating system.

The MUs were functionally isolated by dividing ventral roots of the spinal nerves (L4 or L5) into very thin filaments which were stimulated by 0.1 ms rectangular electrical pulses of variable amplitude (up to 0.5 V), generated by a dual channel square pulse stimulator (Grass Instrument Company, S88). The "all-or-none" appearance of the action potential and the twitch force profile confirmed evoked activity of a single MU. The MUAPs were recorded with a pair of silver, not insulated wire electrodes (150 μm in diameter, 6 mm distance between electrodes) inserted throughout the middle part of the studied muscle, perpendicular to its long axis. The force output and the EMG signals were monitored on the oscilloscope screen and recorded with a 12-bit analog-to-digital converter, with the sampling rate of 1 kHz and 10 kHz for force records and action potentials, respectively. The EMG signals were amplified by a low-noise multi-channel preamplifier (World Precision Instruments, ISO-DAM8-A, with a high-pass filter at 0.1 Hz and a low-pass filter at 3 kHz).

The following stimulation protocol was applied: (1) five single pulses at 1 Hz (twitch forces and MUAPs were averaged); (2) one 500 ms train of pulses at 40 Hz (an unfused tetanus was evoked); (3) one 300 ms train of pulses at 150 Hz (the maximal tetanus was evoked); (4) 14 pulses at 40 Hz repeated every second throughout 3 min (the fatigue test, Burke et al., 1973).

The studied MUs were divided into three types: S, FR, and FF. A sag in unfused 40 Hz tetani was observed in fast, but absent in slow MUs (Grottel and Celichowski, 1990). The fatigue index (FatI, measured as a ratio of the force generated 2 min after the highest force developed at the beginning of the fatigue test to this highest force) below 0.5 enabled us to classify fast MUs as FF, whereas over 0.5 – as FR (Kernell et al., 1983; Gardiner and Olha, 1987).

For each MU, the peak twitch force (TwF), the maximum tetanus force (TetF), the twitch contraction time (CT) and the half-relaxation time (HRT) were measured. For each MUAP recorded during the initial twitch, the following parameters were measured (Fig. 1): the amplitude (from the minimum to the maximum of a MUAP recording), the total duration (time from the beginning to the end of electrical activity), the time peak-to-peak (between the minimum and the maximum deflections of a MUAP recording), and the number of turns, i.e., points of change in direction of the potential with at least 50 μV difference (Stålberg and Ekstedt, 1973; Krutki et al., 2008). For analysis of MUAPs, only MUs with amplitudes of action potentials exceeding 0.01 mV were used to minimize effects of noise and errors from calculations of MUAP parameters.

The ANOVA test (when normal distribution of data was confirmed by Shapiro test) or Kruskal–Wallis ANOVA test (in remaining cases) were used for statistical comparisons of measured parameters between three age groups and properties of the three

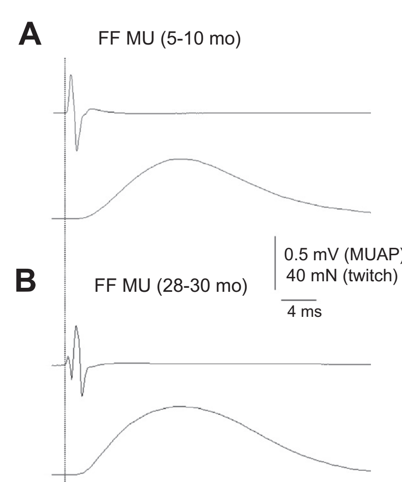

Fig. 1. The typical motor unit action potentials (upper traces) and corresponding twitch contractions (lower traces) recorded from two FF MUs of the young (A, the 5–10 mo group) and old rat (B, the 28–30 mo group). The vertical dotted line indicates onset of MUAPs. The MUAPs in A and B have similar amplitudes, but the longer duration, the longer peak-to-peak time, and higher number of turns (4) can be observed for the old rat (B). Note also higher twitch force and slightly longer twitch contraction time in an FF MU of the old rat in B. Scale bars are valid for A and B.