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Changes of motor unit contractile output during repeated activity

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Abstract. The aim of the study was to evaluate changes in the motor unit output and to determine changes in the optimal stimulation frequency (i.e., giving the maximal output per one pulse) during prolonged contractile activity when, successively, potentiation of force and fatigue developed. The influence of these phenomena was studied on three types of motor units: fast fatigable (FF), fast resistant (FR) and slow (S) in the rat medial gastrocnemius muscle. The motor units were isolated by a method of splitting of L5 ventral root into very thin bundles of axons which were electrically stimulated 10 times with repeated series of 10 trains of stimuli at duration of 500 ms and progressively increasing (1–150 Hz) frequency. The initial (the first series of stimulating trains), potentiated (the second series), as well as fatigued (the tenths series) force recordings were compared. The motor unit output was expressed as the area under the force-time record in response to one stimulus measured at a plateau phase of the tetanic force. The stimulation frequency when the force-time area per one pulse was maximal was accepted as the optimal frequency. In fast motor units, the maximal contractile output increased with potentiation and was reduced with fatigue, and the optimal frequency decreased and increased, respectively. Nevertheless, the fusion degrees of the optimal tetanic contractions were similar in initial state, potentiation and fatigue independently of the changes in force. The applied stimulation protocol had almost no influence on the mechanical activity of slow motor units. The study highlights the physiological importance of force potentiation induced by preceding contractile activity for the economy of motor performance. The observed changes of the optimal stimulation frequency are consistent with the known changes in the motor unit firing rates during voluntary activity when the two phenomena develop.

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INTRODUCTION

The area under the force-time curve is one of important, although rarely studied contractile parameter. This parameter termed in the classical mechanics as an impulse, has been used as a measure of output of whole muscle and motor units' contractions during short periods of development and maintenance of isometric force (Burke et al. 1970, 1976, Celichowski et al. 1998, 2000, Zajac and Young 1980). There is some evidence that this area changes linearly with energy utilized by contracting skeletal muscle fibers during brief periods of isometric activity (De Haan et al. 1986, Jöbsis and Duffield 1967).

The tetanic force of muscle fibers constituting motor unit is modulated by the changes of discharge frequency of motoneurone, i.e., rate coding (Burke et al. 1970, De Luca and Erim 1994). This relation has been studied in a number of experiments as an increase of the motor unit force, evoked by the increase of stimulation rate (Botterman et al. 1986, Hennig and Lømo 1987, Kernell et al. 1983). Studies of the tetani evoked at variable stimulation frequency revealed that force-time area per one pulse within a train of stimuli changes in relation to the stimulation frequency (Celichowski et al. 2000). It has been found, that for three types of motor units, the optimal frequency of stimulation, i.e., when the maximal area per one pulse is obtained, corresponds to subfused tetani with a force of about 75% of the maximum and the fusion index approximately 0.90. Since energy cost of twitch is independent from the contraction frequency, the optimal motor unit tetanic contraction is developed when the maximal area per one contraction is reached (Foley and Meyer 1993, Loiselle and Walmsley 1982, Zajac and Young 1980).

The ability of motor units to maintain the force during prolonged activity depends on a previous activity and is influenced by two main processes: force potentiation and fatigue. These two phenomena modulate not only force but also time parameters, e.g., the contraction and the relaxation times (Celichowski et al. 2006, Gordon et al. 1990, Jami et al. 1983, Vandenboom and Houston 1996, Vandenboom et al. 1995). It can be expected that changes of all these twitch properties (the force, the contraction and the relaxation times) induce changes of the force-time area of twitch as well as the area per one pulse in unfused tetani. However, these relationships have not been studied until now. Therefore, the present study aimed at the following facets: (1) to track the changes of the motor units' contractile output, by means of changes in the force-time area per pulse within 10 times repeated series of tetani at progressively increasing stimulation frequency; (2) to find the optimal stimulation frequency, which gives the maximal output per single contraction in potentiated and fatigued motor units. The predicted results will help to understand the reasons of changes of the contractile output and the economy of three motor unit types during their contractile activity when the two studied phenomena develop. It is expected that potentiation will significantly increase the motor unit output measured as an area under the force recording, and that these changes will be strictly related to reduction of the optimal motoneuronal firing rate. On the other hand, it is hypothesized that fatigue will considerably reduce the effectiveness of motor unit contractions and will be associated with a marked increase of the firing rate necessary to evoke the optimal contraction.

METHODS

Experiments were performed on 7 Wistar female rats (mean body mass 282 ± 94 g). Throughout all procedures, the animals were kept under pentobarbital sodium anesthesia (60 mg/kg i.p., supplemented with additional doses of approximately 10 mg/kg every one hour). The observation of pinna and withdrawal reflexes served as a control of the depth of anesthesia. After the experiments, animals were killed with an overdose of pentobarbital (180 mg/kg, lethal dose). Experiments were performed in accordance with the Polish Law on Animal Experiments and approved by the Local Bioethical Committee.

The employed methodology has been described in detail elsewhere (Celichowski and Grottel 1992, Grottel and Celichowski 1999). In brief, the medial gastrocnemius muscle was exposed by cutting the skin and fascia at the back side of the left hind limb, and isolated from the surrounding muscles. All branches of the sciatic nerve leading to the limb were cut, except the one to the studied muscle. The vessels ensuring the studied muscle blood supply were preserved. Than, after cutting the skin on the back, the laminectomy was performed over L2-S1 vertebra segments. Ventral and dorsal roots were cut near their entrance to the spinal cord. At the zone of laminectomy, a small pool was formed from the skin and filled with paraffin oil, which covered isolated spinal cord and cut spinal nerve roots. The animals were placed on a warm aluminum plate and immobilized with steel clamps on the L1 and S2 spinal processes and the tibia. The studied limb was immersed inside a pool of paraffin oil. The oil temperature was automatically kept at a constant level of 37 ± 1°C. The Achilles tendon was connected to a force transducer (FT-510). The studied muscle was stretched with a passive force up to 100 mN, which ensured development of the highest contractile force for the majority of its motor units (Celichowski and Grottel 1992).

The L5 ventral root was split into thin filaments. Rectangular electrical pulses of 0.1 ms duration and variable amplitudes (up to 0.5 V) generated by stimulator (Grass Instrument Company, model S88), delivered through the bipolar electrodes (silver-wires, 0.5 mm in diameter) to the ventral root filaments were used to evoke contractile activity of the studied motor units. A single motor unit isolation was confirmed by the "all-or-none" appearance of evoked action potentials and force of the twitch (Celichowski and Grottel 1992, Kuffler et al. 1951).

The muscle fiber action potentials were recorded with a silver-wire bipolar electrode (0.1 mm in diameter) inserted into the muscle, perpendicular to its longitudinal axis. Ground electrode was inserted inside the muscles of the opposite limb. Action potentials were amplified using the low-noise multi-channel amplifier (WPI, model ISO-DAM8-A, with the high and low-pass filters set at the frequencies of 0.1 Hz and 10 kHz, respectively). Force was measured under isometric conditions by the force transducer. Throughout the experiments, the force amplitude and the electromyogram were monitored and displayed on an oscilloscope screen, and stored on a computer disc using the analogue-to-digital 12-bit converter (RTI-800) at sampling rate of 1 kHz for force records and 10 kHz for action potentials.

Each isolated motor unit was stimulated according to the following protocol: (1) five pulses at 1 Hz (an average record of the twitch force was obtained); (2) series of stimuli at 40 Hz during 500 ms (the unfused tetanus was obtained); (3) series of stimuli at 150 Hz during 300 ms (the maximum tetanus was obtained); (4) 10 series of constant-frequency trains of stimuli at 1, 10, 20, 30, 40, 50, 60, 75, 100, and 150 Hz (500 ms each). Between all trains of stimuli within these series, 10-second breaks were programmed. The procedure 4 was repeated 10 times. Lastly, the standard fatigue test was implemented (trains of stimuli at 40 Hz and duration of 325 ms, repeated every second for 4 minutes) (Burke et al. 1973). The studied motor units were classified into three types: slow (S), fast resistant to fatigue (FR) and fast fatigable (FF) (Burke et al. 1973). Division of studied units into fast and slow ones was based on the "sag" appearance in unfused tetani at 40 Hz of stimulation (the transient force decrease observed within the unfused tetanus, present for fast and absent for slow units) (Grottel and Celichowski 1990). The fatigue index, expressed as a ratio of the contractile force generated two minutes after the most potentiated contraction at the beginning of the fatigue test to the force of this potentiated contraction, was calculated. Fast motor units were classified as fast fatigable (FF) when the fatigue index was under 0.5 and fast resistant (FR) when the index exceeded 0.5 (Grottel and Celichowski 1990, Kernell et al. 1983). Throughout the entire experimental protocol, the action potentials and force of studied motor unit were continuously monitored and recorded. Stable amplitude and the shape of the potentials, as well as a lack of sudden changes in force, ensured that the same motor unit was tested.

For each averaged twitch record, the force (TwF), the contraction time (CT), the half-relaxation time (HRT), and the force-time area (the area under the force recording curve) were measured. For all tetani (at frequencies from 10 to 150 Hz), the force (measured at the last contraction within tetanus) and the fusion index (expressed as presented in Fig. 1) were measured (Bakels and Kernell 1995, Celichowski and Grottel 1995a). Additionally, for all tetani evoked at different frequencies, the force-time area per one pulse was measured for the next to last contraction within the tetanus at the plateau phase of the force (Fig. 1). The

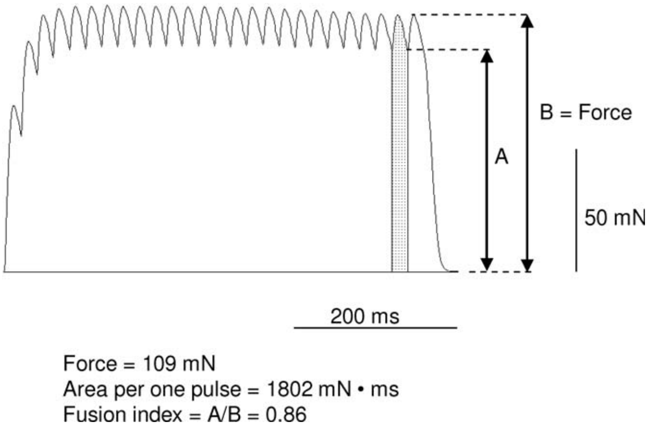


Fig. 1. Example of a tetanus evoked at 50 Hz frequency of stimulation in a FF motor unit. The fusion index was calculated as A/B ratio. Dotted area – the area per one pulse. Force – force amplitude measured at the last contraction within tetanus.

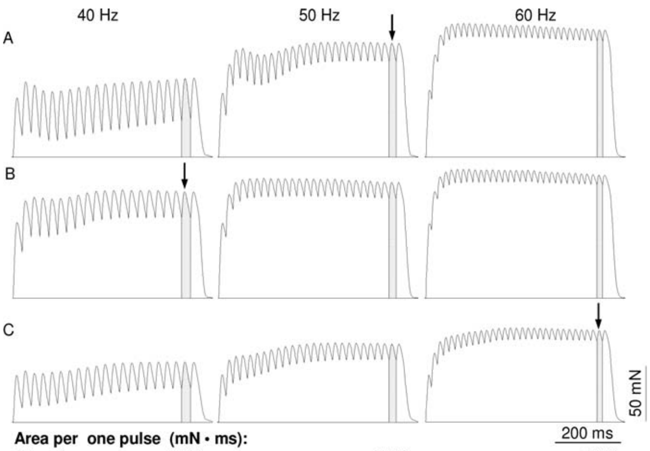
force-time area per single pulse was calculated by a custom-designed computer program (Analog 10 Muscle Force Tracer).

The Student's *t*-test was used for a statistical comparison of measured contractile properties of studied motor units in initial, potentiated and fatigued states. The values of studied parameters were expressed as means and standard deviations (SD).

RESULTS

A total of 38 motor units were studied: 12 FF, 15 FR, and 11 S type units. The contractile properties used for classification of the investigated motor units into the three types, are presented in Table I. The contractile properties of the analyzed motor units and the distribution of motor unit types within the medial gastrocnemius muscle were similar to the previously described, although the number of studied units was relatively small (Grottel and Celichowski 1990).

Changes of motor unit output in response to a single stimulus were analyzed during repeated, constant frequency tetanic stimulations. Three of overall ten series of tetani have been finally chosen for further evaluation: the first (control), which provided the initial values; the second, when for all fast motor units the potentiation at low and middle range stimulation frequencies was observed; and the tenth, with the decrease of force as an effect of fatigue (especially in the FF units) (Celichowski and Grottel 1997). For each tetanus in the first, the second and the tenth series of stimulation trains, the area per one pulse was calculated. The motor unit output was expressed in terms of the force-time area per single pulse measured at the end of the tetanic contraction. Therefore, within each studied stimulation series, the stimulation frequency optimal for the maximal motor unit output (maximal area per one pulse) was determined (Fig. 2). Results of this analysis are summarized in Table II.



Area per one pulse (mN • ms):			
1st series	1342	**1759**	1674
2nd series	**2029**	1802	1516
10th series	1085	1186	**1196**
Force (mN):			
1st series	80	**104**	121
2nd series	**98**	109	117
10th series	54	71	**85**
Fusion index:			
1st series	0.91	**0.96**	0.98
2nd series	**0.91**	0.95	0.97
10th series	0.79	0.89	**0.94**

Fig. 2. Examples of three tetani in a FF motor unit evoked at 40, 50, and 60 Hz frequency of stimulation within the first (A), second (B), and last (C) of ten series of trains of stimuli at increasing frequencies. Dotted areas – areas per one pulse. The maximal area per one pulse was obtained at 50, 40, and 60 Hz frequency of stimulation in the first, second and last stimulation series, respectively (arrows). For all presented tetani belonging to each series of stimulations, the values of the area per one pulse, the force and the fusion index are showed below the records. The bold numbers denote values of the parameters measured for the tetani with the highest area per one pulse.

Table I

Mean values and standard deviations of basic mechanical properties of the three types of studied motor units

Motor unit type	CT (ms)	HRT (ms)	TwF (mN)	TetF (mN)	FI
FF	12.1 ± 1.2	12.6 ± 3.1	38.8 ± 21.8	143.5 ± 59.1	0.28 ± 0.13
FR	14.1 ± 2.4	15.9 ± 3.4	5.9 ± 2.9	38.0 ± 21.4	0.89 ± 0.11
S	26.1 ± 4.7	35.6 ± 6.8	3.9 ± 1.4	32.7 ± 10.5	0.94 ± 0.04

(CT) the contraction time; (HRT) the half relaxation time; (TwF) the twitch force; (TetF) the tetanus force; (FI) the fatigue index

Table II

The changes in studied parameters of the twitch, the optimal tetanus and the maximal tetanus within the three analyzed series of stimulations for the three types of motor units

Motor unit type	1st series	2nd series	10th series
Area under the twitch record (mN·ms)			
FF	872.7 ± 534.0	1399.2 ± 712.5**	744.2 ± 360.6$^{\#\#}$
FR	189.3 ± 73.3	285.5 ± 115.1**	188.8 ± 64.7$^{\#\#}$
S	204.8 ± 93.6	193.9 ± 86.7	205.5 ± 76.3
Maximal area per one pulse (mN·ms)			
FF	1491.1 ± 574.2	1735.9 ± 730.2**	1198.3 ± 444.1** $^{\#\#}$
FR	422.7 ± 186.2	444.0 ± 189.8*	389.2 ± 164.4* $^{\#\#}$
S	797.1 ± 511.8	788.1 ± 525.5	805.7 ± 452.9
Force of the optimal tetanus (mN)			
FF	103.4 ± 30.1	92.8 ± 29.5*	90.3 ± 23.1*
FR	25.7 ± 15.4	26.4 ± 15.8	24.0 ± 12.6
S	25.1 ± 8.9	25.3 ± 9.5	24.7 ± 7.2
Frequency of the optimal tetanus (Hz)			
FF	54.4 ± 13.1	43.3 ± 11.2**	60.0 ± 21.1$^{\#\#}$
FR	51.8 ± 11.7	50.4 ± 11.2	57.1 ± 14.6** $^{\#\#}$
S	24.4 ± 5.3	23.3 ± 5.0	22.2 ± 4.4
Fusion index of the optimal tetanus			
FF	0.90 ± 0.05	0.83 ± 0.08**	0.88 ± 0.09$^{\#\#}$
FR	0.92 ± 0.04	0.93 ± 0.05	0.93 ± 0.05
S	0.94 ± 0.03	0.90 ± 0.09*	0.91 ± 0.03
Maximal tetanus force (mN)			
FF	153.9 ± 46.0	144.4 ± 45.4	137.5 ± 40.5**
FR	38.0 ± 21.4	38.2 ± 21.4	37.1 ± 19.8$^{\#}$
S	35.6 ± 8.7	35.9 ± 9.1	36.0 ± 8.8

The values are expressed as means ± SD. (*) Significantly different from the 1st series, *$P<0.05$, **$P<0.01$, ($^{\#}$) significantly different from the 2nd series, ($^{\#}$) $P<0.05$, ($^{\#\#}$) $P<0.01$ (Student's *t*-test).

Changes of the twitch and the maximal tetanus contractile parameters during activity

In fast motor units, the time parameters of the twitch before each of ten applied series of stimulations were changed. The twitch was prolonged in the second series and shortened in the last one in comparison to the control series. A sum of the contraction and half-relaxation times for twitches recorded before the first, the second and the tenth series amounted to 26.9 ± 4.2 ms, 27.9 ± 3.6 ms, 23.4 ± 3.3$^{\#}$ ms in the FF and 33.3 ± 4.2 ms, 35.6 ± 5.0* ms, 31.1 ± 6.1*$^{\#\#}$ ms in the FR units, respectively [(*) significantly different from the initial series, $P<0.05$, ($^{\#}$) significantly different from the second series, $^{\#}P<0.05$, $^{\#\#}P<0.01$]. In the S units the summed times were insignificantly prolonged in the second and last series in relation to the first one, and amounted to 62.6 ± 10.9 ms, 65.1 ± 13.7 ms, 65.0 ± 11.6 ms, respectively. The twitch force of fast motor units in the second series of stimulations potentiated by 50.3 ± 22.3% and 39.9 ± 22.7% in the FF and FR units, respectively (significantly different from the initial series, $P<0.01$). In the FF units, the maximal tetanus force decreased, while in the FR units did not change significantly (Table II). Thereafter, up to the tenth series a progressive decrease in force amplitudes was observed. In the last series, 2.9% decrease in relation to initial values of the FF motor units twitch force was noted. In the FR units, the twitch force remained still slightly potentiated (by 9.5% – significantly different from the initial series, $P<0.05$). However, in the last series, for both fast unit types the maximal tetanus force decreased (Table II). The force of slow motor units in successive series showed minor changes (Table II). Only the twitch force was a little potentiated (by approximately 3%) in the last series of stimulations, but not in the second one.

Changes of the twitch area during activity

The areas under the twitch record of fast motor units significantly increased in the second series (Table II, Fig. 3A). In the last stimulation series the values of twitch area of the FR units did not differ in comparison to the control one, whereas insignificantly decreased in case of the FF units. In the S units, the mean values of the twitch area remained nearly constant throughout evoked activity (Fig. 3A).

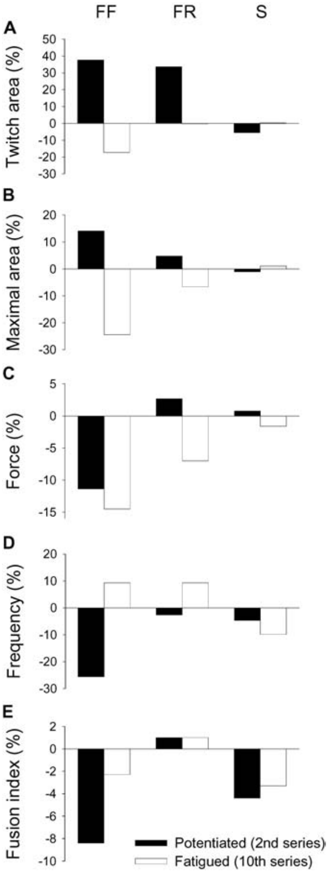


Fig. 3. Relative changes of the studied parameters of the twitch (A) and the optimal tetanus (B–E). Bars represent the differences, in percentage, between the initial and the second or the tenth stimulation series. (A) Twitch area – the area under the twitch record; (B) Maximal area – the maximal area per one pulse obtained with the optimal tetanus; (C) Force – the force of the optimal tetanus; (D) Frequency – the stimulation frequency of the optimal tetanus; (E) Fusion index – the fusion index of the optimal tetanus.

Changes of the optimal tetanus parameters during activity

In the control series, the absolute values of the force-time area per pulse for three types of motor units were arranged in a decreasing order of FF > S > FR. The average values of area per pulse of the S and FR units constituted 53.2% and 28.3% of these calculated for the FF units, respectively (Table II). The optimal frequency of stimulation (i.e., giving the maximal area per pulse) was the highest in the FF motor units and the lowest in the S units (Table II, Fig. 4). However, for all types of units, it corresponded to similarly fused tetani (the fusion index in a range of 0.90–0.94,

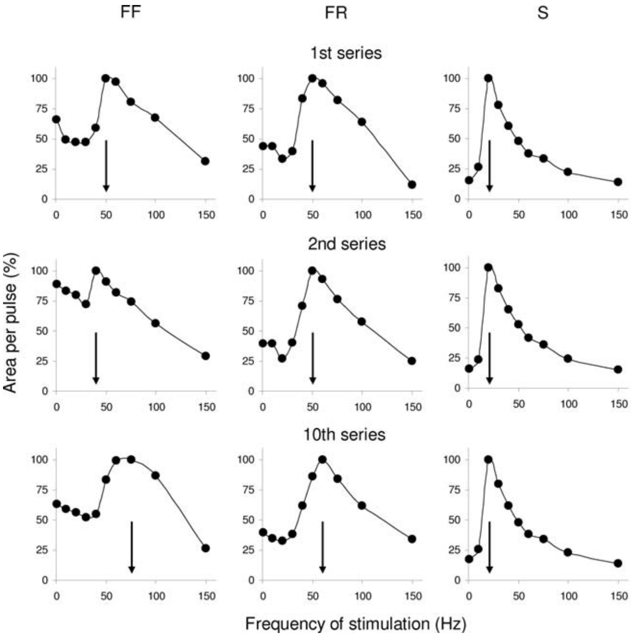


Fig. 4. Normalized areas per one pulse expressed as the percentage of the maximal values as a function of the frequency of stimulation in the three of the ten successive series of stimulation, for representative examples of one of each type of motor units. In the FF unit, the maximum area per one pulse was reached at 50, 40, and 75 Hz stimulation rates within the first, the second and the tenth series, respectively (arrows). The maximal area in the FR unit (middle column) was obtained at 50 Hz frequency of stimulation for the first and second series, whereas in the last series, the optimal frequency increased to 60 Hz. The area-frequency curve for the S motor unit (right column) had a peak at the same stimulation frequency in all 3 series (20 Hz).

Table II), whereas the force of these optimal tetani amounted to 68–75% of the maximal tetanic force (68.7%, 74.3%, and 75.4% in FF, FR and S motor units, respectively).

The motor unit potentiation and fatigue failed to change the relations between the magnitudes of the maximal area per pulse for the three types of motor units which remained in the same order as in the control conditions (FF > S > FR). However, changes in the percentage proportions of the magnitudes were noted. In the second series, the mean values of the maximal area of the S and FR motor units constituted only 45.4% and 25.6% of these calculated for the FF units, respectively. Nonetheless, in the last series, a decrease of the ratio was observed – the mean values of the S and FR units' areas amounted to 67.2% and 32.5% of the area recorded in the FF units, respectively (Table II).

In the second series, the maximal area per pulse of the FF motor units increased (Table II, Fig. 3B), whereas the optimal tetanus was obtained at a lower force (Figs 2 and 3C), lower frequency of stimulation (Figs 3D and 4) and lower degree of fusion (Fig. 3E) in comparison to the first series. In the last series, the magnitude of the area dropped and the force of the optimal tetanus was diminished in comparison to the first one (Table II, Fig. 3B, C). The optimal frequency increased (Figs 3D and 4), while the fusion index of the optimal tetanus was not different when compared to the control values (Fig. 3E). However, in both these series, the force of the optimal tetanus expressed in relation to the maximum tetanus recorded within the present series, was similar to the force measured in the first one (68.7%, 65.8%, and 67.4% of the maximum in the first, the second and the tenth series, respectively).

The changes in the magnitude of the maximal area per pulse of the FR units exhibited very similar trends as in the FF units, but were minor. The magnitude of the maximal area per pulse slightly increased in the second series (Table II, Fig. 3B). Nevertheless, in contrast to the FF units it was obtained at slightly higher force than in the first series (Table II, Fig. 3C) and a little lower frequency and increased fusion of the optimal tetanus (Fig. 3D, E). In the last series, the maximal area and the absolute force of the optimal tetanus decreased. The optimal frequency increased (Figs 3D and 4) but the fusion index remained unaltered (Fig. 3E). The force of the optimal tetanus in relation to the maximal tetanus did not change in either the second (75.9% of the maximum) or the tenth series (73.0% of the maximum) as compared to the first stimulation series (74.3% of the maximum).

The magnitude of the maximal area per one pulse of the S units did not differ significantly among the three series (Table II, Fig. 3B). In the first, the second and the tenth series, the maximal area was obtained in the tetani of comparable force (Table II, Fig. 3C) corresponding to 75.4%, 71.3%, and 67.6% of the maximal tetanus force, respectively (difference significant only between the first and the tenth series, $P<0.05$). Additionally, in the second and the tenth series, a slight decrease of frequency and fusion of the optimal tetanus was detected (Fig. 3D, E).

DISCUSSION

The main interest of the present study was focused on the analysis of changes of the motor unit output during repeated tetanic activity. The main observation concerned the changes of the optimal tetanus, i.e., when the force-time area per one pulse was maximal. It was observed that, in fast motor units, the potentiation increased the analyzed area and decreased the stimulation frequency of the optimal tetanus, whereas the fatigue induced the opposite effects. Moreover, we found that independently of changes in the force-time area per one pulse and changes of the optimal stimulation frequency, the fusion degree of the optimal tetanus remained relatively stable. Analysis of the motor output per single pulse during prolonged activity of the three types of motor units considerably broadens our knowledge concerning dynamic changes and the ability of the smallest physiological units to preserve the skeletal muscle performance during contractile activity. Till now, the data concerning changes of the motor unit output during activity has been limited to force analysis. It should be stressed that force and force-time area are the two partly independent parameters (Celichowski et al. 1998), and that the area seems to be more adequate to predict the effectiveness of muscle contraction (Burke et al. 1970, De Haan et al. 1986).

The applied stimulation protocol induced marked changes in the force output within a single motor unit contraction, which were the most pronounced in fast motor units (particularly FF units). As a result of fast developing potentiation, the maximal area per pulse increased in the second series of trains in comparison to the first one. It is believed that a primary mechanism responsible for augmentation of force after conditioning stimulation is the phosphorylation of myosin regulatory light chains (RLC) (Moore and Stull 1984, Sweeney et al. 1993). This process enhances the rate of force development in fast-twitch skeletal muscles by increasing the sensitivity of the contractile apparatus (i.e., myosin-actin interaction) to Ca^{2+} (Vandenboom et al. 1995). The speed of force development is regulated through Ca^{2+} ions which control the rate of cross-bridges transition from a non-force generating state to force generating state (Sweeney et al. 1993). Since the integral of force over time (force-time area) probably represents the total amount of activated and bound Ca^{2+} during an isometric contraction, an increase of Ca^{2+} sensitivity due to myosin RLC phosphorylation could augment the force output, by increasing the fraction of cross-bridges in the force generating state (Sweeney et al. 1993, Vandenboom and Houston 1996, Vandenboom et al. 1995, Zajac and Young 1980). The enhancement of the area might be also due to prolongation of the time parameters of successive contractions within the tetanus, since for both types of fast units the contraction and relaxation times of the twitch before the second series lengthened (Celichowski et al. 2006).

A prolonged, intermittent stimulation elicited fatigue in the fast motor units. It was mainly manifested by a progressive decrease of the maximal tetanic, twitch and unfused tetanic forces, which was the most pronounced in the last series of stimulation. The maximal force-time area per pulse of fast motor units was markedly decreased. Several reasons responsible for this decrease of output can be considered. It is known that repetitive stimulation is responsible for a reduction in maximum Ca^{2+}-activated force through the decrease of Ca^{2+} sensitivity of the actomyosin complex (Lee et al. 1990, Ørtenblad et al. 2000). In contrast to potentiation, fatigue impairs the sarcoplasmic reticulum Ca^{2+} release rate and reduces its myoplasmic concentration by disturbing intracellular environment, e.g., decreasing pH, metabolic disturbances and altered ion homeostasis. This, in turn, impairs both cross-bridge cycling and sarcoplasmic reticulum function. Additionally, the decrease of twitch time parameters of fast units was observed in the tenth series. It seems therefore, that both attenuation of force and shortening of the twitch time reduced the analyzed areas per pulse by limiting the effective summation of successive contractions into tetanus.

The applied stimulation paradigm had almost no influence on mechanical activity of slow motor units, as they exhibit rather minor changes of mechanical parameters even during long-lasting activity (Burke et al. 1973, Celichowski and Grottel 1995b).

The stimulation frequency is a parameter, which corresponds to motoneuronal firing rate. Therefore, observation of changes in the optimal frequency is relevant for understanding motoneuronal activity during prolonged voluntary contractions. When potentiation developed, the maximal output per pulse was obtained at lower frequencies of stimulation (especially for FF units). This effect was associated with lengthening of contractile time parameters (Celichowski et al. 2006). Indeed, as a result of potentiation, the force-frequency curve of fast units shifts towards lower stimulation rates (Celichowski and Grottel 1997). On the other hand, when fatigue occurred, the maximal force-time area was generated at a higher frequency of stimulation in comparison to the initial conditions. This result might be explained by shortening of the twitch time parameters due to fatigue. Furthermore, during a fatiguing intermittent stimulation, low frequency fatigue develops (Celichowski and Grottel 1997, Cooper et al. 1988, Fuglevand et al. 1999). As a result, higher motoneuronal firing rate must be attained to generate contraction with the maximal force-time area.

In the initial series, for all types of motor units, the maximal area per pulse was reached at the stimulation rates which produced rather well fused tetani (with the fusion index approximately at 0.90), with the force exceeding 70% of the maximum. These values are comparable to the results obtained previously in our laboratory (Celichowski et al. 2000). However, independently of the extent and direction of changes of the optimal frequency during potentiation or fatigue, the maximal output per single pulse was obtained at relatively stable fusion index of around 0.90 in all types of units (except FF motor units in the potentiated state). Therefore, it seems probable that, in response to alterations of muscle fibers physiological properties caused by potentiation and fatigue, motoneurones will adjust their firing frequency (decrease with potentiation and increase with fatigue) to generate tetani optimal for the maximal output (Bigland-Ritchie et al. 1986, Griffin et al. 1998, Klein et al. 2001).

The economy of contraction can be estimated in two ways: by dividing the force alone or by dividing the force-time area by a total energy consumed (De Haan et al. 1986). During activity, skeletal muscle fibers produce certain amount of energy for every contraction in response to each pulse. The force time-area of isometric contraction is proportional to energy expenditure of contracting muscle (Jöbsis and Duffield 1967). However, other authors pointed out that the area under force recordings of the whole muscle tetanic contraction is not the most appropate predictor of energy cost of contracting skeletal muscle (Ameredes et al. 1998). Instead, for estimation of contractile economy, it is better to take into account the area induced by a single pulse, since the energy consumption depends on the number of action potentials delivered to the muscle (Fales et al. 1960).

Studies of rat extensor digitorium longus have shown that the energy expenditure of evoked isometric contractions did not change due to potentiation (Barsotti and Butler 1984). Thus, with potentiation, the increase of force-time area per pulse is not accompanied by an increase of the rate of chemical energy usage (Abbate et al. 2001). Therefore, the energy cost of the potentiated contraction probably does not differ in comparison to unpotentiated contraction. Furthermore, Abbate and others (2002) have reported an increased energy consumption when the mechanical output is increased by higher stimulation frequencies. Hence, during voluntary activity, the decrease of the motoneuronal firing rates in potentiated tetani will act in favor of energy conservation. In addition, the increase of motor unit output due to potentiation is probably greater at low frequencies of stimulation (compare the extent of changes of area under the twitch record and the maximal area per pulse in Table II and Fig. 4A, B). Among all motor unit types, FF units have the greatest potential to increase their motor output, since potentiation induces the highest percentage increase of force-time area. However, due to fatigue, the work of fast motor units is not sustained over prolonged periods of contractile activity. Moreover, in fast units, the extent of changes of motor output is much more pronounced then changes of force. For instance, although with the applied pattern of stimulation, the difference of the force of optimal tetanus of FF motor units in potentiation and fatigue amounted to 2.7% only, the contractile output (area per pulse) differed by 41% and the optimal frequency increased by 38.6%. De Haan and coauthors (1986) discovered that during prolonged intermittent tetanic stimulation of the medial gastrocnemius muscle, the economy of each isometric contraction remained constant irrespective of the total number of contractions. These authors used a stimulation paradigm that comprised only constant frequency tetanic contractions. The observations made in the present study and the previous data (Bigland-Ritchie et al. 1986, Celichowski and Grottel 1997, Griffin et al. 1998) point out that, to keep a constant force level during fatiguing intermittent submaximal isometric contractions, the increasing motoneuronal firing rate is necessary. This, in turn, increases the metabolic expenditure per contraction. Additionally, the augmentation of energy cost with concomitant fatigue-related reduction of contractile output, diminishes the economy of contraction.

CONCLUSIONS

Two studied phenomena: the potentiation and fatigue considerably influence the force output and the economy of contraction of motor units and strongly modulate the optimal rate of motoneuronal firing. However, the fusion degree of the optimal tetanic contractions is rather stable independently of the changes in force. The potentiation augments the economy of contraction, whereas fatigue induces the opposite effect. During prolonged motor unit activity, measurements of the contractile output per pulse may offer a useful tool with which to assess changes in contractile economy.

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