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CHANGES IN THE TIME COURSE OF POTENTIATED AND FATIGUED CONTRACTIONS OF FAST MOTOR UNITS IN RAT MUSCLE

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INTRODUCTION

The force of unfused tetani of active motor units (MUs) depends on a firing rate generated by motoneurons and can be regulated by changes in interpulse intervals in order to adjust the force to current demands (17, 31, 32, 35). On the other hand, it is known that the force of unfused tetani of fast MUs developed in response to the stimulation at a constant frequency is unstable (11, 12, 17). This phenomenon can be observed during the standard fatigue test, in which a motor unit is stimulated with the repeated trains of stimuli at the 40 Hz frequency, every second during at least two minutes (4, 7, 12, 17, 19, 26, 27). The observed initial increase of force – the potentiation, as a consequence of the previous motor unit activity, concerns either twitches or unfused tetani of fast fatigable (FF) and fast resistant to fatigue (FR) MUs (5, 11, 24). The opposite phenomenon, i.e. the decrease of force, develops later during long-lasting motor unit activity and is known as fatigue (4, 18).

The potentiation and fatigue have been studied in numerous investigations (4, 11, 12, 17, 19, 24, 26, 27). In the earlier experiments in our laboratory the course of tetani during the fatigue test in all three MU types of the rat medial gastrocnemius muscle has been analysed and the initial potentiation of MU force has been observed (7). This phenomenon has developed rapidly in FF MUs (within 16 s of the test, on the average) and much more slowly in FR MUs (within 29 s, on the average), amounting to 96% and 77% for FF and FR MUs, respectively. These data are consistent with results obtained in various experiments by other authors (19, 23, 24). In the subsequent series of experiments performed in our laboratory on the rat medial gastrocnemius muscle, relationships between the force and frequency of stimulation have been investigated in fast MUs during repeated 10 times series of trains of stimuli delivered at frequencies between 1 and 150 Hz (11). It has been revealed that up to the 2nd, 3rd or even 4th series, the increase of force in either twitches or unfused tetani develops (the highest potentiation has amounted to 44% for FF MUs and 70% for FR MUs). Within subsequent series of trains in FF MUs this phenomenon disappears, whereas in a majority of FR MUs is still visible. When the fatigue effects develops in FF MUs, the twitch force becomes lower than at the beginning of the

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experiment and the summation of contractions into tetani is considerably limited. The maintenance of a constant level of tetanic force requires increased frequency of stimulation.

The above changes in force are accompanied by changes in the fusion of a tetanus, what has been demonstrated in several studies. Nowak (33) has modified the fatigue test by using 60 Hz frequency of stimulation indicating that at higher frequencies, when tetani become more fused, the potentiation is weaker and the decrease of force is slower than during the standard fatigue test. The author has pointed out in the discussion regarding the influence of the fusion degree on the force that changes in the twitch time parameters, which determine the tetanic fusion: the contraction time (CT) and the half-relaxation time (HRT), also influence the amplitude of potentiation and fatigue (3, 7, 27). However, the detailed analysis of the time parameters during the potentiation and fatigue hasn't been made so far. Kernell *et al.* (25) and Bergström and Hultman (2) have reported the influence of the twitch prolongation on the tetanic force. Further studies have shown that the increase of force may result from the prolongation of twitches (7) whereas the fatigue-related decrease of force may be partly due to the shortening of the twitch time course (7, 19).

The purpose of the present paper was to describe in two types of fast MUs changes in the CT and the HRT between initial, potentiated and fatigued twitches, and between contractile responses to the last pulses within unfused tetani. Moreover, in this study we attempted to find out, whether changes in contraction or relaxation phases, significantly contribute to the changes in development of force during the potentiation and the fatigue.

MATERIAL AND METHODS

Experiments were performed on 4 adult Wistar rats (mean weight 373 ± 153 g). During experiments the animals were anaesthetized with pentobarbital (initial dose of 60 mg/kg, i.p., supplemented as required). The depth of anaesthesia was verified by controlling the withdrawal reflex. The principles of laboratory animal care, as approved by European Union and the Polish Law on Animal Protection were followed. After the experiments, the animals were killed with an overdose of pentobarbital (180 mg/kg).

Fast motor units of the medial gastrocnemius muscle (MG) were investigated. The MG and the respective branch of the sciatic nerve were partly isolated from surrounding tissues; other muscles were denervated. Laminectomy over L2-S1 segments was performed; dorsal and ventral roots were cut proximally to the spinal cord. The animal was immobilized with steel clamps on the tibia, the sacral bone and the L1 vertebra. The operated hind limb and the spinal cord were covered with the paraffin oil; its temperature was kept at 37 ± 1 °C by automatic heating system. The MG muscle, stretched up to 100 mN, was connected to an inductive force transducer to measure the contractile force under isometric conditions (9). The MU action potentials were recorded with a bipolar silver electrode inserted into the muscle. All the recorded data were stored on a computer disc using an AD converter (sampling rate 10 kHz and 20 kHz for force and action potentials, respectively). The functional isolation of single MUs was achieved by splitting the L5 or L4 ventral roots into thin filaments, which were electrically stimulated with suprathreshold rectangular pulses (amplitude up to 0.5 V, duration 0.1 ms). The "all-or-none" appearance of the twitch contractions and MU action potentials in response to stimuli of increasing amplitude indicated the activity of a single MU. In this research only fast MUs, i.e. with the contraction time shorter than 20 ms were approved (20).

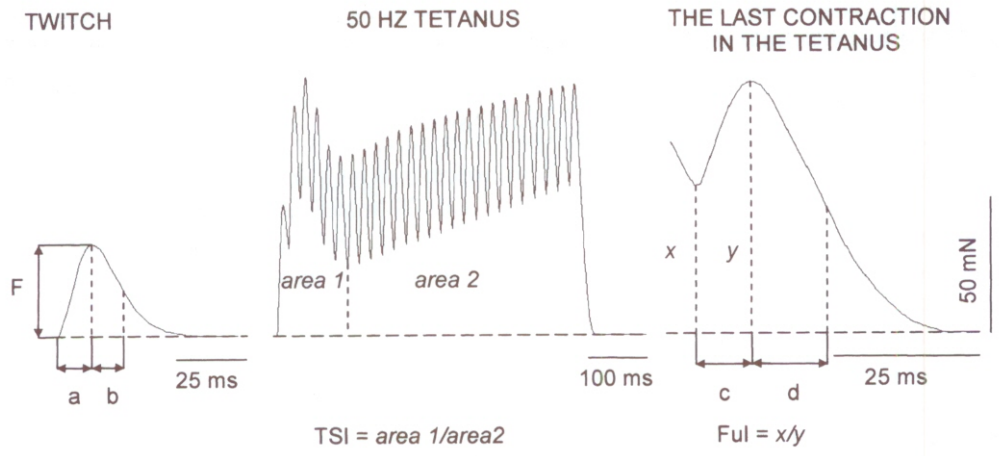

Fig. 1. - Parameters measured in a twitch, unfused tetanus and the last contraction within the tetanus for each of the studied MUs.
F, the force; a and c, the contraction time; b and d, the half-relaxation time; TSI, the tetanus shape index, calculated as a ratio of area 1 to area 2; FuI, the fusion index, calculated as a ratio of x to y. The presented unfused tetanus of FF MUs was evoked at 50 Hz stimulation frequency.

Fast MU were divided into fast fatigable (FF) and fast resistant to fatigue (FR) on the basis of the tetanus shape index (Fig. 1) which was below 0.5 for FF and over 0.5 for FR MUs (13).

During the experiment, MUs were stimulated according to the following protocol: 1) 5 stimuli at 1 Hz (5 single twitches were recorded and then the averaged twitch was estimated); 2) 3 s interval; 3) series of three trains of stimuli at 40, 50 and 60 Hz with 500 ms duration each and 10 s intervals after each successive train (the initial, unfused tetani with variable fusion degree were recorded); 4) 3 stimuli at 1 Hz (3 single twitches were recorded and then the averaged twitch was estimated); 5) 1 s interval; 6) series of three trains of stimuli at 40, 50 and 60 Hz with 500 ms duration and 500 ms intervals after each successive train. The procedures 4-6 were repeated 30 times (first the development of potentiation and then of fatigue were observed in unfused tetani at three initial levels of fusion degree). For each motor unit, the analysis concerned the twitches and tetani at the beginning of the stimulation, at the moment when the maximal potentiation of twitches was achieved and at the end of the stimulation protocol, when the fatigue developed. The following parameters were measured for twitches: the contraction time (CT, from the onset of the force increase to the peak), the half-relaxation time (HRT, from the peak force to the half of its maximum), the twitch force (TwF, from the baseline to the peak) (Fig. 1). For the last contractions within the chosen tetani, the CT, the HRT and the force were also measured (14, 21). Moreover, the fusion index (FuI, the ratio of the initial force of the contraction to the maximal force of this contraction) was calculated for each unfused tetanus (1, 10) (Fig. 1). The data were presented as the means ± SE, and the significance of the changes was analysed using the Wilcoxon's matched pairs test. Significance of differences between FF and FR MUs was determined using Mann-Whitney's U-test. $P < 0.05$ was considered statistically significant.

RESULTS

Motor units of the rat MG muscle representing variable contractile force and time parameters were used in the study. Mean values and variability ranges of basic MU

contractile properties of initial twitches are given in Table 1. They are consistent with results presented in previous papers (8, 14, 21). The mean values of the tetanus shape index for FF and FR MUs amounted to 0.26 ± 0.08 and 4.31 ± 6.45, respectively.

The effects of potentiation and fatigue were studied for MUs with initial tetanic contractions characterized by the fusion indices ranging from 0.2 to 0.9. This condition was fulfilled in 32 tetani of FF MUs and 27 tetani of FR MUs. Figure 2 presents examples of the initial, the most potentiated, and the fatigued twitches and unfused tetani of an FF and an FR MUs. In the whole population studied, the highest potentiation of twitches was visible in FF MUs between the 2nd and the 10th series of stimulation trains (mean 4.5 ± 2.3), whereas in FR MUs between the 7th and 11th series (mean 8.5 ± 1.8). The difference between two types of MUs was significant ($p < 0.01$). The effect of the fatigue at the end of the experiment was noticed evidently in FF MUs, but in FR MUs only slight decrease of force was observed.

The influence of potentiation and fatigue on twitch parameters

Parameters of initial, potentiated and fatigued twitches revealed considerable changes (Fig. 2, Table 1). During the potentiation the mean relative twitch forces increased significantly to 161.97 ± 34.91% in FF MUs and 161.82 ± 31.94% in FR MUs in comparison to the initial values (the difference between two types of MUs non-significant, $p > 0.05$) (Table 1). The mean values of CT were only slightly prolonged, similarly for FF and FR MUs: 101.35 ± 6.40% and 102.67 ± 9.85% of initial values, for FF and FR MUs, respectively (the difference non-significant, $p > 0.05$) (Table 1). On the other hand, the mean values of the HRT in two types of units increased considerably, amounting to 125.54 ± 35.09% and 114.27 ± 17.54% of initial values for FF and FR MUs, respectively (the difference non-significant, $p > 0.05$) (Table 1).

At the end of experimental procedure the force decreased as an effect of fatigue. For FF MUs, the twitch forces decreased below values measured during initial stimulation (the mean 72.71 ± 36.16% of the initial value). However, for FR MUs, the twitch force decreased exclusively in relation to the potentiated force, being still higher than at the beginning (the mean 124.37 ± 38.57% of the initial value) (the difference between two types of MUs significant, $p < 0.01$) (Table 1). The fatigue-related decrease of force was also accompanied by changes in the twitch-time parameters. The mean values of CT for FF MUs decreased slightly to 89.36 ± 10.26% of the initial values, but for FR MUs were nearly unchanged (98.08 ± 14.01%) (the significant difference, $p < 0.05$). The mean values of the HRT shortened slightly below the initial values and amounted to 98.80 ± 20.86% in FF MUs and 94.56 ± 19.73% in FR MUs (the difference non-significant, $p > 0.05$) (Table 1), however, after previous considerable prolongation in potentiated twitches.

The influence of potentiation and fatigue on last contractions within the tetanus

During the potentiation, the mean forces of the last contractions within unfused tetani increased significantly, to the mean 138.29 ± 33.12% of the initial value for

Tab. I. - Changes in the time parameters and force in the twich and the last contraction in the tetanus resulted from the potentiation and the fatigue.

	Twitch			The last contraction in the tetanus			
	CT (ms)	HRT (ms)	F (mN)	CT (ms)	HRT (ms)	F (mN)	FuI
FF (n = 32)							
Initial	11.83 ± 1.23	12.10 ± 2.89	57.75 ± 36.04	8.09 ± 1.12	14.28 ± 4.25	97.47 ± 52.47	0.59 ± 0.24
	10.0 - 14.0	9.0 - 22.0	16.30 - 149.70	6.0 - 10.0	7.0 - 22.0	19.02 - 208.00	0.20 - 0.90
Potentiated	11.97 ± 1.19	14.93 ± 4.13	85.83 ± 43.78	6.91 ± 1.57	23.25 ± 7.70	129.65 ± 68.55	0.80 ± 0.15
	10.0 - 14.0	9.0 - 22.0	26.70 - 202.70	3.0 - 9.0	10.0 - 44.0	27.50 - 324.40	0.30 - 0.94
Fatigued	10.57 ± 1.20	11.75 ± 2.24	35.70 ± 12.93	7.63 ± 1.22	14.73 ± 4.91	38.06 ± 29.28	0.58 ± 0.22
	8.0 - 13.0	8.0 - 16.0	11.88 - 59.99	5.0 - 10.0	7.0 - 23.0	3.08 - 98.70	0.11 - 0.90
FR (n = 27)							
Initial	13.35 ± 1.20	16.31 ± 2.22	29.46 ± 30.38	8.85 ± 1.10	16.11 ± 3.64	61.68 ± 58.93	0.68 ± 0.21
	11.0 - 15.0	14.0 - 21.0	2.10 - 98.00	7.0 - 11.0	10.0 - 23.0	5.30 - 198.70	0.32 - 0.90
Potentiated	13.65 ± 1.38	18.50 ± 2.86	48.99 ± 50.12	7.89 ± 1.48	22.59 ± 4.69	83.06 ± 74.24	0.84 ± 0.10
	1.0 - 16.0	12.0 - 24.0	2.40 - 149.50	5.0 - 11.0	16.0 - 29.0	6.10 - 213.90	0.56 - 0.94
Fatigued	12.96 ± 1.15	15.12 ± 1.99	39.61 ± 42.85	8.33 ± 1.41	20.48 ± 5.06	68.47 ± 66.16	0.78 ± 0.14
	11.0 - 16.0	12.0 - 19.0	2.30 - 117.40	6.0 - 13.0	12.0 - 29.0	4.90 - 183.40	0.43 - 0.94

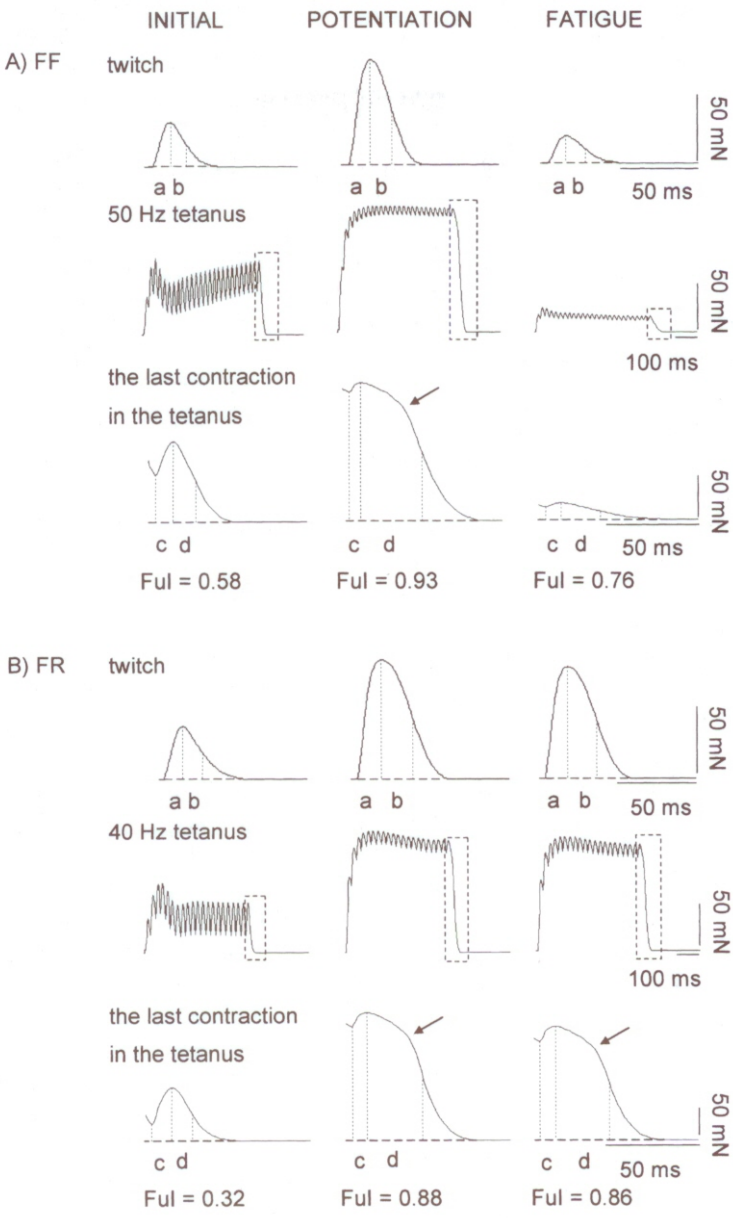

Fig. 2. - Changes in the courses of a single twitch, unfused tetanus and the last contraction within the tetanus during the potentiation and fatigue in FF and FR MUs.
In A and B panels, the upper row presents single twitches, the middle row presents the unfused tetani evoked at 50 Hz in FF MUs (A) and 40 Hz in FR MUs (B), the lower row presents the last contractions from the frames at the enlarged time scale. Note the differences between the initial contraction and contractions during the potentiation and the fatigue with respect to the contraction times (a and c) and the half-relaxation times (b and d). The arrows indicate the biphasic relaxation in the last contractions of tetani with relatively high fusion index (over 0.8).

FF MUs, and 137.93 ± 38.32% for FR MUs (the difference between two types of MUs non-significant, $p > 0.05$) (Table 1). The mean values of CT of these contractions shortened to 85.07 ± 17.11% and 89.56 ± 16.01% of the initial values, for FF and FR MUs, respectively (the difference non-significant, $p > 0.05$), whereas the mean values of the HRT prolonged markedly to 170.11 ± 55.80% and 147.79 ± 50.28% of the initial values, for FF and FR MUs, respectively (the difference non-significant, $p > 0.05$) (Table 1). The potentiation more effectively influenced the HRT than the CT. Figure 3A shows linear relationship between the force increase during the potentiation and the parallel prolongation of the HRT in two types of units. As an effect of the potentiation the increase of fusion indices was also observed, from the mean value of 0.59 to 0.80 in FF MUs, and from 0.68 to 0.84 in FR MUs (Table 1). In both FF and FR MUs, the increase of tetanic fusion was strongly related to the prolongation of the HRT (Fig. 3B). Additionally, this prolongation-related increase of fusion degree was accompanied by the characteristic biphasic relaxation course, which was observed in most of the potentiated tetani (25 unfused tetani of FF MUs with the fusion indices between 0.75 and 0.94 and 22 unfused tetani of FR MUs with the fusion indices between 0.74 and 0.94). In the initial tetani this effect was present only in 7 unfused tetani of FF units with the fusion indices between 0.82 and 0.90 and 14 FR unfused tetani with the fusion indices between 0.79 and 0.90.

When the process of fatigue developed, the decrease of tetanic force was observed in two types of MUs, although in FF MUs the amplitude of this change was higher than in FR MUs. For FF MUs, at the end of the applied stimulation pattern the mean force measured for the last contraction within the tetanus was considerably lower than the initial one (46.00 ± 38.26%), whereas for FR MUs amounted to 107.04 ± 40.74% of the initial value (the difference between two types of MUs significant, $p < 0.01$) (Table 1). The mean values of CT decreased in both types of units and amounted to 94.41 ± 13.49% and 94.93 ± 16.43% of the initial time duration, for FF and FR MUs, respectively (the difference non-significant, $p > 0.05$), but were higher than during the potentiation period. The mean values of HRT considerably shortened in relation to the potentiated tetani, but still amounted to 104.80 ± 35.82% and 134.11 ± 51.23% of initial values, for FF and FR MU, respectively (the difference significant, $p < 0.05$) (Table 1).

In both types of MUs studied, the decrease of fusion indices was noted during the fatigue, in comparison to the potentiated tetani. At the end of the experiment, the mean values of the fusion indices amounted to 0.58 for FF MUs and 0.78 for FR MUs (Table 1) and in many investigated unfused tetani (with the fusion indices below 0.79 or 0.76 for FF and FR units, respectively) the monophasic relaxation was restored (Fig. 2). This concerned 20 unfused tetani of FF units and 4 unfused tetani of FR units.

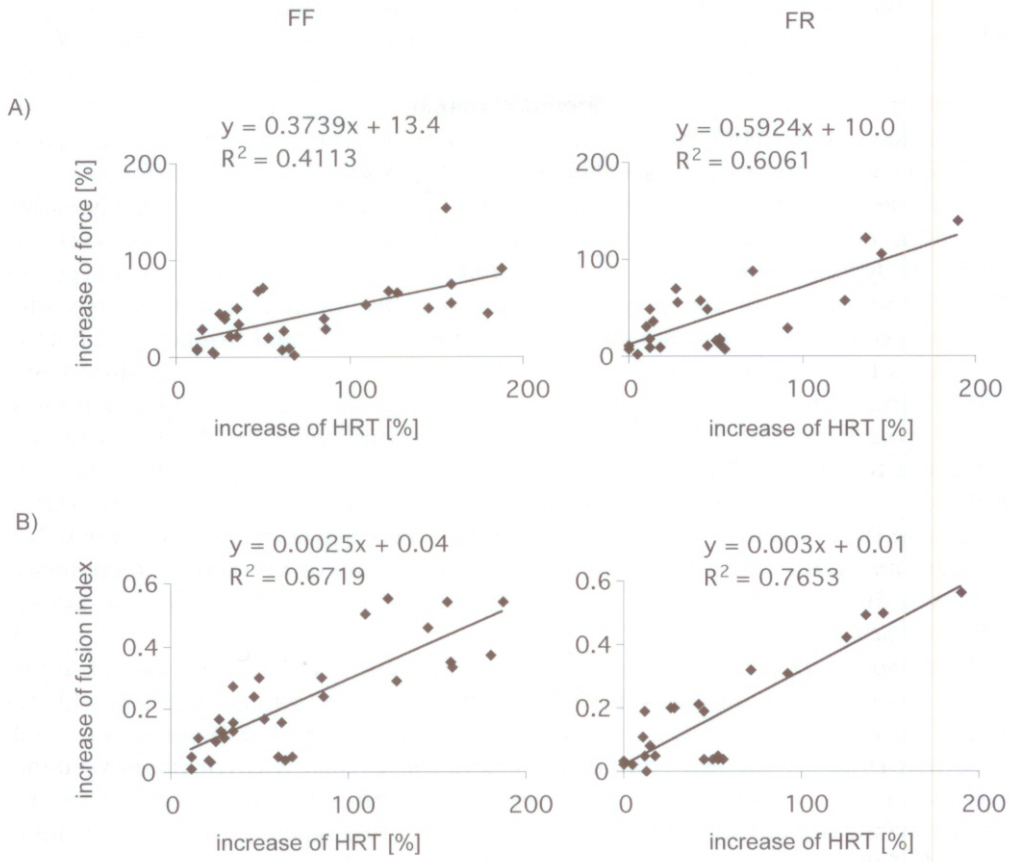

Fig. 3. - The increase of force (A) and the fusion index (B) as a function of the increase of the HRT in the last contractions of potentiated tetani in FF and FR motor units.

DISCUSSION

The phenomena of potentiation and fatigue within unfused tetani of MUs have been commonly observed and discussed in many previous papers. However, the relationship between the twitch time parameters and these two processes hasn't been fully understood. Several authors have stressed the influence of the CT and/or HRT on the fusion degree and suggested the connection of these time parameters with the development of force, its increase or decrease (3, 7, 20, 27). Our results confirm data presented in the former papers that changes in tetanic force, either potentiation or fatigue, develop earlier and are more pronounced in FF than in FR MUs (7, 10). These observations may partly be explained by more dynamic changes of time parameters within successive contractions summating into the tetanus as observed in FF MUs. In the previous papers we have proved that in the rat muscle the course of FF and FR unfused tetani differs considerably, mainly with

respect to the rate of changes in contraction and relaxation times in successive components, which is higher in FF MUs (13, 15). As a consequence, different profiles of FF and FR units' tetani (the rate of force increment at the beginning of a tetanic contraction and its shape related to the appearance of a sag) can be observed. As a likely reason different rates in calcium ions release and removal between two types of MUs have been proposed (15).

The present study has revealed, either for twitches or contractions within unfused tetani, that during potentiation the dominant role play changes in the course of relaxation. Raikova *et al.* (34) have demonstrated greater variability of this parameter in comparison to the contraction time in 115 studied, normalized twitches in both types of fast MUs. However, the relaxation of the twitch is shorter in FF than in FR MUs (8, 13, 14, 21, 29). In this paper we have shown that despite this shorter relaxation in FF MUs, the prolongation of the HRT during the potentiation is more distinct than in FR MUs. Obviously, the HRT prolongation leads to the increase of force produced by potentiated FF MUs and quick changes towards higher fusion degree of successive tetani (Fig. 3). This relationship between the fusion degree and the contraction and relaxation times at variable stimulation frequencies has been investigated and widely discussed in our previous paper (14). It has been concluded that the shape of relaxation in individual components of tetanic contractions depends on the fusion degree and at increasing stimulation frequencies the biphasic course of the relaxation (composed of two parts of force decrease: the slow phase followed by the fast one) appears, what significantly influences the process of summation of successive contractions within the tetanus. Potentiation-related changes of fusion degree, established in the present study, are in agreement with the above cited results – when the HRT is prolonged, the fusion index increases. It has also been observed during the potentiation in both types of the studied MUs that the increase of the fusion index to about 0.75 (even at the same stimulation frequency) coincides with appearance of the biphasic relaxation. The effect of potentiation on the biphasic relaxation course has been more pronounced in FF units, initially presenting less fused tetanic contractions. It is noteworthy to stress that this potentiation-related biphasic relaxation in highly fused tetani is as regular as during high-frequency stimulation of unpotentiated MUs (14). Therefore, this biphasic relaxation is probably strictly related rather to the fusion degree than rate of stimulation. We would hypothesize that the mechanism responsible for the development of biphasic relaxation might be connected to the changes of Ca^{2+} dynamics as well as cross-bridges kinetics (6, 16, 22, 30).

Celichowski and Grottel (11) have reported in the earlier paper effects of motor unit activity evoked by repeated 10 times series of stimulation trains at frequencies increasing from 1 to 150 Hz on the relationships between force and frequency of stimulation. They have observed shifts of the force-frequency curves first left, to lower frequencies and then right, to higher frequencies, accompanied by changes in the twitch time parameters, corresponding first to the potentiation and then to the fatigue. When the potentiation appears and the curve shifts towards lower stimula-

tion frequencies, the unfused tetani with the same level of force may be evoked at lower frequencies. During voluntary activity the force of fast units also undergoes the potentiation at the initial period of the activity. Klein *et al.* (28) have found that the increase in twitch force following the submaximal conditioning contraction is significantly correlated with the decline in firing rate of active motoneurons. The authors have suggested that the twitch potentiation may help maintain a constant force output despite the decrease in a MU firing rate.

As stated above, during fatigue shift of the steep part of the force-frequency curve towards higher stimulation frequencies is observed, indicating that the same stimulation rate is unable to evoke the same force as at the beginning of a MU activity (11). Gardiner and Olha (19), and Celichowski (7) have suggested that a decrease of force visible during fatigue is related to shortening of the twitch time parameters. The present results also confirm these suggestions. For the fatigued twitches, the shortening of time parameters is especially noticeable, not only in relation to the potentiated values but to the initial values as well. On the other hand, for the last contractions within the unfused tetani this shortening concerns exclusively the HRT, and is less markedly visible – in relation to the potentiated values only. It is worth mentioning that in parallel to the shortening of the HRT, the fusion degree of successive tetanic contractions has decreased during fatigue what has resulted in restoring the monophasic relaxation, characteristic for relatively slightly fused tetani. This effect has been mainly observed in FF units – with more pronounced decrease of fusion indices in fatigued tetani, in comparison to FR units.

In conclusion, it has been found that changes of force during the potentiation and the fatigue are accompanied by changes in the time parameters, mainly the HRT. Changes in the twitch time parameters influence the force and the fusion degree of unfused tetani of MUs. During the potentiation, the HRT is significantly prolonged and this prolongation is crucial for the increase of the fusion degree. During the fatigue, the HRT becomes shorter, and the fusion degree is decreased.

SUMMARY

The contraction and relaxation times of the twitches and the last contractions within 32 unfused tetani of FF and 27 unfused tetani of FR motor units in the rat medial gastrocnemius muscle were studied during prolonged activity. The pattern of the MU stimulation included single pulses (to evoke twitches) and series of three trains of stimuli at 40, 50 and 60 Hz (to evoke unfused tetani), repeated 30 times. The analysis concerned changes of force and time parameters at the beginning of activity, during the potentiation and then during the fatigue. It was found that changes of force during the potentiation and the fatigue were mainly accompanied by changes in the course of relaxation. The significant prolongation of the half-relaxation time during the potentiation of either twitches or unfused tetani was revealed in both types of fast MU. The twitch contraction time did not change

markedly, whereas significantly shortened in the last contractions of unfused tetani during the potentiation. These changes of time parameters correlated to the increase of the fusion degree. During the fatigue, the time parameters shortened, however, changes of the half-relaxation times were remarkably higher. The shortening of relaxation was responsible for the decrease of the fusion degree. Changes of the fusion index exceeding 0.75 during the potentiation or decreasing below this value during the fatigue, were accompanied by respective appearance or disappearance of the biphasic relaxation.

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