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MONOPHASIC AND BIPHASIC RELAXATION DURING MOTOR UNIT TETANIC CONTRACTIONS OF VARIABLE FUSION DEGREE

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INTRODUCTION

The unfused tetani of motor units (MUs) consist of successive contractions summating in a nonlinear process (28, 29). Mechanisms underlying this summation, the force development, shape of a tetanus and its fusion degree strictly depend on the time parameters, i.e. contraction time (CT) and half-relaxation time (HRT) (11). The course of the relaxation is one of the main factors determining the effectiveness of summation due to its direct influence on the following contraction in the tetanus (8). Burke *et al.* (4) were the first to study the time course of successive contractions in the unfused tetanus of feline medial gastrocnemius fast MUs. They observed that within several first contractions CT shortened and then remained stable. However, the analysis of relaxation was neglected by these authors. The course of individual contractions within voluntary activity was investigated by Nordstrom *et al.* (26). They studied the time course of single MUs twitches in human muscles using spike – triggered averaging method (STA). Results obtained by this group showed that in better fused tetani CT shortened and the HRT prolonged. Grottel and Celichowski (18) revealed that this observation concerns all three types of MUs in the rat medial gastrocnemius muscle: fast fatigable (FF), fast resistant to fatigue (FR) and slow (S). They analyzed the time course of the last contractions within tetani with variable fusion degree. The gradual prolongation of the relaxation was observed in parallel to the increase of fusion degree and this effect was significantly stronger for slow MUs than for fast ones. Later investigations confirmed that in all three types of MUs, the better tetanus was fused the shorter CT and the longer HRT were measured (8).

In many papers describing the analysis of time parameters, in particular the course of relaxation in reference to the fusion, biphasic relaxation was visible (1, 8, 13, 14, 18, 19, 30). Westerblad and Lännergren (30) observed changes in biphasic relaxation during fatiguing activity and acidosis in unfused and fused tetani of single mouse muscle fibres. They divided the relaxation into initial slow isometric phase, where nearly linear force decline can be observed, followed by a faster exponential phase of force decline. Similar biphasic course was indicated by Fitts and Widrick (14) in rat extensor digitorum longus tetanic contraction. However, this interesting phenomenon hasn't been investigated for individual types of MUs so far. Moreover, the conditions under which the biphasic relaxation appears

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are unknown. In the present paper we investigated the course of relaxation in tetani fused to a variable degree for three main types of MUs in the rat medial gastrocnemius muscle (MG). Our purpose was to describe the relationship between the tetanic fusion degree and the development of biphasic relaxation.

MATERIAL AND METHODS

Experiments were performed on 6 female adult Wistar rats (mean weight 299 ± 31 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 shape of pupils and pinna reflexes. 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).

Motor units of the MG muscle were investigated. The procedure of the surgery was described in details elsewhere (6, 7). Briefly, the MG muscle and the respective branch of 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, 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 was connected to an inductive force transducer to measure the contractile force under isometric conditions. The muscle was stretched up to a passive force of 100 mN, to achieve the maximal twitch force of MUs (9). The muscle fibre 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 ventral root 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 muscle fibre action potentials in response to stimuli of increasing amplitude indicated the activity of a single MU.

All investigated MUs were stimulated according to the following protocol: *1)* 5 stimuli at 1 Hz (averaged twitch was estimated from 5 single twitches), *2)* 10 ms interval, *3)* series of stimuli at 10, 20, 30, 40, 50, 60, 75, 100 and 150 Hz with 500 ms duration and 10 ms intervals between trains (unfused tetani were recorded, except 150 Hz, when fused tetani were obtained), *4)* 10 ms interval, *5)* the fatigue test (14 stimuli at 40 Hz, repeated every second during 180 s) (3).

Properties of the twitch, unfused tetanus and fused tetanus were analyzed for each motor unit. CT (from the onset of the rise in force to the peak), HRT (from the peak to the half of its maximum), the twitch force (TwF, from the baseline to the peak) were calculated from the averaged twitch record and the maximal tetanus force (TetF, from the baseline to the peak) was measured from the 150 Hz tetanus record. For each unfused tetanus, the fusion index (FuI, ratio of the distance from the baseline to the maximal relaxation before the last contraction of the tetanus to the amplitude of this last contraction) (Fig. 1) was calculated (2, 10). The fatigue index (FI, a ratio of force generated in the 120^{th} s after the maximal initial force to this maximal force) was calculated from the standard fatigue test (3, 17, 22).

All MUs tested during experiments were classified as fast or slow ones on the basis of sag appearance in unfused tetani of fast MUs at 40 Hz stimulation. Then fast MUs were divided into fast fatigable (FF) and fast resistant to fatigue (FR) on the basis of the fatigue index, which was below 0.5 for FF and over 0.5 for FR MUs (3, 23).

Besides measurement of HRT, the course of the relaxation was analyzed using a quarter-relaxation time (QRT, time measured from the peak to the quarter of the maximum force) – the new parameter introduced in present paper (Fig. 1). The time course of the relaxation (HRT and QRT) was analyzed in the twitch evoked at 1 Hz and in the last contractions within the unfused tetani evoked at stimulation frequencies in a range between 10 and 100 Hz for fast and between 10 and

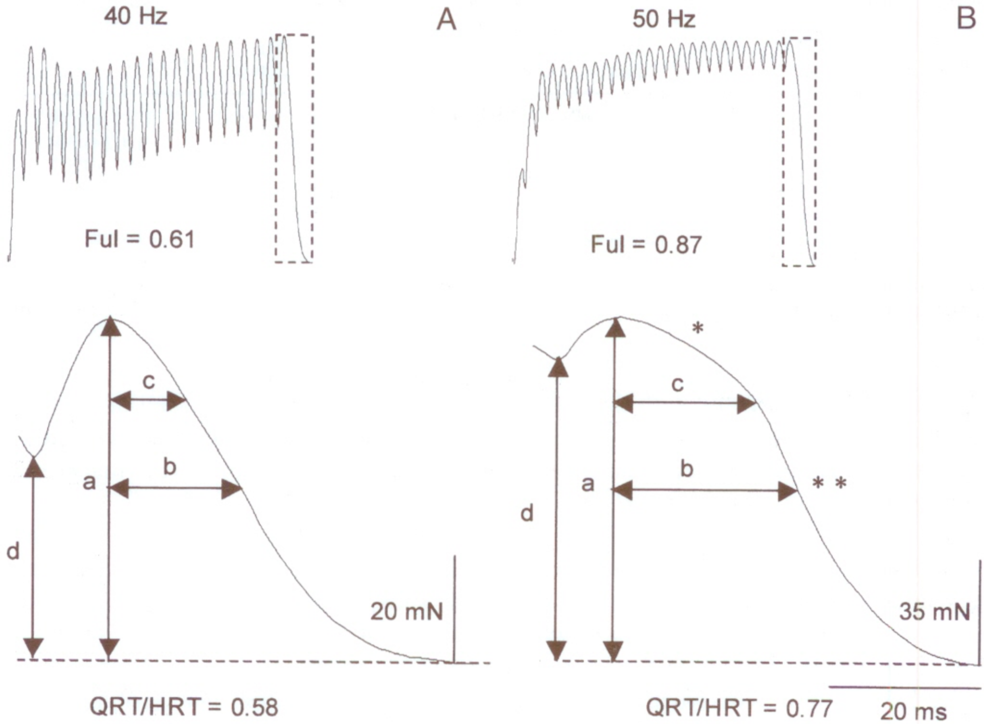

Fig. 1. - *Examples of FF motor unit unfused tetani and the parameters measured.*

Upper records present two unfused tetani evoked at 40 Hz (A) and 50 Hz (B). The last contractions from the frames are presented at lower records, at the enlarged scale; a) the peak force, b) the half relaxation time (HRT), c) the quarter relaxation time (QRT), FuI – fusion index, expressed as a d/a ratio. For the tetanus evoked at 40 Hz (A), the monophasic relaxation course was visible, while at 50 Hz (B) – the biphasic relaxation course (* the first phase, ** the second phase).

40 Hz for slow MUs. Figure 1 presents measured parameters: the peak force (*a*), HRT (*b*), QRT (*c*), and FuI (expressed as a *d/a* ratio). The QRT and HRT reflecting the time course of the relaxation were calculated only for the last contraction within the tetanus because in previous contractions these parameters can not be measured due to the beginning of the next contraction. Moreover, the QRT/HRT ratio was calculated for all MUs as a parameter describing the course of relaxation.

RESULTS

For the purpose of the study, 16 FF, 16 FR and 10 S MUs with low noise-to-signal ratio were used. Mean values and variability ranges of contractile properties of the twitch, unfused and fused tetani for all studied units are given in Table 1. Mean values and ranges of time and force parameters as well as fatigue indices were consistent with previously obtained data for motor units in the rat MG (8, 18).

Figure 2 presents examples of the last contractions recorded within tetani fused to a variable degree of one FF motor unit. The asterisks indicate the biphasic relaxation, which

Table 1. - *Main contractile properties of the three types of motor units.*

	CT (ms)	HRT (ms)	TwF (mN)	TetF (mN)	FI
FF	12.41 ± 1.41	12.08 ± 3.80	41.39 ± 15.81	142.81 ± 55.67	0.21 ± 0.10
(n = 16)	10.6-15.5	8.6-22.4	18.5-81.1	63.7-233.0	0.02-0.30
FR	13.29 ± 1.30	14.77 ± 3.66	15.75 ± 5.70	75.39 ± 17.19	0.87 ± 0.07
(n = 16)	11.1-16.4	9.7-21.1	8.3-27.0	50.7-119.0	0.72-0.98
S	22.44 ± 2.95	35.49 ± 6.69	4.90 ± 1.51	38.66 ± 11.55	0.93 ± 0.04
(n = 10)	17.6-28.0	25.5-43.0	2.4-6.7	21.9-57.3	0.86-0.99

For each type of motor units: upper line – mean values ± standard deviations, lower line – variability range. CT, the contraction time; HRT, the half-relaxation time; TwF, the twitch force; TetF, the tetanus force; FI, the fatigue index.

appeared at 50 Hz tetanus with the FuI of 0.87, but was invisible in tetani at lower frequencies of stimulation, at the FuI 0.61. Similar observations of the monophasic relaxation at lower, and the biphasic relaxation at higher stimulation frequencies were made for all investigated motor units, both fast and slow.

The analysis of QRT to HRT ratios for the last contractions of tetani with increasing fusion indices revealed that gradual changes of this parameter coincided with changes in the shape of the relaxation of the last contraction. Thus, the calculation of QRT/HRT ratio might be useful to classify the relaxation as a monophasic or a biphasic. For each MU of either type, values of QRT/HRT were grouped into two ranges: the first one, corresponding to the lower stimulation frequencies when monophasic relaxation was visible, and the second one, corresponding to the higher stimulation frequencies when biphasic relaxation appeared (Fig. 3). The monophasic relaxation was observed until the values of QRT/HRT amounted up to the means of 0.63 ± 0.04, 0.66 ± 0.04 and 0.59 ± 0.07 for FF, FR and S units, respectively. The biphasic relaxation appeared in tetani with the mean values of QRT/HRT of at least 0.75 ± 0.06, 0.77 ± 0.03 and 0.74 ± 0.04 for FF, FR and S units, respectively (Table 2 and Fig. 4).

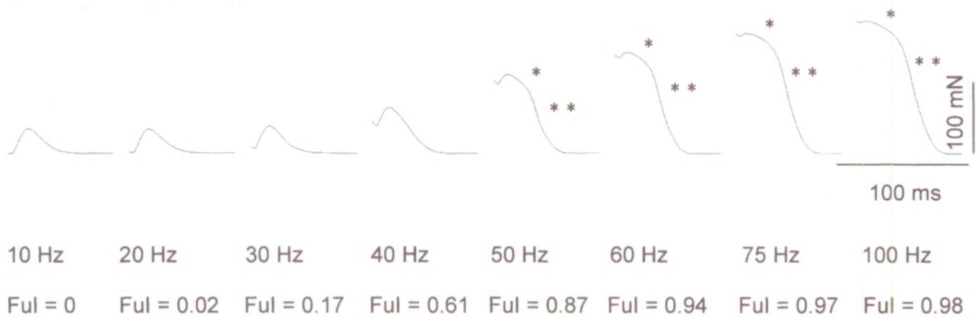

Fig. 2. - *Development of the biphasic relaxation in parallel to the increase of the fusion index (FuI).*

Records of the last contractions from the FF MU unfused tetani, evoked at 10-100 Hz stimulation. The asterisks indicate the biphasic relaxation: the slow and isometric phase (*) followed by the fast and exponential phase (**).

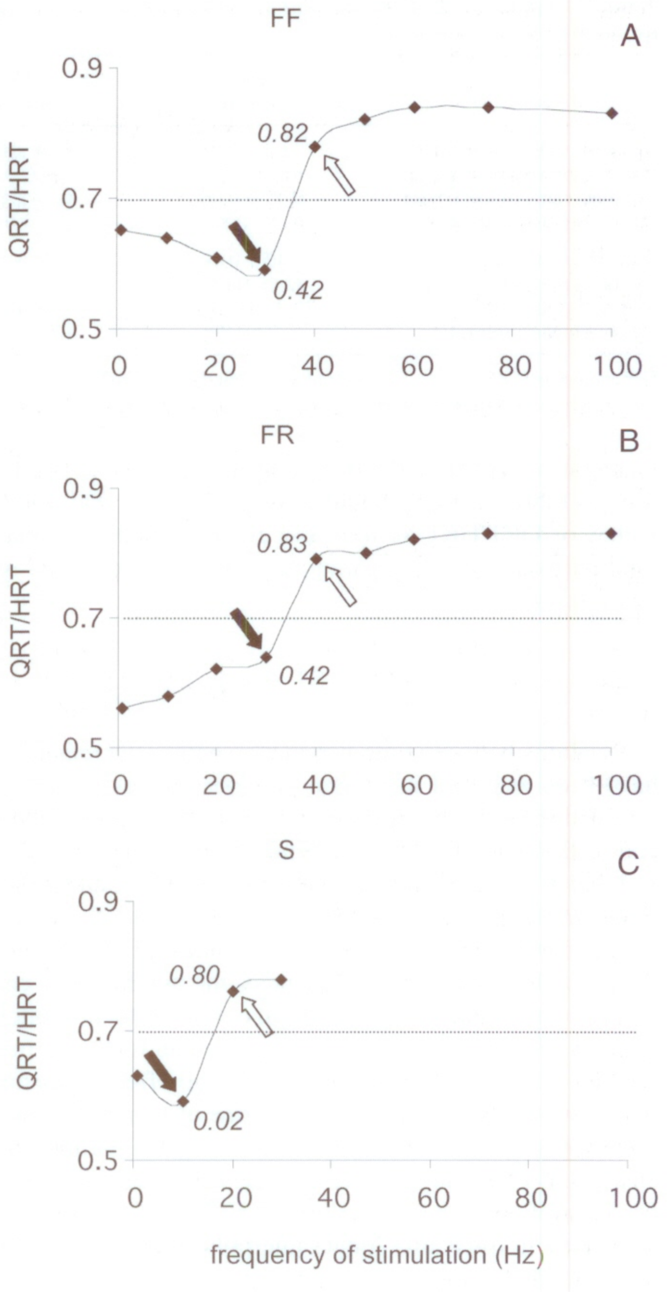

Fig. 3. - *Examples of QRT/HRT values as a function of the stimulation frequency for FF (A), FR (B) and S (C) motor units.*

QRT/HRT values were calculated for twitches and the last contractions in unfused tetani. Data for fused tetani, i.e. for 150 Hz for FF and FR MUs and 40-150 Hz for S MU are not presented. QRT/HRT values under dotted horizontal line correspond to the monophasic relaxation, over this line – to the biphasic relaxation. Black arrows indicate the maximal values of fusion indices (italics) when the monophasic relaxation was still visible, white arrows indicate the minimal values of fusion indices when biphasic relaxation appeared.

For each MU the border values of the fusion index where monophasic relaxation switched into biphasic one could be found (Fig. 3). The monophasic relaxation could be observed for FF, FR and S MUs when the FuI amounted up to the mean of 0.40 ± 0.19, 0.50 ± 0.17 and 0.17 ± 0.26, respectively. The biphasic relaxation could be observed when the

Table 2. - *Values of QRT/HRT and FuI describing the monophasic and biphasic course of the relaxation for three types of motor units.*

	FF (n = 16)	FR (n = 16)	S (n = 10)
Maximal value of QRT/HRT for the monophasic relaxation	0.63 ± 0.04 (0.57-0.70)	0.66 ± 0.04 (0.60-0.75)	0.59 ± 0.07 (0.48-0.68)
Minimal value of QRT/HRT for the biphasic relaxation	0.75 ± 0.06 (0.65-0.82)	0.77 ± 0.03 (0.70-0.82)	0.74 ± 0.04 (0.67-0.78)
Maximal value of FuI for the monophasic relaxation	0.40 ± 0.19 (0.06-0.65)	0.50 ± 0.17 (0.17-0.81)	0.17 ± 0.26 (0.01-0.85)
Minimal value of FuI for the biphasic relaxation	0.80 ± 0.06 (0.70-0.89)	0.86 ± 0.05 (0.72-0.93)	0.89 ± 0.08 (0.75-0.97)

For each panel: upper line – mean values ± standard deviations, lower line – variability range. QRT/HRT, the quarter relaxation time to the half relaxation time ratio; FuI, the fusion index.

mean FuI was over 0.80 ± 0.06, 0.86 ± 0.05 and 0.89 ± 0.08 for FF, FR and S units, respectively (Table 2). One should notice, however, that within each type of MUs, maximal values of QRT/HRT as well as FuI for monophasic relaxation slightly overlapped with minimal values of these parameters measured for tetani with biphasic relaxation (Table 2 and Fig. 4).

DISCUSSION

Relations between time parameters within the tetanic contraction and its fusion degree have been analyzed in several previous studies (8, 18, 26). Nordstrom *et al.* (26) have revealed the close relationship between the degree of the tetanic fusion and the course of contraction and relaxation in human masseter MUs. Using STA method they have observed that at higher motoneuronal firing rate the contraction shortens and the relaxation prolongs in parallel to increasing force and fusion of contractions. Similar results have been obtained in feline soleus and plantaris muscles by Parmiggiani *et al.* (27) who have found that at higher stimulation frequencies the amplitude of contractions is lower and the time course of the relaxation is longer. In our laboratory, Grottel and Celichowski (18) have studied the influence of irregular stimulation on force and fusion of slow and fast MUs in the rat MG muscle and have noticed that the effect of decreasing and increasing interpulse intervals strictly depends on the degree of tetanic fusion. They have revealed that for all three types of MUs in tetani of increasing fusion, the time course of the last contraction changes: CT becomes shorter whereas HRT markedly prolonged. In these experiments they have also showed that in relatively well fused tetani the prolongation of relaxation prevents a decrease in force after longer interpulse interval. This effect is the most prominent when the fusion index exceeds approximately 0.75.

Celichowski and Bichler (8) have also investigated the time parameters of MU contractions in relation to the tetanic fusion. Their analysis has confirmed earlier observations that in parallel with an increase of the FuI a shortening of the CT and the prolongation of the HRT are observed, and authors have stressed that changes of HRT are better developed in comparison to changes in CT. Moreover, these effects are stronger in slow MUs than in fast

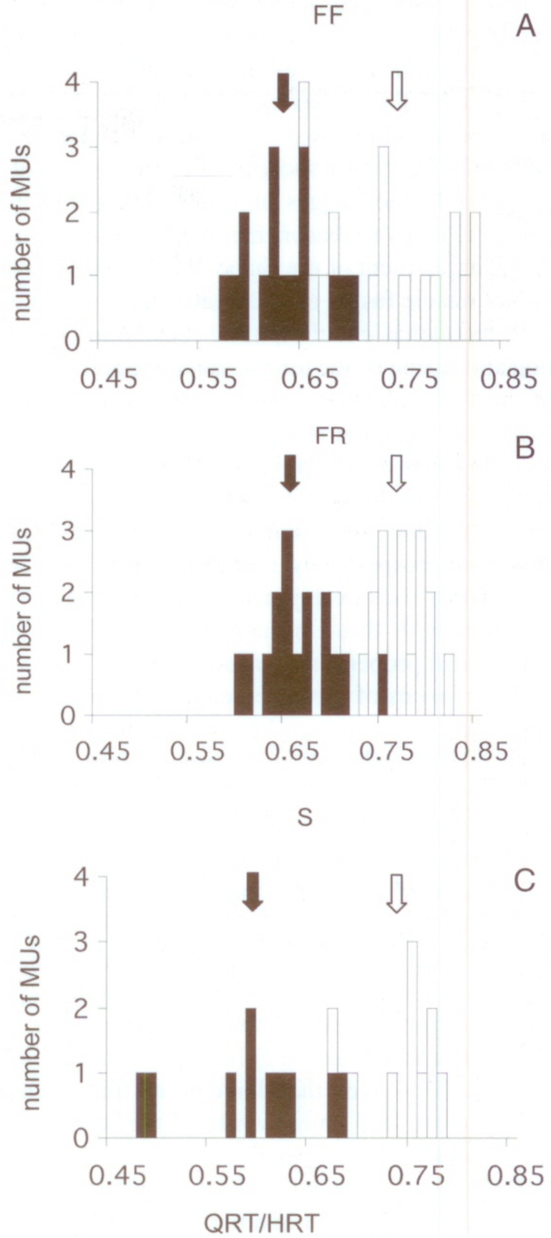

Fig. 4. - *Distribution of QRT/HRT values among FF (A), FR (B) and S (C) MUs.*

Black bars in the histograms refer to the maximal values of QRT/HRT for the monophasic relaxation (black arrows indicate the mean values), white bars refer to the minimal values of QRT/HRT for the biphasic relaxation (white arrows indicate the mean values).

ones. Finally, they have reported changes in force within the last component of tetanic contractions. The maximal velocity of the force decay during the relaxation has been calculated in tetani with fusion indices of 0.79, 0.98 and 0.95 for FF FR and S MU respectively. For only slightly fused tetani (with FuI below 0.5) this velocity has appeared to be significantly lower.

The present paper is the first attempt to describe in detail the biphasic relaxation observed during motor unit activity, although this phenomenon has been obviously visible on figures in numerous previous reports (1, 8, 13, 14, 18, 19, 30). As a tool for this purpose the QRT/HRT ratio has been introduced. The data analysis has proved that this parameter might be useful to classify relaxation as a mono- or biphasic. In each individual motor unit, QRT/HRT ratios for successive tetani evoked at various stimulation frequencies have felt into two distinct ranges of values (compare Fig. 3). Thus, calculations of this parameter appear to be more reasonable in differentiating the mono- and biphasic relaxation than pure visual assessment of the shape. We have investigated the course of the relaxation as a function of tetanic fusion and have attempted to establish the border range between contractions with monophasic and biphasic relaxation. The dependence between the course of relaxation and fusion of the tetanic contraction has been evident in each motor unit: at low values of the FuI relaxation exhibits one phase of force decline, whereas at high values of FuI (over 0.8 for three types of MUs) the biphasic course of relaxation is clearly visible. Despite slight overlapping between border values that has been noticed in few cases within each type of motor units (see Fig. 4), considerable differences have been found between maximum and minimum mean values of fusion indices for monophasic and biphasic relaxation, respectively (compare Table 2). This relationship suggests that mechanisms responsible for development of the biphasic relaxation within the tetanus have much in common with those influencing the effectiveness of summation of twitches and with mechanical properties of muscle fibres.

The reasons of force decrease and biphasic course of the relaxation have been largely discussed in literature (14, 16, 20, 24, 25, 30). Macintosh *et al.* (24) have stressed on main factors influencing the time course of mechanical relaxation: the rate of the dissociation of Ca^{2+} from troponin C preventing reattachment of cross-bridges, the rate of Ca^{2+} removal from sarcoplasm by parvalbumin and accumulation of ions Ca^{2+} by the sarcoplasmic reticulum aiming to decrease free Ca^{2+} concentration and the rate of cycling the cross-bridges. These factors have been also considered as mechanisms responsible for the biphasic relaxation (30). Cannell (5) has studied Ca^{2+} handling and has revealed that during the initial, linear phase of relaxation the intracellular Ca^{2+} concentration falls rapidly, whereas during the later, exponential phase, it declines slowly, remains more or less constant or even transiently increases. Huxley and Simmons (21) as well as Cleworth and Edman (12) have interpreted biphasic relaxation in terms of cross-bridge kinetics. Moreover, they have noted that in unfatigued fibres the relaxation is isometric during the linear phase while at the transition to the exponential phase the intrafibre movements start to appear.

Differentiation between the monophasic and biphasic relaxation is important for studies of processes of the summation of successive contractions into tetanus. The course of contraction and relaxation determines force development and should be considered during modeling. However, in all mathematical studies only monophasic models of relaxation have been used so far (15, 29). Improved model of time parameters during tetanic contractions ensures also more precise extracting individual twitches from tetanus. We expect that phenomenon of the biphasic relaxation might be more precisely analyzed by means of decomposition of unfused tetani with relatively high FuI.

SUMMARY

The phenomenon of transition of the monophasic relaxation into biphasic course in the unfused tetanic contractions was studied on functionally isolated motor units of the rat medial gastrocnemius muscle. The sample consisted of 16 FF, 16 FR and 10 S MUs which were stimulated with the same, digitally controlled patterns. The new parameter – QRT/HRT ratio, was introduced as a convenient tool for the classification of the relaxation into monophasic or biphasic. Analysis of tetani evoked at increasing stimulation frequencies revealed similar relationships between the tetanic fusion degree and the shape of relaxation for all three types of motor units investigated. In each MU, the QRT/HRT ratio fell into two distinct ranges related to either monophasic (lower values) or biphasic (higher values) relaxation. The relationship was also found between the shape of relaxation and degree of tetanic fusion – the biphasic course appeared for better fused tetani when fusion index was over the mean of 0.8. Mechanisms of development of the biphasic relaxation were discussed with respect to importance of this parameter in force development and summation of successive contractions into tetanus. Moreover, it was pointed out that adequacy of mathematical modeling of motor unit contractions should benefit from the precise analysis of the mono- or biphasic course of relaxation.

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