JOURNAL OF HUMAN KINETICS — VOLUME 1, 1999
ANTROPOMOTORYKA NR.19–20

VERIFICATION OF HYPOTHESES OF „MYOFIBRILLIC" AND „CALCIC" POST-TETANIC POTENTIATION OF MUSCLE

by

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The aim of the study was to determine the effect of pattern of tetanic stimulation on the time course of subsequent twitches tension. The quadriceps muscle of healthy men (n=9) (aged 28–37) (weight 74,3±6.2) was studied. The following data were registered: the twitch force of the quadriceps muscle (Pt), muscle contraction time (CT) and half force relaxation time (RT) during twitch. Experimental protocol: a single twitch was evoked before and 500 ms, 1 s, 3 s, 5 s, 10 s, 30 s, 60 s after 5-s of 50 Hz stimulus train. Following muscle stimulation at 50 Hz there was a considerable increase ($p<0.05$) in Pt but RT decreased ($p<0.05$) and didn't recover after 60 s following electrostimulation. Thus, it has been established that in the period from 0.5 s to 3 s immediately after a brief muscle stimulation there occur considerable changes in Pt, CT and RT which should be considered when analysing Pt, CT and RT.

Key words: skeletal muscle, twitch contraction, post-tetanic potentiation

Introduction

The causes of muscle force changes during muscle contraction fall into three categories: 1) reduced Ca^{2+} release from the sarcoplasmic reticulum (SR), 2) changes in Ca^{2+} sensitivity of the myofiiaments, and 3) reduced Ca^{2+} -activated tension (Walker et al. 1992, Westerblad and Lännergren 1991). During brief muscle contractions phosphorylation of myo sin regulatory light chains (RLC) induces an increase in sensitivity of myofilaments to Ca^{2+} (Metzger et al. 1989, Moore and Stull 1984), while there are no significant changes in Ca^{2+} release from SR and Ca^{2+} -activated tension (Westerblad and Lännergren 1991). It has been shown that the mechanism of phosphorylation of myo sin RLC alone can not account for potentiation of muscle fibres (MacIntosh et al. 1993). The effect of preceding muscle activity on force and relaxation rate of brief contractions depends on pause duration in between (Rail 1996). Phosphorylation of

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RLC (Metzger and Moss 1990, Metzger et al. 1989, Moore and Stull 1984), changes in kinetics of Ca^{2+} (Miledi et al. 1983, Rail 1996) and mechanics of sarcomeres (Edman and Johansson 1976, Enoka 1994, Winter and Brookes 1990) during brief and intensive activity can influence (improve or worsen) muscle contraction and relaxation without causing fatigue. It is due to such activities that twitch force increase (Vandervoort et al. 1983), but tetanic force decrease (Shoubridge and Radda 1987). Stimulation of muscle without applying maximum frequencies however may result in decrease of force of contraction (Edman and Johansson 1976). It is the so-called „sag" phenomenon the mechanism of which is still not clear but it is supposed to depend on kinetics of Ca^{2+} (Edman and Johansson 1976). We believe that three main mechanisms, i.e. kinetics of Ca^{2+}, phosphorylation of myosin RLC and muscle mechanics, may influence muscle contraction and relaxation registered immediately afier a brief muscle electrostimulation. The latter circumstance should be taken into account when recording and analysing Pt, CTand RT.

The aim of the study was to determine the effect of pattern of tetanic stimulation on the time course of subsequent twitches tension.

Methods

Subjects. The quadriceps muscle of healthy men (n=9) (aged 28–37) (weight 74,3±6.2) was studied, The subjects were physically active but none took part in any formal of physical exercise or sport.

Apparatus. The equipment and technique of measuring force was the same as that used in our previous study (Ratkevicius et al. 1995). Subjects sat in a straight-backed dynamometer chair with restraining straps placed across the hips. There was 90^0 angle in the knee joint. A cuff was placed around the right lower leg just above malleolus. The cuff was connected with a strain gauge by a metal bar. The output of the transducer, proportional to isometric knee extension force, was amplified and digitised at a sampling rate of 1 kHz by a 12-bit analogue-to-digital converter installed in an IBM-compatible AT 286 personal computer. The digitised signal was stored on hard disk for subsequent analysis.

Electrical stimulation. A voltage stimulator (Medicor MG 440, Hungary) was used for electrical stimulation of the right quadriceps muscle. The square wave pulses of 1-ms duration (voltage 150 V) were delivered to the muscle through lead electrodes (9–18 cm).

Evaluation of contractile properties. The following data were registered: the twitch force of the quadriceps muscle, aroused by electrical stimulation under 1 Hz (Pt), muscle contraction time (CT) and half force relaxation time (RT) during twitch.

The protocol of experiment. Experimental protocol: a single twitch was evoked before (considered as initial) and 500 ms, 1 s, 3 s, 5 s, 10 s, 30 s, 60 s afier 5-s of 50 Hz stimulus train.

Statistics: Paired Student's t-test was used to evaluate cross time changes.

Results

The results of investigation given in fig. 1 indicate that throughout the period registered (60 s) there was a statistically significant ($p<0.05$) increase in Pt (as compared to control values) and there was a decrease ($p<0.05$) in the values of RT following a muscle stimulation and during the period from 500 ms to 3-5 s after muscle stimulation there was a statistically significant ($p<0.05$) increase in the mean values of Pt and RT. It is worth noting that it was only after 3 and 5 s that CT mean values were significantly ($p<0.05$) higher than the control ones.

Discussion

The main conclusion made after our research is that in the period from 0.5 s to 3 s immediately after a brief muscle stimulation there occur considerable changes in Pt, CT and RT (fig. 1). Following muscle stimulation at 50 Hz there was a considerable increase ($p<0.05$) in Pt but RT decreased ($p<0.05$) and didn't recover after 60 s following electrostimulation (fig. 1).

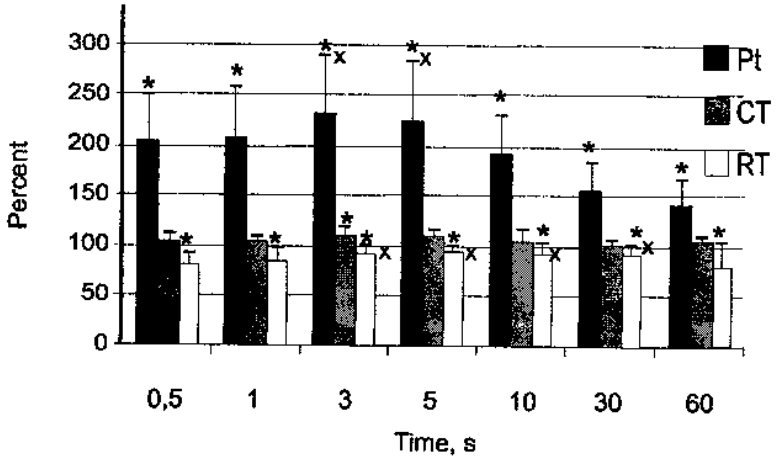

Figure 1. Mean percentage values of Pt, CT and RT (as compared to control ones) following a tetanic muscle electrostimulation (5-s of 50 Hz stimulus train). * – $p<0.05$ from control value, x – $p<0.05$ from 0.5 s average value

"Myofibrillic" hypothesis of post-tetanic potentiation

It was noted that muscle twitch force (Pt) and contraction time (CT) depend on attachment rate of myo sin cross-bridges with actin the process which depends on the amount and speed of Ca^{2+} release from SR, as well as on the sensitivity of

myofibrils to calcium ions (Brenner 1990, Fitts 1994). The latter mechanisms is modified by phosphorylation of myosin RLC, which regulates the transformation rate of myosin cross-bridges attachment to actin from weak state into strong one (MacIntosh et al. 1993, Metzger et al. 1989, Moore and Stull 1984). Due phosphorylation of myosin RLC there is a marked increase in muscle contraction force at low Ca^{2+} concentration (Metzger et al. 1989, Moore and Stull 1984), e.g. during muscle stimulation by one impulse (Vandervoot et al. 1983). The more Ca^{2+} is released from SR, the greater phosphorylation of myosin RLC, the greater the increase in muscle contraction force at consparatively low Ca^{2+} concentration (Metzger et al. 1989, Moore and Stull 1984). Besides, it has been established that even after complete stop of muscle contraction there is immediate discontinuance of phosphorylation of myosin RLC (Moore and Stull 1984).

Thus, according to „myofibrilic" hypothesis of the mechanism of post-tetanic muscle potentiation the greater the frequency of muscle stimulation the greater should be the increase in Pt and decrease in CT while RT should become longer, as it is supposed that there is deterioration in the detachment rate of myosin cross-bridges from actin due to phosphorylation of myosin RLC (Metzger et al. 1989, Moore and Stull 1984). The „myofibrilic" hypothesis is corroborated by the following results of our investigation: gradual decrease in Pt from 3-5 s to 60 s following experiment.

The „myofibrilic" hypothesis however is opposed to by the following results of our experiments:

1. Immediately after stimulation Pt is smaller than 3-5 s after electrostimulation
2. The increase CT 3-5 s after tetanic stimulation with decreased value of RT (fig. 1).

„Calcic" hypothesis of post-tetanic potentiation

It has been established that the rate of development of muscle isometric contraction force is not dependent upon the activity of myo sin ATP-ase while, for instance, CT may depend on the rate and amount of Ca^{2+} release from SR (Cannel and Allen 1984, Cecchi et al. 1984). Besides, the rate of Ca^{2+} release form SR rather modifies Pt and CT than the tetanic muscle contraction force (Cecchi et al. 1984), as during twitch contraction Ca^{2+} do not manage to gain cornplete attachment to troponin-C (TnC) (Cannel and Allen 1984). For instance, with increase in the rate of Ca^{2+} release from SR and with increased sensitiveness of TnC to Ca^{2+} there is an average 65 per cent increase in Pt and but 5 per cent increase in tetanic force (Cecchi et al. 1984). Besides, during twitch Ca^{2+} does not manage to gain complete saturation of TnC (Cannel and Allen 1984). It is believed that the greater the release of Ca^{2+} from SR the greater is the values of Pt and CT (Kugelberg and Thornell 1983).

Muscle relaxation rate depends upon the rate of detachment of myosin cross-bridges from actin which is not directly related to the attachment rate of myosin cross-bridges but is controlled by ATF and the amount of Ca^{2+} (Klein et al. 1991). The regulation of Ca^{2+} concentration in the muscle cell depends upon the capacity of Ca^{2+} pump as well as Ca^{2+} kinetics and the amount of parvalburnin absorbing Ca^{2+}

(Gills et al. 1982, Haiech et al. 1979, Heilmann et al. 1977, Miledi et al. 1983). The longer muscle contraction, the worse muscle relaxation since a more complete absorption of Ca^{2+} by parvalburnin takes place (Haiech et al. 1979, Rail 1996).

The decrease in muscle contraction force when stimulated at low frequencies has been observed by several scholars (Edman and Johansson 1976, Rail 1996). This decrease has been given the name of „sag" phenomenon.

Besides, it has been more markedly expressed in RS of the fast twitch type (Cecchi et al. 1984), but it is questionable though if it manifests itself in human skeletal muscle. The „sag" phenomenon mechanism is still not clear though it is believed to depend upon Ca^{2+} kinetics in myoplasm (Edman and Johansson 1976, Rail 1996). It is supposed that sudden decrease in muscle force at the beginning of activity may depend upon the difference between Ca^{2+} release from SR and its absorption into SR (Edman and Johansson 1976). A number of experiments have been undertaken recently which testify to the fact that the work performed earlier may influence Ca^{2+} kinetics in myoplasm, as a certain period of time is necessary for Ca^{2+} transportation from TnC, parvalburnin to terminal cisternea from which Ca^{2+} is released (Edman and Johansson 1976, Klein et al. 1991, Rail 1996). It has been established that it is only after 3-5 s following muscle contraction that terminal cisternea of SR become fully saturated by Ca^{2+} (Rail 1996) while according to the results of the research carried out by other scientists (Klein et al. 1991) it is only after 20-30 s full recovery of the efficiency of the release of Ca^{2+} from SR takes place. It could be supposed therefore that in our case immediately after electrical stimulation of the muscle this not only calls forth a decrease in the amount of Ca^{2+} release from SR but there is no complete absorption of parvalburnin by Ca^{2+} either. It is due to this reason that immediately after work a decrease in Pt and RT might have taken place. We believe that the lower the frequency of the stimulated muscle the less is Ca^{2+} release from SR and with brief stimulation absorption of parvalbumin by Ca^{2+} is made possible. Provided the muscle were stimulated at higher frequencies and for a longer period of time, as it is well known, it would result in prolonged muscle relaxation (Rail 1996).

Thus, Pt and CT depends upon the amount of the Ca^{2+} release from SR, release rate and sensitiveness of myofibrils to Ca^{2+}. Consequently, decrease in Pt and CT immediately after stimulation at 50 Hz, as compared to values recorded 3-5 s after stimulation, is first of all conditioned by decrease in the amount of Ca^{2+} released from SR.

„Mechanical" hypothesis of regulation of Pt, CT and RT

Muscle contraction and relaxation may also depend upon mechanical factors (Griffiths 1991, Winter, Brookes 1990, Woittiez et al. 1984).

Though in our case the leg was fixed no complete contraction of the muscle at isometric regime was observed, as it has been established (Griffiths 1991) that even applying muscle stimulation at isometric regime myofibrils contract on average by

28 per cent. With contraction of myofibrils some sarcomeres (usually the ones in the centre of myofibrils) may contract considerably while others may become even longer. Furthermore, alongside with changes in the length of sarcomeres there may occur changes in the structure of multiple proteins-surrounding sarcomeres, myofibrils and muscle fibers and it takes time for them to return to the initial state (Enoka 1994). It might be supposed therefore that there exists a certain refractory mechanical period during which even under the same physiological conditions the muscle is not prepared mechanically for developing the greatest contraction force possible. For instance, with decrease in the length of sarcomeres TnC sensitiveness to Ca^{2+} deteriorates (Enoka 1994). It is absolutely not clear however what is the contribution of mechanical factors to the changes of Pt, CT and RT in the case of our experiments.

To conclude, regulation of the changes of muscle contraction and relaxation changes virtually during the first seconds following activity though there is but slight or no muscle fatigue whatever. This may be conditioned by Ca^{2+} kinetics, phosphorylation of myosin RLC, as well as by muscle mechanics and their interaction. The latter circumstance should be taken into account when recording and analysing Pt, CT and RT during the period of their rapid recovery.

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