# Characterization of Tension Decline in Different Types of Fatigue-resistant Skeletal Muscle Fibres of the Frog. Low Extracellular Calcium Effects.

T. RADZYUKEVICH[1], E. LIPSKÁ[2], J. PAVELKOVÁ[2], and D. ZACHAROVÁ[2]

1 *I.M.Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, Thorez av. 44, St -Petersburg 194223, Russia*

2 *Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Vlárska 5, 833 34 Bratislava, Slovakia*

**Abstract.** Twitch and tetanic tension have been measured in single skeletal muscle fibres (m.ileofibularis, *Rana esculenta* and *Rana temporaria*). On the basis of resistance to fatigue produced by repetitive tetanic stable frequency stimulation with various numbers of stimulation trains, twitch fibers were subdivided in three groups (resembling those as described by Westerblad and Lännergren 1986 in *Xenopus*), that is fatigue-resistant (FR), moderately fatigued (MF) and easily fatiguing (EF). It was found futher that the fibres differ in tetanic tension decline resistance i.e. fatiguability relating to some basic contractile parameters including the amplitude, the rates parameters of twitch and tetanus tension as well as the tetanus/twitch tension ratio. The main differences observed concern: 1) The inability to maintain the maximum tetanic tension plateau (IMT) during single tetanus. IMT was 18 times higher in EF fibres and 4 times higher in MF fibres, respectively, in comparison with FR fibres. IMT is the first parameter to change significantly during repetitive tetanic stimulation. 2) The different fibre types show pronounced differences in twitch contraction and tetanus tension during repetitive tetanic stimulation. There is a conspicuous facilitation of twitch tension during and after cessation of repetitive stimulation in FR fibres; the MF and EF fibres show, on the contrary, a depression of twitches. 3) Recovery to original (prefatigue) values is rapid in FR fibres, but slow, however, in EF fibres. 4) Removal of extracellular $Ca^{2+}$ intensified the inability to maintain the maximum tetanic tension (IMT) and the tetanic tension decline, especially in fibres with an initial high fatigue-resistance.

We assume that the results might be explained by a different refractoriness of transmission between the T-tubules and the sarcoplasmic reticulum in examined

Correspondence to Dr. E. Lipská, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Vlárska 5, 833 34 Bratislava, Slovakia

fibres and/or by a different dependency of the T-SR transmission on the extracellular calcium ions. A possible cause of the failure may be an intensification of the inactivation process.

**Key words:** Skeletal muscle — Twitch fibres — Excitation-contraction coupling — Fatigue — Calcium ions — Repetitive contractile activity — Inactivation of contraction

## Introduction

Based on differences in contractile, biochemical and ultrastructural parameters, skeletal muscle fibres may be subdivided generally into twitch and tonic fibres. There are clear differences between these two groups of fibres in almost every respect (for review see Nasledov 1981; Morgan and Proske 1984; Ogata 1988). There is evidence, however, that the differences also exist within the twitch fibres group itself. They concern many quantitative physiological, pharmacological and morphological characteristics. It is difficult to perform classification of this fibre group from the available data. This is mostly due to the fact that the authors have used in their studies different methods and objects and, first of all, different modes of fibre stimulation. The twitch fibres differ not only in their contractile and regulatory myofibrillar proteins (Ohlendieck et al. 1991) but also in structure and surface density of T-tubules and junctional segments of T-tubule network (Franzini-Armstrong et al. 1988).

Among the most substantial physiological characteristics differentiating twitch skeletal muscle fibres of various types is the rate of the tetanic tension decline due to prolonged or repeated activity, i.e. the rate of the development of fatigue (Westerblad and Lännergren 1986). The nature of these differences is still unclear (Westerblad et al.1991). There are correlations between fatigue-resistance and energetics (Van der Laarse et al. 1991), but there are also cases of dissociation of fatigue from metabolic changes (Le Rumeur et al. 1989); the depression of contractile protein force generation per se cannot fully explain mechanical exhaustion (Dawson et al. 1978; Crow and Kushmerick 1982). The continuous frequent stimulation of muscle fibres, leading to dramatical tetanic decline, cannot be also explained by the failure of the membrane excitability or action potential transmission (Grabowsky et al. 1972).

In a classical paper Eberstein and Sandow (1963) proposed that a failure of $Ca^{2+}$ release might contribute to the fatigue. This assumption was based upon a fact that the fatigued muscle showed a substantial recovery when the muscle was rapidly depolarized with a high $K^+$ or upon application of caffeine. These data demonstrate that sarcoplasmic reticulum (SR) $Ca^{2+}$ is still available for the release and activation of contractile proteins. During repeated tetanic stimulation of single

fibres, the fatigue is accompanied by a reduced $Ca^{2+}$ release, although transverse tubular conduction is not altered (Allen et al. 1989; Westerblad et al. 1990). It is supposed that alterations in sarcolemmal $Ca^{2+}$ exchange might influence the fatigue process (Williams and Ward 1991). The essential role of extracellular $Ca^{2+}$ in excitation-contraction (E-C) coupling, in particular in T-tubule-sarcoplasmic reticulum transmission (T-SRT), was also demonstrated (Stefani and Chiarandini 1973; Lüttgau and Spieker 1979; Cota and Stefani 1982; Graf and Schatzmann 1984; Lüttgau et al. 1986). In the past few years, there has been a rapid progress in understanding of the processes that couple the T-tubular action potential to the release of $Ca^{2+}$ into the myoplasm. A version of the hypothesis by Schneider and Chandler (1973), in which depolarization of the T-tubule affects sensor located in the T-tubular membrane and then opens $Ca^{2+}$ channels in the SR, is now widely accepted (for review see Ríos and Pizzarro 1991). There are also reasons to suppose that the primary cellular defect in fatigued skeletal muscle cells is localized in the E-C coupling mechanism, especially in failure of communication between the T-tubule and the sarcoplasmic reticulum and/or in the $Ca^{2+}$ release mechanism proper. Undoubtedly, fatigue is caused by multiple factors acting in various cell sites.

The aim of the present work was to compare the basic contractile characteristics of single twitch muscle fibres, which differ in force, rate of activation and rate of relaxation of tension with their fatiguability. Repeated tetanic stimulation was used to follow the fatigue-resistant properties in different kinds of fibres present in amphibian muscle. We also studied the effects of extracellular $Ca^{2+}$ removal on the tension during repeated tetanic activity in order to explore whether, and how extracellular calcium influences contraction in twitch fibre types characterized by various resistances to fatigue.

Two preliminary abstracts of parts of this work have been published (Radzyukevich et al. 1990, 1992).

## Materials and Methods

The experiments were performed on single fibres dissected from m. ileofibularis of adult frogs *Rana temporaria* and *Rana esculenta*. The dissection and experimental set up have been described in detail elsewhere (Nasledov et al. 1966). The single fibre was stretched by 20% of their slack length. Isometric contractions were elicited by transverse stimulation of the fibre along its whole length with platinum electrodes. Twitches were evoked by single 2 ms pulses of 1.3× treshold voltage to achieve maximal amplitude. Tetanic contractions were produced with single trains of stimuli at 70 Hz for 500 ms (time necessary for reaching a clear tetanic tension plateau). Fatigue-resistance was studied by repetitive stimulation with runs of single trains of tetanic stimulation repeated every 3 s; for a period of 2 to 10 min (i.e. from 40 to 200 single trains in a run). During recovery only single test pulses (twitch and tetanic) were applied. Potassium contractures were induced by rapid application of a high-potassium solution (80 mmol/l $K^+$) keeping the $[K]_0 \times [Cl]_0$

product constant. Sodium was substituted with potassium and chloride was replaced with propionate. The normal Ringer solution (R) of the following composition (in mmol/l) was used: NaCl 115; KCl 2.5; $CaCl_2$ 1.8; and sodium phosphate buffer 3.0 (pH 7.0–7.2). In the calcium-free solution (R-Ca), $Ca^{2+}$ was replaced with 1.8 mmol/l $Mg^{2+}$; the concentration of free $Ca^{2+}$ ions in this solution was 0.02 mmol/l as estimated with a calcium-sensitive electrode. The isometric tension was measured by a silicon tensometer (Marko et al. 1986) and recorded by the transient recorder SE 561 (BBC GOERZ METRAWATT). All experiments were done at room temperature. The inability to maintain the maximum of tetanic tension plateau (IMT) in 500 ms stimulation was calculated as the mean rate of tetanic tension decline from the maximum of amplitude to that in turning-off the stimulation. The values are given as mean ± S.E.M. Where appropriate, the Student's *t*-test was used for statistical evaluation.

## Results

53 single muscle fibres, dissected from different areas of the iliofibularis muscles, were investigated. The slack length of isolated fibres varied from 5 mm to 13 mm (mean value 9.63 ± 0.27 mm). No differences between the fibres dissected from muscles of *R.esculenta* and *R.temporaria* were found, so the results obtained are presented together. The effects of repeated stimulation on tetanic tension in these fibres in normal Ringer solution are documented in Fig. 1*A*. It was possible to reveal three groups of fibres differing in rate and magnitude of the tension decrease. In the first group, the tension fell down to 0.89 ± 0.03 of the original value after a run of 200 single trains of tetani (10 min stimulation; fatigue-resistant fibres, FR fibres, $n = 23$). In the second group, the tension fell down to 0.46 ± 0.5 of the original value after a run of 120–150 single trains of tetani (moderately fatiguable fibres, MF fibres, $n = 9$). The third group is formed by fibres, where the tetanic tension declined much faster than in FR fibres or MF fibres respectively; 0.5 of the original tension was achieved after application of 60 single trains of tetani, i.e. after about 2 min of repetitive stimulation (easily fatiguing fibres, EF fibres, $n = 21$). Prolonged stimulation of EF fibres led to a slow recovery; no recovery was often observed after the end of stimulation, and the experiment had to be stopped. Fig. 1*B* illustrates recovery of tetanic tension for 40 min after the end of repetitive stimulation in three fibre groups whose tension-stimulation curves are shown in Fig. 1*A*. Reduction of tetanic tension in EF fibers in the first minute of recovery represents continuation of tension decline from 0.43±0.03 at the end of the stimulation period to 0.34±0.05 at the start of recovery. The rate of rise of tetanic tension did not change in FR and MF fibers, but was suppressed by repetitive stimulation in EF fibers (Fig. 1*D*). Up to two hours were necessary in some fibres for recovery of the original EF tetanic tension. The recovery of tetanic tension in MF fibres was complete in 40 min, and that in FR fibres in 10 min. These data are comparable with those reported for *Xenopus* muscles by others (Westerblad and Lännergren 1986, Lännergren 1992) in conditions of continuous high-frequency stimulation.

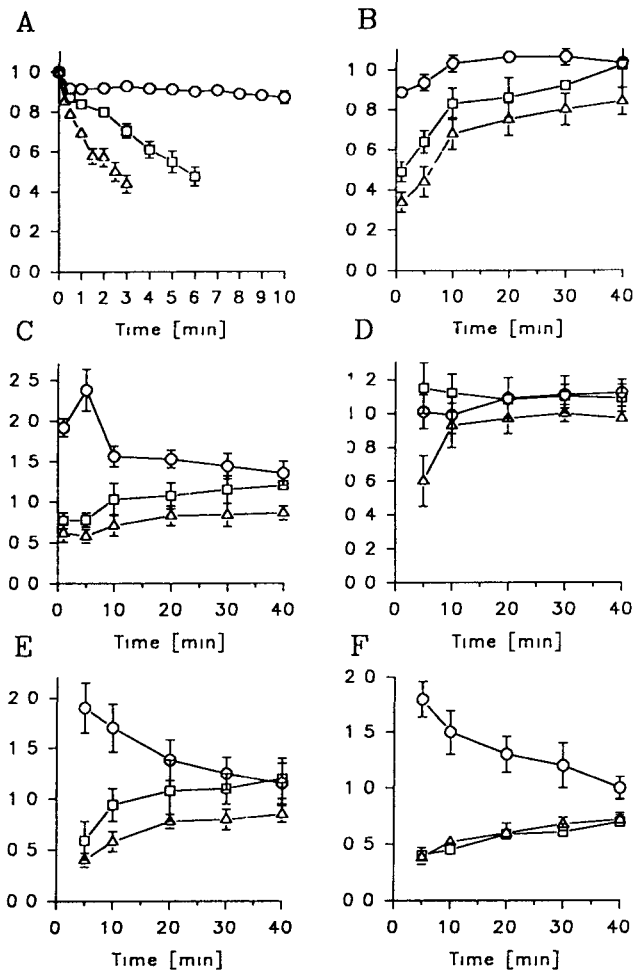


**Figure 1.** Twitch and tetanic tension in different types of skeletal muscle fibres (FR, MF, EF) *A* tetanic tension versus duration of stimulation (in min) *B* recovery of tetanic tension *C* recovery of twitch tension *D* rate of rise of tetanic tension *E* rate of rise of twitch tension after cessation of repetitive stimulation *F* rate of decay to half maximum of twitch tension after stopping the repetitive stimulation (relative to controls before the start of repetitive stimulation) *Circles* FE – fatigue-resistant fibres ($n$=20–22), *squares* MF – moderately fatiguable fibres ($n$ = 3–9), *triangles* EF – easily fatiguing fibres ($n$ = 17 22) muscle fibres Mean ± S E M Standard errors smaller than the size of the symbol are not shown Tension before repetitive stimulation was taken as 1

Changes in twitch contractions do not parallel changes in tetanus tension during repetitive contractile activity, as well as after termination of stimulation

At the end of stimulation period the EF, MF and FR fibres attained 0.6 ± 0.1, 0.8 ± 0.1 and 2.4 ± 0.2 of the prefatigue values of the twitch tension. Fig. 1*C* demonstrates that the twitches in FR fibres were strongly facilitated, sometimes 3–4 times, whereas the tetanic tension was reduced to about 0.89. Facilitation of the twitch tension in FR fibres reached maximum in the 5th minute and decreased slowly afterwards. The twitches in EF and MF fibres were always depressed after repetitive stimulation. Small facilitation of the twitch tension (1.2 of the prefatigue value) was, however, observed also in EF fibres when the amplitude of tetanus fell to 0.88 (comparable to the decline of amplitude at the end of 10 min stimulation of FR fibres). Contraction and relaxation rates of facilitated twitches were increased twice after the end of repetitive tetanic stimulation in FR fibres while those in EF and MF fibres were, on the contrary, reduced to about half (Fig. 1*E*, 1*F*). There was a good recovery of the rate of rise of twitches and a slower restitution of the rate of decay of the twitch tension in EF and MF fibres. Fig. 1*C*, *E*, *F* also show that twitch tension characteristics recovered in 40 min to their respective original values in MF fibres only; recovery in EF fibres was incomplete and the twitch tension in FR fibres, on the contrary, increased.

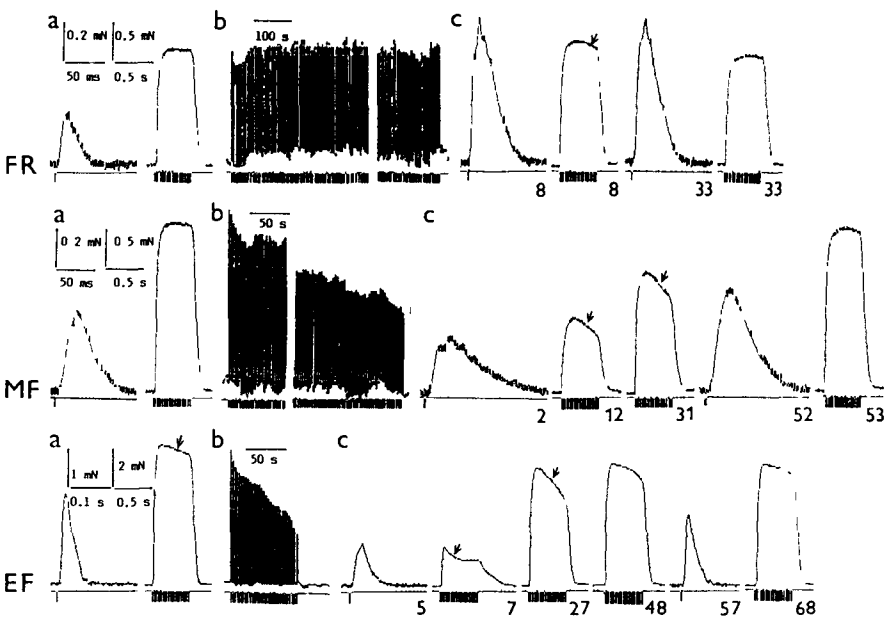


**Figure 2.** Twitch and tetanic tension records from fatigue-resistant (FR), moderately fatiguable (MF) and easily fatiguing (EF) fibres before (*a*), during (*b*) (duration of stimulation was 10 min in FR and 6 min in MF fibres) and after (*c*) repetitive stimulation (numbers below records indicate recovery time in min). Traces below tension records are stimulus markers. *Arrows* indicate inability to maintain maximum tetanic tension, IMT.

**Table 1.** Contractile parameters of fatigue resistant (FR), easily fatiguing (EF) and moderatly fatiguable (MF) single fibres of the frog in normal Ringer solution.

| Parameters | | FR | EF | MF |
|---|---|---|---|---|
| Tetanus Twitch ration | | $4.74 \pm 1.5$ (n=20) | $2.8 \pm 0.3^*$ (n=20) | $4.0 \pm 2.0^{*+}$ (n=9) |
| Tetanus tension | Amplitude [mN] | $2.15 \pm 0.17$ (n=23) | $3.41 \pm 0,42^*$ (n=21) | $3.21 \pm 0.44$ (n=9) |
| | $v_a \cdot 10^3$ [mN/ms] | $9.8 \pm 1.0$ (n=23) | $22.6 \pm 3.9^*$ (n=21) | $14.7 \pm 2.0^+$ (n=9) |
| | IMT$\cdot 10^3$ [mN/ms] | $17.6 \pm 11.8$ (n=23) | $317.8 \pm 1.0^*$ (n=22) | $67.7 \pm 39.2^{*+}$ (n=9) |
| Twitch tension | Amplitude [mN] | $0.52 \pm 0.07$ (n=20) | $1.42 \pm 0.17^*$ (n=20) | $1.07 \pm 0.26^+$ (n=9) |
| | $t_f$ [ms] | $103.6 \pm 5.5$ (n=20) | $114.2 \pm 4.5^*$ (n=20) | $121.5 \pm 12.0^{*+}$ (n=9) |
| | $v_a \cdot 10^3$ [mN/ms] | $18.6 \pm 2.0$ (n=20) | $53.9 \pm 6.9^*$ (n=20) | $37.3 \pm 9.8^{*+}$ (n=9) |
| | $v_{0\,5} \cdot 10^3$ [mN/ms] | $8.8 \pm 1.0$ (n=20) | $22.6 \pm 4.9^*$ (n=20) | $17.6 \pm 4.9^{*+}$ (n=9) |
| | $v_d \cdot 10^3$ [mN/ms] | $6.9 \pm 1.0$ (n=20) | $16.7 \pm 2.0^*$ (n=20) | $12.7 \pm 2.9^{*+}$ (n=9) |

Notes: Mean $\pm$ S.E.M.; $^*$ – statistically significant difference; $^+$ – holds true for both FR and MF fibres respectively; $n$ –number of experiment; $v_a$ – rate of rise; $v_d$ – rate of decay; $v_{0\,5}$ – rate of decay to half maximum; $t_f$ – duration of contraction

Fig. 2 shows representative twitch and tetanic tension records in EF, MF and FR fibres before ($a$), during ($b$) and after ($c$) repetitive tetanic stimulation. Side by side with the tetanic tension decline, as described above, the IMT (inability to maintain maximum tetanic tension) was increased as well (arrows). IMT changes often appeared earlier than the decline of tetanic tension amplitudes during repetitive stimulation. There are some differences in the rate and the magnitude of these changes among the fibre groups. Fig. 3 summarizes the results ($A$) and also shows tension records ($B$) illustrating distinctively the influence of repetitive stimulation on IMT, time course and rate characteristics of the responses. IMT values before and in the third min of repetitive tetanic stimulation were slower in FR than in EF or MF fibres respectively. IMT values were increased up to about 50 and 120 times in FR and MF fibres respectively after 7 min of stimulation. IMT values, close to controls before the beginning of stimulation, were observed both in MF and FR fibres while those in EF fibres were still high enough after 30 min of the recovery.

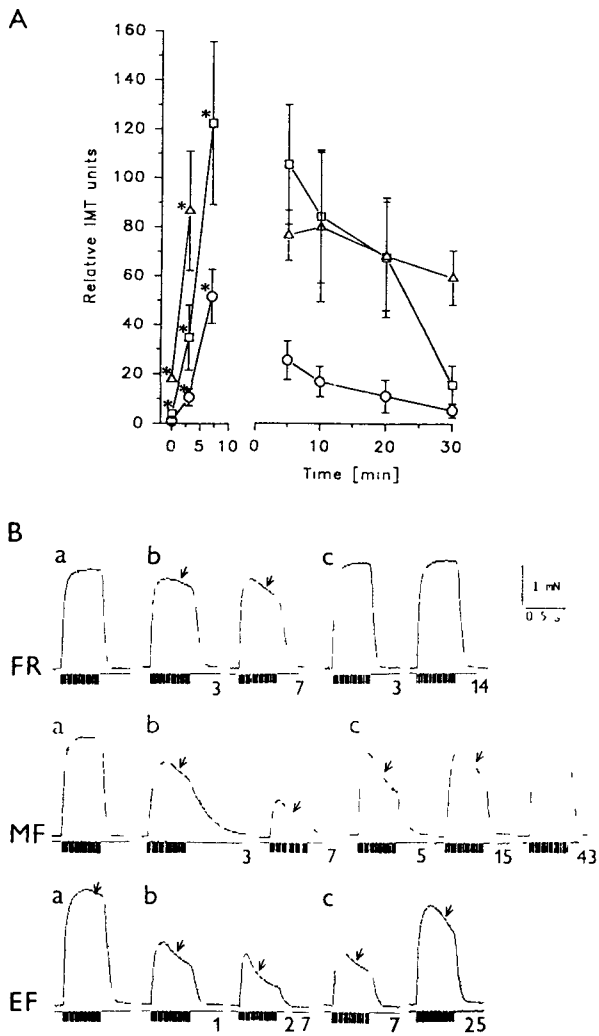


**Figure 3.** The inability to maintain the maximum tetanic tension (IMT) in fatigue-resistant (FR) and fatiguable (MF, EF) fibres *A.* variation of IMT with duration of stimulation (0–10 min) and recovery (0–30 min); *circles*, FR – fatigue-resistant ($n$=7–23); *squares*, MF – moderately fatiguable ($n$ = 4–10), *triangles*, EF – easily fatiguing ($n$ = 5–22) fibres. Mean ± S.E.M. Asterisks indicate statistically significant differences. The value of IMT in fatigue-resistant fibres before repetitive stimulation was taken as 1. *B*: original records (for the legend see text to Fig. 2).

In other words, IMT as well as twitches and tetani in EF fibres recovered more slowly than those in MF and FR fibres. Thus, in conditions of fast depolarization-

repolarization changes in the excitable membrane during both single and repeated tetani, the ability to preserve plateau tension is the first to change.

Three fibre groups differed also in several basic contractile parameters as shown in Table 1; i.e. the rates of contraction and relaxation of twitches and tetanic tension and the twitch/tetanus ratios. The MF parameters are intermediate between those in FR and EF fibres respectively.

The muscle fibres from all three groups investigated, generated similar high potassium contractures with spontaneous relaxation (not shown).

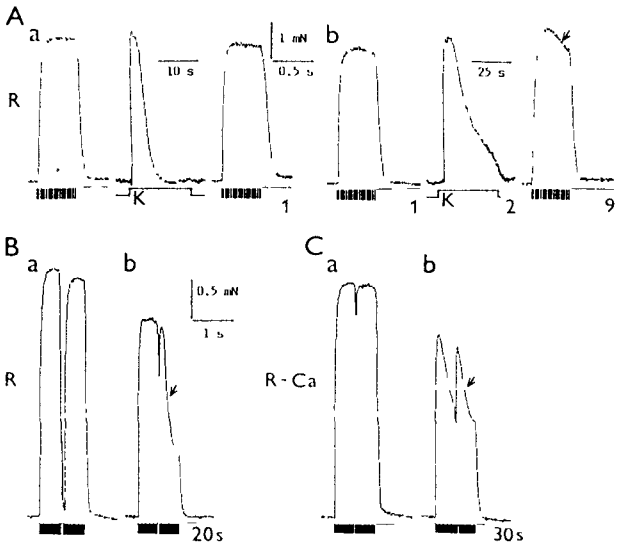


**Figure 4.** The effect of potassium contractures and of double tetanic stimulation on tetanic tension plateau in FR muscle fibres. *A*: tetanic tension recorded after a high potassium contracture (K) (80 mmol/l $K^+$) elicited before the start (*a*) and after the end (*b*) of repetitive tetanic stimulation (not shown) in normal Ringer solution. *B*, *C*: tetanic tension plateau during double tetanic stimulation before (*a*) and after (*b*) the repetitive tetanic stimulation (not shown) in Ringer solution (*B*) and in calcium-free solution (*C*, 20 min incubation). Numbers below the records indicate recovery times (*A* – in min, *B*, *C* – in s).

The subgroups of investigated skeletal muscle fibres thus show pronounced differences in (*i*) several basic contractile characteristics; (*ii*) the ability to maintain steady tetanic tension during prolonged rhythmical stimulation; (*iii*) the rate and the onset of IMT changes and (*iv*) the twitch and tetanus recovery parameters.

It is suggested that IMT changes may be related to an intensified inactivation process. High potassium contracture are accompanied by inactivation as described

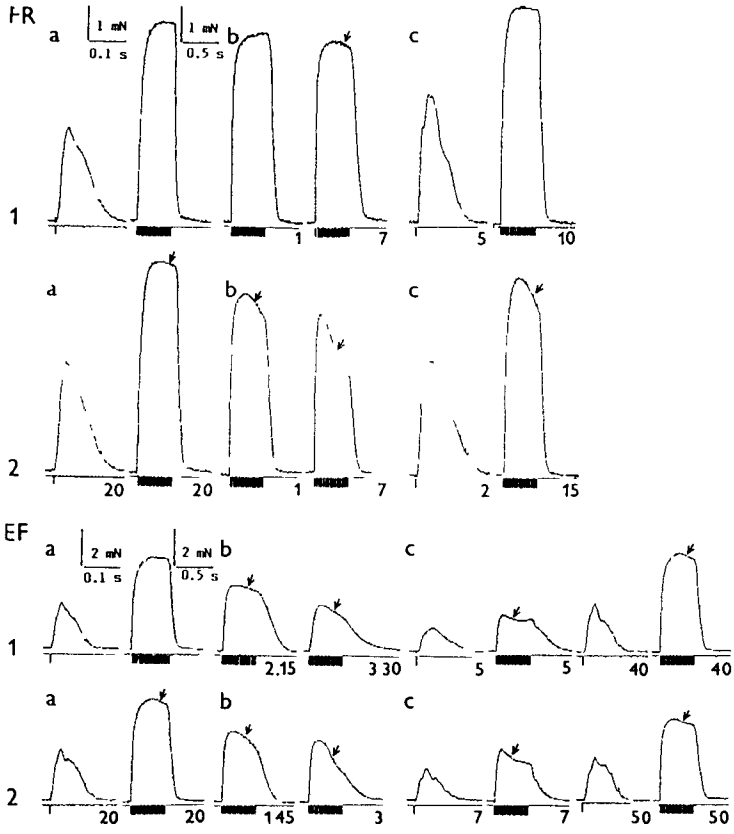


**Figure 5.** The effect of calcium-free solutions on twitch and tetanic tension in FR and EF muscle fibres before (*a*), during (*b*) and after (*c*) repetitive stimulation. 1 – in normal Ringer solution; 2 – in calcium-free solution (for the legend see text to Fig. 2).

elsewhere (Hodgkin and Horowicz 1960; Dulhunty, 1991). That is why we followed IMT changes in FR fibres after high $K^+$ contractures. Fig. 4*A* (*arrow*) illustrates that IMT increased when the $K^+$– contracture was applied after repetitive stimulation. Similar results were found in experiments with a double tetanic stimulation (Fig. 4*Ba* and 4*Bb*; *arrows*); the second tetanus elicited in rapid succession after the first tetanus, was much more depressed if preceeded by a repetitive stimulation. The effect of high potassium contractures and of repetitive tetanic stimulation on IMT is thus similar.

As the extracellular $Ca^{2+}$ ions are believed to play a role in the inactivation of contractility (Luttgau 1963; Lüttgau and Spiecker 1979; Dulhunty and Gage 1988), we studied the effects of extracellular $Ca^{2+}$ ions removal on contractile responses in EF and FR fibres. Fig. 5 shows records of twitch and tetanic tension

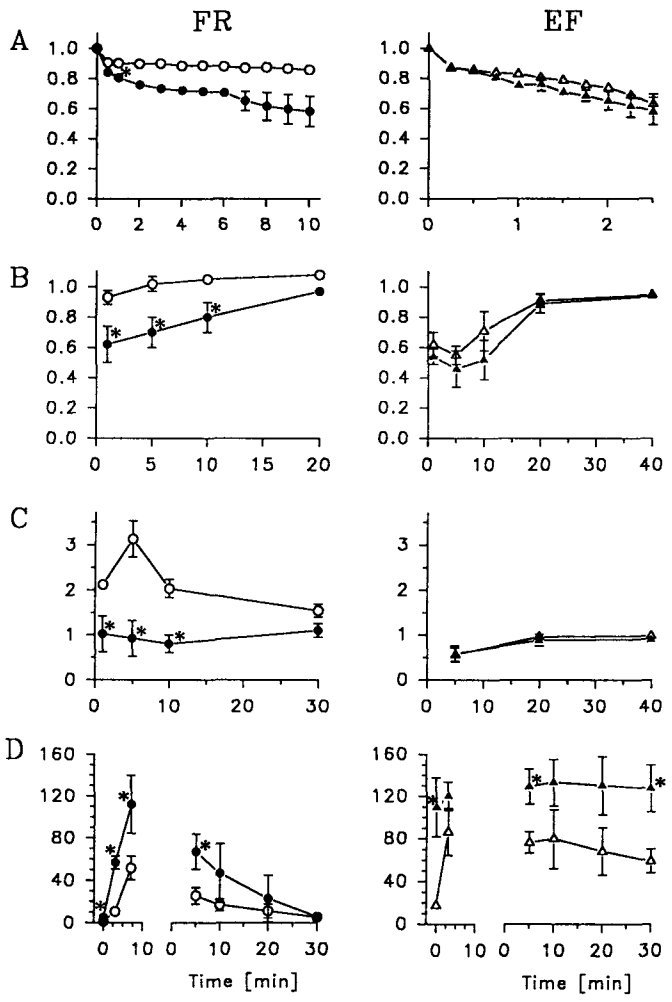


**Figure 6.** The effect of calcium-free solutions on twitch and tetanic tension in fatigue-resistant (FR) and easily fatiguing (EF) muscle fibres. *A* – tetanic tension versus duration of stimulation. *B* – recovery of tetanic tension; *C* – recovery of twitch tension. *D* – inability to maintain the maximum tetanic tension (IMT) during repetitive stimulation (0–10 min) and during recovery (0–30 min) in normal Ringer solution (*hollow symbols*) and in the calcium-free solution (*filled-in symbols*). Mean ± S.E.M.; 4–10 preparations. Asterisks denote statistically significant differences. Tension was normalized to the tension immediately before the repetitive stimulation.

in FR and EF fibres before (*a*), during (*b*) and after (*c*) repetitive stimulation in calcium-free solutions in comparison with Ringer solution. After 20 min in

calcium– free solutions, no changes in twitch and tetanic tension characteristics were encountered (no statistical differences were found) except of IMT. Mean values of IMT were increased about 5–6 times in calcium-free Ringer solution in both EF and FR fibres (Fig. 6*D*). Tetanic tension decline during repetitive stimulation was increased in FR fibres but did not change in EF fibres in calcium-free solutions (Fig. 6*A*). In calcium-free solutions not only the amplitude of tetanic tension was depressed, but also IMT decayed faster during repetitive stimulation in both FR and EF fibres; with the change in FR fibres being more pronounced (Fig. 5*b*, arrows; Fig. 6*D*). It should be underlined that the intensification of IMT in calcium-free solutions is observed still during double tetani (Fig. 4*C*), i.e. the calcium-free medium enhanced both the onset of IMT and fatiguability in FR muscle fibres. Twitch facilitation at the end of repetitive stimulation was also eliminated. It should be noted that the calcium-free solutions did not counteract recovery of tetanic and twitch tension in FR and EF fibres; recovery was, however, slower in FR fibres at the beginning (Fig. 5-*c*, 6*B*). Average values of rate characteristics of the twitch contraction in FR fibres were the same as before the repetitive stimulation, they were, however, reduced twice in EF fibres similarly as in Ringer solution. Fig. 6 summarizes the effects of calcium removal on twitch and tetanic tension during repetitive stimulation and recovery.

Thus, the FR fibres were found to be more sensitive to the absence of extracellular $Ca^{2+}$ ions in comparison with EF fibres; as evidenced by (*i*) the inability to maintain the maximum tetanic tension (increased IMT) before the beginning of repetitive stimulation, (*ii*) significant tetanic tension decline during repetitive stimulation (already in the first minute), (*iii*) increased IMT values at the 7 min of repetitive stimulation in the calcium-free solution which is close to those in EF fibres. In other words the calcium-free solution reduces resistance to fatigue of frog skeletal muscle.

## Discussion

Westerblad and Lännergren (1986) using a fatiguing, intermittent tetanic stimulation with reduced resting interval were able to divide *Xenopus* muscle fibres into three groups on the basis of their resistance to fatigue, e.i. into fast-fatiguing, fatigue-resistant and very fatigue-resistant fibres. The results described in this paper provide information on the effects of a stable frequency tetanic stimulation repeated in several numbers of trains (forming an experimental run) to disclose fatigue resistance and define types within the twitch muscle fibres of *R.temporaria* and *R.esculenta*. It was shown that these fibres might be separated in general, in three subgroups or fibre types respectively as well, i.e. in (*i*) fatigue-resistant (FR), (*ii*) easily fatiguing (EF) and (*iii*) moderately fatigable (MF) fibres. The described fibre types show differences in many contractile parameters. The fibres differ in

resistance to the decline of tetanic tension, i.e. in fatiguability in relation to basic contractile properties, the amplitude, the rates of twitch and tetanic tension development and decay as well as the twitch/tetanus ratio. During the stimulation period, the tension of muscle fibres was not well maintained during the single train of tetanus, which is defined as the inability to maintain the maximum tetanic tension (IMT). This behaviour is characteristic for all types of fibres studied but with definite quantitative differences; e.g. within single train of tetanus (70 Hz, for 500 ms) IMT was 18 times higher in EF fibres than in FR fibers and 4 times higher than in MF fibers. During repetitive stimulation, IMT was the first parameter to change in all fibres types. Twitch and tetani do not change in parallel. No essential tetanic tension decline during the final part of stimulation period was observed in FR fibres with simultaneous enormous potentiation of the twitch. The tetanic and twitch tension in EF and MF fibres was decreased by repetitive stimulation: with a slow recovery after termination of stimulation period. A transient small twitch potentiation was, however, observed even in EF and MF fibres during repetitive tetanic stimulation, if the tetanic tension did not decline more than by 10%. Twitch facilitation, which was observed after the IMT increase indicates that either the propagation of action potential or the twitch activation is probably not altered. It has been shown by various methods that failing membrane excitation did not contribute to fatigue, produced by repeated tetanic stimulation, in spite of the presence of expressed tension decline (for review see Westerblad et al. 1991). Furthermore, the recently published data with measurements of force and calcium intracellular signals also show that the twitch tension depression after fatiguing stimulation was smaller than the tetanic one (Baker et al. 1993). Besides that, fatigued muscle fibres were shown to produce higher tension levels during high $K^+$– and caffeine contractures than during tetani at the end of stimulation period (Lännergren and Westerblad 1989). These data prove that in fatigued muscles the ability to produce tension depends on the kind of stimuli (Edman and Fang Lou 1992). The reduced tetanic tension of fatigued fibres is accompanied by a decreased sarcoplasmic reticulum $Ca^{2+}$ release (Allen et al. 1989; Lee et al. 1991; Györke 1993). These data suggest that the faillure of transmission from T-tubules to sarcoplasmic reticulum (T-SRT), might be an underlaying mechanism of fatigue, which is enhanced by stimulation. Refractoriness in the T-SRT junction has been proposed as a possible mechanisms of T-SRT transmission failure during repetitive stimulation (Lännergren and Westerblad 1989). We assume that the increased IMT found in this study may be the result of the refractoriness (or inactivation) produced by rhythmical stimulation of the T-SRT link. As well known the inactivation develops after contractile activity, especially revealing well after the lasting membrane depolarization and it depends on the value and duration of depolarization (Hodgkin and Horowicz 1960; Lüttgau 1963; Caputo 1972; Nagai et al. 1979). The increase of IMT by high $K^+$–contracture and by double tetani after repetitive stimulation, as described in

this paper, could be explained by intensification of the inactivation process due to repetitive stimulation.

It is well known that twitches may continue in the absence of $Ca^{2+}$ in extracellular medium (Armstrong et al. 1972; Miledi et al. 1984). The role of extracellular $Ca^{2+}$ in contraction is more evident during prolonged membrane depolarization when the impeding restoration after inactivation is observed (Graf and Schatzman 1984; Lüttgau et al. 1986; Dulhunty and Gage 1988). The effects of extracellular $Ca^{2+}$ ions withdrawal were also observed during tetanic stimulation when the inability to maintain tension for more than 0,5 s was seen without any concurrent depression of twitches (Oz and Frank 1991). We have confirmed that the extracellular $Ca^{2+}$ ions play a definite role in the maintenance of tetanic tension especially in FR fibres. In addition, we have found that this effect depends on the duration of stimulation period (see Fig. 5). It is likely that low $Ca^{2+}$ effects during tetanic stimulation are due to changes in the inactivation process. Slower recovery after exposure to repetitive tetanic stimulation (double or prolonged) in calcium-free solutions is thought to reflect the time necessary for the underlying mechanism in the T-SRT transmission to return from inactivated to resting state. It can be supposed that the rate of recovery of that mechanism belongs to inherent properties of an individual type of twitch muscle fibres.

It is assumed that the E-C coupling is done through the T-tubular voltage sensors, i.e. by dihydropiridine receptors according to Ríos and Brum (1987) (see also reviews Fleischer and Inui 1989, Ríos and Pizzarro, 1991), showing intramembrane charge movement during their activity (Schneider and Chandler 1973). However, it was demonstrated recently that intramembrane charge movement was fatigue resistant even though the decrease in sarcoplasmic reticulum $Ca^{2+}$ release was present (Györke 1993). Two possibilities might then explain data showing T-SRT failure during repetitive stimulation. (*i*) Inactivation of T-SRT transmission is located far away from the T-membrane, in later E-C coupling steps. (*ii*) T-tubular "slow" $Ca^{2+}$ channels might play a role in T-SRT transmission. A role of voltage-sensitive "slow" $Ca^{2+}$ channels in muscle contractility was suggested by Oz and Frank (1991). This role also follows from data showing correlation of $Ca^{2+}$ inward current changes with the contractile inactivation (Neuhaus 1986) and rapid activation of the former by repeated stimulation (Feldmeyer et al. 1990). It is possible that this mechanism may be operating in FR fibres as well.

The fibre types, which are documented in this paper, show also significant differences in their behavior in calcium-free solutions. It is assumed that the quantitative differences among the twitch fibres might be explained, at least partly, by differences in the inactivation processes including the role of extracellular $Ca^{2+}$ in the inactivation. The relevant indications are the differences in IMT during 70 Hz short stimulation, in different rates of IMT increase during the repetitive stimulation and in the different sensitivity to the removal $Ca^{2+}$ ions from the solution.

The differences in the original level of inactivation among fibres may provide, in part, a different resistance of some E-C coupling steps to rhythmical stimulation at which the fibres have to transit rapidly from the inactivation state to the next activated state, i.e. they may have different mobility in these processes. There is evidence, that the differences in the inactivation process of contraction between various muscles might exist (Dulhunty and Gage 1985; Dulhunty 1991). We are assuming, that $Ca^{2+}$ ions may modulate inactivation process to a certain degree and that the FR fibres being less inactivated, may have an optimal set-up for their modulatory effects.

It is difficult to explain at present the novel fact of the twitch facilitation during and after cessation of repetitive stimulation and its disappearance in calcium-free solutions, we have observed in FR fibres. This finding is possibly related to the mechanism of fatigue-resistance and may be a good reason for futher inquiry in this field.

In summary, the presented experiments support the notion that tetanic tension decline during repetitive activity (decrease of the fatigue-resistance) is primarily due to failure in transmission from the T-tubules to the sarcoplasmic reticulum. We found, that both the removal of $Ca^{2+}$ ions, the repetitive tetanic stimulation, as well high potassium contractures after repetitive tetanic stimulation, could affect the muscle fibre contractions (mainly the tetanic contraction). These observations may be explained in a straightforward way if it is assumed that calcium ions are needed for a normal functioning of the T-SR transmission, i.e. they may be necessary for the recovery from an inactivation state to a state when the following signal has got ability to pass. Variable fatigue-resistance of the amphibian skeletal muscle fibre types observed in this study might be related to a variable initial down-regulation of the transmission between the T-tubules and the sarcoplasmic reticulum.

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