Muscle contractile properties during intermittent nontetanic stimulation in rat skeletal muscle

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FATIGUE DEVELOPS during maximal or high-intensity exercise and during prolonged submaximal exercise, as evidenced by a decline in the force-generating capacity during maximal (test) contractions (4, 6, 13, 51, 59). It has repeatedly been shown that a considerable component of fatigue is due to processes peripheral to the neuromuscular junction (14, 45, 53), i.e., inhibition of excitation-contraction coupling (2, 27, 54). Most hypotheses on the mechanism of this type of fatigue are based on changes in muscle function and biochemical composition that occur during maximal or high-intensity exercise or during tetanic stimulation. A number of the exercise-induced changes in muscle function and composition seem to be different during prolonged submaximal exercise or low-frequency stimulation. Examples are a slower rate of development of fatigue and slower recovery of force after exercise (4) and a lack of slowing of relaxation (7, 10, 28, 58). A further characteristic of prolonged submaximal exercise is the lack of correlation of the moderate changes in metabolites with fatigue during and after exercise (3, 25, 35, 38, 41, 50, 59). Also, during prolonged submaximal exercise, the increase in extracellular K+ concentration is moderate (50, 55, 57) and is not related to the decrease in maximal force (55). All these differences suggest that the peripheral mechanism of fatigue (the mechanism of inhibition of excitation-contraction coupling) during prolonged submaximal exercise may be different from that during maximal or high-intensity exercise.

In the present study, we have instigated fatigue by prolonged submaximal exercise in isolated perfused skeletal muscles in anesthetized rats. We chose an in situ preparation in which contractions were electrically induced to ensure that the resulting fatigue was located peripherally. The rat model allowed us to examine the effect of prolonged submaximal exercise on the contractile properties of slow-twitch (soleus) and fast-twitch [extensor digitorum longus (EDL)] muscle fibers separately. To mimic fatigue from submaximal exercise, it is important that major changes in intracellular metabolites are avoided. In the rat in situ model, circulation was therefore left intact to prevent ischemia and excessive extracellular accumulation of metabolite breakdown products and electrolytes. Furthermore, the stimulation protocols were constructed so as to promote aerobic metabolism. Because during voluntary prolonged submaximal exercise firing rates of the motoneurons are below the frequencies causing tetanic contractions in individual fibers (13), the applied stimulation frequencies were subtetanic. Temperature is important for muscle performance, and several of the key steps in the excitation-contraction-relaxation cycle have different sensitivities to temperature (48). The in situ model allowed us to maintain a...
constant muscle temperature close to normal body temperature.

Changes in contractile properties of the individual fibers during prolonged submaximal exercise have not been well described, and seemingly contrasting results between studies of high- and low-intensity exercise or stimulation may be due to the use of several different measures of force and contractile speed. The first aim of our study was therefore to examine in detail changes in force generation and other contractile properties, especially relaxation rate, during a fatigue protocol consisting of repeated submaximal isometric contractions. Second, on the basis of the observed combination of changes in contractile properties and the observed changes in metabolic factors, we wanted to formulate a hypothesis on the mechanism of fatigue (excitation-contraction coupling inhibition).

METHODS

All experiments and animals were handled according to the Norwegian Animal Welfare Act. Adult male Wistar rats (Wistar Hannover, Møllegaard Breeding and Research Centre, Skensved, Denmark) were kept in a temperature-, humidity-, and light-controlled (12:12-h light-dark cycle) environment for ≥1 wk after arrival from the supplier. The animals were fed standard rat chow (B & K Universal, Oslo, Norway; standard rat/mouse) and water ad libitum. The rats weighed 326 ± 20 (SD) g on the day of the experiment.

In Situ Muscle Preparation

The rats were anesthetized with 1:3 O2-N2O with 2–4% halothane (Fluothane). A catheter (Venflon 2, 0.8 mm ID, 25 mm long, BOC Ohmeda, Helsingborg, Sweden) was inserted into the carotis communis artery and connected to a Statham pressure transducer (model P23 Gb, Gould Instruments, Hato Rey, Puerto Rico). The skin around the experimental leg was removed, and the soleus or EDL muscle was prepared in situ by a method adapted from Lindinger et al. (42), but with circulation left intact. For the soleus, this was done by making incisions in the fascia between the soleus-gastrocnemius-plantaris muscle group and the lower limb bones up to the knee joint. The Achilles tendon was isolated by cutting off the circulation left intact. For the soleus, this was done by making incisions in the fascia between the soleus-gastrocnemius-plantaris muscle group and the lower limb bones up to the knee joint. The Achilles tendon was isolated by cutting off the calcaneus. The gastrocnemius and plantaris tendons were separated from the soleus tendon and cut. To ensure that movements of the gastrocnemius and/or plantaris muscle did not interfere with the soleus muscle, the thin fascia between the soleus and the gastrocnemius-plantaris was split about halfway up the soleus but distal to the blood vessels supplying the belly of the soleus muscle. The distal soleus tendon with calcaneus was then attached to the force transducer (model PT03, Grass Instrument, Quincy, MA). In the case of the EDL muscle, the tendon was cut distal to the ankle joint; then the thin fascia between the EDL and the surrounding muscles was split, ensuring that the blood vessels supplying the EDL muscle were not damaged. The tendon was then attached to the force transducer. In both cases, care was taken to ensure a “natural” angle of movement for the muscles, and the force transducer was positioned in line with the pull of the muscle. The ischial nerve was cut distal to the hip. Platinum wire electrodes were positioned at the proximal and distal ends of the muscle. The muscle was kept moist and at a constant temperature by constant dripping of warmed Krebs-Ringer solution onto it. Temperature was measured at the surface of the muscle and kept within ±0.5°C by adjusting the temperature of the Krebs-Ringer solution. Muscle surface temperature was, on average, 36°C, although this temperature could vary by 1°C from experiment to experiment.

Stimulation Protocol

The rats were divided into a stimulated (Stim) or a sham-operated (Sham) group. In the Stim group, after equilibration for ~15–20 min, the muscles were stimulated (Pulsar 6bp, FHC Brunswick, ME) with 15-ms pulses at 1 Hz (soleus) or 0.5 Hz (EDL) to find optimum muscle length and stimulation voltage. Optimum muscle length was the length at which the highest single-pulse contraction force was produced. Optimum stimulation voltage was chosen 0.5 V higher than the voltage at which the highest single-pulse force was produced. The resulting optimum stimulation voltage was 5–7 V. A pulse length of 15 ms was chosen, because force production was ~1.7 times the force response resulting from a 1-ms pulse (22) (Fig. 1) but still lower than a tetanic contraction. In the soleus, the same force response can be obtained by inducing three 1-ms pulses within 15 ms (Fig. 1). One 15-ms pulse thus gives a subtetanic contraction, probably resulting from three action potentials at a frequency of ~170 Hz. In the EDL, the same force response caused by a 15-ms stimulus could be induced by four 1-ms pulses within 15 ms (220 Hz). The subtetanic force caused by 15-ms pulses was required to induce fatigue.

Before the start of the fatigue protocol, maximal tetanic force was induced with 15-ms pulses at 50 Hz for 1–3 s. After a 5- to 10-min rest period, the fatigue protocol was started. In Fig. 1. Force traces of single contractions induced by various stimulation pulses. A: typical force traces of a twitch induced by a 1-ms pulse (solid line, lowest force), a single contraction induced by one 15-ms pulse (solid line), and a single contraction induced by one triplet pulse (three 1-ms pulses within 15 ms, dashed line) in soleus muscle. B: same stimulation protocols as in A in extensor digitorum longus (EDL) muscle, but with a quadruplet, instead of a triplet, pulse (dashed line). Peak force of the twitch contraction was 0.43 ± 0.01 N for soleus muscle and 0.94 ± 0.10 N for EDL muscle. Times to peak and one-half relaxation times for the twitches were 28.8 ± 1.1 and 38.0 ± 0.5 ms for soleus muscle and 13.8 ± 0.9 and 17.7 ± 1.0 ms for EDL muscle. Twitch-to-tetanus ratio was 0.25 ± 0.01 for soleus muscle and 0.40 ± 0.03 for EDL muscle (n = 3 for all).
the soleus, the Stim muscles were stimulated intermittently (6 s on, 4 s off) at 5 Hz for 60 min (Stim60, n = 24) or 2.5 min (Stim2.5, n = 6). In the soleus muscles, after 30 and 50 min of the fatiguing protocol, a 1.5-s test 50-Hz tetanus was induced instead of one 6-s contraction. The EDL Stim muscles were stimulated intermittently at 10 Hz (1.5 s on, 1 s off) for 10 min (Stim10, n = 19) or 1 min (Stim1, n = 6). Two Stim60 soleus muscles were allowed to recover for 60 min, and two Stim10 EDL muscles were allowed to recover for 30 min after the fatigue protocol. During recovery, the soleus muscles were stimulated at regular intervals with single 6-s 5-Hz trains or single-pulse contractions and the EDL muscles with single-pulse contractions at regular intervals and a 50-Hz tetanus after 2 min of recovery.

In the Sham group, the muscle was prepared in situ as described above but did not undergo the fatiguing stimulation. Instead, these muscles rested for a period of time corresponding to the stimulation time of the Stim muscles, with no stimulation. In the biochemical analyses, these Sham muscles served as a control for the effects of the in situ preparation.

In all experiments, the contralateral muscle that did not undergo the in situ preparation provided a paired control (CC).

Analysis of Contractile Properties

Force data from the force transducer were sampled at a frequency of 250 Hz on a personal computer (486DX, Metabyte DAS16 analog-to-digital converter) with ASYST software (Asyst/Keithley Software Technologies, Rochester, NY). The 50-Hz test tetani and the partly fused 5- and 10-Hz trains of contractions were analyzed as illustrated in Fig. 2. Maximum force (Fm) was defined as the highest peak force of the whole train or tetanus. Peak force of the first contraction in a train of partly fused contractions (FF_peak) served as a measure of single-pulse force. The increase in resting force between contractions in a train was described with the minimum force before the last contraction of a train of partly fused contractions (Ft_min). Peak force of the last contraction in the train (Ft_peak) was measured, mainly for the purpose of relating changes in relaxation speed to it and of calculating developed force (Ft_peak - Ft_min, Z) at the end of each train. As a measure of the rate of force development, we used contraction time (CT), defined as the time required for force to increase from 5% to 95% of peak force of the first contraction in a train, and the maximum rate of increase of force (maximum contraction dF/dt) during the upstroke of force to peak force in the first contraction. As measures of the rate of relaxation, we used one-half relaxation time (RT50), defined as the time required for the force to decrease from 95% to 50% of peak force after the last contraction in a train, and maximum rate of force decline (maximum relaxation dF/dt) during relaxation from the last contraction in a train (Fig. 2). As a measure of the degree of fusion, the tetanic fusion factor (TFF) was calculated, defined as the rectified force-time integral of the fluctuation of force around the running average of the consecutive peaks and nadirs of force during a train, divided by Fm and the duration of the train (6 s for soleus and 1.5 s for EDL). TFF will decrease with increased fusion and is zero in completely fused tetanic contractions. As another measure of relaxation rate, dF30/dt was calculated for the soleus muscle and dF10/dt for the EDL muscle, defined as the first derivative of force (dF/dt) at an absolute level of force of 30% (soleus) or 10% (EDL) of Fl_peak of the train of contractions at time 0.

Blood Supply

Whether blood supply was intact in the in situ prepared muscle and whether blood supply was functioning sufficiently to allow aerobic energy restoration during the fatigue protocol were established through additional experiments. In three rats, 99mTc-tetrofosmin (Myoview, Amersham International) was given intravenously in the internal jugular vein as a bolus. Tetrofosmin is a small, inert compound that accumulates in the mitochondria of skeletal muscle. It is routinely used in clinical examination of perfusion of heart and skeletal muscle in humans. 99mTc-tetrofosmin uptake into striated muscles has been shown to be highly dependent on blood flow but may also be influenced by mitochondrial respiration (49, 68). Thus any 99mTc-Tc-tetrofosmin found in the in situ prepared muscles would imply that circulation to the muscle was intact. Any increase in the in situ muscle content of 99mTc-Tc-tetrofosmin above that of the non-prepared CC muscle could be due to increased blood flow to the muscle and/or increased mitochondrial respiration. The bolus containing 0.5 ml of NaCl solution (0.9%) with ~80–100 MBq of 99mTc-tetrofosmin was given in 2 min before the start of the fatigue-inducing protocol (n = 1) or a 20-min sham-resting period (n = 2). The frozen muscles were analyzed for relative 99mTc content in a well-type gamma counter (Scaler Timer ST7, Nuclear Enterprises). In addition to the tetrofosmin experiments, a separate series of experiments (Isch) was carried out with three soleus muscles and three EDL muscles. In this series, the blood supply to the muscles was occluded at the femoral artery and vein in the groin of the experimental leg, just before the start of the exercise protocol. The soleus and EDL muscles were then stimulated with their respective fatigue-inducing protocols for 10 min. The force data and the changes in metabolites (ATP, creatine phosphate, and lactate; see below) were compared with the data from the normal Stim groups. Also in these experiments, the contralateral muscle provided a paired control (CC).

Analysis of ATP, Creatine Phosphate, and Lactate Content

Four soleus Stim60 and four Sham muscles, six soleus Stim2.5 muscles, six EDL Stim1 and six Stim10 muscles, three soleus muscles, three EDL Isch muscles, and the CC muscles were analyzed for ATP, creatine phosphate, and lactate. Within 5 s after the stimulation protocol (or rest period), circulation was cut to prevent recovery of metabolite levels. Within 10 s, the Stim, Sham, or Isch muscles were excised and frozen in liquid N2. Then the CC muscles were excised.
and immediately frozen in liquid N₂. Within 24 h after the experiments, the frozen muscles were dissected free from visible blood and tendon, pulverized in a mortar cooled with liquid N₂, and vacuum freeze-dried for 6 h. The freeze-dried powder was stored at −70°C. Within 1 mo, metabolites were extracted from the freeze-dried and pulverized samples with 3 M perchloric acid, then the samples were neutralized with 2 M KHCO₃. The extracts were then analyzed enzymatically with luminescence spectrometry (model LS50B, PerkinElmer, Buckinghamshire, UK) for ATP, creatine phosphate, and lactate (43).

Stability of the Mechanical Properties of the In Situ Model

Stability of the mechanical properties of the in situ model was tested in separate series of experiments with nine soleus and three EDL muscles. Three different tests were done. First, we tested whether the in situ muscle preparation and subsequent period of in situ exposure could have some detrimental effect on the muscle’s ability to generate force. Three Sham muscles, not undergoing the fatigue protocol, were stimulated to induce short test contractions at regular intervals during an 80-min rest period (60 min for the fatigue protocol + 20 min for prefatigue protocol tests). In these Sham muscles, optimum length and stimulation voltage were determined using the method described above for the Stim muscles. Single-pulse contraction force at stimulation voltage varying from 1 to 8 V in 1-V steps was then sampled. Then a single-pulse contraction, a 6-s 5-Hz train, and, after a 5-min rest period, a 1.5-s 50-Hz tetanus were induced. After a further 10-min rest period, the clock was started. At time 0 and 2.5 min, one 6-s 5-Hz train of contractions was induced. At 10, 30, and 60 min, a series of one single-pulse contraction, one 6-s 5-Hz train, and 1 min of rest before a 50-Hz tetanus was induced. At 80 min, single-pulse contraction force at stimulation voltages varying from 1 to 8 V was again sampled and compared with the series of single-pulse contractions at the beginning of the experiment. This test was not done on EDL muscles, since for the EDL muscle the fatigue protocol was relatively short, and a 10-min rest period between two identical test contractions (two 50-Hz tetani or two 1.5-s 10-Hz trains) before the fatigue protocol did not result in any change in force response in six experiments (data not shown).

In the second set of control experiments, we tested whether any changes had occurred in the muscle’s excitability in response to the stimulation voltage. In three soleus muscles, the normal 60-min fatigue protocol was used, except after 55 min the stimulation voltage during the 5-Hz trains was varied from 10 to 1 V in 1-V steps.

The third set of control experiments investigated whether a pulse duration of 15 ms per se had any effect on the changes in contractile properties during the fatigue protocol. In three soleus and three EDL muscles, the fatigue protocol was induced (30 min in the soleus and 10 min in the EDL) with triplets of 1-ms pulses (three 1-ms pulses within 15 ms at 170 Hz) in the soleus muscle and quadruplets of 1-ms pulses (four 1-ms pulses within 15 ms at 220 Hz) in the EDL muscle, instead of 15-ms pulses.

Statistics

Values are means ± SE. Changes in the various contractile property measures over time were tested with one-way repeated-measures ANOVA supplemented with post hoc Student’s paired t-tests (with Bonferroni’s correction for multiple tests) whenever appropriate. Differences between Stim, Sham, or Isch muscles and their respective CC muscles in the content of the various metabolites were tested with Student’s paired t-tests with Bonferroni’s correction for multiple tests. Because of batch differences in the biochemical analyses, the experimental muscles of the Stim, Sham, and Isch groups could not be compared directly with each other. Differences between Stim and Sham muscles and between Stim and Isch muscles were therefore tested by taking the paired differences between the experimental and CC muscles (which were always analyzed together in the same batch) and comparing these for the Stim, Sham, and Isch groups using a one-way ANOVA and post hoc Student’s t-tests with Bonferroni’s correction. Linear regression was calculated for the relation between developed force (FL peak − FL min) and dF 30/dt or dF 10/dt and for the relation between maximum contraction...
dF/dt and maximum relaxation dF/dt. For all tests, the level of significance was 0.05. The statistical analyses were performed with SigmaStat (Jandel Scientific, Erkrath, Germany) and SPSS (version 8.0, SPSS, Chicago, IL).

RESULTS

In the soleus and EDL muscles, the 5- and 10-Hz intermittent stimulation resulted in trains of partly fused contractions (Fig. 3). The peak force of these contractions (F_{peak}, F_{L,peak}, and F_m) was far below the maximal force during test tetani at 50-Hz stimulation. Before the start of the fatigue protocol, the 5-Hz F_m in the soleus muscles and the 10-Hz F_m in the EDL muscles were 53 ± 3% and 64 ± 3%, respectively, of the maximal force of a 50-Hz tetanus (50-Hz F_m). Already after a few trains of contractions, the general pattern of the force recordings during the trains changed. Typical traces of the force response to the intermittent stimulation for the soleus and EDL muscles are shown in Figs. 3 and 4.

Initial Changes of Rates of Force Development and Relaxation

During the first 2.5 min of the fatigue protocol in the soleus muscle and 1 min in the EDL muscle, the most prominent change was that the rate of relaxation slowed. As illustrated in Fig. 4 and averaged for all animals in Fig. 5 and Table 1, maximum relaxation dF/dt from the last contraction in the train declined and RT_{½} increased. In the soleus muscles, maximum relaxation dF/dt decreased to 70 ± 5% of the initial value in the course of 5 min, and in the EDL muscles, it fell to 33 ± 3% of the initial value after 1 min. Also, Fig. 4 shows that the initial slowing of relaxation was evident as an early “shoulder” on the force-relaxation curve, most prominent in EDL muscles. Maximum contraction dF/dt was also reduced especially during the first 5 min in the soleus muscles and the 1st min in the EDL muscles. However, no change occurred in CT in the soleus muscles (P = 0.223), and CT even decreased in the EDL muscles (P < 0.001). Maximum contraction dF/dt and maximum relaxation dF/dt changed in parallel [linear in the soleus (R = 0.679, P < 0.001) and curvilinear in the EDL (R = 0.643, P < 0.001)].

In contrast to the rapid initial changes in the rate of force development and relaxation, in the soleus muscles, F_{L,peak} did not change compared with the initial train during the first 2.5 min (Figs. 5 and 6). Owing to the slowed relaxation rate, force did not return to resting values between contractions during the trains. As a consequence, F_{L,peak} increased (Figs. 3 and 6), and developed force of the last contraction decreased. Thus the contractions started to fuse, and the increased degree of fusion was quantified by the decrease of TFF (Fig. 7). The F_{L,peak} and TFF reached peak and nadir values, respectively, after 2.5 min in the soleus muscles.

In the EDL muscles, in contrast to the soleus muscles, F_{L,peak} showed a rapid decline during the 1st min of the fatigue protocol. Despite the decrease in F_{L,peak}, but in agreement with the relatively larger change in maximum relaxation dF/dt and RT_{½}, F_{L,peak} and the degree of fusion increased (TFF decreased) and developed force decreased even more than in the soleus muscles during the 1st min of the fatigue protocol.

Subsequent Changes in Rates of Relaxation and Force

After the initial rapid slowing of relaxation, the force pattern of the trains changed more gradually over 1 h in the soleus muscles and over the subsequent 9 min in the EDL muscles. In the soleus muscles, maximum relaxation dF/dt still decreased slightly after 5 min (Fig. 5; P < 0.001). In the EDL muscles, maximum relaxation dF/dt showed no further change after the initial decrease during the 1st min (Fig. 5; P = 0.146).

The most prominent change seen after 2.5 min in the soleus muscles was that F_{L,peak} started to decline. The fastest decline was seen between 2.5 and 5 min, but it continued to decrease also after 5 min (P < 0.001), reaching 50 ± 4% of the initial value after 60 min (Fig. 6). In the EDL muscles, F_{L,peak} had already decreased by 31 ± 5% and continued to decrease after 1 min, at a somewhat slower rate than during the 1st min (Fig. 6). After 5 min, F_{L,peak} was 16 ± 5% of the initial value and did not change further.

Fig. 4. Force development and relaxation during the fatigue protocol. Selected typical traces of the upstroke of force development from resting to the 1st peak of the 5- or 10-Hz train of partly fused contractions (left traces) and force relaxation from the last peak of the train (right traces). A: soleus; B: EDL. Rats were the same as those used in Fig. 3. Numbers adjacent to traces, time (in min) during the fatigue protocol. Traces are aligned at peak force.
Also the $F_m$ of the 50-Hz test tetani declined during stimulation and was $81 \pm 4\%$ after 50 min in the soleus muscles ($n = 6$). The relative decline in 50-Hz $F_m$ was smaller than for $F_{F_{peak}}$ and $F_{L_{peak}}$. In the EDL muscles, no 50-Hz tetani force recordings were obtained during the stimulation protocol to avoid interference with the fatigue development, but in two rats a 50-Hz tetanic stimulation was given during recovery, 2 min after the 10-Hz fatigue protocol had ceased. The 50-Hz $F_m$ was then $60 \pm 6\%$ of the initial value.

Because $F_{L_{peak}}$ decreased after the first 5 min in the soleus muscles, resting tension was again eventually reached between each contraction in the train, despite maintained low maximum relaxation $\text{d}F/\text{d}t$ (Fig. 3). In other words, because both $F_{L_{peak}}$ and $F_{L_{min}}$ fell gradually, although not quite in parallel, after the initial 5 min developed force with each contraction ($F_{L_{peak}} - F_{L_{min}}$) first increased and then remained constant from $-20$ min until the end of the protocol (Fig. 6). This is reflected in the calculated TFF, which returned to the initial level after 20 min (Fig. 7). Because RT$_{1/2}$ is a force-dependent calculated variable, it decreased after the initial 5 min in the soleus muscles and was inversely related to TFF (Table 1). The EDL muscles behaved, in general, quite similarly to the soleus muscles after the initial minute (Fig. 2), but TFF and RT$_{1/2}$ never recovered completely in the EDL muscles (Fig. 7, Table 3).

### Table 1. CT and RT$_{1/2}$ in stimulated soleus and EDL muscles

<table>
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<tr>
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<th>Soleus ($n = 22$)</th>
<th>EDL ($n = 12$)</th>
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<td>CT, ms</td>
<td>RT$_{1/2}$, ms</td>
</tr>
<tr>
<td>0</td>
<td>51 ± 3</td>
<td>60 ± 5</td>
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<td>60</td>
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<td>57 ± 4</td>
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Values are means ± SE. CT, contraction time (time from 5% to 95% of force of the 1st peak of the train of unfused contractions); RT$_{1/2}$, one-half relaxation time (time from 95% to 50% of force after the last peak in the train of unfused contractions); EDL, extensor digitorum longus.

### A Different Measure of Relaxation Rate

The various estimates of rate of force development and relaxation provide different information about

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**Fig. 5.** Time-dependent changes of various measures of contractile speed. Average data for all rats during the fatigue protocol show maximum contraction $\text{d}F/\text{d}t$ (A), maximum relaxation $\text{d}F/\text{d}t$ (B), and $\text{d}F_{F/2}/\text{d}t$ for soleus or $\text{d}F_{F/2}/\text{d}t$ for EDL (C). $\text{d}F_{F/2}/\text{d}t$ and $\text{d}F_{F/2}/\text{d}t$ at a fixed force level of 30% and 10% of $F_{L_{peak}}$ at time 0. Values are means ± SE; $n = 22$ for soleus and 12 for EDL.
muscle behavior. Provided that the number of contractile elements (number of muscle fibers) remain constant, RT1/2 and maximum relaxation dF/dt will change when peak force is reduced without any change in the speed of the relaxation processes. CT and RT1/2 may be related to absolute force, and it can be difficult to standardize the two time points used for the calculation. On the other hand, maximum dF/dt provides an estimate of the absolute rates, but the time point on the force curve at which this maximum is achieved is variable. As an adjunct, we present relaxation rates calculated at the same absolute force. This measure will probably reflect the change in the speed of relaxation at a given cytosolic Ca2+ concentration and will be independent of simultaneous changes in peak force (i.e., peak of the Ca2+ transient). For the soleus muscles, we have chosen the force corresponding to 30% of FL peak of the last contraction of the train at time 0, and we have calculated the relaxation rate at the same absolute force level in all succeeding trains. In the EDL muscles, we had to choose a force of 10% to be able to calculate dF/dt also during the later part of the fatigue protocol, when FL peak was reduced below 30% of the initial value.

As can be seen from Fig. 5, the dF/dt at these force levels showed a temporal pattern that at least partly differs from that of maximum relaxation dF/dt. However, relaxation dF30/dt and dF10/dt basically followed the same temporal pattern as TFF (Fig. 7) and, in part, also RT1/2 (Table 1). In both muscles, relaxation rates at these force levels were significantly reduced during the first minutes of the fatigue protocol. In the soleus muscles, recovery of dF30/dt was complete after ~15 min following the nadir at 2.5 min. In the EDL muscles, only a partial recovery was observed after the nadir at 1 min.

Fig. 6. Time-dependent changes of several measures of force. 50-Hz Fm, maximum force; FLpeak, peak force of the last contraction in the train; FLmin, minimum force preceding the last contraction in the train; FFpeak, peak force of the 1st contraction in the train. Values are means ± SE; for soleus, n = 6 for 50-Hz Fm; for EDL, n = 10 for 50-Hz Fm at time 0 and n = 2 for 50-Hz Fm data after 2 min of recovery; otherwise, n = 22 for soleus and n = 12 for EDL. Note difference in time scale before and after the break.

Fig. 7. Degree of tetanic fusion as reflected by tetanic fusion factor (TFF). TFF was calculated as rectified time integral of the fluctuation of force between Fpeak and Fmin throughout the contraction divided by Fm and duration of the train (6 s in soleus and 1.5 s in EDL). Values are means ± SE. Low values reflect high degree of fusion of the contractions. For soleus, n = 22; for EDL, n = 12.
Interestingly, in the soleus muscles, developed force \((F_L \text{peak} - F_L \text{min})\) decreased over the first 2.5 min in parallel with the reduction in \(dF_{30}/dt\) \((R = 0.80; \text{Fig. } 8A)\). Later, when \(dF_{30}/dt\) recovered, the slope of the relationship was less steep. In the EDL muscles, over the 1st min the relationship between developed force and \(dF_{10}/dt\) (Fig. 8B) was slightly curvilinear, but the average linear slope was still close to 1 \((R = 0.82)\).

**FF peak**

In the soleus and EDL muscles, the time course for changes in \(FF \text{peak}\), which represents single-pulse contraction force, differed somewhat from \(F_L \text{peak}\). In the soleus muscles, \(FF \text{peak}\) closely followed the maximum contraction \(dF/dt\) throughout the protocols (Figs. 5 and 6). \(FF \text{peak}\) fell to 68 ± 4% of the initial value after 5 min (Fig. 6). After the initial fast decrease, \(FF \text{peak}\) slowly decreased further in the soleus muscles \((P < 0.001)\), reaching 51 ± 4% of the initial value after 60 min. In the EDL muscles, \(FF \text{peak}\) decreased to 30 ± 3% of the initial value in the course of 1 min, closely paralleling maximal contraction \(dF/dt\). Thereafter, \(FF \text{peak}\) was constant, whereas maximum contraction \(dF/dt\) increased (Figs. 5 and 6).

Initially, in soleus and EDL muscles, \(FF \text{peak}\) was always lower than peak force of the succeeding contractions, and the difference between \(FF \text{peak}\) and \(F_L \text{peak}\) became larger as \(F_L \text{min}\) increased. Subsequently, the difference between \(FF \text{peak}\) and the peak force of the succeeding contractions became increasingly smaller again. In the EDL muscles, however, and in striking contrast to the soleus muscles, \(FF \text{peak}\) was eventually higher than peak force of the succeeding contractions. After 5 min, \(FF \text{peak}\) was two times higher than \(F_L \text{peak}\), and the ratio remained almost unchanged for the rest of the protocol (Fig. 6). In the soleus muscles, the difference between \(FF \text{peak}\) and \(F_L \text{peak}\) became gradually smaller again after 2.5 min, as \(F_L \text{min}\) decreased to resting levels. However, \(F_L \text{peak}\) was never lower than \(FF \text{peak}\).

**Recovery of Force After the Fatigue Protocol**

In the two soleus recovery experiments and the two EDL recovery experiments, single-pulse contraction force showed a rapid, partial recovery during the first minutes after the cessation of the stimulation protocol, but thereafter single-pulse contraction force remained depressed for >30 min. In the soleus muscles, single-pulse contraction force recovered to ~70% of the initial value within 10 min of recovery and reached ~74% after 60 min of recovery. Within 1 min, single-pulse contraction force in the EDL muscles recovered to ~52% of the initial value and showed no further recovery within the 30 min of recovery that were monitored.

**Perfusion and Metabolism**

The three experiments with 99mTc-tetrofosmin showed that the two in situ operated, but resting (Sham) soleus muscles contained ~30% more 99mTc than their CC muscles and that the soleus muscle stimulated for 60 min (Stim) contained 11 times more 99mTc than the contralateral resting muscle. Force records from experiments with soleus muscles where the blood supply was occluded during the stimulation protocol (Isch) showed that the time course of changes in contractile properties of these muscles was very different from that of perfused stimulated muscle. In the ischemic muscles, force and maximum contraction...
analyses and should not be compared.

in situ stimulated with blood supply occluded. Soleus: 60-min Stim and sham, n = 6; soleus and EDL: Isch, n = 6; 2.5-min Stim, n = 6; EDL: 10- and 1-min Stim, n = 6; soleus and EDL: Isch, n = 3. *Significantly different from CC, P ≤ 0.005. †Isch – CC significantly different from 60-min Stim – CC, P ≤ 0.001. ‡1-min Stim – CC significantly different from 10-min Stim – CC, P < 0.001. §Isch – CC significantly different from 1- and 10-min Stim – CC, P ≤ 0.001. Metabolite levels in the different muscles of the different groups may be different because of batch differences in analyses and should not be compared.

Table 2. Contractile properties during stimulation of ischemic muscle

<table>
<thead>
<tr>
<th>Time, min</th>
<th>F_p, N/mm</th>
<th>TFF</th>
<th>Max contraction</th>
<th>Max relaxation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.00 ± 0.11</td>
<td>0.194 ± 0.005</td>
<td>0.388 ± 0.003</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.38 ± 0.03</td>
<td>0.23 ± 0.003</td>
<td>10.98 ± 0.38</td>
<td>3.17 ± 0.47</td>
</tr>
<tr>
<td>5</td>
<td>0.08 ± 0.03</td>
<td>0.24 ± 0.006</td>
<td>3.38 ± 0.78</td>
<td>0.58 ± 0.04</td>
</tr>
<tr>
<td>EDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.16 ± 0.08</td>
<td>0.313 ± 0.013</td>
<td>53.0 ± 7.2</td>
<td>42.0 ± 5.7</td>
</tr>
<tr>
<td>0.5</td>
<td>0.97 ± 0.04</td>
<td>0.056 ± 0.004</td>
<td>33.6 ± 0.6</td>
<td>11.83 ± 0.14</td>
</tr>
<tr>
<td>1</td>
<td>0.23 ± 0.04</td>
<td>0.040 ± 0.003</td>
<td>6.90 ± 1.23</td>
<td>1.38 ± 0.15</td>
</tr>
<tr>
<td>1.5</td>
<td>0.06 ± 0.03</td>
<td>0.052 ± 0.006</td>
<td>2.58 ± 0.92</td>
<td>0.58 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE; F_p, peak force of 1st contraction in train; TFF, tetanic fusion factor; dF/dt, 1st derivative of force.

and relaxation dF/dt fell rapidly during the first trains and did not recover. After 5 min, F_p was 8% of the F_p in the initial train, and maximum contraction dF/dt was 12% and maximum relaxation dF/dt was 6% of the initial values (Table 2). In the EDL muscles, corresponding parameters after 1.5 min were 5%, 5%, and 1%, respectively.

The ATP and creatine phosphate contents of the Stim or Sham soleus muscles after 60 min were not significantly different from CC muscles (Table 3). Lactate content of the 60-min Stim soleus muscles was doubled compared with the CC muscle, but the increase was small compared with the 11-fold increase in lactate in the Isch muscles. Also, after 2.5 min of the intermittent 5-Hz protocol, there were no significant changes in ATP or creatine phosphate. Furthermore, the increase in lactate was as large as the increase after 60 min (Table 3). In the Isch muscles, the contents of ATP and creatine phosphate were significantly lower than in the CC muscles (P = 0.007). The ATP content of the 60-min CC muscles seems low but was not significantly different from the Stim muscles. Variation in ATP content was large in these four CC muscles: 15.2–27.8 mmol/kg wet wt. It seems unlikely that ATP should be reduced in the CC muscles or increased in the Stim muscles, especially since creatine phosphate concentration was unchanged.

In the EDL muscle, in contrast to the soleus muscle, the ATP content of the 10-min Stim muscles was 44% lower than in the CC muscle, creatine phosphate had decreased by 55%, and lactate content had increased sixfold (Table 3). After 1 min of the intermittent 10-Hz protocol, the decrements of ATP and creatine phosphate were not different from those recorded after 10 min. However, the increase in lactate was larger after 1 min than after 10 min (P < 0.001). In three Isch EDL muscles, the changes in ATP and creatine phosphate were not different from those observed in the Stim muscles, but the change in lactate compared with the CC muscle was threefold larger than in the 10-min Stim muscles (Table 3; P < 0.001).

Stability of the In Situ Model

The three Sham soleus experiments revealed no changes in 5-Hz F_m, maximum relaxation dF/dt, dF_30/dt, and developed force of the last peak (F_L peak – F_L min) during the 80-min rest period (P > 0.05). The

Table 3. ATP, creatine phosphate, and lactate content after stimulation or rest vs. their contralateral control muscles in soleus and EDL

<table>
<thead>
<tr>
<th></th>
<th>Soleus</th>
<th>EDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>1-min Stim</td>
</tr>
<tr>
<td>ATP, mmol/kg dry wt</td>
<td>27.1 ± 2.7</td>
<td>26.5 ± 2.5</td>
</tr>
<tr>
<td>CP, mmol/kg dry wt</td>
<td>44.5 ± 1.6</td>
<td>43.4 ± 2.7</td>
</tr>
<tr>
<td>La, mmol/kg dry wt</td>
<td>7.3 ± 1.7</td>
<td>15.1 ± 2.1a</td>
</tr>
</tbody>
</table>

|               | CC     | 1-min Stim | CC | 10-min Stim | CC | 10-min Stim |
| ATP, mmol/kg dry wt | 31.7 ± 2.1 | 23.0 ± 1.9* | 29.0 ± 1.9 | 16.2 ± 1.1* | 43.7 ± 3.3 | 24.6 ± 5.3 |
| CP, mmol/kg dry wt | 56.9 ± 5.8 | 24.9 ± 3.6* | 55.7 ± 1.7 | 25.3 ± 3.2* | 69.8 ± 4.0 | 21.3 ± 3.8* |
| La, mmol/kg dry wt | 3.6 ± 0.8 | 80.6 ± 3.4* | 6.7 ± 1.8 | 41.5 ± 5.1* | 3.4 ± 0.3 | 121 ± 5* |

Values are means ± SE; La–, lactate; CC, contralateral control; Stim, stimulated muscle; Sham, in situ prepared but not stimulated; Isch, in situ stimulated with blood supply occluded. Soleus: 60-min Stim and sham, n = 4; 2.5-min Stim, n = 6; EDL: 10- and 1-min Stim, n = 6; soleus and EDL: Isch, n = 3. *Significantly different from CC, P ≤ 0.005. †Isch – CC significantly different from 60-min Stim – CC, P ≤ 0.001. ‡1-min Stim – CC significantly different from 10-min Stim – CC, P < 0.001. §Isch – CC significantly different from 1- and 10-min Stim – CC, P ≤ 0.001. Metabolite levels in the different muscles of the different groups may be different because of batch differences in analyses and should not be compared.

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50-Hz $F_m$ decreased by $4.0 \pm 1.3\%$ and $RT_{1/2}$ became shorter by $8.4 \pm 2.0\%$ after 60 min of rest. When single-pulse contraction force was tested at several stimulation voltages before and after 80 min of "rest," in two of the three Sham soleus muscles, single-pulse contraction force did not change compared with those before the 80-min rest period. In the third Sham soleus muscle, single-pulse contraction force increased, on average, $21 \pm 3\%$ compared with before the rest period. In the three Stim$_{60}$ soleus muscles, increasing the stimulation voltage from 7 to 10 V at the end of the fatigue protocol did not increase $F_m$ during the 5-Hz trains ($P = 0.187$). Decreasing the stimulation voltage to $<7$ V only decreased $F_m$. Stimulating with triplets or quadruplets of 1-ms pulses instead of 15-ms pulses resulted in changes in contractile properties during the fatigue protocol similar to those during the fatigue protocol induced with 15-ms pulses.

**DISCUSSION**

In this study, repeated submaximal isometric contractions of the rat soleus and EDL muscles resulted in an initial slowing of the relaxation rate, which was subsequently reversed even though stimulation continued. The initial changes in relaxation rate were parallel to the changes in developed force. Interestingly, the pattern of change of the contractile properties was similar in the soleus and EDL muscles; only the rate and the degree of change were larger in the EDL muscles.

**Contractile and Metabolic Changes**

*Circulation in the in situ model.* During prolonged dynamic submaximal exercise, aerobic metabolism is maintained. In the 30% maximal voluntary contraction (MVC) intermittent isometric exercise model in humans, blood flow to the muscles was fluctuating between low flow during the contractions and hyperemia during the pauses between the contractions (60). Even this fluctuating pattern of flow was adequate to support aerobic energy metabolism (59). In our in situ rat model, two observations indicate that circulation was intact. First, $^{99m}$Tc-tetrofosmin uptake was larger in the in situ than in the contralateral muscles. Second, the changes in contractile properties and metabolites in Isch muscles were different from those in the non-occluded in situ muscles. The fact that in the soleus no changes were found in muscle total ATP or creatine phosphate concentration and only minor changes were found in lactate indicates that blood flow was adequate to support aerobic metabolism.

*Fatigue development.* The changes in force development during the 5- or 10-Hz trains, the 50-Hz test tetani, and the test single-pulse contractions in our rat model show that fatigue developed gradually from the start of exercise. Furthermore, although relaxation slowed during the first minute(s) of the fatigue protocol, it did not remain slow. In the soleus muscles, relaxation rate, as represented by $dF_{30}/dt$ and $RT_{1/2}$, had recovered completely by the end of the 60-min fatigue protocol. Interestingly, the changes in the contractile properties during the 5- or 10-Hz trains, the test 50-Hz tetani, and the single-pulse contractions in our in situ rat model showed similarities with the force data from electrically induced 50-Hz, 15-Hz, and twitch contractions during the 30–60% MVC intermittent isometric exercise in humans (58).

*Lack of correlation between change in metabolites and changes in contractile properties.* The lack of change in total muscle ATP and creatine phosphate and only moderate increase in lactate in the soleus muscles suggest that depletion of energy stores or accumulation of metabolic breakdown products did not occur during the fatiguing contractions. Lack of changes in total muscle metabolites in muscle samples does not exclude that local changes in ATP or creatine phosphate or larger local changes in lactic acid concentration occurred or that the metabolite levels recovered within the 5 s required to stop the circulation to the muscle and remove the muscle. However, if such recovery took place and if force generation was related to metabolite levels, we should have seen recovery of force during the pauses between the trains of contractions. Also, during these pauses, the local changes in metabolites could be reversed because of equilibration with the rest of the cytosol. However, in the soleus, force development did not recover within the 4 s of rest between trains of contractions. In the EDL muscles, ATP, creatine phosphate, and lactate changed significantly, and larger local changes may have occurred. Also, in the EDL muscles, peak force recovered partially during the 1.5-s pauses between trains, as reflected by the increased $F_{r, peak}$ compared with the $F_{r, peak}$ of the preceding train of contractions during the later part of the fatigue protocol. However, a substantial part of fatigue seemed to recover more slowly and more slowly than metabolite levels are expected to recover (4, 25, 52).

In ischemic muscles, where metabolite levels changed more drastically, the changes in contractile properties were quite different from those found in the perfused muscles. The changes in metabolites found in the perfused soleus and EDL muscles are therefore not likely the main reason for the development of fatigue and the other changes in contractile properties.

*Relaxation.* Maximal $dF/dt$ is, by definition, located at the steepest part of the relaxation curve and may change simply as a consequence of lower maximum force. Provided that the number of contractile elements (number of active fibers) remain unchanged, $RT_{1/2}$ (and CT) may, to some extent, also be dependent on absolute force, since a shorter absolute time will be required for force to decrease from a lower absolute force level to 50% of this force level if the rate of relaxation at the sarcomere level is unchanged. Initially, in the soleus muscles, while $F_{r, peak}$ was still maintained, the relaxation curves became less steep. The large decrease in maximum relaxation $dF/dt$, the decrease in $dF_{30}/dt$, and the increase in $RT_{1/2}$ and $F_{r, min}$ confirm that the rate of relaxation became slower during this period. Subsequently, however, in the soleus muscles, the
early and late parts of relaxation became faster again, but not the middle, steepest part of the force-relaxation curve. Thus $dF/t$ (soleus) increased and $RT_{10}$ was reduced, whereas maximum relaxation $dF/dt$ did not recover. In the EDL muscles, the same phenomenon occurred, but in addition, maximum relaxation $dF/dt$ showed some recovery after the initial decrease. Also, the restoration of $RT_{10}$ and $dF/10/dt$ after the initial slowing was less complete in the EDL than in the soleus muscles.

Because all fibers were activated from the start, it could be argued that the initial slowing of relaxation was caused by the diminishing contribution of the fast-fatigable fast-twitch fibers in the rat model. However, in ~300-g Wistar rats, the soleus muscles consist of ~99% type I fibers and the EDL muscles consist of ~19% type IIa and 78% type IIb fibers (33). In the soleus muscles, the contribution of faster-fatigable fibers is therefore negligible, yet a slowing of relaxation occurred. In the EDL muscles, even if there might have been a faster development of fatigue in the type IIb fibers, the difference in contractile speed between type IIa and IIb fibers is not large enough (~10%) (23) to explain the large decrease in relaxation speed observed after 1 min. The observed changes in contractile speed in the rat muscles must therefore reflect changes in the properties of the single fibers. Interestingly, in our rat study, the transient slowing of the rate of relaxation from the 5-Hz (soleus) and 10-Hz (EDL) trains of contractions, which elicited ~60% of the 50-Hz tetanic force, compared favorably with the transient slowing of relaxation from the electrically induced test contractions found in the human study at the two higher target forces of 45 and 60% MVC (58).

Temperature. Temperature has a large effect on force and contraction and relaxation rates (15, 21). During the intermittent isometric exercise protocol in humans, muscle temperature increased initially by 3–4°C, which was not paralleled by changes in contractile speed (58). In the present rat experiments, Krebs-Ringer solution at 36°C was dripping onto the muscle surface to keep muscle temperature constant. In control experiments, we found that changing the temperature of the dripping solution by as little as 2°C immediately affected the contractile properties (data not shown), indicating that muscle temperature was highly dependent on the temperature of the dripping solution. Furthermore, an increased temperature, as may occur in muscle at the onset of exercise, cannot explain the initial slowing of relaxation. It is therefore likely that, in these experiments, muscle temperature was constant and that the observed changes in contractile properties were not the result of temperature changes in the muscle.

Mechanical stability of the in situ model. The in situ preparation procedure and the stimulation mode with rather long (15-ms) pulses may in themselves cause changes in the muscle’s contractile properties that have nothing to do with exercise-induced fatigue. However, most contractile properties did not change in the additional experiments of soleus Sham muscles that were given test stimuli only for >80 min. The tetanic force decreased less than during the fatigue protocol, and, if anything, relaxation became faster. The stimulation protocol does not seem to have any effect on membrane excitability, since the voltage sensitivity at the end of the stimulation protocol in the three extra soleus Stim40 muscles did not differ from control. Also changes in total intracellular $K^+$ have been shown to be modest in this model, and no changes were found in intracellular $Na^+$ (56). The tests of stability indicate that the changes in contractile properties observed during and after the fatigue protocol were due to the contractile activity and not other factors inherent to the in situ model and electrical stimulation mode.

Possible Mechanisms Causing the Observed Combination of Changes in Contractile Properties

Three mechanisms have been reported to play a role at different stages during fatigue from repeated tetani in isolated single fibers from mouse (2) and frog (65) or in in vitro whole frog muscle (5): 1) reduced amount of $Ca^{2+}$ released from the sarcoplasmic reticulum (SR) on stimulation, 2) reduced $Ca^{2+}$ affinity of troponin, expressing itself as a reduced force production at certain concentrations of $Ca^{2+}$, and 3) reduced force produced per cross bridge, expressing itself as reduced force production at saturating $Ca^{2+}$ concentration (2). In the present study, we did not measure cytosolic $Ca^{2+}$ concentration, but, as discussed below, relaxation and fatigue seem intimately related and may therefore have a common mechanism: most likely reduced re-uptake and release of $Ca^{2+}$ from the SR.

Relaxation of skeletal muscle is initiated by the release of $Ca^{2+}$ from troponin and the subsequent detachment of the cross bridges (31). The rate of release of $Ca^{2+}$ from troponin is influenced by the cytosolic $Ca^{2+}$ concentration and by the affinity of troponin for $Ca^{2+}$. The rate of relaxation may thus be regulated by the rate of $Ca^{2+}$ reuptake into the SR and has been shown to be influenced by the binding capacity of $Ca^{2+}$ buffers in the cytosol (e.g., parvalbumin) (9, 31, 34). On the other hand, the rate of cross-bridge detachment has been suggested to be independent of the rate of decrease of cytosolic $Ca^{2+}$ concentration and may alternatively be the rate-limiting step in relaxation (64). It has been proposed that the slowing of relaxation during fatigue is caused by the saturation of parvalbumin with $Ca^{2+}$ (18, 47). However, this latter idea has been disputed, since a similar slowing of relaxation also can occur during prolonged tetani in slow-twitch muscle lacking parvalbumin (11). Likewise, in the present study, also in the slow-twitch soleus muscles, relaxation was transiently slowed, and the soleus muscle does not contain detectable amounts of parvalbumin (9). Saturation of parvalbumin with $Ca^{2+}$ therefore cannot explain the transient slowing of relaxation in this muscle. Because in the fast-twitch EDL muscles the pattern of change of relaxation rate showed similarities with that in the soleus muscles, it is likely that mechanisms other than the saturation of parvalbumin.
are needed to explain the transient slowing of relaxation in the EDL muscles. In the present study, the transient slowing of relaxation can therefore be explained by a reduced rate of Ca\textsuperscript{2+} reuptake into the SR or by a slowed rate of cross-bridge detachment. Conflicting results have been reported regarding the mechanism responsible for the slowing of relaxation. Which mechanism is responsible seems to depend on species [frog or mouse (64)] or fiber type [rat soleus or EDL (20)], or both mechanisms are contributing to the slowing of relaxation during fatigue [frog (66)]. Which process is rate limiting has, however, also been shown to depend on temperature of the muscle (29). All the experiments mentioned above were performed at room temperature (20–22°C). Fryer and Neering (29) showed that at >20–25°C the rate of relaxation in rat soleus and EDL muscles seems primarily determined by the rate of Ca\textsuperscript{2+} reuptake into the SR. Several authors have suggested that a slow early phase and a faster late phase on the steep part of the curve can be distinguished in the force-relaxation curve, at least in a single muscle cell after a tetanus (1, 36, 46, 61). It has also been suggested that the rates of SR Ca\textsuperscript{2+} uptake and Ca\textsuperscript{2+} dissociation from troponin are mainly responsible for the initial slow phase and that the rate of cross-bridge detachment has its main influence on the later, steepest part of the force-relaxation curve (46, 61). If this is true also for the whole muscle preparation in the present study, the appearance of an early shoulder during the first minute(s) of stimulation and the subsequent recovery are compatible with a transiently slowed SR Ca\textsuperscript{2+} reuptake rate. On the other hand, the lack of recovery of the maximum relaxation dF/dt in the soleus and its linear relation with maximum contraction dF/dt might indicate a maintained slowed cross-bridge detachment. The changes in contractile properties taken together provide some evidence as to which of these explanations is more likely.

The hypothesis in which the mechanism of fatigue is a reduced Ca\textsuperscript{2+} sensitivity of troponin or reduced force production per cross bridge could certainly explain the reduction in single-pulse contraction force (F\textsubscript{F peak}) and 50-Hz tetanic F\textsubscript{m} but would require a separate mechanism to explain the transient slowing of relaxation and increase in resting force during the trains. The scenario becomes even more complicated when one tries to explain the “leveling off” of peak force or decrease in developed force (F\textsubscript{peak} – F\textsubscript{min}) during the trains, as observed during the initial few minutes of the fatigue protocol. Because contractions are submaximal and, therefore, Ca\textsuperscript{2+} concentration in the cytosol is not maximal, one would not expect a decrease of developed force, provided that Ca\textsuperscript{2+} release was unchanged during the trains. A further reduction in Ca\textsuperscript{2+} sensitivity would have to occur during the trains. At the same time, these changes would have to recover somewhat during the pauses between trains, since during the initial period F\textsubscript{F peak} was larger than developed force (F\textsubscript{L peak} – F\textsubscript{L min}) during the last peak in the preceding train. The reduction in Ca\textsuperscript{2+} sensitivity of troponin or force production per cross bridge would thus require a slow component and a fast, fluctuating component. A reduction in Ca\textsuperscript{2+} sensitivity would, in addition, enhance relaxation rate (i.e., if the rate of relaxation is determined by the rate of removal of Ca\textsuperscript{2+} from troponin), which does not fit with the observed transient slowing of relaxation. Such a complicated multisite mechanism is difficult to imagine.

In light of the observed combination of fatigue and the transiently reduced relaxation rate, the reductions in F\textsubscript{F peak} and the reduction in developed force during the trains seem most easily explained by a reduction in Ca\textsuperscript{2+} release. The linear relation between the reduction in developed force (F\textsubscript{L peak} – F\textsubscript{L min}) and the slowing of relaxation rate (dF\textsubscript{30}/dt or dF\textsubscript{10}/dt) supports the existence of a link between the two mechanisms. This favors a single-site mechanism involving only changes in SR Ca\textsuperscript{2+} handling. Several scenarios may be hypothesized, in which a transiently reduced rate of Ca\textsuperscript{2+} reuptake into the SR can give rise to reduced SR Ca\textsuperscript{2+} release. Many studies report exercise-induced changes in SR Ca\textsuperscript{2+} handling that fit such a scenario, such as reduced SR Ca\textsuperscript{2+} reuptake and release rates or reduced Ca\textsuperscript{2+}-ATPase activity during fatigue (8, 12, 16, 17, 26, 32, 44, 62, 67), inhibition of Ca\textsuperscript{2+} release by normal depolarization-induced increases in cytosolic Ca\textsuperscript{2+} concentration (19), increased Ca\textsuperscript{2+} leak from the SR (39), an effect of increased intracellular inorganic phosphate on Ca\textsuperscript{2+} reuptake (24), precipitation of Ca\textsuperscript{2+} with phosphate in the lumen of the SR (30, 63), and reduction in the releasable pool of Ca\textsuperscript{2+} in the SR (37). Not all these mechanisms may be applicable in the present type of submaximal exercise, since levels of ATP and creatine phosphate are not changed in the soleus. SR Ca\textsuperscript{2+}-ATPase activity is not likely to be reduced because of shortage of energy supply but may still be inhibited by other nonmetabolic mechanisms. Precipitation of Ca\textsuperscript{2+} with phosphate in the SR seems also unlikely, since phosphate levels could not have been elevated.

In conclusion, intermittent stimulation at low frequencies resulted in partly fused contractions. As a result of the intermittent trains of contractions, the soleus and EDL muscles showed an initial but transient slowing of relaxation, which was completely (soleus) or partly (EDL) restored during continued intermittent stimulation. The lack of changes of ATP and creatine phosphate and the moderate increase of lactate in the soleus suggest that metabolic factors cannot explain fatigue or the changes in contraction and relaxation rate with the present type of muscle activity. Developed force (F\textsubscript{L peak} – F\textsubscript{L min}) of the last contraction of the trains was initially reduced in parallel with an increased resting tension between contractions and the reduced rate of relaxation. The three phenomena may therefore be causally related. The observed combination of changes in the different measures of force and relaxation rate seems difficult to explain by a mechanism involving changes in myofilament properties, since it would require changes at multiple sites. We therefore hypothesize that the observed changes in...
contractile properties can be ascribed to a single-site mechanism involving changes in SR Ca\(^{2+}\) handling.

**Perspectives**

Muscle fatigue that limits performance is still an unexplained biological phenomenon. Although it has not reached all textbooks, for a long time it has been quite clear that reduced force generation by the muscle can occur in the absence of glycogen depletion or lactate accumulation. Conspicuous findings in our study were the transient slowing of relaxation early after onset of exercise and the lack of changes of high-energy phosphates when force eventually became reduced. This points to a dynamic control of contractility in the exercising muscle. The perspective of future research in this field will be to describe the mechanisms that control Ca\(^{2+}\) reuptake in the SR and the releasable pool of Ca\(^{2+}\), as well as to investigate the functional consequences of a transient slowing of relaxation for voluntary muscle contractions during submaximal exercise. Possibly, one will find associations with firing patterns of motoneurons and fiber recruitment, or even with more complex phenomena, such as “second wind” (40). Insight into fatigue mechanisms related to SR function could thus provide new approaches to training, warm-up, and competition strategies for athletes, as well as to training and rehabilitation of patients.

The authors thank the Department of Nuclear Medicine (Ulleval Hospital) for assistance with the \(^{99m}\)Tc-tetrofosmin analyses and as well as for training and rehabilitation of patients.

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