Low-frequency fatigue in isolated skeletal muscles and the effects of methylxanthines

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Summary

1. A form of skeletal muscle fatigue was examined with isolated animal and human muscle preparations. The possibility that methylxanthines could overcome this was investigated.

2. Prolonged contractile activity resulted in a long-lasting impairment of force generation at low frequencies of stimulation at times when the force at higher frequencies had substantially recovered. This was seen with both fast-twitch and slow-twitch animal muscles and with samples of isolated human muscle.

3. The decrease in low-frequency force was due to a decrease in twitch amplitude, suggesting damage to the processes involved in excitation–contraction coupling.

4. Caffeine and theophylline at concentrations of 1 mmol/l rapidly and completely reversed the effects of this form of fatigue in both animal and human muscle preparations.

5. Agents that potentiate muscle force production could be an effective means of counteracting the effects of an important form of skeletal muscle fatigue, but a clinically useful compound would need to be more potent than the methylxanthines currently in use.

Key words: skeletal muscle, human muscle, fatigue, methylxanthines.

Introduction

Skeletal muscle fatigue has been the subject of study and speculation for over a century, but there is still much that remains unknown. One clear fact is that fatigue is not a single entity, but that there are a number of forms that have different causes and may be of physiological significance under different circumstances [1]. One such form is often referred to as 'low-frequency' fatigue, and is characterized by the affected muscle generating less than normal forces at low frequencies of stimulation while still generating relatively normal forces at the higher tetanic frequencies; the effects are also very slow to recover. This has been described in human leg and hand muscles as a consequence of repeated high force contractions [2], and has also been described in overstressed respiratory muscles, where it may be a contributory factor in respiratory failure [3–6].

There has not been any thorough investigation of this phenomenon in isolated muscle preparations, where the effects of pharmacological agents might be safely examined. The present work seeks to rectify this situation, describing the occurrence of this form of fatigue in different types of animal muscle as well as in isolated preparations of human skeletal muscle. We have also examined the efficacy of the methylxanthines in reversing this form of fatigue.

Methods

Isolated animal muscles

Soleus, extensor digitorum longus and diaphragm were removed from female albino mice (30–40 g) under pentobarbitone anaesthesia (150 mg/kg). Strips of diaphragm were prepared with parallel fibre bundles running from a portion of rib to the central tendon. Between dissection
and use the muscles were stored in oxygenated incubation medium at room temperature.

**Human muscle preparations**

Samples of sternomastoid were obtained during the course of routine surgery. Viable fibre bundles were identified by careful probing with a small stimulating electrode, and these were dissected out to give preparations with cut and ligated ends [7]. Muscles were incubated in a Krebs bicarbonate medium [8] containing glucose (10 mmol/l) and tubocurarine (20 mg/l) and gassed with O₂/CO₂ (95:5) at 35°C. Preparations were mounted in a holder between platinum plate electrodes set 8 mm apart with the proximal tendon attached to an isometric strain gauge. Muscles were stimulated with square-wave pulses of 0.1 ms duration and 40V. This was 2–3-fold higher than the voltage required to give maximum force. Human muscle preparations that required longer pulse widths for full activation were discarded. Muscle length was adjusted to give maximum isometric twitch tension (l₀).

Force was recorded by using a transient recorder with one output to an oscilloscope and another to a pen recorder. Tetanic contractions and changes in resting tension were recorded on a u.v. oscillograph.

Once l₀ had been set the preparations were allowed to rest for 30 min. During this time the size and shape of the twitch settled to a stable form.

During the initial recovery phase the twitch tension sometimes increased in the case of muscles where there was some delay in setting up the preparation, but more often decreased slightly, with the time course of the twitch becoming somewhat shorter. After this 30 min period both the twitch and the maximum tetanic force, measured every 15 min over the course of several hours, remained constant in the case of the soleus muscle and decreased by approx. 10% h⁻¹ in the case of the extensor digitorum longus and diaphragm. The decrease in tension was not accompanied by any change in the force/ frequency characteristics of the muscles.

Maximum tetanic force was developed at different frequencies for the different types of muscle. For the slow-twitch soleus muscle and the human muscle preparations maximum isometric force was achieved at about 100 Hz, whereas for the extensor digitorum longus and diaphragm 150–200 Hz was necessary. To monitor the high-frequency force, 100 Hz was used for the slower muscles and 200 Hz for the extensor digitorum longus and diaphragm. As an index of low-frequency force, 30 Hz was used as a test for the soleus, 50 Hz for the diaphragm and 70 Hz for the extensor digitorum longus.

Values for twitch and tetanic tension are expressed in some of the Figures as percentages of the control values. The control value was the value measured in the fresh muscle at the end of the 30 min preincubation period.

Caffeine and theophylline were obtained from Sigma Chemical Co. and were made up as 25 mmol/l stock solutions in the incubation medium.

**Results**

**Fatigue studies**

**Soleus muscle.** In the fresh muscle the low-frequency force generated at 30 Hz was 42.3 ± 3.4% (mean ± SD, n = 14) of the maximum tetanic tension (at 100 Hz). The muscle was fatigued by stimulating with alternating periods of high-frequency and low-frequency pulses for 1 s each every 5 s (Fig. 1a). The high-frequency force fell slowly at first and then more rapidly in the second minute to reach a value between 20 and 30% of the initial tension after about 3 min. The low-frequency force at first fell and then increased to become about 50% of the maximum tetanic force at 1 min before declining roughly in parallel with the high-frequency force. Resting tension began to rise after about 1 min and showed a slow but steady increase throughout the remainder of the run. At the end of the fatiguing procedure the low-frequency force was 30.2 ± 3.4% of the high-frequency force.

Under anoxic conditions [gas replaced by N₂/CO₂ (95:5)] a similar but more rapid time course of fatigue was seen, with a larger rise in resting tension.

During recovery the resting tension returned to normal and the force at high frequency was restored, within 10 min, to about 80% of the initial force of the fresh muscle (Fig. 2a; see also Table 1). The force at low frequency, however, recovered to only about 50% of the initial fresh muscle tension in this time (Fig. 2b; see also Table 1). In the next 10 min there was no significant change in the forces generated at either frequency. The consequence of the fatiguing protocol together with 10 min recovery was therefore to decrease the force generated at low frequency as compared with high frequency from 42.3 ± 3.4% in the fresh muscle to 30.0 ± 5.0% in the fatigued muscle, a value from which there was little or no further recovery (see also Table 1).
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Fig. 1. Loss of force during a train of fatiguing contractions. (a) Mouse soleus muscle stimulated with alternating 1 s periods at 30 Hz and 100 Hz, repeated every 5 s. To the left are two control contractions in the fresh muscle and to the right are three contractions measured after 20 min recovery. (b) Mouse extensor digitorum longus muscle stimulated with alternating 1 s trains at 70 Hz and 200 Hz, repeated every 5 s. To the left are two control contractions in fresh muscle and to the right two contractions after 20 min recovery.

Fig. 2. Effects of fatigue and caffeine on force production at two tetanic frequencies. Mouse soleus muscle fatigued as described in the legend to Fig. 1, and allowed to recover for 10 min and then for a further 10 min either in the presence or in the absence of caffeine (1 mmol/l). Muscles were tested with a 1 s tetanus at 30 Hz (b) and 100 Hz (a). , In the absence of caffeine; , in the presence of caffeine (1 mmol/l). Results are for 4–9 muscles; vertical bars indicate ±1 SD.

Extensor digitorum longus muscle. In the fresh muscle the low-frequency (70 Hz) force was 34.9 ± 3.4% of the maximum tetanic force (at 200 Hz). The results of the fatiguing procedure were qualitatively similar to those for the soleus muscles, except that the rate of force loss was greater and there was no rise in the resting tension (Fig. 1b). At the end of the fatiguing run the force at both frequencies had declined to 10–20%. During the recovery phase the high-
TABLE 1. Effects of fatiguing activity, recovery and caffeine on force generation at different frequencies

Results are expressed as percentages of the control values measured with the fresh muscle. The fatiguing procedure was as described in the legend to Fig. 1, and the muscles were tested after 10 and 20 min recovery. In some muscles caffeine (1 mmol/l) was added to the incubation medium immediately after the measurement at 10 min recovery had been made. Results are the means ± 1 SD for 4-11 muscles. For the soleus muscles the low Hz stimulation was 30 s⁻¹ and high Hz 100 s⁻¹. For the extensor digitorum longus (EDL) low Hz was 70 s⁻¹ and the high Hz 200 s⁻¹, and for the diaphragm the low Hz was 50 s⁻¹ and the high Hz 200 s⁻¹.

<table>
<thead>
<tr>
<th>Force as percentage of fresh muscle force</th>
<th>Fatigued</th>
<th>10 min recovery</th>
<th>20 min recovery</th>
<th>20 min + caffeine</th>
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<tbody>
<tr>
<td><strong>Soleus</strong></td>
<td></td>
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<tr>
<td>Twitch</td>
<td>24 ± 10</td>
<td>61 ± 12</td>
<td>68 ± 18</td>
<td>105 ± 7*</td>
</tr>
<tr>
<td>Low Hz</td>
<td>20 ± 10</td>
<td>47 ± 10</td>
<td>55 ± 17</td>
<td>93 ± 20*</td>
</tr>
<tr>
<td>High Hz</td>
<td>30 ± 12</td>
<td>80 ± 12</td>
<td>80 ± 12</td>
<td>88 ± 12</td>
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<tr>
<td><strong>EDL</strong></td>
<td></td>
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<tr>
<td>Twitch</td>
<td>30 ± 12</td>
<td>41 ± 10</td>
<td>44 ± 9</td>
<td>80 ± 17*</td>
</tr>
<tr>
<td>Low Hz</td>
<td>16 ± 7</td>
<td>24 ± 10</td>
<td>25 ± 9</td>
<td>77 ± 20*</td>
</tr>
<tr>
<td>High Hz</td>
<td>11 ± 3</td>
<td>66 ± 11</td>
<td>65 ± 16</td>
<td>74 ± 13</td>
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<tr>
<td><strong>Diaphragm</strong></td>
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<td></td>
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<tr>
<td>Twitch</td>
<td>39 ± 9</td>
<td>66 ± 5</td>
<td>—</td>
<td>100 ± 6*</td>
</tr>
<tr>
<td>Low Hz</td>
<td>23 ± 5</td>
<td>59 ± 8</td>
<td>—</td>
<td>92 ± 9*</td>
</tr>
<tr>
<td>High Hz</td>
<td>27 ± 6</td>
<td>78 ± 10</td>
<td>—</td>
<td>91 ± 8</td>
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* Values differ significantly (P < 0.01) from those at 10 or 20 min recovery, without caffeine.

The frequency force recovered to 60–70% of the initial fresh value, but the low frequency force was 20–30% (Table 1). As with the soleus muscles, there was no further recovery after 10 min.

**Diaphragm preparations.** The diaphragm was intermediate between the soleus and extensor digitorum longus muscles with respect to the speed of contraction, as was the force lost during the fatiguing procedure. The changes in low-frequency force were similar to those obtained with the other muscles (Table 1).

For all the muscles the twitch amplitude showed very similar changes to those reported for the low-frequency force (Table 1).

The loss of force at low frequencies of stimulation was almost entirely due to a decrease in the amplitude of the component twitches. There was a tendency for the time course of the twitches, 10 min after the fatiguing process, to be somewhat faster, the half-time of relaxation being diminished by 15–20% (Fig. 3b), with a similar change in the time to peak twitch tension. Changes in twitch amplitude were similar in all preparations, but the changes in speed of the twitch were significant (P < 0.01) only for the soleus muscles; extensor digitorum longus and diaphragm preparations showed no consistent changes. Thus, although there were small changes in speed, the major change was a decrease in the twitch amplitude (Fig. 3b).

**Effects of caffeine**

After 10 min recovery following the fatiguing procedure caffeine (1 mmol/l) was added to the incubation medium, and twitch and high-frequency and low-frequency tetani were recorded after a further 10 min, although in some cases measurements were also made after 5 min.

With the soleus muscle the effect of caffeine was to potentiate the maximum twitch force (Fig. 3), to increase significantly the tetanic force at the
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![Graph](image)

**Fig. 4.** Effects of fatigue and caffeine on animal and human muscle preparations. The mouse soleus muscle stimulated with a package of frequencies, five twitches at 1 s⁻¹ followed by 2 s stimulation at 10, 30, 70 and 100 Hz. The human sternomastoid muscle preparations was stimulated in a similar fashion except that the frequencies used were 1, 10, 20, 50 and 100 Hz. Both preparations were fatigued by repeating the stimulating packages for approx. 2 min. The preparations were then tested after 10 min recovery and a further 10 min in the presence of theophylline (1 mmol/l).

low frequency (Fig. 2b), but not to affect the maximum tetanic tension at high frequency (Fig. 2a). The low-frequency force increased from 30·0 ± 3·4% of the maximum force at high frequencies in the fatigued muscle to 42·1 ± 3·6% in the caffeine-potentiated muscle. The value of 42% is the same proportion of the maximum tetanic force as that obtained for the fresh unpotentiated muscle (see Table 1).

Results for extensor digitorum longus and diaphragm preparations were essentially the same, with caffeine restoring the low-to-high frequency ratio of the fatigued muscle to a value very similar to that of the fresh unpotentiated muscle (Table 1).

In fresh muscle caffeine caused an increase in peak tension of 9·5 mN, this being an increase of 52% (Table 2). For the muscle that had been fatigued and allowed to recover for 10 min the increment of twitch tension in the presence of caffeine was decreased in size the relative increase was much greater at 88·7%.

Potentiation of twitch force was the major change produced by the caffeine at the concentration used (1 mmol/l). There was some slowing of the relaxation rate of the twitch (Fig. 3b), but potentiation of the low-frequency force was mainly the result of increased height of the

<table>
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<th>Table 2. Potentiation of twitch force in fresh and fatigued mouse soleus muscles at 35°C</th>
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<tr>
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<tr>
<td>Peak force (mN)</td>
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<tr>
<td>Fresh (n = 9)</td>
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<tr>
<td>Fatigued (n = 7)</td>
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<td></td>
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<tr>
<td>Unpotentiated twitch</td>
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<tr>
<td>+ Caffeine (1 mmol/l)</td>
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<tr>
<td>Increment of force</td>
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<tr>
<td>Percentage increase</td>
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</table>

For the fresh muscles the potentiated force was measured 10 min after the addition of caffeine. Other muscles were fatigued as described in the legend to Fig. 1, and the value for the unpotentiated twitch was measured after 10 min recovery. Caffeine (1 mmol/l) was added and the force recorded after a further 10 min. Values for fresh and fatigued muscles were obtained from two batches of mice of slightly different weight. The muscles used in the fatiguing experiments were smaller, the mean twitch tension before the muscles were fatigued being 15·1 mN compared with 18·4 mN for the other group. To compare the absolute increments of force on adding caffeine, the values for the fatigued muscles have been multiplied by the factor 18·4/15·1.

individual twitches rather than increasing the degree of fusion of an unfused tetanus.

Fig. 4(a) shows the mouse soleus preparation tested with trains of pulses at a number of different stimulation frequencies. The records clearly show the disproportionate loss of force at the low frequencies in the fatigued muscle, this
loss being restored by the action of theophylline (1 mmol/l).

At 1 mmol/l caffeine appeared to have no deleterious effects when incubated with a soleus muscle for 6 h. The twitch force remained potentiated and steady for this time, there was no loss of maximum tetanic force and there was no rise in resting tension. Caffeine at 0·1 mmol/l produced a small potentiation of the twitch force (<10%) and an equally small increase in the force of a low-frequency tetanus in a muscle showing low-frequency fatigue.

**Human muscle preparations.** Samples of human muscle were treated in a similar way to the animal muscles and were tested with frequencies similar to that used in testing human muscle function in vivo [9]. As with the animal muscles, fatigue resulted in a proportionately greater loss of force at the lower frequencies of stimulation (Fig. 4b), and this was largely restored by the action of theophylline.

**Discussion**

Low-frequency fatigue, known to be a consequence of severe exercise in human muscle in situ [21], was in the present study examined in isolated animal muscle preparations. The major cause of the loss of force at the low frequencies of stimulation is a decrease in the amplitude of the twitch, and it has been demonstrated that this can be overcome by low doses of caffeine. Similar findings were obtained with isolated preparations of human skeletal muscle, which showed the characteristic relative loss of force at low frequencies that could also be reversed with theophylline.

The force generated by a muscle when stimulated at submaximum tetanic frequencies depends on the amplitude of the individual twitches and on the time course of these. The longer the duration of the twitch the greater will be the degree of fusion and the higher the mean force. During a fatiguing procedure a number of changes occur in the form of the twitch. In the early stages it becomes potentiated and of shorter duration; later, as the muscle fatigues, it becomes smaller with a more prolonged time course. The consequences of these changes for the maintenance of submaximal tetanic force have been discussed by Jones [10]. In muscles allowed to recover for 10 min after the fatiguing procedure there was a tendency for the time course of the twitches to be somewhat faster, both the time to peak and the half relaxation time being shorter. These changes were significant, however, only for the slow soleus muscles. The main consequence of the fatiguing activity was to lower the amplitude of the twitch. It has previously been argued that a decrease in the amplitude of the twitch and low-frequency tetanic response at times when the maximum tetanic tension is relatively unaffected (i.e. a shift to the left of the force/frequency curve) indicates a defect in excitation–contraction coupling [2, 10]. This can be a consequence of either a decrease in the quantity of activator released per impulse or, less likely, a lowered sensitivity of the regulatory proteins to the activator.

The susceptibility of the different types of muscle to this form of fatigue was not systematically studied, but it is notable that, though producing a larger effect in the extensor digitorum longus muscles (low-frequency force decreased to 24% as opposed to 47% for the soleus after 10 min recovery; Table 1), it was the slower muscle that had nevertheless generated a greater integrated force. It appears likely, therefore, that fast-twitch muscle is more susceptible to this form of fatigue, just as it is also more susceptible to loss of force during continuous tetanic stimulation.

The actions of caffeine on skeletal muscle are complex. They depend on the concentration used, the type of muscle (whether it is fast-twitch or slow-twitch) and the temperature. At 35°C we have found no difference in the response of mouse soleus, extensor digitorum longus or diaphragm muscles to low concentrations of caffeine. At 1 mmol/l there is a potentiation of the twitch to about 150% of the fresh muscle force, with little or no change in the relaxation phase. Between 2 and 5 mmol/l the relaxation becomes slower and prolonged with increased potentiation of the peak force, and after about 10 mmol/l the muscle become steadily less excitable, peak force is lost, relaxation becomes even slower and resting tension begins to increase. At 20 mmol/l the muscle becomes electrically inexcitable and goes into a contracture.

The action of caffeine in reversing the effects of low-frequency fatigue is understandable in the light of its known effects on skeletal muscle. The increase in force at the low frequencies is due mainly to potentiation of the force response to each stimulus rather than to any change in the duration of the twitch and change in fusion frequency.

The observation that caffeine gives rise to a similar increment of force in the fresh and fatigued muscle (Table 2) may shed some light on the mode of action of caffeine, as well as on the nature of the changes occurring in low-frequency fatigue. The equal increments of force suggest
that caffeine is acting, not to amplify an existing signal for the release of activator, but to cause the release of an additional source of activator. This would be in keeping with the suggestion [11] that the action of caffeine is to activate a Ca$^{2+}$-induced Ca$^{2+}$ release from the sarcoplasmic reticulum, but that this mechanism is not normally of physiological importance in skeletal muscle. Thus in the fatigued muscle the normal mechanism for release may be damaged, but in the presence of caffeine an alternative mechanism comes into play, augmenting the diminished supply of activator to the contractile elements.

In clinical practice low-frequency fatigue has been demonstrated in the respiratory muscles of patients with chronic airways obstruction [5], and it is known that these patients often benefit from infusions of theophylline [12]. Studies with dogs in cardiogenic shock have shown that partial hypoxia of the respiratory muscles results in low-frequency fatigue [6]. This can be reversed with infusions of methylxanthines, where the concentration in the circulating blood may be as high as 1 mmol/l, and it would be expected that this would have a direct effect on the muscles. In man, however, where toxicity is a problem, it is doubtful whether the blood concentrations should ever be allowed to rise above 0.1 mmol/l [12]. At this concentration caffeine has only a minimal effect on skeletal muscle, and it is therefore difficult to know whether the benefit to the patients is due to a direct effect on the muscle or is a consequence of some of the other actions of caffeine, such as increasing cardiac output, vasodilation, bronchodilation, mobilizing free fatty acids or acting as a purinergic antagonist at the neuromuscular junction.

Most of the actions of caffeine listed above are thought to be mediated via an increase in the concentration of cyclic AMP. It has been argued that the force-potentiating effect of caffeine on skeletal muscle is not mediated by cyclic AMP [13, 14], and it is possible that there may be some compound, possibly an analogue of the methylxanthines, that, while retaining the ability to potentiate skeletal muscle force, may not stimulate cyclic AMP production. Imidazole, which is one portion of the xanthine ring, falls into this category, being a phosphodiesterase activator [15] and also a potentiator of skeletal muscle [14]. It is, unfortunately, 10 times less effective than caffeine. A similar but more potent compound would, however, have considerable potential in the management of conditions where loss of muscle contractility is an important factor.

Acknowledgments

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References