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MAXIMUM TENSION AND FORCE–VELOCITY PROPERTIES OF FATIGUED, SINGLE *XENOPUS* MUSCLE FIBRES STUDIED BY CAFFEINE AND HIGH K^+

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SUMMARY

1. The importance of reduced maximum force-generating capacity in the development of skeletal muscle fatigue has been studied using potassium and caffeine contractures as tools.

2. Single, intact fibres isolated from the lumbrical and iliofibularis muscles of *Xenopus* were fatigued by repeated tetanic stimulations until they produced close to 40% of the original tetanic tension (P_0). Using this stimulation scheme three major types of fibres can be distinguished: easily fatigued (type 1), fatigue resistant (type 2), and very fatigue-resistant (type 3) fibres (Westerblad & Lännergren, 1986).

3. When activated by 8–15 mM-caffeine–Ringer solutions fatigued fibres of all three types developed tensions similar to those of controls ($81{\cdot}0 \pm 6{\cdot}6$ *vs.* $83{\cdot}9 \pm 4{\cdot}2$% of P_0, respectively; means ± S.D.).

4. Tension output also increased markedly when fatigued fibres were depolarized by 190 mM-K^+ solution. The tension produced was in this case fibre type dependent: $71{\cdot}4 \pm 6{\cdot}6$, $81{\cdot}3 \pm 2{\cdot}5$ and $95{\cdot}0 \pm 4{\cdot}4$% of P_0 in fibre types 1, 2 and 3, respectively.

5. Force–velocity measurements were performed during caffeine contractures in fatigued iliofibularis fibres (types 1 and 2) to obtain more information about the functional state of cross-bridges.

6. In fatigued type 1 fibres the shortening velocity was reduced to about 25% of that in controls, while it was not significantly depressed in type 2 fibres.

7. It is concluded that cross-bridges of fatigued fibres can produce nearly full tension, but they may work at a much slower rate in this state.

8. Fibre types 1 and 2 mostly display a long-lasting, reversible state of severely depressed tension production during the recovery period, which has been named post-contractile depression, PCD (Westerblad & Lännergren, 1986). Fibres tested in this state generated full caffeine-activated tension and the shortening velocity was not significantly reduced. The tension output during K^+ contractures was, however, markedly depressed ($12{\cdot}4 \pm 4{\cdot}1$% of P_0).

9. In conclusion, cross-bridges are able to produce close to full tension during PCD as well as in the fatigued state if they are fully activated. The form of functional impairment seems, however, not to be the same in the two cases.

INTRODUCTION

During prolonged activity the tension output of skeletal muscles declines, that is, fatigue develops. Despite a substantial number of investigations there is no generally accepted cause for the loss of contractile performance, probably due to a contribution of several different factors (Edwards, 1981). Fatigue has been suggested to be related to increased energy demand and changes in metabolite levels (e.g. Dawson, Gadian & Wilkie, 1978). This proposal is supported by results obtained with skinned muscle fibres where the solution surrounding the myofilaments can be changed to mimic conditions which are likely to prevail in fatigued fibres. With this technique it has recently been shown that the combination of increased concentrations of hydrogen ions and of inorganic phosphate markedly reduces maximum Ca^{2+}-activated tension and this was suggested to be the major cause of fatigue (Cooke & Pate, 1985; Nosek, Fender & Godt, 1987; Cooke, Franks, Luciani & Pate, 1988).

Others explanations for fatigue development focus on events preceding cross-bridge action. By-passing the normal activation process, either by depolarization with a high-potassium solution or by application of caffeine, has been shown to markedly increase the tension production in fatigued fibres (Eberstein & Sandow, 1963; Grabowski, Lobsiger & Lüttgau, 1972; Kanaya, Takauji & Nagai, 1983). Thus, these results indicate that if fully activated, the contractile elements are still capable of generating almost undiminished tension.

In many studies of skeletal muscle fatigue preparations have been activated by long trains of low-frequency stimuli, whereas repeated, fused contractions of short durations are more common during normal use of muscles *in situ*. Further, in many *in vitro* studies of muscle fatigue temperatures well below the normal muscle temperature of active animals have been used. With this in mind we have worked at room temperature and used repeated tetanic stimulations in our studies of fatigue and recovery in isolated, intact *Xenopus* muscle fibres. In the present investigation we have attempted to evaluate the importance of reduced maximum tension-generating capacity in development of fatigue in general, using the tension response to long-lasting depolarization and caffeine application as tools. Further, by using fibres of different types (Westerblad & Lännergren, 1986) it was possible to investigate whether the mechanism behind the force reduction in the fatigued state is a general one or fibre type dependent.

In order to get more detailed information about the functional state of cross-bridges when the force output is low, fatigued fibres were maximally activated with caffeine and the shortening velocity against various loads measured.

A noteable feature of *Xenopus* type 1 and type 2 fibres is that during the recovery period, after a series of fatiguing tetani, they enter a state of marked, but fully reversible, tension depression. This phenomenon has been named post-contractile depression, PCD (Westerblad & Lännergren, 1986). To further investigate the cause of this delayed force reduction, caffeine and potassium contractures were studied in this state also.

A short account of some of the results has already been given (Westerblad & Lännergren, 1987).

METHODS

Fibre dissection

All experiments were performed on adult, female *Xenopus laevis*. Intact single fibres were isolated from either the iliofibularis muscle or from any of the lumbrical muscles II–IV under dark-field illumination using standard microdissection techniques. After dissection the largest (a) and smallest (b) diameters were measured at different places along the fibre using an ocular scale. From these values the cross-sectional area was calculated as $ab\pi/4$ for each place and the mean taken.

Mounting

Lumbrical fibres. The trimmed-down tendons of an isolated, lumbrical fibre were gripped by platinum-foil micro-clips and the preparation transferred to the perfusion channel of the experimental chamber. This chamber housed a horizontally mounted forced transducer (Akers AE 801, SensoNor, Norway), provided with a glass tube extension with a fine hook at the end. One tendon clip was attached to the transducer hook, the other to an adjustable holder, allowing the fibre to be stretched to a sarcomere length giving maximum tetanic tension.

Iliofibularis fibres. A small stainless-steel hook was tied to each trimmed-down tendon of the dissected fibre. The preparation was then transferred to the experimental chamber where it was suspended between an Akers AE 801 force transducer and the arm of a galvanometer (General Scanning G 100PD, USA). The fibre was stretched to a sarcomere length of 2·3 μm, as determined by microscopy (1000 × magnification).

Stimulation

Transverse stimulation was employed throughout with the fibres flanked by bright platinum plate electrodes. Biphasic current pulses with a total duration of 0·6 ms and with an intensity of about 1·2 × threshold were used. Fatiguing stimulation started with 500 ms stimulation trains delivered every 3·8 s, which gave an initial duty cycle of 0·13 (duty cycle = stimulation time/(resting + stimulation time)). The duty cycle was then successively increased every 2 min by reduction of the resting time; stimulation continued until the tetanic force level was depressed to about 40% of maximum tetanic tension (P_0). Fibres from both muscles were typed according to their fatigue resistances: type 1 = easily fatigued (more than 50% force reduction with a duty cycle $\leqslant$ 0·19); type 2 = fatigue resistant (less than 40% force reduction with a duty cycle = 0·19, more than 50% force reduction with a duty cycle $\leqslant$ 0·42); type 3 = very fatigue resistant (less than 40% force reduction with a duty cycle = 0·42). (For more details of the stimulation scheme and fibre typing see Westerblad & Lännergren, 1986; Lännergren & Westerblad, 1988.) In some experiments force recovery was studied by giving test tetani and recording tension at various times during the recovery period. A stimulation frequency of 70 Hz was used both during fatiguing stimulation and in tests during recovery.

Recording and measurement

General. Signals from the force transducers were displayed on a storage oscilloscope (Tektronix 5111A, USA) and on one channel of a strip-chart recorder (Hewlett–Packard 7402A, USA, or W + W Electronic 310, Switzerland). Measurements of tetanic and contracture tension were made from pen records; the accuracy of these measurements was approximately 1% of maximum tension level. Maximum relaxation rate after tetanic stimulation was measured from pen records. Values are given as means ± S.D. Differences between means were tested for significance using Student's t test; the significance level was set at 0·01 throughout.

Force–velocity (P–V) measurements in iliofibularis fibres. The arm of the galvanometer, to which one tendon was connected (see above), could rapidly be moved by changing the current passing through the galvanometer coil. This current was controlled either by a feed-back system, incorporating the force transducer signal (tension clamp), or by command pulses from a ramp generator (controlled release). Tension and length signals were each recorded by a digital waveform recorder (Wavesaver, Epic Instruments, USA) and then plotted out by an X–Y plotter (Hewlett–Packard 7470A); measurements were made from these plots. The sampling rate was initially 1 kHz and was increased to 2–10 kHz just before fibre shortening started. P–V data were first measured in the rested state using electrical stimulation (100 Hz). Tension clamps were used

in the load range 0·2–0·8 P_0 and controlled releases in the range 0·0–0·25 P_0. P–V values were then obtained during caffeine contractures (see below). To obtain several data points during a single contracture we used a three-step method described by Julian (1971) in which the load is changed in three, successively lower steps and the shortening velocity at each level recorded. The release sequence started when contracture tension had reached its maximum. The measurements during the preceding electrical stimulations included such a three-step release as well and the data points obtained in this way agreed very closely with those obtained during normal, single releases.

Solutions and induction of contractures

Under normal conditions a Ringer solution (mM: NaCl, 115; KCl, 2·5; $CaCl_2$, 1·8; sodium phosphate buffer, 3·0 (pH 7·0–7·2)) was continuously flowing through the perfusion channel at a slow rate. Caffeine contractures were studied (i) in rested fibres, (ii) during continuing stimulation of fibres in the fatigued state (electrical stimulation was stopped as soon as contracture tension started to develop), and (iii) in fibres during PCD (types 1 and 2, at about 20 min of recovery). The contractures were induced by rapidly changing from the normal solution to a caffeine–Ringer solution. The lumbrical fibres were exposed to a caffeine concentration of 15 mM; this concentration was chosen to be well beyond that needed for maximum contraction in fatigued fibres (Kanaya *et al.* 1983). The iliofibularis fibres seemed to be more easily damaged by this high caffeine concentration as shown by incomplete relaxation after removal of caffeine and therefore a concentration of 8 mM was used for these fibres. The use of this lower concentration did not result in any noticeable change in the relation between contracture and tetanic tension. Recovery after caffeine contractures was only followed in some fibres and most showed some loss of contractile capacity which appeared to be irreversible. Therefore, fibres were considered to be abnormal after a caffeine contracture and the experiment was ended. Hence, force and P–V values during such contractures had to be collected from a fairly large number of experiments on individual fibres.

Potassium contractures were studied in lumbrical fibres only. The high-potassium solution (190 mM-K^+) was made up by replacing all NaCl and KCl in the ordinary Ringer solution with 95 mM-K_2SO_4. To obtain a fast solution exchange about 2 ml of the 190 mM-K^+ solution was squirted directly into the perfusion channel (capacity 0·3 ml) with a syringe and excess fluid removed by suction. High-K^+ stimulation was used (i) in the rested state, (ii) in the fatigued state (as above), and (iii) during PCD.

All experiments on lumbrical fibres were performed at room temperature (21–23 °C). For comparison with previous results (e.g. Lännergren, 1987) standard force–velocity measurements were performed at 20·0 °C, using temperature control of the perfusing Ringer solution. The caffeine–Ringer solution was maintained at room temperature and was not passed through the temperature-control system before application; hence during force–velocity measurements the temperature of the caffeine solution might have differed by one or two degrees from that during electrical stimulation.

RESULTS

Tension response to caffeine and to high potassium of fatigued fibres and of fibres during post-contractile depression

The relative tension produced during caffeine contractures is summarized in Fig. 1. From the diagram it is clear that no significant difference existed between the tensions generated by rested fibres, fatigued fibres and fibres during PCD. Moreover, contracture tension was similar in fibres from the two muscles and in fibres of the different types. The relative tension produced by caffeine activation of rested fibres was 83·9 ± 4·2 % (mean ± S.D., $n = 16$) of that obtained with 70 Hz electrical stimulation. In comparison, fatigued fibres produced 81·0 ± 6·6 % ($n = 20$) and fibres during PCD 83·0 ± 5·0 % ($n = 9$).

Figure 2 shows examples of potassium contractures elicited in lumbrical fibres of the different types before and at various times after fatiguing stimulation. Before the stimulation period contracture tension was very close to tetanic tension in all three

fibres. The next series of contractures was induced at the end of the fatiguing stimulation period. The tensions attained at this time were 0·76, 0·80 and 0·98 P_0 for fibre types 1, 2 and 3, respectively, i.e. substantially higher than fatigued tetanic tension (about 0·4 P_0). The clearly shorter duration of contractures in the fatigued than in the rested state displayed by the type 1 and the type 2 fibre in Fig. 2 was a consistent finding in these fibre types.

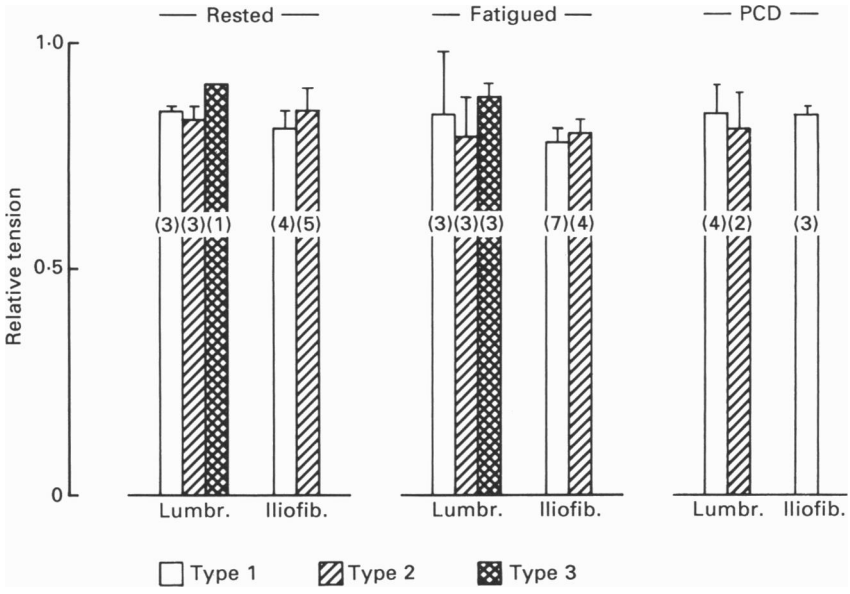

Fig. 1. Relative tension produced during caffeine activation in rested fibres, in fatigued fibres and in fibres during PCD. Values represent mean ± S.D.; the number of fibres is indicated in each column.

During the recovery period both the type 1 and the type 2 fibre displayed PCD. When these two fibres were exposed to the high-K^+ solution at this state they produced a contracture with an amplitude which was only slightly higher than the preceding, severely depressed tension response to electrical stimulation.

All three fibres eventually regained the ability to give close to normal tetanic tension. When tested with high-K^+ depolarization at these times the contracture tensions were close to pre-stimulation values.

In Fig. 3 the results from the K^+ contracture experiments are summarized. Before fatiguing stimulation the contracture amplitude of all three fibre types was close to 1·0 P_0. At the end of the stimulation period, however, the tension produced during contractures differed between the fibre types; the most fatigue-resistant (type 3) fibres still produced almost full tension, while tension output was significantly reduced in type 1 and type 2 fibres.

During PCD a striking difference between the tension produced during potassium and caffeine contractures was noted. This difference is further illustrated in Fig. 4. A type 1 fibre was here fatigued following the normal procedure. The relative tensions produced during K^+ contractures before and at the end of fatiguing stimulation were

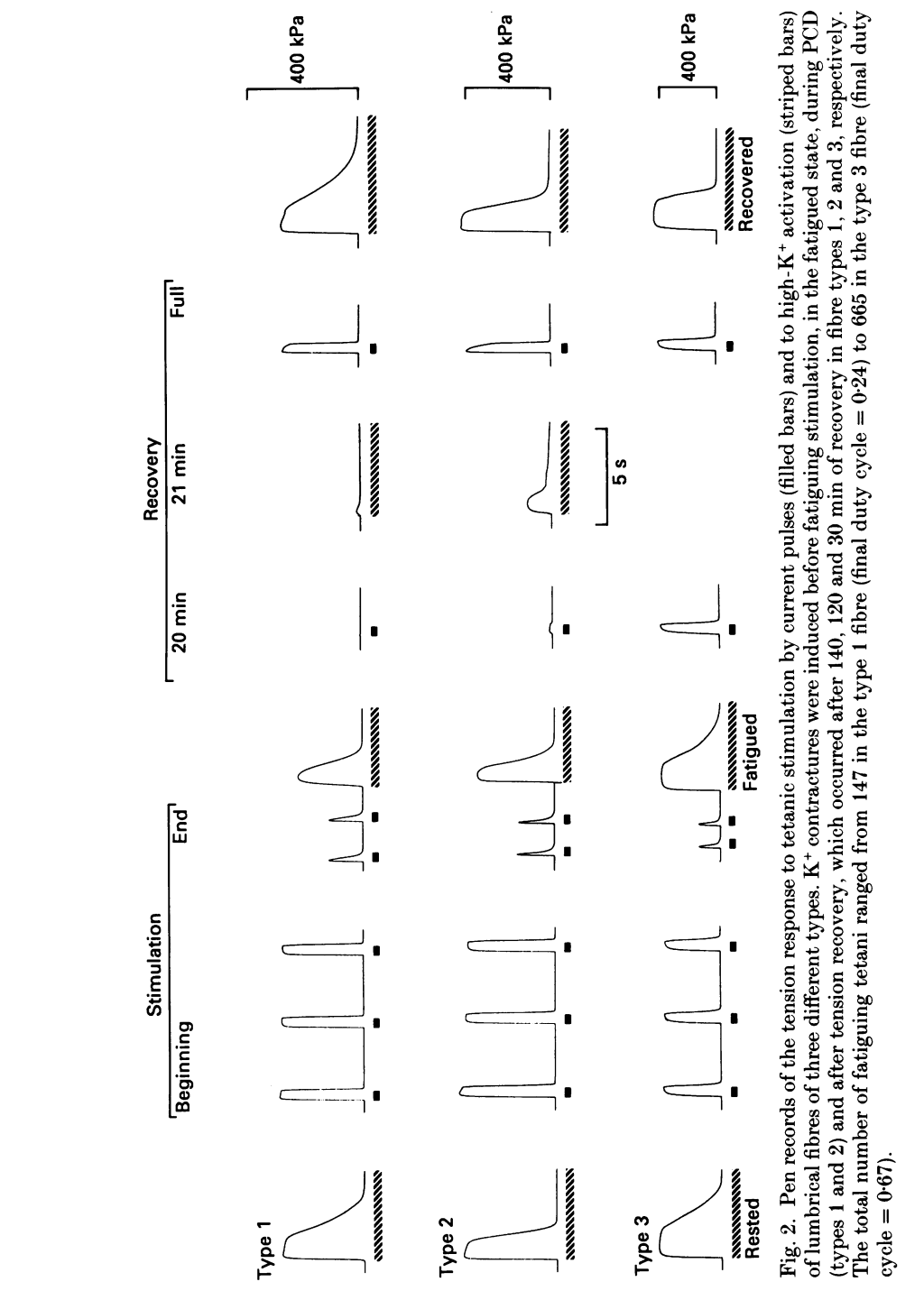

Fig. 2. Pen records of the tension response to tetanic stimulation by current pulses (filled bars) and to high-K^+ activation (striped bars) of lumbrical fibres of three different types. K^+ contractures were induced before fatiguing stimulation, in the fatigued state, during PCD (types 1 and 2) and after tension recovery, which occurred after 140, 120 and 30 min of recovery in fibre types 1, 2 and 3, respectively. The total number of fatiguing tetani ranged from 147 in the type 1 fibre (final duty cycle = 0·24) to 665 in the type 3 fibre (final duty cycle = 0·67).

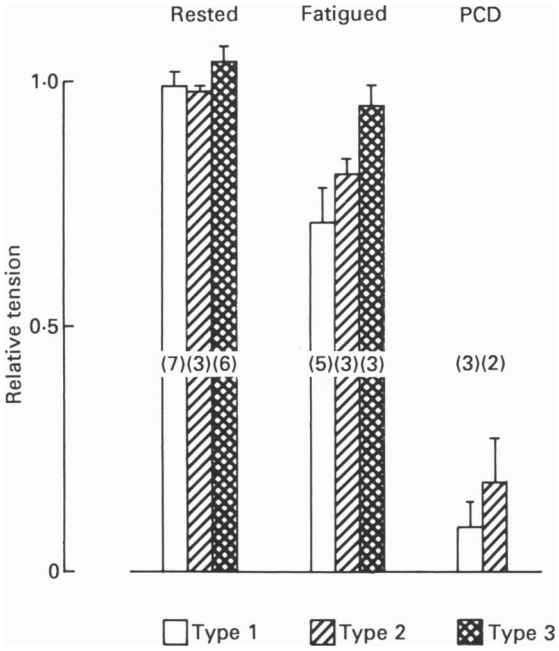

Fig. 3. Relative tension produced during high-K^+ depolarization. Mean ± S.D.; number of fibres indicated.

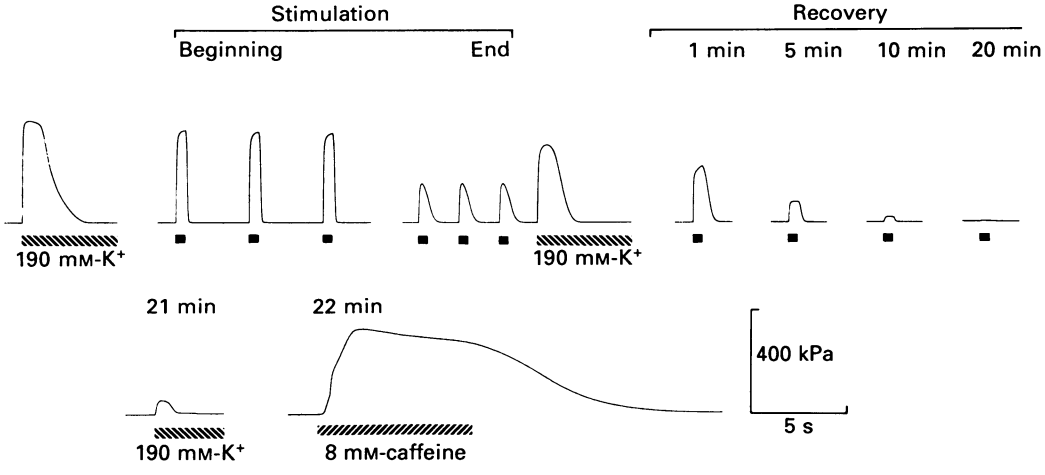

Fig. 4. Pen records from a type 1 fibre showing the tension response to tetanic stimulations (filled bars), to high-K^+ depolarizations, and finally to caffeine activation. Note the differences in tension response to the different activation types during PCD (20–22 min of recovery).

1·0 P_0 and 0·71 P_0, respectively, which are normal values for type 1 fibres. During the recovery period PCD developed and both the tetanic stimulation after 20 min of recovery and a K^+ contracture induced 1 min later resulted in a negligible tension response, while tension was in the normal range in the following caffeine contracture (0·76 P_0).

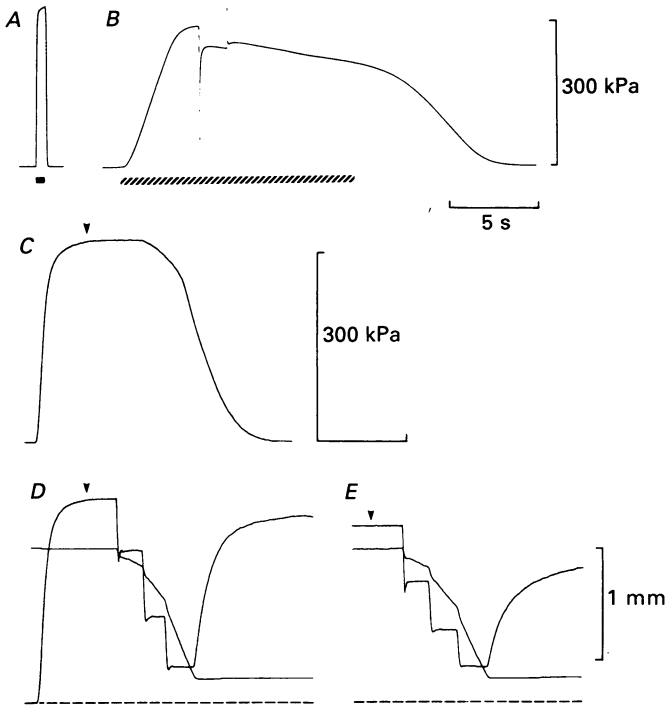

Fig. 5. Force–velocity measurements in a rested type 2 fibre during tetanic stimulation and during caffeine activation. *A* and *C*, the tension response to a 100 Hz stimulation train (indicated by the filled bar in *A*). *B*, contracture tension during caffeine exposure (striped bar indicates exposure time). *D*, a tetanus with a three-step release (for details see Methods). *E*, a similar three-step release during the caffeine contracture shown in *B*. *A* and *B* are pen-writer records; *C*, *D* and *E* represent tension and length (*D* and *E*) records from the *X–Y* plotter. Arrows in *C*, *D* and *E* indicate instants when *A–D* sampling rate was changed from 1 to 2 kHz (*C*) or 5 kHz (*D* and *E*); due to the different sampling rates the unmarked time scale represents 250 ms before arrows and 125 ms (*C*) and 50 ms (*D* and *E*) after arrows. Interrupted lines in *D* and *E* show tension baseline.

Force–velocity (P–V) measurements

Iliofibularis fibres were used to study isotonic shortening properties. When fibres were tested in the rested state their typing had to be based on *P–V* values (Lännergren, 1987). Fibres tested in the fatigued state or during PCD, on the other hand, were typed on the basis of their fatigue resistances. It was found, however, that these fibres would have been grouped in the same way if they had been typed according to their *P–V* values.

Original records of isotonic shortening of an unfatigued iliofibularis fibre (type 2) during electrical stimulation and during caffeine activation are shown in Fig. 5. During the caffeine contracture the fibre produced a peak tension of 0·88 P_0. As soon as the maximum contracture tension was reached the fibre was released (Fig. 5*b*), which allowed it to shorten against three successive loads of decreasing magnitudes (Fig. 5*E*). When the shortening velocities were plotted against the fractional loads the ensuing *P–V* values were close to those obtained during electrical stimulation

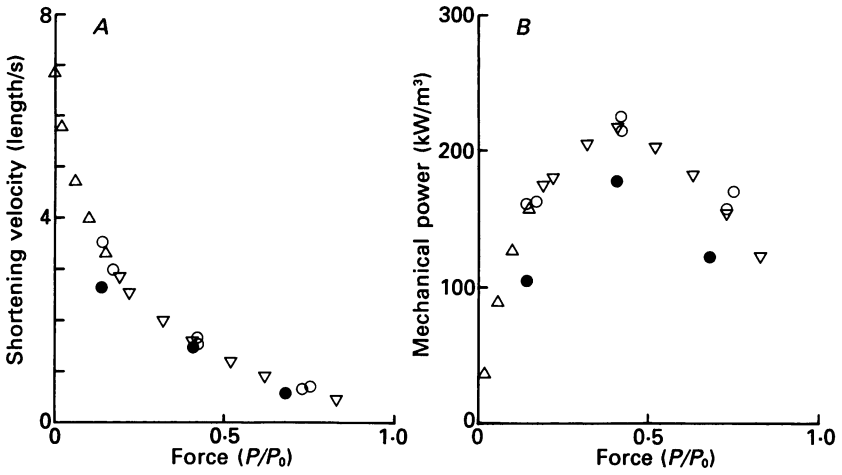

Fig. 6. Force–velocity (*A*) and mechanical power (*B*) data obtained from the experiment partly depicted in Fig. 5. Open symbols refer to electrical stimulation: tension clamp (▽); controlled releases (△); three-step releases (○). ● refers to the three-step release during caffeine exposure.

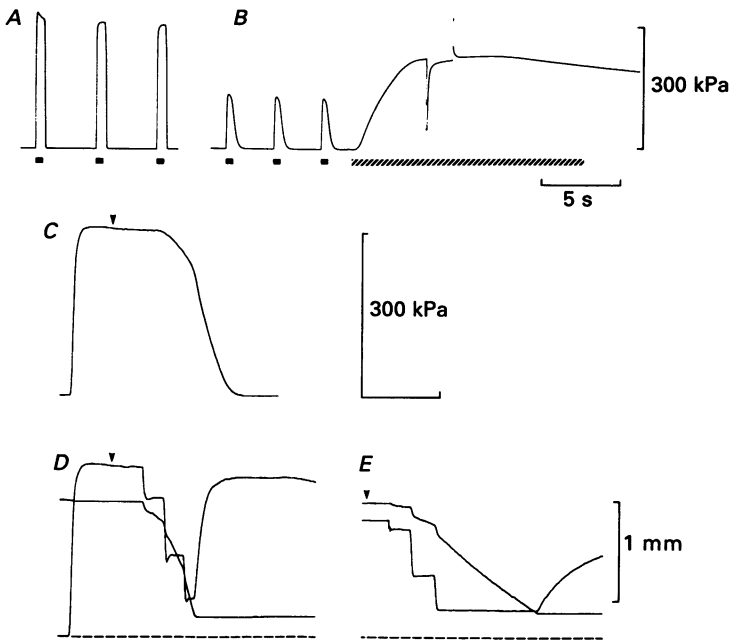

Fig. 7. Records from a type 1 fibre, in which a caffeine contracture was induced in the fatigued state. The meaning of bars, time scale, and interrupted lines same as in Fig. 5. *A*, first three tetani of the fatiguing stimulation period. *B*, last three tetani followed by the caffeine contracture. *C*, tetanus before the fatigue run. *D*, as in *C* but with a three-step release. *E*, a three-step release during caffeine exposure.

(Fig. 6*A*). In Fig. 6*B* mechanical power curves have been constructed from the *P–V* measurements. The somewhat lower power output during caffeine activation is here mainly due to the slightly lower force produced during the contracture.

In Figs 7 and 8 an experiment similar to that in Figs 5 and 6 is illustrated but in this case caffeine was applied when the fibre was fatigued to 0·43 P_0. The shortening

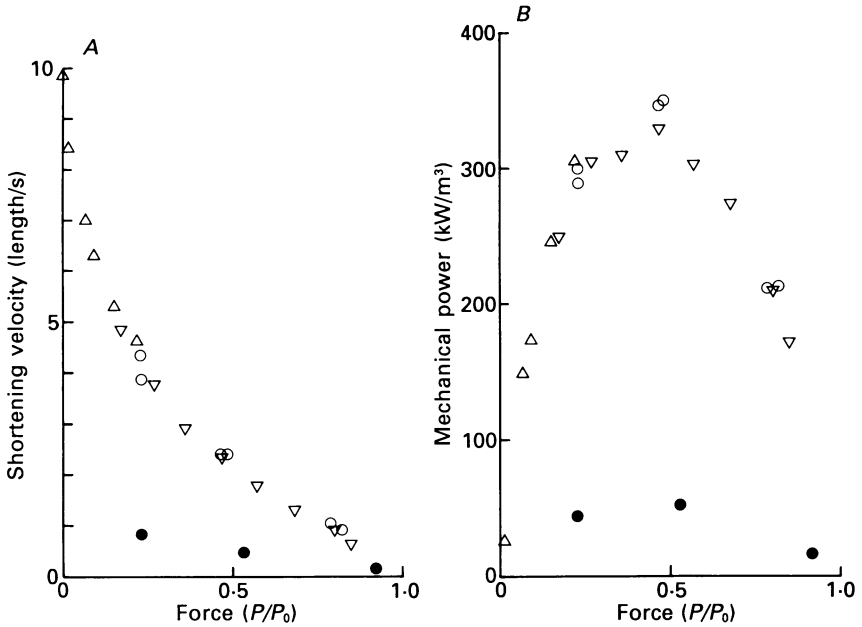

Fig. 8. Plots illustrating the reduced shortening velocity (*A*) and the low mechanical power (*B*) in the fatigued state. Values obtained from the experiment partly shown in Fig. 7. Open symbols refer to electrical stimulation before fatiguing stimulation (for explanation of symbol types see Fig. 6); filled circles are data points obtained from the caffeine contracture.

velocity was then depressed to about one-fourth of that obtained during tetanic stimulation, preceding fatiguing stimulation (Figs 7*D*, *E* and 8*A*); the mechanical power was also substantially reduced (Fig. 8*B*). It may also be noticed that the fibre went into a rigor-like state during the contracture, manifested as failure to relax promptly when a change back to the normal Ringer solution was made (Fig. 7*B*).

Three fatigued type 1 fibres were followed during the recovery period and caffeine contractures were induced after about 20 min of recovery when PCD normally is most prominent; at this time the mean tetanic tension produced by these (iliofibularis) fibres was 0·28 P_0. The isotonic shortening properties of one of the fibres are illustrated in Figs 9 and 10. The contractile speed during the caffeine contracture was here about 80% of the original at corresponding relative loads (Figs 9*E*, *F* and 10*A*).

In Fig. 11 the results from all the *P–V* measurements are summarized. The only significant reduction of shortening velocity during caffeine contractures was obtained in fatigued type 1 fibres. In this group the velocity values ranged from 17 to 40% of the pre-fatigue, tetanic stimulation values at the same relative loads. The duration

of caffeine exposure was approximately 15 s and at this time only one of these six fibres relaxed promptly; this fibre also exhibited the highest shortening speed within the group.

The shortening velocities of the three type 2 fibres tested in the fatigued state varied markedly and values ranged from 44 to 91% of pre-fatigue values. These

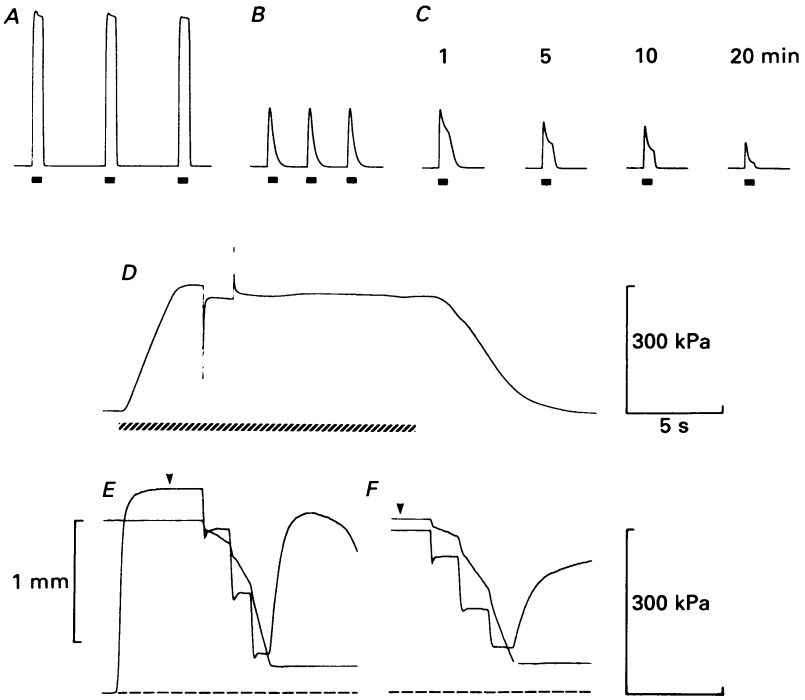

Fig. 9. Tension records from a type 1 fibre, exposed to caffeine during PCD. Lay-out of the figure as in Figs 5 and 7. *A*, the beginning of fatiguing stimulation. *B*, the end of the stimulation period. *C*, tetani at various times during the recovery period. *D*, caffeine contracture induced after 21 min of recovery. *E*, three-step release in the rested state. *F*, three-step release during the caffeine contracture depicted in *D*.

three fibres relaxed rapidly when the solution was changed back to normal Ringer solution. During the final part of the stimulation period their tension was not well maintained during tetani ('sag') and at this time maximum tension decreased in small steps rather than continuously. Also, when observing these fibres in the microscope it was noted that the contraction was slightly inhomogeneous; typically the middle region shortened while the ends were stretched.

Relation between relaxation rate and P–V values in fatigued fibres

Due to the presence of 'sag' towards the end of the stimulation period in iliofibularis type 2 fibres their relaxation rate could not be adequately measured. 'Sag' was also evident in type 1 fibres during the final part of the stimulation period. In five of the six iliofibularis fibres studied a substantial tension was, however, produced also at the end of the last tetanus. In these five fibres the relaxation rate

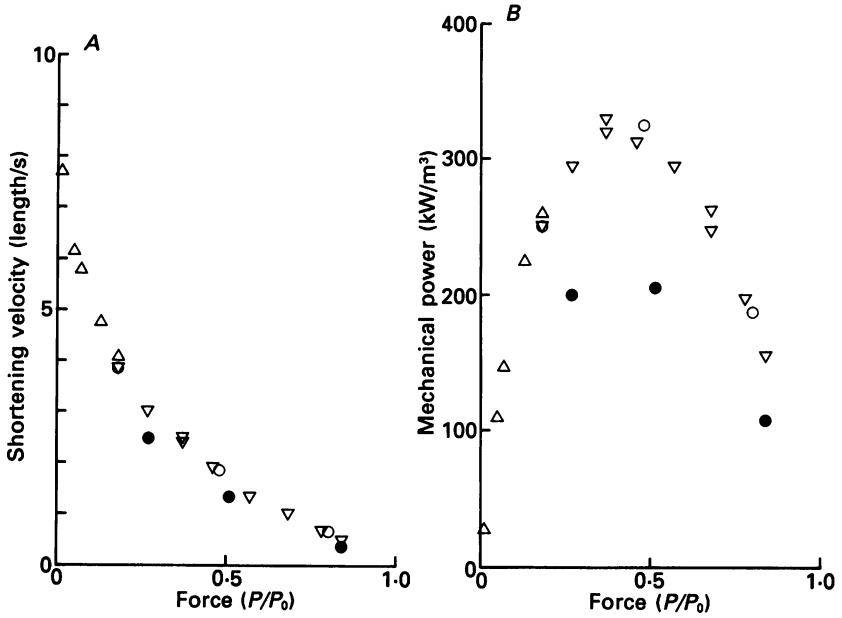

Fig. 10. Force velocity (*A*) and mechanical power (*B*) data obtained from the PCD experiment partly depicted in Fig. 9. Symbols as in Figs 6 and 8. The reduced mechanical power with caffeine activation was here mainly due to the lower tension output during the contracture and not to reduced shortening velocity.

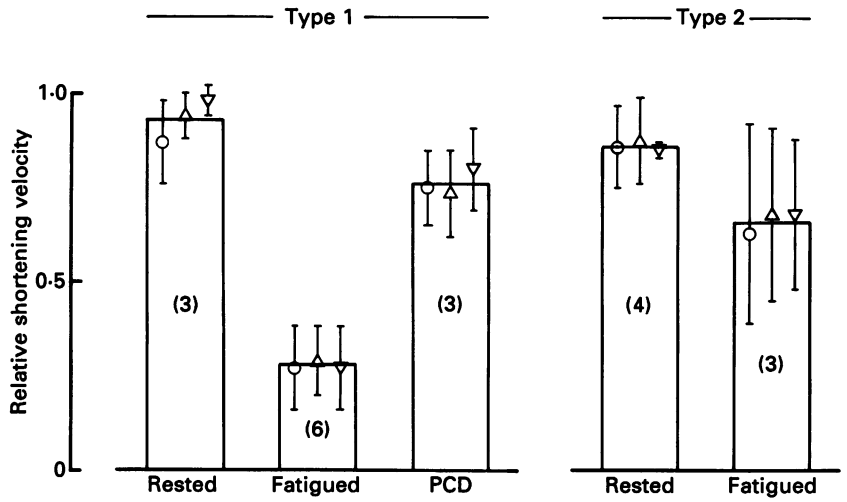

Fig. 11. The relative shortening velocity during caffeine activation in the rested state, in the fatigued state and during PCD (type 1). The relation was obtained by dividing values of the shortening velocities during caffeine exposure with those obtained at the same relative load levels during the preceding electrical stimulation. Symbols represent different load levels: 0·15–0·30 P_0 (○); 0·40–0·55 P_0 (△); 0·70–0·90 P_0 (▽). Column heights represent the mean relative velocity at all load levels; the number of fibres is indicated in each column.

was markedly slower in the fatigued than in the rested state. A direct measurement of the degree of this slowing (and comparison with the decrease in shortening speed) cannot be made since the form of tension fall is different in the two states: biphasic with a clear 'shoulder' in the rested state (Figs 5*C* and 7*C*) and monophasic in the fatigued state. In Fig. 12 we have instead plotted the maximum rate of relaxation of fatigued fibres against their shortening velocity at 0·5 P_0 and a good correlation ($r = 0{\cdot}92$; least-square method) was then obtained.

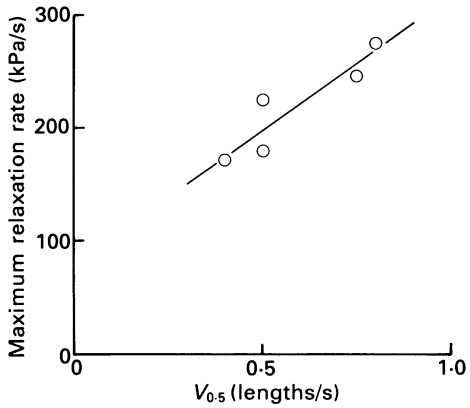

Fig. 12. Maximum relaxation rate *vs.* shortening velocity at 0·5 P_0 ($V_{0·5}$) in five fatigued type 1 fibres. Regression line $y = 234x + 82$, $r = 0{\cdot}92$. (The 0·5 P_0 tension level was arbitrarily chosen; similar plots were obtained when other load levels were used.)

DISCUSSION

The major finding of the present study is that fibres fatigued by repeated tetanic contractions can be made to develop markedly higher tension if they are activated in such a way that the normal activation process is by-passed. This was achieved by the application of high doses of caffeine, which gave tension values indistinguishable from controls, or somewhat less effectively by depolarization with a high-potassium solution. Both kinds of contractures were produced immediately after the last fatiguing tetanus in order to avoid any recovery processes having time to set in. Thus, these results show that the cross-bridges in fatigued fibres are capable of producing almost undiminished tension if they are fully activated. This view contrasts with that which has emerged from skinned fibre experiments (e.g. Cooke & Pate, 1985; Nosek *et al.* 1987; Cooke *et al.* 1988). In these experiments a marked reduction of the maximum Ca^{2+}-activated tension has been obtained in a bathing solution composed to mimic the intracellular milieu of fatigued fibres. The conclusion drawn from these studies was that the force-generating capacity of fully activated cross-bridges is markedly depressed in fatigued fibres. It is not immediately clear how this controversy can be resolved. With the stimulation scheme used here large tension–time integrals are produced and metabolic changes certainly occur as evidenced by e.g. an acidification by up to 0·9 pH units (Westerblad & Lännergren, 1988). We can only speculate that the generally used composition of the bathing media for skinned fibres is such that changes in the levels of some metabolites have a stronger depressive effect than is the case in intact fibres.

Possible causes of tension reduction in fatigued fibres; action of caffeine and high potassium

When direct electrical stimulation of muscles is used the chain of events which finally leads to cross-bridge activity starts with activation of the surface membrane and initiation of action potentials. These then propagate into the T-tubular system, where depolarization, by a poorly understood mechanism, leads to Ca^{2+} release from the adjacent parts of the sarcoplasmic reticulum (SR). The released Ca^{2+} ions bind to troponin-C and cross-bridges are activated. In the present experiments transverse field stimulation was used, which ought to minimize the risk of propagation failure along the surface membrane. The tension reduction in our fatigued fibres is then more likely to be due to failure of one or more of the consecutive steps.

The actions of caffeine and high-K^+ solutions are different: caffeine is assumed to act directly on the SR membrane to release Ca^{2+} (Weber & Herz, 1968), whereas high K^+ causes a step depolarization of the surface membrane and, by electrotonic spread, of the T-tubular membrane (Costantin, 1971). If caffeine contractures are first considered, the result that tension values similar to controls could be obtained in fatigued fibres indicates that the Ca^{2+} content of the SR is not dramatically reduced. The site of failure in fatigued fibres could then be insufficient Ca^{2+} release from the SR (Baylor, Chandler & Marshall, 1983; Schneider, Simon & Szucs, 1987) or decreased sensitivity of troponin-C to Ca^{2+} ions (Fabiato & Fabiato, 1978). In the latter case a supranormal Ca^{2+} release and/or an increased Ca^{2+} sensitivity of the myofilaments induced by caffeine (as shown by Wendt & Stephenson, 1983) has to be assumed.

As discussed above exposure to a high-K^+ solution results in a depolarization of the T-tubular membrane. Since the SR is not considered to be effectively coupled electrically to the T-system (Miledi, Parker & Zhu, 1984), K^+ depolarizations presumably act on the voltage-sensitive sensors claimed to trigger Ca^{2+} release (e.g. Schneider & Chandler, 1973). Fatigued fibres produced markedly higher tension levels during K^+ contractures than during tetani, which indicates an activation failure during tetanic stimulation occurring at an earlier stage than at the SR membrane. The degree of this failure was fibre type dependent. In fatigued type 3 fibres, which generated full tension during K^+ contractures, this failure could then account for all of the loss of force, while a failure at a later stage must be added to explain the tension reduction in type 1 and type 2 fibres. The mechanism behind this high-K^+-reversed, 'early' activation failure could either be blockade of action potential propagation down the T-tubules or refractoriness of the process which transmits the signal across the T-tubule–SR junction.

Under our conditions there is a dramatic difference in fatigue resistance between the different fibre types, which presumably is correlated with differences in metabolic capacity (Lännergren & Smith, 1966). Nevertheless, in all three fibre types fatigue appears to be mainly due to factors other than reduction of maximum cross-bridge tension production. Possible connections between links in the activation pathway and metabolism are thus of interest. Examples of such sites are the Ca^{2+} channels in the SR which are sensitive to the concentration of adenine nucleotides (Smith, Coronado & Meissner, 1985) and the ATP-driven Ca^{2+} pump of the SR.

Force–velocity measurements

As discussed so far, the state of the contractile elements has been judged from the level of isometric tension produced. From this point of view little functional impairment is indicated in the fatigued state. The first-hand conclusion from these results would then be that cross-bridge function is normal in fatigued fibres. However, the isometric tension generated reflects the number and strain of attached bridges at a given moment (Huxley, 1957) but gives no report of their cycling rate. To get information on this point, force–velocity (P–V) properties of rested and fatigued fibres were studied. P–V measurements in fatigued fibres were performed during caffeine contractures for two reasons: (1) towards the end of the stimulation period tension during each tetanus often declined ('sag') and this would have made the interpretation of results difficult; (2) we were interested in the functional state of fully activated cross-bridges and wanted to avoid possible interference from inactive myofibrils. Control experiments on rested fibres, which gave comparable P–V values with electrical and caffeine activation, indicated that a high concentration of caffeine does not itself markedly influence P–V properties.

In single frog fibres exposed to high P_{CO_2} a 30% reduction of the maximum shortening velocity (V_{max}) has been described (Edman & Mattiazzi, 1981); pH_i was not measured in the study, but it would have been reduced to about 6·3 (cf. Renaud, Allard & Mainwood, 1986). We have previously shown a similar reduction of pH_i in fatigued type 1 fibres (Westerblad & Lännergren, 1988). In this fibre type shortening velocity against various loads was decreased by about 75%. If a corresponding reduction of V_{max} is assumed, the shortening velocity was substantially more reduced in our fatigued fibres than in the similarly acidified, unfatigued fibres of Edman & Mattiazzi. Thus, the decreased cross-bridge cycling rate in fatigued fibres is probably not exclusively due to an intracellular acidification. This conclusion is supported by recent results from skinned muscle fibres in which maximum decrease of V_{max} was only about 30% when the pH of the bath solution was reduced from 7·0 to 6·2 (Metzger & Moss, 1987) or to 6·0 (Cooke *et al.* 1988).

Another explanation for the depressed shortening velocity in fatigued fibres, directly related to cross-bridge action, might be reduced ATP concentration and/or an increase of the products of its hydrolysis, ADP and phosphate. Increased phosphate concentration has been shown not to influence the force–velocity relation in skinned fibres (Cooke & Pate, 1985), while a reduced ATP concentration or an increased concentration of ADP markedly reduces the shortening speed (Cooke & Bialek, 1979; Ferenczi, Goldman & Simmons, 1984; Cooke & Pate, 1985; Stienen, van der Laarse & Elzinga, 1988). The reduction in this latter case was in some instances similar to that obtained in our fatigued type 1 fibres.

If we first consider a reduction of the ATP concentration separately, it has been shown to be reduced to about 2·5 mM in fatigued, single frog fibres (Nassar-Gentina, Passonneau & Rapoport, 1981), whereas it must be reduced to below 1 mM before a marked depression of the velocity is obtained (e.g. Cooke & Bialek, 1979). Thus, if a decrease of the ATP concentration contributes to the depressed rate of cross-bridge cycling in our fatigued type 1 fibres it must have been reduced much more than previously reported.

The calculated increase of the ADP concentration from approximately 20 μM in rested to 200 μM in fatigued fibres (Dawson *et al.* 1978) is also too small to have a marked influence on the contractile speed (see Cooke *et al.* 1988). Thus, the change in ADP concentration must also have been more severe in our fatigued type 1 fibres to explain the observed reduction of shortening velocity.

In favour of this low ATP or low ATP/ADP quotient hypothesis is the fact that those type 1 fibres in which the shortening speed was most reduced went into a rigor-like state during caffeine exposure.

The more fatigue-resistant, type 2 fibres exhibited only a small reduction (not significant) of the shortening velocity in the fatigued state. In comparison with the easily fatigued fibres these fibres are less acidified in the fatigued state (Westerblad & Lännergren, 1988). The cross-bridge cycling rate has also been shown to be less sensitive to a reduced ATP concentration in fatigue-resistant fibres (Stienen *et al.* 1988). Moreover, the stepwise tension decrease and the inhomogeneous contractions during the final part of the fatiguing stimulation period in these iliofibularis fibres might indicate a final force reduction without heavy demand on energy metabolism.

Slowing of relaxation

Slowing of relaxation is a well-known but variable feature of fatigued muscle fibres (e.g. Jones, 1981; Sahlin, Edström & Sjöholm, 1987). The slowed relaxation has been suggested to be determined by the rate of Ca^{2+} uptake by the SR (Dawson, Gadian & Wilkie, 1980) and/or by the rate of cross-bridge dissociation after removal of Ca^{2+} (Edwards, Hill & Jones, 1975). Both these mechanisms are dependent on the concentration of ATP and of the products of its hydrolysis (Dawson *et al.* 1980) and they may therefore exist in parallel. The slowed relaxation rate after tetanic stimulation in our type 1 fibres was related to the depression of shortening velocity. This result might then be explained most easily by a slow rate of cross-bridge cycling, since this influences both parameters. Measurements of intracellular Ca^{2+} would, however, be a direct way to resolve this question.

Contractures during post-contractile depression, PCD

During the recovery period after repeated tetanic stimulation PCD develops in most type 1 and type 2 fibres (Westerblad & Lännergren, 1986; Lännergren & Westerblad, 1988). This state of long-lasting, severely depressed tension output is neither related to membrane inexcitability (Westerblad & Lännergren, 1986) nor to sustained intracellular acidification (Westerblad & Lännergren, 1988). Application of caffeine during PCD gave normal tension output and only slightly reduced (not significant) shortening velocity. The latter finding suggests a normalized metabolic state in the fibres at this stage.

Nevertheless, tension production during K^+ contractures was markedly depressed during PCD. At this state morphological changes have been observed at the T-tubular–SR junctions (Westerblad, Lännergren & Flock, 1988). An explanation for the small tension response both to electrical stimulation and to K^+ depolarization might then be disrupted T-tubular–SR communication, resulting in reduced Ca^{2+} release. As discussed above, caffeine, on the other hand, is thought to act directly on the SR membrane and its action should therefore not be afflicted by the suggested disruption of communication.

In summary, we have shown that the tension reduction caused by intermittent tetanic stimulation in three types of *Xenopus* muscle fibres is not due to a marked depression of the cross-bridges' force-generating capacity, since fatigued fibres can produce almost full tension during caffeine contractures. The functional impairment in fatigue is not exactly the same in all fibre types as judged from their different tension response to K^+ depolarization. Although capable of producing a substantial tension, the cross-bridges of fatigued type 1 fibres are affected as judged by dramatically reduced shortening velocity. During the recovery period a severe disruption in the activation process develops in type 1 and type 2 fibres (PCD), which can be overcome by caffeine activation but not by K^+ depolarization.

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