Myofibrillar ATPase activity and mechanical performance of skinned fibres from rabbit psoas muscle

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1. The relationship between energy turnover and mechanical performance was investigated in chemically skinned single fibres from rabbit psoas muscle at 15 °C, pH = 7·1, with MgATP, 5 mM; free Mg^{2+}, 1 mM; ionic strength, 200 mM and sarcomere length, 2·4 μm by measuring force production and myofibrillar ATP turnover during isometric contractions as well as during repetitive changes in length. ATP hydrolysis was stoichiometrically coupled to the breakdown of NADH, which was measured photometrically via the absorption of near UV light at 340 nm.

2. Force and ATPase activity were measured during square-wave length changes of different amplitudes (1–10 % of the fibre length, L_0) and different frequencies (2·5–167 Hz). The average force during the length changes was less than the isometric value and decreased with increasing amplitude and frequency. At full activation (pCa 4·5), the isometric ATP turnover rate (± S.E.M.) was 2·30 ± 0·05 s^{-1} per myosin head. ATP turnover increased monotonically with increasing amplitude as well as with increasing frequency until saturation was reached. The greatest increase observed was 2·4 times the isometric value.

3. Force and ATPase activity were also determined for ramp shortenings followed by fast restretches. The average force decreased with increasing shortening velocity in a hyperbolic fashion. The ATP turnover increased with ramp velocity up to 0·5 L_0 s^{-1} and stayed almost constant (at 2·2 times the isometric value) for larger velocities.

4. Isometric force and ATPase activity both decreased as the calcium concentration was decreased. They did not vary in proportion at low Ca^{2+} concentrations, but this could largely be accounted for by the presence of a residual, Ca^{2+}-dependent, membrane-bound ATPase. At high calcium concentrations ATPase activity during square-wave length changes was higher than the isometric value, but at low calcium concentrations (pCa > 6·1), the ATPase activity during the length changes decreased below the isometric value and reached a minimum of 40 % of the isometric level.

5. ATPase activity and average force obtained during changes in length show a high, movement protocol-independent correlation. During the length changes the rate of ATP turnover divided by the average force level (tension cost) was larger than the isometric tension cost. The largest value found, for 10 % length changes at 23 Hz, was 17 times the tension cost under isometric conditions.

6. The effect of the length changes on energy turnover and of the variation with amplitude, frequency and calcium concentration can be understood in a simple three-state cross-bridge model, consisting of a detached, a non- or low-force-producing, and a force-producing state. In this model, length changes enhance cross-bridge detachment from both attached states, and the calcium concentration determines which of their counteracting contributions to overall ATP turnover prevails.

During muscle contraction, energy turnover associated with the contractile apparatus takes place via the molecular interaction between the myosin heads and the active sites on the actin filament. This continuous process of formation and breakage of bonds, the cross-bridge cycle, is driven by the free energy change of ATP hydrolysis. The coupling between mechanical and chemical events during contraction resides in the influence of cross-bridge distortion on cross-

bridge kinetics (e.g. Huxley, 1957; Eisenberg, Hill & Chen, 1980; Hibberd & Trentham, 1986; Pate & Cooke, 1989). An energetically important feature is the increase in the rate of cross-bridge detachment during muscle shortening. It forms the basis of the Fenn effect (Fenn, 1923, 1924), i.e. the increase in total energy change with shortening. For a review of the energetic aspects of muscle contraction, see Woledge, Curtin & Homsher (1985).

Energy turnover has previously been studied by means of heat measurements, in whole muscles and in single fibres (e.g. Fenn, 1923, 1924; Hill, 1970; Elzinga, Lännergren & Stienen, 1987; Woledge, Wilson, Howarth, Elzinga & Kometani, 1988), and by biochemical determinations on, in most cases, whole muscles frozen at different stages during contraction (e.g. Infante, Klaupiks & Davies, 1964). These measurements yielded valuable information concerning the metabolic pathways involved and the efficiency of muscle contraction. They also provided constraints for models of the molecular mechanism of force production (e.g. Huxley, 1973). These methods, however, provided just an overall picture associated with the total energetic and chemical change of the whole set of reactions involved.

To overcome these inherent difficulties, we studied ATP hydrolysis in single skinned skeletal muscle fibres. In these muscle fibres the sarcolemma has been made permeable such that the composition of the intracellular medium can be manipulated. The myofibrillar ATPase activity was measured photometrically by means of an enzymatic coupling of the resynthesis of ATP to the oxidation of NADH, present in the bathing solution. It is possible in this way: (1) to measure the extent of ATP splitting during contraction directly; and (2) to study the control of energy liberation during movement by varying the composition of the intracellular medium.

As a first step, we have studied the relation between ATP hydrolysis and mechanical performance by imposing abrupt releases and stretches as well as isovelocity shortenings followed by abrupt restretches. Since it was found that Ca^{2+} modulates the effect of movement on ATP turnover in cardiac muscle (Stienen, Papp, Potma & Elzinga, 1992*a*), it was considered of interest to vary the Ca^{2+} concentrations during these measurements as well. The results support the relatively simple concept that myofibrillar ATPase activity and average force (or load) are closely linked and that muscle shortening promotes cross-bridge detachment via the ordinary pathway of splitting ATP but also via reversal of the attachment process.

METHODS

Preparation

The methods for dissection and handling fibres were described by Stienen, Versteeg, Papp & Elzinga (1992*b*). In short, adult New Zealand White rabbits (*ca* 3 kg), were anaesthesized by injection of 60 mg kg^{-1} sodium pentobarbitone or 9·6 mg kg^{-1} fluanisone and 0·3 mg kg^{-1} fentanyl citrate (Hypnorm; Janssen Pharmaceutica B.V., Beerse, Belgium) in the ear vein, and exsanguinated via the carotid artery. Fibre bundles about 2 mm in diameter and 2–3 cm long, from psoas muscles, were chemically skinned and stored in a 50 % glycerol solution for up to two months, as described by Goldman, Hibberd & Trentham (1984*a*). Single fibre segments were separated from these bundles in the storage solution, and subsequently transferred to cold relaxing solution in which either end was crimped into an aluminium T-clip (Goldman & Simmons, 1984). The length of the fibre segment between the clips ranged from 2·1 to 3·2 mm. In some cases the ends of the fibres were chemically fixed by gluteraldehyde as described by Chase & Kushmerick (1988).

Apparatus

The apparatus and the principle of the ATPase measurements were as described by Stienen, Roosemalen, Wilson & Elzinga (1990). The skinned segments were mounted by means of T-clips in the apparatus shown in Fig. 1. One side was attached to a displacement generator (Model 101, Ling Dynamic Systems, Royston, Herts), which was controlled by a Z80-based programmable function generator. The other end was attached to a force transducer (AE801, SensoNor, Horten, Norway), which had a natural frequency of about 2 kHz. The preparation could be transferred manually between baths.

The ATPase activity of the fibre was measured by a coupled enzyme assay (cf. Glyn & Sleep, 1985). The hydrolysis of ATP into ADP and inorganic phosphate (P_i) inside the fibre was coupled to the oxidation of NADH to NAD^+. This reaction sequence is

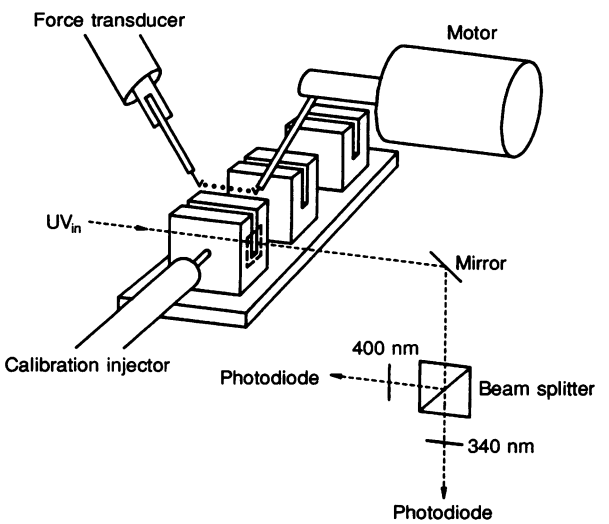


Figure 1. Diagram of the experimental set-up
The preparation was mounted horizontally between the motor arm and a thin carbon fibre glued to the force transducer element. In this way the fibre could be transferred between the baths which contained the relaxing and the pre-activating solution (volume, 80 μl), and the measuring chamber (30 μl, nearest in the figure) which usually contained vigorously stirred activating solution. The NADH absorbance was derived from the light intensity at 340 nm measured by the UV-enhanced photodiodes, after the 50/50 beam splitter. The light intensity at 400 nm was used to correct for intensity variations. To calibrate the absorbance signal small amounts of ADP could be administered by means of a stepper motor-controlled injector.

catalysed by pyruvate kinase and lactate dehydrogenase. NADH breakdown was determined photometrically from the absorbance at 340 nm of near-UV light from a 150 W xenon arc lamp (XBO150, Osram) which passed through the bath beneath the preparation (Fig. 1). The bath that was used for the ATPase measurements (volume 30 μl) was continuously stirred. The temperature of the solutions was controlled at 15 ± 1 °C. The transmitted light intensity was monitored by two UV-enhanced photodiodes at 340 and 400 nm via a beamsplitter and two interference filters. The ratio of the light intensities at 340 and 400 nm from an analog divider was fed into a logarithmic amplifier. This absorbance signal was calibrated at the end of each recording by adding 0·05 μl of 10 mM ADP from an injector, which was controlled by a stepper motor. The rise of phosphate concentration inside the measuring chamber during the measurements was small. Typically about 1 nmol (i.e. 0·03 mM) phosphate was formed upon hydrolysis of ATP into ADP (cf. Fig. 2).

The force and length signals were filtered at 1 kHz (−12dB per octave) and the absorbance signal was filtered at 2·5 Hz (−12dB per octave). The data were recorded on a chart recorder and on a computer at a sampling rate of 5 Hz. When the motor was turned on, force and length changes were also recorded at a sampling rate of 1 or 4 kHz, for 2 s. A 10 W helium–neon laser was used to measure the sarcomere length of the preparations, in relaxing solution. Sarcomere length was adjusted to 2·4 μm.

Experimental protocol

General. During the first contracture the fibre segment was kept isometric and activated maximally (pCa 4·5). Thereafter, if necessary, sarcomere length was readjusted to 2·4 μm and the length of the fibre between the clips as well as its width and depth were measured, from which volume and cross-sectional area were calculated. After the readjustment, sarcomere length usually remained stable throughout the experiment.

Three bathing solutions were used: a relaxing, a pre-activating and an activating solution. All solutions contained 100 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid (Tes), 1 mM free Mg^{2+}, 5 mM MgATP, 0·8 mM NADH, 5 mM phosphoenolpyruvate, 5 mM sodium azide, 10 μM oligomycin B, 0·2 mM p^1,p^5-di(adenosin-5′-)pentaphosphate, 4 mg ml^{-1} pyruvate kinase (470 U mg^{-1}, Sigma, USA) and 0·24 mg ml^{-1} lactate dehydrogenase (710 U mg^{-1}, Sigma). Potassium propionate was added to adjust ionic strength to 200 mM and pH was adjusted to 7·1 (at 15 °C) with potassium hydroxide. The relaxing solution contained 20 mM ethylene glycol-bis(β-aminoethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA), the pre-activating solution 0·5 mM EGTA and 19·5 mM 1,6-diaminohexane-*N,N,N′,N′*-tetraacetic acid (HDTA), and the activating solution 20 mM CaEGTA.

Activating solutions with different Ca^{2+} concentrations were made by appropriate mixture of relaxing and activating solutions, assuming an apparent stability constant for the Ca–EGTA complex of $10^{6.82}$. The preparations were incubated in relaxing solution for 4 min, in pre-activating solution for 3 min, in activating solution for about 2 min (depending on the protocol used for the length changes), and from there they were returned to relaxing solution. In the activating solution, first steady isometric force and ATPase activity were recorded. Then the motor was switched on and the force and ATPase during length changes were obtained, and then the isometric values were again recorded, after the motor had been switched off. Each of these periods lasted about 40 s. In most cases, the motor induced square-wave length changes which were symmetrical with respect to the isometric length. The length changes were performed in 2 ms. The repetition frequency was varied by changing the duration of the pauses between the length changes (cf. Fig. 4). In another set of experiments, ramp shortenings at various velocities followed by fast restretches were imposed (cf. Fig. 6*A*).

The isometric values of force (F_0) and ATPase activity (A_0) obtained before and after the motor had been switched on, were averaged. The values found during the length changes were normalized to these isometric controls. During one activation, occasionally the motor was switched on again to allow for an additional measurement under a different condition. In that case too, force and ATPase for the length changes were compared with the values determined during the two adjacent isometric periods. If the isometric force at full activation became less than 80 % of the force during the first contraction, all measurements from the former full activation (where $F_0 \geqslant 80$ %) onwards, were discarded.

The active force levels were measured relative to the baseline found in relaxing solution. The ATPase activity was derived by linear regression analysis of the absorbance signal. The rate of myofibrillar ATP hydrolysis was derived from the slope of the absorbance signal relative to the baseline obtained after the muscle was taken out of the solution. The slope of the baseline when the preparation was not present in the measuring chamber was mainly due to NADH bleaching under the intense UV light. The Ca^{2+}-activated ATPase activity was corrected for basal ATPase activity measured in relaxing solution (pCa 9). On average this basal activity was 7 ± 1 % (mean ± S.E.M., $n = 28$) of the maximal isometric ATPase activity. Length changes appeared to have no effect on it.

Variation of the mechanical conditions. The following reference conditions were chosen for the square-wave length changes: saturating calcium concentration (pCa 4·5), a repetition frequency (f) of 23 Hz (pauses between the length changes of 20 ms) and a peak-to-peak amplitude (ΔL) of 2·5 % of the initial fibre segment length (L_0). A set of experiments was performed in which the amplitude was varied between 1 and 10 % of the initial length (f was 23 Hz). In another set of experiments the pauses between the length changes were varied between 1 ms ($f = 167$ Hz) and 200 ms ($f = 2{\cdot}48$ Hz) (ΔL was 2·5 % of L_0).

For the experiments in which ramp-shaped shortenings followed by restretches were imposed, an amplitude of 20 % of L_0 was chosen. The restretch always lasted 2 ms but the duration of the shortening ramp, and therefore the shortening velocity (v) during that phase, was varied between 100 ms ($v = 2\ L_0\ s^{-1}$) and 1600 ms ($v = 0{\cdot}125\ L_0\ s^{-1}$). In all experiments, the effects of amplitude, frequency and shortening velocity were tested in random order.

Variation of the calcium concentration. The influence of the level of activation was examined at six different calcium concentrations between pCa 4·50 and 6·55. In addition some experiments were performed for pCa > 6·55, where isometric force is small or absent, to study the calcium dependence of the basal ATPase activity. In most of these experiments, near optimal experimental conditions were used, chosen on the basis of the amplitude and frequency experiments: $f = 23$ Hz; $\Delta L = 5$ % of L_0. In another set of experiments, square-wave length changes were imposed at $f = 12$ Hz; $\Delta L = 2{\cdot}5$ %.

The first activation was carried out at saturating calcium concentration (pCa 4·5; first control). It was followed by a series of activations at lower calcium concentrations, with every third

again at maximum activation, as controls. To correct for the relatively small deterioration of force development and ATPase activity, the isometric force and ATPase were normalized to the nearest control values. The order of different submaximal levels of activation was random.

RESULTS

The average isometric ATPase activity at full activation was $0{\cdot}46 \pm 0{\cdot}01$ mM s^{-1} (mean ± S.E.M., $n = 28$). Assuming a myosin head density of 0·2 mM (e.g. Glyn & Sleep, 1985) this ATPase rate amounts to $2{\cdot}30 \pm 0{\cdot}05\ s^{-1}$ per myosin head. The corresponding average isometric force was 118 ± 4 kN m^{-2} (mean ± S.E.M., $n = 28$).

In Fig. 2*A* the force, length and absorbance signals are shown at low time resolution. They were recorded from a maximally activated fibre which was also subjected to square-wave length changes. During active force development, a steady decline in absorbance occurred, which became larger during the changes in length. In Fig. 2*B*, the force and length signals during the length changes are shown at high time resolution. It can be seen that force recovered after shortening as well as after lengthening (after an initial undershoot), but that it stayed below the isometric level. As a result, the average force during length changes was lower than the isometric force.

Variation of the mechanical conditions

In Fig. 3, typical force and length signals for three different amplitudes are shown at high time resolution. At an amplitude of 1% of the fibre length L_0, the fast force recovery was almost complete, after stretch as well as after shortening. As the amplitude increased, the extent of the force recovery after shortening increased, and the overall rate at which it occurred decreased. For higher amplitudes, force after stretch quickly resumed the level of the recovery after shortening. Fast recovery, especially after shortening, might be truncated because of the 2 ms duration of the length changes, and because force reached its baseline. With increasing amplitude, the average force level during the length changes decreased. Stiffness, derived from the

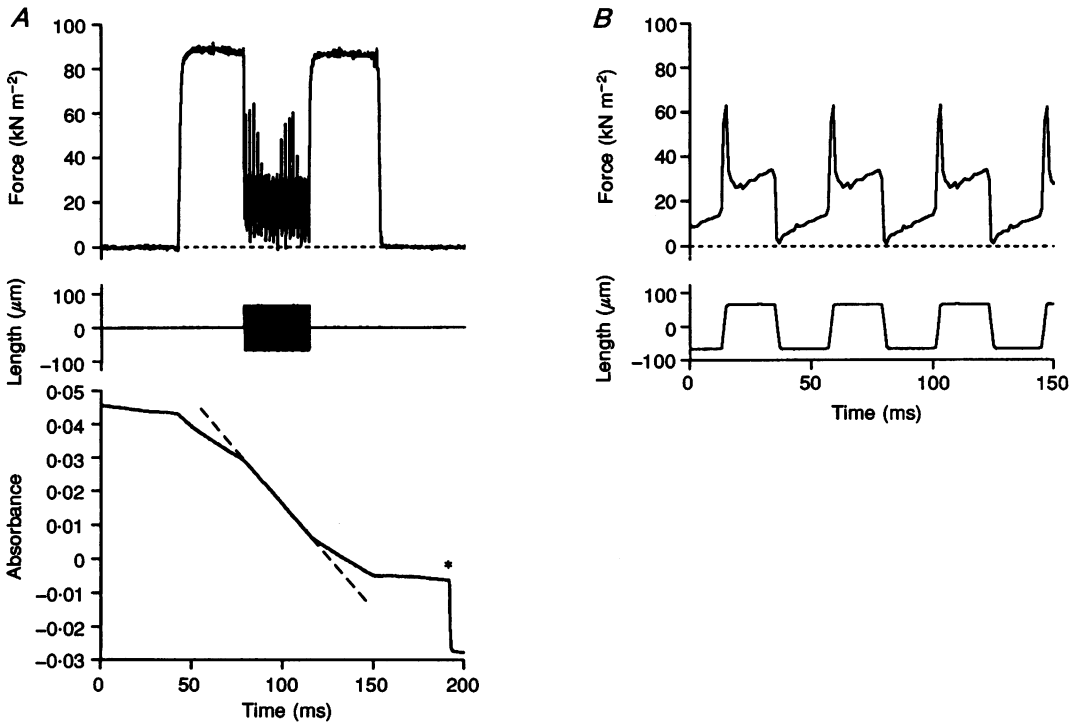


Figure 2. Recordings of force production, fibre length and absorbance for a maximally activated fibre (*A*) and the force responses and the length changes at higher time resolution (*B*)
ATPase activity was determined from the decrease in light absorbance at 340 nm by calculation of the slopes of selected parts of the optical signal, as illustrated. From $t = 42$–152 s, the preparation was immersed in the measuring chamber containing activating solution. After the first isometric period, square-wave length changes of 5% of the initial length were imposed at a repetition frequency of 23 Hz. The fast recordings of the force and length signals (*B*) were obtained during these length changes (note that the apparent changes in the peak force during the length changes in the slow force recording were due to the low sampling frequency of 5 Hz). Subsequently the preparation was kept isometric again and then it was returned to the relaxing solution. Finally 0·5 nmol of ADP was injected into the bath to calibrate the absorbance signal (*). The zero level for the absorbance signal was arbitrarily chosen. Fibre dimensions were: length 2·80 mm and diameters 80/100 μm.

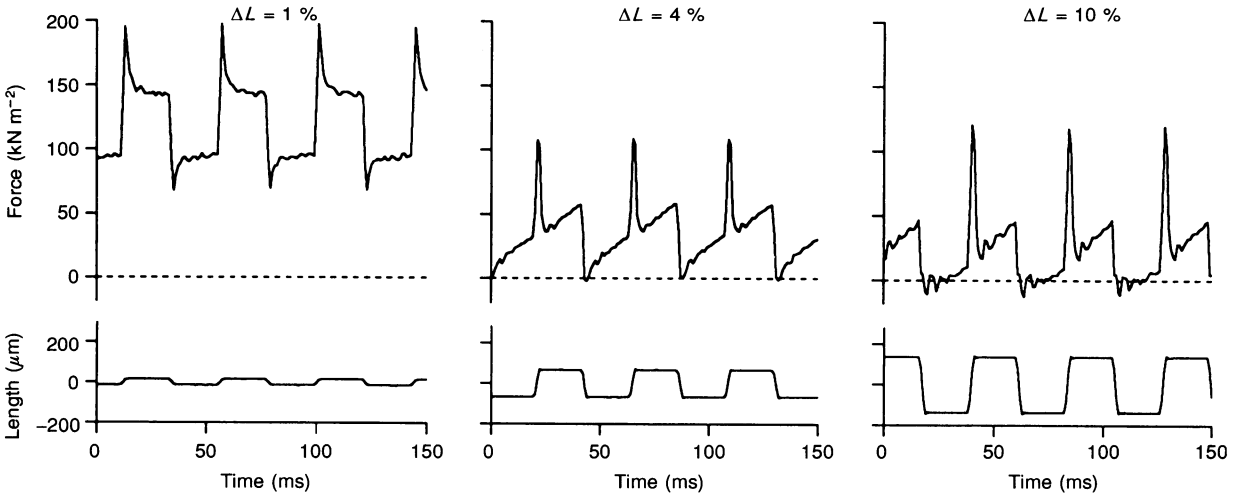


Figure 3. Force responses to repetitive length changes of different peak-to-peak amplitudes
For all experiments $f = 23$ Hz, pCa = 4·5. As the amplitude increased, the speed of the early component of the force response after stretch increased, whereas it decreased after shortening. The fibre became slack during shortening, for amplitudes of about 3 % or higher. Isometric force corresponded to 138 kN m^{-2} at $\Delta L = 1$ % and to 125 kN m^{-2} at $\Delta L = 10$ % (fibre length, 2·65 mm; diameters, 80/65 μm) and to 132 kN m^{-2} at $\Delta L = 4$ % (fibre length, 3·35 mm; diameters, 100/60 μm).

amplitude of the force response during restretch divided by the amplitude of the length change, also appeared to decrease as amplitude increased.

In Fig. 4, the force and length signals are shown for three different repetition frequencies. The force recovery after stretch was faster than after shortening, and these processes showed a similar time course for all frequencies (notice the different time scales). However, as the frequency increases, the time to recover after shortening decreases. The average force during the length changes therefore became smaller with increasing frequency. Apparent stiffness tended to decrease with increasing frequency.

The dependencies on amplitude and on frequency of the ATPase activity and the average force level are summarized in Fig. 5. It can be seen that the ATPase activity during the square-wave length changes was always higher than the isometric value. It increased with increasing amplitude and saturated at about 2·3 times the isometric rate for amplitudes above 5 % of L_0. It also increased with frequency up to 42 Hz, where the ATPase activity appeared to saturate

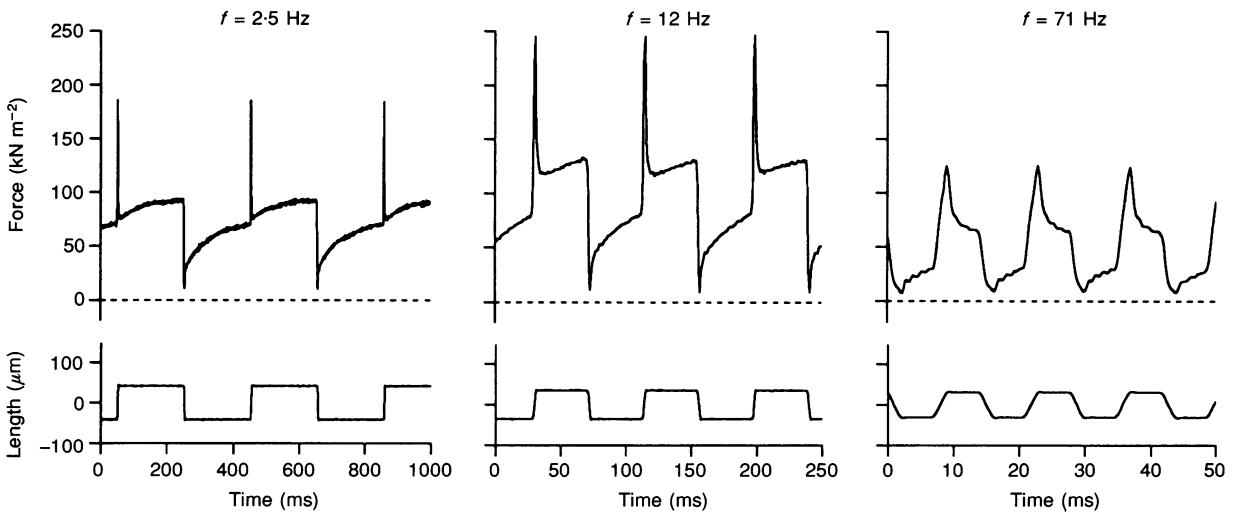


Figure 4. Force responses to length changes of different repetition frequencies
For all experiments $\Delta L = 2{\cdot}5$ % and pCa = 4·5. The recovery rate after lengthening was higher than after shortening. These rates remained approximately the same for all frequencies. The isometric force: 95 kN m^{-2} at $f = 2{\cdot}5$ Hz (fibre length, 3·30 mm; diameters, 75/75 μm), 148 kN m^{-2} at 12 Hz (fibre length, 2·85 mm; diameters, 85/70 μm) and 125 kN m^{-2} at 71 Hz (fibre length 2·50 mm, diameters 80/80 μm).

at about 2·1 times the isometric ATPase ($\Delta L = 2{\cdot}5\,\%$). The values obtained for 42, 71 and 167 Hz were not significantly different ($P < 0{\cdot}05$). It could be argued that this was due to the relatively large scatter in the data points, but paired measurements at the three higher frequencies showed direct evidence of saturation. In these experiments, the differences of the ATPase activities within one fibre were small compared to differences between fibres, and within fibres no systematic trend with frequency was found. It can also be seen in Fig. 5 that the average force during the length changes was always lower than the isometric value. It decreased with increasing amplitude as well as frequency and, over the investigated range, it did not reach a minimum. The paired measurements within one fibre for the three higher frequencies showed a systematic decrease in force with an increase in frequency. For amplitudes and frequencies above the investigated range, average force is expected to remain above zero because the fibre does not bear (large) negative forces. The larger variability in ATPase activity than in corresponding average force values might be due to temperature variation, since ATPase activity is more sensitive to temperature than force.

It appears that the lower panels in Fig. 5 are nearly mirror images of the upper panels. This reflects the fact that average force and ATPase are highly correlated. In a different representation (Fig. 9) the more general nature of this correlation will become apparent.

In a separate series of experiments in which the amplitude ($\Delta L = 5\,\%$) and the frequency ($f = 83$, 125 or 167 Hz) of the square-wave length change were both chosen in the range where the ATPase saturates, a maximum value for the increase in ATPase activity was found of $2{\cdot}4 \pm 0{\cdot}1$ times the isometric value (mean ± S.E.M., 7 fibres).

To estimate the influence of end compliance in the preparations, a series of experiments was performed in which the fibre ends at and underneath the T-clips were chemically fixed by gluteraldehyde (cf. Chase & Kushmerick, 1988; Martyn & Gordon, 1992). The shape of the relationship between the average force level and the amplitude of the length changes was very similar to that for untreated fibres (Fig. 5, lower left panel). The amplitude at which the average force was half of the isometric value, however, decreased from 2·45 % of L_0 to 1·85 % of L_0 when the ends of the fibres were chemically fixed. The shapes of the force responses at corresponding average force levels were very similar for treated and untreated fibres (cf. Fig. 3). This indicates that the contribution of end compliance in these experiments is relatively small.

Previous energetic studies on intact muscle tissue were mainly performed using isotonic or isovelocity shortenings. To allow a comparison with these studies, we imposed large ramp-shaped shortenings (20 % of L_0), followed by fast stretches. An example from such an experiment can be seen in Fig. 6*A*. Apart from an initial fast transient, during and

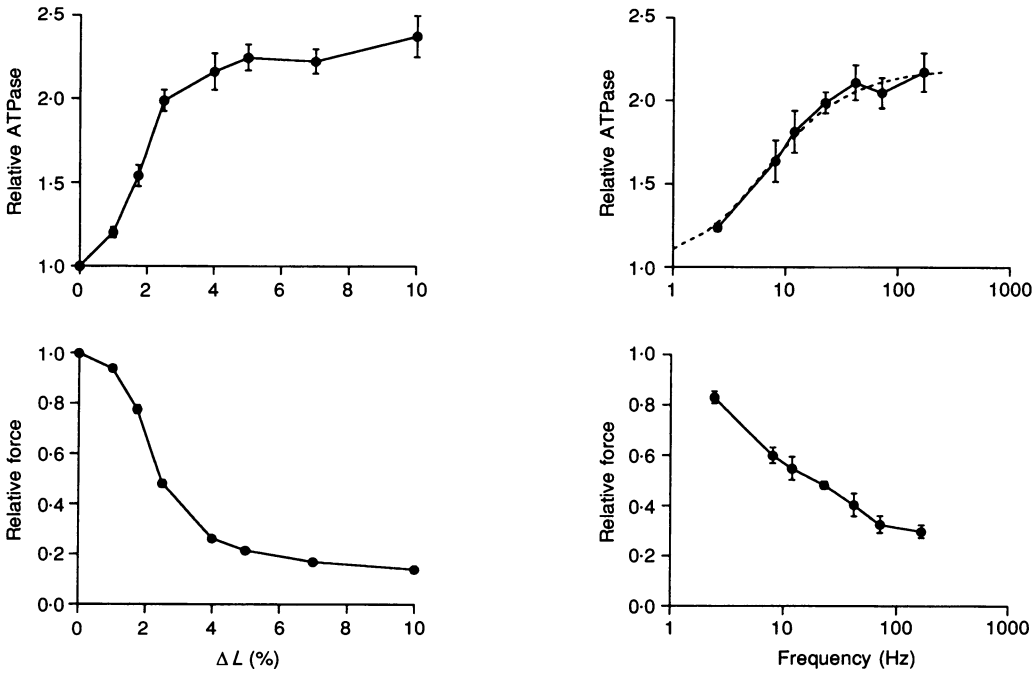


Figure 5. The effect of repetitive length changes of different amplitudes and frequencies on steady-state ATPase activity and on average force level

Amplitude variations were performed with $f = 23$ Hz, frequency was varied at $\Delta L = 2{\cdot}5\,\%$. Data points represent the ratio of the ATPase or force value obtained while the motor was switched on and the corresponding isometric value. All points are given as means ± S.E.M. Error bars are only shown when larger than the symbol size. The number of observations for each data point varied between 4 and 26. The dashed line in the upper right panel represents ATP turnover when the recovery during the length changes takes place according to a monoexponential process with a rate constant of 11 s^{-1}.

after the restretch, force stayed almost constant. The influence of the velocity of the shortening ramp on ATPase activity and average force is shown in Fig. 6*B*. The force level decreased with increasing velocity over the entire investigated range in a hyperbolic fashion, but the ATP turnover already saturated (at about 2·2 times the isometric value) at a ramp velocity of 0·5 L_0 s^{-1}.

A statistical analysis showed that in all cases studied the ATPase activity saturated but the average force level monotonically decreased. The slopes of regression lines through the data points for the three higher amplitudes ($n = 33$), for the three higher frequencies ($n = 13$) and for the five higher ramp velocities ($n = 46$) were not significantly different from zero for ATPase activity, but were significantly negative for average force (Student's t test, $P < 0{\cdot}05$).

Variation of the calcium concentration

In Fig. 7 force, length and absorbance signals are shown at submaximal activation (pCa 6·22). These recordings were obtained from the same fibre as in Fig. 2. Isometric force and ATPase activity were both reduced to about half their maximal values. However, while repetitive length changes caused an increase in the absorbance at maximal activation (Fig. 2), they resulted in a decrease in absorbance, with respect to the isometric level, for submaximal activation. This indicates a reversal of the effect of length changes on ATPase activity at low levels of activity. From the recordings at high time resolution (Fig. 7*B*), it can be seen that at the lower level of activation, active force was almost zero after shortening, indicating that the fibre must have gone slack and therefore probably was shortening at its maximal speed during this period.

A series of experiments was carried out at different calcium concentrations to investigate these effects in more detail. The average isometric force and ATPase activity for the different calcium concentrations are shown in Fig. 8*A*. All values are normalized to the nearest controls (pCa 4·5). A Hill curve, as described in the Appendix, fitted to the force–pCa curve yielded a pK of 6·21 and Hill coefficient, n_H, of 3·5. The relationship between ATPase and pCa had a similar shape, but clearly isometric force and ATPase activity were not proportional for the lower levels of activity. To test how much of this difference could be accounted for by residual membrane-bound ATPase activity, some additional experiments around the threshold of force generation were carried

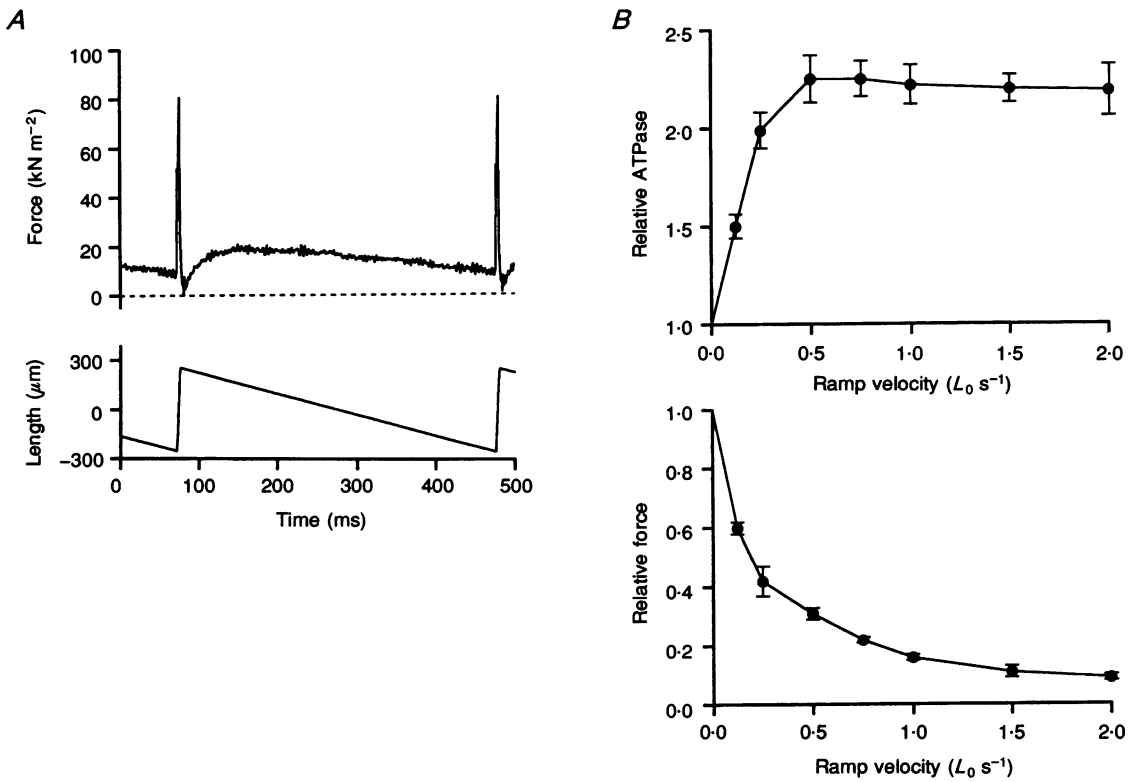


Figure 6. Results of the experiments in which ramp shortenings followed by fast stretches were applied

In *A*, the force response to a saw-tooth with a peak-to-peak amplitude of $\Delta L = 20\,\%$ of L_0 and a ramp shortening velocity of 0·5 L_0 s^{-1} is shown. During most of the shortening phase the fibre was nearly isotonic. Fibre length, 2·55 mm; diameters, 100/80 μm; isometric force 92 kN m^{-2}. In *B*, the effect of different shortening ramp velocities on ATPase activity (upper panel) and force level (lower) is shown. For all experiments $\Delta L = 20\,\%$, pCa = 4·5. Data points were normalized on corresponding isometric values and given as means ± S.E.M. The number of observations per ramp velocity varied between 4 and 12.

out. In these experiments isometric ATPase activity and force were measured from fibres before and after they were immersed in relaxing solution with 1 % (v/v) Triton X-100 (for 1 h, at room temperature). This treatment effectively disrupts membranes and removes ATPase activity of, for example, the sarcoplasmatic reticulum, but leaves the contractile apparatus intact (Kurebayashi & Ogawa, 1991). The results of these measurements are shown in the inset of Fig. 8*A*. Only 25 % (or less) of the force-independent ATPase activity remained after the Triton treatment. So the difference between isometric force and ATPase could be largely explained by a residual pCa-dependent, membrane-bound ATPase.

This non-myofibrillar ATPase activity influences the calcium dependence presented. A correction for it would increase the effect of length changes on ATPase relative to isometric ATPase. At high levels of activation, where the correction would be small, the relative increase in ATP turnover would become larger (about 7 %), and at low levels the effect of a relative decrease with length changes would become stronger too (the ratio of ATPase during length changes and isometric ATPase would decrease by about 20 %). It only plays a minor role (an increase in ratio < 7 %) in the frequency- and length-dependence experiments, since they were all performed at maximum activation. In the simulation described in the Appendix, a correction for this residual ATPase activity has been incorporated.

In Fig. 8*B* the effect of length changes at different Ca^{2+} concentrations is shown as the on : off ATPase ratio, i.e. the ratio of the ATPase activity during the length changes (motor on) for a given pCa and the isometric ATPase (motor off) for the same pCa value. At saturating calcium concentration (pCa 4·5) the maximal increase in ATPase is found: a factor of 2·25 ± 0·08. At pCa 6·1, where the isometric force was about 65 % of the isometric force at full activation, the length changes had no effect on ATPase activity (on : off ATPase ratio ≈ 1·0). The largest reduction was found at pCa 6·34. There the ATPase during length changes was 40 ± 11 % of the isometric value at that pCa. The result of a simulation based on the three-state cross-bridge model, as described in the Appendix, is included as the dashed line in Fig. 8*B*.

Modification of the length change protocol to 2·5 % of L_0 at 12 Hz did not affect the general properties of the relationship between on : off ATPase and calcium concentration. As could be expected on the basis of the amplitude and frequency experiments, the effects of square-wave length changes carried out at $\Delta L = 2{\cdot}5$ % and $f = 12$ Hz were smaller than for the larger and more

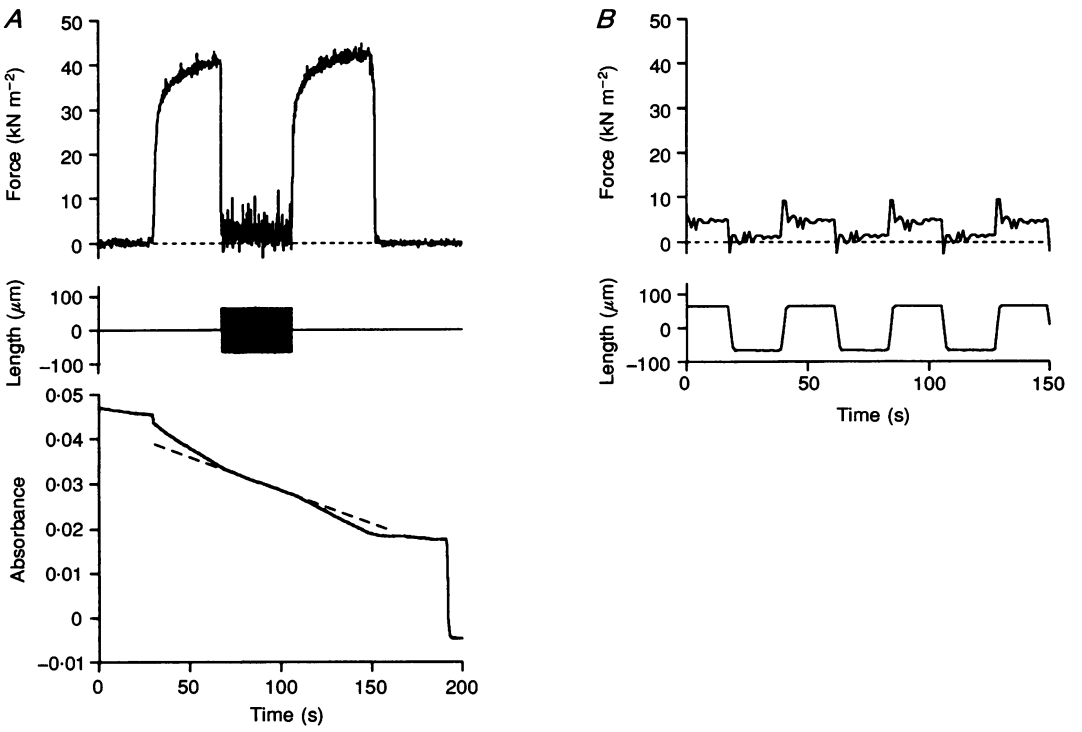


Figure 7. Recordings of force, fibre length and absorbance at low (*A*) and high (*B*) time resolution for submaximal activation (pCa 6·22) from the same fibre as in Fig. 2

The isometric force and ATPase activity were about half of their values for maximal activation (note the difference in scale for the force and absorbance signals in comparison with Fig. 2). The length changes were the same as in Fig. 2. From a comparison with the registrations obtained in relaxing solution it followed that the small force changes seen in *B* are still a result of active force development. The very slow force redevelopment for this submaximal activation agrees well with rates of tension redevelopment at low Ca^{2+} concentrations (about 1–2 s^{-1}) found in slack tests by Brenner (1988) and Metzger & Moss (1991).

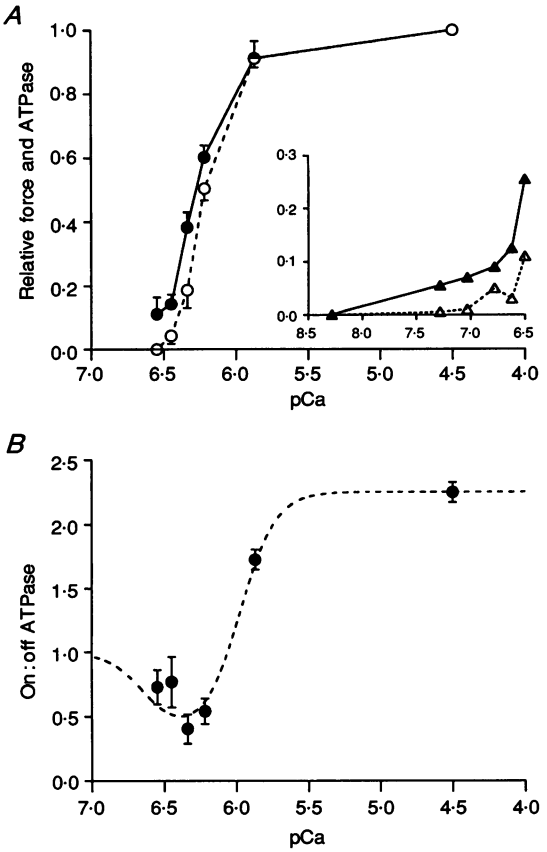


Figure 8. The isometric force and ATPase (*A*) and the effect of length changes on ATPase (*B*), at different calcium concentrations
In *A*, the isometric force (○, S.E.M. downwards) and ATPase (●, S.E.M. upwards) are normalized to corresponding (isometric) control values at maximal activation (pCa 4·5). The inset shows the average results for the additional experiments for values of pCa > 6·5. ▲, the (relative) isometric ATPase before Triton X-100 treatment; △, the isometric ATPase after the fibre was exposed to Triton. In *B*, for each pCa, the ratio (on : off) of the ATPase activity during the length changes and the isometric ATPase at the same pCa is shown. All values are given as means ± S.E.M. of n experiments (n between 4 and 26). The length change protocol resulted in a decrease in ATPase with respect to the isometric value in solutions with a pCa > 6·1 and in an increase when pCa < 6·1. The dashed line represents the best fit to the data based on the model described in the Appendix.

frequent 5 %, 23 Hz length changes. At pCa values at which the 5 %, 23 Hz length changes gave an increase in ATPase activity (on : off ATPase ratio > 1), the 2·5 %, 12 Hz protocol resulted in an increase as well. At the values of pCa at which a decrease (on : off ratio < 1) was found with the 5 %, 23 Hz protocol, a decrease was also found when the 2·5 %, 12 Hz protocol was used.

Correlations between mechanical performance and energy turnover

Figure 9 summarizes the relationships obtained between average force and ATPase activity for repetitive changes in length. During isometric contractions at different calcium concentrations an approximately linear relationship was found between ATPase activity and force. After correction of the observed values for non-myofibrillar ATPase activity (~ 7 % of the total ATPase activity at saturating calcium concentration) the data, normalized to the maximal values obtained, follow the line of identity. Another linear relationship was found between steady-state ATPase activity and the average force level during the 5 %, 23 Hz length changes at different calcium concentrations. It can be noted that the values from the 2·5 %, 12 Hz protocol were positioned along another straight line about half-way between the identity line and the line from the 5 %, 23 Hz protocol. For clarity, these values were not included in the figure. A third, roughly linear relationship was obtained from the results of the square-wave length changes at

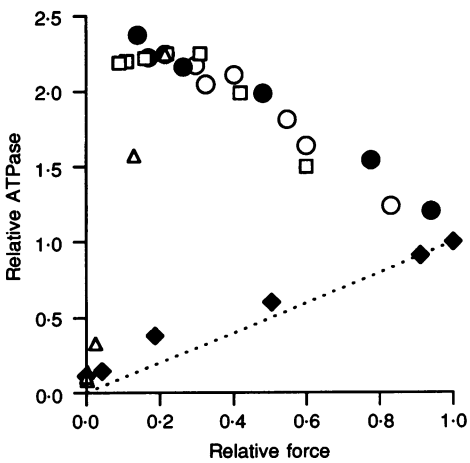


Figure 9. The relationship between the average force level and the steady-state ATPase activity for different experimental protocols
All values were normalized on the corresponding isometric control values at maximal activation. For clarity only the means are shown; S.E.M. values may be found in Figs 5, 8 and 10. For the experiments in which the calcium concentration was varied, ◆ give the isometric values and △ the values obtained during the ($\Delta L = 5$ %, $f = 23$ Hz) length changes. For full activation, ● represent the values during square-wave length changes of varying amplitude, ○ during those of different frequencies and □ during ramp shortenings. The dotted line represents the identity relation.

different amplitudes and frequencies as well as from the results of the ramps/restretches at different shortening velocity, all at maximal activation. As is shown in the Appendix, the relationships which make up the triangle in Fig. 9 may be simulated in a three-state cross-bridge model.

In order to compare our results with those from previous energetic studies during isotonic shortening, we estimated the power output of the fibre by calculating the product of the average force and the velocity of the shortening ramp. The sampling rate of the force signal was not high enough to allow a calculation of the work imposed on the fibre during stretch, but it is likely that most of this is dissipated as heat without net ATP production. The power output is plotted against average force in the lower panel of Fig. 10. In the upper panel, the relationship between ATPase activity and average force is again shown for the ramp shortenings. Obviously, the curves have very similar shapes, suggesting that the extra ATPase activity is proportional to the power output during the shortening phase, reminiscent of the Fenn effect (Fenn, 1923, 1924).

The quantitative results of rectangular length changes and those of ramp shortenings show strong similarities with respect to the maximal increase in ATPase and the relationship between average force and steady-state ATPase.

DISCUSSION

The major findings of this study are: (1) repetitive changes in length result in an increase in ATPase activity above the isometric level at maximal activation, whereas at low levels of activity, these length changes cause a decrease in ATPase activity; and (2) during different types of repetitive length changes, average force level and steady-state ATPase activity show a high correlation.

Comparison with previous studies on ATP turnover

The ATP turnover rate of $2{\cdot}30 \pm 0{\cdot}05\ s^{-1}$ per myosin head obtained for a maximal isometric contraction at 15 °C compares well with values found by others. Glyn & Sleep (1985) found an isometric ATPase activity of $1{\cdot}78 \pm 0{\cdot}2\ s^{-1}$ per myosin head (mean ± S.D.; $n = 6$). Kawai, Güth, Winnikes, Haist & Rüegg (1987) found $3\ s^{-1}$ per myosin head at 20 °C. For the (fast) extensor digitorum longus muscle of the rat, Stephenson, Stewart & Wilson (1989) found an isometric ATPase activity of $3{\cdot}80 \pm 0{\cdot}53\ s^{-1}$ per myosin head (mean ± S.E.M., $n = 8$) at 21–22 °C (sarcomere length 2·7 μm).

There is disagreement in the literature as to whether, for isometric contractions, actomyosin ATPase activity and force are proportional. Levy, Umazume & Kushmerick (1976) found for the skinned semitendinosus muscle of *Rana pipiens* that the Ca^{2+} dependence of force was steeper than that of actomyosin ATPase. They noted that non-actomyosin ATPase activity was low compared to maximally activated actomyosin ATPase but larger than the ATPase activity that could be attributed to the sarcoplasmatic reticulum. Kerrick, Potter & Hoar (1991) found for skinned rabbit adductor magnus fibres (fast twitch) at 21 °C that normalized actomyosin ATPase activity and force did not superimpose for different Ca^{2+} concentrations. They performed several measurements during one activation, with increasing Ca^{2+} concentrations, during which a residual force developed, which might affect the results. In the isometric contractions that we performed at various Ca^{2+} concentrations, the differences between normalized force and ATPase could be accounted for by membrane-bound ATPases. Therefore our findings are in agreement with the results of Brenner (1988) and Metzger & Moss (1991),

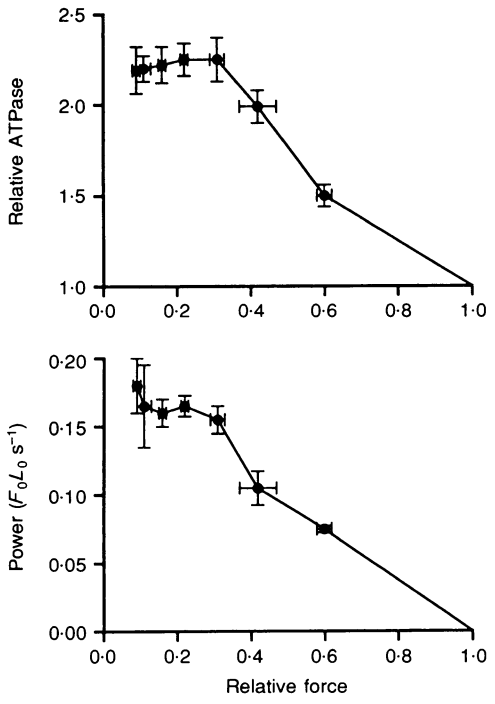


Figure 10. ATPase and power output as a function of force for the ramp shortening experiments
In the upper panel the steady-state ATPase activity during ramp shortenings is plotted as a function of the average force level. In the lower panel the power output of the fibre is shown as a function of the average force level. The power was calculated from the product of the average force over the cycle and the shortening velocity, which is approximately equal to the power output during isotonic shortening. Data points are given as means ± S.E.M.

who found that isometric actomyosin ATPase and force are proportional. As a whole we consider it likely that basal ATPase activity might be involved in the differences found between the various groups. Our figure for the basal ATPase, i.e. 7 ± 1 % of the isometric ATPase at full activation, is in good agreement with values given by Brenner (1988) and Stephenson *et al.* (1989), who found estimates of less than 10 % and 4 %, respectively, for the contribution of non-actomyosin ATPases.

Effects of repetitive changes in length on energy turnover were studied previously by other investigators. The results of Güth, Poole, Maughan & Kuhn (1987) on insect flight muscle are consistent with our data. Their experiments were performed at 22 °C and they normalized the ATPase during length changes to the isometric value after stretch. For pulse-shaped length changes of 1·5 % of L_0 at $f \geqslant 50$ Hz, they found a twofold increase in ATPase with respect to the isometric value. For 1·5 %, 2·5 Hz square waves they found that the ATPase activity was 1·3 times the isometric value, whereas for 2·5 %, 2·5 Hz square waves we found an increase by a factor 1·25. Arata, Mukohata & Tonomura (1979), using various protocols of stretch–release cycles, found at an amplitude of 2·5 % of L_0 in rabbit psoas fibre bundles at 4 °C, a maximal increase in ATP turnover of about 1·9 times the isometric value.

Implications on the molecular level: cross-bridge kinetics

During a maximal isometric contraction, detachment from the force-producing state is considered to be the rate-limiting step. More specifically, ADP release is believed to be the slowest process (Goldman *et al.* 1984*a*; Siemankowski, Wiseman & White, 1985). ATPase measurements probe this slow step.

The effects of repetitive length changes

During changes in length, detachment from the strongly bound, force-generating state will be accelerated (cf. Arata *et al.* 1979; Brenner, 1986; Güth *et al.* 1987). The ATPase activity increases with amplitude of the length changes because the population of attached cross-bridges which experiences a high detachment rate becomes larger (cf. Huxley, 1957). Since the reach of a cross-bridge is limited, a larger amplitude also implies more forcibly detached cross-bridges. Saturation of the increase in ATPase activity is expected because, when the amplitude exceeds a certain value, all cross-bridges will become detached during the length change. The amplitude at which saturation occurred (about 5 % of L_0) is rather large in comparison with current estimates of the length of a single working stroke of the cross-bridge (10 nm), which corresponds to about 1 % of L_0. This estimate of 5 % is likely to be an overestimation in view of the end compliance present in the preparations. The experiments in which the fibre ends were chemically fixed by gluteraldehyde suggest that saturation may occur at about 3 % of L_0, which still exceeds the cross-bridge stroke. Our experiments are therefore in agreement with cross-bridge slippage during shortening (cf. Burton & Simmons, 1991) or the occurrence of multiple power stokes (Ishijima, Doi, Sakurada & Yanagida, 1991; Lombardi, Piazzesi & Linari, 1992), without splitting extra ATP.

The increase in ATPase activity with repetition frequency can be explained as follows. After each change in length recovery starts, i.e. detachment of cross-bridges which are distorted by the length change followed by reattachment. This recovery towards the isometric cross-bridge distribution takes place in an exponential fashion. At very low repetition frequencies, recovery will be almost completed when a new length change occurs. Hence ATP turnover will increase linearly with frequency. At high repetition frequencies there will be no significant change in the speed of recovery before a new change in length takes place. As a result ATP turnover will saturate at high frequencies. The logarithmic increase in ATPase activity at intermediate frequencies is compatible with a monoexponential recovery process, as is illustrated in Fig. 5. The value of the rate constant of this monoexponential recovery (11 s^{-1}) is close to the force redevelopment rates of Brenner (1986) and Metzger & Moss (1991), who found 14·5 and 16·7 s^{-1}, respectively, in rabbit psoas muscle fibres.

The influence of the calcium concentration

At high calcium concentrations, ATPase activity during the length changes was higher than the isometric value, but at low calcium concentrations (pCa > 6·1), the ATPase activity during the length changes decreased below the isometric value. As noted above, the increase in ATPase activity and the decrease in average force at high calcium concentrations may be explained by an increase in the rate of cross-bridge detachment by the changes in length. At low calcium concentrations the opposite effect on ATP turnover occurs. It seems difficult to envisage that the influence of length changes on cross-bridge detachment would be reversed at low calcium concentrations. Therefore, to account for the decrease in ATPase activity at low calcium concentration, an additional effect of length changes is required. We propose that length changes not only increase detachment of force-generating cross-bridges but also of non- or low-force-producing cross-bridges. This later process counteracts ATP hydrolysis and could therefore cause a decrease in ATP turnover. Both processes would be operative at the same time, but the calcium concentration could determine which would be the predominant one.

Model simulations

Data points for various calcium concentrations, during isometric contractions as well as during the two different length change protocols used, lie on straight lines through the origin in the ATPase *versus* force diagram (Fig. 9). This implies that tension cost, ATPase activity divided by average force, is independent of the calcium concentration. This finding is a key point in our modelling efforts.

It follows from Fig. 8*B* that it is possible to simulate our results in a three-state model, described in the Appendix, in which cross-bridges cycle between a detached, a weakly bound and a strongly bound state, with the assumptions

that only the transition from the detached to the weakly bound state is calcium dependent (cf. Brenner, 1988; Metzger & Moss, 1991) and that the detachment rates from both the weakly and strongly bound states are dependent on cross-bridge strain. Good fits can be found for the isometric force, for the isometric ATPase activity and for the on : off ATPase ratio, all as a function of calcium concentration. Also the relation between ATPase activity and average force during various length changes at maximal activation can be fitted. The strain dependence of the detachment rates within this model follows from Fig. 11 in the Appendix, where low average force values correspond to high strain values. The observation that ATPase activity saturated at high amplitudes, frequencies and shortening velocities, while the average force decreased further, is reflected in a detachment rate from the weakly bound state which shows a roughly linear increase with strain and a detachment rate from the force-generating state which exponentially increases with strain. An alternative explanation for this saturation of ATPase activity could be that the detachment rate becomes so high that another, strain-independent, transition becomes rate limiting. However, we consider this alternative less likely because a highly non-linear relationship between average force and ATPase would be expected.

We consider this three-state model to be the simplest possible scheme that explains our results. It should be emphasized that it does not provide a complete picture. For instance, it can be noted that stiffness measurements (Julian & Morgan, 1981) indicate that force during isotonic contractions is linearly related but not proportional to the number of attached cross-bridges. This phenomenon is not taken into account in our model.

The agreement between the effects of the length changes on the rate constants that we found and those found by other investigators is satisfactory. In our simulations detachment rates from the weakly and strongly bound states were increased by a factor of 4 and 10, respectively. Güth *et al.* (1987) used a two-state model containing a detached and an attached state to analyse their ATPase data from experiments on insect flight muscle (at 22 °C). They found that the apparent detachment rate, g_{app}, was enhanced from about 8 s^{-1} during isometric contraction to 200–1000 s^{-1} during length changes and that the apparent attachment rate, f_{app}, was about 20 s^{-1}. Brenner (1986) obtained estimates for the apparent attachment and detachment rates for rabbit psoas muscle at 15 °C. For isometric contraction, f_{app} was about 12 s^{-1} and g_{app} 2 s^{-1}. During isotonic contraction g_{app} increased above f_{app}.

Energy turnover and efficiency under dynamic conditions

From heat measurements it is known that, over a wide range of velocities, the total energy output above the isometric level is proportional to the power output of the fibre (Fenn 1923, 1924; Woledge *et al.* 1985). For ramp shortenings followed by restretches, the ATPase activity above the isometric level was also proportional to the power output of the fibre during the shortening phase. Assuming a free energy change associated with ATP splitting of 23 RT, the efficiency which can be calculated from this proportionality amounts to 36 %. This is somewhat less than the value found in intact frog muscle when the contribution of the ATPase activity of the sarcoplasmatic reticulum is taken into account (Woledge *et al.* 1985). The fact that the curvature of the force–velocity relationship for skinned fibres differs from that for intact fibres (Ferenczi, Goldman & Simmons, 1984), which affects the maximum power output, and the interspecies variation found for efficiency (Woledge *et al.* 1985) may be responsible for this difference.

It may seem contradictory that both the average force and the power (force multiplied by shortening velocity) are linearly related to ATP turnover, but for our data these two correlations do not seem to conflict. In principle it should be possible to discriminate between these two possibilities in experiments in which movement protocols are used which yield lower average force levels. On the basis of the correlation between average force and ATPase, a further increase in ATPase activity would be expected as average force decreases, whereas the correlation between power output and ATPase would predict a decrease in ATPase.

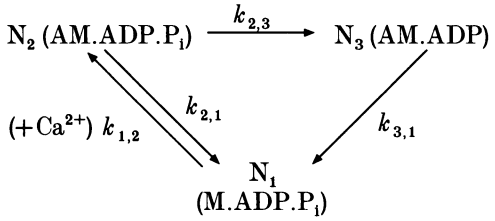
Implications for the chemo-mechanical coupling: the Fenn effect

Since the experiments of Fenn (1923, 1924) a long-standing interest has existed in the chemo-mechanical coupling during muscle contraction. The results for maximally activated mammalian fast skeletal muscle fibres presented here are compatible with earlier findings which show an increase in the energy turnover during isotonic shortening above the isometric value, i.e. the Fenn effect. The basic element in the explanations given for the Fenn effect is the acceleration of cross-bridge detachment. Previous studies showed a decrease in stiffness during and after (rapid) shortening but not during and after stretch (e.g. Julian & Morgan, 1981; Stienen & Blangé, 1981; Lombardi & Piazzesi, 1990). Such a decrease in stiffness reflects an increase in the rate of cross-bridge detachment. Therefore, we consider our repetitive protocol of square-wave length changes an effective means to accelerate cross-bridge detachment. This view is supported by the reduction in stiffness and in the average force level during the repetitive length changes and by the agreement in the results from the square-wave protocol and from the shortening ramps. Moreover, ATP splitting is reduced during stretch (Infante *et al.* 1964) and somewhat enhanced after stretch (Curtin & Woledge, 1979). Therefore, the net energetic effects of the restretches in our repetitive protocols are likely to be small, and the effects of shortenings prevail. On this basis we propose that the decrease in energy turnover during the length changes at low Ca^{2+} concentrations below the isometric value may be due to an apparent reversal of the Fenn effect at low levels of activity. This would explain the disappearance of the Fenn effect during a twitch at room temperature and the absence of it in cardiac muscle (cf. Rall, 1982) since in both cases the contractile apparatus is not maximally activated.

APPENDIX

Simulation of the Ca^{2+} dependence of the ATPase activity during length changes

The two essential observations concerning the Ca^{2+} dependence of the ATPase activity during changes in length were that the ATPase activity during length changes (a) increases at saturating Ca^{2+} concentration above the isometric value, and decreases below the isometric value at low Ca^{2+} concentration (Fig. 7*B*) and (b) is correlated with the average force level during length changes (Fig. 9). The simulations of these observations were based on the following three-state scheme of cross-bridge action:



where M is myosin; AM, actomyosin; N_1, detached cross-bridges; N_2, no-force-producing cross-bridges (weakly bound); N_3, force-producing cross-bridges (strongly bound); $k_{1,2}$, attachment rate; $k_{2,1}$, reverse attachment rate; $k_{2,3}$, rate of myosin isomerization and/or P_i release; and $k_{3,1}$ is the rate of ADP release and/or cross-bridge detachment.

The attachment rate $k_{1,2}$ is taken to be Ca^{2+} dependent. The detachment rates $k_{2,1}$ and $k_{3,1}$ are cross-bridge strain dependent ($k'_{2,1}$ and $k'_{3,1}$, during the length changes). Force is assumed to be proportional to the number of cross-bridges in N_3. The Ca^{2+} dependence of the isometric force production can be described by the so-called Hill equation:

$$F(Ca^{2+}) = F_0 \frac{[Ca^{2+}]^{n_H}}{[Ca^{2+}]^{n_H} + K^{n_H}},$$

in which the Ca^{2+} concentration at which force is half-maximal (K) is $10^{-6{\cdot}21}$ M, the Hill coefficient (n_H) is 3·5 and F_0 is the isometric force at saturating Ca^{2+} concentration (cf. Fig. 8*A*). Since $k_{1,2}$ is the only Ca^{2+}-dependent rate constant, it can be derived from the Hill equation that

$$k_{1,2}(Ca^{2+}) = k_{1,2}\,\alpha, \tag{1}$$

where:

$$\alpha = \frac{[Ca^{2+}]^{n_H}}{[Ca^{2+}]^{n_H} + K^{n_H} \times \beta}, \tag{2}$$

and

$$\beta = 1 + \frac{k_{1,2}(k_{3,1} + k_{2,3})}{k_{3,1}(k_{2,1} + k_{2,3})}. \tag{3}$$

The isometric rate of ATP turnover per myosin head equals:

$$A_{-}(Ca) = \frac{\alpha\, k_{1,2}\, k_{2,3}\, k_{3,1}}{k_{3,1}(k_{2,1} + k_{2,3}) + \alpha\, k_{1,2}(k_{3,1} + k_{2,3})}. \tag{4}$$

To obtain an expression for the ATP turnover during the length changes $A_+(Ca)$, $k_{2,1}$ and $k_{3,1}$ in eqns (3) and (4) have to be replaced by $k'_{2,1}$ and $k'_{3,1}$, respectively. The maximal isometric rate of ATP turnover A_0 is equal to A_- at $\alpha = 1$, and the maximal ATPase rate during the length changes A'_0 equals A_+ at $\alpha = 1$. The ATPase measurements contain a 'basal' component which for simplicity is assumed to be Ca^{2+} independent. Substituting α, A_0 and A'_0 in eqns (3) and (4) and taking the basal component (B) into account yields:

$$A_{-}(Ca) = \frac{A_0 - B}{1 + 10^{n_H(pCa - pK)}} + B, \tag{5}$$

and

$$A_{+}(Ca) = \frac{A_0 - B}{\frac{A_0 - B}{A'_0 - B} + \gamma \times 10^{n_H(pCa - pK)}} + B, \tag{6}$$

with

$$\gamma = \frac{k'_{2,1} + k_{2,3}}{k_{2,1} + k_{2,3}}, \tag{7}$$

and

$$pCa = -\log_{10}[Ca^{2+}],\ pK = -\log_{10} K.$$

Equation (6) has been fitted to the experimental data, yielding a value for B of 0·07 ± 0·04 s^{-1} (mean ± S.D.) and for γ of 2·9 ± 0·2 (mean ± S.D.). The values obtained for B, γ, n_H and pK were substituted in eqns (5) and (6). The result of $A_+(Ca)/A_-(Ca)$ has been included as the on : off ATPase ratio in Fig. 7*B*.

A good fit of the linear relationship between the ATPase activity and average force level during the 5 %, 23 Hz protocol at different Ca^{2+} concentrations (Fig. 9) required that $k'_{3,1}/k_{3,1} = 10$. The complete set of rate constants can now be obtained from eqn (7) by substituting the value for γ of 2·9 assuming that $k_{1,2} = 80\ s^{-1}$, $k_{2,1} = 32\ s^{-1}$. Note that Goldman, Hibberd & Trentham (1984*b*) found values of 100 and 40 s^{-1}, respectively, for these rate constants at 20 °C. We prefer slightly lower values because our experiments were carried out at 15 °C. With A_0 and A'_0 of 2·3 s^{-1} and 5·2 s^{-1}, respectively (this study) and with very few assumptions ($k_{1,2}$ and $k_{2,1}$ only) the essential observations concerning the Ca^{2+} dependence may be explained with the following rate constants:

$k_{1,2} = 80\ s^{-1}$,

$k_{2,1} = 32\ s^{-1} \rightarrow 123\ s^{-1}$ (during length changes),

$k_{2,3} = 17\ s^{-1}$,

$k_{3,1} = 2{\cdot}8\ s^{-1} \rightarrow 28\ s^{-1}$ (during length changes).

With the values assumed for $k_{1,2}$ and $k_{2,1}$, the standard deviations in the rates obtained are between 5 and 10 %.

It follows that the strain dependencies of $k_{2,1}$ and $k_{3,1}$ are quite strong. They increase during the length changes by a factor of 4 and 10, respectively. In more detailed cross-bridge models, $k_{2,3}$ is also strain dependent (Dantzig, Goldman, Millar, Lacktis & Homsher, 1992; Walker, Lu & Moss, 1992). From our model simulations it is, however, not possible to

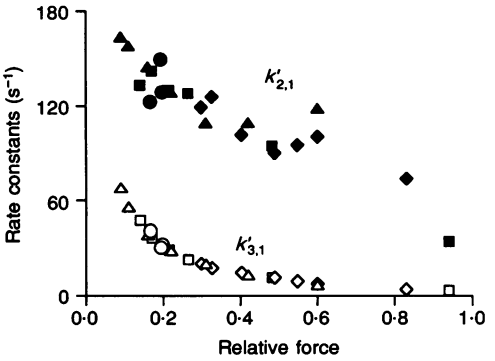


Figure 11. The detachment rates as a function of the average force level, during the various length change protocols
Filled symbols represent the detachment rate $k'_{2,1}$ from the weakly bound state, open symbols the detachment rate $k'_{3,1}$ from the strongly bound state. For the square-wave protocol, squares represent the data points for amplitude variations, diamonds those for frequency variations, and circles those for combined high frequencies and amplitudes. Triangles show the results for ramp shortenings followed by restretches.

arrive at an independent estimate for this dependency. We verified that, for realistic values of $k_{2,3}$ (10–1000 s^{-1}; cf. Hibberd, Dantzig, Trentham & Goldman, 1985; Millar & Homsher, 1990), a strain dependency of $k_{2,3}$ and $k_{3,1}$ only is insufficient to explain the data available. This implies that the three-state scheme presented, with strain-dependent $k_{2,1}$ and $k_{3,1}$, is the simplest model to explain our results.

The Ca^{2+} dependence proposed for $k_{1,2}$ is in fact dictated by the three-state model, via the Ca^{2+} dependence of the isometric force. There exists considerable evidence in the literature that either one or more transitions in the cross-bridge cycle are Ca^{2+} dependent (Julian, 1969; Brenner, 1988). Cross-bridge recruitment is also considered to be a highly Ca^{2+}-dependent, co-operative process (cf. Podolski & Teichholz, 1970; Bremel & Weber, 1972). The proposed Ca^{2+} dependence for $k_{1,2}$ might contain both elements, i.e. Ca^{2+}-dependent recruitment and a Ca^{2+}-dependent attachment rate, but its origin is not essential in the calculations.

It was found that, at saturating Ca^{2+} concentrations, a unique, protocol-independent relationship existed between the average force level attained during the different length change protocols and the increase in ATPase activity (cf. Fig. 9). Within the three-state model it is possible to calculate from this relationship the dependence of $k_{2,1}$ and $k_{3,1}$ during length changes as a function of the average force level. The results of this calculation are presented in Fig. 11. It appeared that $k_{2,1}$ increased almost linearly when the average force decreased, from 32 up to 160 s^{-1} (during high speed ramp shortening) and that $k_{3,1}$ increased in an exponential fashion, from 2·8 up to 70 s^{-1} (high speed ramp shortening).

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