Influence of phosphate and pH on myofibrillar ATPase activity and force in skinned cardiac trabeculae from rat

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1. The effects of inorganic phosphate (P_i) and pH on maximal calcium-activated isometric force and MgATPase activity were studied in chemically skinned cardiac trabeculae from rat. ATP hydrolysis was coupled enzymatically to the breakdown of NADH, and its concentration was determined photometrically. Measurements were performed at 2·1 μm sarcomere length and 20 °C. ATPase activity and force were also determined when square-wave-shaped length changes were applied, with a frequency of 23 Hz and an amplitude of 2·5 %.

2. At pH 7·0 without added P_i, the average isometric force (± S.E.M.) was 51 ± 3 kN m^{-2} ($n = 23$). The average isometric ATPase activity was 0·43 ± 0·02 mM s^{-1} ($n = 23$). During the changes in length ATPase activity increased to 152 ± 3 % of the isometric value, while the average force level decreased to 48 ± 2 %.

3. Isometric force gradually decreased to 31 ± 2 % of the control value when the P_i concentration was increased to 30 mM. Isometric ATPase activity, however, remained constant for P_i concentrations up to 5 mM and decreased to 87 ± 3 % at 30 mM P_i. When P_i accumulation inside the preparation due to ATP hydrolysis was taken into account, a linear relationship was found between isometric force and log [P_i]. The decrease in relative force was found to be 44 ± 4 % per decade.

4. During the length changes, ATPase activity and average force showed, apart from the increase in ATPase activity and decrease in average force, the same dependence on P_i as the isometric values. Stiffness, estimated from the amplitude of the force responses during the length changes, decreased in proportion to isometric force when the P_i concentration was increased. The changes in the shape of the force responses due to the repetitive changes in length as a function of the P_i concentration were relatively small. These results suggest that the effect of P_i on the transitions which influence ATP turnover is rather insensitive to changes in cross-bridge strain.

5. Isometric force, normalized to the control value at pH 7·0, increased gradually from 54 ± 1 % at pH 6·2 to 143 ± 10 % at pH 7·5. ATPase activity remained practically constant for pH values from 6·8 to 7·2 but decreased to 80 ± 1 % at pH 6·2 and to 83 ± 5 % at pH 7·5. ATPase activity during the length changes was reduced more than the isometric ATPase activity when pH was lowered. The average force level during length changes remained almost constant over the entire pH range. However, the shape of the force responses under acidic conditions was pH dependent. These observations suggest that at least one of the pH-sensitive transitions in the cross-bridge cycle which influence ATP turnover is dependent on changes in cross-bridge strain.

6. To mimic the changes which may occur during ischaemia, trabeculae were activated at pH 6·2 in the presence of 30 mM P_i. These experiments showed a decrease in isometric force and ATPase activity to 14 ± 1 % and 47 ± 3 %, respectively. Average force and ATPase activity during the length changes decreased to 32 ± 3 % and 55 ± 3 %, respectively. This indicates that the concurrent changes in [P_i] and pH cause a three- to fourfold increase in isometric tension cost.

It has been well established that the contractile failure which occurs in the myocardium during ischaemia, hypoxia or anoxia is mainly associated with changes in inorganic phosphate concentration (P_i) and pH (Tsien, 1976; Allen, Morris, Orchard & Pirolo, 1985; Allen & Orchard, 1987; Matthews, Taylor & Radda, 1986; Elliot, Smith, Eisner & Allen, 1992). These changes range from about 2 mM P_i and pH 7 in the well-oxygenated heart up to 30 mM P_i and a pH of about 6 during prolonged ischaemia (see e.g. Garlick, Radda & Seeley, 1979; Matthews *et al.* 1986; Allen & Orchard, 1987; Elliot *et al.* 1992).

As changes in pH and [P_i] occur simultaneously, it is difficult to study their individual effects in the intact heart or in isolated papillary muscles. In skinned preparations from which the surface membrane has been removed or made permeable, however, pH and [P_i] may be varied independently.The results from these studies indicate that a rise in [P_i] and a fall in pH both lead to a substantial reduction of isometric force development at saturating Ca^{2+} concentrations and to an even more marked reduction in force at submaximal Ca^{2+} concentrations through a reduction in the Ca^{2+} sensitivity of the contractile apparatus (Herzig & Rüegg, 1977; Fabiato & Fabiato, 1978; Donaldson & Hermansen, 1978; Robertson & Kerrick, 1979; Brandt, Cox, Kawai & Robinson, 1982; Chase & Kushmerick, 1988; Godt & Nosek, 1989; Metzger & Moss, 1990*b*).

Much less is known about the influence of the metabolic changes on energy utilization. Such information is important not only for the understanding of the functional and contractile changes at the cross-bridge level, but also for devising strategies to reduce ischaemic and/or reperfusion damage. For skeletal muscle, it has been shown that the rate of isometric ATP splitting is depressed by P_i but that this reduction is less than the reduction in isometric tension (Kawai, Güth, Winnekes, Haist & Rüegg, 1987; Cooke, Franks, Luciani & Pate, 1988). Some reports indicate that this is also the case for cardiac muscle, but details on the concentration dependence are not available (e.g. Schmidt-Ott, Bletz, Vahl, Saggau, Hagl & Rüegg, 1990).

Whereas the influence of inorganic phosphate now has some experimental and theoretical background (Hibberd, Dantzig, Trentham & Goldman, 1985; Pate & Cooke, 1989; Millar & Homsher, 1990; Stienen, Versteeg, Papp & Elzinga, 1992), insight into the interaction site(s) for H^+ ions in skinned cardiac muscle tissue is still limited (e.g. Kentish, 1991). Recent reports on the effects of pH on isometric force were confined to the combined inhibitory action of acidosis and phosphate via the putative inhibitory action of $H_2PO_4^-$ (Nosek, Fender & Godt, 1987; Nosek, Leal-Cardoso, McLaughlin & Godt, 1990; Kentish, 1991), and relatively little is known regarding the pH dependence of the myofibrillar ATPase activity in cardiac muscle (cf. Blanchard & Solaro, 1984; Godt & Kentish, 1989).

The objective of this study was to investigate the P_i and pH dependence of both isometric force and myofibrillar ATPase activity, to relate these observations to the current scheme of cross-bridge action, and to test whether these dependencies, observed under isometric conditions, may be applied under dynamic conditions, e.g. when length changes are imposed on the preparations. The effects of P_i concentrations between 0 and 30 mM and of pH between 6·2 and 7·5 were studied. To mimic the effects of prolonged ischaemia we also studied the combined effects at 30 mM P_i and pH 6·2. A preliminary account of part of this work has appeared elsewhere (Ebus & Stienen, 1992).

METHOD

Preparation

Adult Wistar rats (~ 380 g body weight) were anaesthetized by inhalation of approximately 2·5 % halothane, and the heart rapidly excised. Cardiac trabeculae were dissected carefully from the right ventricle of the heart in oxygenated Tyrode solution containing 20 mM 2,3-butanedione monoxime (BDM), which inhibits cell contracture and muscle damage during dissection (Mulieri, Hasenfuss, Ittleman, Blanchard & Alpert, 1989). Suitable trabeculae with a diameter of 90–280 μm (173 ± 8 μm, mean ± S.E.M.) and a length of 0·85–3·1 mm (1·77 ± 0·10 μm, mean ± S.E.M.) were then transferred with a smooth glass rod to a dish containing cold relaxing solution to which 1 % (v/v) Triton X-100 was added and were kept at about 3 °C for 2 h to dissolve the membranes. The composition of the relaxing solution was (mM): Na_2ATP, 7·3; $MgCl_2$, 10·6; EGTA, 20; PCr, 10; *N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid (Bes), 100; (pH = 7·0, adjusted with KOH), ionic strength 200 mM (adjusted with KCl). The skinned preparations were mounted in the experimental set-up by means of aluminium T-clips, as described previously by Stienen, Roosemalen, Wilson & Elzinga (1990).

Apparatus

The preparation was mounted between a displacement generator (Ling Dynamic Systems 101, Royston, UK) and a force transducer element (AE801, SensoNor, Norway). Sarcomere length of the preparations was measured in relaxing solution by means of a He–Ne laser (Uniphase, Model 1125) and adjusted to 2·1 μm. The input of the displacement generator originated from a programmable, Z-80-based function generator. During repetitive length changes, a square-wave displacement pattern was used with an amplitude of 2·5 % of the initial length of the preparation (L_0). The average length during the length changes was equal to L_0. The duration of the ramp-shaped shortening and lengthening phases was 2 ms. The duration of the pauses following shortening and lengthening was 20 ms (see e.g. Fig. 2, upper trace). The repetition frequency of the displacement pattern therefore was $(44\ ms)^{-1}$ or 23 Hz. In some cases, quick stretches and releases were imposed with an amplitude of 1 % of L_0 with a time to peak of 1·2 ms. The natural frequency of the force transducer was about 2 kHz. The preparation could be transferred manually between baths, within about 1–2 s. The measuring chamber used for the ATPase assay (volume 30 μl) had quartz windows to allow transmission of near-UV light for the measurement of NADH absorbance. This bath was stirred continuously by motor-driven vibration (frequency about 3 Hz) of a diaphragm at the base of the chamber. The temperature in the bath was kept at 20 ± 1 °C. The absorbance, force and length signals were filtered at 2·5 Hz ($-12\ dB\ oct^{-1}$) and recorded on an Olivetti M280 personal

computer after A–D conversion at 5 Hz. When the motor was turned on, force and length changes (filtered at 1 kHz, −12 dB octave^{-1}) were also recorded for a short period (2 s) at a sampling rate of 1 kHz.

Measurement of ATPase activity

ATPase activity of the skinned trabeculae was measured by a coupled enzyme assay, as described in detail previously (Glyn & Sleep, 1985; Stienen *et al.* 1990). After ATP hydrolysis into ADP and P_i, the ADP formed is resynthesized to ATP by an enzymatic coupling, which eventually results in the oxidation of NADH to NAD^+. This reaction sequence is catalysed by pyruvate kinase and lactate dehydrogenase. The breakdown of NADH was determined photometrically from the absorbance of near-UV light (340 nm) that passed beneath the preparation. The absorbance signal was found to be linearly related to the NADH concentration in the range between 0·2 and 2 mM. The NADH concentration declined from 0·8 mM to approximately 0·7 mM during recording. Calibration of the NADH absorbance signal was carried out by injecting 0·05 μl of 10 mM ADP (i.e. 0·5 nmol) into the measuring chamber.

Experimental protocol

During the experiments, the preparations were incubated in relaxing solution for at least 4 min, in preactivating solution for at least 3 min, in activating solution for about 1·5 min, and then transferred back into relaxing solution. The composition of the solutions was calculated using the equilibrium constants given by Fabiato (1981), and is listed in Table 1. Before the first activation–relaxation cycle sarcomere length, measured in relaxing solution, was adjusted to 2·1 μm. The experiment started with the motor switched off. When the active isometric force became stable, the motor was switched on for about 30 s. Thereafter, the preparation remained in activating solution for another 30 s, and isometric force and ATPase activity were again recorded.

All activations were carried out at saturated calcium concentration. It was ensured that $[Ca^{2+}]$ was saturating by adding extra Ca^{2+} from a concentrated $CaCl_2$ stock solution during activation under the various experimental conditions used. After the first contraction, with no added P_i and pH 7·0, sarcomere length was readjusted if necessary, and the length of the muscle between the clips as well as the width and depth of the preparation were measured (at × 50 magnification). It was found that after this readjustment, sarcomere length in general remained stable throughout the experiment. The second contracture provided the control values for isometric ATPase activity and force. Then, two contractions were carried out in solutions with added P_i or different pH. These measurements were followed by another control contraction without added P_i at pH 7·0. Isometric ATPase activity and force were corrected for fibre deterioration by linear

Table 1. Composition of solutions (mM)

Solution name	$MgCl_2$	Na_2ATP	EGTA	HDTA	CaEGTA	KProp
Control, pH 7·0						
Relaxing	7·85	5·86	10	—	—	75·1
Pre-activating	7·63	5·86	0·5	9·5	—	75·6
Activating (pCa 4·3)	7·57	5·98	—	—	10	75·3
With 30 mM phosphate, pH 7·0						
Pre-activating	8·93	5·86	0·5	9·5	—	—
Activating (pCa 4·3)	8·86	5·98	—	—	10	—
pH 7·5						
Pre-activating	8·02	5·65	0·5	9·5	—	41·1
Activating (pCa 4·5)	7·80	5·67	—	—	10	41·3
pH 6·2						
Pre-activating	6·79	7·61	0·5	9·5	—	102·7
Activating (pCa 3·3)	6·78	7·16	—	—	10	101·2
With 30 mM phosphate, pH 6·2						
Pre-activating	7·21	7·18	0·5	9·5	—	45·9
Activating (pCa 3·5)	7·21	7·60	—	—	10	44·7

In addition, all solutions contained 100 mM *N*,*N*-bis(2-hydroxyethyl)-2-aminoethane-sulphonic acid (Bes), 10 mM phosphoenolpyruvate, 4 mg ml^{-1} pyruvate kinase (500 U ml^{-1}, Sigma), 0·24 mg ml^{-1} lactic dehydrogenase (870 U mg^{-1}, Sigma), 5 mM sodium azide, 10 μM oligomycin B, 0·8 mM NADH, and 0·2 mM p^1,p^5-di(adenosin-5′) pentaphosphate. The free Mg^{2+} and MgATP concentrations were, respectively, 1 mM and 5 mM. Potassium propionate (KProp) was added to adjust ionic strength to 205–210 mM. Ionic strength during the experiments was approximately 200 mM because oligomycin, NADH and p^1,p^5-di(adenosin-5′) pentaphosphate were added from concentrated stock solutions. CaEGTA was made by dissolving equimolar amounts of $CaCO_3$ and EGTA. The pH was adjusted with KOH to the values indicated.

interpolation between control values. The intermediate isometric results were normalized to the interpolated values. ATPase activity and average force during length changes were also normalized to the interpolated isometric control values. When the isometric force of a control measurement was less than 80 % of the first control value, the measurements after the preceding control were discarded.

ATPase activity was derived from linear regression analysis of the absorbance signal. To correct for bleaching of NADH under the intense UV light, the slope of the absorbance signal measured after the fibre had been returned to the relaxing solution was subtracted from the slope of the absorbance signal measured during the contraction.

The Ca^{2+}-activated ATPase activity was corrected for basal ATPase activity measured in relaxing solution (pCa 9). The basal activity amounted to about 5 % of the maximal isometric ATPase activity. No effect of length changes on basal ATPase activity was observed. To verify that the ATPase activity of the sarcoplasmic reticulum (SR) was eliminated by the skinning method used, we compared in four preparations the maximal Ca^{2+}-activated force and ATPase activity in the presence and absence of 10 μM cyclopiazonic acid (CPA), which is an inhibitor of SR ATPase activity (Seidler, Jona, Vegh & Martonosi, 1989). CPA was added to the solutions from a concentrated stock in ethanol (final volume 0·5 % v/v). Neither CPA in 0·5 % ethanol nor 0·5 % ethanol alone had any effect on the force or ATPase activity as compared with the control measurements without CPA and ethanol, indicating that SR ATPase activity had been completely eliminated after 2 h of skinning.

Data values are given as means ± S.E.M. of n experiments. Differences were tested by means of Student's t test at a 0·05 level of significance ($P < 0·05$).

RESULTS

The protocol used during the experiments is illustrated in Fig. 1. In this figure, the force and ATPase activity from a control experiment without added phosphate (*A*) and with 30 mM added phosphate (*B*) are shown. When the fibre was immersed in the measurement chamber where sufficient free Ca^{2+} was present, active isometric force developed and

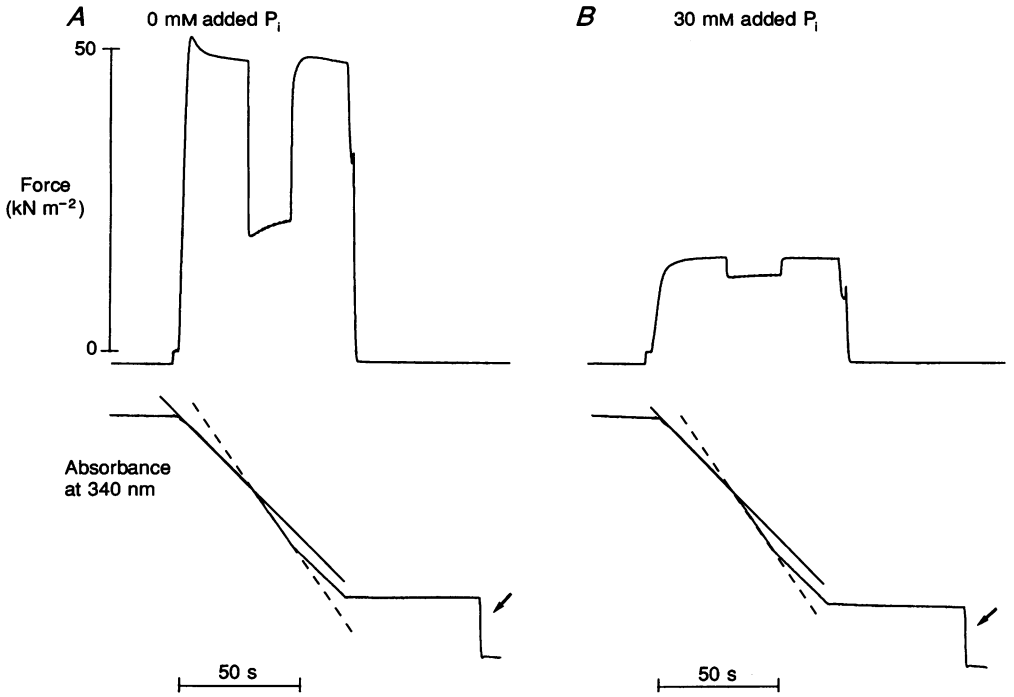

Figure 1. Force production and absorbance
Recordings of force production (upper traces) and absorbance (lower traces) during control conditions (*A*, pH 7·0, 0 mM added P_i) and with 30 mM added P_i and pH 7·0 (*B*). When the preparation was immersed in the activating solution, active isometric force developed, and the absorbance started decreasing. The rate of decrease was a measure of ATPase activity. When a steady state was reached, isometric force and ATPase activity were measured during a period of approximately 30 s. With the motor on, the length of the preparation was varied repetitively at a frequency of 23 Hz by a square wave with an amplitude of 2·5 % of its resting length. This caused an abrupt decrease in the low-pass filtered force signal and an increase in the absorbance. After this period, isometric force and ATPase activity were measured again for about 30 s. After moving the fibre back into relaxing solution, the slope of signal was measured in order to correct for bleaching of NADH under influence of the UV light. Then 0·5 nmol ADP, marked by an arrow, was injected into the measuring chamber to calibrate the absorbance signal. Note that the reduction in isometric ATPase activity at 30 mM added P_i is considerably less than the reduction in isometric force. Dimensions of the preparation: length, 1·80 mm; width, 170 μm; depth, 200 μm.

the absorbance started to decrease. When the motor was turned on and the length changes were imposed upon the fibre, the average force decreased. Simultaneously, the slope of the absorbance signal became steeper, indicating an increase in ATPase activity. After about 20 s the motor was turned off again, and force and ATPase activity returned to the isometric values. Subsequently, the fibre was returned to the relaxing solution. The average isometric force level during the initial control contractions was 51 ± 3 kN m^{-2} ($n = 23$). The average isometric ATPase activity was $0{\cdot}43 \pm 0{\cdot}02$ mM s^{-1} ($n = 23$). Assuming a myosin concentration of 0·16 mM (Barsotti & Ferenczi, 1988), this is equivalent to a rate of ATP turnover per myosin head of $2{\cdot}7 \pm 0{\cdot}1$ s^{-1}.

Isometric contractions are not necessarily representative for the working heart, and we therefore wanted to compare the results during isometric contraction with those obtained under dynamic conditions when the length of the preparation was varied. For this purpose we studied the effects of repetitive changes in length which have a large effect on energy turnover. In experiments (not shown) where the frequency, amplitude and speed of shortening and/or lengthening were varied, we found that the ATPase activity increased with amplitude and frequency, but that it saturated for amplitudes $\geqslant 2{\cdot}5\%$ L_0 and frequencies $\geqslant 23$ Hz. To obtain a large effect on ATPase activity without detrimental effects on the preparation, we used in the P_i and pH experiments described below an amplitude of $2{\cdot}5\%$ L_0 and a frequency of 23 Hz. In experiments in which the type of length change was varied we also found a good, protocol-independent correlation between the average force level and the increase in ATPase activity. We, therefore, used the average force level to quantify the effects of length changes on force production.

Effects of P_i

It can be seen in Fig. 1 that the isometric force in the presence of 30 mM P_i decreased to approximately 30 % of the force under control conditions. ATPase activity was also reduced, but to a lesser degree than isometric force.

The square-wave-shaped length changes and the resulting force responses in solutions with zero and 30 mM added P_i are shown on an extended time scale in Fig. 2. The transient increase in force after lengthening, which was most pronounced in thin preparations, disappeared when more than 2 mM P_i was added and was absent in

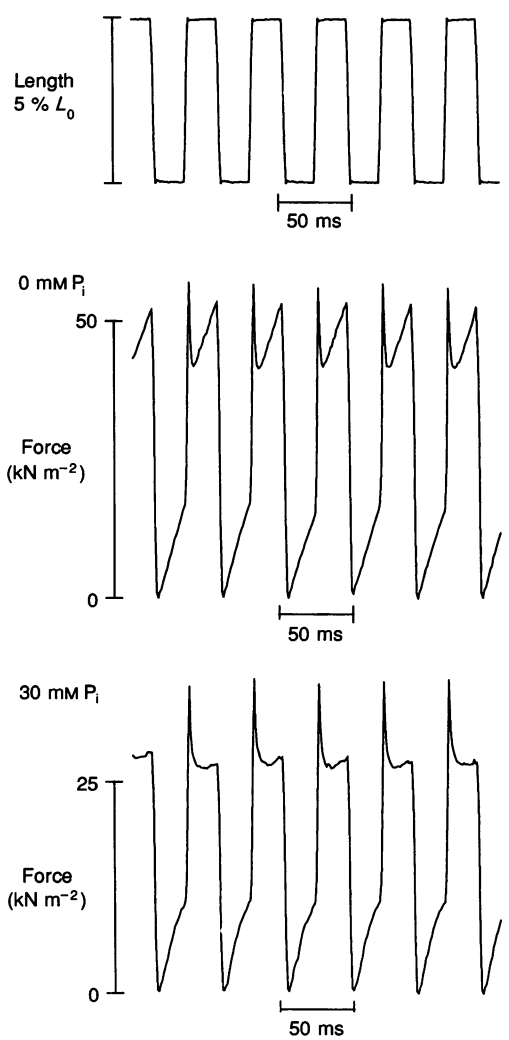

Figure 2. Force responses to length changes with zero and 30 mM added P_i
The upper trace shows the length changes which are imposed upon the fibre. The isometric force levels are 49 kN m^{-2} (0 mM added P_i) and 16 kN m^{-2} (30 mM added P_i). Apart from the disappearance of the transient force recovery after a stretch when 30 mM P_i was added, there are little differences in shape between the responses. Experimental conditions as in Fig. 1.

preparations with a diameter larger than about 200 μm. No other conspicuous differences in the shape of the force responses were observed. The amplitude of the force responses scaled with isometric force when P_i was added. There was a slow increase in the average force level during length changes (cf. Fig. 1), both at zero and at 30 mM added P_i. This change in the average force level was small (about 10 %) in comparison with the amplitude of the force responses due to the length changes.

The dependency of isometric force and ATPase activity on P_i derived from the complete set of experiments is summarized in Fig. 3*A*. This figure shows the normalized isometric ATPase activity and isometric force as a function of the P_i concentration up to 30 mM ($n = 7$). It can be seen that force decreases as a function of the P_i concentration, in agreement with previous observations in skeletal (e.g. Cooke & Pate, 1985) and cardiac muscle (Kentish, 1986). A new finding, however, is that ATPase activity in cardiac muscle decreases only slightly when phosphate is added. When 5 mM P_i was added, ATPase activity did not decrease significantly (98 ± 2 % of the control value, $P = 0{\cdot}95$), whereas force decreased to 65 ± 5 %. With 30 mM added P_i, ATPase activity decreased to 87 ± 3 %, while force decreased to 31 ± 2 %.

The normalized ATPase activity and average force during length changes as a function of P_i concentration are shown in Fig. 3*B*. Without added P_i, ATPase activity during length changes increased to 152 ± 3 % of the isometric value, while the average force decreased to 48 ± 2 % ($n = 23$). The effect of P_i on ATPase activity during length changes is similar to the effect on isometric ATPase activity shown in Fig. 3*A*. The curve representing the ATPase activity during length changes differs from the isometric curve only by a scaling factor of about 1·5. The curve representing the average force level during length changes roughly follows the isometric curve for P_i concentrations between 5 and 30 mM P_i (note the different scales) with a scaling factor of 0·5. At low P_i concentrations, however, the curve is less steep than the isometric curve.

To study the isometric force transients and to allow a comparison with previously reported data, we imposed a 1 % increase in muscle length within 1·2 ms during isometric contraction in five experiments. In this case, force and length of the preparation were sampled at 4 kHz. The force response consisted of a rapid increase in force during the stretch, which was followed by a quick decrease of force, with an undershoot of about 10 % below the isometric force level. Thereafter, force recovered to a steady-state level within about 200 ms in control solution and in about 50 ms when 30 mM P_i was added. The minimum force was reached after about 50 ms in control solution without P_i and after about 20 ms in solution with 30 mM added P_i. During a 1 % shortening, force decreased and after shortening it recovered in an exponential fashion to the initial isometric level. Force recovery was complete within about 150 ms in control solution and in about 80 ms when 30 mM P_i was added. In Table 2 the results of these experiments are summarized. It can be seen that the amplitudes of the force responses during these length changes (a measure of stiffness) are reduced at 30 mM P_i to the same extent as the isometric force. The speed of force recovery, however, is increased by a factor of about three during stretches as well as releases at 30 mM P_i.

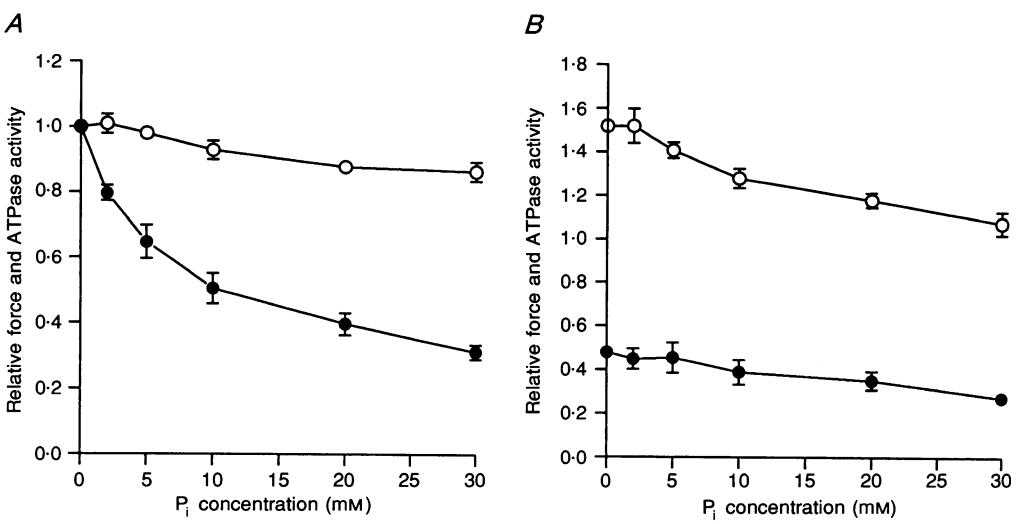

Figure 3. ATPase activity and force as a function of [P_i]
ATPase activity (○) and force (●) were normalized to the nearest control values with zero added P_i. *A* shows isometric ATPase activity and force. *B* shows ATPase activity and force during length changes. Error bars denote S.E.M. Error bars smaller than the symbol are omitted. Number of observations: 0 mM, 23; 5 mM, 6; 30 mM, 12; other values, 7.

Table 2. Force responses and half-times of 1 % length changes

	Control	pH 6·2	30 mM P_i	pH 6·2 + 30 mM P_i
Isometric force (%)	100	54 ± 1	30 ± 5	12 ± 1
Amplitude of the force response during stretch (%)	100	77 ± 3	35 ± 5	29 ± 2
Amplitude of the force response during release (%)	100	66 ± 3	35 ± 2	31 ± 1
Half-time of force recovery after stretch (ms)	4·6 ± 0·3	6·7 ± 0·6	1·6 ± 0·2	6·2 ± 0·5
Half-time of force recovery after release (ms)	17·0 ± 1·5	20·6 ± 1·3	5·4 ± 0·5	20·6 ± 3·1

Amplitude of force response during stretch: 43 ± 1 % of isometric force; during release: 37 ± 5 %.

Effects of pH

The effects of changes in pH were studied in the range from 6·2 to 7·5, which encompasses the values known to occur during hypoxia and ischaemia. In Fig. 4, the force and ATPase activity of a control measurement at pH 7·0 and a measurement at pH 6·2 are shown. Isometric force was decreased to about 60 % at pH 6·2 (in comparison to 31 % when 30 mM P_i was added). The reduction in ATPase activity at pH 6·2 (about 80 %) was less than the reduction in isometric force. Under control conditions, there was a slow increase in the average force level during length changes, but at pH 6·2 the average force level reached a steady state within about 5 s. Unlike isometric force, the average force level during length changes was hardly affected at pH 6·2. It can be seen in Fig. 4 also, that after the fibre was placed back in the relaxing solution, force fell more slowly in comparison with the control measurement. This was probably due to the reduced Ca^{2+} buffering capacity of EGTA at low pH.

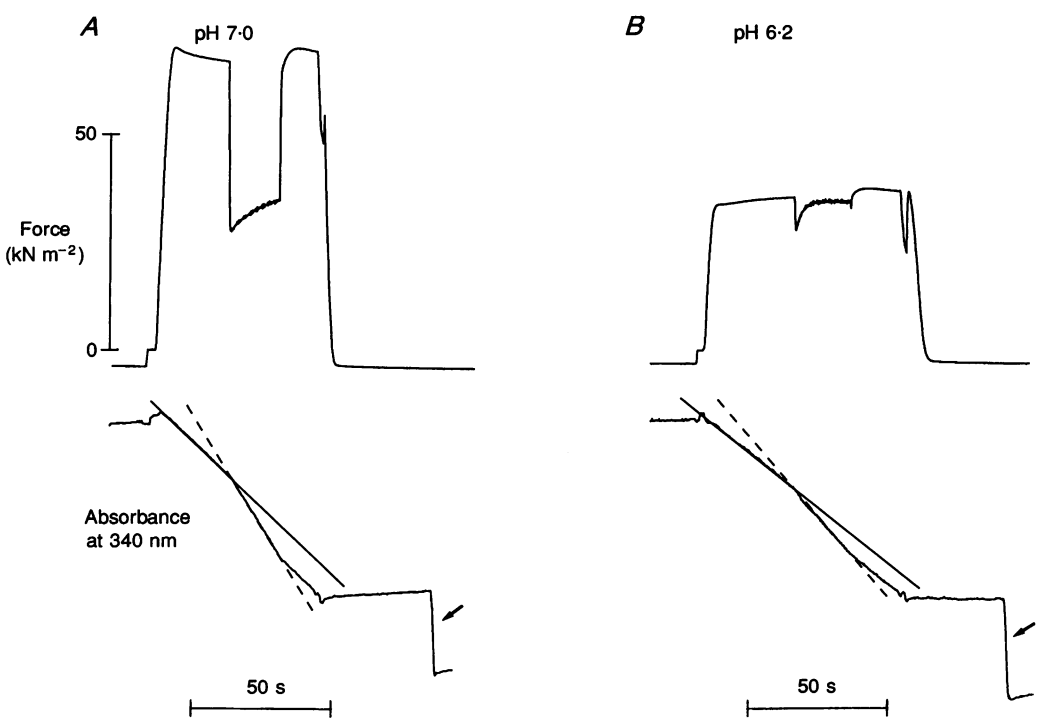

Figure 4. Force production and ATPase activity

Recordings of force production (upper traces) and ATPase activity (lower traces) during control conditions (pH 7·0, *A*) and at pH 6·2 (*B*). Experimental protocol as in Fig. 1. Note that whereas the isometric force was decreased at pH 6·2, ATPase activity was hardly decreased. The average force level during length changes remained constant or increased slightly with respect to the average level at pH 7·0. Dimensions of the preparation: length, 2·00 mm; width, 190 μm; depth, 90 μm.

In Fig. 5, the force responses to repetitive length changes are shown at pH 7·0 and pH 6·2. It can be seen that the shape of the force responses at pH 7·0 and pH 6·2 differed considerably. The undershoot in force after stretch present at pH 7·0 was absent at pH 6·2. The amplitude of the force response during stretch, a measure of stiffness, increased by a factor of 2·20 ± 0·11 at pH 6·2. At pH 6·5 and 6·8, the increases were smaller (on average 1·28 ± 0·15). At pH 7·3 and 7·5, the corresponding values were 1·16 ± 0·05 and 1·19 ± 0·11. The extent of the force recovery after a shortening relative to the value at pH 7·0 was also reduced. At pH 6·2, it was 67 ± 3 % of the value found at pH 7·0. At pH 6·5 and 6·8, the corresponding values were 79 ± 4 and 94 ± 4 %, respectively. The values obtained at alkaline pH were not significantly different from the control values. There were also no conspicuous differences between the shape of the force responses at pH 7·0 and 7·5.

The results for isometric force and ATPase as a function of pH are summarized in Fig. 6*A* ($n = 6$). It can be seen that isometric force gradually decreases to 54 ± 1 % at pH 6·2. The reduction in ATPase activity was less pronounced. At pH 6·8, ATPase activity was not significantly reduced (99 ± 2 %), but decreased significantly to 80 ± 1 % at pH 6·2.

During activation at alkaline pH, the preparations deteriorated much faster than under neutral or acidic conditions. In general, force and ATPase activity were irreversibly reduced by about 35 % after two contractions at alkaline pH. To test whether this rapid deterioration was caused by protease activity, the protease inhibitor leupeptin (0·5 mM) was added during one experiment. This did not improve fibre stability. Fibre deterioration was accounted for by using the same normalization procedure as described above. At pH 7·5 isometric force increased to 143 ± 10 %. Isometric ATPase activity, however, decreased to 83 ± 5 %. The variability in force and ATPase activity between different experiments at alkaline pH is larger than at acidic pH, which is probably related to the deterioration of fibre performance.

In Fig. 6*B*, average force and ATPase activity during length changes are shown. It can be seen that the average force level during length changes increased slightly when pH was reduced from 7·0 to 6·2. The slope of the regression

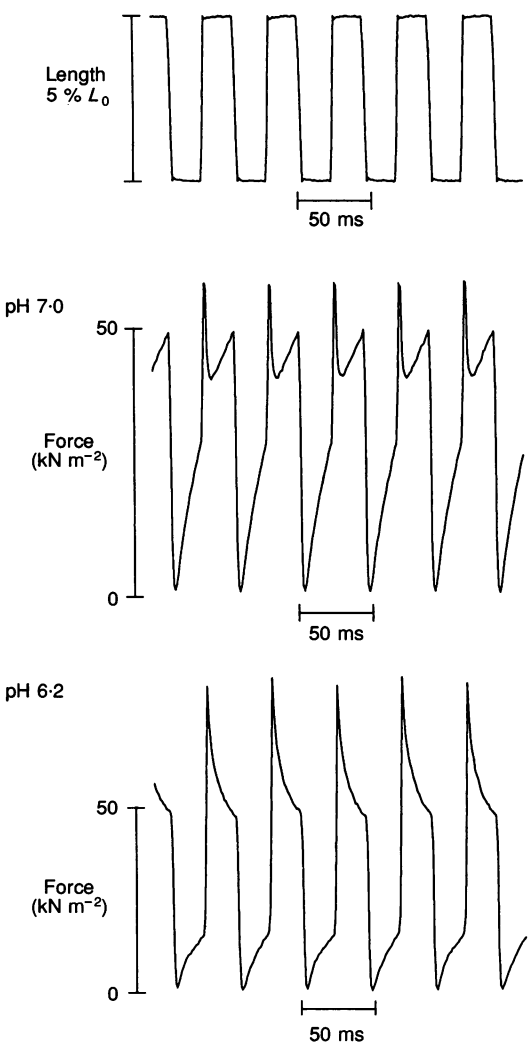

Figure 5. Force responses to length changes at pH 7·0 (middle trace) and pH 6·2 (lower trace)

The upper trace shows the imposed length changes. Isometric force, 69 kN m^{-2} (pH 7·0) and 35 kN m^{-2} (pH 6·2). Note the difference in force scale. The amplitude of the force response during restretch at pH 6·2 is much larger than the amplitude of the response at pH 7·0. Also note the absence of the transient force recovery after stretch, and the slower recovery of force after shortening at pH 6·2. Experimental conditions as in Fig. 4.

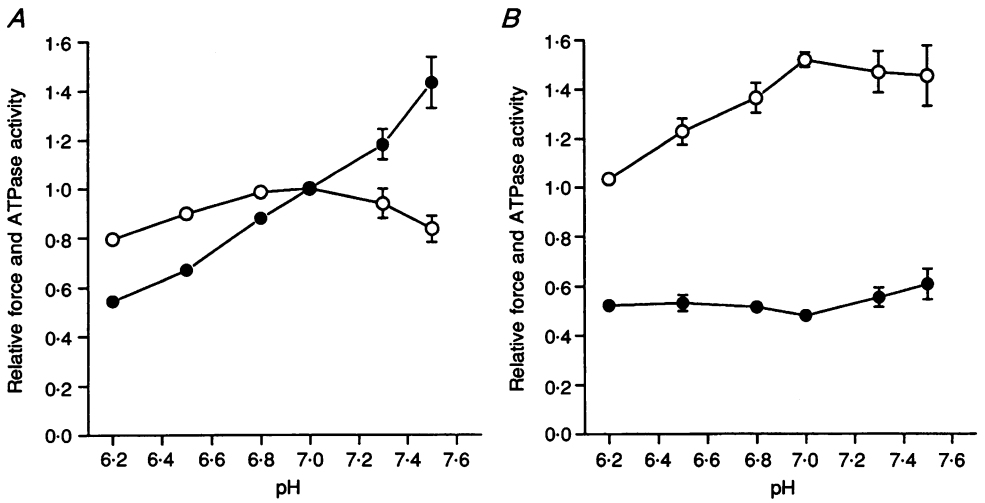

Figure 6. ATPase activity and force as a function of pH
A shows isometric ATPase activity (○) and force (●). *B* shows ATPase activity and average force during length changes. Isometric ATPase activity does not differ significantly from 1 for pH values from 6·8 to 7·3 ($P < 0·05$). Isometric force increases almost linearly as a function of pH in the range from 6·5 to 7·3. Note that the average force during length changes remains constant or increases slightly. Number of observations: pH 7·0, 18; pH 6·2, 11; other values, 6.

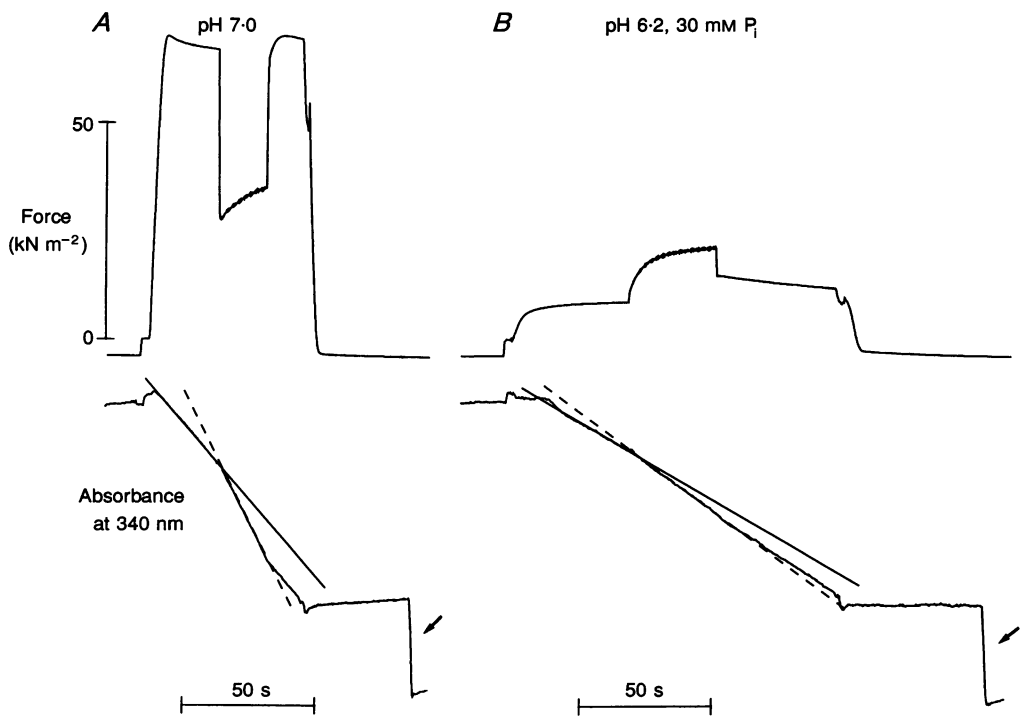

Figure 7. Recordings of force production and ATPase activity with 0 mM added P_i and pH 7·0 (*A*), and 30 mM added P_i and pH 6·2 (*B*)
Same experimental protocol as in Fig. 1. The average force during length changes at pH 6·2 and with 30 mM added P_i is increased above the isometric level. The relative increase in ATPase activity during length changes is less than under control conditions. Same preparation as in Fig. 4.

line fitted to the data points in this range was not significantly different from zero ($P = 0{\cdot}1$). The average force level increased significantly when pH was increased from 7·0 to 7·5 ($P = 0{\cdot}02$). Unlike the situation where P_i was varied, pH affected isometric ATPase activity and ATPase activity during length changes in different ways. At low pH, ATPase activity during length changes was reduced more than isometric ATPase activity, while the reverse was true at high pH values. ATPase activity during length changes remained constant at alkaline pH, whereas isometric ATPase activity decreased when pH was increased.

The effects of changes in pH were also studied during isometric contraction when 1% length changes were applied at pH 6·2. The results of these experiments are listed in Table 2. After a 1% increase in length, a steady-state force level was reached within about 75 ms at pH 6·2 and about 200 ms at pH 7·0. The undershoot which was present at pH 7·0 did not occur at pH 6·2. In general, the speed of force recovery after lengthening was somewhat reduced. The decrease in amplitude of the force response during the length changes (i.e. stiffness) at low pH was less than the decrease in isometric force.

Combined effects of P_i and pH

During ischaemia, pH and the P_i concentration change concomitantly. To mimic this condition, we studied the combined effects of pH 6·2 and 30 mM added P_i. In Fig. 7, the force and ATPase activity of a control measurement without added P_i and pH 7·0 (*A*) and of a measurement with 30 mM added P_i and pH 6·2 (*B*) are shown. Isometric force under these conditions was markedly reduced to 16% of the control value. ATPase activity decreased to 47% of the control value. Surprisingly, the average force during length changes increased with respect to the isometric level. In view of this increase in average force, the increase in ATPase activity observed during length changes was moderate.

The force responses to the length changes in solutions with zero P_i and pH 7·0, and in solutions with 30 mM P_i and pH 6·2 are shown in Fig. 8. The speed of force recovery following length changes at 30 mM P_i and pH 6·2 was lower than under control conditions. The delayed increase in force after a lengthening disappeared at 30 mM P_i and pH 6·2. In addition, the amplitudes of the force responses, especially during releases, were reduced.

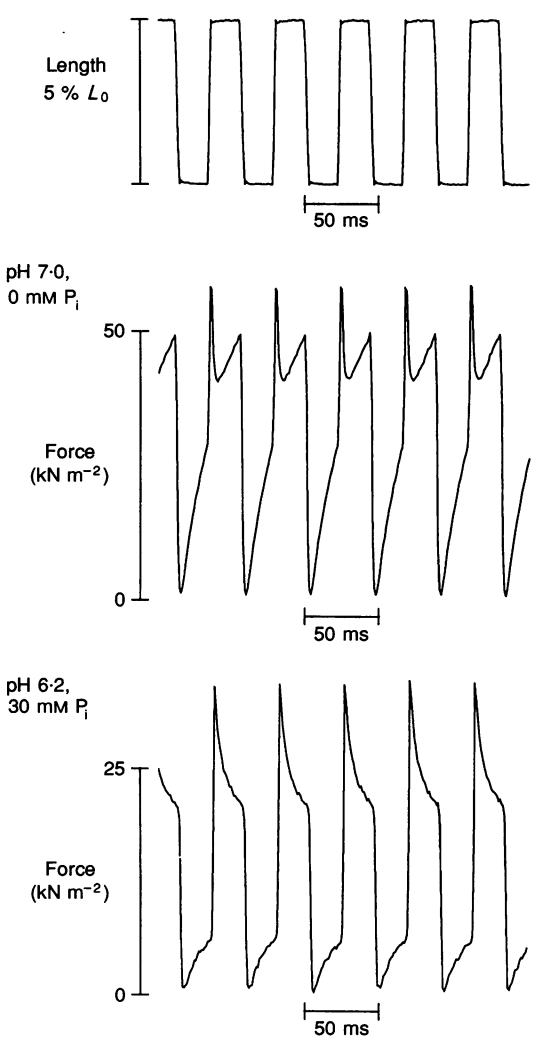

Figure 8. Force responses during length changes at pH 7·0, 0 mM P_i (middle trace) and pH 6·2, 30 mM P_i (lower trace)
Upper trace shows the imposed length changes. Isometric force: 69 kN m^{-2} (pH 7·0, 0 mM added P_i) and 9 kN m^{-2} (pH 6·2, 30 mM added P_i). The shape of the force response at pH 6·2 with 30 mM added P_i resembles that of the force response at pH 6·2 (Fig. 5). Same preparation as in Fig. 4.

The changes in ATPase activity and force under these conditions are summarized in Fig. 9. During these experiments ($n = 12$), isometric force decreased to 14 ± 1 %, whereas isometric ATPase activity decreased to 47 ± 3 %. In this series of experiments, we also investigated the effects of 30 mM P_i and pH 6·2 separately. It was found that the product of isometric force at pH 6·2 with no added P_i and force at pH 7·0 with 30 mM added P_i amounted to 14 ± 2 % of the control value. This is equal to the value (14 ± 1 %) obtained at pH 6·2 and 30 mM P_i. The product of ATPase activity at pH 6·2 and ATPase activity with 30 mM added P_i was 73 ± 7 %. This is significantly different from the value 47 ± 3 % under mimicked ischaemic conditions ($P = 0{\cdot}005$).

The values of ATPase activity and average force during length changes at pH 6·2 with 30 mM added P_i were 38 ± 3 and 71 ± 7 % of the values during length changes under control conditions, respectively. The products of ATPase activity and average force during length changes in the experiments with pH 6·2, 0 mM P_i and pH 7·0, 30 mM P_i were 50 ± 4 and 72 ± 11 %, respectively. The product of relative force during length changes obtained at pH 6·2 and 30 mM P_i separately was not significantly different from the experimental result obtained when the changes in pH and P_i were combined ($P = 0{\cdot}95$). The product of relative ATPase activities at pH 6·2 and at 30 mM added P_i, however, was significantly different from the value obtained at pH 6·2 with 30 mM P_i ($P = 0{\cdot}005$).

In a similar manner to that described for the P_i and pH experiments, 1 % changes in length were applied during isometric contraction in five preparations. The results of these experiments are listed in Table 2. The shape of the force response was similar to that of the responses at pH 6·2 without added P_i. The observed half-times for force recovery after length changes were not significantly different from the values observed at pH 6·2 without added P_i. The amplitude of the force response during the lengthening (i.e. stiffness) decreased to about 30 % of the isometric control value, whereas isometric force decreased to about 12 %.

DISCUSSION

The aim of this study was to investigate the influence of changes in P_i concentration and pH which are known to occur during ischaemia and hypoxia on myofibrillar energy utilization in cardiac muscle. The results obtained during isometric conditions clearly indicate that the effects of P_i and pH on isometric ATP turnover were smaller than on isometric force. These results suggest that tension cost, i.e. ATP turnover divided by force production, is increased under ischaemic and anoxic conditions. Moreover, the experiments indicate that differences exist in the effects of pH on force and ATPase activity under isometric and dynamic conditions. This implies that the energetic and mechanical results obtained during isometric contractions cannot be extrapolated directly to the working heart.

Kinetic scheme for the actomyosin ATPase: interaction sites of P_i and H^+

The basis of the metabolic effects on myofibrillar energy utilization resides in the kinetic scheme for the actomyosin ATPase activity. During muscle contraction there exists a cyclic interaction between myosin and actin, the forming and breaking of cross-bridges, which is driven by the energy available from hydrolysis of ATP into ADP and P_i (see Fig. 10). In this scheme, ATP binds rapidly (step 1) to the rigor cross-bridge (AM) and is hydrolysed in step 3′ or in the series of transitions denoted by step 3, which occur after cross-bridge detachment (step 2). Steps 2 and 4 represent rapid equilibria between detached and weak binding states with little or no force production. Product release takes place after the binding of M*–ADP–P_i to actin (step 4) after the transition from the weak to the strong binding configuration (step 5) in two steps: P_i release

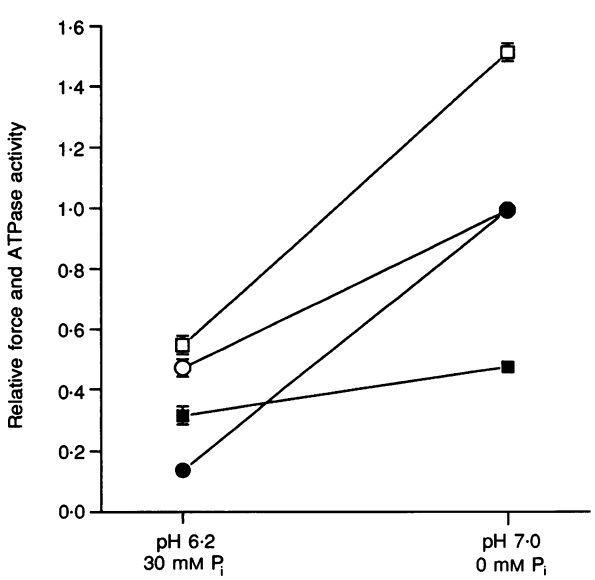

Figure 9. ATPase activity and force at pH 6·2 and 30 mM P_i

□, ATPase activity during length changes; ○, isometric ATPase activity. Filled symbols: corresponding force data. Number of observations: pH 6·2, 30 mM P_i, 12; control, 16.

(step 6) and ADP release (step 7), which completes the cycle. The depression of isometric force by P_i can be explained by mass action which promotes cross-bridges to remain in the weakly bound state or to detach via reversal of steps 6, 5 and 4 (Dantzig, Goldman, Millar, Lacktis & Homsher, 1992). The net amount of H^+ ions released (n) during ATP hydrolysis is pH dependent. For 1 mM Mg^{2+} at 25 °C, $n = 0{\cdot}8$, $0{\cdot}5$ and $-0{\cdot}1$ at pH 7·5, 7·0 and 6·2, respectively (Alberty, 1969). The evidence presented for skeletal muscle (Cooke *et al.* 1988; Metzger & Moss, 1990*a*, *b*) and cardiac muscle (Kentish, 1991) indicates that pH may influence the cross-bridge cycle at more than one transition. Fluorescence measurements showed a release of 0·3 H^+ ions at step 3 and a release of 0·7 H^+ ions during one or more of the subsequent steps (5, 6 and 7) at pH 8 (Chock, 1979). Evidence that it is unlikely that a combination of H^+ release and P_i release occurs during the same transition (step 6) was provided by Kentish (1991).

It is not well established which transition limits the overall cycling rate (the steady-state ATPase rate). It has been argued (e.g. Siemankowski, Wiseman & White, 1985) that ADP release in cardiac muscle might be sufficiently slow to be rate limiting. It has also been shown that ADP inhibits the maximum velocity of shortening (V_{max}) (Cooke & Pate, 1985) and the isometric ATPase rate (Sleep & Glyn, 1986) with a similar concentration dependence. Kentish (1991) proposed a putative H^+ uptake at step 7 (ADP release) of 0·3 H^+ ions. Mass action of protons would thus accelerate cross-bridge detachment via the subsequent steps 1 and 2. However, this is opposite to experimental results regarding the pH dependence of V_{max} (Cooke *et al.* 1988) and ATPase activity (this study). This suggests that ADP release and/or the number of cross-bridges in the AM–ADP state are reduced at low pH, and that association of protons is not rate limiting for cross-bridge detachment.

Effects of added P_i

Our results (Fig. 2) indicate that the isometric ATPase activity in cardiac trabeculae remained constant for P_i concentrations up to 5 mM, and then declined to 87 % when 30 mM P_i was added. Isometric force decreased to 31 % at 30 mM P_i. In fast skeletal muscle fibres, Kawai *et al.* (1987) found a decrease in isometric force to 60 % at 16 mM P_i, whereas isometric ATPase activity decreased to 80 % in comparison with the values without added P_i. This indicates that the effect of phosphate on ATPase activity in cardiac muscle is less pronounced than in skeletal muscle.

Pate & Cooke (1989) reported a linear relationship between force in skeletal muscle and the logarithm of the phosphate concentration. They found a decrease in relative force of 17 % per decade. Stienen *et al.* (1990) reported a reduction of 29 % per decade in frog skeletal muscle fibres. For cardiac muscle, a reduction of 45·6 % per decade was reported by Kentish (1991). We verified by means of ^{31}P-NMR that our control solution, which used phosphoenolpyruvate instead of PCr as ATP regenerating system, was essentially P_i free. During contraction, however, P_i accumulates in the fibre due to ATP hydrolysis. The average additional P_i concentration is proportional to the ATPase activity and the square of fibre diameter. Computation of this P_i concentration (cf. Stienen *et al.* 1990) using a diffusion constant for P_i of $7{\cdot}8 \times 10^{-6}$ cm^2 s^{-1} (Yoshizaki, Seo, Nishikawa & Morimoto, 1982) showed that the average values of additional [P_i] due to ATP hydrolysis inside the fibres ranged between 0·29 and 1·36 mM (mean = 0·53 ± 0·08 mM). This accumulation of P_i has its largest impact at low P_i concentrations ($\leqslant$ 5 mM). Correction of the nominal P_i concentration for P_i accumulation and fitting a logarithmic function to the measured force values yielded:

$$\text{Relative force} = (0{\cdot}96 \pm 0{\cdot}05) - (0{\cdot}44 \pm 0{\cdot}04) \times \log [P_i]$$

(regression coefficient (r) = 0·87). The slope of this relation, $-0{\cdot}44$, is in good agreement with the value of $-0{\cdot}456$ obtained by Kentish (1991) for P_i concentrations in the range from 0 to 20 mM. Our results also show that the logarithmic relationship remains valid for P_i concentrations up to 30 mM. The differences in the reduction of relative force between muscle types indicate that cardiac muscle is more sensitive to P_i than fast skeletal muscle, while slow skeletal muscle is less sensitive than fast.

As stated above, the effects of phosphate on isometric force can be explained by reversal of the P_i-release step in the cross-bridge cycle by mass-action of P_i (see Fig. 10). This notion is supported by our observation that stiffness estimated from the 1 % length changes was reduced approximately in proportion to isometric force at 30 mM P_i

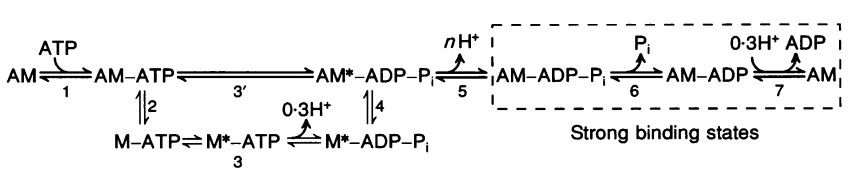

Figure 10. Reaction pathway for the actomyosin ATPase cycle
Modified from Kentish (1991) and Dantzig, Goldman, Millar, Lacktis & Homsher (1992) showing the putative steps of H^+ release and uptake. M, myosin; A, actin. Labels denote different isomerizations of myosin. The stoichiometry of H^+ release or uptake is not necessarily 1:1 (see text).

as well as by related findings in skeletal muscle (e.g. Dantzig *et al.* 1992; Martyn & Gordon, 1992). However, if force is proportional to the number of attached cross-bridges, mass-action would imply that the overall cross-bridge cycling rate and the isometric force would decrease in proportion. Our results show that ATPase activity, which is an indicator of the cross-bridge cycling rate, is reduced considerably less by P_i than is force. Apparently, mass action of P_i cannot be the sole explanation.

The free energy available from ATP hydrolysis is also reduced when $[P_i]$ is increased (e.g. Allen & Orchard, 1987). This causes a decrease in the amount of energy which can be liberated during the power stroke, and hence a reduction of force. Based on this concept, Pate & Cooke (1989) devised a model which predicted approximately linear relationships between force and log $[P_i]$ as well as between ATPase activity and log $[P_i]$ in skeletal muscle. According to this model, the reduction in isometric-ATPase activity was expected to be less than the reduction in isometric force. After correcting for P_i accumulation as described above, we found that isometric ATPase activity as a function of log $[P_i]$ could be fitted by a straight line:

$$\text{Relative ATPase activity} = (1{\cdot}09 \pm 0{\cdot}03) - (0{\cdot}16 \pm 0{\cdot}03) \times \log [P_i]$$

($r = 0{\cdot}70$). Our results suggest that the explanation of the effects of P_i in the Pate & Cooke (1989) model may also be applicable to cardiac muscle.

This dual origin of the effects of P_i, i.e. mass action and change in free energy levels could be responsible for the complex kinetic properties observed. It was observed that the delayed force recovery after stretch during the repetitive length changes was present only in thin preparations and disappeared when P_i was added. Since phosphate accumulation is dependent on fibre diameter, these results indicate that relatively small P_i concentrations are sufficient to suppress the delayed force recovery. The time course of the force responses during the repetitive changes in length, apart from the influence on the delayed force recovery, was not sensitive to phosphate, but the speed of force recovery after the 1% length changes was increased by a factor of about three when 30 mM P_i was added. This enhancing effect of P_i on the speed of force recovery was also observed during 1% releases but not during repetitive changes in length. Apparently, distinct differences exist in the contractile properties during isometric conditions and during repetitive length changes, which reduce the average force level. In this respect, it is interesting to note that the maximum shortening velocity (at zero load) is also not sensitive to changes in phosphate concentration (e.g. Cooke & Pate, 1985). The P_i-dependent changes in speed of force recovery after a 1% stretch during isometric contraction are similar to those reported by Kentish (1991). This similarity in shape of the force responses to 1% length changes indicates that the choice of the ATP regenerating system, PCr based (as used by Kentish) or PEP based (our experiments), does not influence the mechanical behaviour of the preparations.

At low P_i concentrations, the average force level during length changes does not follow the isometric curve (Fig. 3). This difference, however, could result from the increase in ATPase activity during length changes. On average, this causes an increase of the P_i accumulation inside the fibre by a factor of approximately 1·5 (i.e. about 0·8 mM), which may truncate the P_i effects at low concentrations. The length changes caused a reduction in the average force level to 48% and an increase in ATPase activity to 152% of the isometric control value. ATPase activity during repetitive length changes show a similar dependence of $[P_i]$ as isometric ATPase activity (see Fig. 3). In other words, the repetitive length changes, apart from the increase in ATPase activity and the decrease in force, have no (or small) additional effects on force and ATPase activity at various P_i concentrations. This suggests that the effect of P_i on the step(s) in the cross-bridge cycle which affect ATP turnover is hardly influenced by changes in cross-bridge strain. Based on caged P_i experiments in rabbit psoas fibres Dantzig *et al.* (1992) proposed that the forward step of the force-generating transition was strain dependent. Provided that the rate of P_i release in cardiac muscle is too fast to limit the overall rate of ATP turnover, such a strain dependence is compatible with our data.

In a previous study in our laboratory (Stienen, Papp, & Elzinga, 1993), evidence was presented that Ca^{2+} modulates the effects of length changes on ATPase activity and average force level in cardiac muscle, using the same protocol for the repetitive length changes as in this study. To explain these results, it was proposed that the apparent attachment rate was reduced during the changes in length. This reduction became apparent at low Ca^{2+} concentrations, where the cross-bridge attachment rate could be rate limiting for the ATP turnover. In principle this effect could be located in any of the transitions from the weak binding M*–ADP–P_i state to the strong binding states, i.e. steps 4–6 in Fig. 10. Included in these steps is the P_i release step. As stated above, P_i only scaled the effects of length changes in proportion with isometric ATPase activity and force. This suggests that the modulatory role of Ca^{2+} during length changes is not associated with P_i release but with one of the preceding steps (4 or 5). Experiments performed at submaximal activation (near pCa 5·1), where the effect of length changes on ATP turnover is small or absent, indicated that scaling of the responses by 15 mM inorganic phosphate was preserved (Z. Papp, G. J. M. Stienen & G. Elzinga, unpublished observations).

Effects of pH

As with the experiments where $[P_i]$ was varied, isometric force was affected more by pH than isometric ATPase activity. In the range from 6·8 to 7·3 relative isometric force increased almost linearly from 88 to 118%, but the

relative ATPase activity did not differ significantly from 1.

Godt & Kentish (1989) noted a decrease in both ATPase activity and isometric force when pH was decreased from 7 to 6·5, to 70·1 and 62·1 %, respectively. Although our value for the reduction in force at pH 6·5 (65 %) is in good agreement with their data, the decrease in ATPase activity that we found was less pronounced (90 %). Godt & Kentish assessed ATPase activity by measuring changes in fluorescence of NADH, which are dependent on the myosin concentration inside the measuring area. It is not clear whether the authors corrected for expansion of the myofilament lattice due to the increase in H^+ ions at low pH, which causes a lower myosin concentration and hence an apparent reduction in ATPase activity. However, since the myofibrillar lattice expands by only 5–7 % at pH 6·0 in skeletal muscle (Chase & Kushmerick, 1988), this effect probably does not explain the difference completely.

Experiments performed by Blanchard & Solaro (1984) showed that the maximum Ca^{2+}-activated ATPase activity in dog cardiac myofibrils did not vary significantly as a function of pH. These experiments were performed on myofibrils in solution, with 2 mM free Mg^{2+}, 2 mM free MgATP and ionic strength 120 mM. Kentish & Nayler (1979) reported a 20 % decrease in maximal Ca^{2+}-activated ATPase activity in myofibrils from rabbit and guinea-pig when pH was reduced from 7·2 to 6·4. However, the myofibrils did not contract isometrically during these experiments, which makes a direct comparison with our results on isometrically contracting trabeculae impossible.

Metzger & Moss (1990*a*) reported no changes in stiffness as a function of pH in (slow) rabbit soleus muscle fibres, but a reduction of stiffness to about 75 % at pH 6·2 in (fast) rat superficial vastus lateralis fibres at maximal activation. Our experiments in cardiac muscle at pH 6·2 showed a decrease in the amplitude of the force response during 1 % lengthening (a measure of stiffness) to about 75 % of the control value at pH 6·2. In this respect cardiac muscle is similar to fast skeletal muscle.

The effects of pH on the average force and stiffness during the repetitive changes in length are clearly different from the effects on isometric force and stiffness. Under acidic conditions the average force level during the length changes remained more or less constant but stiffness increased up to a factor of 2·2 at pH 6·2, while isometric force and stiffness decreased to 50 and 75 %, respectively. Under alkaline conditions, the changes in the average force level with pH were smaller than in isometric force. During repetitive length changes we also found a decrease in the extent of force recovery. As the duration of the pauses were kept constant this implies that the speed of the recovery is decreased. After the 1 % releases, however, the half-time for force recovery was increased by about 20 %. These observations indicate that the pH effects observed during repetitive changes in length are typical for the dynamic steady state which is reached during movement.

The overall reduction in isometric force and stiffness at low pH could be due to a reduction in the total amount of attached cross-bridges. A possible explanation for the observation that the reduction in isometric force is larger than in isometric stiffness might be that mass action suppresses the release of protons during the transition between detached cross-bridge states (step 3 in Fig. 10). This would promote cross-bridges to follow step 3′ rather than step 3, and thus causes an increase in the fraction of weakly bound cross-bridges. If these weakly-bound cross-bridges contribute less to force than to stiffness this would explain our results. This differential reduction of force and stiffness thus indicates that protons influence cross-bridge kinetics at two or more points in the cycle. Another mechanism through which the relatively small reduction in isometric ATPase activity may be explained is a distortion of the myofilament lattice at low pH, which may affect the force generated per cross-bridge due to a change in geometry, while the ATP cycling rate need not be affected (Matsuda & Podolsky, 1986).

During the length changes ATP turnover is increased above the isometric value. The general shape of the relation between the ATPase activity during length changes and pH, however, does not resemble the isometric curve as was the case for the P_i experiments. The explanation for these observations is clearly rather complex as it was also found that stiffness during the repetitive length changes rose dramatically at low pH. We expect that the shape of the ATPase activity–pH curve will depend on the type of length changes imposed. Nevertheless, the observation that the curve during length changes differs from the isometric curve indicates that the distribution of cross-bridges over the various states during the length changes is different from the isometric distribution. Apparently these distributions are influenced by pH in different ways, which explains why the effects on the overall ATP turnover rate depend on the mechanical conditions. This suggests that the changes in cross-bridge strain, as induced by these length changes, have an additional effect on at least one of the pH-sensitive transitions in the cross-bridge cycle which influence ATP turnover.

Combined effects of P_i and reduced pH

The combined effects of added phosphate and decreased pH were studied to mimic the major metabolic changes occurring during ischaemia, and to investigate further the effects of these changes on ATPase activity. Isometric force decreased on average to 14 % of the control value when pH was lowered to 6·2 and 30 mM P_i was added. It was argued by Dawson, Smith & Wilkie (1986) and Nosek, Fender & Godt (1987), that it is the diprotonated form of P_i ($H_2PO_4^-$) that reduces force in skeletal muscle. However, it has been shown recently that the effects of P_i and pH on isometric force are independent in cardiac muscle (Nosek, Leal-Cardoso, McLaughlin & Godt, 1990; Kentish, 1991). Our

results show that in cardiac muscle both isometric force and the average force during length changes under simulated ischaemic conditions (30 mM P_i, pH 6·2) are not significantly different from the product of the relative reductions in force by 30 mM P_i and pH 6·2 separately. Thus, our results are in agreement with these studies, and indicate that in cardiac muscle the effects of phosphate and pH on force are independent under both isometric and dynamic conditions. However, this is not the case for ATPase activity, because the product of ATPase activity at pH 6·2 and ATPase activity with 30 mM P_i separately, differs significantly from ATPase activity at pH 6·2 with 30 mM P_i, both under isometric and dynamic conditions. This synergistic interaction between P_i and pH is present only in ATPase activity, and thus in the overall cross-bridge cycling rate.

These results show that under these mimicked ischaemic conditions maximal Ca^{2+}-activated isometric tension cost increases about 3·5-fold. However, in the working ischaemic heart the free Ca^{2+} concentration does not reach the levels used in this study. Furthermore, the increase in tension cost is less under dynamic conditions. Therefore, tension cost in the working ischaemic heart is not necessarily identical to the isometric value during maximal activation. An increase in tension cost in the ischaemic heart, however, may be expected to be based upon these results, because in general the reduction in force appears to be larger than the reduction in ATPase activity.

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Acknowledgements

The support of the Dutch Heart Foundation is gratefully acknowledged. We thank I. A. van Graas for expert technical assistance.

Received 18 May 1993; accepted 1 October 1993.