pH MODULATION OF THE KINETICS OF A Ca²⁺-SENSITIVE CROSS-BRIDGE STATE TRANSITION IN MAMMALIAN SINGLE SKELETAL MUSCLE FIBRES

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SUMMARY

1. The rate constant of tension redevelopment (k_{tr}) following a rapid release and subsequent re-extension of muscle length has been demonstrated to be Ca²⁺ sensitive and is thought to reflect the rate-limiting step in the cross-bridge cycle leading to the formation of the strongly bound, force-bearing state. The kinetics of this cross-bridge state transition were investigated at 15 °C over a wide range of Ca²⁺ concentrations while varying pH from 7·00 to 6·20 in rat slow-twitch soleus, rat fast-twitch superficial vastus lateralis (SVL) and rabbit fast-twitch psoas skinned single fibres.

2. At maximal levels of Ca²⁺ activation, k_{tr} was unaffected by changes in pH from 7·00 to 6·20 while isometric tension was depressed to $0·60 \pm 0·02\ P_0$ (mean $\pm$ S.E.M.) at the low pH in fast-twitch fibres and to $0·78 \pm 0·01\ P_0$ in slow-twitch fibres (P_0 is the maximum isometric tension obtained at pH 7·00).

3. At reduced levels of Ca²⁺ activation, corresponding to pCa ($-\log$ [Ca²⁺]) greater than 5·0, k_{tr} was markedly depressed in all fibre types when pH was lowered. The Ca²⁺ sensitivity of steady-state isometric tension was also reduced in all fibres at pH 6·20 compared to pH 7·00.

4. The results suggest that pH has a modulatory effect upon an apparent rate constant which is rate limiting in terms of the formation of the strongly bound, force-bearing cross-bridge state. This effect of altered pH may in part account for the reduction in the Ca²⁺ sensitivity of isometric force at low pH as well as the depression of the rate of rise of tension in living fibres during fatiguing stimulation.

INTRODUCTION

A characteristic manifestation of muscle fatigue is a decrease in the rate of rise of tension during repetitive contractions (Marey, 1868). The basis for this effect is unknown but may in part relate to the stimulation-dependent decline in intracellular pH. Here, we tested whether the recently identified Ca²⁺-sensitive cross-bridge state transition in mammalian skeletal muscle (Brenner, 1988; Metzger, Greaser & Moss, 1989) is modulated by pH. The step in the actin–myosin ATP hydrolysis reaction thought to be sensitive to the concentration of Ca²⁺ is the cross-bridge transition from the weakly bound state to the strongly bound, force-bearing state. In the

present study the kinetics of this state transition were examined in skinned single fibres over a wide range of Ca^{2+} concentrations and at pH 7·00 or pH 6·20. We show here an effect of low pH to reduce the Ca^{2+} sensitivity of this cross-bridge state transition, which may in part provide a mechanism for the reduced rate of rise in tension during fatigue.

Portions of this work have been published previously in abstract form (Metzger & Moss, 1989*a*).

METHODS

Skinned fibre preparation and experimental apparatus

Fast-twitch skeletal muscle fibres were obtained from the superficial portion of the vastus lateralis (SVL) muscles of adult female Sprague–Dawley rats and from psoas muscles of adult male New Zealand rabbits. Slow-twitch fibres were obtained from soleus muscle of adult female Sprague–Dawley rats. Complete details are available in the preceding paper (Metzger & Moss, 1990*a*).

Solutions

Relaxing and activating solutions contained (in mmol l^{-1}): EGTA, 7; free Mg^{2+}, 1; total ATP, 4·4; creatine phosphate, 14·5; imidazole, 20; and sufficient KCl (total Cl^- 68–83) to yield a total ionic strength of 180 mmol l^{-1}. Solution pH was adjusted to 7·00 or to 6·20 with KOH, except for the solution of pH 6·20 and pCa 9·0 in which pH was adjusted with HCl. Complete details are available in the preceding paper. All experiments were conducted at 15 °C.

Rate constant of tension redevelopment

The experimental protocol for measuring the rate constant of tension redevelopment (k_{tr}) was a modification of the multistep protocol developed by Brenner & Eisenberg (1986). The measurement of k_{tr} involves a mechanical manoeuvre leading to the complete dissociation of myosin cross-bridges from actin in a steadily activated fibre, so that the subsequent rate of tension redevelopment reflects the rate-limiting transition in the cross-bridge cycle leading to the formation of the strongly bound, force-generating state. The fibre was first transferred from relaxing solution to an activating solution with controlled pCa in the range 7·0 to 4·5, and steady isometric tension was allowed to develop. The fibre was then rapidly (0·5 ms) shortened by about 200–300 nm (half-sarcomere)$^{-1}$ resulting in an abrupt reduction of force to zero, and the fibre shortened for 5–40 ms under unloaded conditions (i.e. at V_{max}). While shortening at V_{max}, the number of cross-bridges attached at one time is about 20 % of the total (Huxley, 1957; Julian & Sollins, 1975). In order to detach these remaining cross-bridges from actin, the fibre was rapidly (0·5 ms) re-extended to its initial length. Coincident with the restretch, force transiently increased due to positive straining of attached cross-bridges; however, since the amount of restretch (200–300 nm (half-sarcomere)$^{-1}$) was markedly greater than estimates of the working distance of the cross-bridge (about 10 nm (half-sarcomere)$^{-1}$), attached cross-bridges dissociated and force rapidly declined to zero or very nearly zero. The redevelopment of force following this manoeuvre reflects the rate of reattachment of cross-bridges and the transition to the strongly bound, force-producing state. During the redevelopment of force, sarcomere length was held constant since in the absence of sarcomere length control, k_{tr} would be underestimated due to end compliance (Brenner & Eisenberg, 1986). In our experiments sarcomere length was clamped to within 0·5 nm (half-sarcomere)$^{-1}$ of the desired value by servo-control of the position of the first-order line of the laser diffraction pattern (Fig. 2; also see Metzger *et al.* 1989). Records of tension redevelopment were best fitted by a first-order exponential equation: $F_t = F_0 (1 - e^{-k_{tr} \cdot t})$, where F_t is force at time t, F_0 is maximum force, and k_{tr} is the rate constant of tension redevelopment. Complete details of the experimental protocol, curve-fitting procedure, mechanical set-up, and sarcomere length control servo system are available elsewhere (Metzger *et al.* 1989).

Tension–pCa relationship

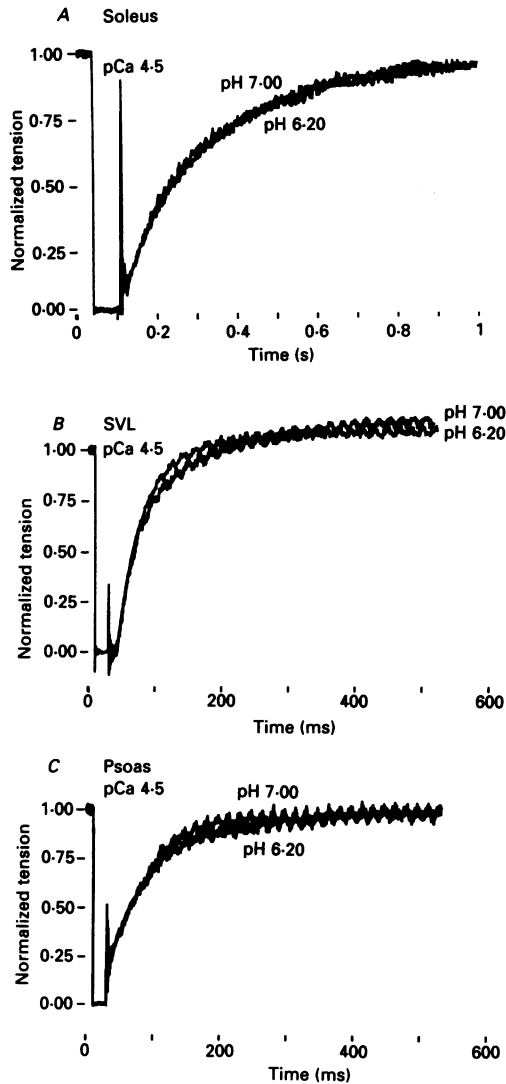Tension–pCa data were collected as described in the preceding paper.

Fig. 1. *A*, tension records obtained during the protocol to determine k_{tr} during maximal levels of Ca^{2+} activation at pH 7·00 and at pH 6·20 in a single soleus fibre. k_{tr} was 3·5 s^{-1} at pH 7·00 and at pH 6·20. In both experimental trials, sarcomere length was clamped to 2·60 μm during the entire phase of tension redevelopment (sarcomere length records similar to those shown in Fig. 2.*A*). P_0, obtained at pCa 4·5 and pH 7·00, was 109 kN m^{-2}. Tension at pCa 4·5 and pH 6·20 was 0·77 P_0. Fibre length was 3·48 mm. *B*, records of tension obtained during the protocol to determine k_{tr} at maximal levels of Ca^{2+} activation at pH 7·00 and at pH 6·20 in a single SVL fibre. k_{tr} was 23·0 s^{-1} at pH 7·00 and 22·2 s^{-1} at pH 6·20. Sarcomere length was clamped at 2·54 μm during tension redevelopment (sarcomere length records similar to those shown in Fig. 2*A*). P_0 was 96·8 kN m^{-2} while maximum tension obtained at pH 6·20 was 0·66 P_0. Fibre length was 2·48 mm. *C*, records of tension obtained during the protocol to determine k_{tr} at maximal levels of Ca^{2+} activation at pH 7·00 and at pH 6·20 in a single psoas fibre. k_{tr} was 19·0 s^{-1} at pH 7·00 and 18·2 s^{-1} at pH 6·20. Sarcomere length was clamped at 2·57 μm during tension redevelopment (sarcomere length records similar to those shown in Fig. 2*A*). P_0 was 138 kN m^{-2}, while maximum tension at pH 6·20 was 0·55 P_0. Fibre length was 2·89 mm.
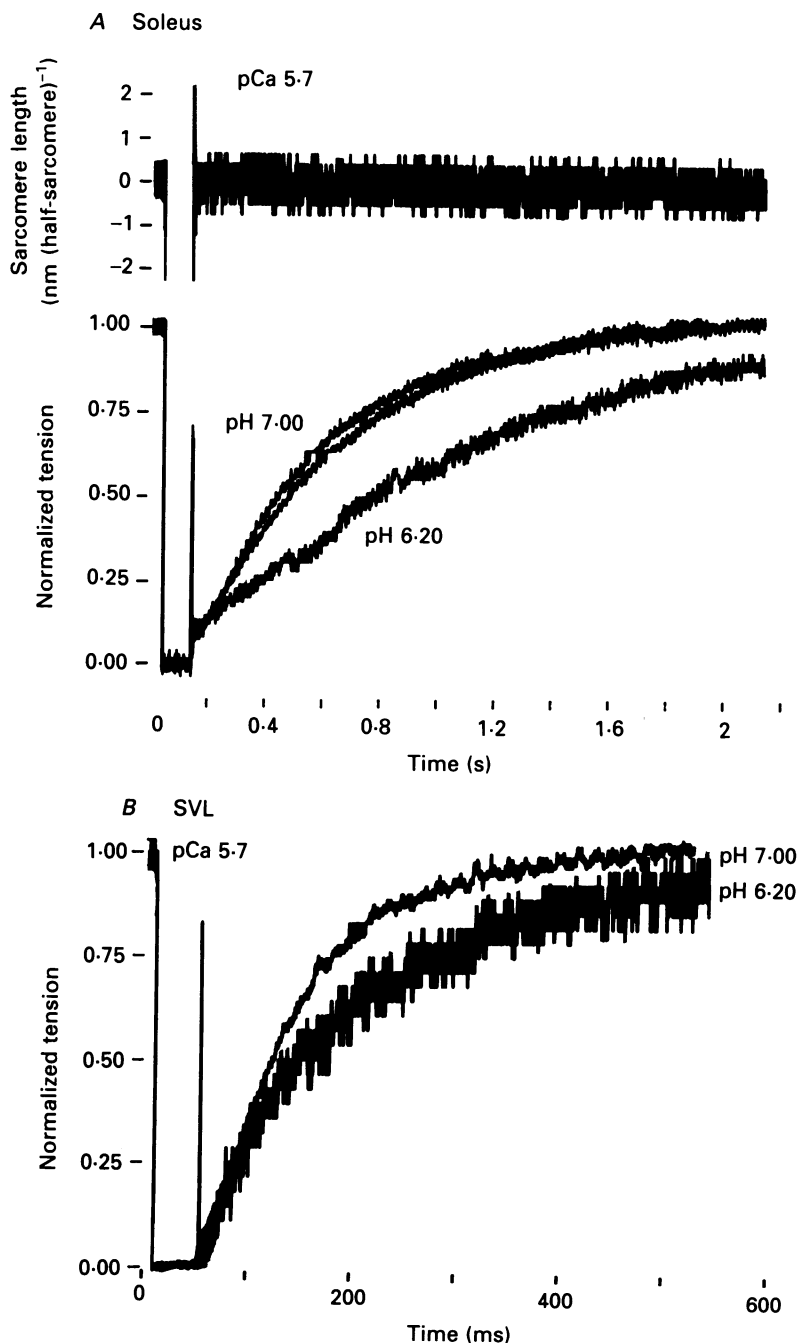
A   Soleus

Fig. 2. For legend see facing page.

Statistics

A two-way analysis of variance (ANOVA) was used to test whether altered pH significantly affected the tension–pCa and/or the k_{tr}–pCa relationships. When a significant interaction between pH and Ca^{2+} was indicated by ANOVA, Student's two-tailed t test was used to determine significant differences between two mean values. A probability level of $P < 0.05$ was selected as indicating significance. Values are reported as means $\pm$ standard error of the mean.
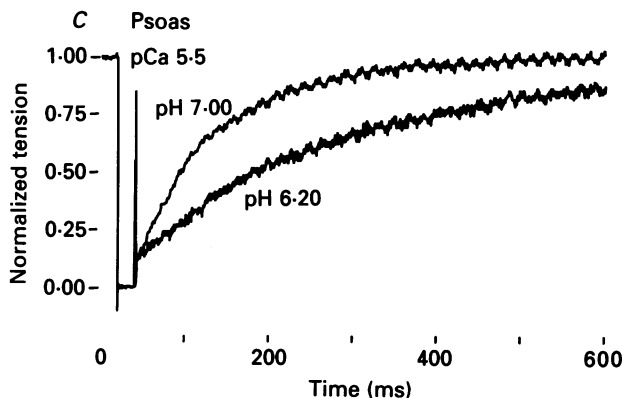
Fig. 2. *A*, records of tension and sarcomere length obtained during the protocol to determine k_{tr} at a submaximal level of Ca^{2+} activation (pCa 5·7) at pH 7·00 and at pH 6·20 in a single soleus fibre. k_{tr} was 2·2 s^{-1} at pH 7·00, 1·0 s^{-1} at pH 6·20 and 2·2 s^{-1} with pH back to 7·00. k_{tr} was 3·3 s^{-1} at pCa 4·5 and pH 7·00. Sarcomere length was clamped at 2·53 μm during tension redevelopment. P_0 was 95 kN m^{-2}. At pCa 5·7, tension was 0·80 P_0 at pH 7·00 and 0·33 P_0 at pH 6·20. Fibre length was 3·08 mm. *B*, records of tension obtained during the protocol to determine k_{tr} at a submaximal level of Ca^{2+} activation (pCa 5·7) at pH 7·00 and at pH 6·20 in a single SVL fibre. k_{tr} was 20·0 s^{-1} at pH 7·00 and 10·0 s^{-1} at pH 6·20. k_{tr} was 24 s^{-1} at pCa 4·5 and pH 7·00. Sarcomere length was clamped at 2·57 μm during tension redevelopment (sarcomere length records similar to those shown in Fig. 2*A*). P_0 was 68 kN m^{-2}. At pCa 5·7, tension was 0·73 P_0 at pH 7·00 and 0·29 P_0 at pH 6·20. Fibre length was 2·00 mm. *C*, records of tension obtained during the protocol to determine k_{tr} at a submaximal level of Ca^{2+} activation (pCa 5·5) at pH 7·00 and at pH 6·20 in a single psoas fibre. k_{tr} was 10·6 s^{-1} at pH 7·00 and 3·7 s^{-1} at pH 6·20. k_{tr} was 19·0 s^{-1} at pCa 4·5 and pH 7·00. Sarcomere length was clamped at 2·57 μm during tension redevelopment (sarcomere length records similar to those shown in Fig. 2*A*). P_0 was 140·7 kN m^{-2}. At pCa 5·5, tension was 0·93 P_0 at pH 7·00 and 0·36 P_0 at pH 6·20. Fibre length was 2·89 mm.

RESULTS

pH modulates isometric tension but not k_{tr} at maximal levels of Ca^{2+} activation

The effect of altered pH on k_{tr} during maximal Ca^{2+} activation of a slow-twitch soleus fibre is shown in Fig. 1. In this fibre, which was activated at pCa 4·5, k_{tr} was 3·5 s^{-1} at both pH 7·00 and pH 6·20. However, as found previously (Metzger & Moss, 1987, 1988) there was a 23% depression of isometric tension at pH 6·20 compared to pH 7·00. There was also no effect of altered pH on k_{tr} during maximal Ca^{2+} activation of fast-twitch SVL fibres and psoas fibres (Fig. 1). In the SVL and psoas fibres shown, the kinetics of tension redevelopment at pH 7·00 virtually superimposed the records obtained at pH 6·20. In agreement with our earlier findings the effect of reduced pH to depress tension was more pronounced in SVL and psoas fibres than that observed in soleus fibres. Table 1 is a summary of the effects of altered pH on k_{tr} and isometric tension at maximal levels of Ca^{2+} activation in each of the fibre types. As noted previously (Metzger & Moss, 1989*b*), k_{tr} measured at maximal levels of Ca^{2+} activation differs markedly between fast-twitch and slow-twitch fibres. At pCa 4·5 and pH 7·00, k_{tr} was 23·4±0·7 s^{-1} ($n = 13$) in rat fast-twitch SVL fibres and 3·1±0·2 s^{-1} ($n = 10$) in rat slow-twitch soleus fibres. The functional significance of

this difference in maximal k_{tr} between fibre types is the subject of another paper (Metzger & Moss, 1990*b*).

Effects of altered pH on k_{tr} and isometric tension at submaximal levels of Ca^{2+} activation

In all fibres examined at submaximal levels of Ca^{2+} activation, k_{tr} was markedly reduced at pH 6·20 compared to pH 7·00. Figure 2 shows tension records obtained from a soleus fibre activated at pCa 5·7. The fibre was first activated at pH 7·00 and

TABLE 1. Summary of the effects of pH on k_{tr} and tension at maximal levels of Ca^{2+} activation

	k_{tr} (s^{-1})		Relative tension (pH 6·20/pH 7·00)
	pH 7·00	pH 6·20	
Soleus	3·1 ± 0·2	3·4 ± 0·2	0·78 ± 0·01
	($n = 10$)	($n = 8$)	($n = 10$)
SVL	23·4 ± 0·7	24·0 ± 0·9	0·60 ± 0·02
	($n = 13$)	($n = 12$)	($n = 13$)
Psoas	17·6 ± 0·8	17·9 ± 0·02	0·60 ± 0·03
	($n = 7$)	($n = 6$)	($n = 7$)

Values are means ± standard error of the mean. Relative tension values were obtained by dividing the tension at pCa 4·5 and pH 6·20 by the tension obtained at pCa 4·5 and pH 7.00 in the same fibre.

a k_{tr} value of 2·2 s^{-1} was obtained. In a subsequent activation at pH 6·20, k_{tr} was reduced to a value of 1·0 s^{-1}. The effect of low pH to decrease k_{tr} was completely reversed by returning the fibre to an activating solution of pH 7·00, indicating that there were no irreversible effects of altered pH on k_{tr}. The effect of low pH to reduce k_{tr} at a submaximal level of Ca^{2+} activation was also observed in SVL and psoas fibres (Fig. 2).

A summary of the effects of altered pH on the k_{tr}–pCa relationships in soleus, SVL and psoas fibres is presented in Fig. 3. In all fibres at maximal Ca^{2+} activation altered pH had no effect upon k_{tr}; however, at lower levels of Ca^{2+} activation corresponding to pCa greater than 5·0, k_{tr} was significantly reduced when pH was lowered. Despite marked differences between fibre types in terms of absolute values of k_{tr} (Table 1), the pCa_{50} (concentration of Ca^{2+} necessary for half-maximal k_{tr}) of k_{tr} was reduced by approximately 0·30 pCa units in all fibres at pH 6·20 compared to pH 7·00 (Table 2).

As reported previously (Metzger & Moss, 1987) the Ca^{2+} sensitivity of isometric tension was significantly depressed at low pH in soleus, SVL and psoas fibres (Fig. 4; Table 2). The concentration of Ca^{2+} necessary for half-maximal isometric tension (i.e. pCa_{50}) was reduced by about 0·4 pCa units in all fibre types.

The results presented in Table 2 also show the differing Ca^{2+} sensitivities of tension and k_{tr}. At pH 7·00, pCa_{50} values obtained from the k_{tr}–pCa relationships were 0·2–0·4 pCa units lower than the pCa_{50} values obtained from the tension–pCa relationships. These differences in pCa_{50} were greater in fast-twitch than in slow-twitch fibres. At pH 6·20, differences between the Ca^{2+} sensitivities of tension and k_{tr} were less and fibre type differences were less apparent. At pH 7·00, values of n_2

obtained from Hill plots of the tension–pCa data were greater in fast- than in slow-twitch fibres, with differences between fibre types being less at low pH, as noted previously (Metzger & Moss, 1987). The steepness of the tension–pCa relationship is thought in part to be a manifestation of molecular co-operativity in the activation of thin filament. Thus, a decrease in n_2 suggests a reduction in co-operative
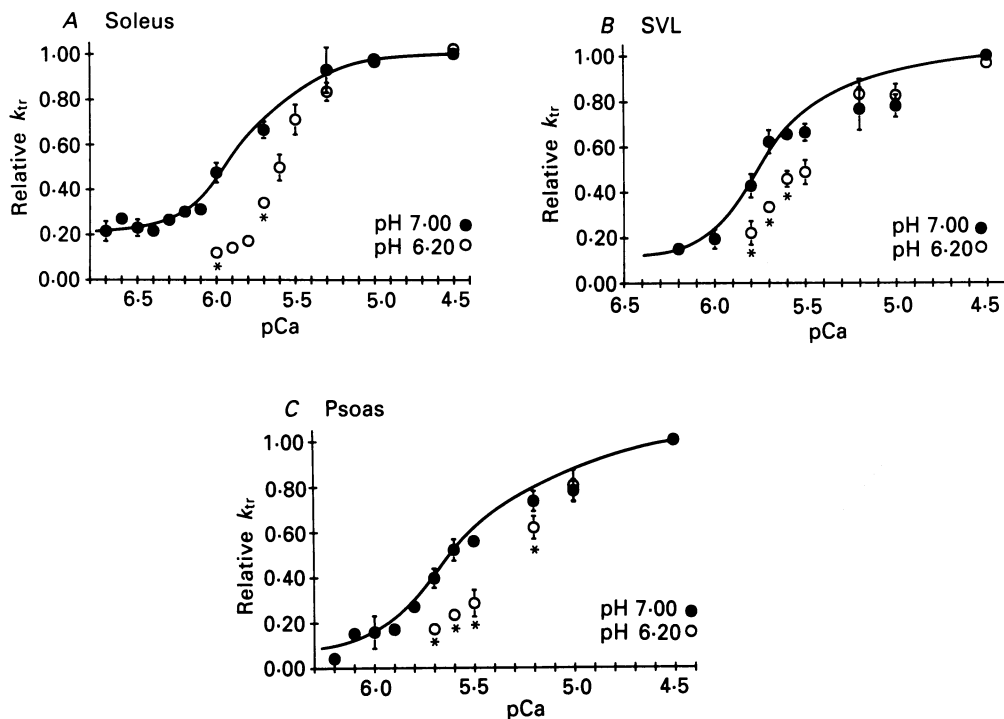

Fig. 3. Summary of the effects of pH upon the k_{tr}–pCa relationships in soleus (A), SVL (B) and psoas (C) fibres. Maximum values of k_{tr} were obtained at pCa 4·5 and average values at pH 7·00 and pH 6·20 were, respectively, $3·1 \pm 0·2$ s^{-1} ($n = 10$) and $3·4 \pm 0·2$ s^{-1} ($n = 8$) for soleus fibres, $23·4 \pm 0·7$ s^{-1} ($n = 13$) and $24·0 \pm 0·9$ s^{-1} ($n = 12$) for SVL fibres, and $17·6 \pm 0·8$ s^{-1} ($n = 7$) and $17·9 \pm 0·02$ s^{-1}($n = 6$) for psoas fibres. Values are means $\pm$ standard error of the mean. In some instances error bars were smaller than the symbol. The curves were fitted to the data by eye. The asterisks denote significant differences between the pH 7·00 and pH 6·20 values at the same pCa.

activation of the thin filament at low pH. In general, the Hill coefficients obtained from k_{tr}–pCa data were lower than those obtained from tension–pCa data, i.e. the k_{tr}–pCa relationship was less steep. At pH 7·00, the transformed k_{tr}–pCa data were clearly biphasic, with n_1 and n_2 values averaging about 1·4 and 2·2, and the difference between values being greater in fast- than in slow-twitch fibres (Table 2). The significance of biphasic Hill plots and n values greater than 1 for k_{tr} data is not known but presumably indicates co-operativity in the system which regulates k_{tr}.

In order to facilitate comparisons of the relative effects on k_{tr} and tension due to reduced pH, results obtained at pH 6·20 have been scaled to the results obtained from the same fibre at the same pCa and pH 7·00 (Fig. 5). Thus, a value less than 1·0

TABLE 2. Summary of the effects of pH on pCa_{50} and Hill coefficients, n, obtained from tension–pCa and k_{tr}–pCa data

	Tension			k_{tr}		
	pH 7·00	pH 6·20	Difference	pH 7·00	pH 6·20	Difference
Soleus						
pCa_{50}	· 6·13	5·64	−0·49	5·95	5·61	−0·34
n_1	1·47	1·54	+0·07	0·96	2·35	+1·39
n_2	1·80	5·12	+3·32	1·27	3·47	+2·20
SVL						
pCa_{50}	6·00	5·62	−0·38	5·75	5·53	−0·22
n_1	1·92	1·60	−0·32	2·15	2·10	−0·05
n_2	4·60	2·10	−2·50	2·55	2·00	−0·55
Psoas						
pCa_{50}	6·04	5·64	−0·40	5·61	5·31	−0·30
n_1	2·39	2·28	−0·11	1·06	1·86	+0·80
n_2	6·20	5·63	−0·57	2·60	1·86	−0·74

The pCa_{50} values were obtained from the tension–pCa and k_{tr}–pCa data shown in Figs 3 and 4. The Hill coefficient n_1 is the slope of the best-fit line for data greater than approximately 50% of the maximum, while the Hill coefficient n_2 is the slope for data less than approximately 50% of the maximum value. The difference value was obtained by subtracting the pH 7·00 value from the pH 6·20 value.
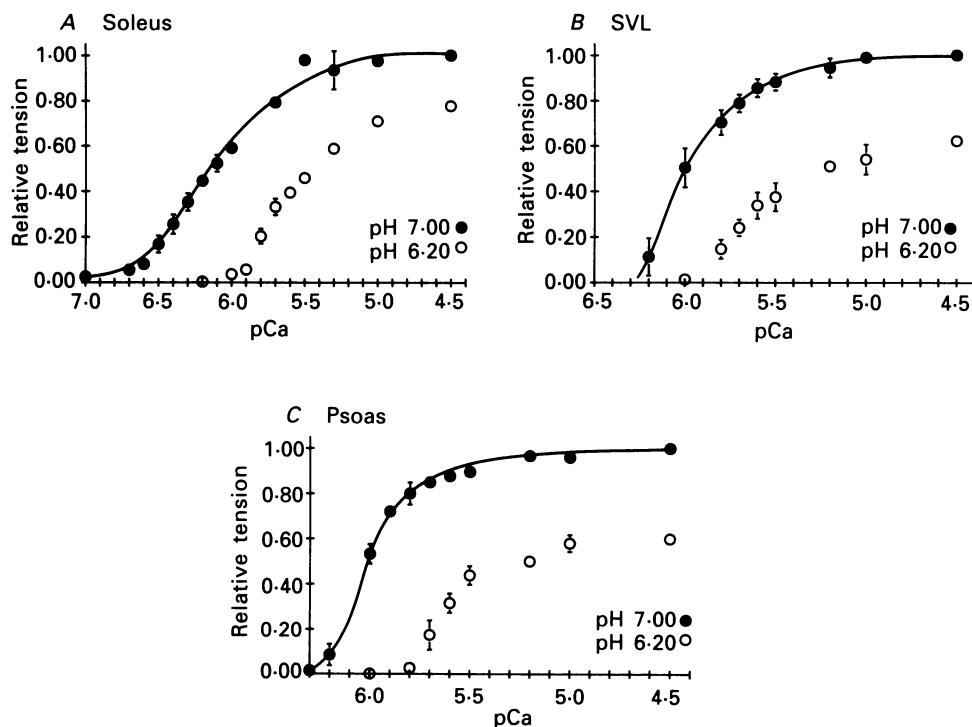

Fig. 4. Summary of the effects of pH upon the tension–pCa relationships in soleus (A), SVL (B) and psoas (C) fibres. Values are means±standard error of the mean; n is given in Table 1. In some instances error bars were smaller than the symbol. The curves were fitted to the data by eye. In all fibres at each pCa, the value at pH 7·00 was significantly greater than the value at pH 6·20.
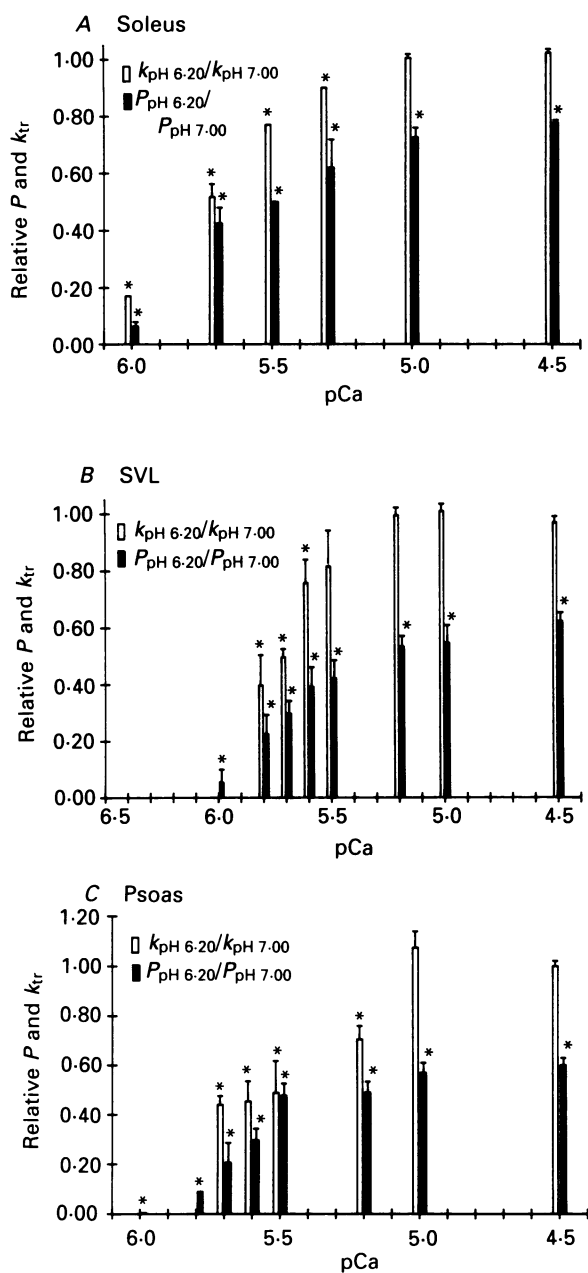
Fig. 5. Summary of the effects of pH upon tension and k_{tr} at various pCa values in soleus (A), SVL (B) and psoas (C) fibres. Values at each pCa were obtained by dividing the value at pH 6·20 by the value at pH 7·00 and are expressed as the means ± standard error of the mean. Asterisks indicate values that are significantly lower than one.

indicates a decrease in tension or k_{tr} when pH was reduced from 7·00 to 6·20. In general, results expressed this way were qualitatively similar in soleus, SVL and psoas fibres. First, at high levels of Ca^{2+} activation (pCa 5·0 or less) there was a dissociation of tension and k_{tr} in that tension was significantly depressed due to low pH while k_{tr} was unaffected. Second, as the concentration of Ca^{2+} was lowered, the effect of low pH to depress tension became more pronounced. Also evident at submaximal $[Ca^{2+}]$ was an effect of low pH to depress k_{tr}.

<div align="center">DISCUSSION</div>

Basis of Ca^{2+} sensitivity of k_{tr}

The mechanism by which pH modulates the Ca^{2+} sensitivity of the rate constant of tension redevelopment (k_{tr}) can be discussed in terms of a kinetic model of actomyosin interaction (Fig. 6). The reaction scheme is derived from current models of the ATP hydrolysis pathway in skeletal muscle (Taylor, 1979; Sleep & Smith, 1981; Eisenberg & Hill, 1985; Goldman & Brenner, 1987; Hibberd & Trentham, 1986). The rate constant of tension redevelopment, k_{tr}, is a manifestation of the step in the reaction pathway which is rate limiting in terms of the cross-bridge transition to the force-bearing state. Following the release and restretch of fibre length during contraction, which results in a drop of tension to zero or very nearly zero, cross-bridges are detached from actin binding sites, and predominantly populate the M–ATP and M–ADP–P_i states. A smaller fraction of cross-bridges populate the M–ADP state (not shown) based on the low rate of P_i ejection when cross-bridges are dissociated from actin (Taylor, 1979; Hibberd & Trentham, 1986). Thus, the rate of rise of tension following the mechanical perturbation reflects the rate-limiting step governing the cross-bridge transition from the detached (M–ATP, M–ADP–P_i) and the weakly bound, low-force states (AM–ATP, AM–ADP–P_i) to the strongly bound, high-force states (AM′–ADP, AM–ADP, AM).

Previous studies have demonstrated that increases in the concentration of P_i depress isometric tension (Rüegg, 1971), increase the rate constant of tension development (Burton & Sleep, 1988), and increase the rate of decay of rigor tension following photolysis of caged ATP (Hibberd, Dantzig, Trentham & Goldman, 1985). These results suggest that: (1) the P_i-release step (Fig. 6, step 5) is coupled to the force-producing step and (2) the AM′–ADP state may be the dominant force-bearing cross-bridge state in an isometrically contracting fibre. Brenner (1987) has shown that stiffness and tension increase with similar time courses following the length release and restretch protocol. These findings strengthen the hypothesis that reaction step 5 in the hydrolysis scheme, i.e. the weak to strong binding transition, is limiting in terms of k_{tr}. Alternatively, the rate-limiting step may occur prior to the P_i-release step. For example, a proposed AM–ADP–P_i isomerization step (Homsher & Millar, 1989) which is not included in the scheme in Fig. 6, may be rate limiting in the transition to the strongly bound, force-bearing state.

In the scheme in Fig. 6, in which all the constituent reactions are reversible, the value of k_{tr} is the arithmetic sum of the forward and reverse rate constants for the rate-limiting steps governing the formation and subsequent dissociation of the dominant force-bearing cross-bridge state. Thus, k_{tr} may be defined by the sum of the apparent rate constants k_{+5}, k_{-5} and possible contributions of k_{+6}, k_{-6}, k_{+7} and k_{-7} (+

and − refer to the forward and reverse reactions, respectively). This definition of k_{tr} is only an approximation since the reaction steps which limit the formation and dissociation of the force-bearing state are currently being debated.
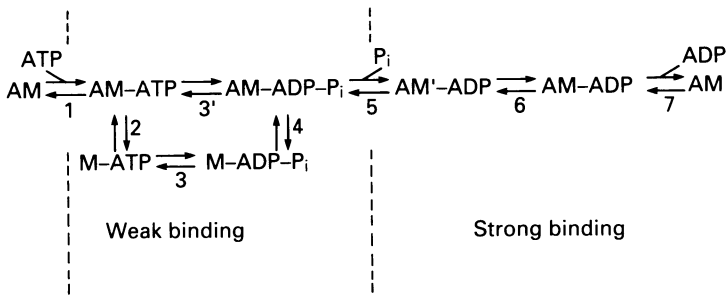

Fig. 6. Schematic model of the kinetics of the actomyosin ATP hydrolysis reaction during contraction in skeletal muscle, where A is actin and M is heavy meromyosin or myosin S1. The scheme is adapted from current models of ATP hydrolysis (Taylor, 1979; Sleep & Smith, 1981; Eisenberg & Hill, 1985; Hibberd & Trentham, 1986; Goldman & Brenner, 1987).

Earlier studies examining the Ca^{2+} sensitivity of k_{tr} provide evidence that the formation of the strongly bound, high-force state, rather than the subsequent dissociation of bound cross-bridges, is the Ca^{2+}-sensitive step (Brenner, 1988). If this view is correct, the Ca^{2+} sensitivity of k_{tr} is likely to be due to an effect of Ca^{2+} on the apparent rate constants k_{+5} and/or k_{-5}. In an attempt to sort out these possibilities the following should be considered. (1) The sum $(k_{+5} + k_{-5})$ increases with increases in the concentration of Ca^{2+} and (2) the ratio $k_{+5}/(k_{+5} + k_{-5})$, a primary determinant of the proportion of cross-bridges in the AM′–ADP state, also increases with Ca^{2+} concentration. Thus, the simplest model that explains the Ca^{2+} sensitivity of k_{tr} is that Ca^{2+} has a direct effect upon the forward apparent rate constant k_{+5}, such that k_{+5} increases with the concentration of Ca^{2+}. However, another interpretation consistent with our results would be that a transition or isomerization step prior to phosphate release is Ca^{2+} sensitive.

Probing the Ca^{2+} sensitivity of k_{tr} by altering pH

In the present study, during maximal Ca^{2+} activation, k_{tr} was unaffected by decreasing pH from 7·00 to 6·20, while isometric tension was depressed at low pH. During submaximal Ca^{2+} activations, both k_{tr} and tension were markedly depressed by reducing pH to 6·20. A possible interpretation of these findings, in keeping with the earlier discussion, is that pH has a modulatory effect upon the sensitivity of k_{+5} to Ca^{2+}. Based on our results reduced pH decreases the Ca^{2+} sensitivity of k_{+5} without influencing the maximum value of k_{+5}. Thus, the apparent rate constant k_{+5} is sensitive to pH at concentrations of Ca^{2+} less than about 10^{-5} M. The depression of force at maximal levels of Ca^{2+} activation appears in part to involve a direct effect of low pH to reduce the force produced by a strongly bound, force-bearing cross-bridge (preceding paper).

If pH modulates the Ca^{2+} sensitivity of an apparent rate constant involved in step 5, it would be expected that following a decrease in pH the new steady-state

distribution of cross-bridges would show an increase in the proportion in the AM–ADP–P_i state and a concomitant decrease in the proportion in the AM′–ADP state. The expectation which follows from such a redistribution of cross-bridge states (i.e. a decrease in the proportion of strongly bound cross-bridges) would be that the stiffness–pCa relationship would be depressed at low pH, a result that is consistent with experimental observations (preceding paper).

Modulation of the P_i release step by pH

Considering that P_i has profound effects on tension and the rate of tension development, one possibility is that the effects of pH presented here are mediated via changes in the protonation of P_i. It has been suggested that the effect of P_i on contractile function may be dependent upon the species of P_i; the diprotonated form of P_i, rather than the monoprotonated form, has been proposed as the biologically active species (Nosek, Fender & Godt, 1987) and the proportions of these two species of P_i depend upon pH. However, subsequent studies provide evidence that force production is critically dependent upon the total concentration of P_i without regard to the species of P_i (Chase & Kushmerick, 1988; Cooke, Franks, Luciani & Pate, 1988). Due to impurities of chemicals in our relaxing and activating solutions it is likely that the concentration of P_i is 0·2–0·5 mM (Pate & Cooke, 1989), a concentration likely to be too low to markedly affect contractile function. Thus, the effects of pH on k_{tr} and isometric tension reported here are independent of any effects of pH on the species of P_i.

Mechanism of rate constant modulation by H^+: mass action or a direct effect

It is known that protons are released during the hydrolysis of ATP by actomyosin in solution, and Chock (1979) provided evidence that H^+ is released at the P_i-release step. At pH 8, one mole of protons is released locally (i.e. prior to buffering by the creatine phosphate–creatine phosphokinase system) per mole of ATP hydrolysed (Taylor, 1979); however, at pH 6·4 the stoichiometry of the reaction is calculated to be 0·03 mol H^+/mol ATP (Hultman & Sahlin, 1980). Thus, at low pH it appears unlikely that the phosphate-release step should be affected by protons via mass action. Additionally, if protons were reversing the phosphate-release step by mass action, force would decline due to a reduction in the proportion of cross-bridges in the AM′–ADP state. Correspondingly, the observed reverse rate constant could be considered pseudo-first order (i.e. $k_{-5} \times [H^+]$) and would therefore increase at low pH. However, our findings showed that k_{tr} declined at low pH. Thus, the effects of H^+ are unlikely to involve a bimolecular effect on the reverse rate constant in reaction 5. Our results obtained at submaximal concentrations of Ca^{2+} showed a depression of tension and k_{tr} at low pH. The simplest explanation for these results is that protons have a direct, depressant effect upon the forward apparent rate constant of reaction 5 in the scheme shown in Fig. 6. This is different from the mechanism that has been hypothesized for effects due to P_i. To account for the effect of P_i to depress steady isometric tension and to increase the rate of relaxation of rigor tension following photolysis of caged ATP, it has been proposed that the observed reverse rate constant is pseudo-first order, i.e. $k_{-5} \times [P_i]$, and increases with increases in the concentration of P_i (Hibberd et al. 1985).

Physiological relevance of an effect of pH upon k_{tr}

The effect of pH on the Ca^{2+} sensitivity of the rate constant governing the transition from the weak to strong binding states could account in part for the reduction in the Ca^{2+} sensitivity of isometric tension at low pH.

Further, our findings may provide a basis for the long-standing observation that the rate of rise of tension is depressed during fatiguing stimulation of skeletal muscle (Marey, 1868). It is well known that during sufficiently intense muscular contraction, intracellular pH declines from a resting value of about 7·00 to values as low as 6·20 (Dawson, Gadian & Wilkie, 1978; Metzger & Fitts, 1987). Our results indicate an effect of low pH to reduce the Ca^{2+} sensitivity of k_{tr}. The pCa values at which k_{tr} was most affected by low pH are compatible with the range of Ca^{2+} concentrations observed during an isometric twitch and tetanus (Blinks, Rüdel & Taylor, 1978).

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