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MECHANICAL PROPERTIES OF SKINNED RABBIT PSOAS AND SOLEUS MUSCLE FIBRES DURING LENGTHENING: EFFECTS OF PHOSPHATE AND Ca^{2+}

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SUMMARY

1. Mechanical properties of permeabilized single fibres from rabbit psoas and soleus muscle were determined by measuring the length responses due to abrupt changes in load and the force responses due to isovelocity length changes at different phosphate and Ca^{2+} concentrations.

2. The length responses due to abrupt increases in load from psoas fibres showed a rapid lengthening during the change in load followed by a phase of lengthening during which the velocity gradually decreased. In soleus fibres an abrupt lengthening during the change in load was followed by a phase of lengthening during which the velocity remained constant or decreased slightly for increases in load to less than 1·45 of the isometric force (F_0). For larger increases in load the velocity during this later phase first increased and thereafter decreased.

3. The initial force–velocity curve, derived from the early part of the isotonic responses after the change in load, as well as the late force–velocity curve derived from the force level attained during isovelocity length changes, were sensitive to phosphate. Phosphate caused a shift of the absolute force–velocity curves of both psoas and soleus fibres towards lower values of force. In psoas fibres, the relative force–velocity curves derived by normalization of the force level to the force developed isometrically was shifted by phosphate to smaller velocities. In soleus fibres, the initial velocity at low and intermediate relative loads ($< 1{\cdot}75\,F_0$) was increased by phosphate but at higher loads it decreased, while the late force–velocity curve showed an overall decrease in velocity.

4. The force responses during isovelocity lengthening of psoas fibres showed an early rapid increase in force followed by a slow rise in force. The position of this break point in force was sensitive to the phosphate concentration. In soleus fibres, the force responses without phosphate showed an overshoot followed by a slow rise in force. The overshoot diminished with increasing phosphate concentration.

5. Phosphate and Ca^{2+} affected the force responses in psoas and soleus fibres in different ways. When the isometric starting levels were the same, force during and after the length change at submaximal activation was always less than at maximal activation in the presence of 15 mM-phosphate.

6. The changes in the mechanical performance during lengthening caused by phosphate in psoas as well as in soleus fibres, are in agreement with a decrease in the average force per attached crossbridge. The results are compatible with a crossbridge model in which phosphate causes a shift of attached crossbridges from a high-force-producing state to a low- or non-force-producing state. However, a decrease in the number of attached crossbridges might contribute to the kinetics of the phosphate effects.

INTRODUCTION

In recent years considerable information has become available on the correlation between the mechanical and biochemical properties of the contractile mechanism in skeletal muscle (e.g. Eisenberg, Hill & Chen, 1980; Hibberd & Trentham, 1986; Pate & Cooke, 1989). This information, however, has been obtained mainly from fast muscle, and the mechanical data were mainly derived during and after shortening.

The release of phosphate is considered to play a key role in the force-generating process because it is likely to be associated with the power stroke of the crossbridge (e.g. Hibberd, Dantzig, Trentham & Goldman, 1985). Phosphate reduces isometric force (e.g. Nosek, Fender & Godt, 1987) but its effect on the rate of ATP splitting (Kawai, Güth, Winnekes, Haist & Rüegg, 1987) and on the shortening velocity at zero load (Cooke & Pate, 1985) is rather small.

This paper examines the mechanical properties of single skinned (fast) rabbit psoas and (slow) soleus muscle fibres by studying the length responses due to abrupt isotonic changes in load as well as the force responses due to changes in length at constant velocity. We focus in particular on the effect of phosphate on the mechanical properties during lengthening. This is of interest from a theoretical point of view because so far most modelling efforts have concentrated on the effects due to shortening, but it is also of practical relevance in view of the changes of muscle performance during eccentric contractions when the intracellular phosphate concentration is increased, as is for instance the case during fatigue.

The mechanical properties during lengthening have been studied previously in intact single (fast) muscle fibres of the frog (e.g. Edman, Elzinga & Noble, 1978, 1979, 1981; Flitney & Hirst, 1978; Sugi & Tsuchiya, 1981; Lombardi & Piazzesi, 1990) and in cardiac preparations (Brutsaert & Housmans, 1977). The mechanical responses of slow muscle during lengthening were studied on whole tetanically stimulated cat soleus muscles (Joyce, Rack & Westbury, 1969; Joyce & Rack, 1969). By using skinned fibres, however, we were able to investigate the properties of the contractile machinery underlying the effects of lengthening in mammalian muscle fibres of different types under different controlled 'intracellular' conditions.

The crossbridge model of Eisenberg *et al.* (1980) and related developments (Hibberd & Trentham, 1986; Bowater, Webb & Ferenczi, 1989; Pate & Cooke, 1989) are based upon a number of biochemically distinct states and take the distribution of crossbridge distortion within each state into account. The experimental results presented here will be discussed within such a framework. In order to distinguish between the effects of phosphate on the number of attached crossbridges and on the shape of the distribution of crossbridge strain within each state, the mechanical

properties were also studied at submaximal Ca^{2+} concentrations. These results will be compared with the results at higher phosphate concentrations.

A preliminary account of part of this work has been given (Elzinga, Stienen & Versteeg, 1989; Versteeg, Stienen, Papp & Elzinga, 1990).

METHODS

Fibre bundles of about 2 mm in diameter and 2–3 cm long were obtained from psoas and soleus muscles of adult New Zealand White rabbits (*ca* 3 kg) which were stunned and killed by bleeding. The bundles were tied to glass rods and chemically skinned according to the procedure described by Goldman, Hibberd & Trentham (1984). The fibres were stored, before use, at −18 °C for up to 2 months in a solution containing 2·5 mM-ATP, 2·5 mM-$MgCl_2$, 5 mM-EGTA, 10 mM-imidazole (pH = 7·0), 170 mM-potassium propionate, 5 mM-sodium azide and 50 % (v/v) glycerol. Single-fibre segments (2·0–5·1 mm in length) were separated from the bundles under the storage solution and transferred, adhering to a glass rod, into a dish containing relaxing solution (Table 1) at about 15 °C. Dissection of the soleus fibres was complicated by the rather dense network of connective tissue surrounding the fibres. Satisfactory results were obtained when fibres, damaged during the isolation of the bundle, were removed to permit a clear view of the remaining fibres over their entire length. Subsequently, a small bundle of about twenty fibres was cut transversely near one tendon and also at a distance of 6–8 mm. A few fibres were torn out of this bundle. In most cases these fibres needed to be stretched considerably, but their removal made it possible for the next fibres to be taken out rather easily. These latter fibres were used in the experiments.

The composition of the relaxing solution and of the other solutions used during the experiments is shown in Table 1. The composition was calculated with a computer program, using the equilibrium constants given by Godt & Lindley (1982). Solutions with a lower concentration of calcium were obtained by appropriate mixing of the relaxing and activating solutions assuming an apparent stability constant for the Ca–EGTA complex of $10^{6·62}$.

The fibre segments were mounted between a thin glass rod extending an Akers AM 801 force transducer element (natural frequency 2 kHz) and a displacement servo-system by means of aluminium T-clips, as described by Goldman & Simmons (1984). The fibres could be incubated in different solutions by changing troughs. The temperature was kept at 15 ± 1 °C. Sarcomere length, measured in relaxing solution by laser diffraction, was adjusted to 2·3–2·4 μm.

During the measurements, the segments were incubated in the relaxing solution for 4 min, in the pre-activating solution for 4 min, in the activating solution until a steady force level was attained, and then returned to relaxing solution.

Two kinds of experiments were carried out. First, we studied the effects of phosphate and Ca^{2+} during isotonic load changes (= load clamp measurements). After force in the activating solution had reached its maximum isometric level (F_0), the displacement response to a change in load was recorded. In subsequent activation–relaxation cycles, different relative loads of 0·5, 0·9, 1·25, 1·45, 1·65 or 1·85 of F_0 were applied by using a force normalizer circuit similar to that described by Ferenczi, Goldman & Simmons (1984). In these experiments, the fibre contracted isotonically until an adjustable, pre-set length was reached. From then onwards, the fibre contracted isometrically at its new length. The fibre was reset to its initial length after the measurement while in relaxing solution. In a second set of experiments, we examined force responses to a change in length (= length clamp measurements). During isometric contraction in the activating solution the fibre was shortened or stretched by 10 % of its initial length at velocities of 0·1, 0·25, 0·5 and 1·0 L_0/s during lengthening and 0·25 and 0·5 L_0/s during shortening. Occasionally lengthening velocities of up to 3 L_0/s were imposed. The results obtained were compatible with those at lower velocities but the fibres deteriorated rapidly. Therefore these higher velocities were not routinely used.

In both kinds of experiment the measurements began at random either with a shortening or a lengthening contraction in an activating solution without phosphate (control). Immediately thereafter the same load or length change was repeated in the presence of 15 mM-phosphate. In general, at least twelve activation–relaxation cycles could be repeated before the isometric force during a control activation was less than 80 % of the force during the first activation. In all cases the experiments were terminated when control force was less than 80 %.

Fibre diameter and length were measured by means of a dissection microscope at a magnification

of respectively 50 and 20 times. Force and lengths signals were recorded with a pen recorder, a digital oscilloscope (Gould 1425), and a computer (Olivetti M24) after A–D conversion (Labmaster) at a sampling rate of 1 kHz.

The velocity after a change in load was derived from the initial slope of the displacement signal and expressed in segment lengths per second, referred to a sarcomere length of 2·4 μm. The initial slope was determined as soon as a constant force level was established, by fitting the early part of

TABLE 1. Compositions of solutions (mM)

Solution name...	$MgCl_2$	Na_2ATP	EGTA	HDTA	CaEGTA	KP
Relaxing	6·92	5·57	20	—	—	41·94
Without phosphate						
Pre-activating	6·54	5·57	0·5	19·5	—	42·72
Activating	6·39	5·67	—	—	20	42·60
With phosphate						
Pre-activating	7·25	5·55	0·5	19·5	—	8·67
Activating	7·10	5·65	—	—	20	8·55

All solutions contained 100 mM-TES, 20 mM-creatine phosphate and 1 mg/ml creatine kinase (350 U/mg at 25 °C, Boehringer). Solutions containing 15 mM-phosphate were obtained by adding KH_2PO_4. Potassium propionate (KP) was added to adjust ionic strength to 200 mM. Solutions with lower calcium concentration were made by mixing activating and relaxing solutions. CaEGTA was made by dissolving equimolar amounts of $CaCO_3$ and EGTA. The pH was adjusted to 7·0 at 15 °C with KOH.

the response with a single exponential. The time derivative of this exponential at the moment at which the change in load occurred was taken as the initial slope. In most cases the analysis was carried out over a period of 100 ms starting at 10 ms after change in load. In some cases however, especially at low load changes in the presence of phosphate, a longer period was used of about 400 ms and the starting point was taken at maximal 60 ms. This was necessary in view of oscillations in the displacement signal.

In psoas fibres under length clamp conditions, the force responses during the length changes showed a break point: a distinct initial rapid increase followed by a slow rise in force. The amplitude of the length change and the force level at this break point were determined at the point of intersection of the initial and final tangents of the force responses during lengthening (cf. Fig. 4). In general, these tangents were drawn by eye. In some cases as a test of this procedure the break point was determined by fitting a double exponential curve to the force signals and calculating the initial and final tangents from the curve obtained. This method yielded similar results. Correction for the changes in passive force was carried out in psoas as well as in soleus fibres by subtracting the relatively small force responses obtained in relaxing solution from the responses in activating solution. The soleus fibres showed an overshoot during lengthening. In these fibres, the break point was also defined by the intersection of the initial and final tangents of the corrected force response. In some of the soleus fibres the overshoot in force was absent. In those fibres the rate of the force change decreased monotonically during the lengthening phase, and the determination of the break point did not seem to be useful.

An estimate for the steady-state force–velocity relationship was derived from the final force level reached during the length changes. These force responses were corrected for the changes in passive force. Since the amplitude of the length changes was kept constant, the time at which this force level was determined varied. Total duration of the phase of the isovelocity change in length was always larger than 100 ms.

Differences between mean values were statistically tested by means of Student's t test at a 0·05 level of significance ($P < 0·05$).

RESULTS

Force development in psoas and soleus fibres

The average isometric force (±S.E.M.) at full activation was 151 ± 10 kN/m^2 ($n = 22$) for psoas fibres and 147 ± 10 kN/m^2 ($n = 21$) for fibres from soleus. In the

presence of 15 mM-phosphate, the average force (±S.E.M.) for psoas ($n = 22$) and soleus ($n = 43$) was 58±2 and 78±1% of the force in the control situation, respectively.

Effects of phosphate in load clamp measurements

Recordings of a load clamp experiment on a psoas fibre are shown in Fig. 1. The results in columns *A* and *B* were obtained in the absence and presence of 15 mM-phosphate, respectively. In each row the results at the same relative load changes are shown. In the fourth row the length responses to isotonic releases are shown. The (elastic) shortening at the moment of release is followed by a length change at nearly constant velocity. The third row shows lengthening responses for a small increase in load in which an abrupt increase in length is followed by a lengthening at nearly constant velocity. However, at larger increases in loads (rows 1 and 2), the length trace following the early quick response was curved and the velocity of lengthening decreased with time. The curvature of the length responses with and without phosphate was rather similar, but the initial velocity of the isotonic lengthening was reduced in the presence of phosphate.

In psoas fibres small damped length oscillations were sometimes visible after small changes in load. These oscillations had a 10–16 Hz frequency and were reminiscent of the oscillations observed in single frog muscle fibres (Armstrong, Huxley & Julian, 1966; Sugi & Tsuchiya, 1981). In our experiments, however, the amplitude of the oscillation increased when the low-frequency gain of the feedback system was decreased. This indicates that special precautions are required for further investigation of such phenomena.

Recordings of load clamp experiments with and without 15 mM-phosphate from a soleus fibre are shown in Fig. 2. In these experiments also, an early quick lengthening or shortening was observed, followed by a slow and more or less curved change in length until a pre-set limit in length was reached. Thereafter the fibre contracted isometrically at its new length. For the two highest load steps (rows 1 and 2), in the absence of phosphate the velocity was, in contrast to the results from psoas fibres, increasing. In other experiments in which lengthening was allowed over a longer range, it appeared that the velocity after some time decreased, resulting in an S-shaped length tracing. In the presence of phosphate a small but distinct transient increase in velocity occurred immediately after the quick lengthening at higher loads (rows 1 and 2).

In soleus fibres at low load changes, the reduction of the initial velocity by adding phosphate was absent. Only the highest load change (row 1) showed a decrease in velocity due to phosphate. After the other changes in load, velocities were nearly the same (row 2) or increased (row 3).

In the initial force–velocity curves (Fig. 3), this comparison of the initial velocity of the isotonic responses is shown in more detail for both psoas and soleus muscle fibres. In the upper row the changes in load both in the presence and absence of phosphate have been normalized to the isometric force developed during each contraction. The results from psoas fibres indicated that the initial velocity of isotonic lengthening was reduced significantly ($P < 0{\cdot}05$) in the presence of phosphate. The initial velocity of isotonic shortening was not influenced by phosphate. In soleus fibres, phosphate increased the initial velocity of lengthening at

low and intermediate relative loads ($< 1{\cdot}75\ F_0$) but at larger loads phosphate decreased it. Both in psoas and in soleus fibres, all mean values during lengthening at high phosphate concentration were significantly different ($P < 0{\cdot}05$) from the control values. The differences during isotonic shortening were not significant. In the

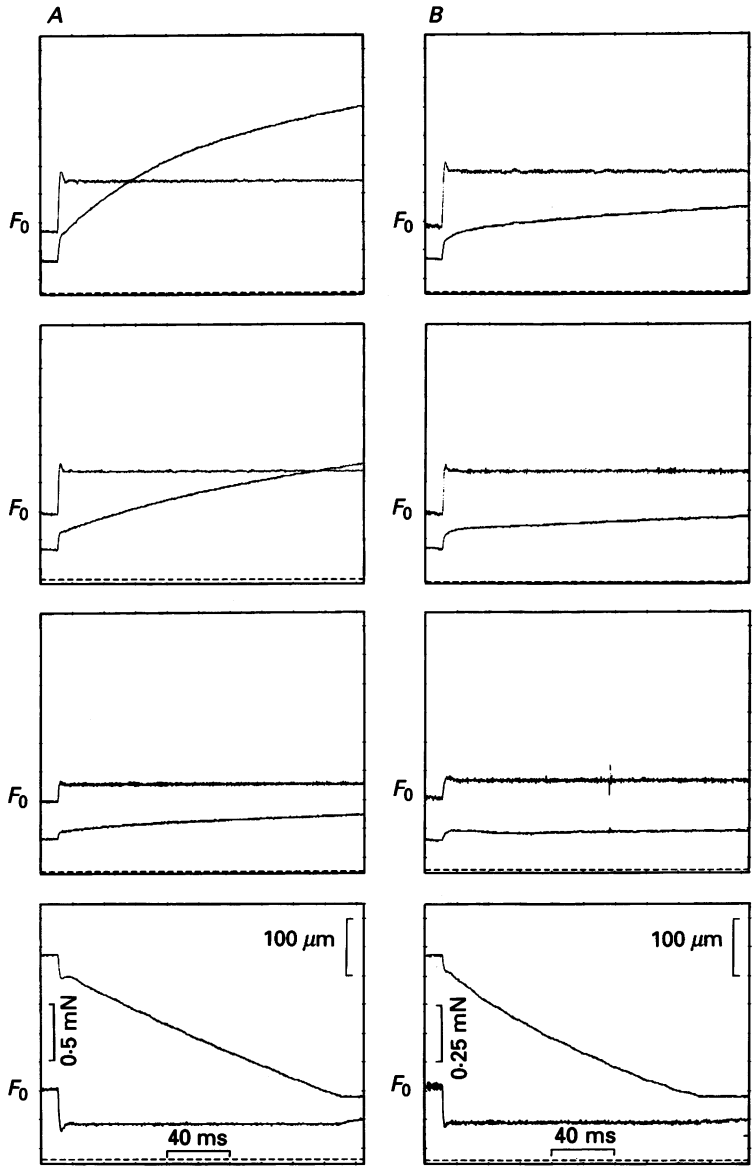

Fig. 1. Load clamp measurements in psoas fibres. Recordings of force and length from a skinned rabbit psoas fibre in the absence (*A*) and the presence (*B*) of 15 mM-inorganic phosphate. In each row the length responses are shown to different changes in load (from top to bottom: to 1·85, 1·65, 1·25 and 0·5 of the isometric force level, F_0). The interrupted lines indicate the force baselines. Note the different force scale in *A* and *B* as indicated. Fibre segment length, 3·85 mm.

lower row of Fig. 3, the changes in load are normalized to the isometric force in the absence of added phosphate. For this purpose the phosphate results are plotted after multiplying the load values of the upper row by the reduction in isometric force, i.e. by 0·58 and 0·78 for psoas and soleus, respectively. These results indicate that

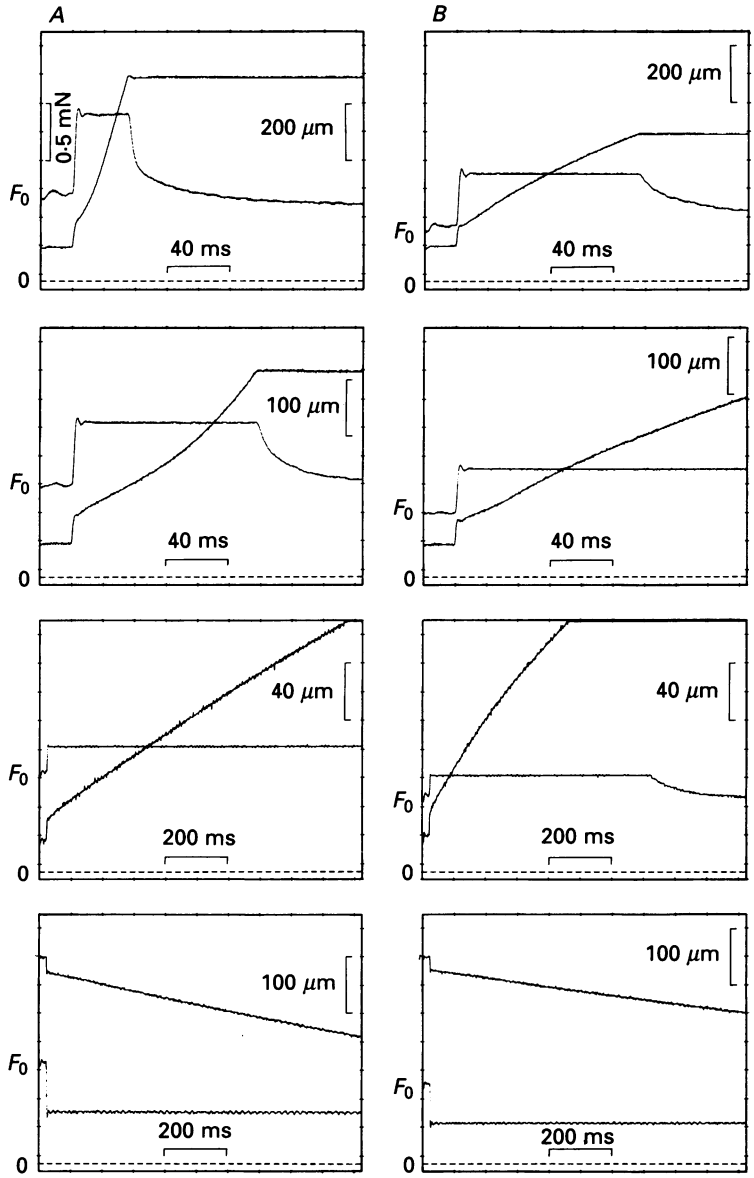

Fig. 2. Load clamp measurements in soleus fibres. Recordings of force and length from a skinned rabbit soleus fibre in the absence (*A*) and the presence (*B*) of 15 mM-inorganic phosphate. In each row the length responses are shown to different changes in load (from top to bottom: to 1·85, 1·65, 1·25 and 0·5 of the isometric force level, F_0). The interrupted lines indicate the force baselines. Fibre segment length, 4·25 mm.

phosphate caused a shift of the absolute force–velocity curves of both psoas and soleus fibres towards lower values of force, i.e. a reduction of the load-bearing capacity of the fibres.

In three psoas fibres, the phosphate sensitivity of the initial lengthening velocity was measured at 1·45 F_0. The phosphate effect was found to be half-maximal at a concentration of 5 mM.

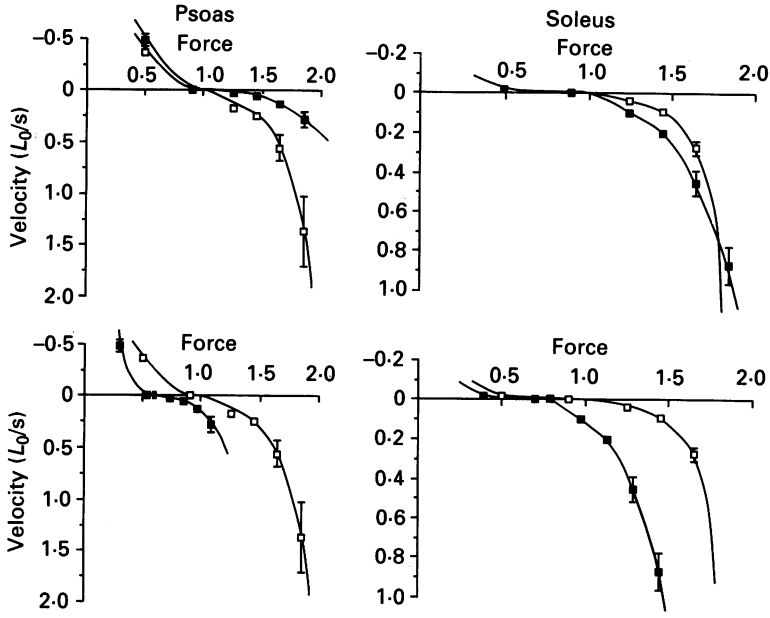

Fig. 3. Initial force–velocity relationship in psoas and soleus fibres in the absence (□) and in the presence (■) of 15 mM-inorganic phosphate. In these curves, the initial velocity of the length changes is shown as a function of the isotonic load. In the lower row, the isotonic load levels reached are normalized to the isometric force in the absence of phosphate while in the upper row they are normalized to the actually developed isometric force. The continuous lines are drawn by eye. The error bars (±S.E.M.) are shown only when they are larger than the symbols used. Number of fibres: eleven (psoas); seven (soleus). The average velocity at a relative load of 1·85 F_0 of soleus fibres in the absence of phosphate was 2·16 L_0/s. This value is out of the range shown, but it can be judged from the curve plotted through the data.

Effects of phosphate in length clamp measurements

In Figs 4 and 5, the force responses are shown, in the presence and absence of 15 mM-phosphate, to changes of 10% of the initial fibre length (L_0), carried out at different velocities in psoas and soleus fibres, respectively. The force response during lengthening in psoas fibres showed a break point: a distinct initial rapid increase followed by a slow rise in force, especially when the length changes were applied at the faster rates. As can be seen from the responses obtained in relaxing solution, part of this slow rise in force was caused by the increase in passive force during lengthening. The break points in the force responses, therefore, were determined after correction for the passive changes. In psoas fibres, the break point (±S.E.M.) in

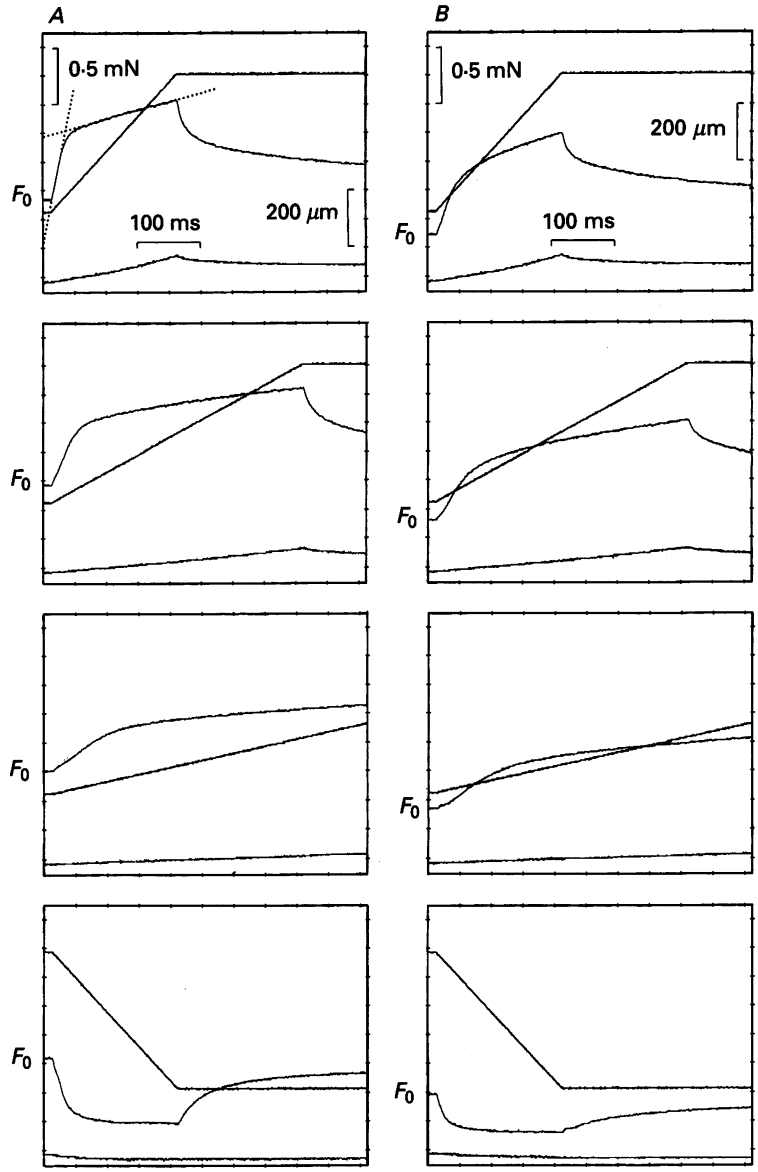

Fig. 4. Length clamp measurements in psoas fibres. Recordings of force and length from a skinned rabbit psoas fibre in the absence (*A*) and the presence (*B*) of 15 mM-inorganic phosphate. In each row the force responses are shown to length changes of 10 % of initial length ($= L_0$), carried out at different velocities (top to bottom: lengthening 0·5, 0·25, 0·1 L_0/s and shortening 0·5 L_0/s). The lower trace in each panel shows the passive force response obtained in relaxing solution. Fibre segment length, 5·1 mm.

the force response to lengthenings at 1 L_0/s occurred, in the absence and presence of added phosphate ($n = 5$), at a relative amplitude of stretch of $0{\cdot}0121 \pm 0{\cdot}0003$ of L_0 and $0{\cdot}022 \pm 0{\cdot}002$ of L_0, respectively. At 0·5 L_0/s the corresponding values, obtained

in the same fibres, were $0{\cdot}0118 \pm 0{\cdot}0006$ of L_0 ($-P_i$) and $0{\cdot}022 \pm 0{\cdot}002$ of L_0 ($+P_i$). The values with and without phosphate differ significantly ($P < 0{\cdot}05$).

In soleus fibres (Fig. 5) the break point in the force records during lengthening at 1 L_0/s was found at a relative amplitude of $0{\cdot}0158 \pm 0{\cdot}0004$ of L_0 ($n = 4$) and at

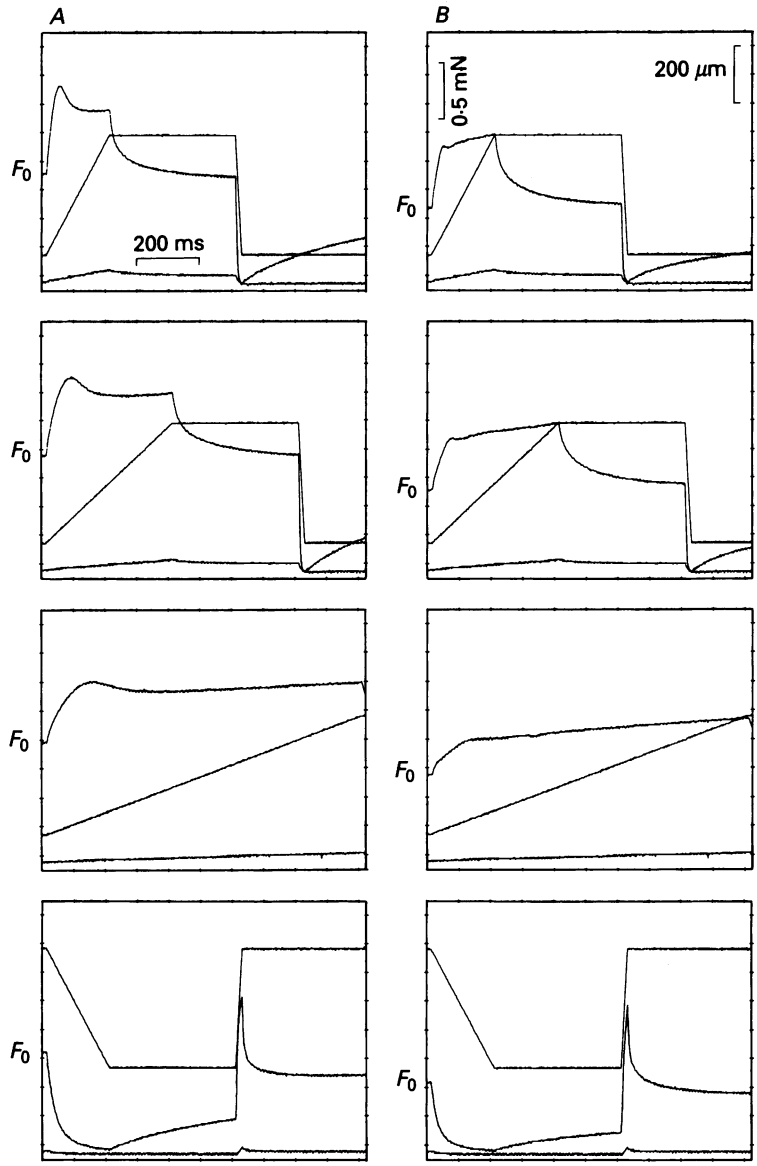

Fig. 5. Length clamp measurements in soleus fibres. Recordings of force and length from a skinned rabbit soleus fibre in the absence (*A*) and the presence (*B*) of 15 mM-inorganic phosphate. In each row the force responses are shown to length changes of 10% of initial length ($= L_0$), carried out at different velocities (top to bottom: lengthening 0·5, 0·25, 0·1 L_0/s and shortening 0·5 L_0/s). The lower trace in each panel shows the passive force response obtained in relaxing solution. Fibre segment length, 4·25 mm.

$0{\cdot}020 \pm 0{\cdot}003$ of L_0 when phosphate was added. At $0{\cdot}5\ L_0$/s the corresponding values were $0{\cdot}0125 \pm 0{\cdot}0002$ of L_0 ($-P_i$) and $0{\cdot}015 \pm 0{\cdot}002$ of L_0 ($+P_i$). In this case the difference in the values with and without was not significant.

Another difference between the results of psoas and soleus fibres is the presence in soleus fibres of an overshoot in the force responses when phosphate is absent (Figs 4 and 5, column *A*). In 23% of the soleus fibres this overshoot was absent and the rate of the force change during lengthening decreased continuously. This type of response might be typical for the fast or mixed fibres present in the soleus muscle (Ariano, Armstrong & Edgerton, 1973; Reiser, Moss, Giulian & Greaser, 1985; Staron & Pette, 1987). However, some of these fibres developed a rather low isometric force per cross-sectional area (~ 100 kN/m^2) and non-uniformity of fibre segments could also be the cause of the deviation.

Effects of phosphate on the late force–velocity curves

In Fig. 3, the relationship is shown between the change in load and the resulting initial velocity of lengthening. The force–velocity relationship is usually considered to characterize the steady-state behaviour of the fibre. The isotonic lengthening responses do not always reach a steady velocity. This could be due to the increase in passive force during lengthening which might act as an internal load, but it could also be an inherent property in skinned fibres since isotonic shortening responses in skinned fibres are also more or less curved.

An estimate for the steady-state force–velocity curve can be obtained from the length clamp experiments. Contrary to the load clamp measurements, here a correction for the passive fibre properties can be obtained by subtracting the force response obtained in relaxing solution from the response in activating solution. Figures 4 and 5, and particularly Fig. 8 in which force responses are corrected, show that a steady state is reached at the end of the length change during the control measurements at saturating Ca^{2+} concentrations without added phosphate. At high phosphate concentration force was still increasing at the end of the length change. In this case larger lengthenings may be required, but a reduction in active force due to the decrease in overlap between the myofilaments could become important.

The relationship between the velocity of the length change and the 'steady-state' force level reached at the end of the displacement is shown in Fig. 6. Here also, as in Fig. 3, the absolute load-bearing capacity is reduced by phosphate, both in psoas and in soleus fibres. The phosphate effects in soleus fibres are smaller than in psoas fibres. Some differences exist between these late force–velocity curves and the initial force–velocity curves in Fig. 3. When the initial and late relative force–velocity curves in soleus fibres are compared, it can be seen that phosphate caused in all cases a decrease in the steady-state velocity, i.e. the opposite of the effects on the initial velocity at low and intermediate relative loads.

Effects of Ca^{2+} concentration

The load and length clamp experiments were also carried out at lower Ca^{2+} concentrations where isometric force and the number of attached crossbridges is reduced. Therefore, these experiments allow us to distinguish between a direct kinetic effect of phosphate on the transition rates between the various crossbridge

states and a change in the number of attached crossbridges. The load clamp experiments were carried out at saturating (pCa = 4·5) and at a submaximal Ca^{2+} concentration corresponding to a pCa value of 6·3. At this pCa, isometric force in psoas fibres was reduced to 58% of its control value at saturating Ca^{2+}

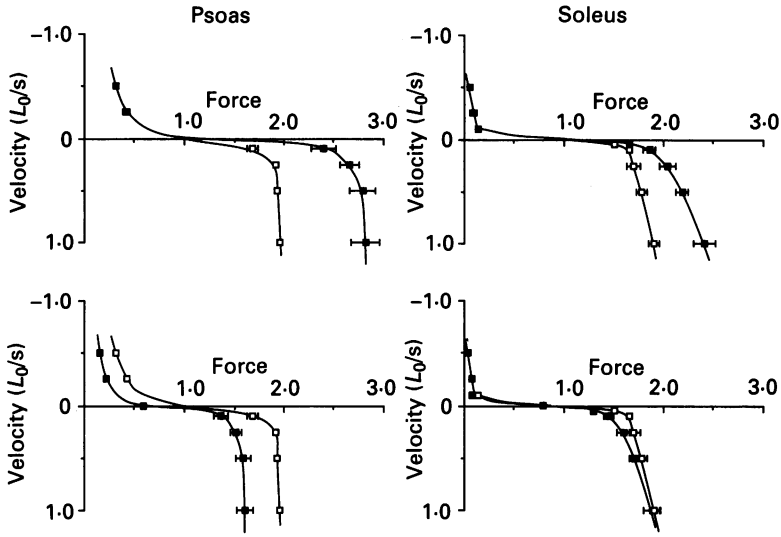

Fig. 6. Steady-state force–velocity relationship in psoas and soleus fibres in the absence (□) and presence (■) of 15 mM-inorganic phosphate. In these curves, the final force level reached at the end of the length change (10% of L_0) is shown as a function of the velocity of the change in length. In the lower row, the force levels reached are normalized to the isometric force in the absence of phosphate while in the upper row they are normalized to the actually developed isometric force. The continuous lines are drawn by eye. The error bars (±S.E.M.) are shown only when they are larger than the symbols used. Number of fibres: seven (psoas); thirty-four (soleus).

concentrations, and thus equal to the average level found at 15 mM-phosphate. The isometric force level in soleus fibres at pCa = 6·3 was reduced to 38%, which was smaller than the isometric force level at 15 mM-phosphate (78% of the control value). The length clamp experiments were carried out in a somewhat different way. In order to compare the kinetics effect of Ca^{2+} and phosphate without interference of a difference in the isometric force levels, the force responses were studied at Ca^{2+} concentrations at which the isometric force was reduced to the value in the presence of 15 mM. These experiments were performed in successive activations of the same fibre.

In Fig. 7, the length responses from a psoas fibre and a soleus fibre are shown to increases in load of 1·45 and 1·65 of the isometric force both at saturating (pCa = 4·5) and at submaximal Ca^{2+} concentrations (pCa = 6·3). It was found that in psoas fibres, at a relative load of 1·45, the initial velocity of lengthening is increased at lower Ca^{2+} concentrations. The degree of curvature of the lengthening response at lower Ca^{2+} concentration was larger than at saturating Ca^{2+} concentration. After some time, the velocity of lengthening became even smaller than the concomitant

velocity of lengthening at high Ca^{2+} concentration. At larger relative loads (e.g. 1·65 F_0), the effect of a reduction in the Ca^{2+} concentration on the initial part of the response was less pronounced, but thereafter the velocity of lengthening at low Ca^{2+} concentrations increased considerably until a certain level of extension was reached

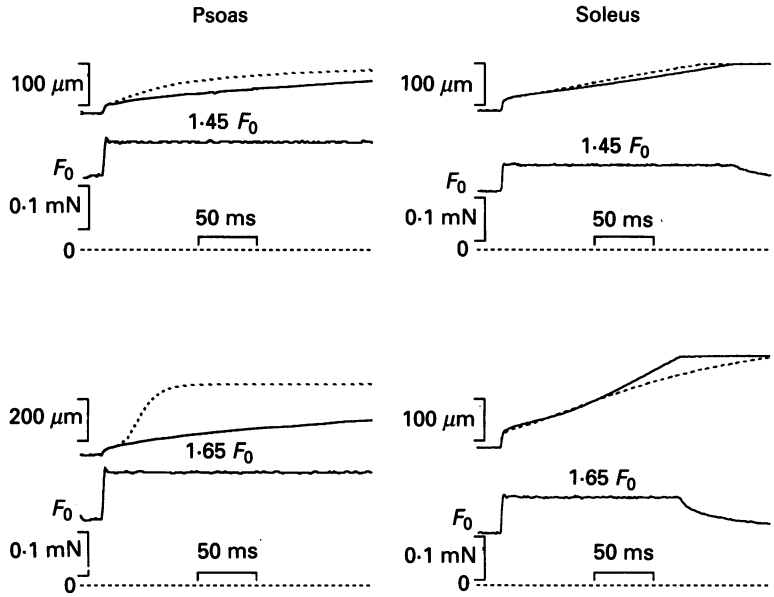

Fig. 7. Effect of Ca^{2+} in load clamp measurements. Isotonic length responses from a psoas fibre (left) and a soleus fibre (right) to a change in load of 1·45 F_0 (upper row) and 1·65 F_0 (lower row) at pCa = 6·3 (dotted length response) and at pCa = 4·5 (continuous length response). At pCa = 4·5, the fibres were fully activated. At pCa = 6·3, isometric force was 0·58 of maximal isometric force in the psoas fibre and 0·38 of maximal isometric force in the soleus fibre. For clarity only the force record at pCa = 4·5 is shown. Fibre segment length, 2·6 mm (psoas) and 3·75 mm (soleus).

at which the velocity became very small. The maximum level of extension (±S.E.M.) was 14±3% of L_0 and could be determined in three psoas fibres only. In the other experiments the limit for the isotonic lengthening was set at lower values because when relaxation of the fibre in load clamp mode was necessary, damage of the fibre and the force transducer could occur.

The overall effects of reduction in Ca^{2+} concentration are less pronounced in soleus fibres than in psoas fibres although the decrease in isometric force was larger in soleus than in psoas fibres. The initial velocity of lengthening in soleus fibres was not influenced by a reduction in Ca^{2+} concentration. At 1·45 F_0, the velocity near the end of the phase of isotonic shortening was also not changed when the Ca^{2+} concentration was reduced. At 1·65 F_0, this final velocity at pCa = 6·3 was smaller than at pCa = 4·5. It should be noted, however, that the final velocity is probably reduced by the increase in passive force during the lengthening and that this effect will be larger at larger relative loads and at low Ca^{2+} concentrations where the active force is relatively small.

In Fig. 8, the force responses of psoas and soleus fibres obtained from the length clamp experiments are shown after correction for the passive force changes. It can be seen that the responses are affected by Ca^{2+} and phosphate in different ways. Force during and after the lengthening at 15 mM-phosphate was larger than at low

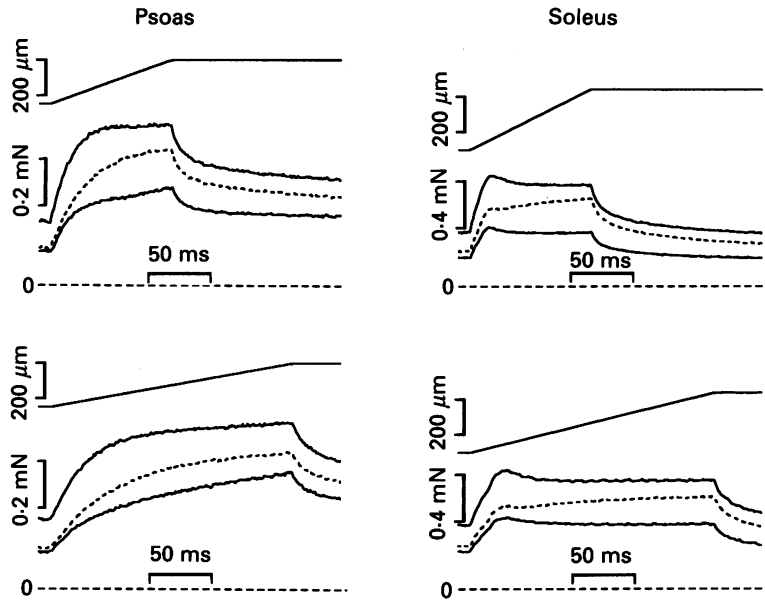

Fig. 8. Comparison of the effects of 15 mM-inorganic phosphate and of a reduction in the Ca^{2+} concentration. Velocity of the length changes: 1 L_0/s (upper row), 0·5 L_0/s (lower row). The Ca^{2+} concentration was adjusted to give an isometric force level equal to that found at 15 mM-inorganic phosphate. In each panel: upper trace length change, followed by the force response at saturating Ca^{2+} concentration, the force at 15 mM-P_i (dotted trace), the force at partial Ca^{2+} activation (pCa ~ 5·7) and the baseline in force (dashed line). All force traces were corrected for the changes in passive force, measured in relaxing solution. Fibre segment lengths, 2·55 mm (psoas) and 3·55 mm (soleus).

Ca^{2+} concentration. These results also illustrate that the increase in relative force during the length change in partly activated psoas fibres was larger than at saturating Ca^{2+} concentrations. In soleus fibres this increase in relative force was smaller. These Ca^{2+} effects were confirmed in additional length clamp experiments at lower Ca^{2+} concentrations where isometric force was about half-maximal. These experiments resulted in the late force–velocity curves shown in Fig. 9. It can be seen that at low Ca^{2+} concentration both in psoas and in soleus fibres, the relative force level attained is increased although in soleus to a lesser extent than in psoas fibres. When the force–velocity curves in Fig. 9 are compared with the phosphate results in Fig. 6, it appears that the steady-state effects of a reduction in Ca^{2+} concentration and a rise in phosphate by 15 mM are rather similar. It should be noted, however, that isometric force at these Ca^{2+} concentrations was reduced to 54% in psoas and to 59% in soleus fibres while in the presence of phosphate it was 58% in psoas fibres and 78% in soleus fibres.

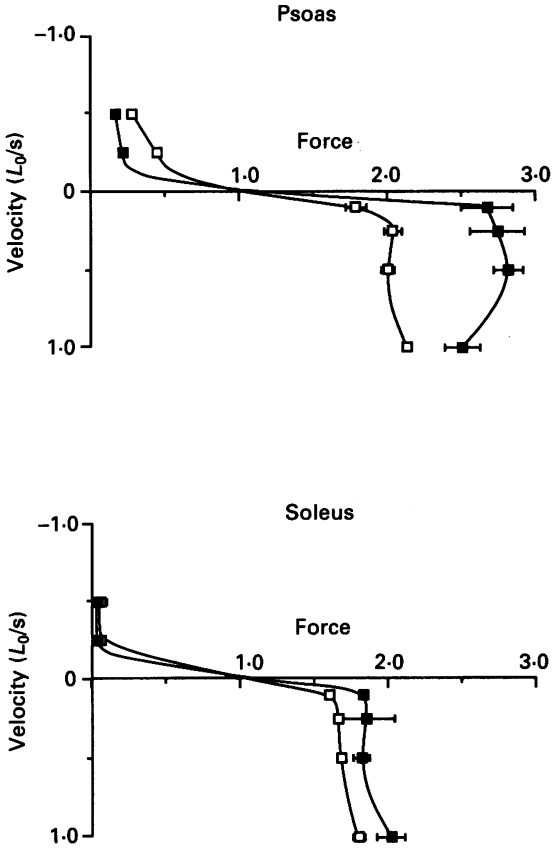

Fig. 9. Late force–velocity curves at saturating (□) and partly (■) activating Ca^{2+} concentrations at which isometric force was reduced to about 50% of the maximal isometric value. The force levels attained at the end of the isovelocity length changes were normalized to the initial isometric starting level. The continuous lines are drawn by eye. The error bars (±S.E.M.) are shown only when they are larger than the symbols used. Number of fibres: five (psoas); four (soleus).

DISCUSSION

General features of the length and force responses

The isotonic length changes, as well as the force responses to isovelocity lengthenings of skinned psoas fibres, show resemblance with the responses of living frog muscle fibres. The sudden increase in lengthening velocity at relative loads of approximately 1·85 F_0, attributed to muscle 'relaxation' (Katz, 1939), and the break point in the force response during steady lengthening are found in both preparations (Edman *et al.* 1978; Flitney & Hirst, 1978). The break points in psoas and soleus fibres ranged between 0·012 and 0·016 of L_0, corresponding to a relative sliding movement within each half-sarcomere (1·2 μm long) of 14 and 19 nm, respectively. These values are likely to be overestimated somewhat due to series elasticity present

in the attachments of the fibre ends, as discussed by Edman *et al.* (1981) in the case of intact fibres. They arrived at an estimate of 15 nm per half-sarcomere obtained in frog fibres after correction for series elasticity. In intact 'tendon-free' segments from anterior tibialis fibres of the frog, the amount of lengthening required to attain the peak value for force ranged between 10 and 14 nm per half-sarcomere (Colomo, Lombardi & Piazzesi, 1988; Lombardi & Piazzesi, 1990). This would suggest that the break point in frog fibres, determined according to our definition, under optimal conditions would be found at a smaller amount of lengthening. In general, therefore, the results derived from fast mammalian muscles seem to be compatible with, though not necessarily identical to, the results obtained on frog fibres.

It is not possible to estimate directly to what extent the responses in our experiments are influenced by non-uniformity of sarcomere length along the fibre segments. Non-uniformity might affect the determination of the break point in a way similar to end-compliance and could also cause a lower force during lengthening. In both cases the variability observed in the experiments was rather small. It seems unlikely that phosphate or Ca^{2+} would affect sarcomere uniformity or the development of non-uniformity during contraction. Therefore we do not consider the degree of uniformity to be an important factor in any of the conclusions presented.

The force responses obtained from the majority of the soleus fibres showed an overshoot which was absent in psoas fibres. The soleus muscle of rabbit consists mainly of slow (type I) fibres, which contain myosin heavy and light chains of the slow type. Some of the soleus fibres, however, contain a mixture of fast and slow myosin heavy and light chains (Reiser *et al.* 1985; Staron & Pette, 1987). The difference in shape of the force responses during isovelocity lengthening observed within soleus fibres – but also between soleus and psoas fibres – could therefore be associated with differences in myosin composition.

Difference between initial and late force–velocity curves

The initial force–velocity curves were derived from the early parts of the length responses during the load clamp experiments. They reflect crossbridge properties about 20–50 ms after a quick change in load. The steady-state crossbridge properties cannot be derived from the load clamp experiments because the increase in passive force during lengthening diminishes the velocity of lengthening. In the length clamp experiments a correction for the contribution of passive force can be made. Therefore, the late force–velocity curves derived from the final force level attained during the isovelocity lengthening can be used to provide an estimate for the late crossbridge properties. There are *a priori* no reasons to suppose that the initial and steady-state crossbridge properties should be equal. We found, however, that the effects of phosphate on the initial and steady-state absolute force–velocity curves are qualitatively the same. One difference was found for soleus fibres in the effect of phosphate after small and intermediate increases in load. In these measurements at equal *relative* load levels (upper right panels in Figs 3 and 6) phosphate induced an increase in initial velocity and a decrease in the late velocity. This difference is in agreement with the disappearance of the overshoot at larger P_i concentrations.

Calcium also affects the initial and late lengthening velocities differently when equal relative load values are compared. In psoas fibres, the initial velocity is increased (at low relative loads) or rather constant (at large relative loads) when the

Ca^{2+} concentration is reduced, while the late relative force–velocity curves show a decrease in velocity at lower Ca^{2+}. In soleus fibres, the initial velocity is hardly affected by a reduction in Ca^{2+} concentration, but the late velocity is also reduced at low Ca^{2+}. The effects of Ca^{2+} on the initial and late force–velocity curves are in agreement with the force changes observed during isovelocity lengthening. This implies that the initial force–velocity curves are of interest mainly as a quantitative description of the early parts of the load clamp experiments. The length clamp experiments are of interest as an illustration of the time dependence of the phosphate and Ca^{2+} effects and provide a quantitative estimate of the effects of Ca^{2+} and phosphate on the steady-state crossbridge properties.

Effects of phosphate and calcium on the mechanical properties

Differences exist in phosphate and calcium sensitivity of the isometric force between fast and slow fibres (e.g. Stephenson & Forrest, 1980; Chase & Kushmerick, 1988). The results presented here show that soleus fibres are less sensitive to phosphate than psoas fibres; the effects of phosphate on the isometric force and on the force–velocity relationships are less marked. This suggests that the affinity for phosphate binding is smaller in soleus fibres than in psoas fibres and provides an explanation for the difference in magnitude of the phosphate effects. However, it does not explain the kinetic effects of phosphate *per se*. Below it will be argued that many of our observations can be explained on the basis of current crossbridge models.

Recently it was found in psoas fibres that the decrease in force by phosphate was larger than the reduction in stiffness (Dantzig, Lacktis, Homsher & Goldman, 1987; Kawai *et al.* 1987). These results and the results of caged ATP experiments in the presence of phosphate (Hibberd *et al.* 1985) indicate that phosphate affects the (reversible) transition of attached crossbridges from a high-force-producing state to a non- or low-force-producing state. If, in the absence of phosphate, crossbridges are mainly present in the high-force-producing state, the distribution of attached crossbridges would become less homogeneous by phosphate. The effect of phosphate, therefore, would then be to reduce the homogeneous or synchronous action of crossbridges and to smooth the force responses to isovelocity length changes. This has been found experimentally because in psoas the break point is less sharp and the overshoot in soleus is diminished in the presence of phosphate.

The presence of the break point in the force responses *per se* implies that the detachment rate rises abruptly at a certain degree of distortion. In the presence of phosphate, the break point in the force responses occurs at a larger amplitude of stretch in psoas fibres and, although less pronounced, also in soleus fibres. This suggests that the yield point of force-generating crossbridges is reached earlier than that of non-force-producing crossbridges. This would indeed be the case if, as has been suggested previously (Hibberd *et al.* 1985), the power stroke is intimately coupled to the release of phosphate. The effects due to phosphate during isovelocity lengthening, therefore, are compatible with a change in the distribution of attached crossbridges towards a non- or low-force-producing state in which crossbridges are less strained. It is of interest to note that Bowater & Sleep (1988) determined a value of 3 mM for the apparent dissociation constant for phosphate from the active site in psoas fibres from ATP–P_i exchange measurements. The effect of phosphate on the

initial velocity of lengthening was half-maximal at about 5 mM. The agreement between the two values indicates a close coupling between the phosphate release step and the mechanical effects.

In psoas fibres, an increase in velocity *during* isotonic lengthening was observed at low Ca^{2+} concentrations and a relatively large increase in load. In soleus fibres this increase in velocity already occurred at saturating Ca^{2+} concentrations after an intermediate increase in load. These results are in agreement with the findings of Joyce *et al.* (1969) who have shown that the effect of lengthening is dependent on the rate of stimulation. In their explanation, based on the Huxley (1957) theory, it was assumed that the attachment rate depended on stimulation frequency, while the detachment rate depended on the distortion of crossbridges. At high rates of stimulation (high Ca^{2+}), the rate of attachment dominates the responses. At low rates of stimulation and high velocity, however, the detachment rate may be involved as well because the attachment rate is reduced and the detachment rate is increased. In principle this explanation is still valid and in fact is supported by the change in shape of the isotonic lengthening response of psoas fibres at low Ca^{2+}. The differences in the responses of psoas and soleus fibres at saturating Ca^{2+} concentration suggest that the difference between the apparent attachment and detachment rates is larger in psoas than in soleus fibres.

The changes in stiffness during and after lengthening (Stienen & Blangé, 1981; Colomo *et al.* 1988; Lombardi & Piazzesi, 1990) suggest a small increase in the number of attached crossbridges. These measurements were made respectively after small rapid stretches on whole frog sartorius muscle or on fast frog fibres during isovelocity lengthening. Under these conditions the increase in the apparent detachment rate, however, is insufficient to outweigh the changes in the transition rates between attached crossbridge states.

Effect of phosphate and calcium on the force–velocity curves

As has been explained previously, the differences between initial and late force–velocity curves are in agreement with observations during isovelocity lengthening. Therefore, we focus in this section only on the steady-state crossbridge properties.

At a given absolute load, the late velocity of lengthening increases with an increase in phosphate concentration. If the only effect of phosphate were to reduce the number of attached crossbridges, this decrease in the load-bearing capacity of the fibre would be explained. In that case the normalized force–velocity curves would superimpose. However, in psoas fibres this clearly is not the case. Therefore, in agreement with the effects of phosphate on the mechanical responses, the phosphate effect on the force–velocity curve in psoas fibres could be caused by a shift of crossbridges to a non- or low-force-producing (attached) state. It should be noted, however, that this shift in the crossbridge distribution could also lead, by mass action, to an increase in the number of detached crossbridges as is suggested by the stiffness measurements during isometric contraction in the presence of phosphate (Dantzig *et al.* 1987; Kawai *et al.* 1987). Our results at lower Ca^{2+} concentration, where the number of attached crossbridges is reduced, displayed a *decrease* in lengthening velocity at a given relative load, i.e. in the same direction as the effect of phosphate. Therefore, the net effect of phosphate is the result of two actions: a

kinetic effect of phosphate and a decrease in the number of attached crossbridges. In view of the difference in the early and late effects of Ca^{2+} the decrease in the number of attached crossbridges might interfere with the kinetic effects of phosphate in a rather complex and time-dependent way.

Our experimental results indicate that a Ca^{2+} concentration which reduces isometric force to 50% causes a reduction in the steady-state lengthening velocity when relative loads are compared. This effect is present in psoas and to a lesser extent also in soleus fibres. These findings are compatible with an increase in curvature of the force–velocity relation during shortening. This behaviour is in agreement with a reduction in the crossbridge attachment rate (Huxley, 1957; Simmons & Jewell, 1974, p. 123). The Ca^{2+} effect on the late force–velocity relation during *lengthening* as well as on the mechanical properties could be due to a reduction in the crossbridge attachment rate when the Ca^{2+} concentration, i.e. the degree of activation of the actin filament, is lower. Further assumptions are required, however, to describe the co-operative nature of this interaction (Bremel & Weber, 1972) and the (phosphate-sensitive) modulatory role of crossbridges already attached to the actin filament (Brandt, Cox, Kawai & Robinson, 1982; Güth & Potter, 1987; Millar & Homsher, 1990).

Differences between psoas and soleus fibres

The experiments on psoas and soleus fibres were performed under identical conditions. Therefore, it is of interest to compare the results from these fast and slow muscle fibres directly. It was observed that soleus fibres differ from psoas fibres in (1) the presence, in the majority of the fibres, of an overshoot in force during isovelocity lengthening, (2) an increase in the initial velocity at small and intermediate loads by phosphate, (3) an increase in the velocity during isotonic lengthening in fully activated fibres at intermediate and large loads, and in that (4) the reduction in isometric force, the increase in relative force during lengthening and the shift in the break point of force by phosphate are smaller.

The overshoot in force, the difference between the phosphate effects on the initial and late force–velocity curves and the increase in velocity during isotonic lengthening (1, 2 and 3) probably reflect, as discussed above, the differences in crossbridge kinetics in fast and slow fibres while the gradual differences summarized under (4) probably are related to the smaller affinity for phosphate binding in soleus as compared to psoas fibres. The dissociation constant for phosphate in solution is found to be much larger in muscle fibres (Bowater & Sleep, 1988). This implies that the biochemical equivalents of the observed differences should also be studied in isolated muscle fibres.

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