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Effects of pH on Myofibrillar ATPase Activity in Fast and Slow Skeletal Muscle Fibers of the Rabbit

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ABSTRACT In permeabilized single fibers of fast (psoas) and slow (soleus) muscle from the rabbit, the effect of pH on isometric myofibrillar ATPase activity and force was studied at 15°C, in the pH range 6.4–7.9. ATPase activity was measured photometrically by enzymatic coupling of the regeneration of ATP to the oxidation of NADH, present in the bathing solution. NADH absorbance at 340 nm was determined inside a measuring chamber. To measure ATP turnover in single soleus fibers accurately, a new measuring chamber (volume 4 μl) was developed that produced a sensitivity approximately 8 times higher than the system previously used. Under control conditions (pH 7.3), the isometric force was 136 and 115 kN/m^2 and the ATP turnover was 0.43 and 0.056 mmol per liter fiber volume per second in psoas and soleus fibers, respectively. Over the pH range studied, isometric force increased monotonically by a factor 1.7 for psoas and 1.2 for soleus fibers. In psoas the isometric ATPase activity remained constant, whereas in soleus it slightly decreased with increasing pH. The pH dependency of relative tension cost (isometric ATPase activity divided by force) was practically identical for psoas and soleus fibers. In both cases it decreased by about a factor 0.57 as pH increased from 6.4 to 7.9. The implications of these findings are discussed in terms of cross-bridge kinetics. For both fiber types, estimates of the reaction rates and the distribution of cross-bridges and of their pH dependencies were obtained. A remarkable similarity was found between fast- and slow-twitch fibers in the effects of pH on the reaction rate constants.

INTRODUCTION

A decline in intracellular pH plays an important role in fatigue and hypoxia, because it causes a reduction in the force-generating capacity, in the maximum shortening velocity, and in the rate of relaxation (Dawson et al., 1978; Edman and Matiazzi, 1981; Renaud et al., 1986). These changes are observed in fast muscle and, to a lesser extent, in slow muscle. Studies on skinned fibers, of which the sarcolemma is removed or made permeable, yielded quantitative information regarding the pH dependency of force development, maximum shortening velocity, stiffness, and the rate of tension redevelopment (Donaldson and Hermansen, 1978; Fabiato and Fabiato, 1978; Robertson and Kerrick, 1979; Chase and Kushmerick, 1988; Cooke et al., 1988; Godt and Nosek, 1989; Metzger and Moss, 1990a, b; Seow and Ford, 1993). However, much less is known about the influence of metabolic changes on energy utilization. Previous studies on the effects of pH on ATPase activity in fast-twitch fibers gave contradictory results (Curtin et al., 1988; Kentish and Nayler, 1979; Godt and Kentish, 1989; Cooke et al., 1988), whereas in slow-twitch muscle fibers no data are available yet.

Energy turnover as well as force production result from the cyclic actomyosin (cross-bridge) interaction. Within the cross-bridge cycle, multiple sites of action of protons have been proposed. To examine and quantify the effects of changes of pH on these transitions in the cross-bridge cycle, we compared the pH dependencies of force and ATPase activity under isometric conditions in single skinned fibers from psoas (fast type IIb) and soleus (predominantly slow type I) muscle. ATPase measurements probe the rate-limiting step in the cross-bridge cycle, whereas the mechanical measurements mainly provide information concerning the fast cross-bridge transitions. Therefore, the energetic data yield quantitative information regarding the influence of pH on the cross-bridge cycle, complementary to those obtained from mechanical measurements.

Our results indicate that a decrease in pH enhances cross-bridge detachment from the force-generating state by the same proportion in fast and slow fibers and that it causes a decline of the force per attached cross-bridge. These findings are consistent with the pH-effects on the mechanical parameters.

MATERIALS AND METHODS

The experimental procedures and equipment used were as described previously (Stienen et al., 1990; Potma et al., 1994). In short, fiber bundles of about 2 mm in diameter were obtained from psoas and soleus muscles of adult New Zealand white rabbits. These bundles were stored at −18°C for up to 2 months in relaxing solution containing 50% (v/v) glycerol (Goldman et al., 1984). Single fiber segments were isolated, treated with the detergent Triton X-100 (1% v/v, for 1 h) to disrupt and to wash out remaining membrane fragments, and mounted, by means of T-clips (Goldman and Simmons, 1984), between a force transducer (AM801, SENSONOR, Horten, Norway) and a micromanipulator. The temperature was kept at 15 ± 1°C. Sarcomere length, measured in relaxing solution by means of helium-neon laser diffraction, was adjusted to 2.4 μm. During the measurements, the fibers were successively incubated in relaxing solution, pre-activating solution, and activating solution. The composition of the solutions was calculated by means of a computer program similar to that of Fabiato and Fabiato (1979) using the equilibrium constants of Godt and Lindley (1982). The relaxing solution contained 20 mM ethylene glycol-bis(β-amino-ethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA), the pre-activating solution, 0.5 mM EGTA and 19.5 mM hexan-diaminotetraacetic acid (HDTA), and the

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activating solution, 20 mM CaEGTA. In addition, the solutions contained: 100 mM *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), 1 mM free Mg^{2+}, 5 mM MgATP, 0.8 mM NADH, 10 mM phosphoenolpyruvate, 4 mg/ml pyruvate kinase (470 units/mg, Sigma Chemical Co., St. Louis, MO), 0.24 mg/ml lactate dehydrogenase (710 units/mg, Sigma), 5 mM sodium azide, 0.2 mM p^1,p^5-di(adenosine-5'-)pentaphosphate, and 10 μM oligomycin B. Potassium propionate was added to adjust ionic strength to 200 mM. The pH of the solutions was adjusted (at 15°C) with potassium hydroxide. The pH-meter was carefully calibrated using a phosphate buffer with an ionic strength of 200 mM (cf. Illingworth, 1981). By adding small amounts of Ca^{2+} from a concentrated $CaCl_2$ stock, it was verified that, for all pHs, the Ca^{2+} concentration of the activating solution was saturating, i.e., pCa < 4.6.

The ATP hydrolysis inside the fiber was stoichiometrically coupled to the breakdown of NADH through a coupled enzyme assay (cf. Glyn and Sleep, 1985). NADH breakdown was monitored photometrically via the absorption of near UV light at 340 nm. The setup that was used for the measurements on psoas fibers was described in detail previously (Potma et al., 1994). It consisted of two anodized aluminium troughs (volume 80 μl, containing relaxing and pre-activating solution, respectively) and a measuring chamber (volume 30 μl, containing activating solution). In this chamber, the ATPase activity was measured. The solution was continuously stirred via a membrane at the base of the chamber. UV light passed through the chamber beneath the fiber, and the transmitted light was monitored by two UV-enhanced photodiodes at 340 and 400 nm. The latter provided a reference signal, independent of NADH concentration. The force and absorbance signals were filtered at 1 kHz and 2.5 Hz, respectively. The data were recorded on a chart recorder and a computer at a sampling rate of 5 Hz. The volume and cross sectional area of the fiber were calculated from its length and its diameters, measured in two perpendicular directions, assuming an elliptical cross section.

In the setup used for the measurements on soleus fibers, the volume of the measuring chamber was reduced to about 4 μl, because ATPase activity for soleus fibers turned out to be so small that a more sensitive device had to be developed. The dimensions of this chamber were: length, 3 mm; width, 0.4 mm; depth, 3 mm. The activating solution inside the chamber was stirred by a syringe, which injected and aspirated 0.4 μl of solution at a rate of 10 s^{-1}. Soleus fibers were activated before being transferred into the measuring chamber, to prevent the fiber from touching the sides of the chamber.

After the first activation (pH 7.3), fiber length was readjusted. After that, sarcomere length usually remained stable throughout the experiment. Thereafter, a series of isometric activation-relaxation cycles was performed in the following order: c (control pH 7.3), pH_1, pH_2, pH_3, c, pH_4, pH_5, etc., with two or three activations between controls. The ATPase activity was obtained by linear regression analysis of the absorbance signal. Force and absorbance signals measured in relaxing solution (pCa 9) served as a baseline for the active force and ATPase activity levels. It was found that the pH of the relaxing solution influenced neither passive force nor the slope of the ATPase baseline. The experiments were stopped when the isometric force during a control activation was less than 80% of the force during the first activation. The results at different pH values were normalized to the interpolated values derived from the two nearest controls. One soleus fiber (from a total of 15) was found to have an ATP turnover (per liter fiber volume) of more than 3 times the average value. Presumably, it was of an intermediate or fast type, and therefore discarded.

To test whether 100 mM BES (pK_a 7.3) gave adequate buffering in the entire pH range studied, control experiments were carried out at pH 6.4, using 100 mM 2(*N*-morpholino)ethane sulfonic acid (MES, pK_a 6.2) and pH 7.9, using 100 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES, pK_a 7.6) (pK_a values at 15°C from Good et al., 1966).

Values are given as mean ± SEM. All statistical statements are based on two-tailed Student's t-tests ($p < 0.05$).

RESULTS

In Fig. 1, experimental recordings are shown of force development and ATPase activity from a psoas fiber and from a soleus fiber, under control conditions (pH 7.3). Force development in soleus fibers was slower than in psoas fibers. This difference may be caused by the difference in fiber type,

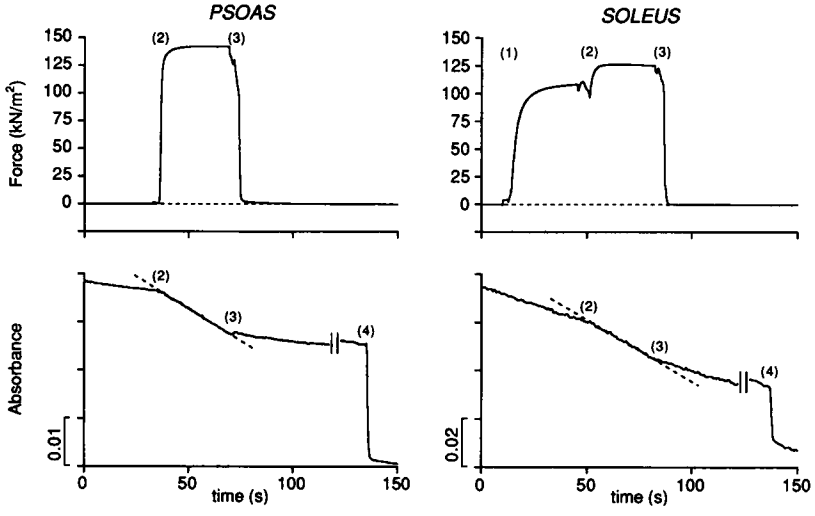

FIGURE 1 Recordings of force (*upper panels*) and ATPase activity (*lower panels*) at pH 7.3 for psoas (*A*) and soleus (*B*) fibers. Psoas fibers were directly transferred from the pre-activating solution (pCa 9) into the measuring chamber (2). Soleus fibers were first transferred from pre-activating solution into activating solution (1) and then into the measuring chamber (2), which also contained activating solution (pCa 4.5). Subsequently (3), the fibers were transferred into relaxing solution (pCa 9). At (4) 0.5 nmol ADP was injected in *A* (psoas) to calibrate the absorbance signal. In *B* (soleus) 0.1 nmol ADP was injected (4). As the fiber was immersed in activating solution, force rose rapidly until the maximum isometric level was reached. For soleus, a further increase in force occurred as the fiber entered the (well stirred) measuring chamber (2). When the fiber was transferred into relaxing solution (3), force returned to the baseline. ATPase activity followed from the change in slope of the NADH absorbance signal seen in the lower panels. After the fiber was immersed in the measuring chamber (2), the rate of NADH breakdown was accelerated (·····), i.e., the fiber hydrolyzed ATP. After removal of the fiber (3), the slope of the absorbance signal returned to its original value. The dimensions of the psoas fiber were: length 3.10 mm and diameters 60/70 μm; and of the soleus fiber: length 2.05 mm and diameters 90/120 μm.

but probably part of it is caused by the fact that the solution in which the soleus fibers were initially activated was not stirred. For soleus, a further increase in force occurred as the fiber entered the measuring chamber. This increase in force occurred because buildup of inorganic phosphate inside the fiber was reduced by stirring. Relaxation appeared equally fast for both fiber types. Even when the preparation was outside the measuring chamber, there was a steady decline in absorbance because of a contaminating ATPase present in the lactate dehydrogenase (LDH), one of the enzymes used, and as a consequence of NADH bleaching under the intense UV light. When the fiber was activated inside the measuring chamber, a faster decline in absorbance occurred as a result of the actomyosin ATPase inside the fiber. The Ca^{2+}-activated ATPase activity, obtained from the difference between the slope of the absorbance signal when the fiber was fully activated inside the measuring chamber and the slope of the baseline, was corrected for a small "basal" ATPase activity, which was measured separately when the measuring chamber contained relaxing solution (pCa 9). For psoas, on average this (pH-insensitive) basal activity amounted to 0.023 ± 0.004 mM/s, i.e., $5 \pm 1\%$ of the maximum Ca^{2+}-activated ATPase activity at pH 7.3 ($n = 30$). For soleus, the basal ATPase activity was 0.005 ± 0.002, i.e., $10 \pm 4\%$ of the maximal activity at pH 7.3 ($n = 14$). It could originate from residual membrane-bound ATPases left after the detergent treatment or, more likely, from Ca^{2+}-independent myosin ATPase activity. Cyclopiazonic acid (10 μM), a specific inhibitor of sarcoplasmic reticulum ATPase activity (Kurebayashi and Ogawa, 1991), did not influence the maximum Ca^{2+}-activated ATPase activity. This indicates that the values obtained after correction for basal activity almost exclusively represent the ATPase activity associated with the actomyosin (cross-bridge) interaction.

Under control conditions (pH 7.3), the mean ± SEM of the force per cross sectional area (F_0) and of the ATPase activity expressed per liter fiber volume (A_0) were, respectively, 136 ± 5 kN/m^2 and 0.43 ± 0.02 mM/s (psoas, $n = 21$ fibers), and 115 ± 6 kN/m^2 and 0.056 ± 0.004 mM/s (soleus, $n = 14$ fibers). Assuming a concentration of myosin heads of 0.2 mM (e.g., Glyn and Sleep, 1985), the ATPase activity, i.e., the rate of cross-bridge turnover per myosin head, corresponds to 2.1 ± 0.1 s^{-1} in psoas and 0.28 ± 0.02 s^{-1} in soleus.

The pH dependence of fully Ca^{2+}-activated isometric force and ATPase activity is shown in Fig. 2. In psoas fibers (Fig. 2 *A*), it was found that force increased almost linearly with pH. As pH was increased from 6.4 to 7.9, isometric force increased by a factor 1.7. ATP turnover was constant over the entire pH range studied. For each pH value, the average ATPase activity was not significantly different from A_0, and a linear regression line fitted through all the data points ($n = 26$) had a slope that was not significantly different from zero. In soleus fibers (Fig. 2 *B*), force also increased monotonically with increasing pH, but the increase was not as linear as it was for psoas, and it was smaller. As pH was increased from 6.4 to 7.9, isometric force increased by a factor 1.2. Although the average ATPase activity was significantly different from A_0 only for pH 7.9, there was an overall decrease of ATPase with increasing pH. A regression line fitted through the individual data points had a (negative) slope, significantly different from zero ($n = 29$), which corresponded to a decrease in ATPase activity by 20% as pH was increased from 6.4 to 7.9.

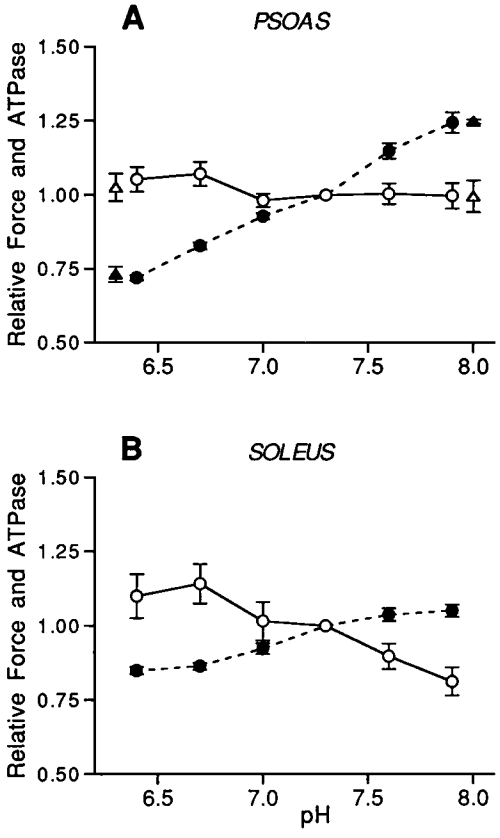

FIGURE 2 Effect of pH on isometric force and ATPase activity in psoas (*A*) and soleus (*B*) fibers. Force (●, ▲) and ATPase activity (○, △) were normalized to control values at pH 7.3. Data points represent means ± SEM. Circles represent the values found for solutions that were buffered with 100 mM BES. Number of observations: for psoas $n \geq 5$, for soleus $n \geq 6$. The triangles represent the values for the control experiments on psoas in a MES-buffered solution at pH 6.4 ($n = 5$), and in a TES buffered solution at pH 7.9 ($n = 8$). For clarity, these points are shifted by 0.1 pH unit.

In Fig. 3 isometric tension cost, ATPase activity divided by force, is shown as a function of pH for both psoas and soleus fibers. For each fiber type, tension cost is normalized on the tension cost at pH 7.3. Absolute tension cost for psoas was 6.5 times higher than for soleus. However, it is obvious that normalized tension cost shows a very similar decrease with increasing pH for both fiber types (a factor 0.57 as pH was increased from 6.4 to 7.9). Only for pH 6.4 normalized tension costs for psoas and soleus were significantly different.

The values found in psoas fibers for force and ATPase activity at pH 6.4 in the solution buffered with MES were not significantly different from those found for the BES-buffered solution. The values found for the TES-buffered solution at pH 7.9 did not differ significantly from those found for the BES-buffered solutions (cf. Fig. 2 *A*). Therefore, the buffering power of 100 mM BES appeared to be adequate for all

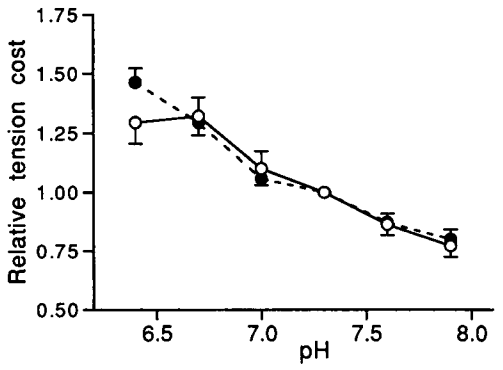

FIGURE 3 Effect of pH on isometric tension cost in psoas and soleus fibers. Tension cost for psoas (●) and soleus (○) were normalized to their respective values at pH 7.3. Data points represent means ± SEM. Number of observations as in Fig. 2. Only for pH 6.4 normalized tension costs for psoas and soleus fibers were significantly different.

pH values investigated. Because ATP turnover in soleus fibers was much smaller than in psoas fibers, the buffering power should be sufficient in soleus fibers too.

For psoas fibers, force deteriorated at alkaline pH values, as was reported before by other investigators (Robertson and Kerrick, 1979; Chase and Kushmerick, 1988). This was also the case for the ATPase activity. At pH 7.9, force and ATPase activity declined during activation by about 15% per min (an activation lasted between 20 and 30 s). Soleus fibers were very stable over the entire pH range investigated.

DISCUSSION

The major findings of this study are as follows. (1) In soleus fibers, the myofibrillar ATP turnover is a factor 7.8 lower than in psoas fibers. (2) ATP turnover in slow fibers decreases with increasing pH, and in fast fibers it remains constant. (3) Although isometric tension cost in soleus fibers is a factor 6.5 lower than in psoas fibers, the relative decrease in tension cost with increasing pH is the same in both fiber types.

Comparison with previous studies

Force and ATP turnover in fast- and slow-twitch fibers under control conditions

The values that we found under control conditions (pH 7.3) for the isometric force and ATPase activity for psoas fibers (136 ± 5 kN/m^2 and 2.1 ± 0.1 per myosin head per second, respectively) and for the isometric force for soleus fibers (115 ± 6 kN/m^2) correspond well with those previously determined by other investigators (Glyn and Sleep, 1985; Kawai et al., 1987; Cooke et al., 1988; Metzger and Moss, 1990a). Previous determinations of energy turnover in intact fibers, by heat production and measurement of metabolites, showed a difference between fast- and slow-twitch muscle similar to what we found. For instance, using heat production, Gibbs and Gibson (1972) and Wendt and Gibbs (1973) found in the rat at 27°C that 5–6 times as much initial heat is produced by the (fast-twitch) extensor digitorum longus (EDL) in the maintenance of an equivalent force as compared with the soleus. Crow and Kushmerick (1982) and Leijendekker et al. (1987) estimated actomyosin ATPase activity during tetani in fast- and slow-twitch mouse muscle at 20°C. Crow and Kushmerick (1982) reported that creatine phosphate breakdown, reflecting ATP turnover, for 1 s tetani in the EDL was threefold greater than in soleus. From the force-dependent heat development during tetani, Leijendekker et al. (1987) found that ATP turnover was about 5 times larger in EDL than in soleus.

The influence of pH on isometric force and ATPase in fast- and slow-twitch fibers

Previous determinations of the effects of pH on isometric force in frog muscle (Fabiato and Fabiato, 1978; Robertson and Kerrick, 1979) and in skinned fibers from mammalian fast-twitch (Donaldson and Hermansen, 1978; Godt and Nosek, 1989; Metzger and Moss, 1990a, b; Nosek et al., 1987; Godt and Kentish, 1989) and slow-twitch muscle (Donaldson and Hermansen, 1978; Metzger and Moss, 1990a, b) are in good agreement with our results. The small differences present may be accounted for by differences in temperature and in some cases by pH inhomogeneity within a fiber caused by inadequate buffering (cf. Chase and Kushmerick, 1988). Chase and Kushmerick (1988) determined the pH dependence of isometric force in skinned psoas and soleus fibers from rabbit in the pH range 6–8 (12°C). Quantitative agreement between their results and ours is excellent for both fiber types.

The pH dependency of isometric ATPase activity was previously determined in fast-twitch muscle fibers only, showed contrasting results, and was studied over a smaller range than shown in this study. In intact fibers of anterior tibialis muscle of frog at 10°C, Curtin et al. (1988) estimated, from the rate of heat production and intracellular pH_i, that the total rate of ATP splitting is reduced by about 20% as pH_i is increased from 6.82 to 7.26. They argued that the actomyosin ATPase activity had to be decreased by a higher amount. Kentish and Nayler (1979) found in myofibrils of rabbit gastrocnemicus little or no effect of pH on MgATPase (37°C). Godt and Kentish (1989) and Cooke et al. (1988) studied ATPase activity of skinned fibers from rabbit psoas muscle. Using a method based on NADH fluorescence, Godt and Kentish found that, as pH was increased from 6.5 to 7.5, ATPase activity increased from 83 to 118% of its value at pH 7.0 (22°C). By measuring NADH absorbance, Cooke et al. (1988) found that, as pH was increased from 6 to 7, ATPase was increased by about 25%, with almost all of the change occurring between pH 6 and 6.5 (10°C). Differences in species, temperature, and perhaps buffering may explain the different effects of pH on ATPase activity found.

In summary, we conclude that in the pH range investigated in this study, an increase in pH results in an increase in isometric force, which is smaller in slow- than in fast-twitch

fibers, and whereas ATP turnover in fast-twitch fibers appears to be pH-independent, it decreases in slow-twitch fibers as pH increases.

Implications for cross-bridge kinetics

The influence of pH on isometric ATP turnover and force found in this study can be accounted for by effects of pH on the kinetics of the cross-bridge cycle. In a simple two-state cross-bridge model with one attached and one detached state and an attachment rate constant f_{app} and a detachment rate g_{app}, force is proportional to the fraction of attached cross-bridges (N_{att}). ATPase activity equals $g_{app} \cdot N_{att}$. Hence, isometric tension cost is proportional to g_{app}. On the basis of ATP turnover and force (this study) and the rate of tension redevelopment (e.g., Brenner, 1986; Metzger and Moss, 1990b; Millar and Homsher, 1992), estimates can be obtained of f_{app} and g_{app} and their pH dependence. For psoas fibers at pH 7.0, f_{app} and g_{app} are about 10 and 3 s^{-1}, respectively. For soleus they amount to about 1 and 0.4 s^{-1}. Our finding that the tension cost in psoas and soleus fibers shows the same pH dependence indicates that the relative decrease in g_{app} is the same for psoas and soleus fibers as pH increases. The attachment rate f_{app} increases steadily from pH 6.4 to 7.3, by a factor 2 for psoas and 1.5 for soleus. For higher pH, f_{app} rises sharply and its increase depends very much on the (literature) value adopted for the rate of tension redevelopment. Certainly, force per cross-bridge must become pH-dependent at high pH, because isometric force does not rise continuously but reaches a peak between pH 7.5 and 8.5 (Robertson and Kerrick, 1979).

This simple two-state model does not take into account the dissociation of force and stiffness when pH is varied (Metzger and Moss, 1990a; Seow and Ford, 1993). In a two-state model, both force and stiffness are supposed to vary in proportion to the number of attached cross-bridges. Moreover, earlier studies suggest that pH influences detachment from the strongly bound state (Chase and Kushmerick, 1988; Bagshaw and Trentham, 1974; Koretz and Taylor, 1975) and the transition from the weakly to the strongly bound state (Chase and Kushmerick, 1988; Metzger and Moss, 1990a, b; Seow and Ford, 1993), whereas so far no pH dependence for cross-bridge attachment has been reported. Therefore, we also performed a quantitative analysis in terms of a three-state cross-bridge model, as described in the Appendix, consisting of a detached state, an attached but non- or low force-producing (weakly bound) state, and a force-producing (strongly bound) state. On the basis of the effects of pH on ATP turnover and tension cost found in this study and rate constants obtained previously (Zhao and Kawai, 1993; Wang et al., 1994; Potma et al., 1994), it is possible to determine the way pH affects the transitions in the three-state model in fast and slow fibers and the consequential effects of pH on the rate of tension redevelopment (cf. Brenner, 1986; Metzger and Moss, 1990b; Millar and Homsher, 1992) and on stiffness (cf. Metzger and Moss, 1990a; Seow and Ford, 1993).

Because the detachment rate remains directly proportional to isometric tension cost, the effect of pH on the detachment rate (from the strongly bound state) is the same as in the two-state model: as pH is increased, the detachment rate shows the same proportional decrease for psoas and soleus. The reaction rate of the transition from the weakly to the strongly bound state appears to be increased by the same proportion in psoas and soleus as pH is increased. This might form the basis of the increase in f_{app} in the two-state model. For either muscle fiber type, an increase in pH results in a shift of cross-bridges from weakly to strongly bound. Therefore, the force per attached cross-bridge, which is proportional to the number of cross-bridges in the strongly bound state divided by the total number of bound cross-bridges, will be increased. The values for the rate of tension redevelopment and stiffness that are predicted by the model are in good agreement with the literature (Metzger and Moss, 1990a, b; Millar and Homsher, 1992; Brenner, 1986; Seow and Ford, 1993).

In conclusion, for a certain change in pH, corresponding reaction rates in psoas and soleus fibers change by practically the same fraction. Nevertheless, the pH-effects on energy turnover and force are different, because the proportions between the different rates for soleus differ from those for psoas.

Relevance to muscle fatigue

During prolonged activity of skeletal muscle, after an initial increase, a decline of intracellular pH caused by lactic acid accumulation occurs, which contributes to muscle fatigue (Dawson et al., 1978; Renaud et al., 1986; Westerblad et al., 1991; Nagesser et al., 1992). By directly affecting the actomyosin interaction, protons inhibit both force and shortening velocity. In this study, we showed that when the muscle becomes acidic, tension cost increases by the same proportion in fast- and slow-twitch fibers. Because for slow muscle energy utilization is less, acidification and its implications are also smaller than in fast muscle. Therefore, our findings are in agreement with the observation that fatigue occurs in slow muscle to a lesser extent than in fast muscle.

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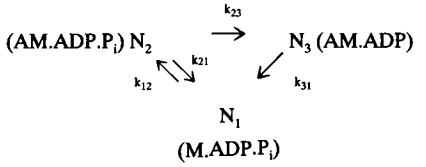
APPENDIX

Influence of pH on the distribution of cross-bridges and on the rate constants in a three-state model

In a three state cross-bridge model (Scheme 1), the relative proportions of cross-bridges in each of the states and the reaction rate constants can be derived at each pH on the basis of rate constants obtained previously (Zhao and Kawai, 1993; Wang et al., 1994; Potma et al., 1994) and the effects of

pH on force and ATP turnover found in this study.



where: M, myosin; AM, actomyosin; N_1, detached cross-bridges; N_2, no-force-producing cross-bridges (weakly bound); N_3, force-producing cross-bridges (strongly bound); k_{12}, attachment rate; k_{21}, reverse attachment rate; k_{23}, rate of myosin isomerization and/or P_i release; k_{31}, rate of ADP release and/or cross-bridge detachment. For each pH value,

$$dN_1/dt = k_{21}N_2 - k_{12}N_1 + k_{31}N_3 \quad (1)$$

$$dN_2/dt = k_{12}N_1 - k_{21}N_2 - k_{23}N_2 \quad (2)$$

$$dN_3/dt = k_{23}N_2 - k_{31}N_3 \quad (3)$$

with normalization:

$$N_1 + N_2 + N_3 = 1 \quad (4)$$

In the isometric steady state, $dN_1/dt = dN_2/dt = dN_3/dt = 0$. Force is assumed to be proportional to the number of cross-bridges in the force-generating state, N_3. It turned out to be unnecessary to incorporate a direct effect of pH on the strength of the bond between actin and myosin (the force per strongly bound cross-bridge), as postulated by Metzger and Moss (1990a). In fact, if this were the case, the effects of pH on the transition rates and on the cross-bridge distribution would be less. The steady-state ATP turnover per myosin head equals $k_{31} \cdot N_3$. Hence, isometric tension cost is proportional to k_{31}, the rate constant of detachment from the strongly bound state. So far no pH dependence for cross-bridge attachment has been reported; therefore, we assume that k_{12} and k_{21} are independent of pH.

In this model, for a given set of rate constants k_{12}, k_{21}, k_{23}, and k_{31}, the time course of tension redevelopment after unloaded shortening is given by the (nonequilibrium) solution $N_3(t)$ of Eqs. 1–4, under the starting condition that at $t = 0$, all cross-bridges are detached, i.e., $N_1(0) = 1$, $N_2(0) = 0$, and $N_3(0) = 0$. The bi-exponential function $N_3(t)$, obtained in this way, is dominated by the slower exponent and therefore can be fit very well by a mono-exponential function, yielding the rate of tension redevelopment, k_{tr}. The influence of pH on stiffness also follows if it is assumed that stiffness is proportional to the total number of attached cross-bridges (i.e., $N_2 + N_3$).

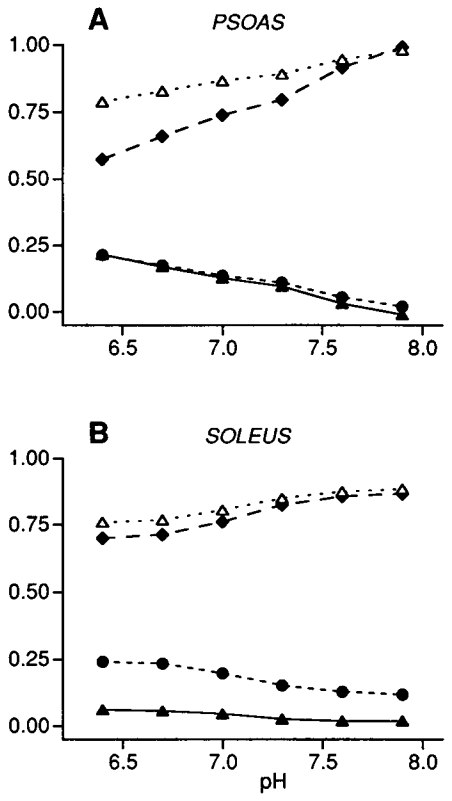


FIGURE 4 Effect of pH on the degree of occupancy of the three states of the cross-bridge model and on stiffness for psoas (*A*) and soleus (*B*) fibers. Filled circles represent N_1, the fraction of detached cross-bridges, filled triangles represent N_2, the fraction of weakly bound cross-bridges, and filled diamonds represent N_3, the fraction of cross-bridges in the force-generating state. Stiffness ($N_2 + N_3$) is represented by the open triangles.

Psoas fibers

The values of k_{12} and k_{21} for psoas fibers, at 15°C, can be estimated from values at 20°C, given by Zhao and Kawai (1993), assuming a Q_{10} of 2: k_{12} = 75 s^{-1}, k_{21} = 64 s^{-1} (cf. Goldman et al., 1984). The detachment rate k_{31} and its pH dependence were calculated from the value at pH 7.1 obtained earlier (Potma et al., 1994) and the pH dependence of tension cost. As pH decreases from 7.9 to 6.4, k_{31} increases from 2.2 to 3.9 s^{-1}. The value of N_3 at each pH now follows from the ATP turnover measured, and Eqs. 1 and 4 yield the corresponding values of N_1 and N_2.

In Fig. 4 *A*, the effects of pH on the degree of occupancy N_1, N_2, and N_3 can be seen for psoas fibers. The pH dependence of k_{23} follows from Eq. 3. From pH 6.4 to 7.3, k_{23} increases from 11 to 23 s^{-1}. At pH 7.9, negative values for N_2, and therefore for k_{23}, were found. Clearly, our assumption that force and N_3 are proportional does not hold at high pH, as was the case in the two-state model. The rate of tension redevelopment (k_{tr}) calculated from the rate constants obtained equals 12 s^{-1} (at pH 7.0). This value compares well with the values found for psoas fibers (15°C, pH 7.0) by Brenner (1986) and Metzger and Moss (1990b): 14.5 and 17.6 s^{-1}, respectively. As pH was decreased to 6.4, the calculated k_{tr} showed only a small decrease to 10 s^{-1}. Metzger and Moss (1990b) found that k_{tr} remained constant as pH was decreased from 7.0 to 6.2. As pH decreases from 7.9 to 6.4, stiffness (cf. Fig. 4 *A*) decreased by 21%, whereas force decreased by 42%. Metzger and Moss (1990a), in rat SVL at 15°C, and Seow and Ford (1993), in rabbit psoas at 1–2°C, both found that the decrease in stiffness was half the decrease in force as pH was lowered. In summary, it can be concluded that the effects of pH on ATP turnover (our study), force, stiffness, and tension redevelopment all may be explained by a pH dependency of k_{23} and k_{31}.

Soleus fibers

For soleus fibers, values of the rate constants k_{12} and k_{21} were estimated from values given by Wang et al. (1994) at 20°C, assuming a Q_{10} of 2: k_{12} = 3 s^{-1}; k_{21} = 6.9 s^{-1}. The pH dependence of the detachment rate k_{31} follows from the pH dependence of tension cost, but a value of k_{31} is not available in the literature. We have chosen k_{31} such that the degree of occupancy as well as the reaction rate k_{23} and the rate of tension redevelopment k_{tr} had positive values and showed a monotonic variation with pH. These criteria determine the k_{31} values with an uncertainty of about 10%. As pH decreases from 7.9 to 6.4, k_{31} increases from 0.26 to 0.44 s^{-1}. Therefore, the value obtained for k_{31} is 8 times lower than for psoas. In this respect, it is interesting to note that Siemankowski et al. (1985) found that the rate constant for dissociation of ADP, which may limit cross-bridge detachment during unloaded shortening, was at least 6 times higher for rabbit psoas than for soleus at 15°C. From pH 7.3 to 6.4, i.e., the range in which k_{23} is well defined for psoas, k_{23} for soleus decreases by a similar factor as for psoas: from 11 to 5 s^{-1}. In Fig. 4 *B*, the resulting cross-bridge distribution is shown as a function of pH. The rate of tension redevelopment k_{tr} calculated from the rate constants obtained equals 1.7 s^{-1} (at pH 7.0), which is low compared with the value of Metzger and Moss (1990b), who found that k_{tr} = 3.1 s^{-1} at 15°C, but is comparable with the values found by Millar and Homsher (1992): 0.6 s^{-1} at 10°C and 2.3 s^{-1} at 20°C (notice that the values given by Millar and Homsher are slight—less than 30%—underestimations, because

of the absence of sarcomere length control). The small decrease in k_{tr} to 1.6 s^{-1} as pH is decreased to 6.4 compares well with the pH independency of k_{tr} that Metzger and Moss (1990b) found between pH 7.0 and 6.2. The effect of pH on stiffness (cf. Fig. 4 *B*) is smaller for soleus than for psoas. Our simulation predicts a 5% decrease in stiffness between pH 7.0 and 6.4. Metzger and Moss (1990a) found that stiffness was constant between pH 7.0 and 6.2.

In conclusion, although the proportions between the different rates in the cross-bridge cycle for soleus are very different from those for psoas, the relative changes in k_{23} and k_{31} with pH are the same in both muscle types.

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