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THE EFFECTS OF METABOLIC FUEL ON FORCE PRODUCTION AND RESTING INORGANIC PHOSPHATE LEVELS IN MOUSE SKELETAL MUSCLE

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SUMMARY

1. The effect of different metabolic fuels (glucose, pyruvate and lactate) and no exogenous metabolic fuel on force production was studied in isolated mouse soleus and extensor digitorum longus (EDL) muscles. Force was measured, at 25 °C, during isometric tetanic contractions and during contractions with isovelocity stretching and shortening. In parallel experiments, measurements were made of the resting phosphorus metabolite levels using ^{31}P NMR.

2. In soleus muscles, the isometric tetanic force was potentiated with pyruvate (20 mM) as metabolic fuel, compared with glucose (11 mM), by $17{\cdot}8 \pm 3{\cdot}6\%$ (mean $\pm$ S.E.M., $n = 6$). The force was the same with no exogenous metabolic fuel, with glucose, or with lactate as metabolic fuel. The force exerted during shortening was also potentiated by pyruvate and by the same proportion as isometric force. However, during rapid stretching there was no force enhancement with pyruvate. The changes in the force seen with pyruvate are qualitatively similar to those produced when inorganic phosphate (P_i) is lowered in skinned rabbit psoas muscle fibres.

3. We tested whether the P_i content decreased in the presence of pyruvate by measuring resting P_i using ^{31}P NMR spectroscopy. We found that, in soleus muscles, resting P_i was present with glucose and absent with pyruvate as metabolic fuel, and the effect was reversible.

4. EDL muscles produced the same isometric force whether the metabolic fuel was glucose, pyruvate, lactate or if no exogenous metabolic fuel was supplied. EDL muscles already had P_i levels below detectability at rest in glucose. There were no changes in the ^{31}P NMR spectrum with pyruvate as metabolic fuel.

5. It appears therefore that the force potentiation in soleus muscles with pyruvate is due to a lowering of P_i. EDL muscles, which have a very low resting P_i in glucose, therefore have very little potential for force enhancement by this mechanism.

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INTRODUCTION

The metabolic fuels used by skeletal muscles include muscle glycogen, glucose and free fatty acids. Although it is well known that cardiac muscle function is influenced by the metabolic fuel supplied (Chapman & Gibbs, 1974; Sweier & Jacobus, 1987; Daut & Elzinga, 1989) little is known of the effects, if any, of different metabolic fuels on the contractile performance of skeletal muscle.

Daut & Elzinga (1989) have shown that, in cardiac muscle, providing pyruvate instead of glucose as exogenous metabolic fuel potentiates force. In the perfused heart, Zweier & Jacobus (1987) found that 10 mM pyruvate, compared to 16 mM glucose, caused an increase in left ventricular developed pressure and oxygen consumption. In addition, phosphorus NMR spectroscopy showed that inorganic phosphate (P_i) was lowered while phosphocreatine (PCr) was higher in hearts perfused with pyruvate compared with hearts perfused with glucose. The increase in left ventricular developed pressure was thought to be due to the lower P_i, and P_i has been shown to depress force in both cardiac and skeletal skinned muscle preparations (Brandt, Cox, Kawai & Robinson, 1982; Kentish, 1986) as well as whole isolated heart (Kusuoka, Weisfeldt, Zweier, Jacobus & Marban, 1986; Ugurbil, Kingsley-Hickman, Sako, Zimmer, Mohanakrishnan, Robitaille, Thoma, Johnson, Foker & From, 1987).

In resting muscle the steady P_i level may be set by a balance between the rate of ATP use and the rates of production via oxidative phosphorylation and glycolysis. A rate-limiting step in glycolysis could for example be the phosphofructokinase (PFK) reaction which is affected by the P_i level (Passonneau & Lowry, 1964). If so pyruvate can lower P_i by bypassing this step. The effect should not be confined to cardiac muscle. We therefore studied the effect of replacing glucose with pyruvate or lactate on force production and phosphate metabolites in isolated skeletal muscle of the mouse (soleus and extensor digitorum longus (EDL)).

Portions of this work have appeared in preliminary form (Phillips & Woledge, 1990) and using a different strain of mouse (Wiseman, Phillips, Woledge & Kushmerick, 1991, 1992).

METHODS

Experiments were performed on mouse soleus and EDL muscles at 25 °C. Both left and right muscles of mice aged 2·5–8 months (C57BL/6 or /10 strain or the tan coat-mutation of the C57 black animal) were dissected after the mice had been killed by cervical dislocation.

Mechanical measurements

The glucose Ringer solution contained (mM): NaCl, 115; KCl, 5; $MgCl_2$, 0·5; $CaCl_2$, 2·5; NaH_2PO_4, 1; $NaHCO_3$, 24; glucose, 11; and curare (15 mg l^{-1}) and was gassed continuously with 95% O_2, 5% CO_2 (pH of 7·4). In addition to this standard solution, Ringer solution was made containing different metabolic fuels instead of glucose or no exogenous metabolic fuel at all. Concentrations of sodium pyruvate or sodium lactate between 5 and 30 mM were used. The concentration of sodium chloride was adjusted to keep the osmolarity of all solutions equal.

Aluminium T-shaped clips were folded around each tendon close to the muscle. A hole in each clip served for attachment to the apparatus. Muscles were mounted between a fixed hook and a hook attached to the lever arm of a Cambridge force transducer/motor (model No. 350). While one muscle was studied the second was kept pinned out, just above slack length, in a Petri dish containing gassed Ringer solution, at room temperature.

Muscles were stimulated directly with supramaximal pulses of 2 ms duration; the tetanic

contractions were 0·5–1·2 s long. Both force and position were measured from the motor system and the output was displayed and stored on an oscilloscope (Nicolet 4094). The optimum length for force (L_o) was determined using isometric tetanic contractions at 50 Hz (soleus) or 70 Hz (EDL) stimulation frequency. Between contractions the muscle rested for 2–3 min.

The effect of Ringer solution containing different metabolic fuels was tested by comparing measurements to those in the standard solution (11 mM glucose), taken at the beginning and end of the experiment. On the addition of a new solution to the bath, half an hour's incubation was allowed before commencing stimulation. This was found to be long enough to achieve reproducible force records. Pyruvate dose–response curves were studied in nine soleus muscles at various stimulation frequencies.

Force–velocity measurements were made as described previously (Phillips, Bruce & Woledge, 1991). Step and ramp movements were produced by a ramp generator signal fed to the motor. Each muscle then underwent a series of releases and stretches at different velocities, during tetanic contractions. Corrections were made for any change in resting force which occurred due to the change in muscle length. Isometric tetanic contractions of sufficient duration to produce maximum force were recorded several times during each experiment at L_o. The force–velocity relation was examined, in six muscles, with 11 mM glucose and again with 20 mM pyruvate as substrate.

The isometric force decline by the end of an experiment was 12% on average. The force rise time and relaxation time for isometric tetanic contractions were measured: the force rise time was the time taken for force to rise from 10 to 90% of plateau force. Relaxation time was the time taken for force to fall from 90 to 10% of plateau force.

At the end of the experiment muscles were fixed at L_o in 2% gluteraldehyde overnight. Small fibre bundles, with tendon at each end, were teased from the fixed muscle and used to estimate fibre length (L_f). Following removal of the tendons, muscles were dried and dry weight (W) was measured. These values were used to express force (in N m g^{-1}; force/(W/L_f)), and velocity was expressed as L_f s^{-1}.

^{31}P NMR spectroscopy experiments

The phosphorus metabolite levels of single muscles, with 11 mM glucose or 20 mM pyruvate as metabolic fuel, were measured using ^{31}P NMR. Measurements of isometric force were also made on these muscles, before and after NMR spectra were obtained, using methods similar to those described earlier; eight soleus and four EDL muscles were studied.

Silk suture tied to muscle tendons was used for mounting to the apparatus. Muscles were maintained up to one hour in Ringer solution before acquisition of spectra. The Ringer solution contained (mM): NaCl, 116; KCl, 4·6; MOPS (titrated to pH 7·4 with NaOH), 26·2; $CaCl_2$, 2·5; $MgSO_4$, 1·2; and gentamycin (10 mg ml^{-1}), gassed with 100% O_2 pH 7·4. The substrate added was either 11 mM glucose or 20 mM pyruvate, with NaCl adjusted to 101 mM in the latter case.

Single muscles were placed in a custom-built NMR probe like that described by Moerland & Kushmerick (1988). The coil used was an eight-turn solenoid of 30-gauge wire. Each muscle was mounted, at L_o, within a 1·5 mm internal diameter capillary glass tube and centred within the coil. The capillary was fixed horizontally in the apparatus, which was made from a block of Delrin. It provided reservoirs at both ends for the superfusate, as well as a mechanically stable foundation for the coil. Ringer solution flow was usually 0·5 ml min^{-1} and the temperature was controlled by a heat exchanger through which thermostated water was pumped to maintain the temperature of the probe (25 ± 2 °C). With a 140 mM Na_2HPO_4 standard in the capillary tube, the signal-to-noise ratio for a single shot ($\pi/2$ pulse, sweep width 5000 Hz, no expontial filtering) was 12:1.

Spectra were acquired on a 7T GN300 General Electric Omega spectrometer. Magnetic field homogeneity was optimized by shimming on the available proton signal from the sample (muscle and superfusate water) and was usually less than 0·1 p.p.m. The $\pi/2$ pulse durations at a nominal power of 50 W were measured for each preparation and were in the range 4–10 μs. Phosphorus spectra were obtained using a $\pi/4$ pulse and a 3 s recycle delay, 2048 complex data points and a 5 Hz sweep width. Data were filtered by a 15 Hz exponential and zero-filled once prior to the Fourier transform. Saturation factors were measured for each muscle type and applied to the raw spectral data. Spectral areas were obtained by integration of each peak within the spectrum using the integration routine available with the spectrometer. The baseline around each peak was flat. Each integral value was expressed as the fraction of the total phosphorus integral in the spectrum. Intracellular pH (pH_i) was calculated from the following formula:

$$pH_i = pK_a + \log(\delta a - \delta P_i)/(\delta P_i - \delta b),$$

for inorganic phosphate $pK_a = 6{\cdot}77$, $\delta a = 0{\cdot}89$, $\delta b = 3{\cdot}19$, where δa and δb are the limiting chemical shifts at low and high pH and δP_i is the chemical shift observed for P_i at experimental pH.

RESULTS

The provision of pyruvate as exogenous metabolic fuel instead of glucose was found to have a clear potentiating effect on soleus muscles. This is illustrated in Fig. 1 which shows that for both 10 and 50 Hz frequency of stimulation, there was an increase in force in the presence of pyruvate. The potentiation caused by pyruvate

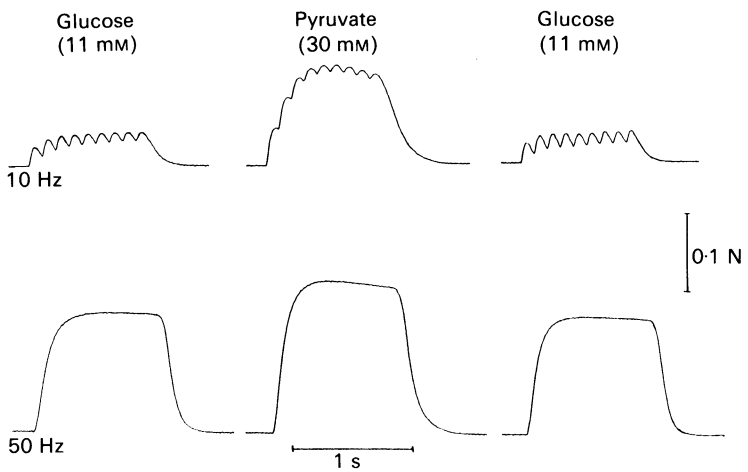

Fig. 1. Records from a soleus muscle at optimum length for isometric force. From left to right: in standard Ringer solution containing 11 mM glucose as substrate, after 30 min incubation in Ringer solution with 30 mM pyruvate as substrate and 30 min after returning to the standard Ringer solution. At 10 Hz (top records) and at 50 Hz (lower records) frequency of stimulation. Muscle dry weight = 1·35 mg with a fibre length of 7·6 mm.

was much greater at the lower frequency. Pyruvate also caused a slowing of relaxation and a decrease in force rise time. All the effects of pyruvate were reversible. Similar effects were seen in all experiments and in Table 1 the results are summarized. Table 1 shows paired data: glucose *versus* different exogenous metabolic fuels. The force produced with pyruvate was significantly greater than that with glucose ($P < 0{\cdot}001$), the force rise time was significantly lower ($P < 0{\cdot}05$) while relaxation slowed significantly ($P < 0{\cdot}01$). In addition to comparing glucose and pyruvate, comparisons were made of glucose with lactate and glucose with no exogenous metabolic fuel. Neither of these comparisons showed any differences. Similar experiments were made with EDL muscles and no significant changes (at 5% level) to force, force rise time or relaxation time were produced by pyruvate, lactate or with no exogenous metabolic fuel (Table 1).

Figure 2 shows the effect of pyruvate on the force–frequency curve of soleus muscles. It is clear that the effect of pyruvate is both to shift the curve to the left and to increase the plateau level of force at maximal stimulus frequencies.

Caffeine potentiates twitch force by inducing Ca^{2+} release from the sarcoplasmic reticulum (Weber, 1968) and therefore is another drug that shifts the force–frequency

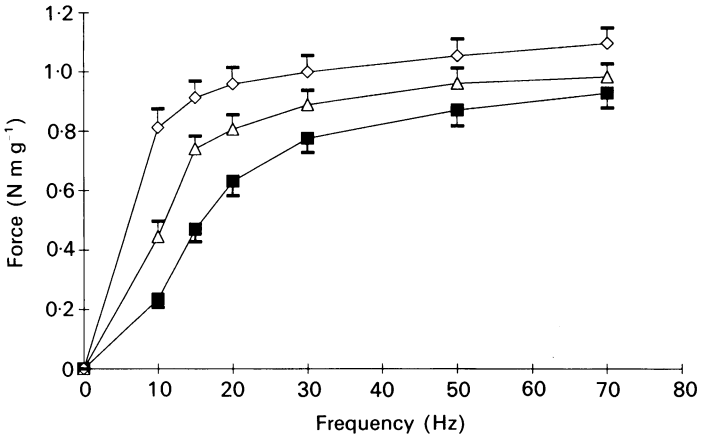

Fig. 2. Mean force–frequency relationship for soleus muscles in the standard Ringer solution containing 11 mM glucose as substrate (■), or with 5 mM (△) or 30 mM (◇) pyruvate as substrate. The results are means±S.E.M. for seven to nine muscles.

TABLE 1. Contractile properties of soleus and EDL muscles are presented, comparing 11 mM glucose as metabolic fuel with either 20 mM pyruvate, no exogenous metabolic fuel, or 20 mM lactate

	Soleus		
	Force	Force rise time	Relaxation time
Glucose	0·902±0·038 (15)	160·0±15·1 (6)	83·1±2·9 (6)
Pyruvate	1·069±0·044 (15)	145·0±11·1 (6)	122·9±11·3 (6)
Glucose	0·890±0·057 (4)	169·9±9·9 (4)	98·8±2·7 (4)
No exogenous metabolic fuel	0·872±0·052 (4)	155·1±16·1 (4)	99·3±5·2 (4)
Glucose	0·877±0·031 (5)	154·8±6·8 (5)	97·8±1·4 (5)
Lactate	0·863±0·037 (5)	151·8±2·9 (5)	99·5±3·2 (5)
	EDL		
	Force	Force rise time	Relaxation time
Glucose	0·971±0·036 (4)	57·2±3·0 (4)	30·9±1·8 (4)
Pyruvate	0·967±0·043 (4)	55·7±2·9 (4)	28·1±1·1 (4)
Glucose	0·930±0·020 (4)	59·0±4·8 (4)	30·8±1·7 (4)
No exogenous metabolic fuel	0·926±0·008 (4)	55·9±4·5 (4)	29·8±1·1 (4)
Glucose	0·971±0·036 (4)	57·2±3·0 (4)	30·9±1·8 (4)
Lactate	0·888±0·081 (4)	55·9±4·8 (4)	30·7±0·9 (4)

Data are given as means±S.E.M. (n) and include normalized force (force/(W/L_f)), relaxation time (90–10% of tetanic force) and force rise time (10–90% of tetanic force) of isometric tetani at 50 Hz (soleus) and 150 Hz (EDL) stimulation frequency.

curve to the left. We compared the effects of caffeine with those of pyruvate. We found that 4 mM caffeine produces a 78, 22 and 9% potentiation at 10, 20 and 30 Hz. However, there was little potentiating effect at frequencies above this; at 50 Hz the increase in force was only $4{\cdot}1 \pm 1{\cdot}8\%$ ($n = 4$) compared with the $18{\cdot}4 \pm 0{\cdot}3\%$ ($n = 15$)

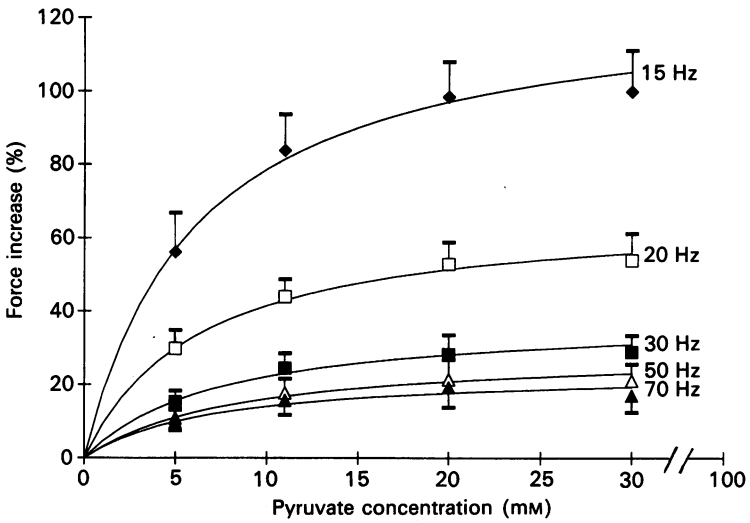

Fig. 3. The relationship between force and pyruvate concentration for soleus muscles at five different frequencies of stimulation. Force is expressed as the percentage increase above that in glucose. The results are means ± S.E.M. for seven to nine muscles. The continuous lines are hyperbolic curves for the data (note the break in the x-axis scale), the half-maximal concentrations of pyruvate at 15, 20, 30, 50 and 70 Hz were 6·1, 6·3, 7·4, 8·3 and 7·0 mM respectively. Data at 100 mM pyruvate are extrapolations of the hyperbolic fits.

increase with pyruvate, and therefore the pattern of its action is different from that of pyruvate.

Figure 3 shows the effect of different pyruvate concentrations on the force developed at five different stimulation frequencies. The relationship between force and pyruvate concentration appears to be hyperbolic. A Lineweaver–Burk plot and regression line of the data provided values of the constants for the Michaelis–Menten equation, from which hyperbolic curves were calculated and shown in Fig. 3. The half-maximal concentration of pyruvate was about 7 mM for each frequency (see legend for values).

Figure 4*A* shows force–velocity (50 Hz stimulation) data for 20 mM pyruvate compared with that for 11 mM glucose. At high velocities of shortening the force exerted is similar with the different metabolic fuels, and V_{max} values were obtained by fitting a hyperbola to the mean data using three-dimensional regression analysis (B. Wohlfart & K. A. P. Edman, unpublished). These values were nearly the same; $4{\cdot}84\ L_f\ s^{-1}$ with glucose and $5{\cdot}20\ L_f\ s^{-1}$ with pyruvate. At all shortening velocities below $2\ L_f\ s^{-1}$ and at all velocities of stretch below $-2\ L_f\ s^{-1}$ the force exerted in the presence of pyruvate is significantly greater than in glucose ($P < 0{\cdot}05$, paired data).

The effect of pyruvate on the shape of the force–velocity curve is revealed more clearly by plotting force relative to the isometric force, as shown in Fig. 4*B*. This shows that pyruvate does not alter the shape of the relation between force and positive (shortening) velocities, but that the relation between force and negative

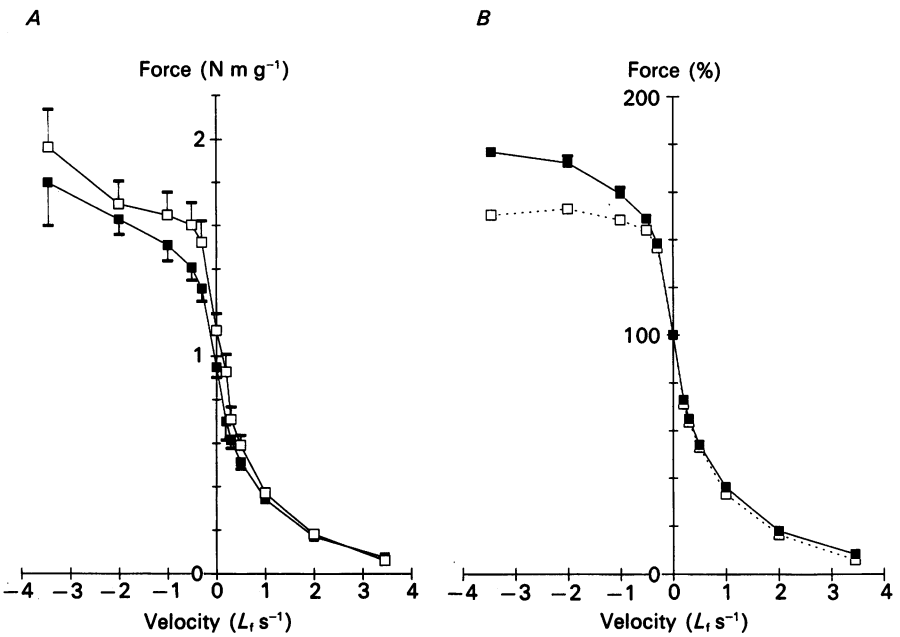

Fig. 4. The mean force–velocity relationship during shortening and stretching (negative values) for soleus muscles with 11 mM glucose (■) or 20 mM pyruvate (□) as substrate. 50 Hz stimulation frequency was used. *A*, force as N m g^{-1}. *B*, the force is expressed as a percentage of the isometric force. The results are means ± S.E.M. (error bars are not included when they fall within the symbol) with the following number of observations (from left to right): glucose 2, 6, 6, 6, 6, 6, 3, 6, 6, 6, 6, 2; pyruvate 2, 6, 6, 6, 6, 6, 2, 6, 6, 6, 6, 1.

(lengthening) velocities is changed. In pyruvate the extra force caused by stretching is less in proportion to the isometric force, than in glucose. The three highest velocities of stretch are all in the region of the force–velocity curve for which force is almost independent of velocity. We therefore combined the results for these three fastest velocities to find the best estimate of the extent to which force during rapid stretch is reduced, relative to isometric force, when pyruvate is the metabolic fuel. This reduction during stretch is 18·2 ± 1·1 %. The effect of pyruvate on the isometric force is to increase it by 17·8 ± 3·6 % (means ± S.E.M., $n = 6$). Thus pyruvate evidently leaves the force exerted at high stretching velocities unaltered. This conclusion is compatible with the results in Fig. 4*A* in which the difference between the pyruvate and glucose results is not statistically significant for the two highest velocities of lengthening (at the 5 % level).

Representative ^{31}P NMR spectra from isolated soleus muscles are shown in Fig. 5*A* and *B*. Spectra from muscles in 11 mM glucose contained peaks from P_i, PCr and the γ-, α- and β-ATP resonances (Fig. 5*A*). The P_i/PCr ratio under these

conditions was 0·61 ($n = 8$) (Table 2). The average ATP content of soleus muscles was 12·6% of the total phosphorus integral. High-performance liquid chromatography analysis of ATP content of mouse soleus muscle gives a value of 3·3 μmol g^{-1} (Kushmerick, Moerland & Wiseman, 1992). Therefore, in this study if 12·6% ATP is equivalent to 3·3 μmol g^{-1} then 21·1% P_i will be 5·5 μmol g^{-1}.

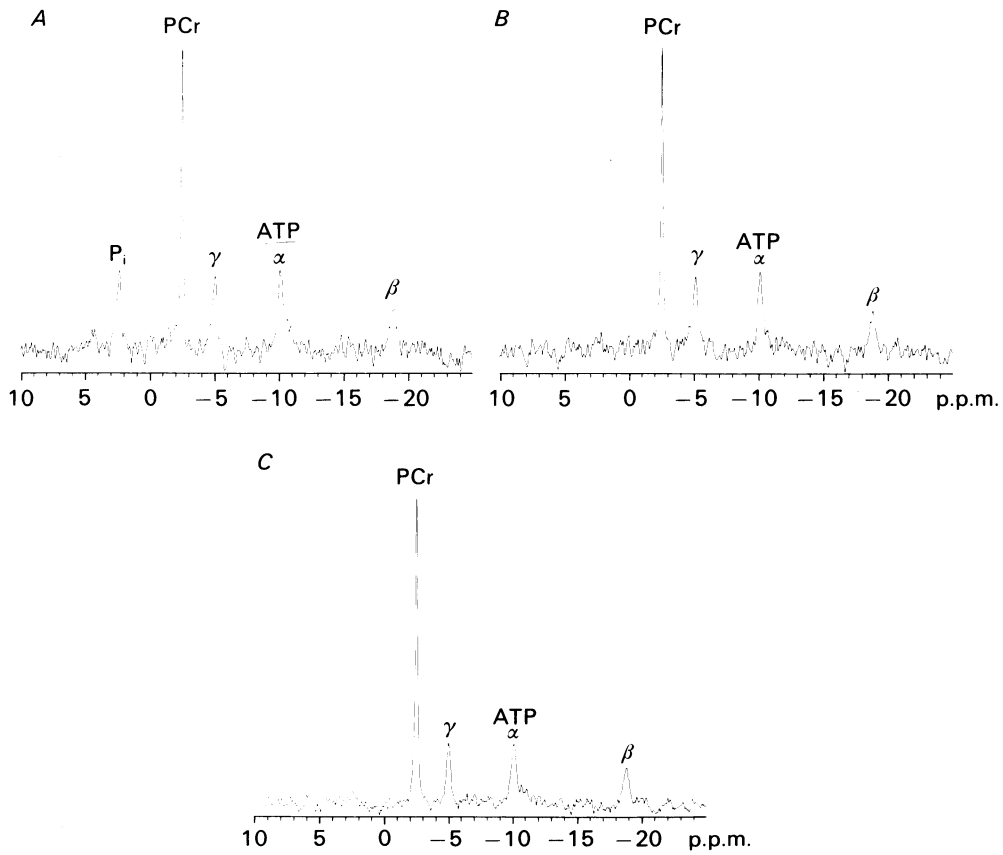

Fig. 5. Typical ^{31}P NMR spectra of resting mouse muscle. Soleus muscle with 11 mM glucose (*A*), 20 mM pyruvate as substrate (*B*) and EDL with 11 mM glucose (*C*). Each spectrum is an average of 600 scans. Spectra have been corrected for saturation factors.

In five of the eight muscles tested, incubation in 20 mM pyruvate resulted in a disappearance of the P_i peak into the baseline noise (Fig. 5*B*). In the remaining three a small P_i peak was visible. The P_i/PCr ratio for these muscles decreased to 0·08 when treated with pyruvate. These muscles also showed a significant increase in isometric force in pyruvate compared to that in glucose (13·4 ± 3·8%, mean ± S.E.M.). On returning the muscle to glucose from pyruvate the P_i peak reappeared in the spectrum. The pH_i was no different with glucose or pyruvate as the metabolic fuel, but this could only be calculated for three out of eight muscles which had above baseline P_i peaks with pyruvate (see Table 2). Qualitatively, the EDL was substantially different. In 11 mM glucose, phosphorus NMR spectra from these

muscles displayed the peaks from PCr and γ-, α- and β-ATP resonances (Fig. 5*C*). The P_i peak was visible in only two out of four muscles. Treatment with pyruvate had no discernible effect on the phosphorus spectra of these muscles (data not shown).

TABLE 2. A summary of the resting ^{31}P NMR data, given as means ± S.E.M. (n), for soleus muscles with 11 mM glucose or 20 mM pyruvate as substrate

	Soleus		EDL
Metabolite	Glucose	Pyruvate	Glucose
P_i (%)	21·1 ± 3·5 (8)	4·3 ± 2·2 (8)*	8·2 (2)
PCr (%)	37·4 ± 4·8 (8)	44·9 ± 1·9 (8)	44·4 ± 4·8 (4)
γ-ATP (%)	12·3 ± 1·2 (8)	18·8 ± 1·2 (8)	12·9 ± 1·5 (4)
α-ATP (%)	19·4 ± 2·8 (8)	20·4 ± 1·0 (8)	19·5 ± 2·5 (4)
β-ATP (%)	12·9 ± 1·9 (8)	11·6 ± 0·8 (8)	14·9 ± 1·1 (4)
P_i/PCr ratio	0·61 (8)		
pH_i	6·9 ± 0·1 (8)	6·9 ± 0·1 (3)	

EDL muscles were little affected by pyruvate and data is only shown for 11 mM glucose as substrate. Phosphorus metabolite levels are expressed as a percentage of the total phosphorus integral in the spectrum. * P_i was detectable in only three out of eight muscles; therefore the mean includes five zeros and may be biased because of the absence of negative values.

DISCUSSION

Pyruvate was found to have a clear reversible potentiating effect on force production in soleus muscles, as it does in cardiac muscle. We found that, as suggested by Zweier & Jacobus (1987) and Daut & Elzinga (1989), the effect is accompanied by, and is probably due to, a decreased level of inorganic phosphate within the cell. There is evidence in cardiac muscle that the reduction in P_i is accompanied by an increase in PCr (Zweier & Jacobus, 1987), as expected; although we did find an increase in PCr (Table 2) it was not statistically significant. Raising P_i depresses force in skinned muscle both by antagonizing the calcium activation process and by depressing the level of force in fully activated muscle (Brandt *et al.* 1982; Kentish, 1986). Both effects also seem to be present in our intact skeletal muscles. The increase of force produced by pyruvate at high frequencies (> 50 Hz) of stimulation can be attributed to the action of P_i on the fully activated myofibrils because caffeine, which potentiates Ca^{2+} release from the sarcoplasmic reticulum, caused no significant potentiation of contraction at these frequencies with glucose as the metabolic fuel. On the other hand at lower frequencies (10, 20 and 30 Hz) at which caffeine did produce substantial potentiation, pyruvate was found to have a much larger potentiating action, presumably because of the enhancement of the degree of activation for a given calcium concentration (Millar & Homsher, 1990). However, there is evidence of a change in calcium handling even at high frequencies of stimulation. This is the significantly faster force rise time and slower relaxation time with pyruvate. A possible explanation for this is that with pyruvate more calcium is released, which then takes longer to be removed, thus altering the time course of a tetanus.

The force–velocity data were obtained with 50 Hz stimulation, which appears to fully activate the muscle because caffeine caused no significant potentiation. The force potentiation by pyruvate must therefore be attributable to an increase in average force per active cross-bridge, rather than an increase in the number of sites active. This explanation is supported by the finding that during rapid stretch there is no effect of metabolic fuel on force developed. The same change in the shape of the force–velocity curve for stretching has been reported on lowering the inorganic phosphate level in skinned rabbit muscle fibres (Elzinga, Steinen & Versteg, 1989). The action of inorganic phosphate in depressing force in fully active myofibrils is generally considered to be due to a shift in the equilibrium between two force producing states (Pate & Cooke, 1989; Millar & Homsher, 1990). Stretching also shifts this equilibrium, forcing nearly all the cross-bridges into the state with P_i bound, thus overriding the action of P_i in shifting the equilibrium, and removing any difference of force.

The absence of any effect of pyruvate in EDL muscle can be explained by the very low resting P_i level. This is in agreement with other reports on muscles *in vitro*, in which at rest fast twitch muscles had very little P_i compared to slow twitch muscles (Meyer, Brown & Kushmerick, 1985; Kushmerick *et al.* 1992). Therefore there is little scope for this to be lowered further by a change in metabolic fuel. Thus all our results are consistent with the effect of pyruvate being on P_i levels.

It is not clear whether there is a difference in P_i levels between fast and slow twitch muscles *in vivo* when the slow twitch muscles probably have a wider range of metabolic fuels available. Muscles chosen for *in vitro* studies are usually not suitable for *in vivo* studies and vice versa. As far as we are aware a P_i peak is always reported as being present *in vivo*, but often the concentration of P_i is low, e.g. 1–2 mM in rat lower leg muscle (Ackerman, Grove, Wong, Gadian & Radda, 1980) which is predominantly fast twitch muscle. A study on three human hand muscles with different proportions of type I fibres found no difference between the muscles, which all had a substantial P_i, about equal to the ATP level of each muscle (concentration not measured; Turner, McIntyre, Jones & Newham, 1992). However, chronic stimulation of canine latissimus dorsi muscle *in vivo* caused a fibre type transformation from fast to slow twitch fibres and a doubling of the P_i/PCr ratio, while ATP/PCr ratio was no different, contralateral muscles acting as controls (Clarke, Acker, McCully, Subramanian, Hammond, Salmons, Chance & Stephenson, 1988). Glycolysis is more important to fast than to slow twitch muscles; it therefore seems reasonable for rate limiting glycolytic enzymes (e.g. phosphofructokinase) of fast twitch muscle to be more sensitive to levels of metabolites, e.g. P_i, which increase their activity. This could result in different P_i levels in fast and slow twitch muscles.

Some authors claim that fast twitch muscle is stronger than slow twitch muscle (Young, 1984; Grindrod, Round & Rutherford, 1987). On the basis of our experiments we might expect a difference in this direction when glucose is the metabolic fuel because of the different resting P_i levels. Although we did find that with glucose EDL muscles were slightly stronger than soleus (Table 1) this difference was not significant. The relative sensitivity of the myofibrils to P_i in mouse soleus and EDL is not known, and this would also be influential in determining any difference in force production at significant P_i levels. A comparison of the intrinsic strength of the

myofibrils between the muscles can be made when pyruvate is the metabolic fuel, when the P_i level is negligible in both muscles. In this case there is still no difference between soleus and EDL muscles (Table 1) therefore our data do not support any intrinsic difference in strength between fast and slow twitch muscle at the cross-bridge level.

This study was designed to investigate whether pyruvate as metabolic fuel was associated with a lowering of P_i in skeletal muscle rather than to determine the mechanism of this action of pyruvate on P_i. The possible mechanism has been discussed by Daut & Elzinga (1989) who studied the effect of pyruvate on cardiac muscle. Their hypothesis of 'substrate control' is that the supply of exogenous pyruvate bypasses the rate-limiting steps of glycolysis (e.g. phosphofructokinase), subsequently the mitochondrial redox potential is increased and so the cytosolic phosphorylation potential increases and P_i decreases. As in the present study Daut & Elzinga also found that lactate did not potentiate force, and suggested it was because lactate dehydrogenase activity is subject to product inhibition.

We have found that the type of exogenous metabolic fuel that is supplied to skeletal muscle can affect its mechanical properties. When pyruvate is supplied as metabolic fuel to soleus muscles the P_i level is lowered and isometric force is increased. Therefore, *in vitro* pyruvate allows the manipulation of P_i levels in whole soleus muscle.

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