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PATTERNS IN MAMMALIAN MUSCLE ENERGETICS

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SUMMARY

A description of cellular energetics of muscular contraction is given in terms of the rates and extents of high-energy phosphate splitting during contractile activity, in terms of high-energy phosphate resynthesis by respiration and net anaerobic glycolysis, and in terms of the associated uptake and/or release of H^+. These chemical changes have been studied quantitatively by rapid freeze-clamping methods and by ^{31}P-NMR methods. The pattern of chemical changes in a fast-twitch glycolytic muscle is rapid depletion of phosphocreatine and later ATP levels, cellular acidification, and a much slower rate of resynthesis of high-energy phosphate compounds during the recovery period afterwards than occurs in the slow-twitch oxidative muscles. In steady-state contractile activity below the maximal, graded levels of high-energy phosphates and of cellular respiration are achieved in both fast-twitch and slow-twitch muscles. Within the metabolic range up to the maximal aerobic capacity, which differs several-fold for different fibre types, this gradation is mediated by the creatine kinase reaction and phosphocreatine stores. Thus while the amount of enzyme present and the content of phosphocreatine differs among muscles of different types, the same general energetic function is seen to occur in all muscle cells. The creatine kinase reaction is both an energy reservoir and a buffer preventing large swings in the ATP/ADP ratios.

INTRODUCTION

The primary function of skeletal muscle is the generation of force, which is essential for animal stance and locomotion. The muscle machine is a chemomechanical converter which can be examined from two points of view. The first characterizes and explains the demands of chemical potential energy utilization placed on the cells by the actomyosin interaction, calcium movements, and other forms of electrical and osmotic work. The second aspect concerns the integrated operation and regulation of metabolic pathways, the net result of which, for an aerobic organism, is the generation of chemical potential energy in the form of ATP and other so-called high energy phosphates, such as phosphocreatine (PCr).

Muscle cells are distinguished from most other cells by their large dynamic range of metabolic activity. For example, the steady rate of ATP production in unstimulated frog sartorius muscle is approximately 1 nmol ATP g^{-1} muscle s^{-1}. During a maximal

tetanic stimulation, the rate of energy utilization can be a thousand times higher. In mammalian skeletal muscle, this range is approximately ten times lower, because the unstimulated so-called resting rate is higher than in amphibians, and because the rate of actomyosin ATP utilization, especially in mammalian slow-twitch muscles, is lower than in frog twitch muscle.

There are thus two sets of relevant questions. The first concerns the rate of energy utilization during contraction of the skeletal muscle types, ranging from fast- to slow-twitch, as is found in mammalian organisms. The second set of questions relates to the generation of high energy phosphate supply. Pathways involved in this metabolism have been discussed elsewhere in this volume (Hochachka, 1985; Gollnick, Riedy, Quintinski & Bertocci, 1985; Pette, 1985). My focus will be on the pattern of cellular respiration or oxidative metabolism in response to contractile activity, its time course and its regulation. The focus will be therefore on the net result, namely oxygen consumption, rather than on the details of substrates being oxidized.

PATTERN OF ENERGY UTILIZATION IN MAMMALIAN MUSCLES

Based on a limited number of heat measurements and a few direct chemical measurements, the energetic pattern in mammalian muscles appears to be basically similar to that in amphibians (for reviews, see Curtin & Woledge, 1978; Homsher & Keane, 1978; Kushmerick, 1983). Recently a detailed comparison has been made between the energetics of a predominantly fast-twitch muscle, the mouse extensor digitorum longus (EDL), and the energetics of a predominantly slow-twitch muscle, the mouse soleus (Crow & Kushmerick, 1982*a*). The energetics of the EDL differed from that of the soleus in two major ways (Fig. 1). First, the energy cost normalized to the isometric force per cross-sectional area was independent of the tetanic duration in soleus. The longest of these contractions (15 s) was sufficient to deplete most of the high-energy phosphate pool. In the EDL, there was a decrease in the energy cost for force maintenance after approximately 9 s of tetanus. It is no longer clear that the mechanism for this decrease in rate of energy utilization is phosphorylation of the so-called regulatory light chain of myosin (18 000 Da light, LC-2f) (compare Crow & Kushmerick, 1982*b*; Butler, Siegman, Mooers & Barsotti, 1983). Because there is definitely a decrease in the rate of cross-bridge turnover as judged from the maximal velocity of shortening (Crow & Kushmerick, 1983), it appears that in mouse EDL as well as in soleus (Edwards, Hill & Jones, 1975) some other factor(s) must be involved in the change in rate of energy utilization; possible mechanisms include regulation by intracellular acidosis, accumulation of inorganic phosphate (Pi) or lowered ATP/ADP ratios. Unfortunately, the mechanism for this important aspect of muscle energetics and mechanics remains unknown.

The second major difference was in the magnitude of the energy cost per unit of force per cross-sectional area. For brief tetani, the energy cost in the fast-twitch EDL was 2·9 times that of the slow-twitch soleus. This was expected from the greater actomyosin-ATPase activity from EDL *versus* soleus muscle. After some 10 s of tetanus, however, the force-normalized energy cost in the EDL was reduced, so that it

was only 1·5 times that of the soleus. Because the mouse soleus contains a significant fraction of fast-twitch fibres, this three-fold dynamic range in ATP utilization rates represents an underestimate of the true range in mammalian muscle cells. These findings were based on recovery metabolism, measured as the total oxygen consumption above the baseline induced by contractile activity.

Another measure of the total energy cost for a contraction is obtained by direct measurements of the extent of chemical reaction in suitably prepared extracts of rapidly frozen muscles. These results (Crow & Kushmerick, 1982*a*) confirm the

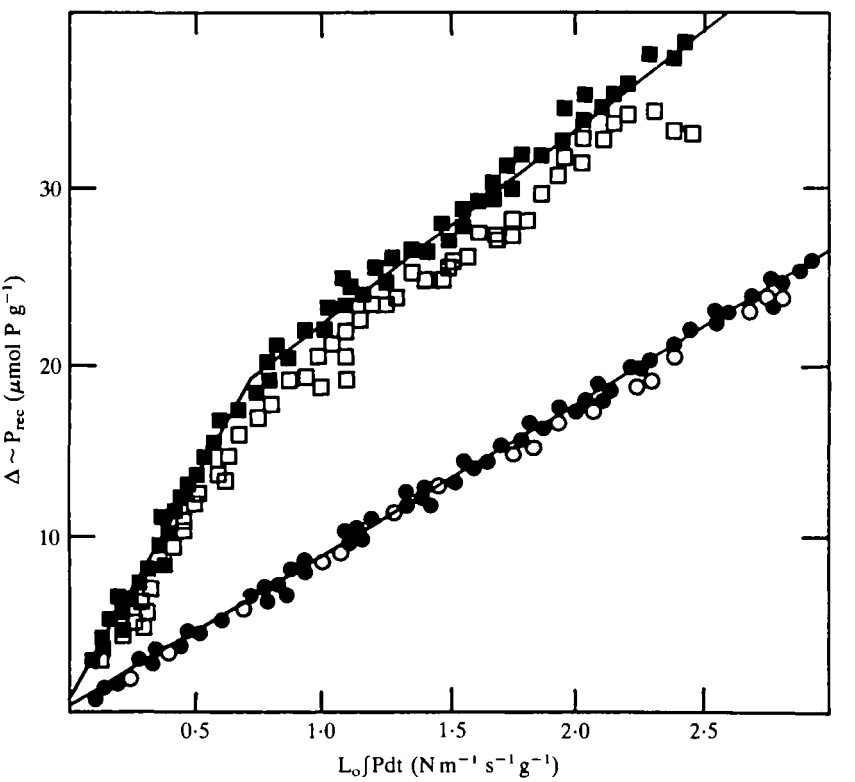

Fig. 1. The relationships between total high-energy phosphate utilization ($\Delta\sim P_{rec}$) and the tension-time integral for isometric tetani of mouse EDL (squares) and soleus (circles) at 20 °C. $\Delta\sim P$ is expressed in μmol P g^{-1}, and is the amount of high-energy phosphate resynthesized as calculated from recovery oxygen consumption alone (open symbols) or from recovery oxygen consumption plus recovery lactate production (closed symbols). The lines drawn through the data are linear functions fitted by least squares:

$$\text{soleus: } \Delta\sim P_{rec} = 0{\cdot}34 \pm 0{\cdot}71 + 8{\cdot}73 \pm 0{\cdot}51\, L_o\textstyle\int Pdt$$
$$\text{EDL: } \Delta\sim P_{rec} = 0{\cdot}68 \pm 0{\cdot}13 + 32{\cdot}4 \pm 3{\cdot}6\, L_o\textstyle\int Pdt$$

for tetani up to 4 s duration, $0 < L_o\int Pdt < 0{\cdot}75$

$$\Delta\sim P_{rec} = 11{\cdot}3 \pm 1{\cdot}5 + 11{\cdot}1 \pm 1{\cdot}2\, L_o\textstyle\int Pdt$$

for tetani longer than 8 s, $1{\cdot}0 < L_o\int Pdt < 2{\cdot}5$. Figure from Crow & Kushmerick (1982*a*), which should be consulted for details.

conclusions already given in Fig. 1. In a fully aerobic soleus muscle, a steady state was possible during a tetanus in which the rate of ATP synthesis matched the rate of ATP splitting. That is, in a continuous tetanic stimulation, the oxygenated muscle could be in a steady state wherein there was no further measurable extent of reaction involving Pi, PCr or ATP. This is illustrated by the data in Fig. 2 (open symbols) and by the oxygen consumption record shown in Fig. 3. If the soleus were anaerobic, the measured extent of high energy phosphate splitting would increase continuously during the maintained tetanus, i.e. chemical changes would not be confounded by aerobic resynthesis. This observation illustrates the wide dynamic range of aerobic capacity in the mouse soleus, and implies that the mitochondrial density and diffusion

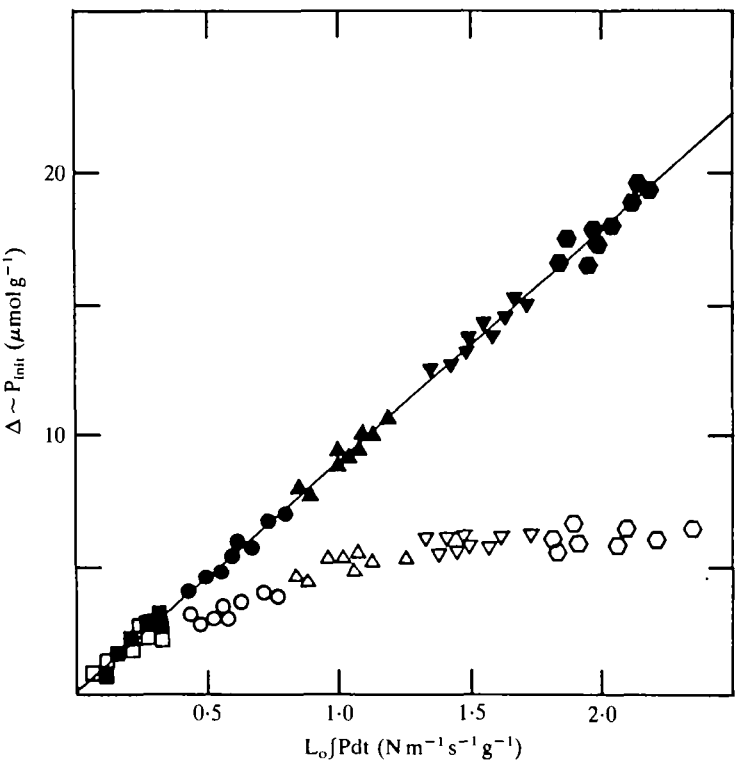

Fig. 2. The relationship between directly-measured initial chemical breakdown and tension-time integral in the soleus. Units are the same as in Fig. 1. Each datum point represents the initial chemical breakdown ($\Delta \sim P_{init}$) in a single muscle compared with its control from observed changes in content of ATP, PCr and Pi. The open symbols represent the initial chemical change assessed under aerobic conditions, whereas the closed symbols represent the initial chemical change assessed under anaerobic conditions. The solid line is the linear regression function fitting the anaerobic data:

$$\Delta \sim P_{init} = 0{\cdot}03 \pm 0{\cdot}34 + 8{\cdot}75 \pm 0{\cdot}98\, L_o\int Pdt.$$

The different symbols represent muscles stimulated for different tetanus durations. The symbols used are (□, ■), 1 s; (○, ●), 3 s; (△, ▲), 6 s; (▽, ▼), 9 s; (⬡, ⬢), 12 and 15 s. From Crow & Kushmerick (1982*a*).

of oxygen and substrates were sufficient to maintain PCr and ATP levels even during an isometric tetanus. This large aerobic capacity was not found in the fast-twitch EDL muscle, which also has a three-fold higher ATPase rate. These two muscles of the mouse illustrate nicely the extremes of energetic patterns in striated muscle (Kushmerick, 1983). The fast-twitch EDL can be described as a 'twitch now, pay later' type of cellular oxidative pattern. The slower soleus, which also has a greater mitochondrial density, follows more of a 'pay as you go' strategy of cellular respiration.

^{31}P-NMR MEASUREMENTS

The ability of modern nuclear magnetic resonance spectrometers to measure the tissue content of phosphate compounds relevant to muscle energetics has been well

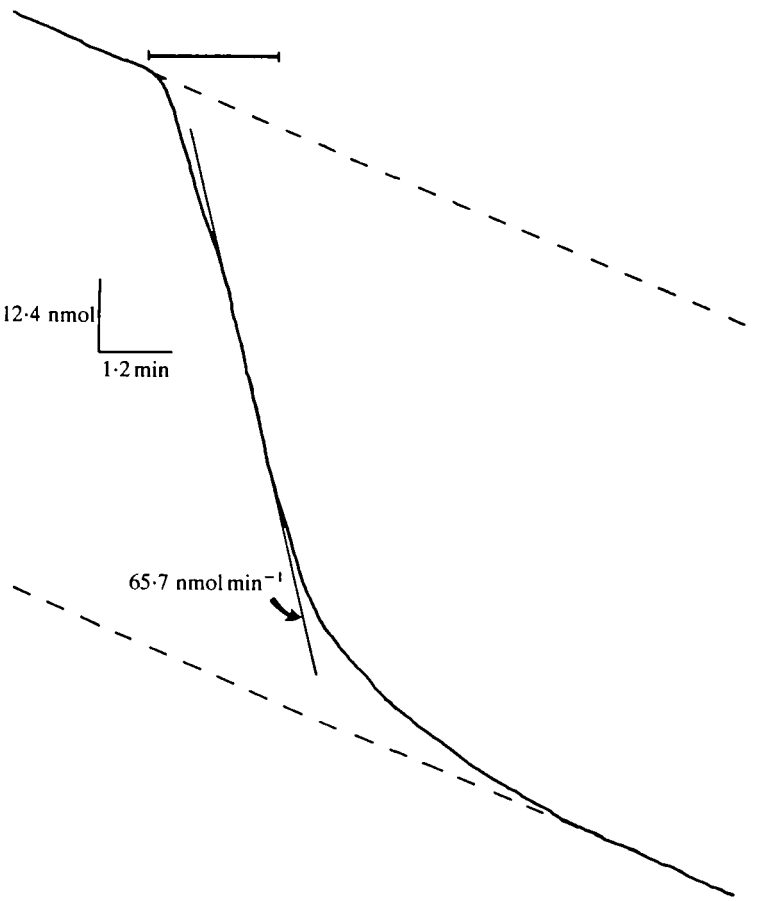

Fig. 3. Record of measurement of rate of oxygen consumption from one isolated mouse soleus muscle in a closed chamber at 20 °C; see Crow & Kushmerick, 1982*a* for experimental details. Partial pressure of O_2 is measured as a function of time; from this value, the solubility of oxygen in the physiological saline, and chamber volume, the oxygen consumption rate was calculated. The horizontal bar marks the time of a continuous isometric tetanus (105 s). The rate of oxygen consumption changed from the rate of the unstimulated muscle to a steady tetanic rate after 45 s of stimulation, and returned to the initial rate approximately 3 min after the end of the stimulation.

established (Gadian, 1982). Sufficiently large magnets exist to monitor these intracellular metabolites in intact human limbs (Ross *et al.* 1981). The first observation based on NMR results which is relevant to our purposes here is that the inorganic phosphate content of muscles in normal human limbs, in rat muscles and in well-perfused fast-twitch muscles (Meyer, Kushmerick & Brown, 1982) is substantially lower (approximately 1 μmol g^{-1}) than is usually reported from analyses of extracts of rapidly frozen tissues (5–7 μmol g^{-1}). It is likely that most of the discrepancy, if not all, is due to artefactual breakdown of phosphocreatine, a much more labile compound than ATP, during the extraction and/or freezing procedures.

The second relevant NMR observation is that there are important qualitative differences in the intracellular pH in response to contractile activity. It is well established that the intracellular pH can be measured from the chemical shift or spectral position of the inorganic phosphate peak (Moon & Richards, 1973), because the doubly protonated species has a chemical shift different from HPO_4^{2-}, and the measured chemical shift is proportional to the average number of each charged species in rapid equilibration with a midpoint at the pK_a. During stimulation and subsequent recovery, the intracellular pH changes are characteristically different in the fast-twitch and slow-twitch muscle. For example, during a 15-min period of steady-state twitches, the purely slow-twitch soleus undergoes a marked intracellular alkalinization from a control value of pH 7·1 to about pH 7·4 (Fig. 4). In these experiments, the extracellular pH was maintained with bicarbonate/CO_2 buffer at pH 7. The mechanism for the alkalinization is proton uptake during net PCr breakdown – a reaction to which we will return later. In the recovery period of the cat soleus, there was a moderate acidification to about pH 6·9. By way of contrast, in the fast-twitch biceps muscle little alkalinization was detected, even though the PCr breakdown was more rapid and proceeded to a greater molar extent per unit volume of muscle (data not shown). The lack of alkalinization is due to a greater intracellular buffer capacity in the fast-twitch than in the slow-twitch muscle, and to a concomitant glycolytic lactate production. During the recovery period, the acidification became quite marked, reaching values as low as pH 6·2. The biceps is a mixed muscle, with approximately equal proportions of fast-twitch oxidative glycolytic (FOG) and fast-twitch glycolytic (FG) fibres. Interestingly, the shape of the Pi peak broadened considerably during the recovery period in the biceps, but not in the soleus. This observation indicates a heterogeneity in fibre-to-fibre intracellular pH not present in the homogeneous soleus muscle but clearly demonstrated in the biceps. Thus there are detectable differences in the kinetics of metabolic responses in FOG and FG fibres, which comprise the heterogeneous cat biceps, whereas there are only slow-twitch fibres in the soleus, and it behaves homogeneously.

The third and most important set of ^{31}P-NMR observations that bears on our topic shows that twitch stimulations at various frequencies produce steady-state graded levels of intracellular PCr and Pi. That is, provided that the maximal aerobic capacity of the muscle is not exceeded, the graded decreased levels of PCr and increased levels of Pi are accompanied by graded levels of oxygen consumption. The data that demonstrate these conclusions were derived from studies of isolated cat biceps and

soleus muscles perfused through their arterial tree by a suspension of red cells in Krebs Henseleit buffer (Meyer, Brown & Kushmerick, 1985). The range of oxygen consumption rates at 27–30 °C in both types of *in vitro* perfused cat muscles are shown in Fig. 5. Contractile activity was induced by applying supramaximal twitches for 5–20 min to obtain the steady-state rate of oxygen consumption measured by arteriovenous differences in the total oxygen content of the perfusate delivered at constant flow. Whereas the biceps reached a maximal oxygen consumption rate of about 60 μmol O_2 min^{-1} 100 g^{-1} muscle at 40 twitches min^{-1}, the maximal rate of oxygen consumption of the soleus was not established – it was probably more than double that of the biceps. Fig. 5B shows that the levels of PCr and Pi attained in the steady state are functions of the oxygen consumption; all these data were obtained under conditions in which we were certain there was no limitation of oxygen supply, as the data in Fig. 5A indicate. Graded levels of intracellular metabolites were also observed

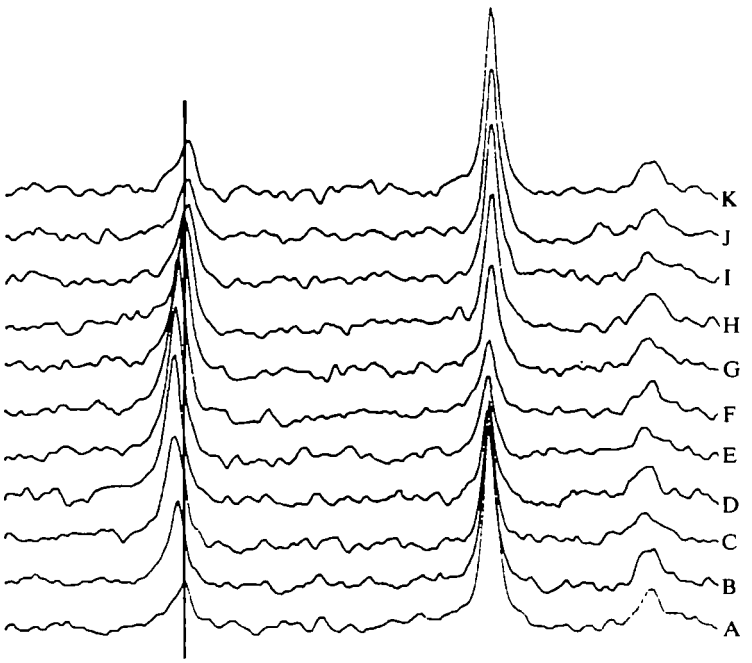

Fig. 4. Portions of ^{31}P-NMR spectra that show the chemical shift of the inorganic phosphate peak as a measure of intracellular pH during a stimulation-recovery cycle. Cat soleus was perfused with an erythrocyte-containing physiological saline (Meyer, Kushmerick & Brown, 1982) at 25 °C and mounted in a Bruker HX-270 spectrometer. The spectra were acquired in the Fourier transform mode using 90° pulses at intervals of 15 s at a frequency of 109·3 MHz. The three peaks shown are, left to right, Pi, PCr and ATP (γ phosphorus). Each set of spectra is aligned vertically at the frequency of PCr that is independent of pH under the conditions of this experiment. The vertical line through the Pi region indicates an intracellular pH of 7·1. A shift to the left indicates an alkalinization, and a shift to the right indicates an intracellular acidification. Sequential spectra taken every 2 min, eight scans averaged. (A) Unstimulated control; (B)–(F), sequential spectra during a 10-min period of maximal isometric twitches at 60 min^{-1}, showing an increase of intracellular pH to 7·4; (G)–(H), sequential spectra during the next 10-min period of recovery. Figure taken from Kushmerick, Meyer & Brown (1983).

in the gastrocnemius muscles of anaesthetized rats studied by ^{31}P surface coils, in which the twitches were delivered to the muscles *via* the sciatic nerve (Fig. 6) (Kushmerick & Meyer, 1985).

THE ROLE OF PHOSPHOCREATINE AND CREATINE KINASE

The rapid equilibration between phosphocreatine and ATP is considered to be important in muscle function as a rapidly-available source of energy. While not analysing this and other possible aspects of the physiological significance of creatine kinase function (Meyer *et al.* 1985; Jacobus & Ingwall, 1980), I would like to discuss what may be the most basic metabolic function of this reaction and the basis for understanding other secondary functions. That creatine kinase serves to buffer the ADP levels in the μmolar range is probably more important than its buffering of cellular ATP levels. Creatine kinase reaction simultaneously generates inorganic phosphate levels in the 1–20 mmol l^{-1} range. The cytosolic ADP level is well known to be an important metabolic regulator, including that of mitochondrial respiration (Lawson & Veech, 1979; Moreadith & Jacobus, 1982), and inorganic phosphate may well prove to be important in the regulation of glycolysis and the modification of actomyosin kinetics.

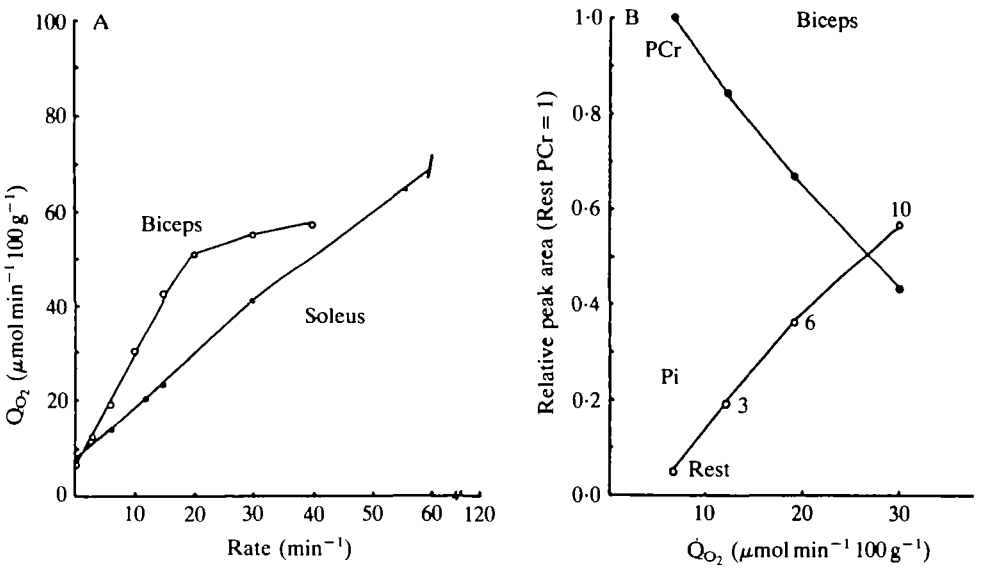

Fig. 5. (A) The steady-state oxygen consumption ($\dot{Q}_{O_2}$) of cat biceps and soleus muscles isolated and arterially perfused with an erythrocyte suspension at various frequencies of isometric twitch stimulation. (B) The relationship between PCr and Pi levels measured by ^{31}P-NMR (in arbitrary units) during steady-state oxygen consumption in the biceps muscle, given in panel A. Numbers by the traces indicated twitches per minute. Data taken from Meyer, Brown & Kushmerick (1985).

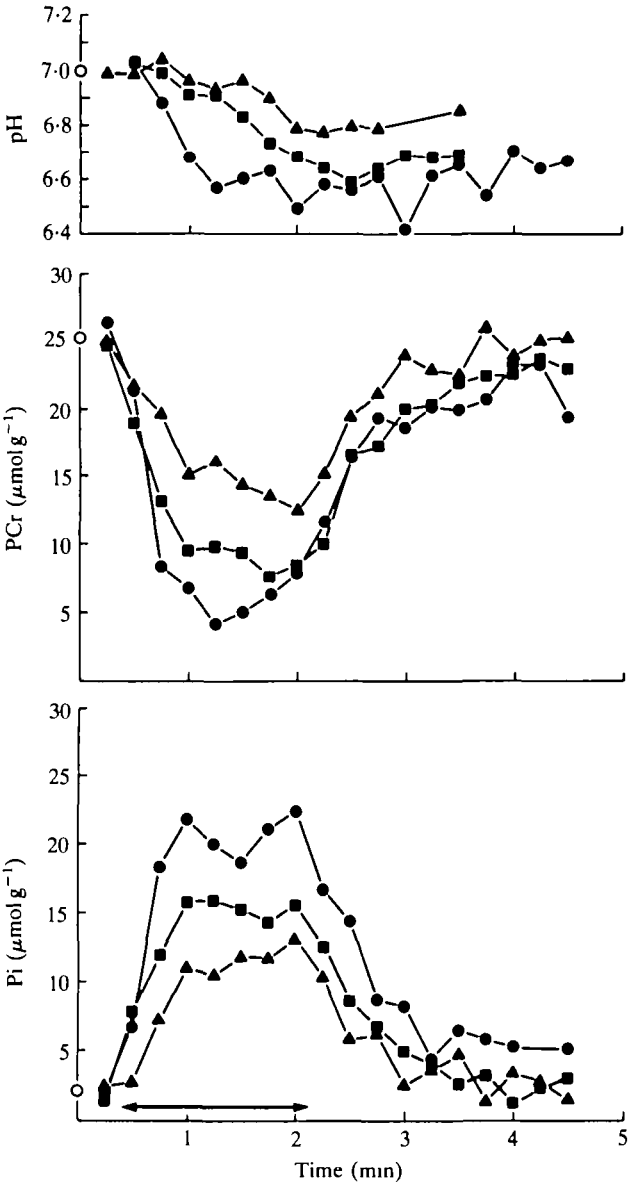

Fig. 6. Chemical changes in a rat lower limb musculature before, during and after 1·8-min stimulation of sciatic nerve at 2 Hz (▲), 4 Hz (■) and 10 Hz (●), obtained from ^{31}P surface coil NMR spectra. Top panel, intracellular pH; middle panel, PCr; bottom panel, Pi. Open symbols at origin represent average of control values for this animal obtained from resting muscle before stimulation and at least 15 min after each stimulation period. Figure from Kushmerick & Meyer (1985).

These aspects of creatine kinase function are easily appreciated by consideration of two sets of reactions. The first is:

$$ATP \leftrightarrow ADP + Pi + \alpha H^+, \quad (1)$$

where α is a function of pH; near pH 7 $\alpha \approx 0{\cdot}5$. This reaction is written reversibly, but of course different enzyme systems catalyse each direction. Actomyosin ATPase and other ATP hydrolases catalyse the reaction to the right. Mitochondrial respiration catalyses the reactions to the left. Substrate level phosphorylation is also a mechanism for ATP generation, but is quantitatively irrelevant for our purposes because the net extent of reaction is quantitatively small in the fully aerobic state.

The second reaction to consider is the truly reversible reaction catalysed by creatine kinase:

$$PCr + ADP + H^+ \leftrightarrow ATP + Cr. \quad (2)$$

The evidence for this reaction being reversible and near equilibrium in muscle cytosol and probably other cells containing creatine kinase has been well discussed elsewhere (Meyer *et al.* 1984). The net reaction, which has been demonstrated to occur in a great variety of muscles during contractile activity and discussed so far in this paper, is the following:

$$PCr + (1 - \alpha)H^+ \leftrightarrow Cr + Pi. \quad (3)$$

Because the forward and reverse fluxes of reaction 2 are faster than the total cellular ATPase activity or ATP synthesis rate (i.e. the fluxes of reaction 1), then reaction 2 can be considered near or at equilibrium. By writing the apparent equilibrium constant for reaction 2 for any particular pH, and rearranging, we obtain:

$$\frac{PCr}{Cr} = \frac{1}{Kck}\frac{ATP}{ADP}. \quad (4)$$

It is seen that the PCr/Cr ratio is directly proportional to the ATP/ADP ratio. At pH 7 and 1 mmol l^{-1} Mg^{2+}, the value of the apparent equilibrium constant (Kck) is approximately 100. The cellular concentration of PCr, Cr and ATP are in the mmolar range, typically 25, 6 and 7 mmol l^{-1} for unstimulated fast-twitch muscles, respectively. However, ADP concentration is in the range of 1–10 μmol l^{-1}. Thus for a ten-fold change in the ratio of PCr/Cr, a ten-fold change in the ratio of ATP/ADP is easily accomplished with no measurable decrement in ATP, and with an increase in cytosolic ADP, perhaps to 100 μmol l^{-1}, a concentration which is less than double the apparent K_m for the nucleo-tide translocase, but below the limits of detectability in typical ^{31}P-NMR experiments. Of course, a small decrease in ATP of about 0·1 mmol l^{-1} must occur when ADP increases.

There is also evidence that the more thermodynamically appropriate quantity that regulates mitochondrial respiration in the cell is the phosphorylation potential (PP) (Erecínska, Wilson & Nishiki, 1978), which is defined as:

$$PP = \frac{ATP}{ADP \cdot Pi} \cdot \tag{5}$$

It should be emphasized that this quantity ignores the large and differential changes in specific fibre types in intracellular pH that are known to occur during and after contractile activity, as I have emphasized in the preceding sections. Thus on thermodynamic as well as factual grounds, the phosphorylation potential has its limitations for mechanistic interpretation. Nonetheless, there are expected and observed correlations between the magnitude of the phosphorylation potential and the PCr, Cr and Pi levels and metabolic regulation, i.e. between any metabolic function coupled to the phosphorylation potential and equation 3.

Combining equation 1 with equation 3, we get:

$$\frac{PCr}{Cr} \cdot \frac{1}{Pi} = \frac{PP}{Kck} \cdot \tag{6}$$

The point of all of these quantitative aspects is the view that the PCr/Cr ratio bears a predictable relationship to the ATP/ADP ratio in the cell. Thus the observed simple relationship between the steady-state levels of PCr and Pi and oxygen consumption described in our particular examples in Figs 4 and 5 is easily understood.

POORLY-METABOLIZABLE SUBSTRATES FOR THE CREATINE KINASE REACTION

For some years, several laboratories (e.g. Fitch *et al.* 1975; Mahanna, Fitch & Fischer, 1980; Woznicki & Walker, 1980) have studied analogues of creatine. The most common of these is β-guanidinopropionate (β-GPA), which can be phosphorylated by the reversal of reaction 2, but very slowly, since the K_m for β-GPA is higher, and the V_m is substantially lower than that of creatine under comparable conditions. When the analogue is fed to animals over the course of 6–12 weeks, the analogue is incorporated into the muscles, hearts and brains of the animals, displaces much of the creatine phosphate and creatine normally present, and is itself phosphorylated. In prolonged contractile activity, it can be shown that the phosphorylated β-GPA can be split very slowly, but in bursts of brief contractile activity this does not occur. One might argue that the survival of these animals demonstrates that the creatine kinase reaction plays no role in normal muscle function; however, but this view is incorrect.

On the basis of the previous discussion, I propose that it is relatively easy to understand the energy metabolism of these animals, as well as in normal animals. If no other metabolic adaptation has occurred (and this has not been tested systematically),

contractile activity should induce large changes in the ATP/ADP ratio even with relatively mild contractile efforts because of the absence or near-absence of equation 2 in the analogue-fed animals. In these analogue-fed animals, one would expect the oxygen consumption not to show the graded response obtained in normal animals, but to be turned on to a muscle's maximal capacity, and more rapidly in time following the onset of contractile activity precisely because of the absence of the creatine kinase reaction (equation 2). We have shown in preliminary experiments that analogue-fed animals in which the phosphorylated form of β-GPA accumulates in rat skeletal muscle and heart, and in which the creatine phosphate levels are no more than 5 % of normal values, can be induced to run on a treadmill and have nearly the same maximal oxygen consumption as control animals. There is therefore no obvious limit to the normal maximal oxygen consumption in the presence of the analogue. To date, no one has evidence regarding the kinetics of oxygen consumption following exercise either in these animals or in their isolated muscles. The prediction is straightforward, and the experiment is relatively easy to do.

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