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An investigation of arterial insufficiency in rat hindlimb

A combined ^{31}P-n.m.r. and bloodflow study

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1. A small animal model of arterial insufficiency is presented which involves unilateral femoral artery ligation and section. 2. Invoked alterations in metabolism and perfusion of the affected muscle mass have been investigated 12 h, 4, 7 and 14 days post-ligation by ^{31}P-n.m.r. and microsphere infusion, both at rest and during isometric muscle contraction at 1 Hz. 3. At rest, the concentration of phosphocreatine was similar to the mean control value (36.0 ± 1.0 mM) from 4 days post-ligation, but was significantly lower at 12 h (28.5 ± 3.6 mM). Inorganic phosphate concentrations were significantly elevated for 7 days post-ligation. No significant differences were noted in intramuscular pH. 4. Upon stimulation of the affected muscle mass, a time-dependent improvement in phosphocreatine utilization was observed such that 14 days post-ligation phosphocreatine utilization was not significantly different from mean control values. A similar amelioration was noted for the contraction-induced fall in intramuscular pH. 5. At rest, no significant differences in bloodflow to the muscles of the ligated limb compared with the unaffected contralateral limb were observed. However, isometric contraction of the affected muscle mass resulted in a markedly reduced hyperaemic response 12 h post-ligation. Thereafter, a time-dependent improvement in tissue perfusion during stimulation was observed which paralleled the improvements in phosphocreatine utilization and intramuscular pH changes. 6. The results presented are discussed with respect to the interrelationship between oxygen delivery, high energy phosphate utilization and force maintenance.

INTRODUCTION

During the course of development, mammalian skeletal muscle fibres become specialized for the performance of different physiological tasks. However, the physiological and biochemical features of mature skeletal muscle fibres retain some degree of plasticity and can be influenced by external factors; for example, indirect stimulation of muscles has been shown to produce a fundamental rearrangement of the physiological, metabolic and ultrastructural characteristics (Pette *et al.*, 1975; Eisenberg & Salmons, 1981) and that this response is primarily due to the increased contractile activity (Hudlická *et al.*, 1982) has been demonstrated in rats subjected to a prolonged endurance exercise-training programme (Green *et al.*, 1983, 1984). The increase in contractile activity results in increased capillary density (Andersen & Henriksson, 1977) and maximal activities of the oxidative enzymes (Baldwin *et al.*, 1972; Pette *et al.*, 1973; Holloszy & Booth, 1976; Heilig & Pette, 1980).

It has been suggested that the signal triggering mitochondrial proliferation/enzyme induction and increased capillarity may be invoked by transient hypoxic insults which occur in the initial stages of increased contractile activity; this view is supported by the observations that patients with arterial insufficiency have increased maximal activities of the oxidative enzymes and increased capillary supply to muscle fibres of the affected limb (Bylund *et al.*, 1976; Hammarsten *et al.*, 1980; Bylund-Fellenius *et al.*, 1981). However, when the relationship between the delivery of oxygen to contracting skeletal muscle, force maintenance and ATP turnover has been addressed, conflicting conclusions have been drawn. Monitoring cellular redox status of NAD by surface fluorescence in dog gastrocnemius and gracilis muscles suggested that oxygen delivery never limits the mitochondrial respiratory chain (Jöbsis & Stainsby, 1968), whereas a recent investigation by Idström *et al.* (1985) using the perfused rat hindlimb preparation has demonstrated a correlation between oxygen supply, force maintenance and the rate of high-energy phosphate utilization. ^{31}P-n.m.r. has been used to investigate intramuscular changes in high-energy phosphates and pH in patients with arterial insufficiency both at rest and during voluntary exercise (Keller *et al.*, 1985; Hands *et al.*, 1986). In both studies claudicants were seen to utilize more phosphocreatine and acidify to a greater degree than healthy age-matched controls during exercise; slower recovery rates were also observed for both parameters on cessation of exercise. In the present study, we have used unilateral femoral artery ligation of rats as a model of arterial insufficiency in which to investigate the importance of oxygen delivery to the hypoperfused muscle mass. ^{31}P-n.m.r. has been used to monitor high-energy phosphate status of skeletal muscle of sham-operated and ligated animals both at rest and during isometric contraction in conjunction with measurement of bloodflow by the microsphere technique.

MATERIALS AND METHODS

Animal preparation

Male Wistar rats (200–240 g) were obtained from Bantin and Kingman, Hull, U.K., and were maintained in the Department's animal house for at least 5 days

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before use. Under light anaesthesia with ether, the artery–vein–nerve bundle of the left thigh was exposed and the femoral artery was isolated by blunt dissection. Two silk ligatures were tied around the artery about 1 cm proximal to the epigastric bifurcation and the artery was cut between the ligatures. Sham-operated controls were treated likewise except that the artery was not ligatured or cut. The wound was sutured and animals allowed to recover.

Microsphere or ^{31}P-n.m.r. studies were carried out 12 h–14 days after operation. Anaesthesia was induced with pentabarbital (60 mg/kg, intraperitoneal). The left sciatic nerve was exposed and two Teflon-coated silver electrodes were sewn into place in contact with the nerve. The wound was sutured and brass pins forced through the knee and ankle joints. The prepared limb was placed in a specially designed perspex cradle. The distal tendon of the gastrocnemius muscle was attached to a force transducer and the muscle was adjusted to the optimal length for maximal isometric contraction; the whole apparatus was then placed in the vertical bore of the magnet. Rectal temperature was monitored and maintained at 37 °C by blowing warm air over the animal. Anaesthesia was maintained using 0.5–1% halothane in N_2O/O_2 (1:1) delivered via a face mask.

N.m.r spectroscopy

All experiments were performed at 73.84 MHz in a vertical wide bore 4.3 T magnet. A three-turn surface coil (14 mm outer diameter) was placed over the gastrocnemius muscle and served both as transmitter and receiver. As the signal intensity is negligible a radius away from the plane of the surface coil (Ackerman *et al.*, 1980), gastrocnemius muscle is predominantly observed with a small contribution from plantaris and soleus muscles. Spectra were collected as 1024 data points using a sweep-width of $\pm$2000 Hz, a pulse width of 15 μs (nominal tip-angle of 70° at centre of coil) and a recycle time of 2 s (10 s for fully-relaxed spectra). Spectra were the result of 64 transients and were zero-filled; all spectra were referenced to the proton resonance of tissue water which had been set to a zero-offset on resonance after adjusting the magnetic field homogeneity (Gadian *et al.*, 1979). At the beginning of each experiment, a fully-relaxed spectrum was collected for quantification of phosphorus-containing metabolites. At least two resting spectra were taken and the muscle was then stimulated supramaximally (50 μs, 45 V) at 1 Hz for 7 min (three spectra), the muscle was allowed to recover for 15 min (seven spectra) and was then restimulated at 1 Hz for 15 min followed by a 15 min recovery period. Any variations in this protocol are detailed in the Figure legends. At the end of the final recovery period, gastrocnemius muscles were freeze-clamped for subsequent metabolite analysis.

Physiological studies

Experimental protocols were as described above, except that animals were anaesthetized with urethane [25% (w/v); 5 ml/kg, intraperitoneal] and mounted in a rigid animal frame (Byrne *et al.*, 1985).

Metabolite analysis

Freeze-clamped muscle samples were stored at 77 K and analysed for ATP and creatine-plus-phosphocreatine by using standard chemical techniques (Bergmeyer, 1974).

Bloodflow measurements

Bloodflow was assessed by a modification of the microsphere method of Armstrong & Laughlin (1983). In the present study we were only concerned with assessing the relative impairment in bloodflow caused by unilateral section of the femoral artery; because of this, no attempt was made to convert bead number into a quantitative index of bloodflow. The bilateral symmetry of microsphere distribution was confirmed by comparison of the bead content of kidneys and testes.

Anaesthesia was induced and maintained as described above (n.m.r. section); animals were studied either at rest or during bilateral nerve stimulation of 1 Hz. In each case a fine cannula was passed down the left carotid artery into the left ventricle. Pre-mixed microspheres (^{46}Sc; 15 μm, NEN-TRACTM microspheres, 10 mCi/g; 400000 beads/ animal) were infused at rest or in the fifth min of bilateral nerve stimulation (i.e. at a point in the stimulation at 1 Hz when the phosphocreatine concentration and pH had become constant). The cannula was flushed with warm saline and the animal was killed 1 min later. Tissue samples were dissected and counted in a Packard Auto-gamma 5320 scintillation spectrometer.

Materials

All enzymes and cofactors used were obtained from Boehringer, Lewes, E. Sussex, U.K.

RESULTS AND DISCUSSION

Resting muscle

^{31}P-n.m.r. spectra of the rat gastrocnemius muscle at rest are presented in Fig. 1. Spectrum (*a*) was obtained from a sham-operated animal; spectrum (*b*) was obtained from an animal 7 days after ligation and section of the left femoral artery. Biochemical analysis of freeze-clamped gastrocnemius muscle from sham-operated rats gave an

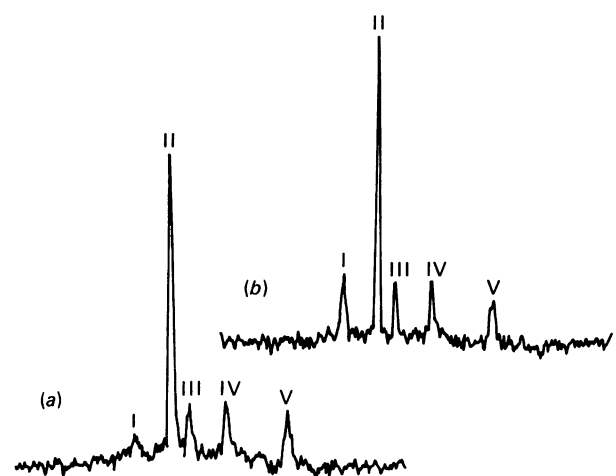

Fig. 1. ^{31}P-n.m.r. spectra of rat gastrocnemius muscle at rest

Fully-relaxed spectra were obtained as described in the Materials and methods section. The lower trace (*a*) is from a sham-operated animal and the upper trace (*b*) is from an animal 7 days after unilateral femoral artery ligation and section. Spectral assignments are: I, P_i; II, phosphocreatine; III, IV and V, the γ, α and β resonances of ATP respectively.

Table 1. Resting concentrations of phosphocreatine and P_i and pH in the gastrocnemius muscle determined by ^{31}P-n.m.r. for sham-operated rats and rats studied 0.5–14 days after unilateral femoral artery ligation and section

Concentrations of phosphocreatine and P_i were determined from peak areas of fully-relaxed muscle spectra, where the ATP concentration had been determined in neutralized $HClO_4$ extracts of the muscle. Intracellular pH was assigned from the chemical shift of P_i relative to the phosphocreatine resonance. Values are means ± S.E.M. for at least four separate determinations. Statistical significance was determined using Student's *t*-test and is indicated by $*P < 0.05$, $**P < 0.01$.

Ischaemic period (days)	[Phosphocreatine] (mM)	[P_i] (mM)	pH_i
Sham-operated	36.0±1.0	3.6±0.4	7.02±0.02
0.5	28.5±3.6**	13.3±3.7*	6.84±0.13
4	38.7±0.6	6.4±1.0*	6.98+0.06
7	41.2±2.2	6.6±1.3*	6.95±0.02
14	39.1±5.1	5.3±1.2	7.00±0.06

ATP concentration of 6.65±0.15 μmol/g of tissue and a total creatine (creatine-plus-phosphocreatine) concentration of 27.3±0.8 μmol/g of tissue. There is good evidence that chemically determined ATP is entirely n.m.r. visible (Dawson *et al.*, 1980; Meyer *et al.*, 1982); thus, using the respective chemically determined values for each animal studied and assuming that the volume of intracellular water is 0.67 ml/g wet wt. in gastrocnemius (Sahlin, 1978; Sjogaard & Saltin, 1982), the concentrations *in vivo* of phosphocreatine and inorganic phosphate (P_i) in resting gastrocnemius muscle have been calculated from fully-relaxed ^{31}P-n.m.r. spectra and are presented in Table 1.

The concentrations of phosphocreatine and P_i and pH_i for resting muscle from the four femoral artery ligated groups studied (i.e 0.5, 4, 7 and 14 days post-ligation) displayed some variations from control values. The concentration of phosphocreatine was significantly lower in the 0.5 day group and P_i was significantly elevated in the 0.5, 4 and 7 day groups (Table 1). The intramuscular pH (pH_i, which was calculated from the chemical shift of P_i; see Gadian *et al.*, 1979) was not significantly different to the sham-operated group at any time-point (Table 1). Thus, femoral artery ligation and section produces only mild alterations in the concentrations of metabolites and pH in gastrocnemius muscle at rest (see also Hayes *et al.*, 1985). In contrast, acute restriction of bloodflow by a tourniquet placed around the upper part of the rat hindlimb results in complete, irreversible depletion of high-energy phosphates in 4 h (Thulborn, 1981). This suggests that the restriction of bloodflow at the level of the femoral artery permits perfusion of the muscles of the lower leg via a collateral circulation.

Metabolic and physiological response to muscle stimulation

Isometric contraction of the gastrocnemius/plantaris muscles at a frequency of 1 Hz revealed marked differences in phosphocreatine utilization and pH_i in the four groups studied compared with sham-operated control animals. The time-course of changes in phosphocreatine concentration and pH_i are shown in Figs. 2 and 3 respectively.

Sciatic nerve stimulation for 7 min for animals 12 h after femoral artery ligation resulted in a 70% fall in phosphocreatine concentration and a fall in pH_i to 6.41±0.09 in the gastrocnemius/plantaris muscles; at this point in the protocol, sham-operated animals depleted phosphocreatine by 25% with no change in pH_i (7.02±0.02).

In the other three time groups (i.e. 4, 7 and 14 days post-femoral artery ligation), stimulation provoked similar, but less extreme, increases in phosphocreatine utilization and falls in pH_i. A clear time-dependent post-operative improvement was apparent; this improvement is perhaps most clearly observed in Fig. 2. During the second period of stimulation (for 15 min at 1 Hz), the phosphocreatine concentration fell to constant levels of 40%, 55% and 65% of the resting phosphocreatine concentration for the 4, 7 and 14 days femoral artery ligated groups respectively. A similar time dependence was observed for pH_i (Fig. 3).

Animals 7 days after femoral artery ligation have been studied in greater depth. Fig. 4 shows twitch-tension and half-relaxation time data for sham-operated and 7 day post-operative animals. In sham-operated animals, stimulation of the sciatic nerve at 1 Hz produced a staircase response (i.e. the developed force was greater than the initial twitch-tension) with a concurrent decrease in half-relaxation time (Fig. 4). In the 7 day ligated group a staircase response was also observed; however, continuation of stimulation caused a rapid decline in twitch-tension and a prolongation of the half-relaxation time (an index of fatigue; see Edwards *et al.*, 1975*a*,*b*).

Thus, subjecting animals that had 7 days previously undergone femoral artery ligation and section to isometric muscle contraction at 1 Hz causes a 45% decrease in phosphocreatine concentration, a 0.25 unit fall in pH_i and an inability to maintain force development.

Recovery from stimulation

In sham-operated control animals, phosphocreatine is rapidly resynthesized on termination of stimulation (Fig. 2). Resynthesis of phosphocreatine in the sarcoplasm is catalysed by the near-equilibrium enzyme creatine phosphokinase (Beis & Newsholme, 1975; Veech *et al.*, 1979; Gadian *et al.*, 1981; Shoubridge *et al.*, 1984); thus, the sarcoplasmic phosphocreatine concentration will depend on the concentrations of the other species participating in the reaction equilibrium:

$$[\text{phosphocreatine}] = \frac{[\text{Mg}\cdot\text{ATP}][\text{creatine}]}{K_{\text{eq.}}[\text{Mg}\cdot\text{ADP}]\,[\text{H}^+]}$$

The concentrations of ATP and total creatine

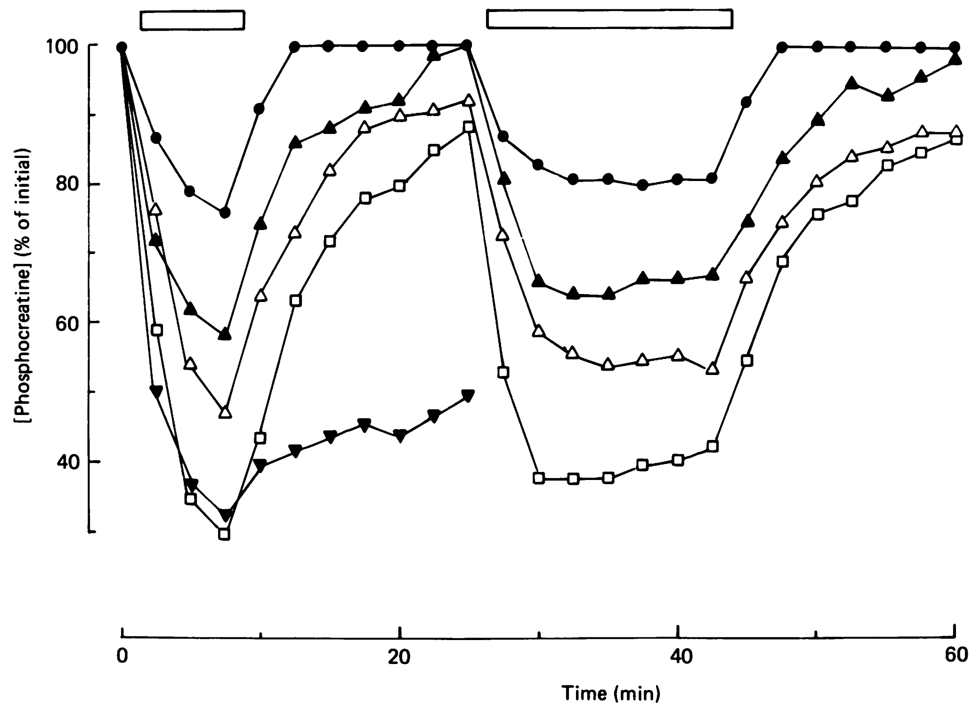

Fig. 2. Relative changes in phosphocreatine concentration in the gastrocnemius muscle during stimulation at 1 Hz and during recovery in sham-operated control animals (●) and those that underwent femoral artery ligation and section 0.5 (▼), 4 (□), 7 (△) and 14 days (▲) prior to the experiment

Stimulation periods are indicated by the open boxes. Values shown are means of six, three, twelve, seven and three experiments respectively. Error bars have been omitted for clarity. Experiments were discontinued after the first recovery period in the 0.5 day group.

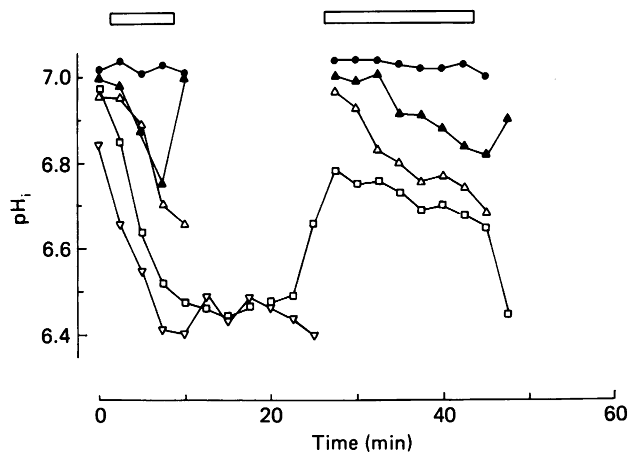

Fig. 3. Changes in intracellular pH in gastrocnemius muscle during stimulation at 1 Hz and during recovery in sham-operated control animals (●) and those that underwent femoral artery ligation and section 0.5 (▽), 4 (□), 7 (△) and 14 days (▲) prior to the experiment

Stimulation periods are indicated by the open boxes. The pH was determined from the chemical shift of P_i with reference to the phosphocreatine resonance as described in the Materials and methods section. Values shown are means of six, three, twelve, seven and three experiments respectively. Error bars have been omitted for clarity. Experiments were discontinued after the first recovery period in the 0.5 day group.

(creatine + phosphocreatine) do not change significantly during 1 Hz muscle stimulation; it therefore follows that the concentration of phosphocreatine at any time during the recovery period will be determined by the cytosolic free ADP concentration and pH_i.

Considering the recovery data presented in Figs. 2 and 3, it can be seen that recovery from stimulation of the muscle mass of animals 12 h after ligation of the femoral artery is very slow, the intracellular pH remains low (6.48 ± 0.05 after 10 min recovery) and the phosphocreatine resynthesis rate is markedly reduced, suggesting an inhibition or substrate limitation of oxidative processes (Horstman *et al.*, 1976; Harris *et al.*, 1976; Sahlin *et al.*, 1979) and a failure of secondary active transport mechanisms responsible for recovery of pH_i (Roos & Boron, 1981; Arnold *et al.*, 1984). Recovery from stimulation of the muscle mass 4 days after femoral artery ligation (Figs. 2 and 3) again reveals a very slow recovery of pH_i; however, in this case, there is phosphocreatine resynthesis (recovery to $85 \pm 4\%$ of the pre-stimulation phosphocreatine concentration in 15 min, half-recovery time 4–5 min; see Fig. 2) and, upon restimulation at 1 Hz, phosphocreatine and pH_i approach steady-state values (Figs. 2 and 3).

For 7 and 14 day post-operative animals our data on pH_i recovery are incomplete. This is a consequence of the fact that in both groups the recovery of inorganic phosphate (P_i) concentrations to pre-stimulation values was rapid (half-recovery time $\leqslant 2$ min for both groups) and in some experiments the P_i concentration fell to

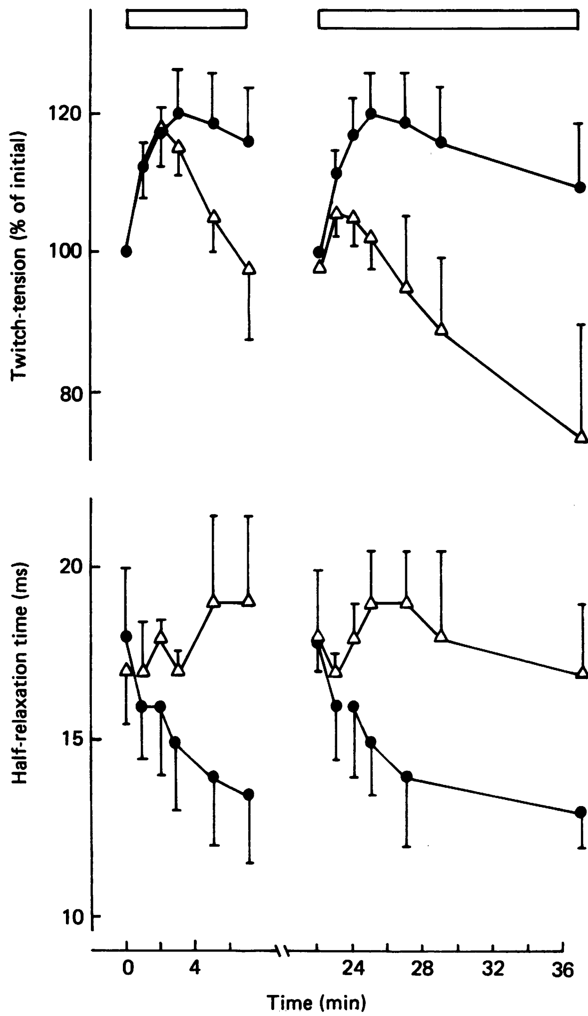

Fig. 4. Changes in twitch-tension and half-relaxation time in the gastrocnemius muscle of control (●) and 7 day femoral artery ligated and sectioned (△) rats during periods of stimulation at 1 Hz

Stimulation periods are indicated by the open boxes. The values are means ± S.E.M. for six sham-operated animals and seven animals 7 days after femoral artery ligation and section.

below pre-stimulation levels such that it disappeared into spectrum noise (results not shown). The half-recovery time of phosphocreatine appeared to be slower (4 min for 7 day group, 3 min for 14 day group) than that for P_i and free ADP (Hayes *et al.*, 1985); although the time-resolution of the current experiments (2 min spectral acquisition) was inadequate to quantify this difference, similar discrepancies in the rates of phosphocreatine resynthesis and P_i and ADP disappearance have been reported and discussed for human muscle after voluntary exercise (Taylor *et al.*, 1983; Arnold *et al.*, 1984).

Bloodflow at rest and during stimulation

A full interpretation of the observations made above using the ^{31}P-n.m.r. technique requires a knowledge of bloodflow to the affected limb both at rest and during (steady-state) muscle contraction. The radiolabelled microsphere technique has been used to determine the relative bloodflow to the gastrocnemius, plantaris and

Table 2. Relative bloodflow assessed using ^{46}Sc-labelled microspheres to the right (C) and left (I) gastrocnemius, plantaris and soleus muscles and testes of sham-operated rats or rats that underwent unilateral left femoral artery ligation and section 0.5–14 days prior to study

Microspheres were infused at rest or 5 min after commencing stimulation at 1 Hz (see the Materials and methods section). For each experiment, variations of bilateral microsphere distribution to the kidneys were less than 10% (results not shown). Values are means ± S.E.M. with the number of experiments shown in parentheses. Statistical significance (C versus I) was assessed using Student's *t*-test (for paired observations) and is indicated by $*P < 0.05$, $**P < 0.01$.

Ischaemic period (days)	Relative blood flow (beads/g of muscle)							
	Gastrocnemius		Plantaris		Soleus		Testis	
	C	I	C	I	C	I	C	I
At rest								
0	118 ± 14 (4)		162 ± 32 (4)		380 ± 32 (4)		303 ± 64 (4)	
0.5	125 ± 10 (7)	140 ± 20 (7)	130 ± 8 (7)	103 ± 17 (7)	425 ± 64 (7)	467 ± 84 (7)	277 ± 42 (7)	276 ± 38 (7)
7	116 ± 28 (4)	86 ± 19 (4)	142 ± 41 (4)	90 ± 21 (4)	337 ± 100 (4)	250 ± 79 (4)	342 ± 100 (4)	320 ± 89 (4)
14	127 ± 11 (6)	84 ± 10 (6)*	156 ± 25 (6)	102 ± 16 (6)	513 ± 58 (6)	501 ± 75 (6)	231 ± 43 (6)	255 ± 44 (6)
Stimulation								
0	600 ± 55 (6)		596 ± 29 (6)		1561 ± 215 (6)		365 ± 82 (6)	
0.5	722 ± 69 (5)	276 ± 45 (5)**	795 ± 35 (5)	294 ± 57 (5)**	1678 ± 244 (5)	500 ± 87 (5)*	202 ± 18 (5)	211 ± 23 (5)
7	668 ± 98 (7)	394 ± 59 (7)*	647 ± 145 (7)	410 ± 79 (7)*	1054 ± 141 (7)	517 ± 107 (7)**	329 ± 40 (7)	305 ± 44 (7)
14	670 ± 56 (5)	620 ± 71 (5)	775 ± 95 (5)	599 ± 66 (5)	1396 ± 221 (5)	939 ± 274 (5)	295 ± 38 (5)	301 ± 49 (5)

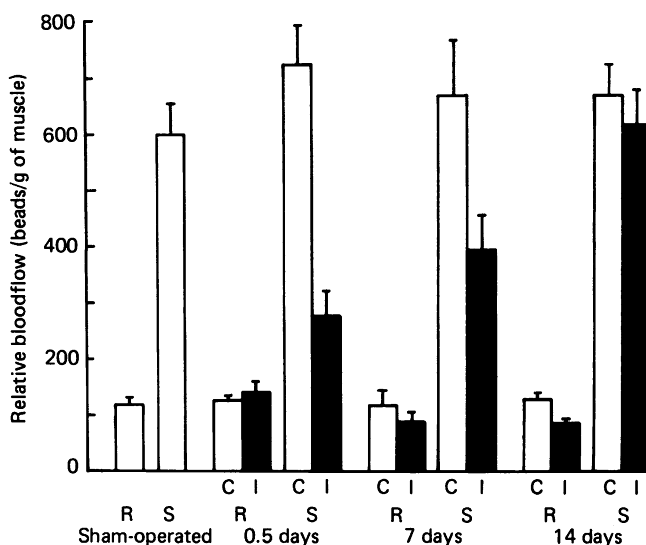

Fig. 5. Relative bloodflow assessed using the ^{46}Sc-microsphere technique to the left (I) and right (C) gastrocnemius muscles of sham-operated control rats or rats that underwent unilateral left femoral artery ligation and section 0.5–14 days prior to study

Microspheres were infused either at rest (R) or 5 min after commencing stimulation at 1 Hz (S). The results are shown as means ± S.E.M. The number of experiments performed and the statistical analysis are given in Table 2.

soleus muscles of the hindlimb both at rest and during stimulation in sham-operated animals and in animals 12 h, 7 and 14 days after unilateral femoral artery ligation and section (Table 2). It is emphasized that because flow is not quantified by withdrawal of a reference blood sample (see Armstrong & Laughlin, 1983), the only valid statistical analysis which can be made is a comparison of the bead number trapped in the muscles of the ligated and contralateral limb of a given experimental animal. However, referral to Table 2 shows the extremely low variability for control values obtained for each tissue investigated. Thus, it is felt appropriate to make some comments with regard to the increase in bloodflow to each muscle caused by stimulation at a frequency of 1 Hz (Table 2, Fig. 5). In addition, the kidneys and testes were taken from each animal studied to confirm the bilateral distribution of microspheres; in each of the 44 individual experiments reported there was less than 10% variation in bead distribution to these organs (see Table 2 for testes data).

Bloodflow at rest to the ligated and contralateral limbs are given in Table 2. At 12 h after unilateral ligation, bloodflow to the affected limb was 112%, 79% and 110% of the control values for gastrocnemius, plantaris and soleus muscles respectively. This may reflect the reactive hyperaemia of acute onset due to vasodilatation of the peripheral vascular bed reported by Thulesius (1962) to be a haemodynamic consequence of arterial occlusion. For animals 7 and 14 days after unilateral ligation, bloodflow to the affected limb was generally reduced to 65–90% of the blood flow to the contralateral limb; this reduction in resting perfusion only reached statistical significance in the gastrocnemius muscle of 14 day post-operative animals ($P < 0.05$, Table 2).

Bloodflow during stimulation at 1 Hz to the ligated and contralateral gastrocnemius, plantaris and soleus muscles are given in Table 2. From the data obtained for control animals it can be seen that stimulation at this frequency caused a 4–5-fold increase in bloodflow (Fig. 5).

At 12 h after unilateral ligation, the stimulation-induced increase in bloodflow is severely restricted in all three muscles investigated. Thus, the hyperaemic response in the ligated limb is 38%, 37% and 30% of that observed for the contralateral gastrocnemius, plantaris and soleus muscles respectively. As has been discussed above, this reduction in the stimulation-induced hyperaemic response has marked metabolic consequences as revealed by ^{31}P-n.m.r. For 7 day post-operative animals the increase in bloodflow to the ligated limb is 59%, 63% and 49% of the hyperaemia observed in the contralateral gastrocnemius, plantaris and soleus muscles. At 14 days post-ligation these figures are 92%, 77% and 67% respectively and there is no longer a statistical significant difference in bloodflow to the two limbs (Table 2).

The time-dependency of the improvement in perfusion of the ligated limb is illustrated histographically for the gastrocnemius muscle in Fig. 5.

Concluding remarks

In the present study ^{31}P-n.m.r. spectroscopy and the microsphere technique have been used to elucidate changes in muscle metabolism and bloodflow to the hindlimb of the rat afer ligation of the left femoral artery. A remarkable adaptability has been revealed; it has been shown that a muscle caused to contract 12 h after ligation experiences a greatly diminished hyperaemic response in muscles distal to the ligation and the muscle cannot maintain phosphocreatine concentration and experiences a marked fall in pH_i; furthermore, recovery from stimulation has not been observed. In contrast, 14 days after ligation, stimulation of the distal muscle mass caused a hyperaemic response which is approx. 70–90% of that of the non-ligated limb and consequently the changes in phosphocreatine concentration and pH_i observed by ^{31}P-n.m.r. are modest and recovery after termination of stimulation is rapid.

How does this time-dependent improvement in muscle performance come about? Direct circumvention of the ligation by new vascular growth seems very unlikely: the femoral artery is double ligatured and sectioned, therefore there is a 5–8 mm section of artery removed from the artery–vein–nerve bundle. In similar studies to the current investigation, Thulesius (1962) observed that acute occlusion of the femoral artery in the cat caused a reactive hyperaemic response which was due to vasodilatation of the peripheral vascular bed. However, collateral vasodilatation was slower in onset (Thulesius, 1962; Sanne & Sivertsson, 1968). Activation of collateral vasodilatation also raises certain questions pertaining to the nature and origin of the vasodilatatory factor(s), particularly as vasodilatation of the larger collateral vessels proximal to the ligation would be necessary to improve perfusion of muscle distal to the ligation. In addition to vasodilatation of the collateral vessels and the peripheral vascular bed, at least two other adaptations have been reported previously and warrant discussion.

Surprisingly little work has been carried out to investigate the stimulation of capillary growth in skeletal muscle of adult animals (see Hudlická, 1982, for review).

Both exercise-induced transient hypoxia and hypoxia *per se* cause increases in the activity of oxidative enzymes and increase capillary density (Cotter *et al.*, 1973; Honig, 1977); however, such changes in capillarity may occur over a period of weeks rather than days (Cassin *et al.*, 1971; Cotter *et al.*, 1973; Ljungsquist & Gunnar, 1977; Hudlická & Schroeder, 1978; Pette & Tyler, 1981) and may therefore contribute little to the improvements in bloodflow during stimulation observed in the present study.

Discussion so far has centred on adaptation of the local circulation to overcome the applied constraint. It is also possible that adaptation may occur in the affected muscles (Bylund *et al.*, 1976; Bylund-Fellenius *et al.*, 1981); thus, in the accompanying paper (Hayes *et al.*, 1986) results of investigations on possible enzymic adaptive mechanisms are presented.

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