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EXHAUSTIVE EXERCISE IN THE SEA LAMPREY (*PETROMYZON MARINUS*): RELATIONSHIP BETWEEN ANAEROBIC METABOLISM AND INTRACELLULAR ACID–BASE BALANCE

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Summary

We measured intracellular acid–base balance and indicators of carbohydrate and high-energy phosphate metabolism as lampreys recovered from exhaustive exercise.

A combined respiratory and metabolic acidosis was observed in the locomotory muscle following 'burst' exercise. Muscle pH decreased from approximately 7.2 to 6.7, whereas intracellular P_{CO_2} rose from approximately 0.6 to 1.6kPa. Unlike the situation in similarly stressed teleost fish such as rainbow trout, the respiratory acidosis in muscle persisted for several hours. This apparent CO_2 retention in lamprey muscle may be the result of a restricted ability of the circulatory system to transport CO_2 due to reduced erythrocyte anion exchange, or it could represent a restricted ability of the muscle itself to clear the intracellular pool of CO_2 due to reduced carbonic anhydrase activity.

Maximal lactate dehydrogenase activity of lamprey muscle exhibited a marked dependence on pH, increasing in activity by 30% as pH decreased from 7.2 to 6.7 (reflecting the 'resting' to 'post-exercise' pH change observed *invivo*).

Following exhaustive exercise, the acid–base balance of the muscle is influenced by both proton-consuming (e.g. AMP deamination, glycogen replenishment) and proton-producing (e.g. rephosphorylation of creatine) metabolic processes. The net effect is that, although intracellular pH is maximally depressed, energy stores such as phosphocreatine and glycogen are partially restored within 1h of exhaustive exercise, placing the animal in good stead for further locomotory work.

Introduction

The sea lamprey *Petromyzon marinus* L. is an anadromous form that spends the parasitic phase of its life either in the ocean or in other large bodies of water, and then migrates upstream to spawn and die in freshwater rivers and streams. During such migrations the animals must not only sustain aerobic swimming, but must also use burst activity to negotiate turbulent waterfalls. Although much maligned because of its parasitic nature, the migratory prowess of the lamprey rivals that of the salmonids.

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Burst swimming for more than a few minutes generally results in a marked lactacidosis (Milligan and Wood, 1986; Tang and Boutilier, 1991) and a decrease in muscle energy stores such as glycogen and high-energy phosphates (Dobson and Hochachka, 1987; Schulte *et al.* 1992) in the working white muscle of teleost fish. While ATP in white muscle can be rapidly generated by phosphocreatine (PCr) hydrolysis to support intense muscular activity for tens of seconds, anaerobic glycolysis must be recruited to sustain several minutes of high-intensity work (Dobson *et al.* 1987).

The intracellular accumulation of metabolic H^+ (H^+_m) resulting from anaerobiosis must subsequently be restored to normal by (i) buffering the excess protons, (ii) excreting them from the cell, or (iii) utilizing them in proton-consuming metabolism. In a compartmental analysis of H^+_m clearance from white muscle of exhaustively exercised trout, Tang and Boutilier (1991) concluded that H^+-consuming metabolism was quantitatively the most important mechanism for correcting the intracellular acidosis. Moreover, in a similar study on exercised trout, Schulte *et al.* (1992) suggest that the retention of H^+_m by muscle may be necessary to set up optimal conditions for gluconeogenesis, in itself a proton-consuming process.

In erythrocytes of agnathans, the membrane equilibration of chloride and bicarbonate (i.e. Cl^-/HCO_3^- exchange) is very slow, of the order of several hours (Nikinmaa and Railo, 1987; Ellory *et al.* 1987). Thus, the bulk of the CO_2 which diffuses from the tissues enters the erythrocytes where it is catalyzed to HCO_3^- and H^+ in the usual fashion; but because HCO_3^- cannot then be exported to the plasma in a physiologically relevant time scale, the *majority* of blood CO_2 is transported to the gills *inside* the erythrocyte (Tufts and Boutilier, 1989, 1990; Nikinmaa and Mattsoff, 1992). Whether this 'primitive' form of blood CO_2 transport actually limits CO_2 excretion is questionable, however, since total CO_2 carriage by lamprey blood *in vitro* is the same with or without effective Cl^-/HCO_3^- exchange (Tufts and Boutilier, 1990). Indeed, the lack of rapid anion exchange may be compensated for entirely by a high erythrocyte pH together with large oxygenation-linked variations in total CO_2 content (Nikinmaa and Mattsoff, 1992).

The present study examines post-exercise changes in the muscle acid–base status of lamprey and compares them with those seen in the more 'advanced' teleosts. To this end, we have estimated exercise-induced increases in muscle P_{CO_2} from measurements of intracellular pH and HCO_3^- (cf. Pörtner *et al.* 1990) and compared this with similar estimates made on rainbow trout (Tang and Boutilier, 1991), whose erythrocytes exhibit rapid anion exchange (Romano and Passow, 1984; Nikinmaa, 1990).

In addition to intracellular acid–base balance, indicators of carbohydrate and high-energy phosphate metabolic status were measured as lamprey recovered from an exercise-induced acidosis. As the jawless fishes are often taken to represent the ancestral vertebrate condition, this study examines whether the metabolic basis for anaerobic burst exercise was present in the earliest vertebrate forms. The time course and nature of the recovery of intracellular pH and energy metabolism following exhaustive exercise in lamprey suggest that these processes are intimately linked in the restoration of muscle acid–base balance, as they are in the more advanced teleost fishes (Dobson and Hochachka, 1987; Tang and Boutilier, 1991; Schulte *et al.* 1992).

Materials and methods

Animals

Adult sea lamprey, *Petromyzon marinus* (approximately 300–400g, *N*=30) were collected in early May 1991 during their spring spawning run at Shelter Valley River in Eastern Ontario. The animals were transported to the laboratory in aerated, chilled ice chests, and held thereafter in 1 m^3 freshwater tanks at 8–10˚C for at least 1 month before experimentation. Water was continuously supplied to the tanks at 5 $l\,min^{-1}$ and the animals were kept on a 12h:12h L:D photoperiod. Lamprey do not feed during this migratory phase of their life history and were thus maintained in this naturally starved state. The ion concentrations of the water in which the animals were held, and for all experiments, were as follows (in mequiv l^{-1}): [Na^+] 2.1, [K^+] 0.05, [Ca^{2+}] 1.1, [Cl^-] 1.3, [HCO_3^-] 1.5 (pH7.4).

Experimental procedures

Lamprey were kept in individual Perspex chambers supplied with aerated flowing fresh water at 10˚C. After 24h in this condition, the animals were either sampled (control animals) or transferred to a large cylindrical chamber in which they were exercised to exhaustion (i.e. until they were refractory to further stimulation) by manual chasing for 5 min. The exercise protocol was identical to that of Tufts (1991). Following exercise, the animals were returned to their individual light-proof boxes and sampled either immediately (0min of recovery) or at 30min, 1h or 4h after exhaustion. At each sampling time, water flow to the chamber was stopped and a stock solution of buffered MS-222 was slowly introduced to produce a final concentration of 0.2 $g\,l^{-1}$. After 2–3min, during which the animals remained quiescent, the lamprey were fully anaesthetized. At this time, a 3–4cm fillet of muscle was quickly taken from the side of the fish, starting at the midline of the dorsal fin and cutting backwards. Samples were immediately freeze-clamped in tongs pre-cooled in liquid nitrogen, and then stored in liquid N_2 until analysis. The time between removing the fish from the box and freeze-clamping the tissue was less than 10s. Animals were then killed by anaesthetic overdose. A critique of this procedure against other methods of sampling muscle tissue *in vivo* can be found in Tang and Boutilier (1991).

Analytical procedures

Muscle pH, total CO_2 and P_{CO_2}

Muscle samples were ground to a fine powder under liquid nitrogen using a pre-cooled porcelain mortar and pestle. Care was taken to avoid contamination of the tissue with CO_2 (e.g. from one's breath). Intracellular pH and tissue concentrations of CO_2 (C_{CO_2}) were estimated using the tissue homogenate method of Pörtner *et al.* (1990). In brief, approximately 200mg of tissue powder ('wet' with liquid N_2) was transferred to a pre-weighed 0.5ml Eppendorf tube containing 0.2ml of an ice-cold medium consisting of potassium fluoride (KF) at 150mmol l^{-1} and nitrilotriacetic acid (NTA) at 6mmol l^{-1}. This medium inhibits homogenate metabolism (Pörtner *et al.* 1990). The tube (containing the powder and medium) was quickly weighed, filled to capacity with additional medium,

briefly stirred with a needle, capped and reweighed. The mixture was then vigourously shaken on a Vortex mixer for 3–4s, and centrifuged for 3–5s. Samples of the supernatant were taken immediately for measurement of pH and total CO_2. The pH of a 0.1ml sample of supernatant was determined at 10˚C using a microcapillary pH electrode (Radiometer G279/G2) coupled with a Radiometer PHM 84 pH meter. Supernatant total CO_2 concentration (C_{CO_2}) was measured, using 0.2ml samples, with a Corning model 965 CO_2 analyser. Total CO_2 of tissue water (C_{tw,CO_2}) was calculated using the equation:

$$C_{tw,CO_2} = C_{h,CO_2} \frac{VM + (FW \times F_{tw})}{FW \times F_{tw}} \ (\text{mmoll}^{-1}\,\text{tw}), \tag{1}$$

where FW is the fresh mass in milligrams, C_{h,CO_2} is the total CO_2 of the homogenate, F_{tw} is the fraction of water in tissue (tw is tissue water) and VM is the volume of the medium in microlitres.

For estimation of tissue water, muscle samples of approximately 1g were removed from storage in liquid N_2 and placed in a dried, tared Eppendorf tube. Following determination of the wet mass, the samples were dried to a constant mass at 80˚C, and re-weighed for determination of percentage water content. Over the course of the experiments, the water content of muscle tissue varied from 77.7±0.7 to 78.7±1.1 % (mean ± S.E.M.), and no significant changes were observed. The equation for calculation of total CO_2 concentration in cell water (cw) is:

$$C_{cw,CO_2} = \frac{C_{tw,CO_2} - Q \times C_{ew,CO_2}}{1 - Q}, \tag{2}$$

where ew is extracellular water (interstitial fluid+plasma) and Q is the fraction of extracellular water in tissue water (estimated to be 15% of total tissue H_2O based on measurements in *Petromyzon marinus* by Thorson, 1959). Intracellular lactate concentrations were calculated using the same principles.

Based on the analysis of intracellular pH in the homogenates and of appropriate values for the apparent first dissociation constant of carbonic acid (pK′′′ as determined empirically below), and CO_2 solubility, α_{CO_2} (Heisler, 1986), intracellular partial pressures of CO_2 (P_{CO_2}) were calculated using the modified Henderson–Hasselbalch equation:

$$P_{CO_2} = C_{CO_2}/[(10^{pH - pK'''} \times \alpha_{CO_2}) + \alpha_{CO_2}]. \tag{3}$$

Values for pK′′′ were obtained in an experimental analysis of non-bicarbonate buffering capacity of tissue homogenates under metabolic control (cf. Pörtner, 1989). In brief, tissue was powdered under liquid nitrogen as before and added to three times its weight of the KF–NTA medium as above. The homogenate was tonometered in intermittently rotating flasks against humidified CO_2 gases ranging from 1 to 9% CO_2 in air. Samples taken anaerobically at each CO_2 level were rapidly centrifuged in a capped Eppendorf tube and the supernatant was analysed for pH and C_{CO_2}. Non-bicarbonate buffer values were determined by estimating the slope of the relationship between pH and HCO_3^-, where $[HCO_3^-] = C_{CO_2} - \alpha_{CO_2} \times P_{CO_2}$. For calculation of α_{CO_2}, we assumed

[*M*]=0.210mol l^{-1} as molarity of dissolved species (Heisler, 1986) based on intracellular ion concentrations for agnathans (Brodal and Fänge, 1963). The pK′′′ was then determined by rearrangement of the Henderson–Hasselbalch equation (see Boutilier *et al.* 1984).

Muscle metabolites

Samples of lamprey muscle were ground to a fine powder as before. Approximately 1.5g of the powder was weighed in a test tube to which 4 vols of ice-chilled 8% perchloric acid (PCA) containing 1mmol l^{-1} EDTA was added. This mixture was quickly vortexed to form a slurry. A 10min extraction period followed, during which the slurry was slowly inverted at 4°C. 500 μl of the extracted homogenate was set aside at this time for the determination of tissue glycogen. The remainder was neutralized with an ice-chilled 2mol l^{-1} KOH solution containing 400mmol l^{-1} imidazole and 400mmol l^{-1} KCl. The neutralized extract was frozen under liquid nitrogen until analysis (1–2 weeks). For both metabolite and enzyme activity determinations (see below) care was taken to remove all evidence of skin from the frozen muscle prior to homogenization.

Unless otherwise stated, assays were performed using NAD- or NADH-coupled enzymatic reactions with absorbance changes being recorded on a Varian DMS 200 UV/VIS spectrophotometer at 340nm. All solutions were prepared using double-distilled and de-ionized water and analytical grade reagents. All chemicals were purchased either from Boehringer-Mannheim or Sigma. Metabolite determinations were routinely made on 1–1.5ml reaction volumes in disposable polystyrene cuvettes.

Muscle lactate, pyruvate, glucose, glucose 6-phosphate (G6P), adenosine triphosphate (ATP) and phosphocreatine (PCr) were determined by the methods outlined in Lowry and Passoneau (1972). Glycogen was determined by the method of Keppler and Decker (1974). Inosine monophosphate (IMP) was quantified by treating the extracts with 5′-nucleosidase prior to inosine determination by the method of Coddington (1965; Driedzic and Hochachka, 1976) at 295nm. Appropriate metabolite standards were used routinely with each assay.

Muscle lactate dehydrogenase maximal activity

The maximal activity (Act_{max}) of lactate dehydrogenase (LDH) was determined at 0.5 pH unit increments over a pH range of 5.2–10.2 at 10°C. Frozen muscle samples (approximately 0.75g) were homogenized using an Ultra Turrax tissue homogenizer in 5 vols of ice-cold homogenization buffer (5mmol l^{-1} EDTA, 5mmol l^{-1} EGTA, 15mmol l^{-1} β-mercaptoethanol in 50mmol l^{-1} K_2HPO_4, pH7.5 at 20°C). The resulting slurry was centrifuged for 15min at 5°C (13000 ***g***), and the supernatant stored on ice until analysis.

LDH activity (Act_{max}, corrected for non-specific activity) was determined at 10°C with the following cuvette conditions: NADH, 0.15mmol l^{-1}; pyruvate, 1.0mmol l^{-1}; imidazole–HCl, 50mmol l^{-1}. For the determination of non-specific activity, pyruvate was omitted from the cuvette. The imidazole was adjusted with HCl to obtain the various pH values (5.2–10.2) at 10°C. For each analysis, 2 μl of diluted homogenate (the original supernatant mixed 1:30 vol:vol with homogenization buffer) was used in a 1ml reaction

volume. LDH activity was determined at 10˚C with a Gilford UV/VIS recording spectrophotometer set at 340nm. LDH Act_{max} was also determined at 20˚C (pH6.7) under optimal substrate conditions as detailed above. The typical reaction rate caused a change in recorded absorbance of approximately 0.03–0.06min^{-1}. LDH activity is reported as enzyme units, with one unit being the amount of activity that causes the reduction of 1 μmol of pyruvate per minute at 10˚C.

Statistical analysis

Mean values ±1 S.E.M. are reported throughout. Differences between groups were analysed statistically using unpaired one-tailed Student's *t*-test, 5% being taken as the fiducial limit of significance.

Results

Acid–base status

Following exercise, muscle pH (pHi) decreased from approximately 7.2 to 6.6, and remained at this level for the first hour of recovery (Fig. 1). Though pHi increased significantly over the next 3h, the values were still significantly lower than those of pre-exercise controls. In contrast, the extracellular acidosis (Fig. 1; Tufts, 1991) was corrected within 1h. Intracellular and extracellular bicarbonate concentrations decreased following exercise and, as with pH, recovery of the blood compartment preceded that of the muscle (Fig. 1B). The acidosis in the intracellular compartment was accompanied by a large and prolonged elevation of intracellular P_{CO_2} (Fig. 1C), which did not reach pre-exercise control levels even after 4h recovery. The much smaller elevation in arterial P_{CO_2} was corrected within 1h. Clearly, the acidosis in the intracellular compartment was much greater, and persisted longer, than that in the extracellular compartment.

Carbohydrate metabolism

Glycogen concentration in muscle was reduced to one-third of its resting level at exhaustion (Fig. 2A). Within the first hour post-exercise, glycogen levels began to be restored and, after 4h, the levels reached were not significantly different from those seen at rest. Muscle glucose levels began to increase following exercise and continued to rise by three- to fourfold over the 4h of recovery examined (Fig. 2B). Similarly, G6P levels in muscle were elevated following the post-exercise period (Fig. 2C).

Intracellular lactate reached peak values immediately following exercise and remained at these levels for the first hour of recovery (Fig. 3A). By 4h post-exercise, lactate levels were approaching, but had not reached, pre-treatment control levels. Muscle pyruvate levels increased steadily over the first hour following exercise, reaching values fivefold higher than those at rest (Fig. 3B). After 4h of recovery, muscle pyruvate concentrations were not significantly different from the pre-exercise controls.

Adenylates and phosphocreatine

Exercise caused a significant decrease in PCr (71%), which was restored to levels not significantly different from control values 30min after exercise (Fig. 4A). After 1h of

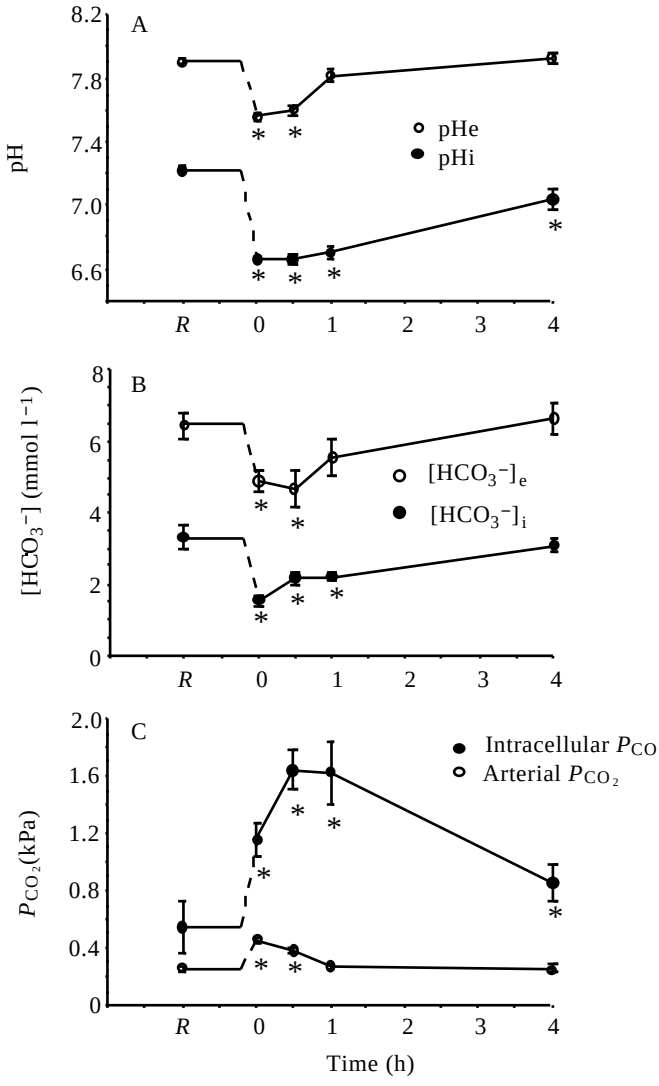

Fig. 1. (A) *In vivo* extracellular and muscle pH values (pHe, pHi); (B) bicarbonate concentrations ($[HCO_3^-]_e$, $[HCO_3^-]_i$) and (C) carbon dioxide partial pressures (P_{CO_2}) for *Petromyzon marinus* at rest (*R*) and after exercise to exhaustion. Extracellular values are from Tufts (1991), determined on a different batch of lampreys collected from the same river 1 year previously (see Materials and methods). The exercise protocol in the present study was identical to that of Tufts (1991). N=6 for intracellular values, N=8 for extracellular values. All values are means ±1 S.E.M. Asterisks denote significant differences from values at rest determined by unpaired one-tailed Student's t-test (P<0.05).

recovery, PCr levels became slightly depressed, but after 4h they were not significantly different from those seen prior to exercise (Fig. 4A). ATP concentration declined to a lesser extent (30%) and remained depressed at 1h post-recovery (Fig. 4B). Between 1

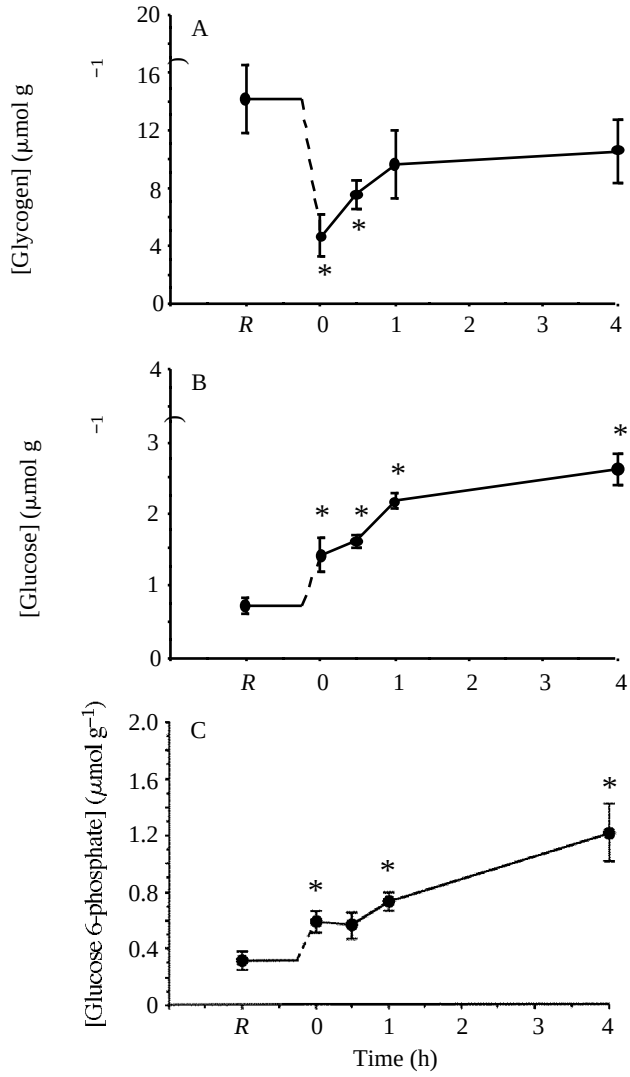

Fig. 2. Muscle tissue concentrations of (A) glycogen, (B) glucose and (C) glucose 6-phosphate for *Petromyzon marinus* at rest (*R*) and after exercise to exhaustion. Values are means ±1 S.E.M. (*N*=6). Asterisks denote significant differences from values at rest determined by unpaired one-tailed Student's *t*-test ($P<0.05$).

and 4h of recovery, ATP concentrations increased to levels not significantly different from those at rest. The changes in ATP over the first hour of recovery were mirrored by changes in IMP, the latter being about half the magnitude of the former. As with ATP, IMP levels were re-established by 4h of recovery.

Lactate dehydrogenase activity

Reductive lactate dehydrogenase activity of lamprey muscle exhibited a maximal value

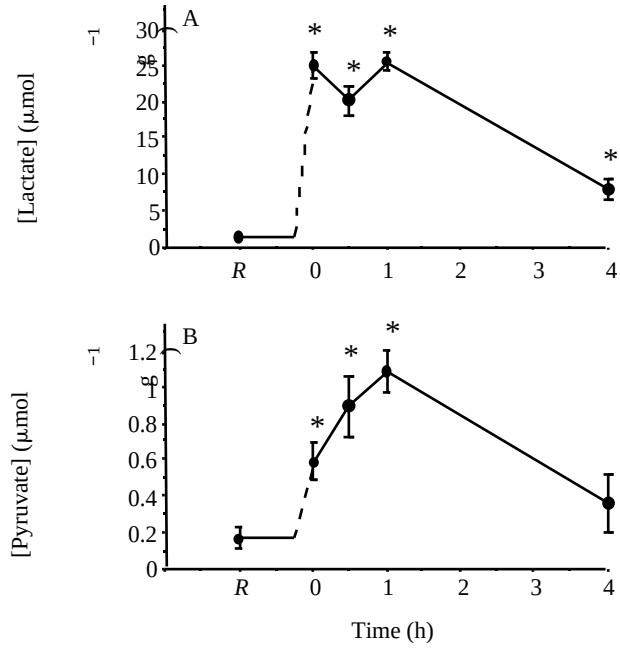

Fig. 3. Muscle tissue concentrations of (A) lactate and (B) pyruvate for *Petromyzon marinus* at rest (*R*) and after exercise to exhaustion. Values are means ±1 S.E.M. (*N*=6). Asterisks denote significant differences from values at rest determined by unpaired one-tailed Student's *t*-test ($P<0.05$).

(LDH Act_{max}) of 581±61units g^{-1} wetmassmuscletissue at 10°C (Fig. 5). LDH Act_{max} showed a marked dependence on pH, exemplified by the 30% increase in activity which was observed as pH decreased from 7.2 to 6.7 (reflecting 'resting' to 'post-exercise' muscle pH changes, respectively; Fig. 1). At 20°C and pH7.0, LDH Act_{max} was 812±87units g^{-1} wetmass. This compares well with the value of 868±32units g^{-1} determined previously for trout white muscle LDH Act_{max} at 20°C (Ferguson and Storey, 1992). The Q_{10} for lamprey muscle LDH Act_{max} is 1.4±0.1.

Non-bicarbonate buffering capacity of muscle

Four of the muscle samples from the control animals were used to estimate non-bicarbonate buffering capacity (β_{NB}). The regression coefficients (r^2) of buffer lines constructed at five P_{CO_2} levels were between 0.92 and 0.97. The mean ± S.E.M. buffer slope ($\Delta[HCO_3^-]/\Delta pH$) of the four curves was −45.4±4.5mmol l^{-1} pHunit^{-1}.

H^+ and lactate loads

The post-exercise intracellular metabolic proton load ($\Delta H^+_{m,i}$) of muscle (mmol l^{-1}) was calculated as:

$$\Delta H^+_{m,i} = \{[HCO_3^-]_{i,1} - [HCO_3^-]_{i,2} - \beta(pHi_1 - pHi_2)\}, \quad (4)$$

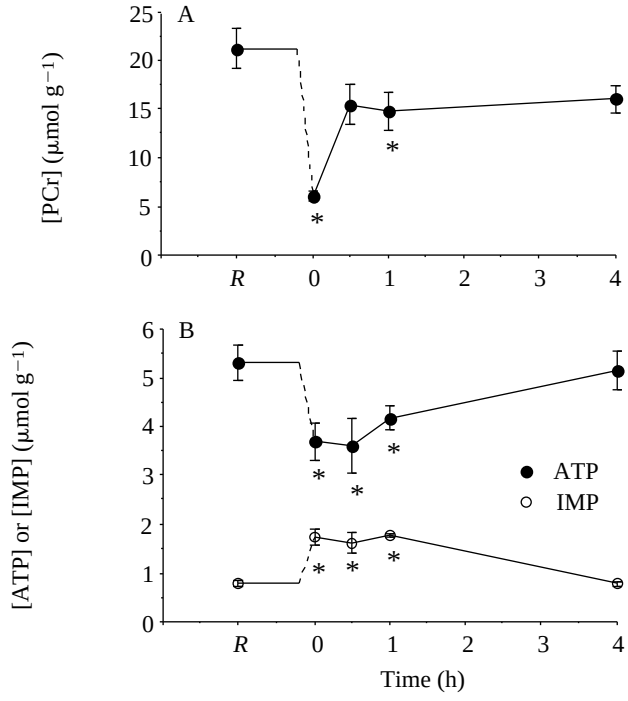

Fig. 4. Muscle tissue concentrations of (A) creatine phosphate (PCr) and (B) adenosine triphosphate (ATP) and inosine monophosphate (IMP) for *Petromyzonmarinus* at rest (*R*) and after exercise to exhaustion. Values are means ±1 S.E.M. (*N*=6). Asterisks denote significant differences from values at rest determined by unpaired one-tailed Student's *t*-test ($P<0.05$).

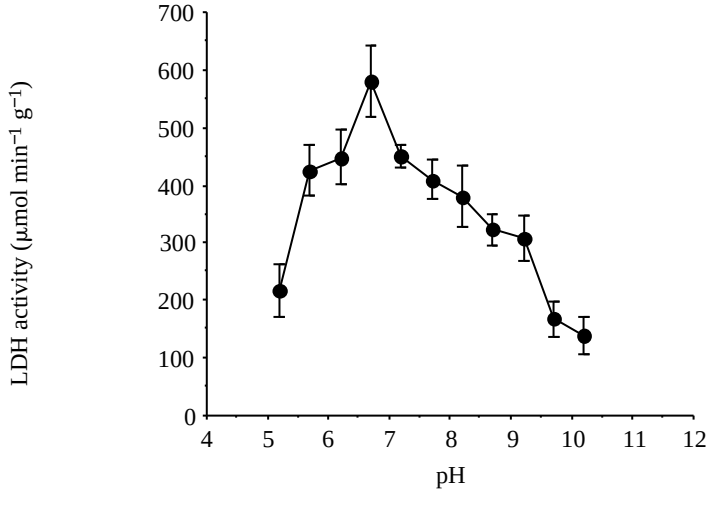

Fig. 5. Lactate dehydrogenase (LDH) activity of lamprey muscle as a function of assay pH at 10°C (see text for assay conditions). Values are mean ±1 S.E.M. (*N*=6).

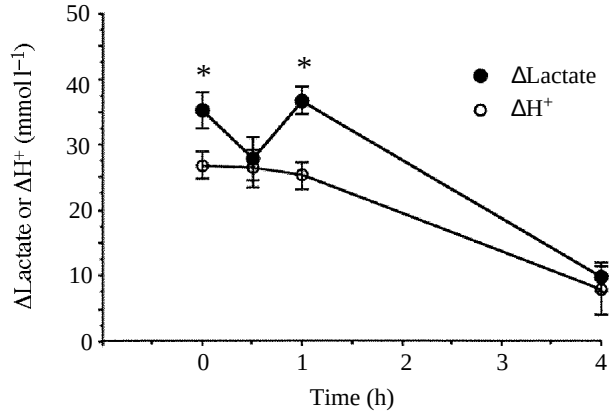

Fig. 6. Estimated changes in muscle tissue concentrations of metabolic protons ($\triangle H^+$) and lactate ($\triangle$Lactate) for *Petromyzonmarinus* during recovery from exhaustive exercise. Values are means ±1 S.E.M. (*N*=6). Asterisks denote sigificant differences between $\triangle H^+$ and $\triangle$Lactate.

where β is the non-bicarbonate buffer value of muscle. The post-exercise intracellular lactate load ($\Delta[\text{lactate}]_i$) was calculated (mmol l^{-1}) as:

$$\Delta[\text{lactate}]_i = \{[\text{lactate}]_{i,1} - [\text{lactate}]_{i,2}\}. \quad (5)$$

Fig. 6 illustrates the intracellular metabolic proton load and lactate load in lamprey muscle following exhaustive exercise. Both proton and lactate loads increase sharply following exercise and remain elevated for the first hour of recovery. An apparent stoichiometric clearance of approximately 75% of the proton and lactate loads from muscle occurred between 1 and 4h of recovery (Fig. 6).

Discussion

Intracellular acid–base and lactate status

Resting status

The intracellular pH (pHi) values for muscle from lamprey at rest are comparable (temperature effects considered) to those recorded for rainbow trout white muscle (Milligan and Wood, 1986; Pörtner *et al.* 1990; Tang and Boutilier, 1991) and for the musculature of carp and goldfish as determined *in vivo* by ^{31}P-NMR spectroscopy (van den Thillart and van Waarde, 1991). The corresponding intracellular bicarbonate concentrations ($[HCO_3^-]_i$) of lamprey muscle are virtually identical with those of freshwater rainbow trout white muscle (Tang and Boutilier, 1991). Calculated intracellular P_{CO_2} levels of lamprey muscle at rest were 0.28kPa higher than the corresponding venous P_{CO_2} values recorded by Tufts *et al.* (1992), suggesting near-equilibrium conditions between CO_2 production and elimination.

Lactate concentrations in muscle of lamprey at rest (Fig. 3) were within 1mmol l^{-1} of the concentrations found in the blood (Tufts, 1991), a situation we also found to occur in

rainbow trout (Tang and Boutilier, 1991). However, much larger muscle to blood lactate concentration gradients (approximately 10mmol l^{-1}) have been reported (e.g. Johnston, 1975; Turner *et al.* 1983; Milligan and Wood, 1986), all using techniques that involve handling the awake animal for a short time. Anaesthetization prior to muscle sampling, if carried out without causing the fish to struggle, invariably reveals much lower muscle lactate concentrations (e.g. Schulte *et al.* 1992; Fig. 3) than with other methods (see critique of sampling methods in Tang and Boutilier, 1991). Based on our observations, it seems probable that lactate is in a near-equilibrium state across the muscle cell membrane of resting lamprey, as in other higher vertebrates.

Post-exercise status

The post-exercise acidosis in lamprey has both a respiratory and a metabolic component, though the former accounts for only a small fraction of the acidosis in comparison to the total proton load. Certainly the post-exercise increase in intracellular P_{CO_2} reflects dehydration of $[HCO_3^-]_i$ by H_m^+. Such exercise-induced increases in P_{CO_2} often occur in venous and arterial blood of fish, but generally subside over 1–2h post-exercise (Wood and Perry, 1985; Tang and Boutilier, 1991; Tufts, 1991). Calculations of intracellular P_{CO_2} for exhausted trout white muscle, based on pHi and $[HCO_3^-]_i$ values in Tang and Boutilier (1991), show that the increase in muscle P_{CO_2} is neither as large, nor as prolonged, as in lamprey. In freshwater trout, muscle P_{CO_2} increases from 0.4kPa at rest to 1.4kPa immediately following exhaustive exercise. Unlike lamprey, however, intracellular P_{CO_2} in rainbow trout returns to values between 0.5 and 0.3kPa within the first hour post-exercise. Indeed, the persistent elevation of intracellular P_{CO_2} in the case of lamprey may be a result of (i) a restricted ability of the circulatory system to transport CO_2, (ii) a restricted ability of the muscle itself to clear the intracellular pool of CO_2, and/or (iii) a persistently high CO_2 production rate in muscle following exercise.

Erythrocytes of agnathans show both limited anion exchange (Nikinmaa and Railo, 1987; Ellory *et al.* 1987) and very low levels of carbonic anhydrase (CA) activity compared with trout (Nikinmaa, 1990). Thus, the clearance of intramuscular CO_2 may be limited by the transport capacity of the erythrocyte. Furthermore, the very low non-bicarbonate buffering capacity of the true plasma of lamprey (Tufts and Boutilier, 1989, 1990; see below) would result in an exaggerated decrease in pHe if muscle CO_2 were to be cleared more rapidly.

Conversely, CO_2 transport from muscle to blood may be the rate-limiting step. Muscle CA is believed to be important for CO_2 excretion at the cellular level in mammalian muscle (Gros and Dodgson, 1988) and, if CA activity is as correspondingly low in lamprey muscle as it is in the erythrocytes (R. P. Henry, B. L. Tufts and R. G. Boutilier, unpublished data), then cellular CO_2 excretion may be impaired. Little is known about the cellular role of CA outside mammalian systems, and the explanation for this apparent CO_2 retention in muscle of lamprey must await further experimentation.

The total proton and lactate load measured after exhaustive exercise in lamprey (Figs 1, 3 and 6) is 30–50% lower than in rainbow trout white muscle (Milligan and Wood, 1986; Tang and Boutilier, 1991; Schulte *et al.* 1992). Despite the lower H^+ load, the pHi change between exercise and exhaustion in lamprey (approximately 0.65 units) is

similar to that seen in trout (Tang and Boutilier, 1991; Schulte *et al.* 1992). Presumably, this reflects the lower intracellular H^+ buffering capacity of lamprey ($\beta = -45\ \mu molpHunit\ g^{-1}$), than of trout ($\beta = -74\ \mu molpHunit\ g^{-1}$; Milligan and Wood, 1986). Castellini and Somero (1981) have suggested that the potential for anaerobic work (i.e. indexed as LDH activity) is positively correlated with the non-bicarbonate buffering capacity of muscle from a wide variety of teleost fishes. Lamprey and trout, however, have roughly the same LDH activities (Fig. 5; Ferguson and Storey, 1992), but their corresponding buffering capacities clearly differ in their potential for acid–base regulation, and these differences might set limits on H^+ accumulation.

Apart from intracellular nonbicarbonate buffers, H^+_m can be cleared from muscle by exporting the protons to the extracellular space or by H^+-consuming metabolism. Though we know little about the excretion of H^+ either to the extracellular space or to the water by lamprey, there is reason to suspect that this accounts for only a limited amount of clearance. In freshwater rainbow trout, for example, H^+ clearance to the environmental water accounted for only 5% of the total H^+_m produced (Tang and Boutilier, 1991). The absence of rapid Cl^-/HCO_3^- exchange on the erythrocyte membrane of lamprey also means that haemoglobin cannot participate effectively in buffering H^+_m addition to blood. Thus, the only non-bicarbonate buffering capacity (β_{NB}) available in the blood must come from plasma protein buffers. Indeed, blood of lamprey *in vivo* should have an effective β_{NB} of only 1–2 $\mu molpHunit\ g^{-1}$ (Tufts and Boutilier, 1990; Tufts, 1991) and, if there were substantial H^+ export (or CO_2 excretion; see above) from muscle, the pH of blood would be depressed to extraordinarily low levels. Since this does not occur (Fig. 1), it seems likely that the bulk of the H^+_m produced is probably retained in the muscle and that the main strategy for H^+ clearance is with H^+-consuming metabolic processes. This, incidently, was the conclusion also reached by Tang and Boutilier (1991) for rainbow trout white muscle, after quantitative analysis of H^+ clearance in intracellular, extracellular and extracorporeal compartments.

Anaerobic energy metabolism and acid–base balance

There are three primary stores of energy available for use by white muscle during anaerobic ‘burst exercise’: PCr, ATP and glycogen. The metabolism of each also plays a role in acid–base balance.

Phosphagen utilization

Under resting conditions PCr accounts for four times the amount of high-energy phosphate as does ATP (Fig. 4), and it is generally the first store called upon during exercise (Dobson *et al.* 1987; Dobson and Hochachka, 1987) or hypoxia (van den Thillart and van Waarde, 1991) in fish. The utilization of phosphagen is a proton-consuming process:

$$PCr + ADP + H^+ \rightleftharpoons ATP + Cr. \quad (6)$$

Hydrolysis of ATP results in the overall reaction:

$$PCr \rightarrow Cr + P_i. \quad (7)$$

The accumulated inorganic phosphate P_i, apart from triggering the phosphorolysis of glycogen, also acts to offset further pH changes by increasing the intracellular non-bicarbonate buffering capacity (Pörtner, 1989). During recovery, synthesis of phosphagen occurs more rapidly than the metabolism of accumulated end products (Fig. 4A). Because the rephosphorylation of creatine produces one acid equivalent (Hochachka and Mommsen, 1983; Pörtner *et al.* 1984), this probably contributes to the persistent metabolic acidosis for the first hour of recovery (Fig. 1A). The rapid repletion of PCr following exercise has the adaptive advantage, however, of restoring the highest and most quickly mobilized energy store first, thereby positioning the animal in better stead to access such stores rapidly for subsequent locomotion.

Adenine nucleotide utilization

The purine nucleotide cycle is clearly in operation in this primitive vertebrate as shown by the rise in IMP level as ATP levels decrease (Fig. 4B). That IMP does not increase in an amount stoichiometrically equivalent to the decrease in ATP is in contrast to the situation found in white muscle of trout (Driedzic and Hochachka, 1976; Mommsen and Hochachka, 1988; Schulte *et al.* 1992). This interspecific difference in the magnitude of IMP accumulation may reflect differences in muscle fibre composition. Trout 'white muscle', for example, makes up about 90% of the total body musculature (Johnston, 1981), and although it is considered to be a mosaic of red and white fibres, it is a predominantly fast-twitch muscle. Thus, trout white muscle is far more homogeneous, both biochemically and functionally, than many mammalian fast-twitch muscles (Dobson *et al.* 1987). The body musculature of lamprey is a true mosaic of red and white fibres and, as such, it is classified as a heterogeneous muscle (O'Boyle and Beamish, 1977). Recent studies on contraction-induced ATP depletion in different muscle fibre types of rat have shown that, in slow-twitch muscle, the extensive deamination of AMP to IMP+NH_3, characteristic of fast-twitch muscle, does not occur (Terjung *et al.* 1985; Whitlock and Terjung, 1987). In human muscle, however, ATP depletion and IMP accumulation during voluntary short-term exercise occurred in both type 1 (slow-twitch) and type 2 (fast-twitch) fibres, although the changes were somewhat larger in the latter (Jansson *et al.* 1987). These variable responses between fibre types, as well as intra- and interspecific differences in IMP accumulation, presumably reflect differences in muscle fibre recruitment, the intensity of the intracellular chemical signal(s), and/or the underlying metabolic organization of the fibre types.

The significance of a stoichiometric accumulation of IMP with falling ATP is that the ratio ATP/(ADP+AMP) remains constant, so that decreases in energy charge in the cell are minimal. As the differences in absolute changes between ATP and IMP are significantly different (Fig. 4B), ATP depletion is not stoichiometric with IMP accumulation. Indeed, IMP accumulates to approximately half the amount that ATP is depleted (Fig. 4B). This means that the ATP/(ADP+AMP) ratio should be lower after exercise and that the relative increases in ADP and AMP might signal enhanced energy production through oxidative metabolism in slow-twitch muscle fibres; indeed, increases in [P_i] stimulate O_2 consumption by isolated mitochondria of trout (Moyes *et al.* 1992). Moreover, as resting P_i in fish muscle is approximately 1mmol l^{-1} (van Waarde, 1988),

and as 2 mol of P_i are released per mol of ATP, the decrease in [ATP] by approximately $2\,mmol\,l^{-1}$ (Fig. 4B) would lead to an approximately $4mmol\,l^{-1}$ increase in P_i from rest to exercise, a potentially large signal to increase oxidative metabolism. In addition to the falling adenlyate pool in lamprey, a stimulation of oxidative metabolism is also indicated by the increased availability of pyruvate (Fig. 3B), since fish muscle mitochondria are poised to oxidize pyruvate (Mourik, 1983; Moyes *et al.* 1989).

Exercise-induced accumulation of IMP also serves two other purposes. First, because the ammonia accumulated with IMP does not leave the muscle cell (Terjung *et al.* 1985), this supports its role as a proton buffer:

$$AMP \rightarrow IMP + NH_3 \rightarrow NH_4^+ . \quad (8)$$

Assuming that all of the cellular ATP were converted to IMP and ammonia, this yields a buffering capability for most cells of approximately $5\,\mu mol\,g^{-1}$ (Hochachka and Guppy, 1987). In lamprey muscle, IMP levels increase by approximately $1mmol\,l^{-1}$ following exercise, reflecting a quantitatively modest contribution to the buffering of the total proton load (Fig. 6). Second, the accumulation of IMP also provides a pool of purine nucleotide which remains in the muscle during and following exercise and is immediately available for reamination when conditions permit recovery of the adenine nucleotide pool (Fig. 4: Terjung *et al.* 1985; Dobson and Hochachka, 1987).

Glycogen utilization

The amount of lactate accumulated is approximately what one would expect on the basis of glycogen depletion alone (i.e. 2 mol of lactate produced per mol of glycogen utilized; compare Figs 2A and 3A). The intracellular lactate and proton loads in muscle of lamprey appear to be cleared at the same rate following exercise (Fig. 6), suggesting a metabolic link in the recovery process.

Replenishment of glycogen over the first hour of recovery (Fig. 2A) occurs without any apparent utilization of muscle lactate, since concentrations of the latter remain unchanged during this time (Fig. 3A). Increases in muscle glucose and G6P (Fig. 2B,C) may play a role in the early replenishment of muscle glycogen, though the process remains obscure. In the longer term, however, glyconeogenesis could also occur *in situ*, as has been observed for various other vertebrate muscles (Bonen *et al.* 1989, 1990). The enzymes required for glyconeogenesis from lactate are present in lamprey muscle (Savina and Plisetskaya, 1976; Savina and Wojtczak, 1977; Hardisty and Rovainen, 1982) and the pH conditions during recovery (Fig. 1A) are near optimal for gluconeogenic flux through the principal regulatory locus of this pathway (i.e. the fructose-1,6-biphosphatase, 6-phosphofructo-1-kinase locus) as shown by Ferguson and Storey (1992) for trout white muscle. Maximal flux through this locus, in the gluconeogenic direction, occurs at pH6.5, similar to that seen in lamprey muscle for much of the recovery period (Fig. 1A). Bonen *et al.* (1989) argue that low muscle pH (i.e. proton retention) is a necessary prerequisite for increased glyconeogenic flux, and that this may be a feature common to all vertebrates.

In conclusion, the post-exercise acid–base balance of lamprey muscle is influenced both by proton-consuming processes, such as AMP deamination and gluconeogenesis,

and by the proton-producing rephosphorylation of creatine. The net effect is that while pHi is maximally depressed, glycogen and creatine phosphate stores are replenished within the first hour of recovery from exhaustive exercise, readying the animal to access such stores rapidly should further muscular work be required.

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