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MECHANISMS OF ACID–BASE REGULATION IN MIGRANT SEA LAMPREYS (*PETROMYZON MARINUS*) FOLLOWING EXHAUSTIVE EXERCISE

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Summary

The life cycle of the sea lamprey (*Petromyzon marinus*) is characterized by a terminal upstream spawning migration that may be associated with brief bursts of high-intensity swimming. Such activity usually leads to pronounced blood acid–base disturbances in fishes, but lampreys rapidly correct these perturbations within 1 h. In the present study, patterns of post-exercise H^+ excretion (J^H_{net}) and ion movements were followed in sea lampreys to test the hypothesis that dynamic manipulation of Na^+ *versus* Cl^- movements across the animal's body surface, presumably at the gills, accounted for the rapid restoration of blood pH following exercise. The first hour of post-exercise recovery in sea lampreys was associated with marked stimulation of J^H_{net} (equivalent to base uptake), which approached $-500\,\mu mol\,kg^{-1}\,h^{-1}$. After 1 h, J^H_{net} patterns had returned to resting rates. Analyses of net Na^+ and Cl^- movements (J^{Na}_{net}, J^{Cl}_{net}) suggested that elevated net Cl^- losses, which greatly exceeded net Na^+ losses, accounted for most of the J^H_{net}. Subsequent experiments, using radiotracers ($^{22}Na^+$, $^{36}Cl^-$), indicated that differential increases in Cl^- *versus* Na^+ permeability accounted for the greater post-exercise Cl^- losses and the corresponding stimulation of net proton excretion. Finally, metabolic acid budget analyses confirmed our hypothesis that rapid excretion of metabolic protons was the primary means used by sea lampreys to correct post-exercise extracellular acidosis.

Key words: gill, Cl^-/HCO_3^- exchange, net H^+ excretion, acid excretion, acid–base, ionoregulation, Na^+ influx, outflux, net flux, sea lamprey, *Petromyzon marinus*.

Introduction

The terminal spawning phase of the life cycle of the sea lamprey (*Petromyzon marinus*) is distinguished by migrations up many of the freshwater streams that are found in the Great Lakes watershed or along Atlantic coastal regions of North America and Europe (Scott and Scott, 1988). This upstream migration may be characterized by brief periods of high-intensity burst swimming as the animals are compelled to swim against strong currents and/or are forced to overcome natural and man-made obstacles. As with salmonids and other fishes (e.g. Black *et al.* 1962; Milligan and Wood, 1986; Schulte *et al.* 1992; Wang *et al.* 1994), such brief periods of high-intensity exercise result in marked post-exercise depressions in blood pH, approaching 0.3–0.5 units, due to the metabolic and respiratory generation of H^+ (Tufts, 1991; Tufts *et al.* 1992; Boutilier *et al.* 1993). Despite the severity of these disturbances, lampreys rapidly correct blood acid–base balance within the first hour of recovery (Tufts, 1991; Tufts *et al.* 1992; Boutilier *et al.* 1993).

Virtually nothing is known about how lampreys correct exercise-induced blood acid–base disturbances. The few studies to examine lamprey acid–base regulation indicate that the low permeability of red blood cells (RBCs) to acid equivalents, due to the absence of significant RBC membrane Cl^-/HCO_3 exchange (Nikinmaa and Railo, 1987; Tufts and Boutilier, 1989, 1990; Cameron *et al.* 1996), reduces the overall contribution of haemoglobin to non-bicarbonate buffering by lamprey blood (Nikinmaa *et al.* 1995). As a result, the total non-bicarbonate buffering capacity of lamprey blood is relatively low compared with that of teleosts (Matsoff and Nikinmaa, 1988; Tufts and Boutilier, 1990), which makes them more susceptible to exogenously induced acid–base disturbances (e.g. exposure to water at pH 4.0; Matsoff and Nikinmaa, 1988). The ability of the sea lamprey to correct post-exercise acidosis rapidly (Tufts, 1991; Boutilier *et al.* 1993), despite their blood buffering limitations, suggests that they use other highly efficient strategies to correct acid–base perturbations. Accordingly, the central objective of this study was to determine the physiological attributes that contribute to the rapid post-exercise correction of acid–base balance in sea lampreys.

Many teleost fishes correct acid–base perturbations by differentially manipulating the influx and outflux patterns of Na^+ and Cl^- across their gills, to facilitate H^+ or HCO_3^- excretion, respectively (e.g. Cameron, 1976; Holeton *et al.*

1983; Wood, 1988; McDonald *et al.* 1989; Goss *et al.* 1992). At present, it is not known whether lampreys use similar acid–base regulatory strategies, but the similarities between lamprey and teleost gills suggest that this is likely. Indeed, other than the fact that the gills of juvenile and adult lampreys are tidally ventilated, as opposed to the unidirectional flow design of other fish gills (Randall, 1972; Lewis, 1980), lamprey gills are structurally similar to those of teleosts. Common features include the presence of paired gill filaments ornamented with numerous lamellae (for a review, see Laurent, 1984), respiratory cells (pavement cells) and mitochondria-rich cells (Youson and Freeman, 1976; Morris and Pickering, 1975, 1976; Bartels, 1989; Mallat *et al.* 1995; Bartels *et al.* 1996). The latter are morphologically similar to, but distinct from, the chloride cells of freshwater species (Youson and Freeman, 1976; Morris and Pickering, 1976; Bartels *et al.* 1996), which are probably the sites of Cl^-/HCO_3^- exchange in freshwater teleosts (Perry and Laurent, 1989; Goss *et al.* 1992). Thus, this study was designed to test the hypothesis that sea lampreys clear large amounts of metabolic acid from their blood following exercise by differentially modulating trans-branchial Cl^- *versus* Na^+ movements. Accordingly, net H^+ excretion and net and unidirectional ion (Cl^- and Na^+) movements across sea lamprey gills were measured as the animals recovered from exhaustive exercise.

Materials and methods

Experimental animals and apparatus

Mature, migrating sea lampreys of both sexes (*Petromyzon marinus* Linnaeus; mass 259±8 g; mean ± S.E.M., N=26) were captured during their spring spawning run up the Shelter Valley River, Ontario, and held in 750 l of flowing (2.0 l min^{-1}), dechlorinated tap water ($[Na^+]$=0.4 mmol l^{-1}; $[Cl^-]$=0.6 mmol l^{-1}; $[Ca^{2+}]$=2.2 mmol l^{-1}; titration alkalinity 2.0 mmol l^{-1}) at Queen's University. Since spawning is a non-trophic life phase for lampreys, feeding was not necessary. At least 2 weeks prior to experimentation, subgroups (10–12) of animals were taken from their holding tanks at 5 °C and transferred to a Living Stream (Frigid Inc.) thermostatted to 10 °C, the experimental temperature. One day prior to experimentation the animals were transferred, one at a time, to individual, darkened, rectangular 5.0 l flux chambers, each of which received water at a rate of 0.5 l min^{-1}. The flux chambers, based on a design originally described by McDonald and Rogano (1986), consisted of an inner chamber, containing the animal, and a well-aerated outer chamber to ensure adequate mixing for accurate determinations of titratable acid flux (J^{TA}), total ammonia excretion (J^{Amm}) and net and unidirectional ion movements (Na^+, Cl^-) across the gill. Water samples were withdrawn from the box using an external sampling port mounted on the anterior side of the chamber. The sampling port consisted of a three-way stopcock, inserted through a rubber stopper, connected to a piece of rubber tubing that ran into the inner chamber. This permitted a syringe to be attached or detached from the external valve without disturbing the animal. Two series of experiments were performed.

Experimental protocol

Net ion movements and acid excretion following exercise

Flux measurements, for the determination of J^{TA}, J^{Amm} and net Na^+ (J^{Na}_{net}) and Cl^- (J^{Cl}_{net}) movements, were made by cutting off the water flow to each flux container, reducing the volume to 3.0 l, and subsequently taking water samples (30 ml) at regular intervals to determine the rate of changes in water titratable acidity and Na^+, Cl^- and ammonia concentrations in the container water.

Initial control measurements were made on resting sea lampreys over a 1 h pre-exercise period. The lampreys were subsequently chased one at a time for 5 min in a 2 m diameter, plastic exercise tank at 10 °C (see Tufts, 1991) and then returned to their respective flux containers. Water samples (30 ml) were then taken to determine flux rates at 0–0.5 h, 1–2 h, 4–5 h, 8–9 h and 12–13 h of the post-exercise period. Water samples (10 ml) for determination of J^{TA} were refrigerated and analyzed the same day, and the remainder was frozen and stored at –20 °C for later analyses of water total ammonia, Na^+ and Cl^-.

Unidirectional ion movements following exercise

The unidirectional movements (net flux, influx and outflux) of Na^+ and Cl^- were determined using radiotracers ($^{22}Na^+$, $^{36}Cl^-$). Briefly, 10 min prior to each flux determination, water flow to each flux chamber was cut off, and the volume was adjusted to approximately 3.0 l, after which 4 μCi of $^{22}Na^+$ or 8 μCi of $^{36}Cl^-$ was added to each container. After a 10 min mixing period, water samples (30 ml) were taken at regular intervals (0, 2 h, 4 h, 8 h, 12 h) over a 12 h control (resting) period. The containers were then thoroughly flushed to remove the radiotracers, and then water flow was re-established to each container.

The 12 h control flux period confirmed that there were no diurnal fluctuations in Na^+ or Cl^- flux patterns or in J^{Amm}. Owing to basal ammonia excretion by the animals, however, water total ammonia concentration in the container increased significantly to approximately 50–100 μmol l^{-1} over the 12 h period, but these concentrations were well below resting blood ammonia levels (approximately 300 μmol l^{-1}; M. P. Wilkie and B. L. Tufts, unpublished data) and unlikely to inhibit J^{Amm}. This premise is supported by the stable rates of J^{Amm} that were observed.

Approximately 12 h after resting unidirectional ion flux rates had been measured, lampreys were removed from their containers and exercised (see above). The procedures used to determine post-exercise unidirectional Na^+ and Cl^- movements were virtually identical to those used to determine resting rates, but because it was necessary to ensure that the radiotracers added to the flux chamber were adequately mixed, it was not possible to take an initial water sample immediately following the exercise period (i.e. 0 h). Accordingly, the nominal 0 h water sample was actually taken 10 min following exercise.

Overall, unidirectional flux determinations were made at 10 min (0 h) to 1 h, 2–3 h, 3–4 h, 4–5 h, 8–9 h and 12–13 h following exercise. After the 5 h post-exercise sample, each container was thoroughly flushed, and water flow was then re-established until 10 min before the 8–12 h sample period. Blood samples were then taken following this final post-exercise sampling period for later determination of the mean specific activity of $^{22}Na^+$ and $^{36}Cl^-$.

Analytical techniques and calculations

Ion and acid fluxes

Water Na^+ and Cl^- concentrations were determined using flame photometry (Corning clinical flame photometer) and the mercuric thiocyanate assay (Zall *et al.* 1956), respectively. Water total ammonia (sum of $[NH_3]+[NH_4^+]$) concentrations were determined using the salicylate hypochlorite assay (Verdouw *et al.* 1978).

Estimates of J^{Amm}, J^{Cl}_{net} and J^{Na}_{net} were based on the following equation:

$$J^X = \frac{([X]_i - [X]_f) \times V}{M \times T}, \quad (1)$$

where [X] represents the concentration ($\mu mol\,l^{-1}$) of total ammonia, Na^+ or Cl^-, measured at the beginning (i) and end (f) of a flux period, V is the volume of the container, M is the animal's mass (kg) and T is the duration of the flux interval (h). Using this approach, positive values represent net *inward* movement or gain of ions, while negative values indicate net *outward* movement or loss of ions.

The titratable acid flux (J^{TA}) by lampreys (see above) was determined by measuring the titration alkalinity of water samples. Titration alkalinity was determined by adding 0.02 mol l^{-1} HCl (Sigma) to 10 ml water samples until an end-point pH of 4.0 was reached. Equation 1 was also used to determine the titratable base flux, which was based on the initial minus final titratable alkalinity of water samples. As above, net base gains or losses are also represented by positive and negative signs, respectively. Accordingly, it was possible to express the titratable base flux rates as J^{TA} simply by switching positive titratable base flux values to negative values, and *vice versa* (for further details, see Wood, 1988). The sum of J^{Amm} and J^{TA} was then used as a *direct* measure of *net* acid flux (J^H_{net}) and is described by:

$$J^H_{net} = J^{Amm} + J^{TA}. \quad (2)$$

Differences between J^{Cl}_{net} and J^{Na}_{net} were used as an *indirect* index of J^H_{net} to predict how post-exercise modifications to *actual* J^H_{net} were achieved (e.g. Wood, 1988; McDonald *et al.* 1989). Accordingly,

$$Predicted\ J^H_{net} \approx J^{Cl}_{net} - J^{Na}_{net}. \quad (3)$$

The radioactivity of water samples was determined, in triplicate, on 5 ml water samples that were added to 10 ml of scintillation fluor (Amersham, ACS). The samples were shaken vigorously and then left overnight to minimize chemiluminescence before being counted on an LKB Rackbeta scintillation counter. The respective concentrations of 'cold' (non-radioactive) Na^+ or Cl^- in each sample were determined as described above.

Rates of Na^+ and Cl^- influx (J^{Na}_{in}, J^{Cl}_{in}) were determined from reductions in water radioactivity during each flux determination period, the known container volume, and the fish's mass, using the following formula (Wood, 1988):

$$J^{Ion}_{in} = \frac{(CPM_i - CPM_f)}{MSA} \frac{V}{W \times T}, \quad (4)$$

where J^{Ion}_{in} represents J^{Na}_{in} or J^{Cl}_{in}, CPM_i and CPM_f are the respective $^{22}Na^+$ or $^{36}Cl^-$ radioactivities (cts min^{-1} ml^{-1}) at the start and end of a flux determination period, *MSA* is the mean water specific activity (cts min^{-1} μmol^{-1}) and M, V and T are as previously stated. Back-flux correction was not necessary as the maximal internal specific activity of each animal, based on plasma measurements taken immediately after the 12 h post-exercise sample period, never exceeded 10 % of the water specific activity (Wood, 1988). Initial water specific activities were approximately 9×10^3 cts min^{-1} μmol^{-1} for $^{22}Na^+$ and 17×10^3 cts min^{-1} μmol^{-1} for $^{36}Cl^-$.

Estimates of J^{Na}_{net} and J^{Cl}_{net} were based on the respective differences between the 'cold' (non-radioactive) Na^+ and Cl^- concentrations in the water as described in equation 1. Outward movements (J^{Ion}_{out}) for Cl^- (J^{Cl}_{out}) and Na^+ (J^{Na}_{out}) were calculated from differences between J^{Ion}_{net} and J^{Ion}_{in}, as described by:

$$J^{Ion}_{out} = J^{Ion}_{net} - J^{Ion}_{in}. \quad (5)$$

Metabolic proton loads to the intracellular and extracellular compartments and to the environmental water

Another goal of the present study was to quantify the proportion of the lamprey's total peak metabolic acid load that was metabolized within its muscle, as well as the fraction of the acid load that was excreted to the water during the post-exercise period. Accordingly, levels of metabolic acid excreted to the water in the present study were compared with the cumulative internal post-exercise metabolic acid loads previously measured by Boutilier *et al.* (1993) and Tufts (1991).

To describe the fate of metabolically generated protons due to exercise, the total metabolic proton load ($\Delta H^+_{m,Total}$) was calculated using the following formula:

$$\Delta H^+_{m,Total} = \Delta H^+_{m,ICF} + \Delta H^+_{m,ECF} + \Delta H^+_{m,Water}, \quad (6)$$

where $\Delta H^+_{m,ICF}$ is the metabolic proton load to the muscle intracellular compartment (from Boutilier *et al.* 1993), $\Delta H^+_{m,ECF}$ is the extracellular metabolic proton load (from Tufts, 1991) and $\Delta H^+_{m,Water}$ denotes the cumulative appearance of metabolic acid (protons) in the environmental water, as measured in the present study.

Since the $\Delta H^+_{m,ICF}$ values reported by Boutilier *et al.* (1993) were originally expressed as mmol l^{-1} tissue water, their values were converted to mmol kg^{-1} body mass using the following equation:

$$\Delta H^{+}_{m,ICF}\ (mmol\ kg^{-1}) = (\Delta H^{+}_{m,ICF}) \times (\text{muscle } H_2O \text{ content}) \times (\%\ \text{muscle in body}), \quad (7)$$

where the measured muscle H_2O content was $0.78\ l\ kg^{-1}$ (Boutilier *et al.* 1993) and it was assumed that the muscle comprised 70 % of the total body mass of the lamprey (Hardisty and Rovainen, 1982). Similarly, the values of Tufts (1991) were converted to mmol kg^{-1} body mass using:

$$\Delta H^{+}_{m,ECF}\ (mmol\ kg^{-1}) = (\Delta H^{+}_{m,ECF}) \times (\text{extracellular fluid volume}), \quad (8)$$

where $\Delta H^{+}_{m,ECF}$ was originally expressed as mmol l^{-1} tissue water and the extracellular fluid volume was assumed to be $0.239\ l\ kg^{-1}$ body mass (Thorson, 1959).

Statistics

All data are expressed as means (±1 S.E.M.). Statistical significance was evaluated using repeated-measures analysis of variance (ANOVA), and Dunnett's *post-hoc* tests were applied, where appropriate, to determine statistical significance at the $P \leqslant 0.05$ level.

Results

Net ion movements and acid excretion following exercise

Resting ammonia excretion (J^{Amm}) and titratable acid (J^{TA}) flux rates in spawning sea lampreys averaged $-85\ \mu mol\ kg^{-1}\ h^{-1}$ and $70\ \mu mol\ kg^{-1}\ h^{-1}$, respectively (Fig. 1A,B). As a result, the net acid excretion rates were not significantly different from zero (Fig. 1C), indicating that the lampreys were in 'net' acid–base balance prior to exercise. Immediately following exercise, however, there was a threefold stimulation of J^{Amm} and a reversal of J^{TA}, which led to high rates of net acid excretion. During the first hour following exercise, the net acid excretion rate exceeded $-500\ \mu mol\ kg^{-1}\ h^{-1}$ (Fig. 1C), but by 2 h J^{H}_{net} had returned towards resting values as J^{Amm} and J^{TA} approached pre-exercise levels. Notably, there was a reversal of J^{H}_{net} after 8 h, which led to a small net gain of acid by the animals at that time (Fig. 1B,C).

Resting lampreys were also in net ion balance, as reflected by net Cl^- (J^{Cl}_{net}) and net Na^+ (J^{Na}_{net}) flux rates that were not significantly different from zero (Fig. 2A,B). Following exercise, J^{Cl}_{net} became highly negative, leading to net Cl^- loss rates that approached $-600\ \mu mol\ kg^{-1}\ h^{-1}$ during the first hour of the post-exercise period. These pronounced Cl^- losses were not, however, accompanied by significant changes in J^{Na}_{net} (Fig. 2A,B) and, as a result, there were significant increases in the predicted net acid losses by the lamprey during the first hour following exercise (Fig. 2C). By 2 h post-exercise, however, J^{Cl}_{net} and predicted J^{H}_{net} had returned to resting levels (Fig. 2A,C). By 8 h, there appeared to be net Na^+ gains by the animal, when inwardly directed J^{Na}_{net} approached $300\ \mu mol\ kg^{-1}\ h^{-1}$ (Fig. 2B).

Unidirectional ion movements following exercise

Resting lampreys were also in net ion balance in the second experimental series (Figs 3, 4). In general agreement with observations made in the first series, the first hour following exercise was characterized by a marked stimulation of net Cl^- losses, reflected in J^{Cl}_{net} rates that approached $-400\ \mu mol\ kg^{-1}\ h^{-1}$ (Fig. 3). By 2 h, J^{Cl}_{net} had returned to resting levels, where it remained until 4 h. There then appeared to be a net inward movement of Cl^-, which persisted until 12 h, but these changes in J^{Cl}_{net} were not significant. The greater net Cl^- loss resulted from a marked eightfold stimulation of Cl^- outflux (J^{Cl}_{out}) during the first hour following exercise (Fig. 3). The greater Cl^- outflux was short-lived, however, and by 2 h net Cl^- losses and Cl^- outflux rates had returned to resting levels (Fig. 3). Post-exercise Cl^- influx patterns were unaltered, except at 8 h where there was a net influx of Cl^- (Fig. 3).

Compared with J^{Cl}_{net} patterns, changes in J^{Na}_{net} following exercise were much less pronounced; net Na^+ losses were approximately $-90\ \mu mol\ kg^{-1}\ h^{-1}$ and $-170\ \mu mol\ kg^{-1}\ h^{-1}$ at 1 h and 2 h, respectively (Fig. 4). These greater net losses of Na^+ were solely the result of marked increases in Na^+ outflux in the first 2 h of the post-exercise period (Fig. 4). Beyond 2 h, no further significant changes in net outward Na^+ movement were

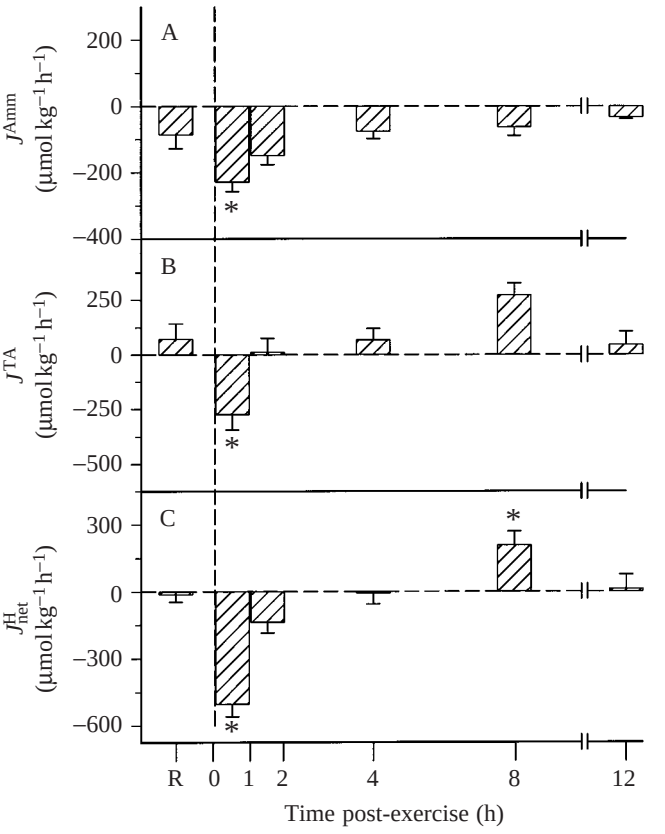

Fig. 1. Changes in (A) ammonia excretion (J^{Amm}), (B) titratable acid flux (J^{TA}) and (C) net proton flux (J^{H}_{net}) across the body surface (gills + renal pathways) of migrant sea lampreys following 5 min of exhaustive exercise. Values are means ± 1 S.E.M.; N=10. An asterisk denotes a statistically significant difference (P<0.05) from the value for resting (R) animals.

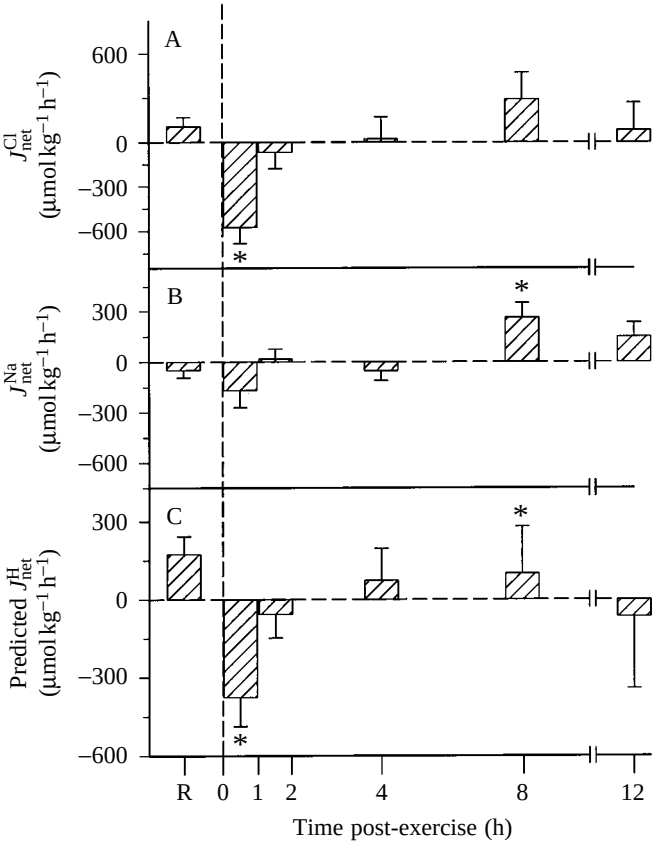

Fig. 2. Changes in (A) net Cl^- flux (J^{Cl}_{net}), (B) net Na^+ flux (J^{Na}_{net}) and (C) the *predicted* net proton flux rates across the body surface of migrant sea lampreys. The differences between J^{Cl}_{net} and J^{Na}_{net} were used as an indirect index to *predict* net branchial proton movements (J^{H}_{net}). Values are means ± 1 S.E.M.; N=10. An asterisk denotes a statistically significant difference (P<0.05) from the value for resting (R) animals.

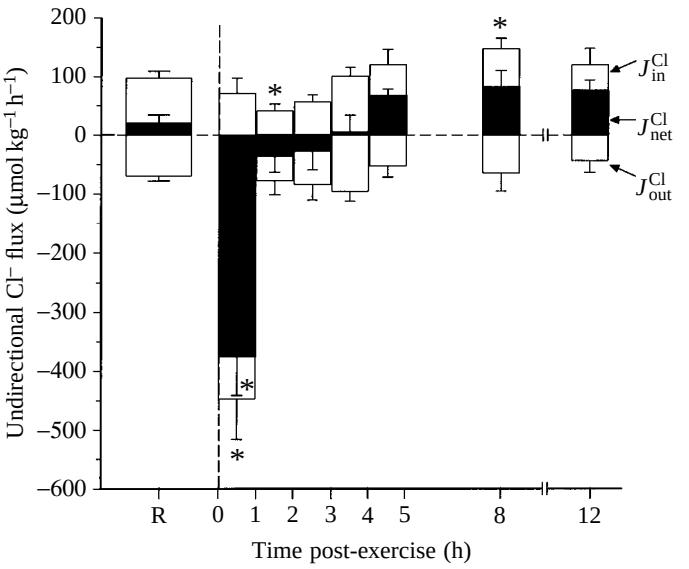

Fig. 3. Changes in rates of Cl^- influx (J^{Cl}_{in}, upward-facing bars), outflux (J^{Cl}_{out}, downward-facing bars) and net movement (J^{Cl}_{net}, shaded bars) across the body surface of migrant sea lampreys following 5 min of exhaustive exercise. Values are means ± 1 S.E.M.; N=9. An asterisk denotes a statistically significant difference (P<0.05) from the rate for resting (R) animals.

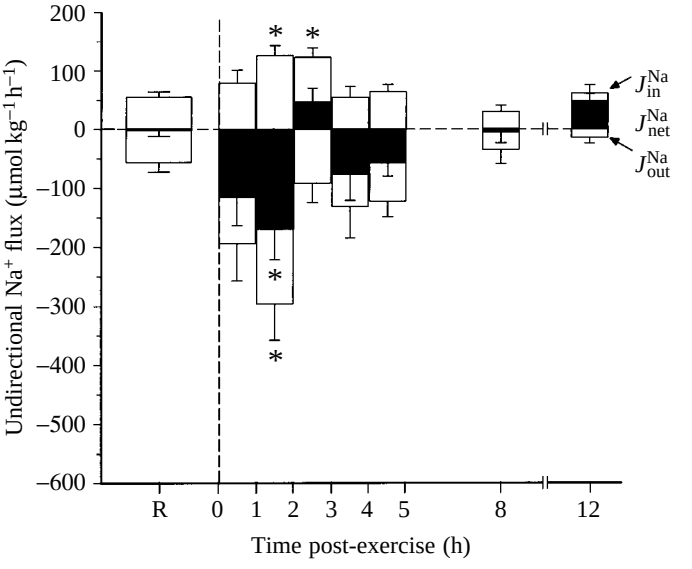

Fig. 4. Changes in rates of Na^+ influx (J^{Na}_{in}; upward-facing bars), outflux (J^{Na}_{out}, downward-facing bars) and net movement (J^{Na}_{net}, shaded bars) across the body surface of migrant sea lampreys. Values are means ± 1 S.E.M.; N=7. An asterisk denotes a statistically significant difference (P<0.05) from the rate for resting (R) animals.

observed. There was a temporary twofold stimulation of Na^+ influx between 1 and 3 h of the post-exercise period, however, which led to small, but significant, net Na^+ gains in the lamprey (Fig. 4).

Discussion

Sites of acid–base regulation

In freshwater fishes, the major site of acid–base regulation is the gills, which operate through the differential modulation of net Na^+ and Cl^- influx and outflux patterns (e.g. Wood, 1988; McDonald *et al.* 1989; Goss *et al.* 1995). Generally, teleost gills account for more than 90 % of net acid (or base) equivalent movements (McDonald and Wood, 1981). To our knowledge, acid–base regulation across lamprey gills has not been studied, although there have been some detailed examinations of ion-exchange processes in larval lampreys (Morris and Bull, 1968, 1970; Stinson and Mallat, 1989; Mallat and Stinson, 1990). In the present study, most of the observed alterations in acid flux and ion flux patterns were probably mediated by the gills because the ultrastructure of the lamprey gill is similar to that of teleosts (e.g. Morris and Pickering, 1976; Peek and Youson, 1979; Bartels *et al.* 1989, 1996). Ions may also be excreted *via* renal pathways, however, in addition to diffusive efflux across the gills (e.g. McDonald and Wood,

1981; Wheatley *et al.* 1984). Since renal routes of ion excretion were not isolated from branchial routes of diffusive ion efflux in the present study, the alternative term 'outflux' describes outward ion movements across the lamprey's body.

Post-exercise acid excretion

The pronounced acidosis experienced by lampreys following exercise is corrected through marked stimulations of net acid excretion (Fig. 1). Like other fishes, sea lampreys experience post-exercise metabolic and respiratory acidoses which are characterized by 0.4–0.5 unit depressions in blood pH (Tufts, 1991; Tufts *et al.* 1992). Unlike freshwater salmonids, in which correction of the post-exercise extracellular acidosis generally requires 4–12 h (Holeton *et al.* 1983; Milligan and Wood, 1986; Wang *et al.* 1994; Kieffer *et al.* 1994), lampreys restore blood pH within 1 h (Tufts, 1991; Tufts *et al.* 1992). This rapid correction is probably related to the lamprey's very high post-exercise rates of acid excretion.

The peak rates of acid excretion of lampreys are approximately 50 % greater than rates measured in exhaustively exercised freshwater rainbow trout (*Oncorhynchus mykiss*) (Wood, 1988; McDonald *et al.* 1989; Kieffer and Tufts, 1996) and approximately twice as high as rates measured in acidotic rainbow trout and eels (*Anguilla rostrata*) under hyperoxic conditions (Hyde and Perry, 1987; Wood, 1991; Goss and Wood, 1990). Post-exercise rates of acid excretion by lampreys were also 2–3 times higher than rates seen in other acidotic freshwater fishes including hypercapnic carp (*Cyprinus carpio*) (Claiborne and Heisler, 1984), killifish (*Fundulus heteroclitus*) injected with HCl (Patrick *et al.* 1997), goldfish (*Carassius auratus*) exposed to acidic water (Maetz, 1973) and channel catfish (*Ictalurus punctatus*) infused with ammonium sulphate (Cameron and Kormanik, 1982).

Notably, the lamprey's rates of acid excretion were lower than values measured in freshwater rainbow trout injected with ammonium sulphate (McDonald and Prior, 1988). In addition, the post-exercise rate of acid excretion was approximately 50 % lower than the peak post-exercise values measured in the dogfish (*Scyliorhinus stellaris*; Heisler, 1993). This difference is probably related to the dogfish's saltwater habitat. Indeed, Tang *et al.* (1989) suggested that the leakier gill epithelium of saltwater- *versus* freshwater-acclimated rainbow trout probably promoted faster rates of metabolic proton removal following exercise.

The low non-bicarbonate buffering capacity of lamprey blood (Tufts and Boutilier, 1990; Matsoff and Nikinmaa, 1988) should also be considered when comparing rates of post-exercise recovery of blood pH between lampreys and other fishes. Although the reduction in post-exercise blood pH is similar in lamprey and trout (e.g. Milligan and Wood, 1986; Tufts, 1991), the anaerobic capacity and production of metabolic protons are lower in lamprey (Tufts, 1991; Boutilier *et al.* 1993). In fact, exercise-induced metabolic proton production by trout far exceeds metabolic proton production

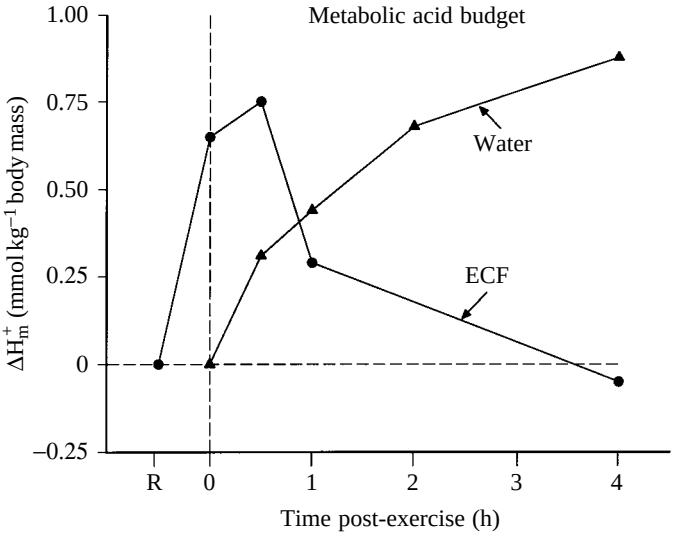

Fig. 5. Compartmentalized metabolic acid budget analysis (ΔH_m^+) relating the correction of the post-exercise metabolic acid load in the extracellular fluid (ECF) of migrant sea lamprey blood to the appearance of metabolic H^+ in the water. Blood acid–base data are taken from Tufts (1991). Extracellular fluid space of the lamprey was estimated to be 0.239 l kg^{-1} body mass (Thorson, 1959).

by lampreys, as reflected by twofold higher peak extracellular metabolic acid loads (Milligan and Wood, 1986; Tang and Boutilier, 1991; Tufts, 1991; Boutilier *et al.* 1993; Kieffer *et al.* 1994; Wang *et al.* 1994).

To resolve how lampreys correct internal post-exercise acidosis, their internal metabolic acid loads, as measured by Tufts (1991), were compared with the total post-exercise net acid excretion rates reported here. This analysis revealed that virtually all of the post-exercise removal of metabolic acid from the extracellular fluid of the lamprey took place *via* proton excretion to the environmental water (Fig. 5). Although different groups of sea lamprey were used to measure acid–base balance (Tufts, 1991) and ion flux rates (present study), the degree of extracellular acidosis experienced by each group was probably comparable, since both sets of lampreys were exercised under virtually identical experimental conditions (e.g. temperature, water quality, season) and were in the same non-trophic, spawning phase of their life cycle. This premise is supported by different studies conducted in our laboratory, which demonstrate that changes in sea lamprey blood pH following exercise are reproducible (Tufts, 1991; Tufts *et al.* 1992). Ideally, blood acid–base status and ion flux rates should have been measured on the same group of animals, but this is undesirable because surgically implanted arterial catheters tend to compromise ion balance and flux patterns (Wood, 1988).

As in the trout (Milligan and Wood, 1986; Tang and Boutilier, 1991; Kieffer *et al.* 1994; Kieffer and Tufts, 1996), the vast majority of the metabolic protons, along with lactate, produced during exercise were probably retained in the intracellular compartment of the muscle and consumed during

Table 1. *Cumulative metabolic proton load to the muscle intracellular compartment ($\Delta H^{+}_{m,ICF}$), extracellular compartment ($\Delta H^{+}_{m,ECF}$) and environmental water ($\Delta H^{+}_{m,Water}$) in sea lamprey following 5 min of exhaustive exercise*

Time after exercise (h)	$\Delta H^{+}_{m,ICF}$† (mmol kg^{-1} body mass)	$\Delta H^{+}_{m,ECF}$†† (mmol kg^{-1} body mass)	$\Delta H^{+}_{m,Water}$††† (mmol kg^{-1} body mass)	$\Delta H^{+}_{m,Total}$* (mmol kg^{-1} body mass)
0	14.74 (95.8 %)	0.65 (4.2 %)	0	15.39
0.5	14.21 (93.4 %)	0.69 (4.5 %)	0.31 (2.0 %)	15.21
1.0	13.65 (94.9 %)	0.29 (2.0 %)	0.44 (3.1 %)	14.38
4.0	4.06 (83.4 %)	−0.07 (−1.4 %)	0.88 (18.1 %)	4.87

Numbers in parentheses represent the percentage contribution of each compartment to $\Delta H^{+}_{m,Total}$.
*$\Delta H^{+}_{m,Total} = \Delta H^{+}_{m,ICF} + \Delta H^{+}_{m,ECF} + \Delta H^{+}_{m,Water}$.
†Data taken from Boutilier *et al.* (1993).
††Data taken from Tufts (1991).
†††Estimates of $\Delta H^{+}_{m,Water}$ were based upon the cumulative appearance of metabolic acid in the environmental water following exercise.

glycogen resynthesis (Boutilier *et al.* 1993). To illustrate this point, the cumulative metabolic acid load to the muscle of exercised sea lampreys from Boutilier *et al.* (1993) was compared with the post-exercise extracellular metabolic acid load reported by Tufts (1991) and with the levels of metabolic acid excreted to the water in the present study. This analysis revealed that more than 90 % of the total acid load appearing in the lampreys' whole body was restricted to the muscle (Table 1). Further, almost 70 % of this whole-body metabolic acid load was metabolized in the muscle during the first 4 h of the post-exercise recovery period, but only approximately 6 % of this acid load was cleared *via* excretion to the water (Table 2). Similar observations have also been reported for rainbow trout (Tang and Boutilier, 1991; Kieffer and Tufts, 1996).

Analysis of the net ion movements across the body surface of the lamprey indicated that the post-exercise stimulation of acid excretion resulted from significantly higher losses of Cl^- than of Na^+ during the first hour (Fig. 2). According to the strong ion difference theory (Stewart, 1983) and the constraints of electroneutrality, net Na^+ or Cl^- uptake must be accompanied by simultaneous acid (equivalent to base uptake) or base equivalent excretion (equivalent to acid uptake), respectively (Wood, 1988; McDonald *et al.* 1989). Using these criteria, it is possible to use net Cl^- and Na^+ flux patterns to predict net acid excretion patterns. Accordingly, the predicted proton flux rates calculated here (Fig. 2), based upon differences between J^{Cl}_{net} and J^{Na}_{net}, confirm our hypothesis that a stimulation of net Cl^- over Na^+ losses during the first hour of recovery accounted for most of the net acid excretion we observed. In fact, when *actual* J^{H}_{net} was plotted against *predicted* J^{H}_{net}, a significant linear relationship was observed (Fig. 6). The slope of this relationship, approximately 0.7 (r=0.82; P=0.02), was very close to values obtained by Wood (1988) in rainbow trout recovering from exhaustive exercise. The lack of complete (i.e. 1:1) agreement between the *estimated* and *actual* rates of J^{H}_{net} might have been due to the slightly higher variability of net ion compared with *actual* H^+ movements, but other ions (e.g. Ca^{2+}, $H_2PO_4^-$, HPO_4^{2-}) also probably contributed to the outward acid flux. In fact, ions such as $H_2PO_4^-$ and HPO_4^{2-} may have been excreted *via* renal pathways (Wheatley *et al.* 1984; Wood, 1988).

Table 2. *Percentage of the total metabolic acid load ($\Delta H^{+}_{m,Total}$) cleared via* in situ *metabolism within the muscle intracellular compartment (muscle ICF) or via excretion to the environmental water*

Time after exercise (h)	Percentage clearance	
	Muscle ICF*	Excretion to water†
0.5	3.2	2.0
1.0	7.1	2.8
4.0	68.8	5.7

*Percentage of $\Delta H^{+}_{m,Total}$ metabolized in muscle ICF = $\dfrac{(\Delta H^{+}_{m,ICF} \text{ at } 0\,h - \Delta H^{+}_{m,ICF} \text{ at } t\,h)}{(\Delta H^{+}_{m,Total} \text{ at } 0\,h)}$.

†Percentage of $\Delta H^{+}_{m,Total}$ excreted to water = $\dfrac{(\Delta H^{+}_{m,water} \text{ at } t\,h)}{(\Delta H^{+}_{m,Total} \text{ at } 0\,h)}$.

Post-exercise unidirectional ion movements

Radiotracers were used to determine how changes in post-exercise net acid flux took place. Under resting conditions, the lamprey's body surface was relatively impermeable to both Na^+ and Cl^-, as illustrated by net outflux rates for each ion that were less than $-75\,\mu mol\,kg^{-1}\,h^{-1}$. Outflux rates in other teleosts, maintained under similar conditions, are typically 2–4 times higher (e.g. Maetz and Garcia-Romeu, 1964; Cameron, 1976; Wood, 1988; Perry *et al.* 1992). In all resting lampreys, the small Na^+ and Cl^- outflux rates were countered by matching

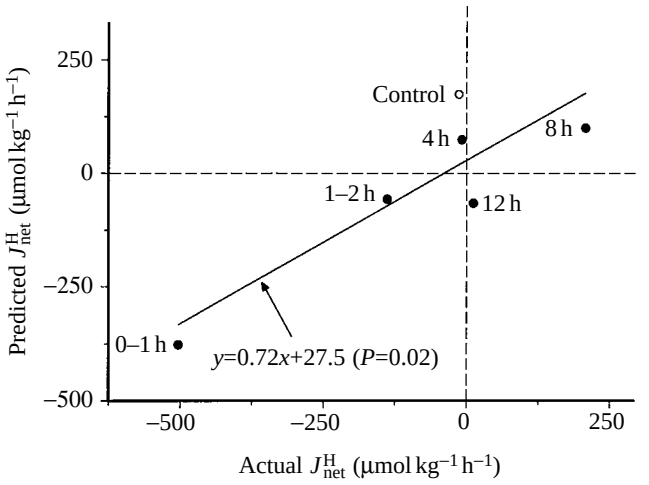

Fig. 6. *Actual* versus *predicted* net proton flux (J^{H}_{net}) rates across the body surface of migrant sea lampreys following 5 min of exhaustive exercise. *Predicted* J^{H}_{net} is based on calculated differences between net Cl^- and net Na^+ flux rates (predicted $J^{H}_{net} \approx J^{Cl}_{net} - J^{Na}_{net}$) under resting (control) conditions (open circle) and after 0–1 h, 1–2 h, 4 h, 8 h and 12 h of post-exercise recovery. The solid line represents the best fit line, as determined using least-squares analyses (y=0.72x+27.5; P=0.02, r=0.82). A significant correlation was observed, as determined using Pearson's linear correlation coefficient (r).

rates of ion influx. Interestingly, Cl^- and Na^+ influx rates in larval sea lampreys and river lampreys (*Lampetra fluviatilis*) are 2–3 times higher than the rates in the spawning sea lampreys examined in the present study (Morris and Bull, 1968; Stinson and Mallat, 1989). These apparent discrepancies might be due to factors such as life stage, body size, water quality or water temperature and should be investigated further.

The unidirectional Cl^- flux data indicate that the net Cl^- losses following exercise were almost exclusively due to the large increases in outflux (branchial plus renal excretion) rather than to a reduced Cl^- influx. Although J^{Cl}_{in} was significantly lower at 2 h than in resting lampreys, this reduction was slight and was unlikely to have had any major effects on post-exercise acid–base balance in these animals.

Stimulation of Na^+ influx is associated with increased H^+ excretion in many fish species (Cameron, 1976; McDonald and Wood, 1981; Wood, 1988; Hyde and Perry, 1989; Tang and Boutilier, 1991; Goss *et al.* 1992). In the present study, however, increases in Na^+ influx were relatively small compared with net Cl^- losses over the first hour of recovery. The twofold greater Na^+ influx rates observed between 2 and 4 h suggest that some additional acid excretion took place, but it was masked by the simultaneously high Na^+ outflux rates (Fig. 4). It is curious that Na^+ influx would be stimulated at a time when net acid excretion had probably returned to resting levels (Fig. 1; Tufts, 1991), but post-exercise intramuscular pH (pHi) remains depressed beyond 4 h in sea lampreys (Boutilier *et al.* 1993). This suggests that H^+ arising from the muscle intracellular compartment after 1 h may have led to additional H^+ excretion in the later stages of post-exercise recovery.

In conclusion, adult sea lampreys correct exercise-induced decreases in extracellular pH by differentially modulating Cl^- *versus* Na^+ movements across their body surface. The high rate of net metabolic H^+ excretion, which clears all of the metabolic acid from the animals' extracellular fluid by 2 h, is facilitated by higher post-exercise Cl^- *versus* Na^+ excretion rates. As in the rainbow trout, the majority of metabolic acid produced during exercise is retained and metabolized within the muscle compartment and only approximately 6 % of the whole-body metabolic acid load is excreted, presumably *via* the gills. Overall, the general mechanisms of acid removal by sea lampreys resemble the strategies used by teleosts, despite the fact that these groups have had separate phylogenetic lineages for at least 400 million years (Forey and Janvier, 1993). Future studies, aimed at precisely elucidating the mechanisms of acid–base and ion regulation in sea lampreys, should improve our understanding of the evolution of these physiological processes in vertebrates.

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