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ACID–BASE REGULATION AND BLOOD GAS TRANSPORT FOLLOWING EXHAUSTIVE EXERCISE IN AN AGNATHAN, THE SEA LAMPREY *PETROMYZON MARINUS*

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Summary

Exhaustive exercise in cannulated sea lampreys, *Petromyzon marinus*, resulted in a marked extracellular acidosis in the arterial blood which had both a respiratory and a metabolic component. Blood CO_2 tension (P_{CO_2}) returned to control levels within an hour after exercise, but the metabolic acidosis had a somewhat longer time course and the extracellular pH (pHe) did not fully recover until the 4 h recovery sample. The magnitude and duration of the changes in both the plasma lactate concentration and the concentration of metabolic protons were very similar and the maximal proton deficit after exercise was, therefore, only 1.5 mequiv l^{-1}. In contrast to the changes in pHe, there were no significant changes in the erythrocyte pH (pHi) following the exercise period. The regulation of pHi was apparently not adrenergically mediated, however, since addition of catecholamines to lamprey blood *in vitro* had no significant effect on pHi. In addition, the period of exhaustive exercise *in vivo* was not associated with any significant changes in the mean cellular hemoglobin concentration. The total carbon dioxide concentration in the arterial whole blood and true plasma were both significantly reduced after exercise, but the total carbon dioxide concentration within the erythrocytes was transiently increased. Finally, there was a marked decrease in the arterial P_{O_2} immediately after exercise, which was associated with a significant reduction in the amount of oxygen bound to hemoglobin; however, within 30 min, these values had both returned to normal. The maintenance of pHi presumably contributes to the regulation of oxygen transport in lampreys and it may be particularly important during the brief period immediately after exercise when oxygen transport is clearly compromised. Although several studies have provided evidence that chloride/bicarbonate exchange limitations may exist in agnathan blood *in vitro*, the present results demonstrate that the characteristics of carbon dioxide transport and acid–base regulation after exercise in *P. marinus* are not markedly different from those in other lower vertebrates.

Introduction

The sea lamprey *Petromyzon marinus* usually spends the parasitic phase of its

Key words: lamprey, *Petromyzon marinus*, acid–base regulation, exercise, blood gas transport.

life in the ocean, but spawns in freshwater rivers and streams. Landlocked forms of this lamprey also now exist. The parasitic phase of landlocked lampreys develops in large bodies of fresh water, but then migrates into rivers and streams in the early summer months prior to spawning. In many cases, the lampreys' spawning streams are the fast-flowing type cohabited by anadromous salmonids such as *Salmo salar*. Thus, the spawning migration in sea lampreys may also involve periods of burst activity to scale obstacles and ascend through the turbulent flow to the actual spawning site.

The physiological characteristics of exhaustive exercise in salmonids have been extensively studied. In contrast, there is a paucity of information on the physiology of exhaustive exercise in relatively primitive vertebrates such as the agnathans. Moreover, *in vitro* studies on agnathan blood have described several unique characteristics of their erythrocytes which may influence gas transport and acid–base regulation in these animals after exercise *in vivo*. The erythrocytes of both *Lampetra fluviatilis* and *P. marinus* regulate pH *in vitro via* a sodium/proton exchange mechanism on the erythrocyte membrane (Nikinmaa, 1986; Nikinmaa *et al.* 1986; B. L. Tufts, unpublished results). In *L. fluviatilis*, the erythrocyte pH is also regulated *in vivo* during chronic acid exposure (Mattsoff and Nikinmaa, 1988). In salmonids, regulation of erythrocyte pH contributes to the regulation of oxygen transport after exhaustive exercise (Primmett *et al.* 1986). Clearly, erythrocyte pH regulation may also have important consequences for the regulation of oxygen transport in lampreys after exercise, but this has not been investigated. Recent *in vitro* investigations have also demonstrated that chloride/bicarbonate exchange activity across the erythrocyte membrane may be absent or very limited in agnathan blood (Nikinmaa and Railo, 1987; Ellory *et al.* 1987; Tufts and Boutilier, 1989, 1990). This exchange mechanism is an integral part of the CO_2 transport system in most vertebrates (Roughton, 1964; Randall and Daxboeck, 1984; Cameron, 1978; Perry, 1986). Rapid chloride/bicarbonate exchange also provides a link between carbon dioxide and oxygen transport *via* the Bohr effect and enables hemoglobin to buffer metabolic acid rapidly during acid–base disturbances in the true plasma. Thus, any chloride/bicarbonate exchange limitations in agnathan erythrocytes might be expected to have profound consequences on gas transport and acid–base regulation in these animals after exercise. The purpose of the present investigation was, therefore, to exercise cannulated sea lampreys, *P. marinus*, exhaustively, and determine whether the unique features that have been previously described in agnathan erythrocytes *in vitro* impact significantly on the characteristics of gas transport and acid–base regulation during recovery from exercise *in vivo*.

Materials and methods

Animals

Adult lampreys, *Petromyzon marinus* Linnaeus (320±12.0 g: N=20), were collected during their spawning migration from the Shelter Valley River in Eastern

Ontario. The animals were transported to the Biology Department at Queen's University where they were held in freshwater tanks at 8–10°C for at least 2 weeks prior to the experiments. The composition of the water used to hold the animals and in the experiments was as follows: $[Na^+]=2.1$; $[K^+]=0.05$, $[Ca^{2+}]=2.2$, $[Cl^-]=1.3$, $[HCO_3^-]=1.5$ mequiv l^{-1}, pH 7.4.

Surgery

The lampreys were anesthetized in an aerated and pH-balanced solution of tricaine methane sulfonate (66.7 mg l^{-1} MS-222 and 133.3 mg l^{-1} $NaHCO_3$). The animals were then transferred to a surgical table and a mid-ventral incision (3–4 cm) was made approximately half-way down the body to allow access to the dorsal aorta. A cannula of PE 50 tubing was implanted in the dorsal aorta and secured to the body wall. The cannula extended through the incision, which was then closed with sutures. During the surgical procedure, the lamprey's head and gills were kept moist by intermittent immersion in the anesthetic solution and the body was wrapped in a damp cloth. Following the 5–10 min of surgery, the lampreys recovered in a lightproof Perspex box with aerated flowing fresh water at 10°C for 24–48 h prior to the experiment.

In vivo *experiments*

Following recovery, a 1.0 ml sample of blood was taken into a Hamilton gas-tight syringe. Whole-blood carbon dioxide content (C_{CO_2}), oxygen content (C_{O_2}) and oxygen tension (P_{O_2}) were measured immediately using about 400 μl of the sample. Triplicate hematocrit measurements were made with approximately 200 μl of blood and the remaining blood was dispensed into a 0.5 ml Eppendorf tube and centrifuged at 10 000 *g* for 4 min at 10°C. The C_{CO_2} of true plasma was determined on a 100 μl sample of plasma taken from the hematocrit tubes using a 100 μl gas-tight Hamilton syringe. Following centrifugation, the plasma pH (pHe) was measured immediately from the supernatant of the Eppendorf tube. A further 200 μl of the plasma supernatant was added to 200 μl of chilled 8 % perchloric acid (PCA) and then frozen in liquid nitrogen for the subsequent determination of plasma lactate concentrations. The remaining plasma was removed from the red cell pellet and the pellet was immediately frozen in liquid nitrogen prior to the determination of erythrocyte pH (pHi).

After the control sample had been taken, the lamprey was moved to a cylindrical tank containing aerated water at 10°C, where it was exhaustively exercised by manual chasing. Following the exercise period, the exhausted lamprey was quickly returned to the Perspex container and a second 1.0 ml blood sample was removed from the cannula. Identical analyses to those described for the control sample were performed on this blood sample. Samples were also taken at 0.5, 1, 4 and 8 h of recovery from exercise. Throughout the experiment, blood samples were replaced with a similar volume of heparinized (20 i.u. ml^{-1}) saline.

In vitro *experiments*

In a second series of experiments, 4 ml of blood was removed from a cannulated lamprey and equilibrated with a humidified 1% CO_2:air mixture in paired intermittently rotating tonometers at 10°C with 2 ml of blood per tonometer. Following a 60 min equilibration period, a 0.5 ml blood sample was removed from each tonometer and analyzed for pHe and pHi. At this point, 100 μl of either saline (sham) or saline plus catecholamines (final concentrations 10^{-5} mol l^{-1} adrenaline and 10^{-5} mol l^{-1} noradrenaline) was added to each blood pool. The blood was then equilibrated for a further 15 min, at which time a final 0.5 ml of blood was removed from each tonometer for another determination of pHe and pHi.

Analyses

Plasma (pHe) and erythrocyte (pHi) pH were determined with a PHM 73 pH meter and associated micro-pH unit (Radiometer, Copenhagen, Denmark) thermostatted at 10°C. Erythrocyte pellets were frozen and thawed twice in liquid nitrogen prior to the determination of pHi according to the method of Zeidler and Kim (1977). The whole-blood P_{O_2} was measured with an E5046 oxygen electrode (Radiometer, Copenhagen, Denmark), also thermostatted at 10°C, and an associated oxygen meter (Cameron Instrument Co., Texas, USA). Another E5046 oxygen electrode was used to determine the total oxygen content (C_{O_2}) of whole-blood samples using the Tucker method (Tucker, 1967). Total CO_2 contents (C_{CO_2}) of whole blood and plasma were measured with a Corning model 965 CO_2 analyzer (Ciba Corning Canada Inc.). Analysis of hemoglobin concentration was performed by Drabkin's method (Drabkin and Austin, 1935) with Sigma reagents. The concentration of plasma lactate was measured using the L-lactate dehydrogenase method (Loomis, 1961), also using Sigma reagents.

Measured values of true plasma total CO_2 and pHe were used to determine P_{CO_2} and true plasma bicarbonate concentration ($[HCO_3^-]_{t.pl}$) *via* a rearrangement of the Henderson–Hasselbalch equation with the values for pK′ determined according to Boutilier *et al.* (1984) and a CO_2 solubility coefficient (αCO_2) of 8.29×10^{-3} mmol l^{-1} kPa^{-1} (Tufts and Boutilier, 1990). The concentration of metabolic protons added to the plasma ($\Delta[H^+]_m$) over any given time period (e.g. time 1 to time 2) was calculated according to McDonald *et al.* (1980) using the following equation:

$$\Delta[H^+]_m = [HCO_3^-]_{t\,pl,1} - [HCO_3^-]_{t.pl,2} - \beta(pHe_1 - pHe_2)\,,$$

where β is the nonbicarbonate buffer value of true plasma (-3.1 mequiv l^{-1} pH unit^{-1}; Tufts and Boutilier, 1990). Finally, the erythrocyte C_{CO_2} (C_{CO_2eryth}) was determined from the C_{CO_2} of whole blood (w.bl) and true plasma (t.pl.) and the hematocrit (Hct) according to the following equation:

$$C_{CO_2eryth} = [C_{CO_2w.bl} - C_{CO_2t.pl} \times (1\text{-Hct})]/\text{Hct}\,.$$

Mean cellular hemoglobin concentrations (MCHC) were determined from the

hematocrit (Hct) and the blood hemoglobin concentration (Hb) using the following equation:

$$MCHC = Hb/(Hct/100).$$

Statistics

All values are presented as means ± standard error (*in vivo*, control, $N=6$, exercise, $N=8$; *in vitro*, $N=6$). A repeated-measures analysis of variance (ANOVA) was used to assess the significance of observed differences in both the *in vivo* and the *in vitro* experiments. If the ANOVA indicated significance ($P<0.05$), a Dunnett's multiple comparisons test was then used to determine significant differences ($P<0.05$) between resting values and post-exercise values in the *in vivo* experiment. An unpaired Student's *t*-test was also used to compare the exercise values to the control values in Fig. 3C.

Results

Exhaustive exercise in *Petromyzon marinus* resulted in an immediate drop in the extracellular pH (pHe) of 0.350 units (Fig. 1A). Thereafter, pHe began to recover and the extracellular acidosis only persisted until the 1 h sample. The acidosis was not apparent, however, at the level of the erythrocyte and throughout the recovery period the erythrocyte pH (pHi) was not significantly different from the pre-exercise resting value of 7.502±0.030 (Fig. 1B). The changes in the pH gradient (ΔpH=pHe−pHi) across the erythrocyte membrane reflect the considerable difference between extracellular and erythrocyte pH regulation after exhaustive exercise in the lamprey (Fig. 1C). There is a reduction of 0.322 units in this gradient immediately after exercise as the values for pHe and pHi become almost identical. The pH gradient steadily increases during the recovery period; however, owing to the recovery of pHe by the 1 h sample, the pH gradient is no longer significantly different from the resting value. Further evidence of the consistent regulation of erythrocyte pH in *P. marinus* is obtained when the individual data points for the relationship between pHe and pHi are plotted (Fig. 2). The slope of the regression line through these points is only 0.12; again demonstrating that pHi is hardly influenced by changes in pHe *in vivo*.

The regulation of erythrocyte pH *in vivo* did not appear to be adrenergically mediated. No significant change in erythrocyte pH was observed after catecholamines had been added to lamprey blood *in vitro* (Table 1). It should also be noted that there were no significant changes in the mean cellular hemoglobin concentration of lamprey erythrocytes after exhaustive exercise (Table 2). Thus, the *in vivo* regulation of erythrocyte pH in *P. marinus* does not appear to be associated with any significant changes in erythrocyte water content.

There were significant reductions in the hematocrit in both the control and exercised animals during the course of the experiment (Table 2). This is probably an effect of blood sampling, since removed whole blood was replaced with saline. The changes in hematocrit had no significant effect on any of the measured

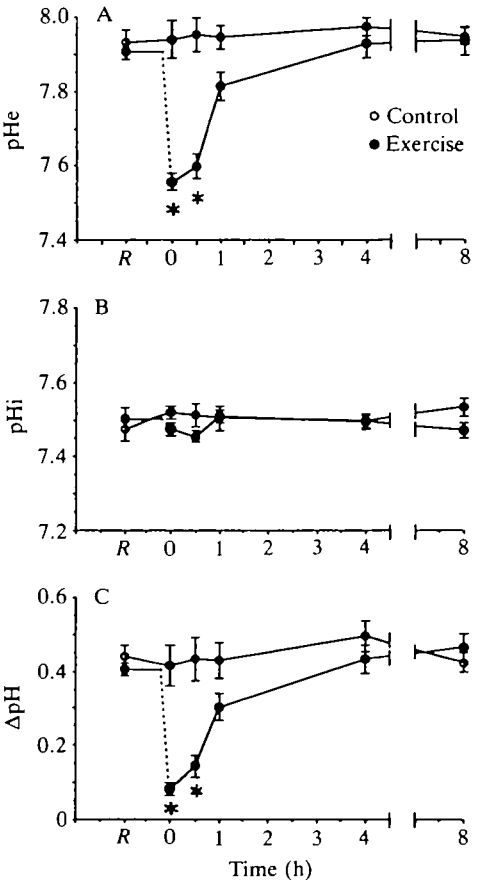

Fig. 1. (A) Extracellular pH (pHe), (B) erythrocyte pH (pHi) and (C) the pH gradient (ΔpH=pHe−pHi) across the erythrocyte membrane at rest (*R*) and 0, 0.5, 1, 4 and 8 h following exhaustive exercise in *Petromyzon marinus*. The dashed line represents the 10–15 min period of exhaustive exercise. Values are means ± standard error (control, $N=6$; exercise, $N=8$). Asterisks denote a significant difference from the resting value.

variables in the control animals, however, and the control hematocrit values were not significantly different (unpaired *t*-test; $P<0.05$) from the exercise hematocrit values at any of the sample times. Moreover, all the measured variables had returned to resting levels well before the largest reductions in hematocrit were observed. Thus, it is unlikely that the reductions in hematocrit had a siginificant impact on the observed differences in the exercised animals.

The extracellular acidosis observed in the lampreys after exercise was due to increases in both the arterial P_{CO_2} and the concentration of metabolic protons ($[H^+]_m$) in the plasma (Fig. 3). P_{CO_2} increased to a maximal value of 0.46±0.02 kPa immediately after the exercise period, but values not significantly different from the resting value (0.26±0.02 kPa) were restored by 1 h into the recovery period. At this point, the fact that the P_{CO_2} values in the present study are calculated values

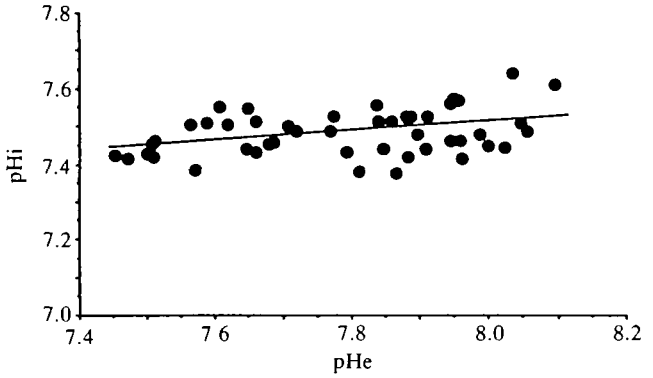

Fig. 2. Extracellular pH (pHe) *versus* erythrocyte pH (pHi) for blood from *Petromyzon marinus in vivo*. The regression equation for this relationship is pHi=0.117pHe+6.575, r^2=0.126.

Table 1. *Effect of catecholamines on extracellular pH (pHe), erythrocyte pH (pHi) and the pH gradient (ΔpH) across the membrane of* Petromyzon marinus *erythrocytes*

	Control	Treatment
Sham		
pHe	7.631±0.046	7.606±0.034
pHi	7.374±0.016	7.399±0.019
ΔpH	0.258±0.044	0.207±0.039
Catecholamines		
pHe	7.652±0.049	7.632±0.048
pHi	7.407±0.032	7.403±0.029
ΔpH	0.246±0.045	0.229±0.051

Values are means ± standard error (N=6).
No treatment values were significantly (P<0.05) different from the control values.

should be taken into consideration. Certainly, any relative differences in arterial P_{CO_2} will be reflected in these values, but it is important to note that the absolute values may be somewhat different if, for any reason, the CO_2 reactions in the plasma have not reached equilibrium. The increase in the plasma lactate concentration, [lactate], and the associated increase in $[H^+]_m$ occurred somewhat more slowly than the rise in P_{CO_2}. These values did not peak until 0.5 h into the recovery period and had only fully recovered at the 4 h sample time. The maximal change in plasma [lactate] was 4.4 mequiv l^{-1}, whereas that for $[H^+]_m$ was only 2.9 mequiv l^{-1}. These values can be used to calculate the proton deficit, which is the difference between the change in [lactate] and the change in $[H^+]_m$ at any given time. In *P. marinus*, the proton deficit at any time after exercise is very small and only reaches a maximal value of 1.5 mequiv l^{-1} 0.5 h into the recovery period.

Table 2. *Effect of exhaustive exercise on the hematocrit and mean cellular hemoglobin concentration (MCHC) in* Petromyzon marinus

	Time					
	Rest	0 h	0.5 h	1 h	4 h	8 h
Hematocrit (%)						
Control	24.6±3.3	22.5±3.5*	21.8±3.6*	20.0±3.1*	18.3±2.7*	17.7±3.0*
Exercise	28.4±1.5	26.7±1.6	25.6±1.6*	23.8±1.3*	20.6±1.2*	16.8±1.3*
MCHC ($g\,Hb\,dl^{-1}$ erythrocytes)						
Control	26.3±0.5	26.6±0.9	27.4±0.7	27.4±0.9	26.7±1.0	27.8±1.0
Exercise	25.2±1.2	27.8±0.3	27.4±0.5	25.6±1.6	24.7±1.7	24.9±2.1

Values are means ± standard error (control, $N=6$; exercise, $N=8$).
Asterisks denote values that are significantly ($P<0.05$) different from the resting value.

The total CO_2 concentration in the plasma ($C_{CO_2t.pl}$) fell significantly after exercise, but had recovered by 1 h (Fig. 4A). The lowest value for $C_{CO_2t.pl}$ was observed 0.5 h into the recovery period and amounted to a $1.8\,mmol\,l^{-1}$ change from the resting value. In contrast, erythrocyte total CO_2 concentration (C_{CO_2eryth}) was significantly elevated for the first 30 min after exercise (Fig. 4C). In this case, the maximal change ($2.4\,mmol\,l^{-1}$) was observed at 0 h. Immediately following the exercise period, the changes in the plasma and the erythrocytes appeared to offset each other and there was no significant change in the whole-blood total CO_2 concentrations ($C_{CO_2w.bl}$) (Fig. 4B). Thereafter, the $C_{CO_2w.bl}$ decreased, but the magnitude of this decrease was smaller than that observed in the plasma.

Exhaustive exercise in *P. marinus* was also associated with significant changes in the oxygen transport characteristics of the arterial blood The partial pressure of oxygen (P_{O_2}) had fallen by 36 % immediately after exercise (Fig. 5A). P_{O_2} quickly recovered to values that were not significantly different from the resting value, but the large initial decrease was also associated with a significant decline in the amount of oxygen bound to hemoglobin ($Hb:O_2$; Fig. 5B). $Hb:O_2$ had also returned to normal within 30 min.

Discussion

Exhaustive exercise in the agnathan *P. marinus* causes a significant extracellular acidosis which largely resembles that observed in the phylogenetically more recent jawed fishes (Fig. 1A). In fish, the extracellular acidosis following exercise has both a respiratory and a metabolic component (Heisler, 1986; Wood and Perry, 1985). The significant increases in arterial P_{CO_2} and plasma [lactate] observed in the present study are evidence that these two factors also contribute to the acidosis observed after exercise in the sea lamprey *P. marinus* (Fig. 3A,B). Depending on the species, the arterial P_{CO_2} in fish increases by 50–400 %

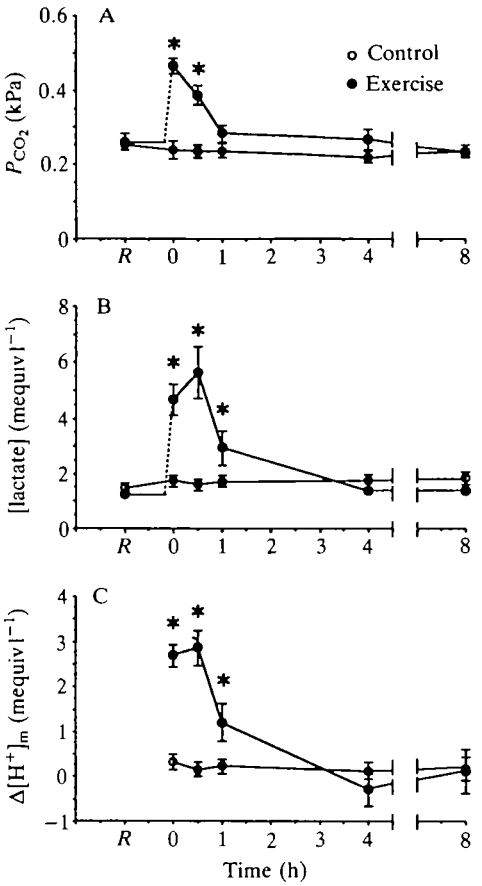

Fig. 3. (A) Arterial CO_2 tension (P_{CO_2}), (B) plasma lactate concentration ([lactate]) and (C) plasma metabolic proton load ($\Delta[H^+]_m$) at rest (R) and 0, 0.5, 1, 4 and 8 h following exhaustive exercise in *Petromyzon marinus*. The dashed line represents the 10–15 min period of exhaustive exercise. Values are means ± standard error (control, N=6; exercise, N=8). Asterisks denote significant differences from resting values in A and B and a significant difference from the control values in C.

immediately after exhaustive exercise (Wood and Perry, 1985). The 78 % increase observed in lampreys in the present study is comparable with the lower portion of this range. The magnitude of the increase in blood [lactate] following exercise also covers a broad range in fish and is largely dependent on species. Maximal values range from less than 2 mmol l^{-1} in benthic species like the flounder *Platichthys stellatus* and sole *Hippoglossoides elassodon* to more than 25 mmol l^{-1} in very active species like the salmon *Salmo salar* (Turner *et al.* 1983; Milligan and Wood, 1987; Black, 1957; Tufts *et al.* 1991). The peak plasma [lactate] after exercise in *P. marinus* also falls within the documented range for fish, but [lactate] only reaches 5.6±0.9 mmol l^{-1} (Fig. 3B). It is noteworthy that the maximal [lactate] in *P. marinus* is observed only 30 min into the recovery period, whereas in most

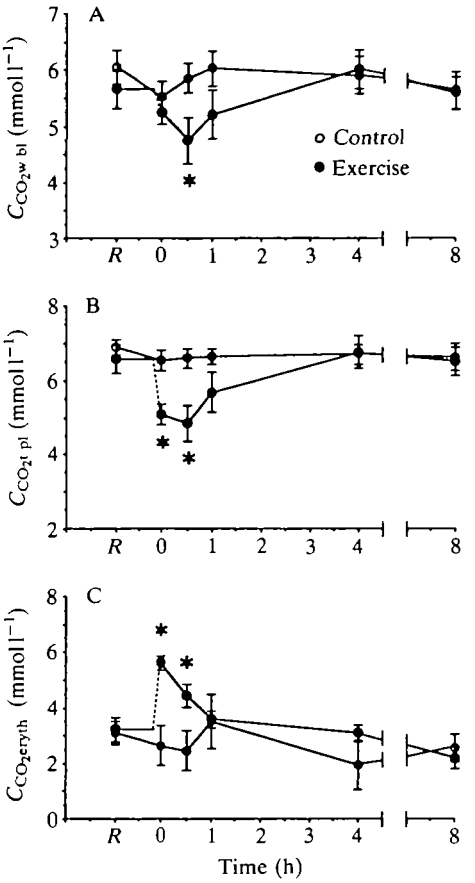

Fig. 4. Total carbon dioxide concentration C_{CO_2} in (A) whole blood (w.bl), (B) true plasma (t.pl) and (C) erythrocytes (eryth) at rest (R) and 0, 0.5, 1, 4 and 8 h following exhaustive exercise in *Petromyzon marinus*. The dashed line represents the 10–15 min period of exhaustive exercise. Values are means ± standard error (control, N=6; exercise, N=8). Asterisks denote a significant difference from the resting value.

species of fish, peak lactate concentrations are not observed until about 2 h (Heisler, 1986; Wood and Perry, 1985). The relationship between $[H^+]_m$ and [lactate] in the plasma of *P. marinus* is intermediate between the two general patterns described by Wood and Perry (1985) for benthic *versus* active fishes. In the present study, the calculated plasma Δ[lactate] marginally exceeds $\Delta[H^+]_m$ during the entire recovery period, but the dynamics of these two variables are quite similar and the maximal proton deficit calculated for the plasma only reaches 1.5 mequiv l^{-1} (Fig. 3B,C). Finally, although there is a substantial drop in pHe after exercise in *P. marinus*, there are relatively few metabolic protons added to the plasma (Fig. 3C). Since the increase in P_{CO_2} is also relatively low, the magnitude of the extracellular pH disturbance (0.350 units) probably reflects the

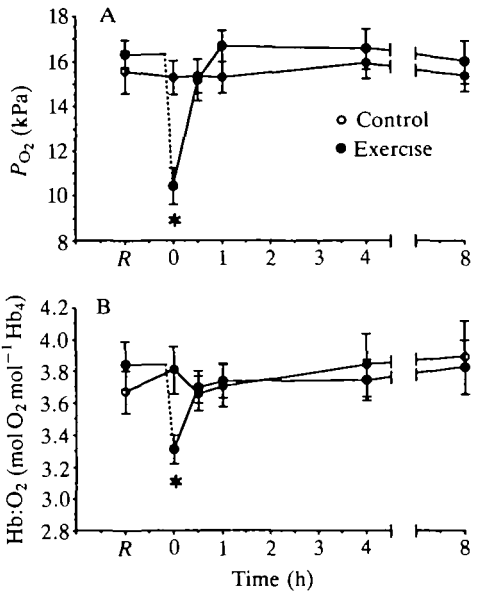

Fig. 5. (A) Arterial oxygen tension (P_{O_2}) and (B) hemoglobin:oxygen carriage (Hb:O_2) at rest (R) and 0, 0.5, 1, 4 and 8 h following exhaustive exercise in *Petromyzon marinus*. The dashed line represents the 10–15 min period of exhaustive exercise. Values are means ± standard error (control, N=6; exercise, N=8). Asterisks denote a significant difference from resting values.

relatively low nonbicarbonate buffer value of the true plasma in *P. marinus* (Tufts and Boutilier, 1989, 1990).

In contrast to the extracellular compartment, there was no significant acidosis observed within the erythrocytes of *P. marinus* following exercise (Fig. 1B). Thus, the marked difference between extracellular and erythrocytic pH (pHi) regulation in the lamprey resulted in large changes in the pH gradient (ΔpH) across the erythrocyte membrane during the course of the experiment (Fig. 1C). In certain species of fish, it has been demonstrated that pHi may be regulated by catecholamines after exercise (Nikinmaa *et al.* 1984; Primmett *et al.* 1986; Milligan and Wood, 1987). This mechanism has been well described and involves the adrenergic stimulation of sodium/proton exchange on the erythrocyte membrane (Cossins, 1989; Hoffman and Simonsen, 1989; Nikinmaa and Tufts, 1989). In the sea lamprey *P. marinus* the regulation of erythrocyte pH after exercise does not, however, appear to be adrenergically mediated, since adrenergic stimulation had no significant effect on the pHi of lamprey blood *in vitro* (Table 1). The absence of any adrenergic response in the present experiments is further supported by the observation that there were no significant changes in MCHC after exercise (Table 2). In fish, adrenergic regulation of pHi is associated with cell swelling and, therefore, a drop in MCHC (Nikinmaa *et al.* 1984; Primmett *et al.* 1986; Milligan and Wood, 1987). Thus, the agnathans may be similar to vertebrate groups like elasmobranchs and amphibians, which appear to lack this adrenergic response

(Tufts *et al.* 1987*a*,*b*; Tufts and Randall, 1989). In both *L. fluviatilis* and *P. marinus*, pHi is regulated *in vitro*, however, by a sodium/proton exchange mechanism that is not dependent upon catecholamine levels, but which appears to be stimulated by changes in pHe (Nikinmaa, 1986; Nikinmaa *et al.* 1986; B. L. Tufts, unpublished observations). The present results suggest that this mechanism is probably also operating in *P. marinus* after exercise *in vivo* (Figs 1B, 2). Moreover, this study demonstrates that the mechanism of pHi regulation in the sea lamprey, although independent of catecholamines, is clearly as effective, if not more effective, at regulating pHi *in vivo* than the adrenergic systems that have been described in many species of fish. Indeed, the slope of the *in vivo* relationship between pHe and pHi is only 0.12 in the present experiments, whereas it is 0.20 in a similar series of experiments carried out on *Oncorhynchus mykiss*, a species with a relatively large adrenergic response (Fig. 2; Milligan and Wood, 1987; Salama and Nikinmaa, 1989).

In most vertebrates, CO_2 transport is largely dependent on erythrocyte chloride/bicarbonate exchange (Roughton, 1964; Randall and Daxboeck, 1984; Cameron, 1978; Perry, 1986). In agnathans, however, it has been suggested this anion exchange protein may be absent or only present in very limited quantities within the erythrocyte membrane (Ellory *et al.* 1987; Nikinmaa and Railo, 1987). The *in vitro* CO_2 transport properties and ion distributions in *P. marinus* blood also provide evidence to support this view (Tufts and Boutilier, 1989, 1990). In fish, it has been suggested that temporary inhibition of bicarbonate flux through the erythrocytes may contribute to the P_{CO_2} rise in the arterial blood immediately after exercise (Wood and Perry, 1985). In a similar manner, the observed increase in arterial P_{CO_2} after exercise in the present study could, therefore, reflect a chloride/bicarbonate exchange limitation in *P. marinus* erythrocytes (Fig. 3A). In both fish and lampreys, however, titration of plasma bicarbonate by metabolic protons from muscle and by protons extruded from the erythrocyte *via* sodium/proton exchange would also be expected to contribute to the temporary rise in arterial P_{CO_2}. Since the relative importance of these potential sources of change in P_{CO_2} cannot be differentiated in these experiments, it is impossible to determine whether the P_{CO_2} increase does, in fact, reflect an *in vivo* anion exchange limitation in *P. marinus* blood. Further study into the nature of the CO_2 reactions in agnathan blood *in vivo* is required before any conclusions can be made on this point.

The observed changes in C_{CO_2} in arterial blood after exercise do not provide any indication that CO_2 transport is adversely effected *in vivo* by possible chloride/bicarbonate exchange limitations (Fig. 4). Indeed, the only significant change observed in the $C_{CO_2w.bl}$ was a decrease between 0.5 and 1 h of the recovery period (Fig. 4A). This was apparently due to an even larger reduction in the $C_{CO_2t.pl}$ in *P. marinus* blood after exercise (Fig. 4B). Again, as in fish, the reduction in $C_{CO_2t.pl}$ can be explained as the titration of plasma HCO_3^- by protons arising from the previously described sources. In the erythrocytes of *P. marinus*, there is a significant increase in C_{CO_2} during the first 30 min of the recovery period,

Significant quantities of carbonic anhydrase (CA) have been found within the erythrocytes of agnathans (Nikinmaa *et al.* 1986). Thus, the increase in C_{CO_2eryth} in the arterial blood of *P. marinus* after exercise probably does not reflect any inhibition of CO_2 transport at the level of the erythrocyte. During passage of blood through the gills, any bicarbonate within the erythrocyte would already have had access to CA and could have been dehydrated to CO_2 for excretion. Rather, the increase in C_{CO_2eryth} would be expected if erythrocyte sodium/proton exchange extruded protons from the erythrocyte after exercise at the same time that arterial P_{CO_2} was transiently elevated (Fig. 3A). Together, these two factors would increase the apparent bicarbonate concentration of the erythrocyte and result in the observed increase in C_{CO_2eryth}.

In contrast to CO_2 transport, it is clear that O_2 transport is adversely affected by exhaustive exercise in *P. marinus*. In exhausted lampreys, there is a substantial drop in the arterial P_{O_2} immediately after the exercise period (Fig. 5A). Transient decreases in arterial P_{O_2} have also been observed immediately after exercise in fish and have been attributed to a reduction in ventilation frequency in particularly exhausted animals (Primmett *et al.* 1986; Milligan and Wood, 1987). The present reduction in P_{O_2} probably also reflects a brief reduction in ventilatory frequency in exhausted lampreys. The drop in P_{O_2} was sufficient to have a significant effect on arterial Hb:O_2 carriage (Fig. 5B). Concurrent with the 36 % fall in P_{O_2}, there was a 14 % reduction in the amount of O_2 bound to hemoglobin. The hemoglobin of *P. marinus* has a significant Bohr effect (Manwell, 1963). Thus, the maintenance of pHi in the sea lamprey probably enhances oxygen uptake at the gills after exercise and the decline in Hb:O_2 carriage would probably have been even greater in these animals had the pHi not been so well regulated.

In summary, exhaustive exercise in the agnathan *P. marinus* results in a significant extracellular acidosis, which is very similar to that observed in other lower vertebrates. In addition, despite reports that chloride/bicarbonate exchange limitations exist in agnathan erythrocytes *in vitro*, the present results provide no evidence that acid–base regulation or carbon dioxide transport is adversely affected after exercise *in vivo*. Finally, the extracellular acidosis is not transferred to the erythrocyte and the regulation of pHi may have an important role in the transport of oxygen immediately after exercise in the sea lamprey.

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