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RELATIVE EFFECTS OF CARBONIC ANHYDRASE INFUSION OR INHIBITION ON CARBON DIOXIDE TRANSPORT AND ACID–BASE STATUS IN THE SEA LAMPREY *PETROMYZON MARINUS* FOLLOWING EXERCISE

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Summary

***In vivo* experiments were carried out to determine the relative effects of carbonic anhydrase (CA) infusion or inhibition on carbon dioxide (CO_2) transport and acid–base status in the arterial and venous blood of sea lampreys recovering from exhaustive exercise. Infusion of CA into the extracellular fluid did not significantly affect CO_2 transport or acid–base status in exercised lampreys. In contrast, infusion of the CA inhibitor acetazolamide resulted in a respiratory acidosis in the blood of recovering lampreys. In acetazolamide-treated lampreys, the post-exercise extracellular pH (pHe) of arterial blood was significantly lower than that in the saline-infused (control) lampreys. The calculated arterial and venous partial pressure of carbon dioxide (P_{CO_2}) and the total CO_2 concentration in whole blood (C_{CO_2wb}) and red blood cells (C_{CO_2rbc}) during recovery in the acetazolamide-infused lampreys were also significantly greater than those values in the saline-infused control lampreys. These results suggest that the CO_2 reactions in the extracellular compartment of lampreys may already be in equilibrium and that the access of plasma bicarbonate to CA is probably not the sole factor limiting CO_2 transport in these animals. Furthermore, endogenous red blood cell CA clearly has an important role in CO_2 transport in exercising lampreys.**

Key words: carbon dioxide, erythrocyte, blood, acetazolamide, carbonic anhydrase, lamprey, *Petromyzon marinus*, exercise.

Introduction

There is now considerable evidence indicating that functional chloride/bicarbonate exchange is either present at very low levels or is absent in agnathan red blood cells (Ellory *et al.* 1987; Nikinmaa and Railo, 1987; Tufts and Boutilier, 1989, 1990). This red blood cell anion exchange mechanism is considered to be a potential rate-limiting step in the process of CO_2 transport in vertebrates (Swenson, 1990). Not surprisingly, the strategy for CO_2 transport in sea lampreys is also markedly different from that in other vertebrates (Tufts and Boutilier, 1989; Ferguson *et al.* 1992; Tufts *et al.* 1992; Nikinmaa and Matsoff, 1992; Cameron and Tufts, 1994). In sea lampreys, blood CO_2 transport appears to rely heavily on CO_2 carriage within the erythrocyte rather than in the plasma, as is typical of most other vertebrates (Tufts *et al.* 1992).

As for most vertebrates, agnathan red blood cells possess significant quantities of carbonic anhydrase (CA; Maren *et al.* 1980; Nikinmaa *et al.* 1986; Henry *et al.* 1993). According to Henry *et al.* (1993), the CA present in lamprey red blood cells displays kinetic properties similar to the type I, slow turnover, CA isoenzyme. Inhibition of red blood cell CA in resting lampreys also results in only a minor respiratory acidosis in the extracellular fluid (Henry *et al.* 1995). To date, however, the relative importance of red blood cell CA in agnathans under conditions when metabolic rates are elevated (i.e. following exercise) has not been investigated.

In mammals, carbonic anhydrase is also available to catalyze the CO_2 reactions in the extracellular fluid because of its presence on the capillary endothelial cells of the lungs (Crandall and O'Brasky, 1978; Effros *et al.* 1978; Klocke, 1978; Henry *et al.* 1986). In contrast, similarly situated CA that would be available to catalyze the CO_2 reactions in the extracellular fluid appears to be absent from the gills in lower vertebrates such as teleost fish (Henry *et al.* 1988) and sea lampreys (Henry *et al.* 1993). Since agnathan red blood cells are also deficient in functional anion exchange, one might therefore predict that the accessibility of CA for plasma bicarbonate hydration/dehydration reactions may be an important factor limiting CO_2 transport and excretion in these primitive vertebrates. Notably, inhibition of anion exchange in trout red blood cells by 4,4′-diisothiocyano-2,2′-stilbene disulphonic acid (DIDS) increases the relative amount of CO_2 carried within the red blood cell and reduces that carried by the plasma, thereby causing the *in vitro* CO_2 transport

properties of trout blood to become more similar to those of the lamprey (Cameron and Tufts, 1994). In addition, treatment with the red blood cell anion exchange inhibitor 4-acetamido-4′-isothiocyano-2,2′-stilbene disulphonic acid (SITS) in a blood-perfused isolated trout head significantly reduced CO_2 excretion across the gills (Perry *et al.* 1982).

The fact that the red blood cells of primitive vertebrates such as the sea lamprey lack significant levels of functional anion exchange provides a unique opportunity to examine the potential selective pressures that have given rise to the pattern of CO_2 transport typical of most modern vertebrates. Thus, the purpose of the present investigation was to examine further some of the potential factors limiting CO_2 transport and excretion in the sea lamprey. More specifically, we have examined the effects of (i) extracellular CA infusion and (ii) inhibition of red blood cell CA by acetazolamide on arterial and venous acid–base status and CO_2 transport properties following exhaustive exercise. We hypothesize that CA infusion should enhance CO_2 excretion and therefore recovery from exercise if the access of plasma bicarbonate to CA is the sole factor limiting CO_2 transport in lampreys. We also propose that the relative importance of red blood cell CA in lampreys following exercise will be far greater than that previously demonstrated in resting animals.

Materials and methods

Adult sea lampreys, *Petromyzon marinus* (L.) (250–400 g; *N*=18), were collected during their spawning migration in 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 before the experiments. The composition of the water used to hold the animals and in the experiments was as follows (mmol l^{-1}): [Na^+] 2.1; [K^+] 0.05, [Ca^{2+}] 1.1, [Cl^-] 1.3, [HCO_3^-] 1.5, pH 7.4, at 8–10 °C.

Surgery

Lampreys were anaesthetized in an aerated and pH-balanced solution of tricaine methane sulphonate (66.7 mg l^{-1} MS-222 and 133 mg l^{-1} $NaHCO_3$). The animals were then transferred to a surgical table where a mid-ventral incision was made approximately half way down the length of the body. Cannulae of polyethylene tubing (PE 50) prefilled with heparinized (20 i.u. ml^{-1}) saline were implanted in the dorsal aorta and posterior cardinal vein (Tufts *et al.* 1992). Following the 5–10 min of surgery, the lampreys were allowed to recover in a lightproof Perspex box containing aerated fresh water for 4–6 h prior to the experiments. The duration of this recovery time was less than that in previous studies (Tufts, 1991; Tufts *et al.* 1992) but, since the cannulae often became blocked overnight, shorter recovery times were necessary to ensure that the cannulae could be frequently monitored and would therefore remain open. This approach also considerably reduced the number of animals that were ultimately required for these experiments.

Protocol

After recovery, 600 μl blood samples were taken into Hamilton gas-tight syringes from both the arterial and venous cannulae. Whole-blood total carbon dioxide concentration (C_{CO_2wb}) was measured immediately on 100 μl of the sample. Triplicate haematocrit measurements were performed using 200 μl of blood and the remainder was dispensed into 0.5 ml Eppendorf tubes and centrifuged at 10 000 ***g*** for 4 min. The true plasma total carbon dioxide concentration (C_{CO_2tpl}) was then determined on a 100 μl sample of plasma taken from the haematocrit tubes using a 100 μl gas-tight Hamilton syringe. Plasma pH (pHe) was measured immediately from the supernatant in the Eppendorf tube. An additional 100 μl of plasma was also added to 200 μl of 8 % perchloric acid (PCA) and frozen in liquid nitrogen for the subsequent measurement of lactate concentration. The remaining plasma was removed from the tube and the pellet was frozen in liquid nitrogen for later determination of erythrocyte pH (pHi). Throughout the experiment, blood samples were replaced with a similar volume of heparinized (20 i.u. ml^{-1}) saline after both the arterial and venous sample had been taken. The composition of the saline used in these experiments was as follows: 124 mmol l^{-1} NaCl, 10 mmol l^{-1} $NaHCO_3$, 5 mmol l^{-1} KCl, 0.5 mmol l^{-1} $MgCl_2$, 1.0 mmol l^{-1} $CaCl_2$ and 5.0 mmol l^{-1} glucose, pH 8.2 (in air). It should also be noted that the acetazolamide stock solution had a composition identical to that of regular saline with the only exceptions being that the acetazolamide was first dissolved in a 0.01 mol l^{-1} NaOH solution prior to addition of the other saline components and that the pH was adjusted to that of the regular saline using orthophosphoric acid.

After the control blood sample had been taken, one group of lampreys (*N*=6) was slowly infused with carbonic anhydrase (CA II from bovine erythrocytes, Sigma; 20 mg kg^{-1}) in a saline vehicle (2 ml kg^{-1}) into their arterial cannulae, while another group (*N*=6) was similarly infused with an equivalent amount of saline and served as a control. A third treatment group (*N*=6) consisted of lampreys infused with acetazolamide in a saline vehicle (2 ml kg^{-1}) into their arterial cannulae. The initial circulating concentration of acetazolamide was calculated to be 10^{-4} mol l^{-1} on the basis of an extracellular fluid volume of 24 % (Robertson, 1974). However, acetazolamide was not infused until after the exercise period (see below) since pharmacological inhibition of CA has been found to reduce muscle performance in vertebrates (Geers and Gros, 1988). Each lamprey was then moved to a cylindrical tank containing aerated water at 10 °C, where it was manually chased to exhaustion in 5 min. The exhausted lamprey was then returned to the Perspex container and a second 600 μl sample was removed from each cannula. Identical analyses to those described for the control sample were performed on this blood sample. At this point, the remaining group of lampreys received the infusion of acetazolamide. Additional samples were then taken after 20 min and 1 h of recovery from exercise.

Analyses

Plasma pH (pHe) and erythrocyte pH (pHi) were determined using a PHM 73 pH meter and associated micro-pH unit (Radiometer, Copenhagen, Denmark) thermostatted at 10 °C. Erythrocyte pellets were frozen in liquid nitrogen and then thawed immediately prior to the determination of pHi according to the method of Zeidler and Kim (1977). Total CO_2 concentrations of whole blood (C_{CO_2wb}) and true plasma (C_{CO_2tpl}) were measured with a Corning model 965 CO_2 analyzer (Ciba Corning Canada Inc.).

Erythrocyte C_{CO_2} (C_{CO_2rbc}) was determined from the C_{CO_2wb} and C_{CO_2tpl} values and the haematocrit (Hct), as in Tufts and Boutilier (1989), according to the following equation:

$$C_{CO_2rbc} = C_{CO_2wb} - C_{CO_2tpl} \times (1 - \text{Hct})/\text{Hct}\,.$$

Measured values of C_{CO_2tpl} and pHe were used to determine the partial pressure of CO_2 (P_{CO_2}) *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 kPa^{-1} (Tufts and Boutilier, 1990).

The concentration of metabolic protons ($\Delta[H^+]_m$) over any given period (i.e. time 1 to time 2) was calculated according to the following equation:

$$\Delta[H^+]_m = ([HCO_3^-]_1 - [HCO_3^-]_2) - \beta(pH_1 - pH_2)\,,$$

where β is the nonbicarbonate buffer value of true plasma (–3.1 mequiv l^{-1} pH unit^{-1}; Tufts and Boutilier, 1990).

Plasma lactate concentrations were measured on acidified PCA extracts using the method of Lowry and Passonneau (1972).

Statistics

Means and standard errors (S.E.M.) of all values are presented. A repeated-measures analysis of variance (ANOVA) was used to assess the significance of observed differences between sample times. If the ANOVA indicated significance (P<0.05), a Dunnett's multiple comparison's test was then used to determine significant differences (P<0.05) between resting and post-exercise values. In addition, a factorial ANOVA was used to assess the significance of observed differences between treatment groups. If significant, a Scheffe's test was then used to determine significant differences between treatment groups at individual sample times.

Results

Despite the relatively short recovery period following surgery, plasma lactate concentrations in resting lampreys were very low in all treatment groups (Table 1). Following exercise, plasma [lactate] and the associated metabolic proton load ($\Delta[H^+]_m$; Table 1) both increased and remained elevated for the duration of the experiment. However, there were no significant differences in either plasma [lactate] or $\Delta[H^+]_m$ between treatment groups.

Table 2 shows the effect of the sampling protocol on the haematocrit values of the lampreys used in these experiments. Although there were significant reductions in the haematocrit values in all treatment groups after 60 min of recovery, there were no significant differences in haematocrit between treatment groups at any time during the experiment.

As for plasma [lactate], there were no significant differences in resting pHe between treatment groups in arterial blood (Fig. 1A). Exhaustive exercise resulted in an extracellular

Table 1. *Plasma lactate concentration and metabolic proton concentration ($\Delta[H^+]_m$) in resting and exercised sea lampreys infused with saline (control), carbonic anhydrase or acetazolamide*

	[Lactate] (mmol l^{-1})			
Treatment	Rest	5 min post-exercise	20 min post-exercise	60 min post-exercise
Saline	0.5±0.1	4.4±0.3*	5.1±0.4*	4.5±0.7*
Carbonic anhydrase	0.3±0.1	4.9±0.2*	6.5±0.3*	5.2±0.9*
Acetazolamide	0.5±0.2	5.1±0.3*	5.4±0.4*	5.2±0.6*
	$\Delta[H^+]_m$ (mmol l^{-1})			
Saline	NA	4.4±0.6	6.1±0.5	4.5±0.7
Carbonic anhydrase	NA	5.6±0.5	7.3±0.8	5.9±1.5
Acetazolamide	NA	5.4±0.6	5.3±1.3	5.0±1.5

All values are presented as means ± S.E.M. (N=6).

NA, not applicable.

An asterisk indicates that a recovery value is significantly different (P<0.05) from the resting value within the same treatment group. There were no significant differences (P<0.05) between the saline treatment group and the other treatment groups at any sample time.

In all treatment groups, lampreys were exercised to exhaustion following the resting sample and then blood samples were taken after 5, 20 and 60 min of recovery.

Table 2. *Haematocrit in resting exercised sea lampreys infused with saline (control), carbonic anhydrase or acetazolamide*

	Haematocrit (%)			
Treatment	Rest	5 min post-exercise	20 min post-exercise	60 min post-exercise
Saline	33.2±2.5	30.7±2.7	27.7±2.6	26.2±2.7*
Carbonic anhydrase	31.8±2.8	30.3±3.1	28.8±2.9*	25.8±3.0*
Acetazolamide	31.0±2.1	30.4±2.1	28.2±1.9	24.4±2.1*

An asterisk indicates that a recovery value is significantly different (P<0.05) from the resting value in the same treatment group. There were no significant differences between groups at any given sample time.

Other details as in Table 1.

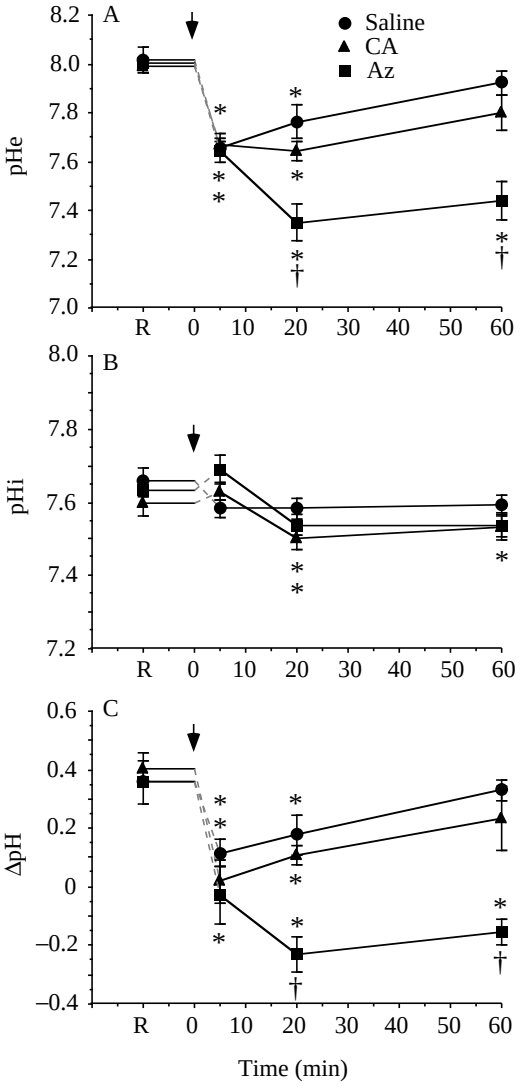

Fig. 1. (A) Extracellular pH (pHe), (B) erythrocyte pH (pHi) and (C) the pH gradient (ΔpH=pHe−pHi) across the erythrocyte membrane of arterial blood at rest (R) and 5, 20 and 60 min following exhaustive exercise in sea lampreys infused with saline (●), carbonic anhydrase (CA; ▲) or acetazolamide (Az; ■). The arrow designates the beginning of the 5 min period of exercise (dashed lines). All values are means ± S.E.M. (N=6 in each treatment group). Asterisks denote a significant difference (P<0.05) from the resting value. A dagger (†) denotes that a value is significantly different (P<0.05) from the saline treatment value at any given sample time.

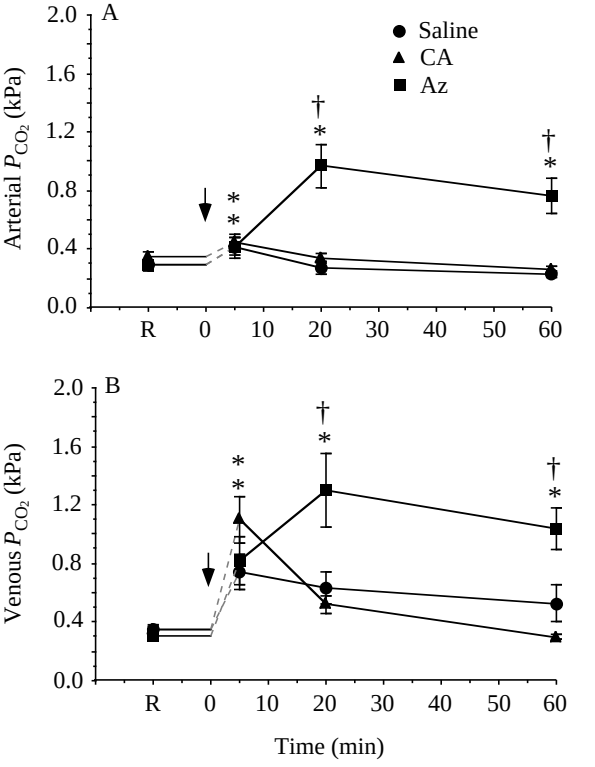

Fig. 2. Calculated arterial (A) and venous (B) P_{CO_2} of blood at rest (R) and 5, 20 and 60 min following exhaustive exercise in sea lampreys infused with saline (●), carbonic anhydrase (CA; ▲) or acetazolamide (Az; ■). Other details as in Fig. 1.

acidosis that was initially similar in magnitude for each treatment group (Fig. 1A). The pattern of recovery for pHe was identical in the saline and CA treatment groups, and arterial pHe in these lampreys was no longer significantly different from the resting value at 1 h post-exercise. In contrast, arterial pHe in the acetazolamide-treated lampreys was significantly lower than that in the saline treatment group at both the 20 min and the 1 h recovery sample times (Fig. 1A). Even at 1 h post-exercise, arterial pHe remained significantly lower than resting values in acetazolamide-treated animals. In venous blood, there were no significant differences in pHe between the saline control group and either the CA or acetazolamide treatment groups (data not shown).

Small, but significant, decreases in pHi were observed in arterial blood of both CA- and acetazolamide-treated lampreys following exercise (Fig. 1B). Similar changes were also seen in the venous blood of all treatment groups during recovery (data not shown). In contrast to pHe, there were no significant differences in pHi between treatment groups at any time during the experiment.

The pH gradient (ΔpH) across the red blood cell membrane decreased significantly after exercise (Fig. 1C). Recovery of ΔpH was similar in both the saline- and CA-treated lampreys, and this variable had returned to values that were not significantly different from resting values within 1 h (Fig. 1C). In contrast, arterial ΔpH in the acetazolamide-treated animals was significantly lower than that in the saline treatment group at both the 20 min and the 1 h recovery sample times and did not fully recover to resting levels before the end of the experiment (Fig. 1C). As for pHe, there were no significant differences in venous ΔpH between the saline treatment group and either of the CA or acetazolamide treatment groups (data not shown).

It should be noted that the calculated P_{CO_2} values in this study assume equilibrium conditions. These values will therefore be subject to some degree of error if, for any reason,

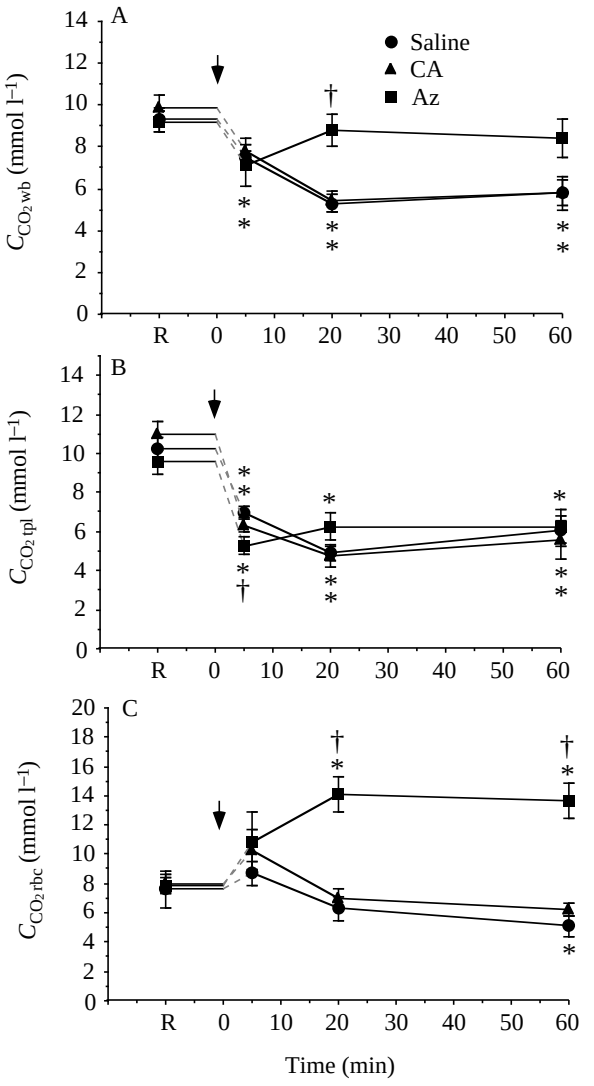

Fig. 3. Total CO_2 concentration of (A) whole blood (C_{CO_2wb}), (B) true plasma (C_{CO_2tpl}) and (C) red blood cells (C_{CO_2rbc}) in lampreys infused with saline (●), carbonic anhydrase (CA; ▲) or acetazolamide (Az; ■). Other details as in Fig. 1.

the CO_2 reactions in the blood of these animals are not in chemical equilibrium. Nonetheless, exercise resulted in an increase in the calculated blood P_{CO_2} (Fig. 2). In lampreys treated with saline or CA, these changes were short-lived and the P_{CO_2} in both arterial and venous blood had returned to values that were no longer significantly different from resting values within 20 min. In the acetazolamide treatment group, however, the calculated blood P_{CO_2} continued to increase until 20 min post-exercise, and the 20 min and 1 h recovery values were significantly greater than both the resting value and their corresponding values in the saline treatment group.

In lampreys treated with saline or CA, arterial C_{CO_2wb} was reduced immediately after exercise and it remained depressed throughout the remainder of the experiment (Fig. 3A). The venous C_{CO_2wb} of these lampreys also became significantly depressed after the 20 min sample and did not recover within 1 h (Table 3). In contrast, the post-exercise C_{CO_2wb} of the acetazolamide-treated group was never significantly different from the resting value at any time during the experiment (Fig. 3A; Table 3). In addition, both the arterial and venous C_{CO_2wb} of acetazolamide-treated lampreys were significantly greater than those of the saline-treated group after 20 min of recovery. The trends observed in C_{CO_2tpl} were somewhat different from those seen in whole blood. Exercise caused a significant decrease in the C_{CO_2tpl} of both arterial and venous blood in all experimental groups that persisted throughout the experiment (Fig. 3B; Table 3). In this case, the only significant difference observed between groups was at 5 min of recovery, when the arterial and venous C_{CO_2tpl} in the acetazolamide-treated group were found to be significantly lower than their corresponding values in the saline-treated group. In comparison, major differences were found between acetazolamide-treated lampreys and the other treatment groups with regard to C_{CO_2rbc} (Fig. 3C; Table 3). During the recovery period, the C_{CO_2rbc} in acetazolamide-treated lampreys rose to values that were almost double those measured in the other treatment groups.

The arteriovenous difference in C_{CO_2wb} increased in all treatment groups immediately after exercise, but the increase was only significant in the acetazolamide and CA groups

Table 3. *The effects of saline (control), carbonic anhydrase or acetazolamide infusion on the total carbon dioxide concentration in venous whole blood, true plasma and red blood cells from resting sea lampreys and during recovery from exhaustive exercise*

	C_{CO_2} (mmol l^{-1})			
Treatment	Rest	5 min post-exercise	20 min post-exercise	60 min post-exercise
Whole blood (C_{CO_2wb})				
Saline	10.6±0.4	10.2±0.4	7.3±0.5*	7.6±0.5*
Carbonic anhydrase	10.3±0.6	10.4±0.6	6.3±0.4*	6.6±0.8*
Acetazolamide	9.7±0.5	10.6±1.1	9.9±1.0†	9.1±1.0
True plasma (C_{CO_2tpl})				
Saline	10.9±0.6	8.4±0.4*	6.1±0.2*	6.7±0.6*
Carbonic anhydrase	11.2±0.6	7.4±0.4*	5.1±0.5*	6.0±1.0*
Acetazolamide	9.8±0.6	6.8±0.2*,†	7.0±0.7*	7.0±0.9*
Red blood cells (C_{CO_2rbc})				
Saline	10.1±0.8	15.1±1.8*	10.6±1.5	9.8±1.4
Carbonic anhydrase	8.5±0.7	17.7±1.3*	9.2±0.4	8.2±0.8
Acetazolamide	9.4±0.5	19.6±2.5*	18.2±1.8*,†	16.6±1.8*,†

An asterisk indicates that a recovery value is significantly different (P<0.05) from the resting value in the same treatment group.

A dagger denotes a significant difference (P<0.05) from the saline treatment value at any given sample time.

Other details as in Table 1.

Table 4. *The effects of saline (control), carbonic anhydrase or acetazolamide infusion on the arterio-venous difference in the total CO_2 content of whole blood from resting sea lampreys and during recovery from exhaustive exercise*

	C_{CO_2wb} (mmol l^{-1})			
Treatment	Rest	5 min post-exercise	20 min post-exercise	60 min post-exercise
Saline	1.3±0.3	2.6±0.4	2.0±0.6	1.8±0.5
Carbonic anhydrase	0.4±0.1†	2.7±0.7*	0.9±0.2	0.8±0.2
Acetazolamide	0.6±0.1	3.1±1.3*	1.2±0.3	1.0±0.2

C_{CO_2wb}, total CO_2 content of whole blood.

An asterisk indicates that a recovery value is significantly different ($P<0.05$) from the resting value in the same treatment group.

A dagger denotes a significant difference ($P<0.05$) from the saline treatment value at any given sample time.

Other details as in Table 1.

(Table 4). During the recovery period, no significant differences were found between groups for this variable.

Discussion

At the time of the initial resting sample, the plasma lactate concentrations in all three groups of lampreys were very low (Table 1). Indeed, our 'resting' values for plasma [lactate] were lower than those observed in lampreys 24 h after surgery (Tufts, 1991). The initial values for plasma acid–base status were also almost identical to those reported for resting lampreys in previous studies (Tufts, 1991; Tufts *et al.* 1992). Thus, although the recovery period following surgery was relatively short in the present study, it was sufficient to obtain resting values for the variables of interest that were very similar to those in previous studies.

The main focus of the present investigation was to compare blood acid–base status and CO_2 transport properties between lampreys in the various treatment groups following exercise. Exhaustive exercise resulted in an increase in plasma lactate concentration similar in magnitude to that previously documented for this species (Tufts, 1991). More importantly, exercise performance was the same in all of our treatment groups since there were no significant differences in either plasma [lactate] or the plasma metabolic proton load ($[H^+]_m$) between groups at any time during the recovery period (Table 1). In addition, the impact of the sampling protocol was approximately the same in each group since there were no significant differences in haematocrit between treatment groups at any time during the experiment (Table 2). In view of these results, we are confident that any significant differences in acid–base status and CO_2 transport observed between groups during the recovery period can be attributed to the experimental treatments.

As expected, there was a significant extracellular acidosis in all lampreys following the exercise period (Fig. 1). Despite this marked extracellular acidosis, red blood cell pH was largely maintained after exercise (Fig. 1). As explained by Tufts and Boutilier (1989, 1990), the absence of functional red blood cell anion exchange will effectively increase the observed buffer value of the red blood cells and reduce that of the plasma in sea lampreys. In the present study, this results in a large reduction in the pH gradient (ΔpH) across the red blood cell membrane in all treatment groups immediately following exercise and even a reversal of ΔpH in acetazolamide-treated animals during the remainder of the recovery period (Fig. 1C). These results provide further support for the view that the pH of the red blood cell in sea lampreys is extremely well maintained even during substantial extracellular pH disturbances.

In rainbow trout, the magnitude of post-exercise acidosis in the plasma of arterial blood is attenuated by infusion of carbonic anhydrase prior to exercise (Wood and Munger, 1994; Currie *et al.* 1995). This was not the case in sea lampreys (Fig. 1), however, even though the concentration of CA infused before exercise was double that infused into rainbow trout in previous studies. In previous studies, the concentration of CA used was enough to double the net HCO_3^- dehydration activity in whole blood of rainbow trout (Wood, 1991). The twofold higher concentration of CA used in the present study should therefore have been more than adequate to cause a large increase in the rates of the CO_2 reactions in lamprey plasma. While naturally occurring CA inhibitors are known to exist in the plasma of some lower vertebrates, they do not show crossreactivity with mammalian CA (Haswell *et al.* 1983; Dimberg, 1994); therefore, it is unlikely that the lack of effect of infused CA was due to inactivation.

Current evidence indicates that there is no CA on the endothelial membrane of the gill vasculature in teleost fish that could enhance the rate of CO_2 formation from bicarbonate and protons in the plasma (Henry *et al.* 1988, 1993, 1995). This uncatalyzed CO_2 dehydration reaction is also relatively slow (half-time of approximately 25 s at 25 °C; Edsall, 1969) compared with the normal residence time of blood in fish gills (0.5–1.0 s, Cameron and Polhemus, 1974). In species such as the rainbow trout, the effects of infused CA on arterial pHe and P_{CO_2} can therefore be explained by the fact that CA eliminates the slow uncatalyzed dehydration reaction or disequilibrium acid–base disturbance that exists in the plasma of arterial blood (Gilmour and Perry, 1994; Gilmour *et al.* 1994) which appears to be increased after exercise (Wood and Munger, 1994; Currie *et al.* 1995). The observation that CA infusion had no significant effects on acid–base status or CO_2 transport in the blood of sea lampreys (Figs 1–3; Table 3) suggests that the CO_2 reactions in the extracellular compartment of these animals may already be in equilibrium. These results further indicate that access of plasma bicarbonate to CA is probably not the sole factor limiting CO_2 transport in sea lampreys following exercise, even though the red blood cells of these animals appear to be deficient in chloride/bicarbonate exchange (Nikinmaa and Railo, 1987; Tufts and Boutilier,

1989, 1990). However, experiments incorporating more sensitive techniques to evaluate directly the existence of acid–base disequilibria in the blood of lampreys may still be warranted.

In contrast to CA, infusion of the CA inhibitor acetazolamide had a profound impact on both blood acid–base status (Fig. 1) and CO_2 transport (Figs 2, 3) in sea lampreys recovering from exercise. As in other vertebrates, agnathans have significant quantities of CA within their erythrocytes (Maren *et al.* 1980; Nikinmaa *et al.* 1986; Henry *et al.* 1993). In sea lamprey red blood cells, the CA present displays kinetic properties similar to the type I, slow-turnover, CA isoenzyme (Henry *et al.* 1993). According to the predictive model proposed by Tufts and Boutilier (1989), lamprey erythrocyte CA should have an important function in CO_2 transport even in the absence of red blood cell anion exchange. However, Henry *et al.* (1995) recently demonstrated that acetazolamide infusion resulted in only a very minor extracellular acidosis in the extracellular fluid of resting sea lampreys. In contrast, infusion of acetazolamide into exercised lampreys in the present study resulted in a much larger acidosis during recovery compared with that in lampreys infused with saline (Fig. 1). Since the plasma lactate and metabolic proton loads were similar in all three treatment groups following exercise (Table 1), the additional post-exercise acidosis in the lampreys treated with acetazolamide is probably of respiratory origin. Indeed, the previously documented time course of maximal red blood cell CA inhibition (>98 % within 15–30 min) in lampreys infused with this concentration of acetazolamide (Henry *et al.* 1995) correlates well with the observed effects of acetazolamide in the present study. The calculated P_{CO_2} values in acetazolamide-treated lampreys after 20 and 60 min of recovery further support this view (Fig. 2). Taken together, these results suggest that the need for red blood cell CA may be most critical during the periods of exercise that are probably a common feature of the lifestyle of these active vertebrates.

The respiratory acidosis after acetazolamide infusion was associated with significant changes in C_{CO_2wb} in lampreys recovering from exercise (Fig. 3; Table 3). When these changes are compartmentalized, it is apparent that the differences in C_{CO_2wb} between the acetazolamide and saline treatments are almost entirely due to large differences in C_{CO_2rbc} (Fig. 3C; Table 3). Since lamprey red blood cells have an extremely high nonbicarbonate buffer value and have been found to be essentially impermeable to bicarbonate (Tufts and Boutilier, 1989, 1990), these differences can probably be explained by the fact that more bicarbonate will be trapped within the red blood cells at the higher predicted blood P_{CO_2} values that probably exist in acetazolamide-treated lampreys (Fig. 2). In contrast, lamprey plasma has a relatively low nonbicarbonate buffer value (Tufts and Boutilier, 1989, 1990). It is therefore not surprising that there were no detectable differences in C_{CO_2tpl} between the saline- and acetazolamide-treated lampreys (Fig. 3B) since one would only expect a small amount of additional plasma bicarbonate to be formed at the elevated blood P_{CO_2} values that probably exist in the lampreys treated with acetazolamide.

From the measurements of both arterial and venous C_{CO_2} in the present study, the effects of both exercise and the various pharmacological treatments on the arteriovenous difference in whole-blood C_{CO_2} across the gills could be examined. As observed by Tufts *et al.* (1992), exercise appeared to cause an increase in the arteriovenous difference in whole-blood C_{CO_2}, although this increase was only significant in two of the three treatment groups (Table 4). However, there were no significant differences in the arteriovenous C_{CO_2} observed between the various treatment groups that could be attributed to acetazolamide or CA. In the case of CA, this is not surprising since CA had virtually no effects on any of the variables measured in arterial or venous blood. In contrast, one would predict that acetazolamide should have caused a decrease in the arteriovenous C_{CO_2} difference, since the uncatalyzed dehydration of bicarbonate is too slow to reach completion during the relatively short residence time of blood in the gills. The respiratory acidosis observed in the blood of these animals confirms that acetazolamide must have caused a reduction in CO_2 excretion. Once the initial retention of CO_2 has shifted the equilibrium to a higher blood P_{CO_2}, however, a normal level of CO_2 excretion will be restored. Thus, the absence of any measurable effect of acetazolamide on the arteriovenous C_{CO_2} difference across the gills can be explained by the fact that a new equilibrium for CO_2 excretion had probably been established by the time our measurements were taken.

In summary, our results indicate that the relative importance of red blood cell CA in lampreys following exercise is greater than that previously demonstrated in resting animals. Since CA infusion had no significant effect on any of the measured variables, our results also suggest that the CO_2 reactions in the extracellular compartment of sea lampreys may already be in equilibrium. Furthermore, access of plasma bicarbonate to CA is probably not the sole factor limiting CO_2 transport in these animals even though agnathan red blood cells appear to be deficient in functional anion exchange.

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