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EFFECTS OF EXTERNAL ACIDIFICATION ON THE BLOOD ACID–BASE STATUS AND ION CONCENTRATIONS OF LAMPREY

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Summary

We studied the effects of acute external acidification on the acid–base status and plasma and red cell ion concentrations of lampreys. Mortality was observed within 24 h at pH 5 and especially at pH 4. The main reason for the high sensitivity of lampreys to acid water appears to be the large drop in blood pH: 0·6 and 0·8 units after 24 h at pH 5 and pH 4, respectively. The drop of plasma pH is much larger than in teleost fishes exposed to similar pH values. The difference in the plasma pH response between lampreys and teleosts probably results from the low buffering capacity of lamprey blood, since red cells cannot participate in buffering extracellular acid loads. Acidification also caused a decrease in both Na^+ and Cl^- concentrations and an elevation in K^+ concentration of plasma. The drop in plasma Na^+ concentration occurred faster than the drop in plasma Cl^- concentration which, in turn, coincided with the decrease in total CO_2 concentration of the blood.

Introduction

The river lamprey, *Lampetra fluviatilis* (L.), is a commercially important species in Finland. In 1982 the value of the lamprey catch in Finland was estimated to be 7·7 million Fmk (1·8 million US dollars). During the 1970s the catches of lampreys declined markedly: the main reason was probably the building of water courses (Ikonen, Kokko, Tuunainen & Auvinen, 1983). In addition, in a few of the rivers in western Finland in which lampreys spawn, low pH values have been observed (Ikonen *et al.* 1983; Hilden, Hudd & Lehtonen, 1982); this is largely because of drainage from fields containing sulphide-rich soils.

The effects of acidification in adult teleost fish include changes in the blood acid–base status (Neville, 1979; Booth, Jansz & Holeton, 1982; Holeton, Booth & Jansz, 1983), ionic balance (McWilliams & Potts, 1978; McDonald & Wood, 1981; McDonald, 1983) and oxygen transport (Packer & Dunson, 1972; Ultsch & Gros, 1979; Ultsch, Ott & Heisler, 1981). Regulation of the acid–base balance and

Key words: lamprey, acidification, acid–base status, ion concentrations, low water pH.

oxygen transport of lampreys differs from that of teleost fishes in several respects. First, their red cells lack the anion exchange pathway (Nikinmaa & Railo, 1987). As a consequence, haemoglobin, the most important intracellular buffer of blood, cannot participate in buffering extracellular acid loads (Nikinmaa & Railo, 1987). Second, lamprey red cell pH is controlled by a Na^+-dependent proton extrusion (Nikinmaa, 1986*a*; Nikinmaa, Kunnamo-Ojala & Railo, 1986). At present, the effects of extracellular acidification on the activity of this transport system and red cell pH *in vivo* are not known. Notably, in the absence of an organic phosphate effect on haemoglobin O_2 affinity, the red cell pH appears to be a very important determinant of the oxygen-binding properties of lamprey haemoglobin (e.g. Nikinmaa & Weber, 1984; Nikinmaa, 1986*a*).

In view of these differences between lampreys and teleost fishes, the effects of external and internal acid loads on the ionoregulation, acid–base status and oxygen-binding properties of lamprey blood need to be studied. In the present study we report the effects of acute external acidification induced by mineral acids. CO_2-induced acidification and internal acidification induced by stress are subjects of later reports.

Materials and methods

Adult lampreys (*Lampetra fluviatilis*, mass 44 ± 11 g, $\bar{x} \pm$ S.D., $N = 66$) were obtained from the river Simojoki in northern Finland. They were maintained in dechlorinated Helsinki tap water (8–10°C) for at least 1 month before experiments.

To allow undisturbed blood sampling during the experiments, anaesthetized (MS 222, 2 g l^{-1}) lampreys were cannulated (Portex PP 50) using a modification of the technique described by Soivio, Nyholm & Westman (1975) for dorsal aortic catheterization in fish. The dorsal aorta or posterior cardinal vein was penetrated from the side of the animal at the front end of the dorsal fin, and the cannula stitched in position. Lampreys were allowed to recover from the operation for about 20 h in dark containers.

After recovery, lampreys were exposed to pH 5 (maximal variation 4·95–5·35) and 4 (4·05–4·25) in water containing (in mg l^{-1}) Ca^{2+}, 18·5; Na^+, 4·6; Cl^-, 5·8; K^+, 1·4; and Al^{3+}, 0·07. Acid water was prepared by titration with 1·0 mol l^{-1} HCl 24 h before experiments, whereafter the water was bubbled with air to expel CO_2 until the experiments started. The water pH could be readjusted accurately at the onset of and during the experiments. The water pH of the control series was 7·8. Exposure times were 3 and 24 h and the sizes of groups were as follows: control, $N = 19$; pH 5, 3 h, $N = 10$; pH 4, 3 h, $N = 15$; pH 5, 24 h, $N = 6$; pH 4, 24 h, $N = 16$. Only the animals that survived through the experiments are included in data presented.

For the determination of red cell pH, 50 μl of [^{14}C]DMO (5,5-dimethyloxazolidine-2,4-dione; 1 μCi ml^{-1}; 98 % purity; Amersham) in physiological saline was injected *via* the cannula into the circulation of lampreys before the experiment.

Lampreys were then quickly transferred from neutral to acidic water in their containers.

Blood samples (0·5 ml) were taken through the cannula into a heparinized syringe and analysed immediately for pH, total CO_2 content and haematocrit (Hct). The extracellular pH was measured using Radiometer BMS 2 Mk 2 and PHM 73 apparatus. Blood CO_2 content was measured using the method of Cameron (1971).

For the determination of red cell pH and ion concentrations of both plasma and red cells, a portion of plasma and red cells was separated by centrifugation (10 000 *g*; 2 min) immediately after sampling, deproteinized with 0·6 mol l^{-1} perchloric acid and stored in a refrigerator. Red cell pH was determined from the extracellular pH and from the distribution of radioactively labelled weak acid, DMO, between plasma and red cells as described by Nikinmaa & Huestis (1984) using the formula:

$$pHi = pK_{DMO} + \log\{[DMO]_i/[DMO]_e \times (10^{pHe-pK_{DMO}}+1) - 1\}.$$

The samples were analysed for ^{14}C by liquid scintillation counting (LKB Wallac 1211 Minibeta). The cellular water content was determined from the difference between the wet and dry (+80°C, 24 h) mass of red cells.

Sodium and potassium concentrations were determined using flame photometry (FLM 3, Radiometer, Copenhagen) and chloride concentration using a Radiometer CMT 10 coulometric titrator. Red cell concentrations are given in mmol l^{-1} water.

The non-bicarbonate buffering capacity of blood was measured by direct titration. 10 ml of blood (pooled samples from two trout and 10 lampreys) was acidified to pH 6·8 and gassed with N_2 until CO_2 was removed (total CO_2 concentration < 1 mmol l^{-1}). Thereafter blood was titrated with 100 mmol l^{-1} NaOH from pH 6·9 to 8·9, and the mean base consumption in mmol l^{-1} blood pH $unit^{-1}$ taken as the non-bicarbonate buffering capacity of the blood.

Statistical differences between the means were determined with Student's *t*-test. The best-fit curve describing the relationship between intra- and extracellular pH was calculated using Spain's (1982) curfit program, and the least squares method. In the text, the *F*-statistics [$= SS_{regression} \times SS_{residual}^{-1} \times (n-K-1) \times K^{-1}$, in which n = number of data pairs and K = number of constants fitted to the equation] are additionally given.

Results and discussion

Lampreys are highly sensitive to environmental acidification, since acidic water caused deaths on the first day of exposure. At a water pH of 5, 20 % (one out of six) of the lampreys died and at pH 4, the percentage mortality increased to 50 % (four out of eight). A 3-day exposure to acidic water at pH 4 caused 100 % (eight out of eight) mortality.

The first and most pronounced physiological change observed in lampreys was the marked drop of plasma pH value within 3 h at both acid exposures (Fig. 1). It is likely that blood acidosis results mainly from the diffusion of external H^+ along the concentration gradient into the blood of lampreys. The pHe continued to decrease with time. After 24 h the drop in pHe was approx. 0·6 units at pH 5 and 0·8 units at pH 4. This is a very large decrease compared with the situation in teleost fishes, in which the acidification-induced decrease in pHe is usually 0·2 units or less

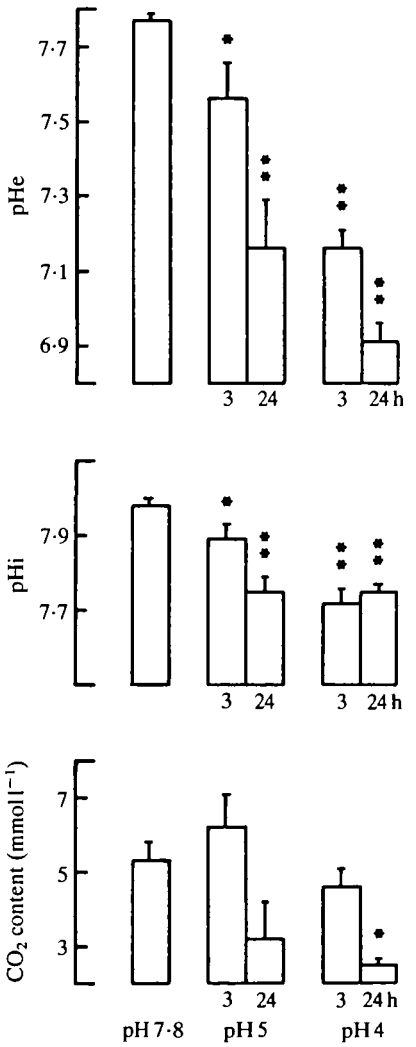

Fig. 1. Blood pH (pHe), intraerythrocytic pH (pHi) and total CO_2 content of blood in control lampreys and in lampreys exposed to pH 5 or pH 4 for 3 and 24 h. Means ± S.E.M. (control, $N = 18–19$; pH 5, 3 h, $N = 10$; pH 5, 24 h, $N = 5$; pH 4, 3 h, $N = 15$; pH 4, 24 h, $N = 4$) are given. Significant differences determined using the *t*-test between the exposed and control groups are indicated by asterisks ($^*P < 0·05$, $^{**}P < 0·01$).

(Table 1). To show if the differences in buffering capacity of blood could explain why the pH drop is larger in lampreys than, for example, in trout at similar water pH values, we determined the non-bicarbonate buffering capacity for both species by direct titration. The mean buffering capacity of trout blood (pooled sample from two trout, Hct = 29) in the pH range 6·9–8·9 was 9·7 slykes (= mmol $H^+ \Delta pH^{-1} l^{-1}$), which is similar to the non-bicarbonate buffering capacity, measured by the CO_2 equilibration method, in carp (Albers, Götz & Hughes, 1983), tench (Jensen & Weber, 1982) and sheatfish (Albers, Götz & Welbers, 1981), whereas that of lamprey blood (pooled sample of 10 lampreys, Hct = 37) was 2·5 slykes. Thus, for a given acid load, a much greater effect on plasma pH would be expected in lamprey than in trout. This strongly suggests that intraerythrocytic haemoglobin does not participate in the buffering of extracellular acid loads.

The marked drop in plasma pH may be the primary reason for the deaths of lampreys. Ultsch *et al.* (1981) have measured final pH values of 6·907 ± 0·046 before death in carp in acidic water of pH 3·5, and the extracellular pH of dying rainbow trout decreased at least 0·4 units below normal before death at a water pH of 4·2 and a high calcium concentration (McDonald, Höbe & Wood, 1980). A drop in extracellular pH increases the magnitude of the pH gradient across the cell membranes. Since most cells maintain their intracellular pH by active acid

Table 1. *Reported data on blood pH of teleost fishes exposed to low water pH for approx. 24 h*

Control water pH 7·0–7·5	Acid water pH 4·0–4·3	Species	Water $[Ca^{2+}]$ ($mmol\,l^{-1}$)	Acid	Reference
7·90	7·70	Rainbow trout	0·5	H_2SO_4	1
7·85	7·65	Rainbow trout	0·5	H_2SO_4	2
7·83	7·69	Rainbow trout	1·6	HCl	3
7·78	7·51	Rainbow trout	1·93	H_2SO_4	5
7·81	7·77	Rainbow trout	0·24	H_2SO_4	4
7·85	7·76	Rainbow trout	0·3	HCl	3
7·96	7·82	White sucker	1·78	H_2SO_4	7
Control water pH 5·1	Acid water pH 4·0				
7·81	7·69	Carp	High Ca^{2+}, low Na^+	HCl	6
Control water pH 8·4	Acid water pH 5·0				
8·03	7·93	Tench	3·0	H_2SO_4	8

References: (1) Booth, Jansz & Holeton (1982); (2) Holeton, Booth & Jansz (1983); (3) McDonald, Höbe & Wood (1980); (4) McDonald (1983); (5) McDonald & Wood (1981); (6) Ultsch, Ott & Heisler (1981); (7) Höbe *et al.* (1983); (8) Jensen & Weber (1987).

Tabulated values are taken from figures in each of the references, and are thus approximate.

extrusion mechanisms (see, for example, Roos & Boron, 1981), the acid extrusion becomes increasingly difficult as the proton gradient across the membrane increases. This is particularly important for the most common intracellular pH-regulatory pathway in vertebrate cells, the Na^+/H^+ exchanger, which uses the actively maintained Na^+ gradient to extrude protons (e.g. Mahnensmith & Aronson, 1985). If, owing to the extracellular acidification, the proton gradient becomes large enough, no net proton excretion can occur and pHi starts to decrease. Since accurate regulation of pHi is required for normal cellular functions (see Roos & Boron, 1981; White & Somero, 1982; Somero, 1986; Nikinmaa, 1986*b*), a drop of pHi may cause disturbances in cellular function and ultimately cause the death of the animals. However, the sensitivities of different cell types and different cellular functions to intracellular acidification are poorly understood.

Since lamprey red cells regulate their intracellular pH by a Na^+-dependent acid extrusion mechanism (Nikinmaa, 1986*a*; Nikinmaa *et al.* 1986), their behaviour during an extracellular acidification may give further insight into the changes occurring in other cell types regulating pHi by Na^+/H^+ exchange. During the initial phase of the acidification, the pH of the red cells changes little, despite the drop in the extracellular pH, because they are very impermeable to acid (and base) equivalents (Nikinmaa & Railo, 1987). However, the transmembrane proton gradient increases (see Fig. 1) and, because of this, the electrochemical driving force for proton extrusion, provided by the Na^+ gradient, diminishes and disappears (Table 2). This leads to a larger decrease in pHi per unit change in pHe at low than at high pHe values (see Fig. 2), giving a hyperbolic best-fit relationship between red cell and blood pH ($N = 52$; $F = 127$; df = 50):

$$\text{pHi} = 8{\cdot}45 \times (\text{pHe} - 5{\cdot}8)/[0{\cdot}12 + (\text{pHe} - 5{\cdot}8)].$$

Acid exposure caused a decrease in plasma sodium and chloride concentrations (Fig. 3). The largest drop was observed at pH 4 after the 24 h of exposure. These changes are similar to those observed in many fishes (McWilliams & Potts, 1978; Fromm, 1980; McDonald *et al.* 1980; McDonald & Wood, 1981). Since Na^+ is taken up actively in exchange for H^+ (or NH_4^+)(see, for example, Heisler, 1980), the unfavourable proton gradient makes Na^+ uptake increasingly difficult with

Table 2. *The sodium, $[Na^+]_i/[Na^+]_o$, and proton, $[H^+]_i/[H^+]_o$, ratios across the red cell membranes of lampreys exposed to low water pH*

Group	$[Na^+]_i/[Na^+]_o$	$[H^+]_i/[H^+]_o$
pH 7·8	0·290 ± 0·012	0·645 ± 0·035
pH 5, 3 h	0·288 ± 0·018	0·520 ± 0·069
pH 5, 24 h	0·272 ± 0·018	0·290 ± 0·067
pH 4, 3 h	0·329 ± 0·013	0·286 ± 0·018
pH 4, 24 h	0·284 ± 0·039	0·148 ± 0·013

Means ± S.E.M. are given.
Number of animals as in Fig. 1.

decreasing pH (see, for example, Heisler, 1984). Additionally, the diffusion of H^+ into the fish changes the transepithelial potential of the gill epithelial cells from inside negative to inside positive. This accelerates the diffusion of sodium ions out of the fish (McWilliams & Potts, 1978).

The decrease of plasma Cl^- concentration was slower (Fig. 3) than that of Na^+ concentration, and coincided with the decrease of total CO_2 concentration (Fig. 1). Since HCO_3^- is the counterion for Cl^- uptake, this observation suggests that the decrease in plasma chloride concentration is caused by decreased Cl^- uptake, resulting from limited counterion availability (see Heisler, 1984).

Calcium is an important modulator of the ion and water permeability of the gills (Isaia & Masoni, 1976). The ionic imbalance caused by acid stress observed in different fish species is shown to be dependent on the Ca^{2+} concentration of the water (McDonald *et al.* 1980; McDonald, 1983). Acidification accelerates the losses of ions in fishes at low Ca^{2+} concentrations (McDonald *et al.* 1980) and is expected to increase the passive penetration of protons through the gills (McWilliams & Potts, 1978). Acidic water, moreover, detaches surface-bound calcium from the gill epithelium (McWilliams, 1983), which also leads to higher permeability. Plasma chloride relative to sodium losses are also dependent on water Ca^{2+} concentration: in moderately hard water ($Ca^{2+} = 1{\cdot}6\,mmol\,l^{-1}$) the ratio of plasma Na^+ loss to Cl^- loss is 3·7:1 (McDonald *et al.* 1980), in soft water ($Ca^{2+} = 0{\cdot}22\,mmol\,l^{-1}$) the losses are nearly equimolar (McDonald, 1983), and in extremely soft water chloride loss even exceeds sodium loss (Leivestad, Muniz & Rosseland, 1980). In the present study, water Ca^{2+} concentration was approx.

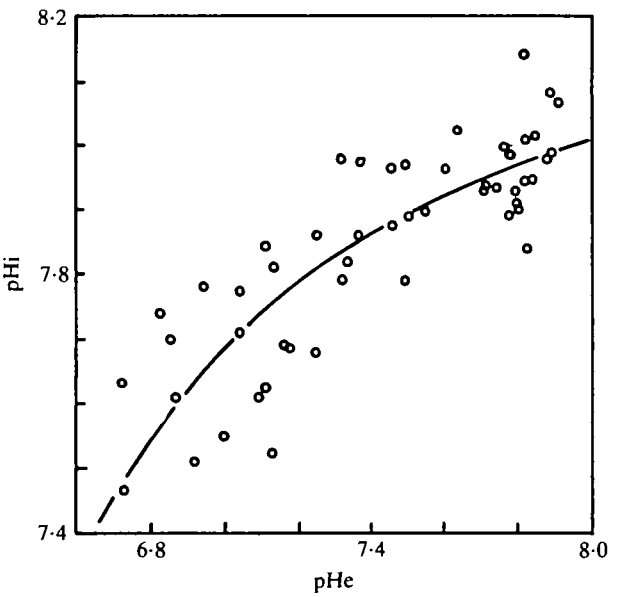

Fig. 2. The relationship between blood (pHe) and red cell (pHi) pH for the control and acid-exposed lampreys. See text for further details.

0·5 mmol l^{-1} and the ratio of Na^+:Cl^- loss was 1·33:1. The lower efflux of chloride compared with sodium can thus be explained by the Ca^{2+} concentration of the water.

The potassium concentration in the plasma of lampreys increased during acid stress (Fig. 3), which is also noted, for example, in rainbow trout despite the losses in gills and kidney (McDonald & Wood, 1981; Ultsch *et al.* 1981; Booth *et al.* 1982). The reason for increased plasma K^+ concentration is unknown. However, we observed that the haemolysis increased, suggesting that red cell breakdown may contribute significantly. Booth *et al.* (1982) suggest that it may be at least partially due to reduction in the extracellular space which is seen, for example, in

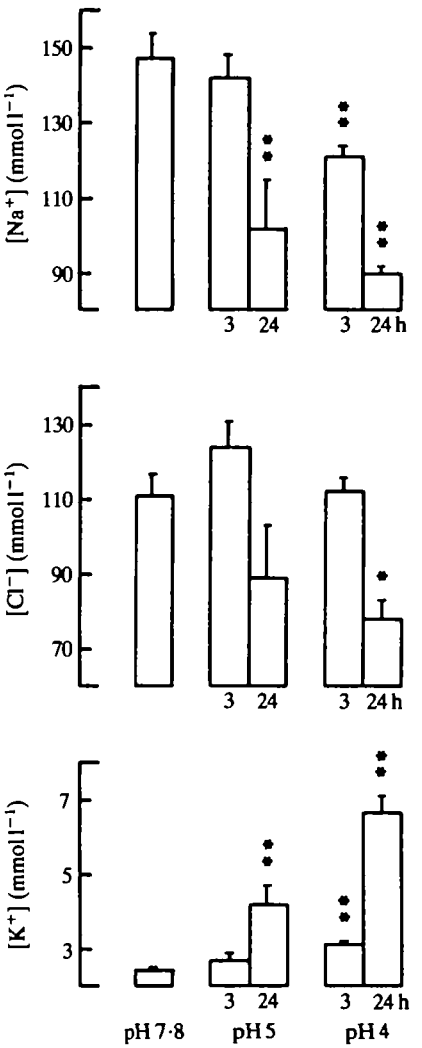

Fig. 3. Plasma Na^+, Cl^- and K^+ concentrations of control lampreys and lampreys exposed to pH 5 or pH 4 for 3 and 24 h. Legend as in Fig. 1.

rainbow trout exposed to a pH of 4·2 (McDonald & Wood, 1981). Also, K^+ may be released from cells involved in buffering H^+ to maintain the cell's normal electrical potential (McDonald & Wood, 1981).

The decrease in red cell pH, observed in acid water (Fig. 1), will decrease the haemoglobin oxygen affinity (see Johansen, Lenfant & Hanson, 1973; Nikinmaa & Weber, 1984). *In vitro*, the decrease of extracellular pH from 7·9 to 7·3, a change of similar magnitude to that observed in the present study after 24 h of exposure to pH 5, caused an increase in P_{50} from approx. 2 to approx. 3·3 kPa (Nikinmaa & Weber, 1984). Thus, at this pH range it is unlikely that O_2 transport would be affected seriously enough to cause the deaths of the lampreys in air-saturated water. However, at lower extracellular pH values intracellular pH decreases more rapidly, and the effects of acidification on oxygen transport may be larger. Changes in the volume of red cells also affect the O_2-binding properties of lamprey haemoglobins (Nikinmaa & Weber, 1984). However, in this study the red cell water content remained unchanged, being 72·9 ± 1·8 (18) % in controls, 72·6 ± 2·3 (5)% after 24 h at pH 5 and 71·6 ± 3·3 (4) % after 24 h at pH 4. In addition to the effect on haemoglobin, acidification may affect respiration by causing an increased secretion of mucus (see Ultsch & Gros, 1979) or gradual necrosis of gill epithelium (Daye & Garside, 1976). If large amounts of mucus were secreted from lamprey gills, the mucus could seriously affect respiration by increasing the diffusion distance for O_2. However, to evaluate this possibility, changes in postbranchial P_{O_2} during acid exposure must be measured.

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