HYPOXIC ACCLIMATION IN THE LAMPREY, *LAMPETRA FLUVIATILIS*: ORGANISMIC AND ERYTHROCYTIC RESPONSES

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SUMMARY

Acute exposure of *Lampetra fluviatilis* to hypoxia (P0₂ = 40–50 mmHg) resulted in a large increase in ventilation frequency and a significant increase in O₂ consumption (from 40 to 60 mg kg⁻¹ h⁻¹ at 8°C). After 1 week’s hypoxia, the O₂ consumption decreased (from 60 to 50 mg kg⁻¹ h⁻¹), indicating the existence of slow, acclimatory changes that remove some of the strain from the ventilatory response. The hypoxic animals had a higher blood O₂ affinity than the normoxic controls. This acclimatory response is not the result of a decreased allosteric interaction between the haemoglobin and erythrocytic organic phosphates, as in teleost fish, but is attributable partly to dilution of haemoglobin within the red cells and partly to an increase in the intracellular pH. The intraerythrocytic pH of hypoxic animals, measured with a freeze-thaw method, was higher than the plasma pH, suggesting that protons are not passively distributed.

INTRODUCTION

In acute hypoxia, the ventilatory frequency of lampreys increases markedly (Johansen, Lenfant & Hanson, 1973; Claridge & Potter, 1975). The resulting increase in ventilation cost raises the standard O₂ consumption in *Lampetra fluviatilis* (Claridge & Potter, 1975). This response to acute hypoxia is similar to that seen in some teleosts, for example rainbow trout (Hughes & Saunders, 1970).

In teleosts, prolonged hypoxia additionally evokes energetically less costly adaptive mechanisms which involve the enhancement of blood O₂ loading in gills (cf. Nikinmaa, 1981; Weber, 1982). In eel (Wood & Johansen, 1972), plaice (Wood, Johansen & Weber, 1975), carp (Weber & Lykkeboe, 1978) and rainbow trout (Soivio, Nikinmaa & Westman, 1980; Tetens & Lykkeboe, 1981; Nikinmaa & Soivio, 1982) blood oxygen affinity increases because of large decreases in the erythrocytic concentrations of nucleotide triphosphates (NTP) and resultant increases in the red cell pH.

It is not known whether the respiratory properties of the blood of cyclostomes adapt to prolonged hypoxia. The O₂ affinity of *in vitro* preparations of lamprey haemoglobin

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is not influenced by the organophosphates which depress haemoglobin O₂ affinity in teleost and mammalian red cells (Johansen et al. 1973; R. E. Weber & M. Nikinmaa, in preparation), suggesting that a hypoxic response, if present, will involve other cellular and molecular mechanisms. In the present study we have investigated the whole blood O₂-binding properties in *Lamptera fluviatilis*, their modification in hypoxia, and the cellular mechanisms underlying these modifications. The oxygenation reactions of the erythrocytes and haemoglobin of this species will be the subject of a separate report (R. E. Weber & M. Nikinmaa, in preparation).

**MATERIALS AND METHODS**

The lampreys, *Lampptera fluviatilis*, were caught in Simojoki river in northern Finland during their spawning run (August–November), and transferred either to Helsinki or Odense, where they were allowed to acclimate to laboratory conditions for at least 1 month. During this period, the P₀₂ of the water was in excess of 130 mmHg and P₅CO₂ was 0.5–1.5 mmHg. The pH varied between 7.2 and 7.6 in Helsinki, and 7.7 and 8.0 in Odense. Altogether 37 animals (36.7 ± 1.5 g, X ± s.e.m.) were used.

**Oxygen consumption studies**

Eight animals were transferred from the holding tanks into 2-L glass respirometers (kept at 8°C). The animals were allowed to acclimate for 2 h, after which the O₂ concentration in the respirometer was determined. The flow of water into the respirometer was then stopped for 1 h, and the O₂ concentration measured at the end of this period. The O₂ consumption rate was calculated from the difference in O₂ concentrations, the water volume and the weight of the fish. The measurements were repeated three times in normoxic water; between the measurements water was allowed to flow through the respirometers for 30 min. After the third normoxic measurement the respirometer was flushed with hypoxic water (P₀₂, 40–50 mmHg) for 30 min, and the O₂ consumption measured three times as above, giving the values for acute hypoxic stress. The fish were then kept in the hypoxic water for a week, after which the O₂ consumption rates under chronic hypoxia were measured as above.

**Studies on the respiratory properties of blood**

These experiments were carried out at 15 and 2°C. In the 15°C-experiments, eight animals were transferred into a 60-L aquarium where they were allowed to consume part of the dissolved oxygen and gradually induce environmental hypoxia. The animals were prevented from contact with air by a perforated Plexiglass sheet positioned about 2 cm below the water surface. The P₀₂ of the water was kept at 40–50 mmHg by a flow of water through the aquarium and mixing of the water in the aquarium; if the O₂ tension increased or decreased beyond these limits, the flow or mixing rates were adjusted. Although the flow-through system prevented excessive accumulation of ammonia or CO₂, water P₅CO₂ increased from about 1 mmHg to 3 mmHg in the course of the experiment. The normoxic control animals (nine in number) were kept in the original, aerated, holding tank throughout the experiment.

The procedure in the 2°C-experiments was the same as at 15°C, except that the
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Water PO2 was decreased by bubbling N2 into the aquarium. Seven hypoxic and five normoxic (control) animals were used at this temperature.

After 7 days' acclimation the fish were anaesthetized in MS 222 solution (2 g l-1) and blood samples taken from caudal vessels into heparinized syringes. The following were then measured:

- plasma pH (immediately after sampling, using Radiometer's BMS 3 and PHM 71 & 72);
- red cell pH (after separating plasma and red cells, and freezing and thawing the red cell mass twice, as described by Jensen & Weber, 1982);
- blood P50 values (at Pco2 values of 0·8 and 3·7 mmHg, with a mixing method after 30 min equilibration in Radiometer BMS 2 tonometers);
- blood haemoglobin concentration (Hb; with the cyanmethaemoglobin method);
- mean cellular Hb concentration (MCHC; calculated from blood Hb and haematocrit values);
- plasma and red cell lactate concentrations (with Boehringer test kit no. 124842);
- red cell nucleoside triphosphate (NTP) concentration (with Boehringer test kit no. 123897);
- ATP and guanosine triphosphate (GTP) (by thin layer chromatography, after Johansen, Lykkeboe, Weber & Maloiy, 1976);
- plasma glucose concentration (with Boehringer test kit no. 123896);
- plasma and red cell Na+ and K+ concentrations (with a Perkin Elmer atomic absorption spectrophotometer, or an EEL flame spectrophotometer);
- plasma and red cell Cl- concentration (with a Radiometer CMT 10 chloride titrator);
- plasma and red cell Mg2+ concentration (with Wako test kit no. 273-32809);
- plasma Ca2+ concentration (with Wako test kit no. 276-21809).

RESULTS

Ventilatory frequency and oxygen consumption

The ventilation frequency at 15 °C (Fig. 1) increased drastically (P < 0·001) from 99 ± 14 (8) in normoxia to 241 ± 37 (8) at 40–50 mmHg PO2 in acute hypoxia. Below 40 mmHg PO2 the animals were not able to maintain the high ventilation frequency, but started to show apnoeic periods of varying length, resulting in a decreased number of ventilatory movements per minute. After 7 days of hypoxic exposure the ventilation frequency at 40–50 mmHg had decreased slightly to 224 ± 40 (8). The O2 consumption of the animals increased significantly (P < 0·01) from 40 ± 3·7 mg kg-1 h-1 (x ± s.e.m.; number of fish 8) in normoxia to 60 ± 5·2 mg kg-1 h-1 in acute hypoxia. In prolonged hypoxia, the O2 consumption of the animals decreased significantly (P < 0·05) from that in acute hypoxia, to 53 ± 3·5 mg kg-1 h-1, presumably reflecting the decrease in ventilatory activity compared to that in the non-acclimated specimens.

Blood oxygen transport

Blood Hb concentration and MCHC

At both 15 and 2 °C the blood Hb concentration was practically the same in the hypoxic as in the normoxic lampreys (Tables 1, 2), indicating that, unlike some
teleosts such as killifish (Greaney & Powers, 1978) and eel (Wood & Johansen, 1972), the lamprey is unable to modify the blood O2-carrying capacity in response to hypoxia. On the other hand, MCHC fell by more than 20% at 15 °C from approximately 17 to 14 mmol monomeric Hb l⁻¹ packed cells; the decrease was most probably due to cellular swelling. At 2 °C the slight decrease in MCHC was not statistically significant.

**Plasma and red cell pH**

There was practically no pH gradient across the red cell membrane in normoxia at either temperature (Tables 1, 2). In hypoxia, however, the measured red cell pH was approximately 0.2 units higher than the plasma pH both at 15 and at 2 °C (Fig. 2). In the normoxic specimens, plasma pH was inversely related to acclimation temperature (between 2 and 15 °C, ΔpH/Δ °C values were -0.016 and -0.011, for the cells and the plasma respectively), reflecting conformity with the relative alkalinity concept applicable to ectotherm vertebrates (Howell, Baumgardner, Bondi & Rahn, 1970). No similar pH regulation is seen in the hypoxic specimens (Fig. 2).

**Oxygen equilibria**

The log P_{50} vs pH diagrams are given in Fig. 3. The Bohr factor in the blood of the hypoxic animals (−0.14) was significantly (P < 0.02) lower than that in the blood of normoxic animals (−0.34) in the pH range from 7.3 to 8.2. These regression coefficients were compared using the t-test as described by Goldstein (1964). The regression equations were:
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Table 1. Blood values of hypoxic and normoxic lampreys at 15 °C

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hypoxic</th>
<th>P</th>
<th>Normoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g l⁻¹)</td>
<td>86.5 ± 8.2 (8)</td>
<td>NS</td>
<td>95.5 ± 3.3 (8)</td>
</tr>
<tr>
<td>MCHC (g l⁻¹)</td>
<td>227.6 ± 12.9 (8)</td>
<td>0.01</td>
<td>283.6 ± 15.9 (8)</td>
</tr>
<tr>
<td>plasma pH</td>
<td>7.605 ± 0.056 (7)</td>
<td>NS</td>
<td>7.596 ± 0.015 (5)</td>
</tr>
<tr>
<td>red cell pH</td>
<td>7.751 ± 0.030 (7)</td>
<td>0.01</td>
<td>7.561 ± 0.030 (8)</td>
</tr>
<tr>
<td>plasma lactate (mg l⁻¹)</td>
<td>854 ± 304 (7)</td>
<td>0.01</td>
<td>235 ± 35 (8)</td>
</tr>
<tr>
<td>red cell lactate (mg l⁻¹)</td>
<td>373 ± 273 (7)</td>
<td>0.05</td>
<td>96 ± 11 (8)</td>
</tr>
<tr>
<td>red cell NTP (mmol l⁻¹)</td>
<td>1.36 ± 0.06 (7)</td>
<td>NS</td>
<td>1.91 ± 0.35 (8)</td>
</tr>
<tr>
<td>plasma K⁺ (mmol l⁻¹)</td>
<td>3.64 ± 0.06 (5)</td>
<td>NS</td>
<td>3.32 ± 0.04 (5)</td>
</tr>
<tr>
<td>red cell K⁺ (mmol l⁻¹)</td>
<td>71.2 ± 2.5 (5)</td>
<td>0.05</td>
<td>76.8 ± 1.6 (5)</td>
</tr>
<tr>
<td>plasma Na⁺ (mmol l⁻¹)</td>
<td>128.0 ± 3.1 (5)</td>
<td>NS</td>
<td>131.8 ± 5.7 (5)</td>
</tr>
<tr>
<td>red cell Na⁺ (mmol l⁻¹)</td>
<td>40.4 ± 2.9 (5)</td>
<td>NS</td>
<td>32.8 ± 2.5 (5)</td>
</tr>
</tbody>
</table>

The means, standard errors of the mean with the number of animals (in brackets) are given.
Student’s t-test was used for statistical comparisons of the groups.
In the red cell determinations, values are per litre of packed red cells.
NS = not significant.

log P5₀ = -0.14 pH + 2.29, r = 0.64, N = 19 and
log P5₀ = -0.34 pH + 3.88, r = 0.88, N = 14, respectively.

Calculating the P5₀ values for in vivo plasma pH at 15 °C (7.6 both in normoxia and hypoxia) yields values of 20 mmHg in normoxia and 16 mmHg in hypoxia, indicating a higher O₂ affinity in the hypoxia-acclimated specimens. The P5₀ and Bohr factor values of the hypoxic and normoxic specimens are lower and higher, respectively, than those obtained by Bird, Lutz & Potter (1976) for L. fluviatilis blood at a slightly lower temperature: (log P5₀ = -0.22 pH + 2.70; r = 0.71, N = 16 at 10 °C).
Organic phosphate and lactate concentrations

At both temperatures, the erythrocytic NTP concentration tended to decrease.
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Fig. 3. Log $P_{so}$ vs pH plot for the blood of (A) normoxic and (B) hypoxic lampreys at 15°C, $\Phi = $ Bohr factor. The regression equations are given in the text.

during hypoxic exposure (Tables 1, 2). The decrease was mostly due to red cell swelling, as indicated by the fact that the NTP/Hb (monomer) molar ratio remained largely unaltered. In normoxia and hypoxia, the ratio was 0·11 and 0·10, respectively, at 15°C, and 0·10 and 0·12, respectively, at 2°C. Thin layer chromatography experiments showed that the erythrocytic NTPs consist largely of ATP but also contain some GTP (respective concentrations were 0·70 ± 0·02 S.D. and 0·33 ± 0·05 mmol l$^{-1}$ cells, $N = 3$). Bartlett (1982) similarly demonstrated the presence of GTP (at concentrations of 6–66% of that of ATP) in red cells of individuals of the lamprey Entospherus tridentatus. That organophosphates other than NTPs may occur in lampreys follows from the observations (Johansen et al. 1973; Bartlett, 1982) that $E.$ tridentatus red cells contain 2,3-diphosphoglycerate (DPG) at concentrations of 9–40% of the ATP + GTP pool.

At 15°C, both the red cell and plasma lactate concentrations increased markedly in hypoxia (Table 1; Fig. 2). The increase in the cellular concentration was proportionally greater than that in plasma; the red cell/plasma lactate ratio thus increased from 0·41 to 0·67. At 2°C the plasma lactate concentration remained unchanged although the red cell lactate concentration increased significantly (Table 2). As a result the red cell/plasma ratio increased from 0·37 in normoxia to 0·49 in hypoxia.

Inorganic ion concentrations

Hypoxic exposure at 15°C slightly increased the plasma concentration of $K^+$ while the red cell $K^+$ level showed a pronounced decrease. On the other hand, a slight
decrease in plasma Na⁺ concentration was concomitant with a marked increase in red cell Na⁺ (Table 1). This resulted in a distinct fall in red cell K⁺/Na⁺ ratio from 2·9 to 1·8 (Fig. 2). A similar decrease in this ratio, from 2·6 to 1·5, took place in hypoxia at 2°C as red cell K⁺ concentration decreased and the Na⁺ concentration increased without significant changes in plasma concentrations (Table 2; Fig. 2). Also, the plasma Mg²⁺ concentration was lower in hypoxia than in normoxia at 2°C (Table 2).

**DISCUSSION**

The blood responses were remarkably similar at 15 and at 2°C. At both temperatures hypoxic exposure induced lower red cell K⁺/Na⁺ ratios, higher intracellular than plasma pH, and increased red cell/plasma lactate ratios. However, the changes in MCHC, and in plasma and red cell lactate concentrations were more pronounced at 15°C than at 2°C, demonstrating that the same degree of environmental hypoxia is more stressful at the higher temperature, which is ascribable to the higher metabolic rate under these conditions (see Claridge & Potter, 1975).

As in teleosts, the initial response of *Lampetra fluviatilis* to hypoxia is hyperventilation (see also Claridge & Potter, 1975). The resulting increase in the cost of ventilation is reflected in higher O₂ consumption rates. If in prolonged hypoxia the high cost of ventilation had to be maintained, the energy stores of the fish could be seriously depleted. However, in 7-day hypoxia the O₂ consumption was about 15 % lower than in acute hypoxia, showing that some other adaptive responses had occurred, reducing the energy-demanding contribution from the ventilatory response.

The increased blood O₂ affinity which will enhance O₂ loading in gills of the hypoxic animals appears to be part of this adaptation. The oxygen-binding properties of the haemoglobin (R. E. Weber & M. Nikinmaa, in preparation) suggest involvement of two mechanisms: a decrease in cellular haemoglobin concentration, as would result from red cell swelling, and an increase in the intracellular pH. A third factor that favours a higher blood O₂ affinity in hypoxic animals at low pH is that the Bohr effect is lower in hypoxic than in normoxic animals.

Although no data are available for the physiological concentration range, the O₂ affinity of lamprey haemoglobin seems to be strongly dependent on its concentration. Briehl (1963) showed that an increase in Hb concentration from 0·25 to 15·8 mmol l⁻¹ (haem basis) decreased the O₂ affinity 10-fold in *Petromyzon marinus*. In *Entosphenus japonicus*, Dohi, Sugita & Yoneyama (1973) observed that the P₅₀ value increased from about 15 mmHg to 35 mmHg as Hb concentration increased from 1 to 8 mmol l⁻¹ at pH 6·9. The increase in the O₂ affinity with dilution may be attributable to dissociation of haemoglobin to monomers, which have a higher O₂ affinity than dimers or tetramers (Riggs, 1972). R. E. Weber & M. Nikinmaa (in preparation) will show that dilution of *L. fluviatilis* haemoglobin in solution not only increases its O₂ affinity but also decreases the Bohr effect, supporting the view that the corresponding changes observed in the whole blood may result from erythrocytic swelling.

The increase in the red cell pH in the hyperventilating hypoxic specimens at 15°C will increase the O₂ affinity of the blood via the Bohr effect. The parallel reduction in the Bohr effect will favour maintenance of a high O₂ affinity. Dilution increases the proportion of monomeric haemoglobins which lack heterotropic interactions basic...
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The Bohr effect. The low pH sensitivity of dilute *L. fluviatilis* haemoglobin in solution (R. E. Weber & M. Nikinmaa, in preparation) supports this view.

An intriguing finding was that red cell pH was higher than plasma pH in the hypoxic animals. Some methodological factors may affect red cell pH measurements and deductions about the pH gradient. Firstly there was a time lag between plasma and red cell pH measurement. Secondly, the samples were taken from anaesthetized fish, and this may influence the pH gradient across the red cell membrane. Thirdly, according to Roos & Boron (1981) the freeze-thaw method for intracellular pH is reliable only for mammalian red cells. However, the intracellular pH values estimated from the freeze-thaw measurements are generally lower than those obtained with other methods (see Roos & Boron, 1981). Our finding of a higher pH in the red cells than in the plasma is furthermore in accordance with the calculations of Riggs (1972) [based on Manwell’s (1963) and Briehl’s (1963) data from *P. marinus*] which reflect an intracellular pH that is 0.7 units higher than the extracellular one. Such a difference could exist if the majority of impermeable polyions in the cell were positively charged (cf. Riggs, 1972). This is, however, unlikely, as the chloride ratio (Cl⁻cell/Cl⁻plasma) is less than one, and the same for both normoxic (0.60) and hypoxic (0.63) animals at 2°C. At anion and volume equilibrium, the anion ratio (for those anions that can exchange across red cell membrane) can be calculated from the relation (cf. Hladky & Rink, 1977):

$$r = \frac{2B + ZP}{2B + (Z + 1)P},$$

where $r =$ anion ratio (for exchangeable anions), $B =$ number of cations in the cell (mostly Na⁺ and K⁺), $P =$ number of impermeable polyions in the cell (mostly haemoglobin and organic phosphates) and $Z =$ the charge on impermeable polyions. $Z$ can be solved by substituting data from Table 2 into the above equation (anion ratio taken as 0.6; all other values converted into number of particles/cell). According to these calculations the charge of impermeable polyions is −3.

Red cell pH could also be higher than plasma pH, if protons were not passively distributed. A sodium/proton exchange mechanism operates in *Amphiuma* red cells (Kregenow, 1981). If such an exchange was functional in lamprey, the existing sodium gradient across the red cell membrane could drive the exchange and raise extracellular H⁺ concentration above the intracellular level.

Red cell swelling and changes in intracellular pH may be achieved by related mechanisms. In rainbow trout red cell swelling and increase in intracellular pH seem to occur simultaneously as a result of beta-adrenergic stimulation (Nikinmaa, 1982). In rainbow trout (Nikinmaa & Soivio, 1982; Nikinmaa, 1982) as well as in lamprey (this study), the change in intracellular H⁺ concentration seems to be twice the change in cell volume.

The red cell swelling could, however, also be explained by decreased activity of the K⁺/Na⁺ pump, which would explain the observed changes in K⁺ and Na⁺ concentrations (Tables 1, 2). Often a decreased pump activity is the result of anoxia, and a resultant depletion of ATP stores (cf. Macknight & Leaf, 1980). It is, however, questionable if the P0₂ in hypoxic blood is low enough to cause decreased ATP synthesis and impairment of the pump function. Greaney & Powers (1978) showed
that the ATP concentration of killifish red cells decreased only in complete anoxia, and Tetens & Lykkeboe (1981) concluded that hypoxia does not impair ATP synthesis by rainbow trout red cells, suggesting that a hormonal mechanism could regulate the NTP concentration and O₂ affinity of these cells. Hormonal control by adrenalin seems, indeed, to be implied at least in the rapid O₂ affinity changes of rainbow trout blood (Nikinmaa, 1982). Adrenalin moreover influences the cation concentrations of nucleated red cells by changing the cation permeability of the red cell membrane (see Palfrey, Alper & Greengard, 1980).

We conclude that hypoxic exposure in the lamprey does increase blood O₂ affinity, despite insensitivity of the Hb-O₂ affinity to organic phosphate concentration and ionic strength, and that the change in blood O₂ affinity appears to be mediated by changes in the red cell membranes resulting in lower cellular Hb concentration and increased cellular pH.

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