THE ADRENERGIC RESPONSES OF CARP (CYPRINUS CARPIO) RED CELLS: EFFECTS OF PO2, AND pH

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Summary

Carp (Cyprinus carpio) red cells do not show β-adrenergic responses when incubated with 10^-5 mol l^-1 adrenaline at atmospheric oxygen tension and a pH value close to the in vivo resting pH (approx. 8.1). However, when either the pH or the oxygen tension of the incubation medium is decreased, the adrenergic responses appear, showing that oxygen or an oxygen-linked phenomenon has a direct influence on the response. Once present, the adrenergic red cell response is similar to that of trout: cellular water content, sodium content and intracellular pH all increase. Quantitatively the effect appears to be much smaller in carp than in trout. Adrenaline induces an increase in red cell oxygen content when the oxygen content is plotted as a function of extracellular pH. This effect coincides with the onset of the Root effect and is caused by the adrenaline-induced increase in intracellular pH, since it disappears when the oxygen content is plotted as a function of intracellular pH. The red cell ATP content decreases metabolically during adrenaline incubations. In contrast, cellular GTP content is not metabolically reduced in adrenaline-treated cells, showing that the rapid and selective decrease in red cell GTP concentration, observed in hypoxic cyprinids, is not adrenergically induced.

Introduction

The adrenergic response of trout (Salmo gairdneri) red cells is well characterized. In vitro results (e.g. Nikinmaa & Huestis, 1984; Baroin, Garcia-Romeu, Lamarre & Motais, 1984; Cossins & Richardson, 1985) have shown that adrenaline activates Na^+/H^+ exchange across the red cell membrane. Proton extrusion via this pathway leads to a decrease in the transmembrane pH gradient. The influx of Na^+, and Cl^- influx through the anion exchange pathway, cause cell swelling. Also, the concentrations of red cell organic phosphates and haemoglobin decrease. All the above responses shift the oxygen dissociation curve to the left (Nikinmaa, 1983). The in vitro response occurs at atmospheric PO2, at normal plasma pH, PCO2, and bicarbonate concentration, but is markedly enhanced by a decrease in pH (Borgese, Garcia-Romeu & Motais, 1987; Heming, Randall &
Mazeaud, 1987; Nikinmaa, Steffensen, Tufts & Randall, 1987b). Borgese et al. (1987) have shown that the pH-dependence of the adrenergic activation of sodium/proton exchange follows a bell-shaped curve, reaching a maximum at pH 7.3. In vivo, adrenaline-activated Na\(^+\)/H\(^+\) exchange occurs both in hypoxia (Fievet, Motais & Thomas, 1987) and in exercise (Primmett, Randall, Mazeaud & Boutiliel, 1986; Milligan & Wood, 1986).

In contrast, much less is known about the adrenergic responses of cyprinid red cells. The adrenergic response is absent in exercised tench, Tinca tinca (Jensen, 1987), but present in hypoxic carp, Cyprinus carpio (Nikinmaa, Cech, Ryhänen & Salama, 1987a). However, carp red cells swelled and their intracellular Na\(^+\) concentration increased only when blood P\(_O_2\) decreased below 10 mmHg (1 mmHg = 133.3 Pa). These observations suggest, albeit indirectly, that P\(_O_2\) may have a direct influence on the adrenergic response. Recently, Motais, Garcia-Romeu & Borgese (1987) have shown that, although present at atmospheric P\(_O_2\), the adrenergic response of trout is enhanced by decreased O\(_2\) tension.

We have investigated the role of P\(_O_2\) and pH on the adrenergic response of carp red cells. Particular emphasis was paid to the effect of adrenaline on the oxygen content of the red cells and the relationship between the Root effect and the adrenergic response.

**Materials and methods**

Female carp (Cyprinus carpio, 1.2–2.5 kg, \(N = 30\)) were obtained from Evo Fisheries Experimental Station, transported to the Department of Zoology, University of Helsinki, and acclimated to laboratory conditions (11–12°C, oxygen tension >120 mmHg, pH 7.2–7.4, 12 h:12 h L:D rhythm) for at least a month before use.

Blood samples were taken into heparinized syringes by caudal puncture from anaesthetized (MS-222, 0.1 g l\(^{-1}\), 5 min) fish. The blood was centrifuged (2 min, 10,000 g), and the buffy coat and plasma were discarded. The red cells were washed twice in the physiological saline used and left overnight in the saline at 5°C. This ensured that the cells were not in a catecholamine-stimulated condition (see Bourne & Cossins, 1982). The following day the red cells were again washed and then suspended in the physiological saline at a haematocrit of about 20%. The composition of the saline (in mmol l\(^{-1}\)) was: NaCl, 128; KCl, 3; MgCl\(_2\), 1.5; CaCl\(_2\), 1.5; and NaHCO\(_3\), 10.

Each 1.2-ml sample was divided in two parts: \(10^{-5}\) mol l\(^{-1}\) adrenaline (final concentration) was added to one, the other served as control. A high concentration of adrenaline was used to ensure a maximal response. 10 μl of \(^{14}\)C-labelled DMO (5,5-dimethyl-2,4-oxazolidine-dione, 10 μCi ml\(^{-1}\)) was added to each subsample at the onset of the incubations. The subsamples were incubated in a shaking tonometer for 30 min at 20°C in the conditions given in Table 1. The humidified gas mixtures were obtained using Wösthoff gas-mixing pumps.
The extracellular pH and the oxygen content of the suspensions were measured immediately after the end of the incubation. The extracellular pH (pHe) was measured using Radiometer BMS 3 Mk 2, PHM 72 apparatus, and the oxygen content using Tucker's (1967) method and subtracting the oxygen dissolved in the incubation medium. The O\textsubscript{2} solubility coefficient of the ferricyanide reagent at 20°C was extrapolated from Tucker's data and the values for 0.119 mol\textsuperscript{-1} NaCl (Altman & Dittmer, 1971) were used as the solubility coefficient of the incubation medium. 10\mu L of the suspension was taken for measurement of the haemoglobin concentration by the cyanmethaemoglobin method.

The rest of the suspension was then centrifuged for 2 min at 10,000 g in two Eppendorf tubes, and the supernatants transferred into a third Eppendorf tube. The topmost layer of the red cells was then discarded to ensure effective removal of the incubation medium. One of the red cell pellets was used for determination of cell water. It was weighed, dried to a constant weight and reweighed. The water content of the cell pellet is given in kg water kg\textsuperscript{-1} dry mass of the cell pellet. The other cell pellet was deproteinized in 0.6 mol\textsuperscript{-1} perchloric acid and stored in liquid nitrogen until the determinations of red cell ATP, GTP, Na\textsuperscript{+}, K\textsuperscript{+}, Cl\textsuperscript{-} and DMO contents were made. The supernatant was also deproteinized in 0.6 mol\textsuperscript{-1} perchloric acid and the Na\textsuperscript{+}, K\textsuperscript{+}, Cl\textsuperscript{-} and DMO concentrations were measured. Na\textsuperscript{+} and K\textsuperscript{+} were determined by flame photometry (Radiometer FLM3), and Cl\textsuperscript{-} by Radiometer CMT10 chloride titrator. All the intracellular ion contents are given in mmol kg\textsuperscript{-1} dry mass of the cell pellet. ATP and GTP contents were measured enzymatically as described by Albers, Goetz & Hughes (1983) and are

<table>
<thead>
<tr>
<th>Table 1. Incubation conditions used in the experiments</th>
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</table>

The pH values given are those measured at the end of the incubation.
given in mmol kg\(^{-1}\) dry mass of the cell pellet. Intracellular pH (pHi) was calculated from the distribution of \([^{14}\text{C}]\text{DMO}\) across the cell membrane using the formula:

\[
\text{pHi} = \text{pK}_{\text{DMO}} + \log\left(\frac{[\text{DMO}]_i}{[\text{DMO}]_e} \times 10^{\text{pHe} - \text{pK}_{\text{DMO}} + 1} - 1\right),
\]

in which \(\text{pK}_{\text{DMO}}\) at 20°C was taken as 6.19 (Boutilier, Heming & Iwama, 1984) and \([\text{DMO}]_i\) is taken as disint min\(^{-1}\) ml\(^{-1}\) H\(_2\)O in the red cell pellet.

Since our estimations of water and ion contents included the contribution from the extracellular fluid, we estimated, in a separate experiment, the proportion of the extracellular fluid in the cell pellets of adrenaline-treated cells at low pH (7.1) and control cells at high pH (8.1), using \([^{14}\text{C}]\text{polyethylene glycol}\) as a marker. With the centrifugation used, the proportion of extracellular fluid was independent of the treatment and was 6.5 ± 0.2% of the packed cell volume for the whole material \((N = 16)\). Thus, the adrenaline-induced changes observed in the water and ion contents give a realistic picture of the changes taking place in the intracellular compartment. Small effects of adrenaline may be lost in methodological inaccuracies. With regard to the estimation of pHi, the use of the water content of the cell pellet in the calculation of \([\text{DMO}]_i\), instead of the water content of the red cells (corrected for 6.5% trapped medium), gives values that are 0.001–0.015 units higher than the corrected values. Since the proportion of trapped extracellular fluid was similar for both control and adrenaline-treated cells, the conclusions about the effects of adrenaline on the red cell pH are not affected.

### Results and discussion

#### Cellular water content

At atmospheric \(P_{\text{O}_2}\) and at pH 7.5 or above, adrenaline did not cause a significant increase in the cellular water content. However, adrenergic swelling appeared either if pH was decreased below 7.5 at atmospheric \(P_{\text{O}_2}\) or if at pH 7.5 the \(P_{\text{O}_2}\) of the incubation was decreased to 30 mmHg or below (Fig. 1). These results show that the adrenergic response in carp is dependent on both pH and \(P_{\text{O}_2}\), and may explain why, in tench, neither exercise nor adrenaline infusion caused adrenergic swelling of red cells (Jensen, 1987). In the exercised tench, the plasma pH values remained higher than 7.5 and blood \(P_{\text{O}_2}\) was above 30 mmHg.

It should be noted that different individuals were used at different times for determinations at the different \(P_{\text{O}_2}\) and pH values. Thus, in addition to the effects of \(P_{\text{O}_2}\) or pH on the water content per se, the water content was affected by differences in, for example, the organic phosphate and haemoglobin concentrations among the different individuals and sampling times. The apparently bell-shaped curve in Fig. 1B may be caused by such variation. In contrast, at a given pH or \(P_{\text{O}_2}\), the effects of adrenaline were always tested using a single blood sample, divided into two portions, and thereby give a realistic picture of the effects of adrenaline on the red cell function at any experimental condition.
Adrenaline and carp red cells

Ion contents

Similar to the effect of adrenaline on the cellular water content, and as expected from the role of Na⁺/H⁺ exchange in the adrenergic response of trout (e.g. Borgese et al. 1987), the adrenaline-induced increase in the red cell Na⁺ content was dependent on both pH and P_O₂ (Fig. 2). Also the red cell Cl⁻ content increased as a result of adrenergic stimulation, although much more marked

![Graph](image1)

**Fig. 1.** The water content (kg H₂O kg⁻¹ dry mass of the cell pellet) of adrenaline-treated (●) and control (○) carp red cells as a function of extracellular pH at atmospheric oxygen tension (A, N = 6–8) and as a function of oxygen tension at extracellular pH 7.5 (B, N = 8). Asterisks indicate statistically significant (P < 0.05) differences between the means of adrenaline-treated and control samples; bars show s.e. Paired t-test was used for comparisons.

![Graph](image2)

**Fig. 2.** The sodium content (in mmol kg⁻¹ dry mass of the cell pellet) of adrenaline-treated and control carp red cells as a function of extracellular pH at atmospheric oxygen tension (A) and as a function of oxygen tension at extracellular pH 7.5 (B). Symbols otherwise as in Fig. 1.
changes in Cl$^-$ level were caused by decreases in pH or oxygen tension (Fig. 3). As a result of either a fall in pH or deoxygenation, the negative charge of haemoglobin decreases, and Cl$^-$ enters the cell; such pH- and P$_O_2$-dependent changes in Cl$^-$ content are a characteristic feature of red cells (for a review see Hladky & Rink, 1977). The intracellular K$^+$ content (Fig. 4) increased significantly in the adrenaline-treated cells at atmospheric P$_O_2$ when pH decreased below 7.5, suggesting that the sodium pump is activated as a result of Na$^+$ influx.

![Figure 3](image-url)  
Fig. 3. The chloride content (in mmol kg$^{-1}$ dry mass of the cell pellet) of adrenaline-treated and control carp red cells as a function of extracellular pH at atmospheric oxygen tension (A) and as a function of oxygen tension at extracellular pH 7.5 (B). Symbols otherwise as in Fig. 1.

![Figure 4](image-url)  
Fig. 4. The potassium content (in mmol kg$^{-1}$ dry mass of the cell pellet) of adrenaline-treated and control carp red cells as a function of extracellular pH at atmospheric oxygen tension. Symbols otherwise as in Fig. 1.
Adrenaline and carp red cells

Table 2. Effects of $10^{-5}$ mol$^{-1}$ adrenaline on the ATP and GTP contents (in mmol kg$^{-1}$ dry cell mass) at constant carbon dioxide and varying oxygen tension, and at varying carbon dioxide and constant oxygen tension

<table>
<thead>
<tr>
<th>$P_{O_2}$ (mmHg)</th>
<th>$P_{CO_2}$ (mmHg)</th>
<th>ATP (mmol kg$^{-1}$)</th>
<th>GTP (mmol kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Adrenaline</td>
<td>Control</td>
</tr>
<tr>
<td>60</td>
<td>7-6</td>
<td>16.30 ± 1.03*</td>
<td>14.19 ± 0.57</td>
</tr>
<tr>
<td>30</td>
<td>7-6</td>
<td>13.63 ± 0.70</td>
<td>12.75 ± 0.71</td>
</tr>
<tr>
<td>15</td>
<td>7-6</td>
<td>14.92 ± 0.56</td>
<td>14.64 ± 0.53</td>
</tr>
<tr>
<td>8</td>
<td>7-6</td>
<td>10.98 ± 1.00*</td>
<td>9.14 ± 0.75</td>
</tr>
<tr>
<td>3</td>
<td>7-6</td>
<td>14.17 ± 0.73*</td>
<td>12.99 ± 0.72</td>
</tr>
<tr>
<td>0</td>
<td>7-6</td>
<td>12.33 ± 0.40*</td>
<td>10.85 ± 0.39</td>
</tr>
<tr>
<td>8</td>
<td>3-8</td>
<td>7.59 ± 0.44*</td>
<td>5.90 ± 0.39</td>
</tr>
<tr>
<td>8</td>
<td>7-6</td>
<td>10.98 ± 1.00*</td>
<td>9.14 ± 0.75</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>11.67 ± 0.53*</td>
<td>10.35 ± 0.60</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>11.96 ± 0.28*</td>
<td>10.84 ± 0.66</td>
</tr>
</tbody>
</table>

$N = 8$; the statistical significances of the differences between the means of the control and adrenaline-treated cells were determined using paired $t$-test ($* = P < 0.05$).

The incubations did not significantly affect extracellular Na$^+$ and Cl$^-$ concentrations (not shown). The extracellular K$^+$ concentrations in all control incubations were slightly higher (approx. 3.5 mmol l$^{-1}$) than the initial concentration in the incubation medium (3 mmol l$^{-1}$), indicating either a net efflux of K$^+$ during incubation or a very slight haemolysis of the cells during the final centrifugation. In contrast, the extracellular K$^+$ concentrations of the adrenaline-treated cells were not different from the initial concentration in the incubation medium.

Organic phosphate contents

The adrenaline-induced metabolic decrease in red cell ATP content observed previously in trout (Nikinmaa, 1983) was also observed in carp (Table 2). Cellular GTP contents were not affected by adrenaline. Thus, the rapid and selective decrease of GTP concentration in hypoxic carp (Lykkeboe & Weber, 1978) and hypoxic-hypercapnic tench (Jensen & Weber, 1985) is not caused by adrenergic stimulation.

Extra- and intracellular pH

At atmospheric $P_{O_2}$, at a given extracellular pH (below pH 7.7) adrenaline-treated cells has a smaller proton gradient across the membrane than control cells (Fig. 5A). However, the magnitude of the adrenaline-induced decrease in the proton gradient was smaller than in trout, in which a decrease of up to 0.2 units has been observed (Cossins & Richardson, 1985; Heming et al. 1987). In carp, the corresponding change was only 0.03–0.05 pH units. Changes in the oxygen tension of the incubation had little effect on the adrenaline-induced drop in the pH
gradient when pHe was 7.5 (Fig. 5B). The drop in the pH gradient is partly caused by a decrease in cellular ATP content and partly by the apparent activation of the Na\(^+\)/H\(^+\) exchange.

**Oxygen content**

At atmospheric oxygen tension, adrenaline-treated cells had a higher oxygen content than control cells at and below a pHe of 7.5 (Fig. 6A). This effect coincided with the appearance of the Root effect (i.e. a decrease of oxygen content with decreasing pH at atmospheric P\(_{O_2}\)). At a P\(_{O_2}\) of 8 mmHg the adrenaline-treated cells had a higher oxygen content than control cells at all the pH values studied.

The effect of adrenaline on the O\(_2\) content is largely due to its effects on intracellular pH. When the O\(_2\)/haemoglobin molar ratio is plotted as a function of pH\(_i\), the difference in the oxygen content between the control and adrenaline-treated cells almost completely disappears (Fig. 6B) at both atmospheric and 8 mmHg oxygen tension. This observation agrees with the *in vitro* data on trout red cells (Nikinmaa, 1983), and strongly suggests that the effects of adrenaline on the pH of the microenvironment of haemoglobin are the decisive factors in the adrenergic enhancement of O\(_2\) transport. However, the observations do not rule
Fig. 6. The oxygen content (in mol oxygen mol⁻¹ haemoglobin) of adrenaline-treated (filled figures) and control (empty figures) carp red cells as a function of extracellular pH (A) and intracellular pH (B) at atmospheric (circles; \( N = 4-8 \)) and 8 mmHg (triangles; \( N = 5-8 \)) oxygen tension. Legend otherwise as in Fig. 1.

out the possibility that within the red cell, local pH changes in the vicinity of the haemoglobin molecules would be greater than the measured mean changes in pHi.

From the \( O_2 \) content data as a function of \( P_{O_2} \) at pH 7.5 and 7.1, we have calculated the Hill plots for both adrenaline-treated and control cells. At pH 7.5 the Hill equation for the control cells was:

\[
\log\left[\frac{Y}{(1-Y)}\right] = 1.12 \log P_{O_2} - 1.21, \quad r = 0.862, \quad N = 45;
\]

and for adrenaline-treated cells:

\[
\log\left[\frac{Y}{(1-Y)}\right] = 1.19 \log P_{O_2} - 1.27, \quad r = 0.860, \quad N = 45.
\]

These equations are very similar (\( Y = \) fractional \( O_2 \) saturation), giving \( P_{50} \) values of 12.0 mmHg for control and 11.7 mmHg for adrenaline-treated cells. Thus, at pH 7.5, the effect of adrenaline on the \( O_2 \)-binding behaviour of red cells is minimal. However, the Hill plot calculated for the whole material underestimates the effect of adrenaline on the \( O_2 \)-binding behaviour, as at \( P_{O_2} \) values of 8 and 15 mmHg adrenaline-treated cells had significantly higher oxygen contents than control cells [1.47 ± 0.05 (\( N = 8 \)) vs 1.33 ± 0.05 (\( N = 8 \)) at 8 mmHg and 2.44 ± 0.15 (\( N = 7 \)) vs 2.30 ± 0.13 (\( N = 7 \)) mol \( O_2 \) mol⁻¹ haemoglobin at 15 mmHg]. At pH 7.1 the effects of adrenaline were much more pronounced. The Hill equation for adrenaline-treated cells was:

\[
\log\left[\frac{Y}{(1-Y)}\right] = 1.17 \log P_{O_2} - 1.93, \quad r = 0.985, \quad N = 29,
\]
giving a $P_{50}$ value of 45·1 mmHg, and for control cells the equation was:

$$\log\left[\frac{Y}{(1 - Y)}\right] = 1·16\log P_{O_2} - 2·01, \quad r = 0·982, \quad N = 29,$$

giving a $P_{50}$ value of 53·1 mmHg. Thus, adrenaline-induced changes in the general $O_2$-binding behaviour are minimal at normal blood pH values (7·8–8·2), and certainly much smaller than in trout (cf. Nikinmaa, 1983), in which at an extracellular pH of 7·3–7·4 the $P_{50}$ values of adrenaline-treated and control cells were 82 and 118 mmHg, respectively. This corresponds with the observed difference in the effect of adrenaline on pH. However, the above comparison may not be altogether realistic, since Nikinmaa (1983) used Tris-buffered saline instead of the $CO_2$-$HCO_3^-$ buffered saline used in this study.

**General discussion**

Our results show that the adrenergic response of carp red cells is dependent on both pH and $P_{O_2}$. This observation may explain the lack of adrenergic response in exercised tench (Jensen, 1987) and the $P_{O_2}$ dependence of the hypoxia-induced red cell swelling and the increase in red cell $Na^+$ concentration of carp (Nikinmaa et al. 1987a). The response in carp appears to be quantitatively much smaller than in trout, and at atmospheric $P_{O_2}$ occurs only at non-physiological pH values. Both tench and carp have haemoglobins with relatively high $O_2$ affinities (Weber & Lykkeboe, 1978; Jensen & Weber, 1982). Thus, in normoxic conditions, oxygen loading could be little affected by adrenaline-induced shifts in the $O_2$ dissociation curve. In contrast, in trout and striped bass, such an effect appears quite important (Nikinmaa, Cech & McEnroe, 1984; Primmett et al. 1986; Milligan & Wood, 1986). In both cases, however, the effect at atmospheric $P_{O_2}$ may be associated with the onset of the Root effect.

Carp may utilize the hypoxia-induced shift of the adrenergic response to a higher pH value to some extent in the first hours of acclimation (Nikinmaa et al. 1987a). However, even in this case the effect is small and, during adaptation, is certainly overshadowed by the marked drop in cellular GTP concentrations (Weber & Lykkeboe, 1978).

The cellular mechanisms by which pH and $P_{O_2}$ exert an influence on the adrenergic response are not known. However, Borgese et al. (1987) have shown that the pH-dependent activation of sodium/proton exchange in trout above external pH 7·3 is largely due to a marked effect of intracellular protons. At extracellular pH values below 7·3, the inhibitory effect of external protons starts to predominate over the activation by internal protons and the activity decreases. Also, in trout, deoxygenation of haemoglobin enhances the adrenergic response of red cells (Motais et al. 1987). This effect is not due to $O_2$ lack, since the treatment of cells with carbon monoxide does not enhance the adrenergic response (Motais et al. 1987). Thus, the mechanism by which $O_2$ affects the adrenergic response may be related to the concentrations of the deoxy and oxy forms of haemoglobin within the cell. This possibility is also supported by the present results in which the
adrenergic swelling and the Root effect (i.e. extreme stabilization of the deoxy form of haemoglobin) are closely connected.

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References


