Experimental Panel 15.2 How do more active teleost fish maintain oxygen delivery to their oxidative muscle fibres during exhaustive exercise?

Background

The acidosis that accompanies exhaustive exercise in teleost fish potentially causes a reduction in oxygen affinity of the haemoglobin via the Bohr effect and reduction in the oxygen-carrying capacity of the blood via the Root effect[1]. Both of these effects reduce the amount of oxygen delivered to the oxidative muscle fibres. However, unlike more sluggish species of fish, such as starry flounder (*Platichthys stellatus*), more active species, such as rainbow trout (*Oncorhynchus mykiss*) show little or no change in pH within their red blood cells, so delivery of oxygen to their red muscle fibres is not compromised

The objective of these experiments was to answer the questions:

- What is the physiological basis for the different responses of more active and more sluggish fish to exhaustive exercise?
- In particular, how are the more active species able to maintain oxygen delivery to their oxidative muscle fibres?

Experimental approach

Two series of experiments were performed to answer these questions.

Experiment 1

A small tube, a cannula, was surgically inserted into an artery of rainbow trout and starry flounder. The fish were allowed at least 2-3 days to recover from the surgery, after which they were exercised in large tanks for 6-10 min until they showed signs of exhaustion. Sequential blood samples were then taken from the fish at fixed time intervals, as illustrated in Figure A. A number of variables were then measured, including concentrations of the catecholamines, adrenaline and noradrenaline (also known as epinephrine and norepinephrine, respectively), pH of the blood, intracellular pH (pHi) of the red blood cells and concentration and partial pressure of oxygen in arterial blood (CaO_2 and PaO_2, respectively). Similar data were also obtained from a group of control fish which did not undergo exercise.

Exhaustive exercise causes large increases in the concentrations of circulating catecholamines in rainbow trout, whereas there is no such increase in the starry flounder, as shown in Figure A(i). The significance of this difference is that, although there is a large decrease in arterial pH (pHa) following exhaustive exercise in both species, there is only a prolonged decrease in intracellular pH (pHi) of the red blood cells (RBCs) in the flounder, as shown in Figure A(ii). In contrast, after an initial small drop, pHi of the red cells of the trout did not differ from the pre-exercise value.

The concentration of oxygen in the arterial blood of trout following exhaustive exercise is no different from the pre-exercise value (after an initial dip due to a brief fall in PaO_2), whereas that in the control fish was significantly lower after 2 h, as illustrated in Figure A(ii). At 8 h after the period of exercise, CaO_2 did decline as a result of the repeated blood sampling.

The maintenance of CaO_2 in trout following exhaustive exercise enables the animals to provide sufficient oxygen to the oxidative muscle fibres for them to be able to perform sustainable swimming activity. Catecholamines did not play such a role in the starry flounder although, as shown in Figure A(ii), CaO_2 declined only modestly after 4 h following exhaustive exercise in this species. Consequently, other, as yet unknown, mechanisms must have been involved.

Experiment 2

The involvement of catecholamines in the responses to exhaustive exercise by trout and founder was demonstrated by constructing oxygen equilibrium curves (OEC) on blood pooled from a number of fish of each species. Two batches of blood were equilibrated with either nitrogen, to desaturate the haemoglobin, or air, to saturate the haemoglobin with oxygen. Different proportions of the two batches were then mixed together to give a range of percentage oxygen saturations. Oxygen concentration and partial pressure were then measured in each mixture.

The results of these experiments are shown in Figure B(i). The blood was equilibrated with one of two different partial pressures of carbon dioxide (PCO_2). The higher PCO_2 acidified the blood. Catecholamines were added to half of the blood from each species to simulate the levels present at the end of exhaustive exercise. At normal levels of catecholamines in the blood, acidification led to both a Bohr effect (reduction in affinity of the haemoglobin for oxygen—rightward shift of the OEC) and a Root effect (reduction in oxygen concentration in the blood), in both species. However, elevated levels of catecholamines abolished these responses to acidification in trout blood, but not in flounder blood.

The mechanism by which catecholamines maintain pHi in trout is shown in Figure B(ii). By using drugs such as amilioride which inhibits Na^+/H^+ exchange, it has been demonstrated that catecholamines act via β-adrenergic receptors in the cell membranes of the RBCs and stimulate a unique Na^+/H^+ exchanger. This exchanger causes extrusion of protons from the red cells and influxes of Na^+ and water, which cause the cells to swell. In addition to causing swelling of the RBCs, the catecholamines also cause a reduction in the synthesis of the allosteric organic phosphate in fish, ATP[2] and/or an increase in its degradation. Both of these processes lead to a reduction in the concentration of ATP in the red cells.

Although the adrenergic stimulation of the Na^+/H^+ exchanger has been observed in other species of fish, it is generally relatively large in salmonids and more modest in cyprinids.

Overall findings

Catehcolamines maintain pHi of the red cells of active fish following exhaustive exercise which means there is no Bohr or Root effect on the haemoglobin. Consequently, the concentration of oxygen in the arterial blood (CaO_2) is maintained. Also, a reduction in the concentration of ATP leads to an increase in the affinity of the haemoglobin for oxygen. Catecholamines do not have similar effects on red cells from the flounder, so the Bohr and Root effects are still present in these animals at the end of a period of exhaustive exercise although there is only a modest decrease in CaO_2.

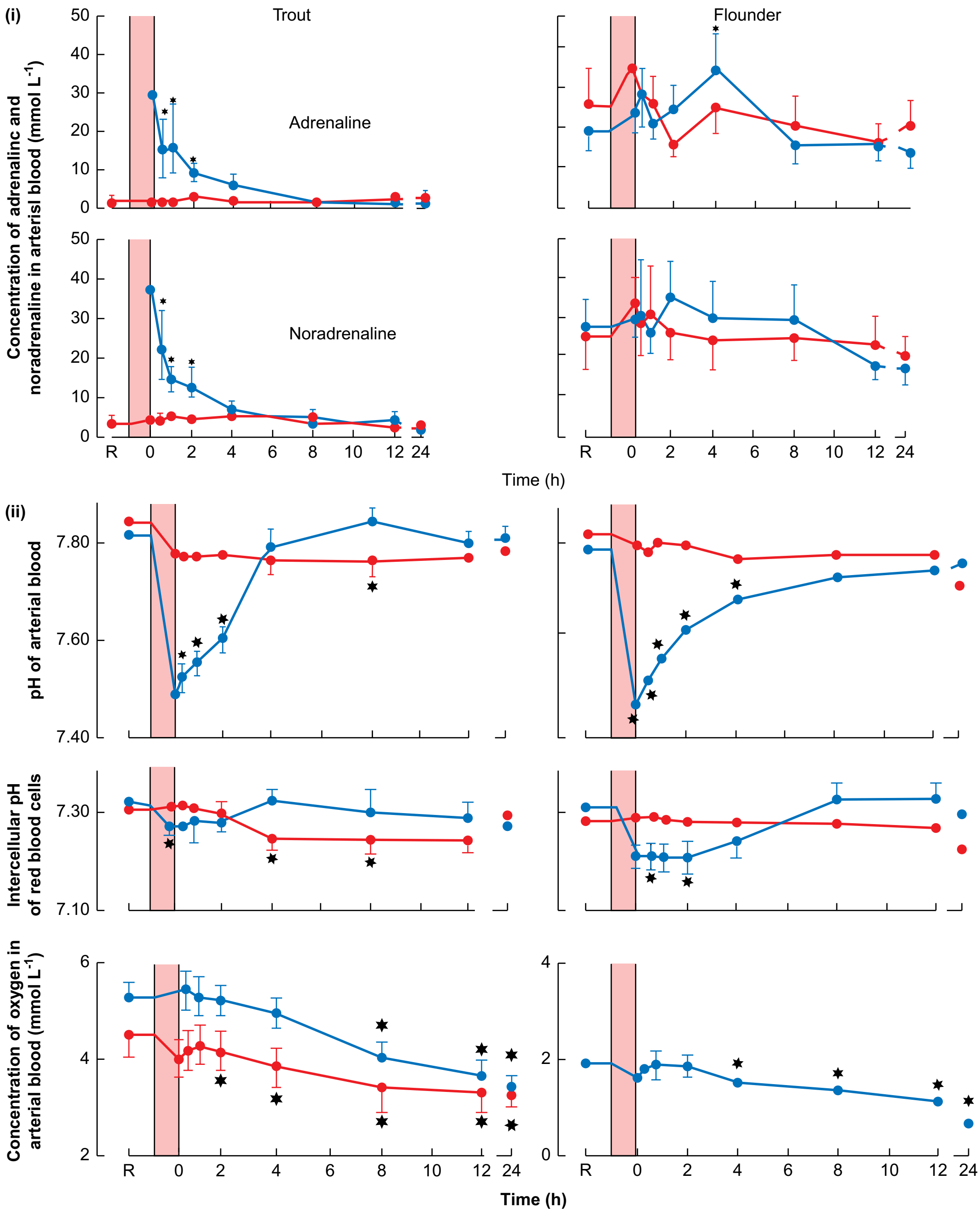

Figure A The effect of exhaustive exercise on release of catecholamines and blood oxygen transport properties of rainbow trout (*Oncorhynchus mykiss*) and starry flounder (*Platichthys stellatus*).

For all panels, exhaustive exercise occurred during the period denoted by the shaded bar, which was 6 min for trout and 10 min for flounder, R, rest; 0 immediately after exercise. ● control group (trout, n = 7; flounder, n = 8); ● exercise group (trout and flounder, n = 8). Asterisk indicates a significant difference from the corresponding value at rest. Values are mean ± SEM.

(i) Levels of the catecholamines adrenaline (epinephrine) and noradrenaline (norepinephrine) in arterial blood.

(ii) pH in arterial blood, intracellular pH of red blood cells and concentration of oxygen in arterial blood

Reproduced from: Milligan CL and Wood CM (1987). Regulation of blood oxygen transport and red cell pH_i after exhaustive activity in rainbow trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). Journal of Experimental Biology133, 263–282.

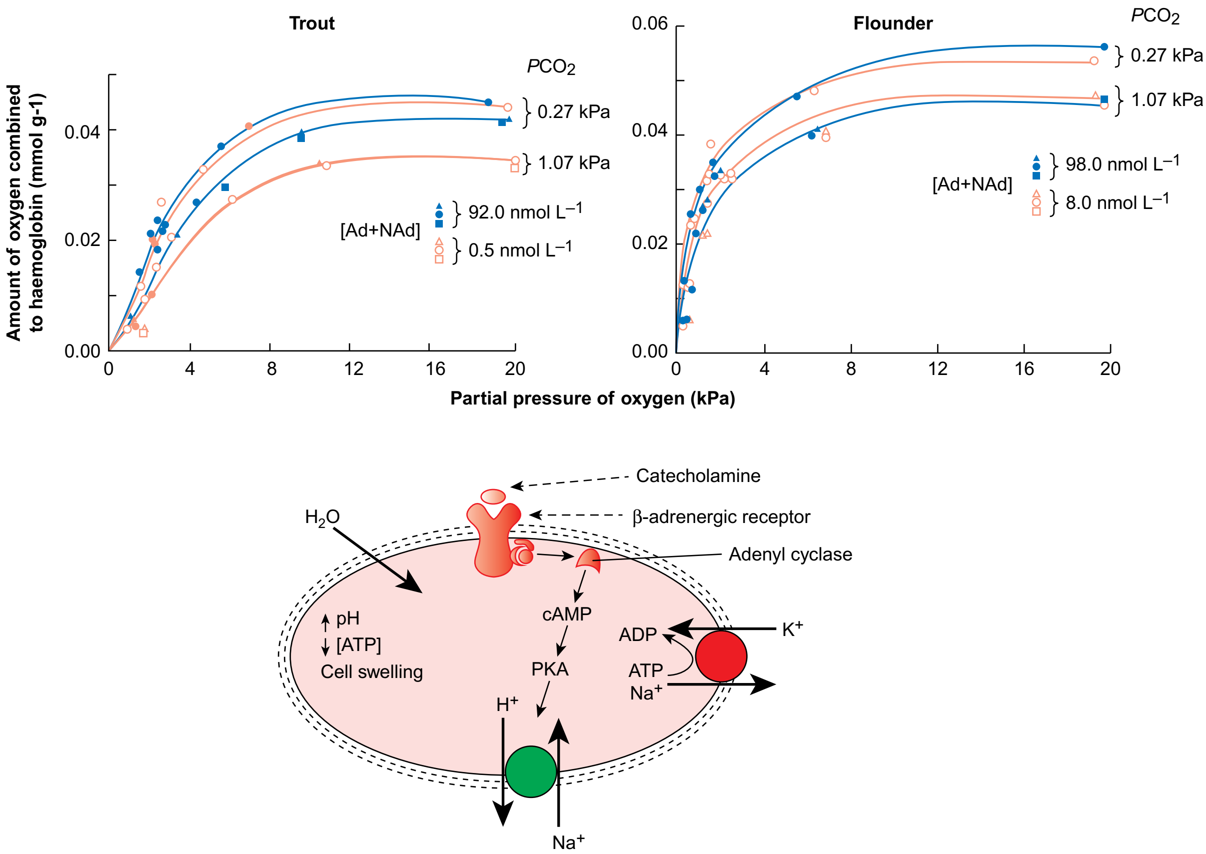

Figure B Influence of catecholamines on the response of the oxygen equilibrium curves (OEC) of rainbow trout (Oncorhynchus mykiss) and starry flounder (Platichthys stellatus) to acidification.

(i) Increase in PCO_2 causes acidification of the blood and, at low levels of catecholamines, both trout and flounder show a rightward shift of their OEC (Bohr effect) and a decrease in the amount of oxygen in the blood at a given partial pressure of oxygen (Root effect). However, in the presence of catecholamines at similar concentrations to those present after exhaustive exercise, the OEC of flounder still exhibits Bohr and Root effects, whereas the OEC of trout does not. Different shaped symbols indicate 3 separate experiments.

(ii) Diagram showing how catecholamines influence intracellular pH (pHi) in the red blood cells of some species of teleosts such as rainbow trout. Catecholamines bind to β-adrenergic receptors and activate adenyl cyclase which causes an increase concentration of cAMP. cAMP activates Na^+/H^+ exchange via protein kinase A (PKA). There is then a net efflux of H^+ and, therefore, an increase in pHi. The increased intracellular concentration of Na^+ causes the osmotic inflow of water and stimulates $Na^+ – K^+$ exchange leading to a decrease in intracellular concentration of ATP [ATP].

(i) Modified from Milligan CL and Wood CM (1987). Regulation of blood oxygen transport and red cell pH_i after exhaustive activity in rainbow trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). Journal of Experimental Biology 133, 263–282. (ii) reproduced from Perry SF and Gilmour KM (2010). Oxygen transport in water breathers. In: Nilsson G (ed) Respiratory Physiology of Vertebrates. pp 49–94 Cambridge University Press, Cambridge.

Find out more:

Milligan CL and Wood CM (1987). Regulation of blood oxygen transport and red cell pH_i after exhaustive activity in rainbow trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). Journal of Experimental Biology 133, 263–282.

Nikinmaa M (1990). Vertebrate Red Blood Cells. Adaptations of Function to Respiratory Requirement. 262 pp. Springer-Verlag, Berlin.

Nikinmaa M and Huestis WH (1984). Adrenergic swelling of nucleated erythrocytes: cellular mechanisms in a bird, domestic goose, and two teleosts, striped bass and rainbow trout. Journal of Experimental Biology 113, 215–224.

Perry SF and Gilmour KM (2010). Oxygen uptake and transport in water breathers. In: Nilsson GE (ed). Respiratory Physiology of Vertebrate Life with and without Oxygen. pp 49–94. Cambridge University Press.

1 The Bohr and Root effects are discussed in section 13.1.2

2 Section 13.1.2 discusses details of the influence of organic phosphates on the binding of oxygen to haemoglobin in vertebrates