EXTENSIVE ERYTHROCYTE DEFORMATION IN FISH GILLS OBSERVED BY IN VIVO MICROSCOPY: APPARENT ADAPTATIONS FOR ENHANCING OXYGEN UPTAKE

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Summary

The secondary lamellae of the gills are the primary sites of oxygen uptake in fish. We have used epi-illumination microscopy in vivo to observe the microcirculation directly in secondary lamellae of rainbow trout (Oncorhynchus mykiss) and roach (Rutilus rutilus).

Our observations point at previously unrecognised factors in fish respiratory physiology. Erythrocytes passing through secondary lamellae became greatly deformed. We propose that this deformation plays a role in oxygen uptake by diminishing the diffusion boundary layer (adhesion layer) of fluid around the erythrocyte and possibly also by mixing intracellular haemoglobin molecules. Moreover, the erythrocytes were apparently slowed down and forced to travel over an extended path to pass through the lamella, making it tempting to speculate that plasma passes more readily than erythrocytes through the lamella, resulting in a local elevation of the haematocrit.

Key words: blood rheology, gas exchange, lamellae, Oncorhynchus mykiss, rainbow trout, roach, Rutilus rutilus, secondary circulation.

Introduction

The secondary lamellae make up most of the surface area of the gills and are the primary sites of oxygen uptake in fish. A secondary lamella is a vascular sheet that has the appearance of a pillared hall, where the pillars are made up of specialised cells, appropriately called pillar cells (Farrell et al. 1980; Laurent, 1984). Our present knowledge of respiratory mechanisms in fish gills is mainly derived from histological studies and physiological measurements of flow variables (Houlihan et al. 1982; Satchell, 1991). In addition, there have been two in vivo studies of lamellar flow rates using laser-Doppler flow measurements (Hughes et al. 1981) and digital radiographic imaging (Bhargava et al. 1992).

However, we have been unable to find any published record of direct visual observations of lamellar blood flow in vivo or any descriptions of the appearance of erythrocytes in vivo when passing through the lamellae.

In the present study, we have used epi-illumination microscopy in vivo to observe the microcirculation directly in secondary lamellae of rainbow trout [Oncorhynchus mykiss (Walbaum), family Salmonidae] and roach (Rutilus rutilus L., family Cyprinidae), two distantly related teleosts.

Materials and methods

Animals

The rainbow trout used for the study weighed 540–590 g. They were obtained from a local fish dealer near Uppsala, Sweden, and kept indoors in tanks (50 fish per tank) containing 2000 l of aerated Uppsala tap water (10 °C). The water was continuously exchanged at a rate of 3 l min⁻¹. The roach studied weighed 89 g. Roach were caught in a pond near Uppsala. They were kept indoors in a 300 l tank (20 fish per tank) continuously supplied (2 l min⁻¹) with aerated water (10 °C). Both species were fed daily with commercial trout food (Ewos, Sweden). The artificial light automatically followed the light/dark cycle of latitude 54 °N. All experiments were carried out between 09:00 and 17:00 h in March after at least 6 months of acclimation to the indoor conditions.

Experimental design

The fish were anaesthetised with an intraperitoneal injection of Mebumal (pentobarbital, 45 mg kg⁻¹) and immobilised with Alloferin (alcuronium, 6 mg kg⁻¹). Half of the right operculum was removed and the fish was placed on its side in a rectangular acrylic box equipped with a stand-pipe adjusted so that the respiratory water (aerated Uppsala tap water, 10 °C, 500 ml min⁻¹) rose to a level that covered the fish. This box
Erythrocyte deformation in fish gills was mounted on the stage of a Leitz Ortholux microscope equipped with a Leitz Ultropak epi-illuminator and a water immersion objective (22× magnification). See Nilsson et al. (1994) for further details of the microscope. Background light was obtained by placing a piece of reflecting aluminium foil below the gill filament to be observed. This greatly increased the light level and contrast of the picture. All observations were made on the distal quarter of the filaments of the second gill arch. Micrographs were obtained with a Sony 3-CCD video camera (720 lines resolution) with a 10× ocular and recorded on a professional Sony Betacam SP video recorder. Video frames were imported into the program Adobe Photo Shop on a Macintosh Quadra 800 equipped with a Falcon video capture card (Graphics Unlimited, Cambridge, UK) for enhancing

Fig. 1. Sequence of micrographs showing the passage of erythrocytes through the efferent portion of a secondary lamella of a rainbow trout (weighing 540 g). Approximately one-fifth of the total length of the lamella was available for observation. Erythrocytes are seen as dark elongated sausage-like objects. The colour micrograph shows an overview, in which it can be seen that the erythrocytes that are squeezed between the pillar cells tend to form a honeycomb pattern. Numbers show the time (in s) that has passed from the first frame. The main flow direction is from right to left. Three erythrocytes have been outlined with different colours. Note the deformation of the erythrocytes and how they affect each other’s passage way through the lamella by temporarily blocking available passages (e.g. at 1.48 s). After 2.52 s, the erythrocyte marked with red reaches the marginal channel/efferent lamellar arteriole (seen as a dark curved band). Scale bar, 100 μm.

Fig. 2. Sequence of micrographs showing the passage of erythrocytes through the efferent portion of a secondary lamella of a roach. Note that, after 0.80 s, the erythrocyte marked with green temporarily blocks the passage for the erythrocyte marked with red, whereupon the latter appears to be affected by an erratic flow during the following 0.48 s, causing it to deform and to make short movements in different directions. The erythrocyte marked with green reaches the marginal channel/efferent lamellar arteriole just before 1.28 s. Scale bar, 100 μm.
Results

The in vivo microscopic observations of secondary lamellae of rainbow trout and roach show that the erythrocytes are forced by the blood flow to become greatly deformed when passing between the pillar cells, acquiring G- or S-shapes, and sometimes even Y-shapes (Figs 1, 2), thus taking on a worm-like appearance and pattern of movement.

At least two factors seem to contribute to these striking rheological features of the erythrocytes in the lamella. First, the placing of the pillar cells is such that few straight passages are available through the lamella. This is revealed by the honeycomb-like pattern formed by erythrocytes moving through the lamellae (Fig. 1). Second, perhaps most importantly, it was apparent from our observations that erythrocytes affect the routes of neighbouring erythrocytes by constantly changing local flow directions when they temporarily become squeezed between pillar cells and thus block local flows (shown in the micrograph sequences in Figs 1, 2). Each erythrocyte is guided by numerous irregular changes in the flow direction during a passage through the lamella, rather than experiencing a steady direction of flow. Indeed, several times we observed that erythrocytes even reversed their course and travelled for a short distance against the prevailing direction of movement. The distance that an erythrocyte travelled to get from one point to another was 49.2±9.6% (trout) or 50.0±14.7% (roach) longer than the shortest distance between these points (mean ± s.d. from 10 measurements from five lamellae of two trout or one roach on a stretch of 68–93 μm) (Fig. 3). Hence, rainbow trout and roach lamellae appeared very similar with respect to the increase in distance that the erythrocytes had to travel.

The erythrocytes became more elongated when squeezed between pillar cells and, in this respect also, rainbow trout and roach were very similar. Inside the efferent arterioles and arteries, the erythrocytes of both fish species were oval-shaped, the maximum and minimum diameters being 13.3±1.2 and 8.3±0.9 μm for trout and 13.6±1.9 and 8.4±1.7 μm for roach (mean ± s.d., N=15 erythrocytes in two rainbow trout and one roach). The thickness of the erythrocytes was 3.9±0.3 μm (trout, N=24 erythrocytes in two fish) or 3.9±0.4 μm (roach, N=11 erythrocytes in one fish). During their passage through the lamella, the mean erythrocyte length was increased to 18.4±2.1 μm for trout (N=15 erythrocytes from two fish) and to 18.1±2.7 μm for roach (N=15 erythrocytes from one fish). These values are significantly greater than the maximum erythrocyte lengths (diameters) in arteries/arterioles (Student’s t-test, P<0.001 for both species). At the same time, the thickness of the erythrocytes, as seen from above (i.e. 90° from the plane of the lamella) was 3.6±0.5 μm (trout) or 3.7±0.6 μm (roach). This suggests that the erythrocytes were on edge when passing through the lamella, an observation that is also supported by previous histological observations of fish secondary lamellae (Soivio and Tuurala, 1981; Laurent, 1984).

Discussion

Erythrocyte deformation has previously been studied in the mammalian microcirculation, where it has been shown to play an important role in oxygen uptake and release to tissues by diminishing the diffusion boundary layer of fluid (also referred to as the stagnant layer or adhesion layer) around the erythrocyte and possibly also by causing a mixing of intracellular haemoglobin molecules (Maeda and Shiga, 1994). That the diffusion boundary layer retards oxygen uptake is supported by convincing experimental evidence (Huxley and Kutchai, 1981; Hook et al. 1988). With regard to intracellular haemoglobin mixing, less evidence seems to be available, and it is possible that this phenomenon is of minor quantitative importance since very high oxygenation rates have been measured in experiments with human or eel erythrocytes kept completely at rest (Heidelberger and Reeves, 1990; Pelster et al. 1992).

We suggest that erythrocyte deformation plays a similar role for oxygen uptake in fish gill secondary lamellae. The present observations clearly reveal a high degree of deformability in fish erythrocytes. It has been suggested that the biconcave disc shape of mammalian erythrocytes is important for their deformability (Maeda and Shiga, 1994). Fish erythrocytes, in contrast to mammalian erythrocytes, are nucleated and are
therefore slightly biconvex rather than biconcave. However, their shape and the presence of a nucleus do not stop them from undergoing extensive deformation. In fact, a study of the time required for fish erythrocytes (yellowtail, *Seriola quinqueradiata*) to pass a 5 μm Nucleopore filter, a measure thought to be highly dependent on deformability, has indicated that these red cells are at least as deformable as human erythrocytes, despite having a 30% greater cell volume (Hughes et al. 1982). Similar filter passage times were obtained in a subsequent study of rainbow trout erythrocytes (Hughes and Kikuchi, 1984).

Although outside the scope of the present study, we noted that the flow through the lamellae varied both spatially and temporally. Thus, some lamellae could at times show a complete stop in flow, or flow only through the marginal channel, while high flow rates were simultaneously seen in others. Moreover, we often noted whole filaments without channel, while high flow rates were simultaneously seen in complete stop in flow, or flow only through the marginal temporally. Thus, some lamellae could at times show that the flow through the lamellae varied both spatially and temporally.

These are the first *in vivo* observations of microcirculation in fish gills. In the literature, we have only been able to find one statement which relates to the phenomena presently described: when pressing on freshly excised trout gill filaments and observing the lamellae under a microscope, Wood (1974) noted that the erythrocytes were undergoing 'squeeze'.

To conclude, we believe that the present observations point to three previously unrecognised factors in fish respiratory physiology. The extensive deformation of fish erythrocytes when passing through the lamellae will, first, counteract the formation of a diffusion boundary layer around the erythrocyte and, second, cause mixing of intracellular haemoglobin molecules, two factors that should decrease the distance that oxygen has to diffuse to reach haemoglobin. The importance of the first of these factors for oxygen uptake is well documented in the mammalian literature. Third, apparently as a result of the placing of the pillar cells and the narrow passages they create, erythrocytes temporarily block these passages, causing neighbouring erythrocytes to experience constantly changing flow directions, thereby slowing them down and extending the distance that they have to move through the lamella. It is tempting to speculate that this phenomenon also plays a role in oxygen uptake, since it may cause the erythrocytes to pass through the lamella at a lower speed than the blood plasma, resulting in a local increase in intralamellar haematocrit.

During lamellar recruitment, a consequence of a possible retardation of erythrocytes in the lamellae would be an initial temporary fall in efferent filamental artery haematocrit. In such a situation, the non-respiratory secondary blood vessel system of the gills might play a role in maintaining the haematocrit of outgoing arterial blood. These secondary vessels are known to perform plasma skimming of the respiratory vessels and have been shown to attach to the efferent filamental arteries through ‘arteriovenous anastomoses’. A function of the secondary vessels in regulating haematocrit has been hypothesised previously (Soivio and Hughes, 1978) and is one of several functions suggested for this non-respiratory blood vessel system (see Olson, 1991, for a review).

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References


