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RAPID RESPIRATORY CHANGES IN TROUT RED BLOOD CELLS DURING Na^+/H^+ EXCHANGE ACTIVATION

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Summary

Respiratory variables of rainbow trout (*Oncorhynchus mykiss*) blood were monitored continuously *in vivo* using an extracorporeal circulation or *in vitro* using blood flowing in a semi-closed loop. The experiments were designed to assess the rapid effects of endogenous catecholamines on red blood cell (RBC) O_2 and CO_2 transport. The addition of catecholamines (nominal final blood concentrations were 250nmol l^{-1} adrenaline and 20nmol l^{-1} noradrenaline) caused activation of RBC Na^+/H^+ exchange both *in vivo* and *in vitro* as indicated by the reductions in whole-blood pH.

In both experimental systems, the activation of Na^+/H^+ exchange was associated with a rapid reduction of blood P_{CO_2}, indicating a sudden net movement of plasma CO_2 into the RBC. *In vitro* the initial reduction of blood P_{CO_2} was followed by a pronounced elevation as a result of the titration of plasma HCO_3^- by H^+ extruded from the RBC. Blood P_{O_2} fell markedly in a transitory manner after the addition of catecholamines. The decreases in P_{O_2} were probably caused by rapid increases in the affinity/capacity of haemoglobin for O_2 which, in turn, caused O_2 molecules to enter the RBC from the plasma. The results are discussed with reference to the role of circulating catecholamines in rapidly modifying blood O_2 and CO_2 transport in rainbow trout.

Introduction

In rainbow trout [*Oncorhynchus mykiss* (Walbaum)] and several other teleost species, the elevation of catecholamine levels in the blood is known to activate a Na^+/H^+ exchanger in the red blood cell (RBC) membrane both *in vitro* after addition of exogenous catecholamines (Baroin *et al.* 1984; Nikinmaa and Huestis, 1984; Cossins and

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Richardson, 1985) and *in vivo* after release of endogenous catecholamines (Thomas *et al.* 1986; Fievet *et al.* 1987; Perry and Thomas, 1991). The activation of the Na^+/H^+ exchanger elicits the immediate dissociation of RBC buffers and an abrupt extrusion of protons. The extruded protons combine with extracellular HCO_3^- to form H_2CO_3, the subsequent dehydration of which produces CO_2 at the uncatalysed rate in the plasma. Owing to the maximal activity of the Na^+/H^+ exchanger during the first minutes, an acid–base disequilibrium develops as H^+ accumulates in the plasma (Motais *et al.* 1989). It has been shown (Motais *et al.* 1989) that, after the initial abrupt phase of extracellular disequilibrium, the Na^+/H^+ exchange activity is sustained by a process of 'CO_2 recycling' between the plasma and the RBC.

The pathways or events responsible for CO_2 re-entry into the RBC have not been elucidated. Thus, a primary goal of the present study was to provide experimental evidence of this phenomenon and to observe its consequences on the respiratory status of the blood. In the normal process of respiration, CO_2 molecules diffuse according to P_{CO_2} gradients and thus it is likely that activation of RBC Na^+/H^+ exchange causes a temporary inwardly directed P_{CO_2} gradient between the plasma and RBC. The inwardly directed gradient could result from a transient and rapid decrease in P_{CO_2} inside the RBC or an increase outside. Owing to the ease of diffusion of CO_2 molecules between the intra- and extracellular compartments, any variation in either compartment would tend to be immediately followed by a similar variation in the other. This study continuously monitored extracellular P_{CO_2}, before and during activation of Na^+/H^+ exchange. CO_2 recycling, if it occurs, should be associated with a rapid reduction of intracellular P_{CO_2} and then with a reduction of extracellular P_{CO_2} as CO_2 molecules enter the RBC by diffusion. The immediate and short-term reduction in P_{CO_2} is likely to have a dramatic effect on haemoglobin-O_2 binding. This possibility was assessed by continuous monitoring of blood P_{O_2}.

The present investigation deals only with the respiratory consequences of the acid–base disequilibrium during the activation of the Na^+/H^+ exchanger which lasts approximately 10–20min after a single bolus injection of catecholamine. This is in contrast to previous studies that have focused almost exclusively on the consequences of the final equilibrium corresponding to a new acid–base steady state.

Materials and methods

Experimental animals

Rainbow trout (*Oncorhynchus mykiss*) of either sex weighing between 700 and 950 g were obtained from Linwood Acres Trout Farms (Campbellcroft, Ontario). Fish were held indoors in large fibreglass tanks supplied with flowing dechlorinated city of Ottawa tapwater ($[Na^+]$=0.12mmol l^{-1}; $[Cl^-]$=0.15mmol l^{-1}; $[Ca^{2+}]$=0.35–0.40mmol l^{-1}; $[K^+]$=0.03mmol l^{-1}; pH7.5–8.0). The temperature of the holding and experimental waters was 12–13˚C, photoperiod was kept at 12h:12h light:dark. Fish were fed daily with a commercial salmonid diet (Martin Feed Mills Inc.) but were not fed for 48h before experimentation.

*Animal preparation for blood sampling (*in vitro *experiments)*

Fish were anaesthetized in a solution of 1:10000 (w/v) ethyl-*m*-aminobenzoate (MS 222; adjusted to pH7.5 with $NaHCO_3$; gassed with oxygen to achieve a final Pw_{O_2} of 67–80kPa) and placed onto an operating table that permitted continuous irrigation of the gills with the same solution. An indwelling cannula (Clay Adams PE 50 polyethylene tubing; internal diameter 0.580mm, outer diameter 0.965mm) was implanted into the dorsal aorta (Soivio *et al.* 1975). After surgery, fish were placed into individual opaque acrylic boxes supplied with aerated flowing water, where they were allowed to recover for 24h prior to sampling. At the end of this period, 5ml of blood was withdrawn slowly (approximately $1 ml min^{-1}$) from the dorsal aortic cannula into a pre-heparinized syringe (0.05ml of $2500 units ml^{-1}$ ammonium heparin). The blood was then transferred to a 50ml round-bottomed tonometer flask, placed on ice, and gassed for 3h with humidified air. The flasks were shaken frequently during this 3h period preceding the experiment.

*Animal preparation for extracorporeal circulation (*in vivo *experiments)*

Fish were anaesthetized (see above) and an indwelling cannula (Clay Adams PE 50 polyethylene tubing) was implanted into the dorsal aorta. Two cannulae (PE 50) were inserted into the coeliac artery in the orthograde and retrograde directions slightly posterior to the pectoral fin (Thomas and Le Ruz, 1982). The cannulae were filled with $140 mmol l^{-1}$ NaCl (containing $10 units ml^{-1}$ ammonium heparin) and heat-sealed. The wound was treated with topical antibiotic (streptomycin) before suturing. After surgery, fish were placed into individual opaque boxes (5l volume) supplied with aerated flowing water, where they were allowed to recover for 24h prior to experimentation.

Protocol for in vitro *experiments*

The blood (5ml) was placed in a tonometer flask (50ml; Eschweiller tonometer), which was immersed in a water bath kept at 12–13°C and gassed continuously with the appropriate humidified gas mixture (normoxic–hypercapnia: 0.9% CO_2, 20% O_2, remainder N_2) supplied by a Wösthoff gas-mixing pump (model M301-A/F). During the experiments, the blood was pumped by the same pump as used for the extracorporeal circuit (see below), at the same flow rate ($0.4 ml min^{-1}$) and passed through the same cuvettes holding P_{O_2}, P_{CO_2} and pH electrodes.

Protocol for in vivo *experiments*

The extracorporeal circuit was established by connecting the two coeliac artery cannulae in series with cuvettes holding P_{O_2}, P_{CO_2} and pH electrodes. Blood flow in the circuit was maintained by a peristaltic pump at $0.4 ml min^{-1}$ (see Thomas and Le Ruz, 1982). The volume of blood contained in the extracorporeal circuit (1.0ml) represented less than 3% of the total blood volume of the fish. The tubing and cuvettes of the external circuit had previously been rinsed with heparinized ($1000 units ml^{-1}$ ammonium heparin) $140 mmol l^{-1}$ NaCl to prevent clotting. This circuit allowed continuous recording of arterial P_{O_2} (Pa_{O_2}), P_{CO_2} (Pa_{CO_2}) and pH (pHa) for as long as 4–5h without interruption. In addition, P_{CO_2} of the inflowing water (Pw_{CO_2}) was continuously monitored by pumping

(with a peristaltic pump) a small volume of water from the inflow tube supplying the fish-holding box through a cuvette containing a P_{CO_2} electrode. After stabilization of the measured blood respiratory variables (usually within 20–30min of commencing the extracorporeal circulation), the fish was exposed to hypercapnia by gassing a water equilibration column supplying the holding box with an appropriate gas mixture of CO_2, N_2 and O_2. A series of preliminary experiments established the necessary flow rates of water and the proportions of the three gases to achieve a final Pw_{CO_2} of 0.73–0.80kPa (5.5–6.0mmHg) within 5min.

Experimental protocol

The same protocol was used for *in vivo* and *in vitro* experiments. The blood respiratory/acid–base variables (P_{O_2}, P_{CO_2}, pHe) were recorded continuously for 1 h under conditions of normoxic hypercapnia. Then, each fish (*in vivo* experiments) or each flask (*in vitro* experiments) was given a bolus injection (0.3ml kg^{-1}) of a catecholamine mixture (2.5×10^{-4} mol l^{-1} adrenaline bitartrate/2.0×10^{-4} mol l^{-1} noradrenaline bitartrate dissolved in Cortland saline, pH7.8) to yield final nominal circulating concentrations of 250nmol l^{-1} adrenaline and 20nmol l^{-1} noradrenaline. The blood respiratory/acid–base variables were recorded for a further 30min. The absolute levels and the proportions of the catecholamines used in the present study were chosen on the basis of our previous studies, which have measured similar levels and ratios of the catecholamines, adrenaline and noradrenaline, in the blood of trout during severe stresses including hypoxia (Thomas *et al.* 1992; Perry and Reid, 1992*a*) or hypercapnia (S. F. Perry and S. Thomas, in preparation).

Numerous previous studies from our own laboratory and from others have clearly shown that injections of saline either *in vitro* (e.g. Reid and Perry, 1991; Perry *et al.* 1991; Perry and Reid, 1992*b*) or *in vivo* (Fritsche *et al.* 1992) are totally without effect in such preparations. Thus, control experiments employing injections of carrier vehicle (saline) were not performed in the present study.

Analytical procedures

Blood pH [pHe (*in vitro*) or pHa (*in vivo*)] was measured using a Metrohm EA120 combination electrode in conjunction with a Radiometer PHM73 acid–base analyzer. Blood P_{O_2} and P_{CO_2} were measured by Radiometer PHM73 analyzers (E5036-E5046 electrodes). The electrodes were calibrated by pumping either saline equilibrated with appropriate gas mixtures (obtained by Wösthoff pumps) or buffer solutions for pH calibration. Measuring cells were kept at the same temperature as the fish by thermostatting water at 12–13 °C. Pw_{CO_2} was continuously monitored from the output of the water equilibration column using a Radiometer P_{CO_2} electrode (housed in a thermostatted cuvette) and PHM73 meter.

Data acquisition

An analog–digital interface (software AD/DATA; P. Thoren, Department of Physiology, University of Göteborg, Sweden), in conjunction with a data acquisition

board (DT2801-DT707, Data Translation Inc.), was used to read P_{O_2}, P_{CO_2} and pH each 20ms and store at regular intervals (every 5s in the present study) the average values calculated from the last 50 measured values. In other words, there were 12 data points per minute, taken at 5s intervals.

The transit time of the blood in the measuring loops was approximately 45s and there was an approximately 10s blood transit time delay between each of the three measurement electrodes. Thus, asynchrony between the three measured variables arising from spatial separation of the electrodes was corrected by deleting the first four data points (data collected over 20s) from the pH traces (the pH electrode was the last electrode in series) and the first two data points (data collected over 10s) from the P_{CO_2} electrode (the P_{CO_2} electrode was the second electrode in series). However, differences in response times of the P_{O_2}, P_{CO_2} and pH electrodes probably prevent absolute synchrony of the continuously recorded blood variables. No attempt was made to correct the continuous traces for electrode response time differences. In addition, time zero does not refer to the time at which catecholamines were introduced in the experimental preparation but to the time at which blood pH started decreasing. This process allowed ‘real-time’ visualization of the immediate, potentially subtle, and transient changes in the respiratory/acid–base status of the blood after addition of exogenous catecholamines.

Results

In vitro experiments were conducted on seven blood samples taken from seven different fish. The addition of catecholamines elicited rapid changes in the three recorded variables (Fig. 1). Whole-blood pH (pHe) decreased from 7.64±0.01 to 7.31±0.03 within 5 min and then gradually returned toward the initial (pre-addition) values, although pHe had still not totally recovered at the conclusion of the recording period. These catecholamine-mediated changes in pHe were indicative of activation of RBC Na^+/H^+ exchange activity (e.g. Thomas *et al.* 1991). P_{CO_2} decreased from 0.90±0.001kPa (6.75±0.01mmHg) to 0.85±0.001kPa (6.41±0.01mmHg) within 2min of the addition of catecholamines. This reduction of P_{CO_2} was followed immediately by a secondary elevation, P_{CO_2} reaching 0.99±0.007kPa (7.45±0.05mmHg) within 10min. Then, owing to the effects of tonometry, P_{CO_2} returned progressively towards the initial equilibration value. The application of catecholamines was associated with an immediate reduction of P_{O_2}, which decreased from 20.0±0.03kPa (150.2±0.2mmHg) to 17.8±0.13kPa (133.7±1.0mmHg) within 2.5min; P_{O_2} returned to its initial value within 10min.

Although the actual levels of catecholamines were not measured in the present study, the results of a previous study (S. F. Perry and S. Thomas, unpublished observations) utilizing a similar protocol showed that adrenaline and noradrenaline levels increased to 225nmol l^{-1} and 60nmol l^{-1}, respectively, 5min after addition and remained constant for 30min thereafter.

The *in vivo* experiments, conducted on eight fish, showed exactly the same sequence of events after injection of catecholamines into the dorsal aorta (Fig. 2) as were seen in the *in vitro* experiments (see above), although the variations had a different amplitude and

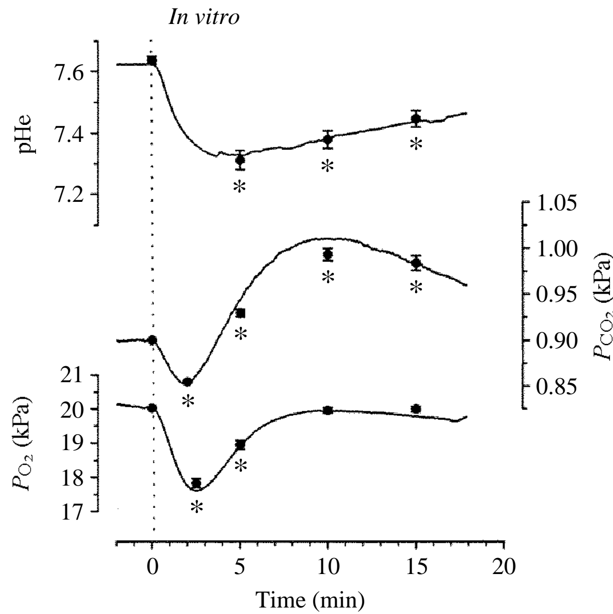

Fig. 1. Temporal changes in whole-blood pH (pHe), P_{CO_2} and P_{O_2} *in vitro* after addition of an adrenaline/noradrenaline mixture (final nominal concentrations 250nmol l^{-1} and 20nmol l^{-1}, respectively). All values shown are means ±1 S.E.M. (N=7). The continuous recordings of P_{O_2}, P_{CO_2} and pHe in each panel are typical examples that effectively illustrate the dynamic nature of the responses. The blood was initially equilibrated and remained in contact with a gas mixture (0.9% CO_2, 20% O_2, remainder N_2) continuously supplied to the tonometer flask, before and after the addition of catecholamines. * indicates a significant difference from the pre-catecholamine value (P<0.05; Fisher's LSD test).

time course. The rate of reduction of arterial pH (pHa) was more rapid (from 7.53±0.03 to 7.19±0.03 within 2min) and complete recovery of pHa was achieved within only 10–15min. P_{CO_2} decreased from 0.93±0.001 to 0.90±0.001kPa within 2min, whilst P_{O_2} was reduced by 2.9kPa (22mmHg) within 2.5min. The secondary elevation of P_{CO_2} was less obvious *in vivo* and there was a greater degree of variability in the data. The continuous recording shown in Fig. 2 clearly illustrates a secondary elevation of Pa_{CO_2} in that particular fish, although the mean values were not statistically elevated.

Using identical protocol, we (S. F. Perry and S. Thomas, unpublished observations) showed that plasma adrenaline and noradrenaline levels increased to 200nmol l^{-1} and 25nmol l^{-1}, respectively, 5min after injection into the dorsal aorta. Unlike the situation *in vitro*, the levels had returned to pre-injection values after 30min.

Discussion

The change in extracellular pH as an index of Na^+/H^+ exchange activity

The transitory reduction of extracellular pH (pHe) after addition of catecholamines to the blood is indicative of the activation of a Na^+/H^+ exchanger in the RBC membrane

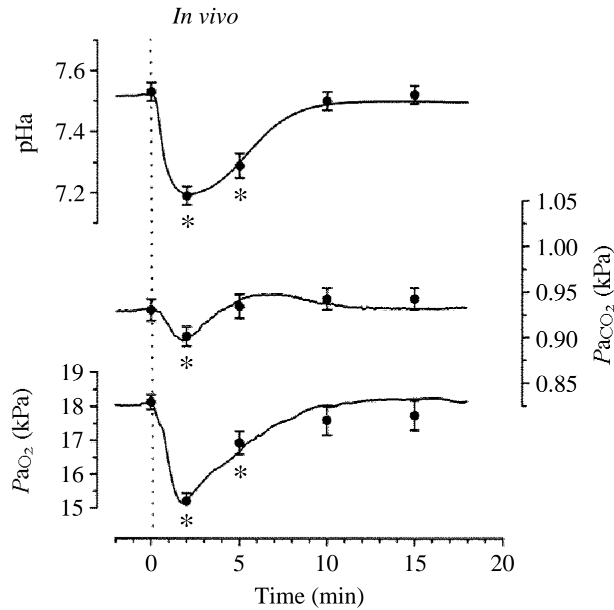

Fig. 2. Temporal changes in arterial blood pH (pHa), P_{CO_2} (Pa_{CO_2}) and P_{O_2} (Pa_{O_2}) after injection of an adrenaline/noradrenaline mixture (final nominal circulating levels 250nmol l^{-1} and 20nmol l^{-1}, respectively). All values shown are means ±1 S.E.M. (N=8). The continuous recordings of Pa_{O_2}, Pa_{CO_2} and pHa in each panel are typical examples that effectively illustrate the dynamic nature of the responses. The fish were breathing hypercapnic water during the experiment. Flow rates of water and proportions of the three gases (CO_2, O_2, N_2) were chosen to achieve a final water P_{CO_2} of 0.73–0.80kPa (5.5–6.0mmHg). * indicates a significant difference from the pre-catecholamine value (P<0.05; Fisher's LSD test).

(Motais *et al.* 1989; Thomas *et al.* 1991). It has previously been shown that the magnitude of the pHe reduction is proportional to the amount of catecholamine added (Tetens *et al.* 1988; Thomas *et al.* 1991). Thus, measuring variations in pHe is a simple method to quantify the extent of adrenergic activation of the RBC Na^+/H^+ exchanger. In the present experiments, both the *in vitro* and the *in vivo* data demonstrated pronounced activation of RBC Na^+/H^+ exchange after addition of catecholamines.

The experimental conditions

The aim of this work was to investigate the *immediate* consequences of the adrenergic activation of RBC Na^+/H^+ exchange on the transport of CO_2 and O_2 by the blood. The specific hypotheses were (i) that blood P_{CO_2} would decrease rapidly after activation of RBC Na^+/H^+ exchange, owing to a transient inwardly directed P_{CO_2} gradient between the RBC and plasma, and (ii) that blood P_{O_2} would rapidly decrease, owing to the entry of O_2 into the RBC as haemoglobin oxygen-affinity/capacity increases. The experimental protocol relied upon continuous measurements of P_{CO_2} and P_{O_2}. In order to control the experimental conditions, it was necessary that the blood should be continuously in contact with a medium of constant P_{CO_2} and P_{O_2}. This was achieved *in vitro* by a

tonometer supplied with a gas mixture of constant composition and *in vivo* by placing the fish in water with a constant Pw_{CO_2} and Pw_{O_2}. In addition, the closed component of our system (the tubing circuit) was designed to detect rapid changes in the intracellular acid–base/respiratory status. Only in such a closed system would changes in the intracellular acid–base/respiratory status be mirrored by changes in the extracellular blood gas status. Of course, if the blood transit time in the tubing were altered, the results would presumably differ. Increasing the rate of flow would probably diminish the magnitude of the responses. Furthermore, absolute values as they exist in the blood of the fish would be distorted during the blood circuit if the variables being measured were not in equilibrium. It must be stressed, however, that the intention of this paper was not to provide absolute values of dorsal aortic blood gases or absolute values as they exist in the tonometry flask nor was it our intention to provide direct evidence of potential adrenergic effects on CO_2 excretion (difficult to assess in a semi-closed system). Instead, the goal was to provide experimental evidence for rapid intracellular acid–base changes during RBC Na^+/H^+ exchange activation, an idea clearly supported by the present data. Finally, in this study we attribute the effects of catecholamines on P_{CO_2} or P_{O_2} to adrenergic activation of RBC Na^+/H^+ exchange rather than to other effects of catecholamines on RBC function. This assumption is based upon the absence of any changes in blood gases induced by catecholamines after prior administration of propranolol (β-adrenoceptor blocker) or amiloride (an Na^+/H^+ exchange blocker).

The initial reduction in P_{CO_2} *and* P_{O_2} *after addition of catecholamines*

The hypothesis of a temporary reduction in blood P_{CO_2} after adrenergic activation of RBC Na^+/H^+ exchange originated from the suggestion of Motais *et al.* (1989) that a portion of the CO_2 molecules produced by uncatalyzed H_2CO_3 dehydration in the plasma re-enter the RBC, are rapidly hydrated in the presence of carbonic anhydrase to form H^+ and HCO_3^- and thus sustain the activation of the Na^+/H^+ and Cl^-/HCO_3^- exchangers. Our present observation of a rapid reduction in the P_{CO_2} of the blood (Fig. 1) provides evidence for a pronounced and temporary disappearance of CO_2 molecules from the plasma and their appearance in the RBC. In theory, this phenomenon can be explained by a rapid left shift of the intracellular equilibrium $H^+ + HCO_3^- \rightleftharpoons CO_2 + H_2O$, occurring at the catalysed rate, which is a direct result of the massive extrusion of H^+ *via* Na^+/H^+ exchange. Thus, P_{CO_2} within the RBC must decrease and allow CO_2 to enter the RBC by diffusion. Owing to the long response time of the P_{CO_2} electrode at 12˚C, it can reasonably be assumed that the true P_{CO_2} reduction in the plasma was much larger than was actually measured.

In theory, during this rapid phase of intracellular adjustment, RBC intracellular pH (pHi) must rise and, owing to Root and Bohr effects (depending on the initial degree of oxygenation), the oxygen-carrying capacity or affinity of haemoglobin must increase accordingly. Again, the results (Figs 1 and 2) demonstrate very clearly that the entry of O_2 molecules into the RBC was such that P_{O_2} decreased by approximately 2.7–4.0kPa (20–30mmHg) after the stimulation of RBC Na^+/H^+ exchange both *in vivo* and *in vitro*. In the same way as for P_{CO_2} electrodes, one can assume that the slow response time of the P_{O_2} electrodes has led to an under-estimation of the actual reduction of P_{O_2}.

The *in vitro* experiments were particularly important in demonstrating that the changes in P_{CO_2} or P_{O_2} were not related to changes in gill ventilation, diffusive conductance or cardiac output. Ventilation was not measured in the present experiments, although a previous study employing similar methodology (Perry and Thomas, 1991) found no effect of catecholamine injections on ventilatory frequency or amplitude. The potential involvement of circulating catecholamines in the control of ventilation in fish is currently debated (for opposing views, see reviews by Randall and Taylor, 1991; Perry *et al.* 1992). In any case, any change in gill ventilation or gill diffusive conductance would be expected to cause rapid dissimilar effects on Pa_{O_2} and Pa_{CO_2}, which was not the case in the present study. It is likely that cardiac output increased after injection of catecholamines. Such a response, by reducing the transit time of blood within the gill vasculature, might be expected to affect arterial blood gas tensions, although not in the manner observed in this study.

The secondary rise in P_{CO_2}

Shortly (2min) after addition of catecholamines, P_{CO_2} began to increase. It is likely that the inwardly directed gradient between the plasma and the RBC was of short duration because the activity of the Na^+/H^+ exchanger decreases considerably after 2min (Garcia-Romeu *et al.* 1988) and the continuous supply of CO_2 molecules by tonometry or metabolic production can overwhelm the intracellular deficit. Thus, the enormous quantity of H^+ extruded from the RBC during the initial phase is titrated gradually by plasma HCO_3^- and progressively produces a large amount of CO_2, which is the origin of the secondary elevation of P_{CO_2} that tonometry is unable to clear rapidly. *In vivo*, a secondary elevation of Pa_{CO_2}, although obvious in certain fish (e.g. the continuous recording in Fig. 2), was not evident when analysing the mean data. The attenuation of this response *in vivo* can be attributed to several factors, including (i) a shorter duration of Na^+/H^+ exchange activation, owing to the more rapid metabolic degradation of plasma catecholamines *in vivo,* and (ii) the greater efficiency of the gills at equilibrating CO_2, especially during the hypercapnia-mediated hyperventilation. In contrast to the present study, Perry and Thomas (1991) demonstrated a significant elevation of Pa_{CO_2} in trout after the injection of exogenous catecholamines or the release of endogenous catecholamines. Differences between the two studies may reflect differing ventilation volumes. It has also been demonstrated that intra-arterial infusion of HCl is able to lower blood pH in a similar way to activation of RBC Na^+/H^+ exchange and also to cause a significant elevation of blood P_{CO_2} (Tang *et al.* 1988).

Consequences for blood O_2 *and* CO_2 *transport*

Most previous studies that have investigated adrenergic effects on blood gas transport have focused solely on the physiological benefits of the new equilibrium state on blood O_2 transport. The results of the present study, however, indicate that the sudden extrusion of H^+ from the RBC after adrenergic activation of the Na^+/H^+ exchanger induces a sudden change in the intracellular acid–base status that has important *immediate* consequences on both O_2 and CO_2 transport by the blood. First, the release of H^+ from haemoglobin buffers is triggered by the sudden removal of H^+ from the RBC.

Consequently, the affinity/capacity of haemoglobin O_2-binding is enhanced, which results in an instantaneous reduction in Pa_{O_2} as O_2 molecules enter the RBC. Second, the immediate deficit of H^+ within the cell shifts the CO_2 equilibrium in the direction of net hydration, yielding H^+ and HCO_3^-, which are exchanged for Na^+ and Cl^-, respectively. Thus, P_{CO_2} within the RBC must decrease, allowing CO_2 molecules to diffuse into the RBC as demonstrated by the initial reduction of blood P_{CO_2} reported in this study after addition of catecholamines *in vitro* or *in vivo*.

These observations apply to blood flowing in a closed system. *In vivo*, the blood arriving at the gills is flowing in a closed system prior to entering the lamellar circuit and, indeed, catecholamine levels are initially elevated in the pre-branchial blood owing to their release from chromaffin cells associated with the posterior cardinal vein and anterior kidney (see review by Randall and Perry, 1992). Therefore, we propose that the intracellular acid–base changes immediately following the adrenergic activation of RBC Na^+/H^+ exchange have important beneficial consequences for O_2 loading at the gill. The initial intracellular changes in P_{CO_2} are likely to be transient, but O_2 loading at the gills presumably remains enhanced owing to the cumulative effects of Na^+/H^+ exchange activation on RBC pHi, cell volume and organic phosphate levels (see reviews by Boutilier and Ferguson, 1989; Nikinmaa and Tufts, 1989; Randall, 1990; Thomas and Motais, 1990; Thomas and Perry, 1992).

After the gills, the blood flows in a closed system and during this transit the P_{CO_2} must rise as HCO_3^- is titrated by H^+ originating from the RBCs. This elevation of P_{CO_2} in the systemic circulation will serve to offset the otherwise expected increase in haemoglobin O_2-affinity and thus sustain the delivery of O_2 to the tissues at normal blood P_{O_2} values. This rise in venous P_{CO_2} may partially explain the secondary elevation in arterial P_{CO_2} observed by Perry and Thomas (1991).

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