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Pre- and post-branchial blood respiratory status during acute hypercapnia or hypoxia in rainbow trout, *Oncorhynchus mykiss*

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Abstract. Simultaneous venous (pre-branchial) and arterial (post-branchial) extracorporeal blood circulations were utilized to monitor continuously the rapid and progressive effects of acute environmental hypercapnia (water partial pressure of CO_2 4.8 ± 0.2 torr) or hypoxia (water partial pressure of O_2 25 ± 2 torr) on oxygen and carbon dioxide tensions and pH in the blood of rainbow trout *(Oncorhynchus mykiss)*. During hypercapnia, the CO_2 tension in the arterial blood increased from 1.7 ± 0.1 to 6.2 ± 0.2 torr within 20 min and this was associated with a decrease of arterial extracellular pH from 7.95 ± 0.03 to 7.38 ± 0.03; the acid-base status of the mixed venous blood changed in a similar fashion. The decrease in blood pH in vivo was greater than in blood equilibrated in vitro with a similar CO_2 tension indicating a significant metabolic component to the acidosis in vivo. Under normocapnic conditions, venous blood CO_2 tension was slightly higher than arterial blood CO_2 tension (1.7 ± 0.1 torr). This arterial-venous CO_2 tension difference was abolished or reversed during the initial 25 min of hypercapnia indicating that CO_2 was absorbed from the water during this period. Arterial O_2 tension remained constant during hypercapnia; however, venous blood O_2 tension decreased significantly (from 22.0 ± 2.6 to 9.0 ± 1.0 torr) during the initial 10 min. Hypercapnia elicited the release of catecholamines (adrenaline and noradrenaline) into the blood. The adrenaline concentration increased from 6 ± 3 to 418 ± 141 nmol $\cdot l^{-1}$ within 25 min; noradrenaline concentration increased from 3 ± 0.5 to 50 ± 21 nmol $\cdot l^{-1}$ within 15 min. During hypoxia arterial blood O_2 tension declined progressively from 108.4 ± 9.9 to 12.8 ± 1.7 torr within 30 min. Venous blood O_2 tension initially was stable but then decreased abruptly as catecholamines were released into the circulation. The release of catecholamines occurred concomitantly with a sudden metabolic acidosis in both blood compartments and a rise in CO_2 tension in the mixed venous blood only.

Key words: Acide-base status – Blood gases – Catecholamines – Hypercapnia – Hypoxia – Trout, *Oncorhynchus mykiss*

Introduction

The biochemical and physiological responses of teleost fish to environmental disturbances such as hypoxia (Hughes 1973; Wood 1980; Randall 1982; Thomas and Motais 1990; Fritsche and Nilsson 1992) and hypercapnia (Cameron and Randall 1972; Janssen and Randall 1975; Eddy et al. 1977; Thomas and Le Ruz 1982; Perry et al. 1988; Vermette and Perry 1988; Perry and Kinkead 1989) have been extensively studied. These studies have demonstrated that an array of adaptive cardiovascular, ventilatory, and respiratory responses are elicited during such disturbances which serve to optimize branchial O_2 uptake and blood O_2 transport. Thus, in several teleosts including trout, aerobic metabolism is largely conserved despite the reduced availability of ambient O_2 (hypoxia) or the acidosis-induced (hypercapnia) lowering of blood O_2 carrying capacity (the Root effect). Many of the adaptive physiological responses are triggered by the release of catecholamines from chromaffin tissue (Perry and Wood 1989; Randall 1990; Randall and Perry 1992; Thomas and Perry 1992; Thomas and Motais 1990). In particular, the elevation of circulating catecholamine levels causes an increase in the number of circulating rbc owing to contraction of the spleen (Perry and Kinkead 1989; Wells and Weber 1990) and enhances haemoglobin O_2 (Hb-O_2) binding affinity/capacity by triggering the activation of a β-adrenergic Na^+/H^+ exchanger on the rbc

Abbreviations: CCO_2, plasmatotal carbondioxide; CtO_2, blood oxygen content; PO_2, partial pressure of oxygen; PCO_2, partial pressure of carbon dioxide; PaO_2, arterial blood PO_2; $PaCO_2$, arterial blood PCO_2; $PvCO_2$, venous blood PCO_2; PwO_2, water PO_2; $PwCO_2$, water PCO_2; Hb, haemoglobin; $SHbO_2$, haemoglobin oxygen saturation; HPLC, high-performance liquid chromatography; rbc, red blood cell(s); Hct, haematocrit

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membrane (Nikinmaa 1986; Boutilier and Ferguson 1989; Nikinmaa and Tufts 1989; Motais et al. 1990; Nikinmaa 1992). The extrusion of H^+ by the Na^+/H^+ exchanger causes a relative alkalization of the rbc and the inward movement of Na^+ ultimately leads to a decrease in the levels of intracellular organic phosphates owing to acceleration of the Na^+ pump (Ferguson et al. 1989; Ferguson and Boutilier 1989). The alkalization of the rbc and the lowering of organic phosphate levels enhance Hb O_2 binding. Other catecholamine-mediated responses include an enhancement of branchial O_2 diffusive conductance (Pettersson 1983; Perry et al. 1985), increased glucose availability (Perry et al. 1988; Wright et al. 1989), and ventilatory adjustments (Randall and Taylor 1991; Perry et al. 1992).

Our understanding of the respiratory responses to hypoxia or hypercapnia are based almost exclusively on analyses of arterial (post-branchial) blood. Relatively few studies have evaluated the effects of hypoxia on the venous (pre-branchial) blood compartment (e.g. Holeton and Randall 1967; Saunders and Sutterlin 1971; Peyreaud-Waitzenegger and Soulier 1989; Perry et al. 1991) and to date there are only scant data concerning the effects of hypercapnia on the venous system of teleost fish (Eddy et al. 1977). Further, all previous in vivo assessments of the effects of elevated catecholamines on blood respiratory function have been conducted with arterial blood. The consequences of catecholamine release on the venous blood may be markedly different owing to its relatively long residence time in a closed compartment.

The goal of this study was to simultaneously evaluate the respiratory status of mixed venous and arterial blood of rainbow trout during acute hypoxia or hypercapnia using two independent extracorporeal circulations. Specifically, it was intended to characterize the respiratory status of the blood before and after the stress-induced release of catecholamines into the circulation.

Materials and methods

Experimental animals. Rainbow trout *(Oncorhynchus mykiss)* of either sex weighing between 700 and 950 g were obtained from Linwood Acres Trout Farm (Campbellcroft, Ontario, Canada). Fish were held indoors in large fiberglass tanks supplied with flowing dechlorinated city of Ottawa tapwater ($[Na^+] = 0.12$ nmol $\cdot l^{-1}$; $[Cl^-] = 0.15$ mmol $\cdot l^{-1}$; $[Ca^{2+}] = 0.35$–0.40 mmol $\cdot l^{-1}$; $[K^+] = 0.03$ mmol $\cdot l^{-1}$; pH = 7.5–8.0) for at least 2 weeks before beginning experiments. The temperature of the holding and experimental water varied between 8 and 10 °C (April). Photoperiod was kept at 12 h light: 12 h dark. Fish were fed daily with a commercial salmonid diet but were not fed for 48 h before experimentation.

Animal preparation for extracorporeal circulation. Fish were anaesthetized in a solution of 1:10000 (w/v) ethyl-*m*-aminobenzoate (MS 222; adjusted to pH 7.5 with $NaHCO_3$; gassed with O_2) and placed onto an operating table that permitted continuous irrigation of the gills with the same solution. A small incision (2–3 cm) was made just behind (1 cm) the pectoral fin. The coeliac artery generally was found in contact with the gall bladder. Two indwelling cannulae (Clay Adams PE 50 polyethylene tubing; i.d. = 0.580 mm, o.d. = 0.965 mm) were implanted in the orthograde and retrograde directions according to the basic method of Thomas and Le Ruz (1982). For the venous loop, the mixed venous blood was pumped from the cannulated afferent artery of the third gill arch (right or left), and reinjected into the fish by using a T-connection in the coeliac return cannula. After surgery, fish were placed into individual acrylic boxes that were supplied with aerated flowing water and allowed to recover for 24 h.

Animal preparation for sampling of blood for catecholamine measurements and in vitro experiments. Fish were anaesthetized as above, but surgery was limited to the cannulation of the dorsal aorta. An indwelling cannula (Clay Adams PE 50 polyethylene tubing; i.d. = 0.580 mm, o.d. = 0.965 mm) was implanted according to the technique of Soivio et al. (1975).

Experimental set up. The arterial extracorporeal circuit was established by connecting the two coeliac artery cannuale in series with cuvettes holding PO_2, PCO_2 and pH electrodes. Blood flow in the circuit was maintained by a peristaltic pump at 0.4 ml $\cdot$ min^{-1}. The pre-branchial extracorporeal loop was established by connecting the afferent branchial artery cannula and coeliac artery return cannula in series with a second set of cuvettes also holding PO_2, PCO_2 and pH electrodes, and the blood flow in the circuit was maintained in an identical manner to the arterial one by using the second channel of the same peristaltic pump. The volume of blood contained in the two extracorporeal circuits was identical (1.0 ml) and represented less than 5% of the total blood volume of the fish. The tubing and cuvettes of the external circuits were pre-rinsed with a solution of heparinized (1000 units $\cdot$ ml^{-1} sodium heparin) NaCl (140 mmol $\cdot l^{-1}$) to prevent clotting. In addition, the PCO_2 or PO_2 of the inflowing water ($PwCO_2$, PwO_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 PCO_2 or PO_2 electrode. After stabilization of the measured blood respiratory variables (usually within 20–30 min of commercing the extracorporeal circulations) the fish was exposed to hypercapnia or hypoxia by gassing a water equilibration column supplying the holding box with an appropriate gas mixture (supplied by a Wösthoff gas-mixing pump) of CO_2, N_2 and O_2.

Analytical procedures. Blood pH was measured using a combination electrode in conjunction with a Radiometer PHM73 acid base analyzer. Blood PO_2 and PCO_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.

Blood respiratory/acid-base variables (PO_2, PCO_2, pH) were continuously acquired using customized data acquisition software (AD/DATA; P. Thoren, Department of Physiology, University of Göteborg) in concert with a commercial analog-digital interface (DT2801-DT707, Data Translation). This process allowed real-time detailed visualization of the developments in the plasma acid-base and respiratory variables during the experiments.

Plasma adrenaline and noradrenaline levels were measured on alumina-extracted plasma samples using HPLC in conjunction with electrochemical detection according to the basic method of Woodward (1982).

Experimental series. Series 1 utilized a group of six fish fitted with arterial and venous catheters for the sole purpose of continuously recording blood respiratory variables during hypercapnia without disturbing the animals by repeated blood withdrawal (removal). Series 2 was devoted to catecholamine measurements in the plasma of arterial blood sampled through the dorsal aortic catheter in six fish submitted to the same experimental conditions as for series 1. Series 3 and 4: same as for series 1 and 2 but in fish exposed to hypoxia. Series 5 was an additional group of fish cannulated in the dorsal aorta. The purpose of this series was to establish O_2 dissociation curves. Two curves were constructed at two PCO_2 levels corresponding to normocapnia (2.3 torr) and hypercapnia (6.0 torr).

Determination of O_2 dissociation curves. Blood was withdrawn from the dorsal aortic cannula into heparinized syringes. Samples were divided into 1-ml aliquots and placed into tonometry flasks (Eschweiller tonometer) and kept on ice until required. Blood samples were then equilibrated for 20 min at 10 °C. Appropriate mixtures of O_2, CO_2 and N_2 were delivered to the tonometer flask by cascading two gas mixing pumps (Wösthoff). The blood O_2 content (CtO_2) was determined by the method of Tucker (1967). The dissociation curves were established by plotting the degree of haemoglobin O_2 (HbO_2) saturation ($SHbO_2$) as a function of PO_2. The dissolved fraction of O_2 was calculated using the Bunsen solubility coefficient of Christofforides and Hedley-Whyte (1969) and was subtracted from the measured total CtO_2 values. The blood O_2 capacity $cHbO_2$max was calculated as 0.989[Hb], where 0.989 is a correction coefficient to account for the difference in O_2 capacity determined by the Tucker method and the cyanmethemoglobin method (Tetens and Lykkeboe 1985). Haemoglobin concentration measurements as cyanmethemoglobin were performed in duplicate on 20-µl blood samples using a commercial spectrophotometric assay kit (Sigma). For each PCO_2, five curves were constructed using blood withdrawn from five different fish.

Statistical analysis. All data are presented as means ±1 standard error of the mean (SEM). As indicated above, in series 1 and 3 corresponding to Figs. 1 and 3, the values of PO_2, PCO_2 and pH were continuously monitored and stored on computer. However, for the sake of clarity and ease of statistical analysis, the figures present only the mean values ±1 SEM obtained at times 0, 5, 10, 15, 20, 25, 30 min after the imposition of hypercapnia or hypoxia. The significance of differences relative to control levels within a group ($P<0.05$) was assessed by the paired Student's two-tailed *t*-test.

Results

Hypercapnia

Equilibrating the inflowing water with a normoxic-hypercapnic gas mixture for 30 min resulted in a progressive increase in water PCO_2 reaching 4.8±0.1 torr within 20 min; thereafter $PwCO_2$ remained stable within a very narrow range. $PaCO_2$ increased in a parallel fashion from 1.7±0.1 to 6.2±0.2 torr within 20 min. Under normocapnic conditions, $PvCO_2$ was greater than $PaCO_2$ by 0.5 torr but during the first 25 min of hypercapnia this $PvCO_2$-$PaCO_2$ difference was abolished or reversed. Although the PCO_2 of the water and arterial blood reached equilibrium after 20 min, the PCO_2 of the venous blood was still rising at the termination of the experiment (30 min; Fig. 1B). Predictably, the blood pH decreased as the PCO_2 increased; however, the decrease in the blood pH was markedly larger than in blood equilibrated with similar levels of PCO_2 (6.0 torr) in vitro, and in this case blood pH decreased to 7.58 (Fig. 1C). Thus, the decrease in blood pH in vivo must have resulted from a mixed respiratory/metabolic acidosis.

Despite the obvious reversal of the $PvCO_2$-$PaCO_2$ difference (Fig. 1B), there was no similar reversal of the arterial-venous pH difference (Fig. 1C). Arterial PO_2 did not change throughout the duration of hypercapnia (Fig. 1A). However, venous PO_2 decreased from 22.0±2.6 to 9.0±1.0 torr within 10 min.

Figure 2 shows that catecholamines were released abruptly into the circulation between 5 and 10 min of hypercapnia. The maximal values were obtained at

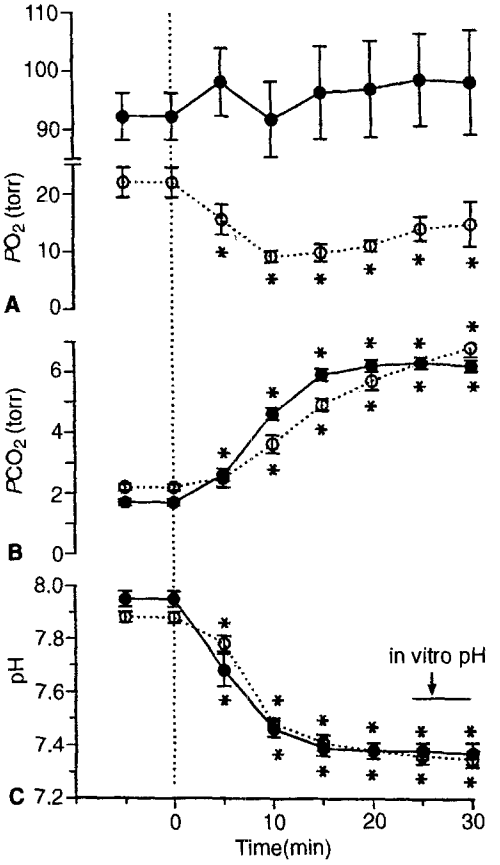

Fig. 1A–C. The temporal changes in **A** PO_2, **B** PCO_2 and **C** pH of rainbow trout blood during acute exposure to hypercapnic water ($PwCO_2$ varying from 0.25 to 4.8 torr within 25 min). Arterial blood: *solid circles, solid line;* venous blood: *open circles, dotted line. Vertical bars* indicate ±1 standard error of the mean (SEM), $n=6$. The *solid line* drawn on the pH panel at 25 min is the pH value obtained from measurements of pH in blood that was submitted in vitro to an identical change in PCO_2. *Asterisks* indicate experimental means significantly different ($P<0.05$) from the mean of the control measurements

$t=25$ min for adrenaline (418±141 nmol · l^{-1}) and at $t=15$ min for noradrenaline (50±21 nmol · l^{-1}).

In series 2, devoted exclusively to blood sampling for assaying the levels of plasma catecholamines in arterial blood, the haemoglobin concentration decreased insignificantly from 5.95±0.27 to 5.32±0.45 g · 100 ml^{-1} after seven successive samplings.

Hypoxia

The effects of acute hypoxia on the PO_2 and PCO_2 of the arterial and mixed venous blood were markedly different (Fig. 3). PaO_2 decreased with the onset of hypoxia reaching 12.8±1.7 torr after 30 min of exposure (Fig. 3A). In contrast, PvO_2 was relatively stable for the initial 10 min of hypoxia (the reduction from 19.9±1.5 to 17.0±0.8 torr was not statistically significant) and then declined abruptly, re-stabilizing at a value of approximately 5 torr after 15 min.

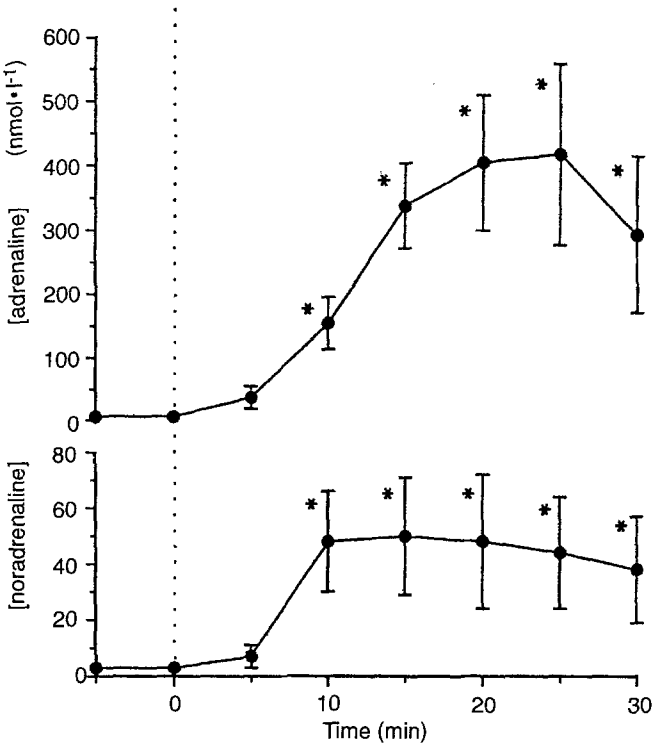

Fig. 2. The effects of external hypercapnia on arterial plasma adrenaline and noradrenaline levels. *Vertical bars* indicate ±1 standard error of the mean (SEM). Where not shown the SEM lies within the symbol. Other details as in Fig. 1. *Asterisks* indicate experimental means significantly different ($P<0.05$) from the mean of the control measurements

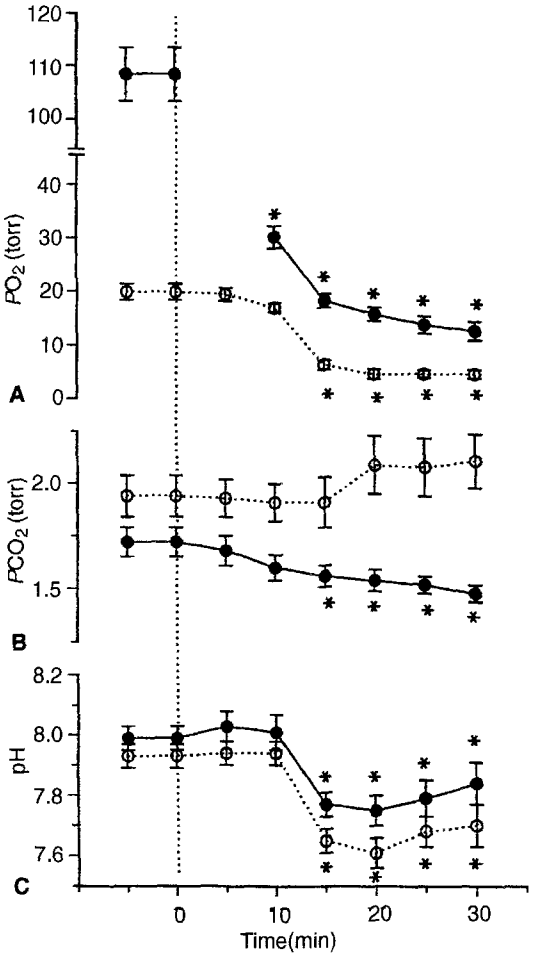

Fig. 3A–C. Temporal changes in arterial (solid circles, solid line) and venous (open circles, dotted line) in **A** PO_2, **B** PCO_2 and **C** pH of trout blood during acute exposure to hypoxic vater (PwO_2 varying from 155 to 35 torr within 25 min). Arterial blood: *solid circles, solid line;* venous blood: *open circles, dotted line. Vertical bars* indicate ±1 standard error of the mean (SEM); $n=6$. *Asterisks* indicate experimental means significantly different ($P<0.05$) from the mean of the control measurements

A clearly visible hyperventilation (based on visual observations) developed during hypoxia and presumably increased the washout of CO_2 and reduced the $PaCO_2$ (from 1.72±0.07 to 1.48±0.04 torr after 30 min). $PvCO_2$ did not change in a similar manner; initially $PvCO_2$ was stable but suddenly increased after 15 min and then restabilized. Although the increase in $PvCO_2$ was not statistically significant, it is nevertheless obvious that the patterns of PCO_2 changes in the arterial and mixed venous blood were dissimilar (Fig. 1B).

Blood pH initially was constant and then fell rapidly in a similar fashion in both blood compartments after 10 min of hypoxia (Fig. 3C). This sudden metabolic acidosis was associated with the release of catecholamines into the circulation (Fig. 4). Further, the release of catecholamines coincided with the sudden reduction of PvO_2 (Fig. 3A) and the delayed rise in $PvCO_2$ (Fig. 3B).

As in the hypercapnia experiment, the impact of the frequent sampling of blood in series 4 on the Hb concentration was minimal owing to the large size of the animals; [Hb] decreased from 5.75±0.20 to 5.17±0.35 g · 100 ml^{-1}.

In vitro O_2 dissociation curves

Figure 5 illustrates in vitro Hb-O_2 dissociation curves established for two blood PCO_2 values (2.25 and 6.00 torr). Thus, the two curves corresponded to the in vivo experimental conditions of normocapnia and hypercapnia. They show the predicted in vivo arterial and mixed venous blood O_2 values under the various experimental conditions. Under normocapnic conditions *(solid symbols)*, the arterial PO_2 of 92.2 torr resulted in 91% Hb O_2 saturation, while the venous PO_2 of 22 torr was associated with approximately 38% saturation. These curves, although empirical, can only estimate in vivo Hb O_2 saturation because they do not take into account the Bohr effect related to the arterial-venous PCO_2 difference nor a variety of other regulatory factors. Thus, the calculation of a decrease in saturation by 53% is an approximation. Regardless, under hypercapnic conditions *(open symbols)*, this analysis predicts a similar arterial-venous Hb-O_2 saturation difference (55%) despite the CO_2-induced Bohr and Root effects. The maintenance of a constant arterial-venous Hb-O_2 saturation difference was associated primarily with the lowered PvO_2 (14.7 torr) and to a lesser extent the slightly increased PaO_2 (98.4 torr).

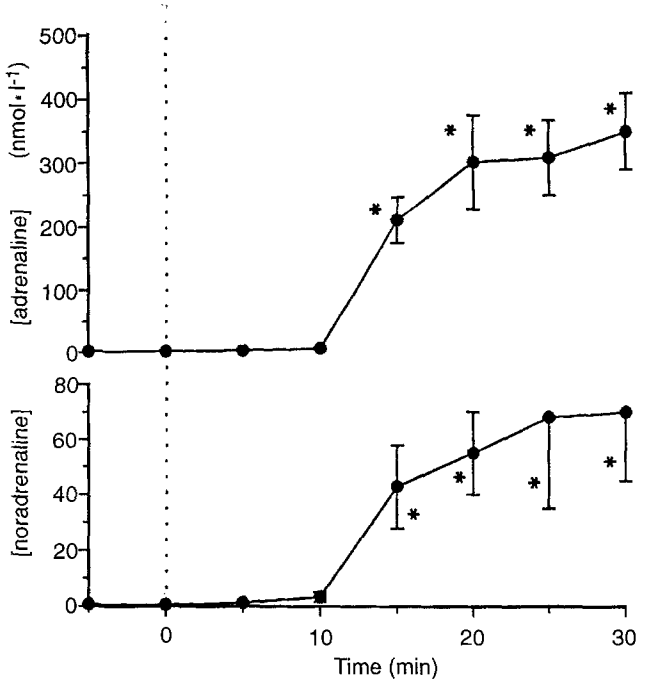

Fig. 4. The effectes of hypoxic exposure on plasma adrenaline and noradrenaline levels. *Vertical bars* indicate ±1 standard error of the mean (SEM). Where not shown the SEM lies within the symbol. Other details as in Fig. 3. *Asterisks* indicate experimental means significantly different ($P<0.05$) from the mean of the control measurements

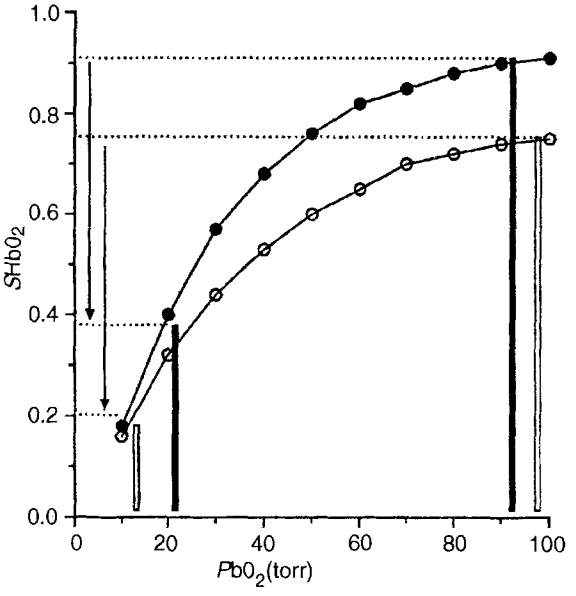

Fig. 5. In vitro Hb-O_2 dissociation curves established at two levels of PCO_2 (normocapnia: 2.25 torr, *solid symbols;* hypercapnia: 6.00 torr, *open symbols*). Each point is the mean of five determinations performed using blood from five different fish. For the sake of clarity, the SEM values are not shown (range 0.01–0.02). The *solid bars* show the degree of saturation in arterial and mixed venous blood during the initial situation of normocapnic normoxia. The *open bars* present the degree of saturation in arterial and mixed venous blood after imposition of the normoxic-hypercapnia. We have not taken into account the Bohr effect related to the arterio-venous PCO_2 difference and thus the *arrows* are only an estimation of the arterio-venous saturation difference

Discussion

The experimental set up

In this study we have utilized a double extracorporeal circulation in rainbow trout in conjunction with periodic blood sampling to assess arterial and venous blood respiratory status as well as the levels of circulating catecholamines. The double loop and the blood sampling were performed on separate groups on fish in order to avoid any interference between the two techniques. The usefulness and validity of a **single** extracorporeal arterial circulation and blood gas measurement technique has been demonstrated and discussed previously (Perry and Thomas 1991; Fievet et al. 1987; Thomas and Le Ruz 1982). In the case of a **double** loop, it is important that the arterial and the venous circuits be as similar as possible in all respects. As an added precaution in the present study, each circuit was used alternatively for arterial blood in one experiment and for venous blood in the following experiment.

Simultaneous measurements of Hct, Hb concentration, blood O_2 content, and plasma HCO_3^- levels would have provided valuable additional information on the mechanisms of blood gas transport during hypercapnia and hypoxia. However, such measurements require periodic blood removal from the extracorporeal loops and it is our experience that such blood removal compromises the measurements of the blood respiratory gases. Thus, we opted not to perform such measurements in the present study.

Respiratory variables before and after catecholamine release

i) Hypercapnia. The simultaneous monitoring of mixed venous and arterial blood provided new and interesting data on the physiological responses of rainbow trout to external hypercapnia. We are aware of one other study (Eddy et al. 1977) which has reported the respiratory characteristics of mixed venous blood, yet the low numbers of animals used in the earlier study (as few as two in some instances), the much higher $PwCO_2$ (15 versus 4.8 torr) and the duration of the hypercapnia (1 week versus 30 min) make comparisons between the two studies difficult.

Despite a constant PaO_2, the exposure of fish to hypercapnia elicited a rapid and significant fall in venous PO_2. The in vitro O_2 dissociation curves revealed that the blood O_2 capacity was reduced by approximately 17% during hypercapnia owing to the Root effect (Root 1931). Assuming a similar Root effect in vivo, in order to achieve the same rate of O_2 delivery to the tissues during hypercapnia, there must be either an elevation in the arterial-venous PO_2 difference as demonstrated here and/or an increase in cardiac output. We are unaware of any studies that have examined the effects of hypercapnia on cardiac output in trout although it was shown in dogfish *(Squalus acanthias)* that cardiac output is lowered (Kent and Peirce 1978). Thus, it seems likely that in the present

study the fall in PvO_2 was required to maintain O_2 delivery.

As in several previous studies (Eddy et al. 1977; Perry et al. 1989; Kinkead and Perry 1991), the marked hyperventilation that is known to accompany hypercapnia (Janssen and Randall 1975) did not elicit a significant rise in PaO_2. It is conceivable that the absence of any effect of the hyperventilation on raising PaO_2 simply reflected the low pre-branchial PO_2. Thus, the significance of the hyperventilatory response during hypercapnia may be to maintain PaO_2 in the face of the lowered PvO_2.

During the initial 25 min of hypercapnia CO_2 appeared to be absorbed across the gill from the water as indicated by the abolishment or reversal of the normocapnic arterial-venous PCO_2 difference. Although mathematical estimates of CCO_2 are complicated by the probable non-equilibrium state during the initial phases of hypercapnia, the calculations revealed that the arterial-venous CCO_2 difference changed from -0.72 mmol $\cdot$ l^{-1} before hypercapnia to $+0.75$ mmol $\cdot$ l^{-1} after 15 min of hypercapnia, thus supporting the notion of CO_2 uptake into the arterial blood. During this initial period of apparent CO_2 uptake from the water, there would likely be additional detrimental effects on branchial O_2 uptake and delivery owing to the reversal of the usual Bohr effects at the gill and tissues. In other words, the uptake of CO_2 into the blood during transit through the gills would lower the affinity of Hb-O_2 binding in contrast to the usual increase in Hb-O_2 affinity associated with CO_2 excretion. In a similar fashion, the entry of CO_2 into the tissues would presumably compromise O_2 delivery.

The reduction in pH after 30 min of hypercapnia was the result of a mixed respiratory/metabolic acidosis. Although we did not perform a detailed quantitative analysis of the blood acid-base status, a metabolic component to the acidosis was obvious from comparisons of the blood in vivo and in vitro. In the absence of a metabolic component in vitro, equilibration of the blood to achieve the same degree of hypercapnia as in vivo, caused a smaller decrease in pH. The metabolic acidosis in vivo probably was the consequence of sudden activation of rbc Na^+/H^+ exchange by the rapid elevation of circulating catecholamine levels between 5 and 10 min of hypercapnia.

The elevation of plasma catecholamines was much greater than previously reported for trout exposed for similar duration and to similar levels of hypercapnia (Perry et al. 1987, 1989); however, reasons for the differences among the studies are unclear. On the basis of the known potency of catecholamines to stimulate the β-adreneregic rbc Na^+/H^+ exchange (Tetens et al. 1988) and the additional positive influence of acidosis on the activity of the exchanger (Borgese et al. 1987; Nikinmaa et al. 1987; Cossins and Kilbey 1989), it is reasonable to assume that rbc Na^+/H^+ exchange was indeed activated during hypercapnia as proposed in an earlier study (Perry and Kinkead 1989). Further evidence for its activation was a pronounced metabolic component to the predominantly respiratory acidosis. The physiological consequences of rbc Na^+/H^+ exchange activation have been discussed in detail elsewhere [reviews: Thomas and Perry (1992); Randall and Perry (1992)]. Aside from the metabolic acidosis, there were no obvious effects of catecholamine release on the measured blood respiratory variables. On the basis of theory and previous studies, the release of catecholamines probably caused an increase in Hb-O_2 binding affinity and capacity [reviews: Nikinmaa (1992); Thomas and Perry (1992)].

ii) Hypoxia. In agreement with previous studies [review: Thomas and Motais (1990)], the initiation of hypoxia caused an immediate and pronounced reduction in PaO_2. Of particular interest, however, was the fact that PvO_2 did not change significantly during the first 10 min of hypoxia despite an approximate 80 torr decline in PaO_2. Similar results were reported by Holeton and Randall (1967) for trout exposed simultaneously to hypoxia and hypercapnia. Assuming that O_2 uptake remained constant (Holeton and Randall 1967) and that cardiac output was unaltered (Fritsche and Nilsson 1993), the stable PvO_2 can be explained by an increase in the affinity of Hb-O_2 binding and/or an increase in the O_2 carrying capacity of the blood. An increase in the Hb-O_2 binding affinity is expected as rbc pHi rises owing to the Haldane effect associated with deoxygenation (Jensen 1986).

Catecholamines were released abruptly into the circulation after approximately 10–15 min of hypoxic exposure. In agreement with previous studies (Perry and Reid 1992; Thomas et al. 1992), the release of catecholamines occurred after PaO_2 had fallen to approximately 20–30 torr or about 50–60% $SHbO_2$. The release of catecholamines into the circulation surely activated rbc Na^+/H^+ exchange and the marked metabolic acidosis at the time of release was indicative of such activation (Fievet et al. 1987). Owing to the infrequent blood sampling for catecholamine determinations (every 5 min), the sudden reduction in blood pH was used to pinpoint catecholamine release. Thus, it was obvious that the release of catecholamines did not provoke any obvious changes in arterial blood gases but that the PO_2 of the venous compartment was markedly affected. It was recently demonstrated (Perry and Thomas 1993) that the addition of catecholamines to trout blood in vitro causes an immediate reduction in plasma PO_2 because of the diffusive entry of O_2 into the rbc as Hb-O_2 binding is enhanced. Thus, in the present study, a component of the PO_2 reduction presumably also reflected the catecholamine-induced enhancement of Hb-O_2 binding, the sudden fall in rbc intracellular PO_2 and the subsequent movement of O_2 into the rbc from the plasma to re-equilibrate O_2 across the rbc membrane. Unlike the earlier in vitro study (Perry and Thomas 1993), the change in PvO_2 was not transient and thus other factors must have also contributed to the persistent reduction in PvO_2.

In the present study $PaCO_2$ continued to decline throughout the period of hypoxia with no obvious effect of catecholamine release. This observation is in contrast to a previous study that showed an elevation of $PaCO_2$ after the release of catecholamines during hypoxia (Perry and Thomas 1991). Thus, despite the higher pre-branchial PCO_2 (Fig. 3) and the immediate inhibitory effects of Na^+/H^+ exchange activation on rbc HCO_3^- de-

hydration (Wood and Perry 1991; Petty et al. 1991) $Pa\text{-}CO_2$ remained constant.

Control of catecholamine release

Several theories have been proposed to explain the abrupt release of catecholamines into the circulatory system during acute periods of stress. At least three factors have been suggested as possible catecholamine releasing stimuli, including blood acidosis (Tang and Boutilier 1988), lowering of arterial blood PO_2 (Fievet et al. 1990) or depression of arterial O_2 content (Perry and Reid 1992). During hypoxia, the lowering of blood O_2 content rather than PO_2 per se appears to be the proximate controlling stimulus [review: Randall and Perry (1992)]. Several studies have reported that the O_2 content threshold for catecholamine release during hypoxia corresponds to approximately 50–60% $SHbO_2$ (Perry and Reid 1992; Thomas et al. 1992). A similar relationship between arterial blood O_2 content and catecholamine release was witnessed in the present study.

During hypercapnia, the reduction of blood O_2 content (caused by the acidosis-induced Root effect) is believed to elicit the release of catecholamines (Perry et al. 1989). Clearly, however, catecholamine release occurs well above the O_2 content threshold of 50–60% $SHbO_2$ reported for normocapnic hypoxia. There are at least two possible explanations: first, it is conceivable that the acidotic condition, while not a stimulus per se, modifies the O_2 content release threshold. Second, the release process may be controlled, at least in part, by the O_2 status of the venous blood. Prior to catecholamine release, PvO_2 fell by approximately 13 torr while PaO_2 remained constant (Fig. 1). This reduction in PvO_2 coupled with the hypercapnic acidosis would have a marked impact on the O_2 content of the venous blood (Fig. 5). The resultant hypoxemia in the venous compartment could stimulate catecholamine release either by exerting a local effect as previously demonstrated for Atlantic cod, *Gadus morhua* (Perry et al. 1991) or by initiating the afferent limb of the neural reflex control of catecholamine release (Randall and Perry 1992) by stimulating putative venous O_2 chemoreceptors.

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