CARDIOVASCULAR EFFECTS OF HYPERCARBIA IN RAINBOW TROUT 
(ONCORHYNCHUS MYKISS): A ROLE FOR EXTERNALLY ORIENTED CHEMORECEPTORS

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Summary

In situ and in vivo experiments were performed on rainbow trout (Oncorhynchus mykiss) to examine (i) the direct effect of CO2 on the systemic vasculature and (ii) the influence of internal versus external hypercapnic acidosis on cardiovascular variables including blood pressure, cardiac output and systemic vascular resistance. Results from in situ saline-perfused trunk preparations indicated that CO2 (0.6, 1.0 or 2.0 % CO2) elicited a significant vasodilation, but only in the presence of pre-existing humoral adrenergic tone. In the absence of pre-existing vascular tone, CO2 was without effect on systemic resistance. In contrast, hypercarbia in vivo triggered a statistically significant increase in systemic resistance (approximately 70%) that was associated with elevated ventral aortic (approximately 42%) and dorsal aortic (approximately 43%) blood pressures and with a significant bradycardia (approximately 12%); cardiac output was not significantly affected.

To determine the potential roles of internal versus external chemoreceptors in mediating the cardiovascular responses to hypercarbia, experiments were performed to elevate the endogenous arterial partial pressure of CO2 (Paco2) without an accompanying increase in external PCO2 (PwCO2). In one series, trout were given a bolus injection of the carbonic anhydrase inhibitor acetazolamide (30 mg kg^-1) to inhibit CO2 excretion, and thus raise Paco2, 5–7 h prior to being exposed to an acute increase in PwCO2 (maximum PwCO2=6.3±0.4 mmHg; 1 mmHg=0.133 kPa). Despite a marked increase in Paco2 (approximately 7 mmHg) after injection of acetazolamide, there was no increase in dorsal aortic blood pressure (PDA) or systemic resistance (RS). The ensuing exposure to hypercarbia, however, significantly increased PDA (by approximately 20%) and RS (by approximately 35%). A second series of experiments used a 5–7 h period of exposure to hyperoxia (PwO2=643±16 mmHg) to establish a new, elevated baseline Paco2 (7.8±1.1 mmHg) without any change in PwCO2. Despite a steadily increasing Paco2 during the 5–7 h of hyperoxia, there was no associated increase in PDA or RS. Ensuing exposure to hypercarbia, however, significantly increased PDA (by approximately 20%) and RS (by approximately 150%). Plasma adrenaline levels were increased significantly during exposure to hypercarbia and, therefore, probably contributed to the accompanying cardiovascular effects.

These findings demonstrate that the cardiovascular effects associated with hypercarbia in rainbow trout are unrelated to any direct constrictory effects of CO2 on the systemic vasculature and are unlikely to be triggered by activation of internally oriented receptors. Instead, the data suggest that the cardiovascular responses associated with hypercarbia are mediated exclusively by externally oriented chemoreceptors.

Key words: Oncorhynchus mykiss, rainbow trout, hypercapnia, hypercarbia, systemic resistance, catecholamine, carbon dioxide, chemoreceptor, hyperoxia, acetazolamide, cardiovascular.

Introduction

It is generally accepted that O2 (rather than CO2/H+) chemoreceptors predominate in controlling physiological function in fishes (e.g. Cameron, 1989). Certainly, their important role in mediating cardio-respiratory adjustments during hypoxia is universally recognized (for a review, see Fritsche and Nilsson, 1993). An equivalent role for CO2/H+ receptors is less obvious. Although there is extensive documentation on the effects of hypercarbia on modulating ventilation (e.g. Janssen and Randall, 1975; Thomas and Le Ruz, 1982; Thomas, 1983; Wood and Munger, 1994; Burleson and Smatresk, 2000), the effects have generally been attributed to changes in blood O2 status arising from Bohr and Root effects, rather than to specific effects of CO2 (e.g. Randall, 1982; Smith and Jones 1982). This traditional view, however, has been challenged (see Perry and Wood, 1989) for a variety of reasons. These include pronounced hyperventilatory
responses to CO\textsubscript{2} in (i) elasmobranchs (Heisler et al., 1988; Wood et al., 1990; Graham et al., 1990; Perry and Gilmour, 1996), species that lack a Root effect (Butler and Metcalfe, 1988), and (ii) teleosts displaying no significant impairment of blood O\textsubscript{2} status (e.g. during mild hypercapnia or hypoxic hypercapnia; Kinkead and Perry, 1991; Burleson and Smatresk, 2000). Thus, models of the control of breathing in fishes have been revised extensively in recent years to include a role for CO\textsubscript{2}/H\textsuperscript{+} receptors (Milsom, 1995a; Milsom, 1995b). Experiments incorporating denervation techniques (Burleson and Smatresk, 2000; Reid et al., 2000; Sundin et al., 2000) have revealed that the gill is a significant site of CO\textsubscript{2} chemosensitivity.

In contrast, there is a paucity of data concerning the effects of CO\textsubscript{2} on piscine cardiovascular physiology. Recently, however, Perry et al. (Perry et al., 1999) demonstrated that hypercapnia specifically elicits profound cardiovascular adjustments in rainbow trout that include increased blood pressure and systemic resistance. Although Perry et al. (Perry et al., 1999) successfully identified CO\textsubscript{2}/H\textsuperscript{+} as the stimulus for the cardiovascular responses during hypercapnia, that study was unable to determine whether the effects were caused by the environmental hypercapnia acting on externally oriented chemoreceptors or by the ensuing rise in endogenous levels of CO\textsubscript{2} influencing internally oriented receptors. Moreover, the possibility that the pressor response to hypercapnia was related to specific constrictory effects on the systemic vasculature, although suggested by Perry et al. (Perry et al., 1999), has not been tested.

The goals of this study, therefore, were (i) to evaluate whether the cardiovascular effects of hypercapnia in rainbow trout are attributable to external and/or internal elevation of P\textsubscript{CO\textsubscript{2}} and (ii) to investigate the potential role of CO\textsubscript{2} acting directly on the systemic vasculature. The experiments were performed in situ using a saline-perfused trunk preparation (Wood and Shelton, 1975) and in vivo using an extracorporeal arterial blood shunt (Thomas, 1994).

**Materials and methods**

**Experimental animals**

Rainbow trout *Oncorhynchus mykiss* (Walbaum) of either sex were obtained from Linwood Acres Trout Farm (Cambellcroft, Ontario, Canada) and transported in oxygenated water to the University of Ottawa. Trout were maintained on a 12 h:12 h L:D photoperiod in 1300 l fibreglass tanks supplied with flowing, aerated and dechlorinated City of Ottawa tapwater (14 °C). They were fed a commercial pelleted diet and allowed to circulate for 4 min. The animal was then completely transected at the anterior end of the heart, the atrium and ventricle were removed, and the dorsal aorta was cannulated using polyethylene tubing (Clay Adams, PE 160). A circular incision was made (approximately 0.5 cm in depth) around the spine and dorsal aorta to allow for a sunken silk ligature to secure the cannula and to prevent leakage due to back pressure. The preparation was then immediately submerged in a constant-level bath containing 0.9 % NaCl saline (14 °C) and perfused with aerated Cortland saline (Wolf, 1963).

The reservoir of perfusate was contained in a temperature-controlled constant-level water bath atop a Mettler top-loading balance. The preparation was supplied with a constant flow of perfusate via an adjustable-speed polysylastic pump (Buchler Instruments) preset to supply approximately 5 ml kg\textsuperscript{-1} total body mass min\textsuperscript{-1}; the change in mass of the saline reservoir was used to calculate actual flow rates. The pump output was noticeably pulsatile, so an inverted 500 ml Erlenmeyer flask containing an air bubble (300–400 ml) was inserted as a Windkessel to dampen the pulsatility between the trunk and the pump (Wood and Shelton, 1975). Perfusion back-pressure from the trunk was monitored via a T-joint accessing a UFI (model 1050BP) pressure transducer pre-calibrated daily against a static column of water. Analogue pressure signals were converted to digital data and collected at 10 samples s\textsuperscript{-1} on a computer accompanied by a data-acquisition system (Biopac) using AcqKnowledge 3.03 software. The entire system, from trunk to perfusate, was connected using polyethylene tubing (PE 160).

**Series I: investigation of potential direct constrictory effects of CO\textsubscript{2} on the systemic vasculature**

Environmental hypercapnia in vivo has recently been shown to elicit an increase in systemic resistance (Perry et al., 1999). This experimental series was designed to assess whether this effect was due, at least in part, to CO\textsubscript{2} acting directly on the systemic vasculature.

After the trunk preparation had stabilized and a constant baseline pressure had been established (approximately 30 min), experimental recordings were begun. After an additional 10 min of perfusing the trunk with aerated saline, the perfusate was switched (via a three-way valve) to saline gassed with 0.6 %, 1.0 % or 2 % CO\textsubscript{2} to yield P\textsubscript{CO\textsubscript{2}} values of approximately 4.5, 7.5 and 15 mmHg (1 mmHg=0.133 kPa). The preparation was perfused with hypercapnic saline for 20 min before switching back to aerated saline for the remaining 10 min. As the perfusate left the water bath on top of the top-loading scale, mass/flow measurements were recorded manually at regular intervals to facilitate calculations of R\textsubscript{S}.
Series II: investigation of potential direct dilatory effects of CO₂ on the systemic vasculature

Initial procedures were identical to those used in series I. However, after the initial 10 min of data recording using aerated saline, the perfusate was switched (via a three-way valve) to aerated saline containing 10⁻⁴ mol⁻¹⁻¹ adrenaline (bitartrate salt) to induce vasoconstriction. Within 40 min, the preparation had reached a new stable, elevated baseline level of vasocostriction, and the perfusate was switched once more to saline containing 10⁻³ mol⁻¹⁻¹ adrenaline gassed with air (control), 0.6 %, 1.0 % or 2 % CO₂. Experiments were terminated after a further 70 min.

In vivo experiments

Surgical procedures

Rainbow trout, weighing between 707 and 1450 g (experimental N=27), were anaesthetized using benzocaine (0.10 g l⁻¹ ethanol-p-aminobenzoate, Sigma) until breathing movements stopped. The fish were then placed on an operating table, where their gills were force-ventilated with the same oxygenated anaesthetic solution. To permit drug injections and measurement of dorsal aortic pressure (P_D), a polyethylene cannula (Clay Adams, PE 50) was implanted into the dorsal aorta via percutaneous puncture of the roof of the buccal cavity (Soivio et al., 1975). To allow an extracorporeal blood shunt, a lateral incision was made in the caudal peduncle immediately below the lateral line to expose, separate from the surrounding tissue and cannulate (PE 50) the caudal artery and vein in the anterograde direction. The incision was closed using sutures, and both cannulae were secured to the caudal peduncle independently via silk ligatures.

A small ventral incision was made to expose the pericardial cavity, and the pericardium was dissected to expose the bulbus arteriosus. To allow measurement of central venous pressure (P_Ven), a non-occlusive silicone cannula (0.51 mm internal diameter; 0.94 mm external diameter) was implanted into the right horn of the ductus of Cuvier and secured using cyanoacrylate glue (Olson et al., 1997). The silicone cannula was then attached to a polyethylene tube (Clay Adams, PE 90), approximately 75 cm in length, filled with heparinized (50 i.u. ml⁻¹ sodium heparin) Cortland saline. To allow measurement of ventral aortic pressure (P_VA), the ventriculo-bulbar junction was temporarily clamped shut to restrict blood flow while the bulbus arteriosus was cannulated with silicone tubing. The clamp was removed, and an 3S or 4S ultrasonic flow probe (Transonics Systems Inc., Ithaca, NY, USA) was placed non-occlusively around the bulbus to enable the measurement of cardiac output (V₆; Olson et al., 1997). The incision was sutured and sealed with Vetbond (3M Animal Care Products, MN, USA). The bulbus and ductus cannulae and the flow probe cable were secured externally to the ventral surface of the fish using silk ligatures and Vetbond. After surgery, the fish were revived and placed in opaque acrylic boxes supplied with flowing water, where they were left to recover for approximately 24 h prior to experimentation.

Experimental protocol

Measurement of blood/water respiratory variables

The extracorporeal blood shunt employs a peristaltic pump to pull blood continuously from the caudal artery, passing it over P_O₂, pH and P_CO₂ electrodes before returning it to the fish via the cannulated caudal vein (Perry and Gilmour, 1996). O₂ and CO₂ electrodes (Radiometer) were housed in temperature-controlled cuvettes and connected to a Radiometer blood gas analyzer. To reduce the chance of blood clotting in the tubing or electrodes, the entire loop was flushed for 10 min with a sodium heparin solution (1000 i.u. ml⁻¹ in saline) immediately prior to experimentation. A similar pump-driven loop continuously withdrew water from just in front of the mouth of the fish and passed it over a separate set of temperature-controlled P_O₂, P_CO₂ and pH electrodes connected to a Radiometer and Cameron Instruments meters. All electrodes were calibrated prior to each individual experiment (Perry et al., 1999).

Measurement of cardiovascular variables

The dorsal aorta, ductus of Cuvier and bulbus arteriosus cannulae were connected to UFI (model 1050BP) pressure transducers pre-calibrated against a static column of water. Mean blood pressure was calculated as (systolic pressure + diastolic pressure)/2. Cardiac output was determined by connecting the 3S or 4S ultrasonic flow probe to a Transonic T106 small-animal blood flow meter. Analogue signals were converted to digital data and collected at 40 samples s⁻¹ on a computer accompanied by a data-acquisition system (Biopac Systems) using AcqKnowledge 3.03 software. Recordings began upon stabilization of blood and water levels.

Series I: cardiovascular effects of hypercarbia

Once blood pressures and water/cardiovascular values had stabilized, an initial blood sample (0.5 ml) was drawn from the return line of the extracorporeal blood shunt. After 10 min of normocarbic normoxia, the water supplying the fish box was rendered hypercarbic for 20 min by gassing a water equilibration column with 1.5 % CO₂ in air (Cameron flowmeter; model GF-3/MP). A second blood sample (0.5 ml) was drawn at maximum hypercarbic exposure, immediately prior to switching the inflow water back to normocarbic normoxia. In this series, systemic resistance (R_S) was calculated using the formula:

\[
R_S = \frac{(P_D - P_Ven)}{V_6}. \tag{1}
\]

Gill resistance (R_g) was calculated using the formula:

\[
R_g = \frac{(P_VA - P_D)}{V_6}. \tag{2}
\]

Series II: cardiovascular effects of hypercarbia in acetzolamide-treated fish

The carbonic anhydrase inhibitor acetazolamide was used to investigate the cardiovascular effects of external versus internal hypercapnia. Once stable blood pressures and
water/cardiovascular values had been achieved, the fish were administered a bolus injection of acetazolamide (30 mg kg\(^{-1}\)) via the return line of the extracorporeal blood shunt and data were recorded for 5–7 h, at which time \(P_{\text{a}CO_2}\) had risen to a new elevated baseline level. At this point, an initial blood sample (0.5 ml) was drawn from the return line of the extracorporeal blood shunt. After 10 min of normocarbic normoxia, the water supplying the fish box was rendered hypercarbic for 20 min (see above). A second blood sample (0.5 ml) was drawn at maximum hypercarbic exposure, immediately prior to switching the inflow back to normocarbic water. Because of the potential stress of the substantial surgical load during these prolonged experiments and the lack of effect of \(CO_2\) on \(R_g\) in series I, dorsal aortic and ductus of Cuvier cannulae were not implanted for series II and III. In turn, \(R_g\) was not calculated and \(R_S\) was calculated as \(R_S = P_{DA}/V_b\) for series II and III.

**Series III: cardiovascular effects of hypercarbia in hyperoxic fish**

Because of potential non-specific effects of acetazolamide, a second ‘non-drug-induced’ method (i.e. hyperoxia) was used to elevate endogenous levels of \(CO_2\). Once blood pressures and water/cardiovascular values had stabilized, the water supplying the fish box was rendered hyperoxic by gassing a water equilibration column with 100% O\(_2\) for 6–8 h, at which time \(P_{aCO_2}\) had established a new elevated baseline level. At this point, an initial blood sample (0.5 ml) was drawn from the return line of the extracorporeal blood shunt. Following 10 min of normocarbic normoxia, the water supplying the fish was rendered hyperoxic for 20 min as in series I and II. A second blood sample (0.5 ml) was drawn at maximum hyperoxic exposure, immediately prior to switching the inflow water back to hyperoxic normocarbia.

**Analytical techniques**

All blood samples collected for measurements of catecholamines were centrifuged immediately at 12 000 \(g\) for 1 min and flash-frozen in liquid N\(_2\) before being placed in storage at −80 °C. Plasma noradrenaline and adrenaline levels were determined on alumina-extracted samples (200 \(\mu\)l) using high-pressure liquid chromatography (HPLC) with electrochemical detection (Bernier and Perry, 1997). The HPLC consisted of a Varian Star 9012 solvent-delivery system (Varian Chromatography Systems) coupled to a Princeton Applied Research 400 electrochemical detector (EG & G Instruments). The extracted samples were passed through an Ultratechsphere ODS-\text{C}\(_{18}\) 5 \(\mu\)m column (HPLC Technology Ltd), and the separated amines were integrated using the Star Chromatography software program (version 4.0, Varian). Concentrations were calculated relative to appropriate standards and with 3,4-dihydroxybenzylamine hydrobromide (DHBA) as an internal standard in all determinations. All figures were plotted using the SigmaPlot 4.0 commercial graphics software package (SPSS Inc.).

**Statistical analyses**

Data are presented as mean values ± 1 standard error of the mean (S.E.M.). For the *in situ* experiments (Figs 1, 2), the statistical significance of the observed effects of a given treatment over time were tested using a repeated-measures analysis of variance (ANOVA) followed by a *post-hoc* pairwise comparison with the final control point (\(t=10\) min). For the *in vivo* experiments (Table 1; Figs 3–8), the statistical significance of the observed effects was determined using paired *t*-tests or repeated-measures ANOVA followed by a *post-hoc* pairwise comparison. All statistical analyses were performed using SigmaStat 2.0 commercial software package (SPSS Inc.) with a fiducial limit of 5%.

**Results**

**In situ experiments**

Direct effects of \(CO_2\) on systemic vasculature

Because of the large degree of variability amongst the initial \(R_S\) values of the different perfused preparations, the absolute change in \(R_S\) is depicted in Fig. 1. Absolute changes were determined by subtracting the value at the time of switching perfusate \(CO_2\) composition (10 min) from all previous and subsequent data points. In the absence of humoral and neuronal tone, perfusing the systemic vasculature of decapitated trout with saline equilibrated with 0.6–2.0% \(CO_2\) had no vasoconstrictory effect. In contrast, trunks pre-perfused with aerated saline containing 10\(^{-4}\) mol l\(^{-1}\) adrenaline (creating an increase in \(R_S\) of 56.4±3.2 mmHg ml min\(^{-1}\) kg\(^{-1}\)) displayed a significant decrease in \(R_S\) upon adding 0.6–2.0% \(CO_2\) to the perfusate (Fig. 1B–D). In the absence of \(CO_2\) (i.e. continued perfusion with aerated saline containing 10\(^{-4}\) mol l\(^{-1}\) adrenaline), \(R_S\) remained stable (Fig. 1A). While this \(CO_2\)-induced dilation of the systemic vasculature was not dose-dependent in the range studied (0.6–2.0%), the overall direct effect of \(CO_2\) was clearly vasodilatory.

**In vivo experiments**

Series I: cardiovascular effects of hypercarbia

Exposing rainbow trout to an acute increase in \(PW_{CO_2}\) (approximately 5.5 mmHg) caused a statistically significant increase in \(P_{DA}\) and \(P_{VA}\) with no effect on cardiac output despite a significant decrease in heart rate (Fig. 2A–D). Furthermore, there was no increase in central venous pressure (normocapnia, \(P_{Ven}=3.9±1.3\) mmHg; maximum hypercapnia, \(P_{Ven}=4.7±1.2\) mmHg). The significant increases in arterial pressure with virtually no change in venous pressure or cardiac output during hypercarbia resulted in stable gill resistance (\(R_g\)) but a significant increase in systemic resistance of approximately 70% (Fig. 2E,F).

Series II: cardiovascular effects of hypercarbia in acetazolamide-treated fish

Fig. 3 depicts typical data-acquisition traces from a single representative fish and illustrates the effects of increasing...
internal $P_{\text{ACO}_2}$ in the absence of elevated external $P_{\text{CO}_2}$ and the cardiovascular response to hypercarbia in these carbonic-anhydrase-inhibited trout. The single bolus injection of acetazolamide triggered an immediate increase in $R_S$ that was followed by a rapid and sustained increase over the ensuing 7 h. Importantly, the increase in $R_S$ was not preceded by any changes in $P_{\text{ACO}_2}$, and there was clearly no correlative relationship between $P_{\text{ACO}_2}$ and $R_S$ as long as $P_{\text{WCO}_2}$ remained low. However, upon exposure to hypercarbia, there was a marked and progressive increase in $R_S$ that correlated with both $P_{\text{WCO}_2}$ and $P_{\text{ACO}_2}$.

The quantitative data from these experiments are shown in Fig. 4. Acetazolamide injection and the ensuing increase in $P_{\text{WCO}_2}$ did not statistically affect $P_{\text{DA}}$, $f_H$ or $R_S$; heart rate ($f_H$) was significantly reduced by approximately 35–40%. The decrease in $f_H$ occurred within a few minutes of acetazolamide injection and was not correlated with $P_{\text{ACO}_2}$ (data not shown). Rainbow trout subjected to virtually the same degree of hypercarbia as in series I (Table 1) were still able to increase $P_{\text{DA}}$ (by approximately 20%) and $R_S$ (by approximately 35%) (Fig. 4A,D, respectively) despite the pre-existing acetazolamide-induced elevated $P_{\text{ACO}_2}$ (Table 1). It is worth noting (see Table 1) that the mean increase in $P_{\text{ACO}_2}$ during hypercarbia (3.3 mmHg) was much less than the increase caused by acetazolamide (7.0 mmHg).

**Series III: cardiovascular effects of hypercarbia in hypoxic fish**

Fig. 5 represents excerpts from a typical data-acquisition trace, illustrating both the lack of hyperoxia-induced cardiovascular adjustments and the vasoconstrictory response to hypercarbia in these hyperoxic trout. Although there was no change in $P_{\text{WCO}_2}$, $P_{\text{ACO}_2}$ gradually increased during hyperoxia with no correlative increase in $R_S$. The quantitative data from

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**Fig. 1.** Effects of acute elevations of perfusate $P_{\text{CO}_2}$ on systemic resistance ($R_S$) in pre-contracted perfused trunk preparations of rainbow trout (*Oncorhynchus mykiss*). In all preparations, adrenaline (10$^{-4}$ mol l$^{-1}$) was first added to the perfusate to attain an increase in baseline $R_S$ prior to raising $P_{\text{CO}_2}$ in the continued presence of adrenaline. The interval of elevated $P_{\text{CO}_2}$ is to the left of the dashed vertical lines. (A) Control (air only; N=5), (B) 0.6 % CO$_2$ (N=6), (C) 1.0 % CO$_2$ (N=5), and (D) 2 % CO$_2$ (N=5). Values are means ±1 s.e.m.; significant differences ($P<0.05$) from the normocarbic values (time=10 min) are indicated by horizontal lines with arrowheads. 1 mmHg=0.133 kPa.

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**Fig. 2.** The effects of external hypercarbia ($P_{\text{WCO}_2}$=6.2±0.4 mmHg) on cardiovascular variables in the rainbow trout (*Oncorhynchus mykiss*) including (A) dorsal aortic blood pressure ($P_{\text{DA}}$; N=9), (B) ventral aortic blood pressure ($P_{\text{VA}}$; N=6), (C) heart rate ($f_H$; N=9), (D) cardiac output ($f_H$; N=8), (E) gill resistance ($R_G$; N=6) and (F) systemic resistance ($R_S$; N=6). Filled columns represent pre-hypercarbic (i.e. normocarbia) values; open columns represent maximal responses obtained during hypercarbia. Values are means ±1 s.e.m.; an asterisk denotes a significant difference between normocarbia and hypercarbia ($P<0.05$). 1 mmHg=0.133 kPa.
these experiments are shown in Fig. 6. Rainbow trout subjected to the same degree of hypercarbia as in series I increased their $P_{DA}$ (by approximately 20%) and $R_S$ (by approximately 150%) (Fig. 6A,D, respectively) despite a hyperoxia-induced elevated $P_{a CO_2}$ (Table 1). There was no effect of hypercarbia on heart rate or cardiac output (Fig. 6B,C).

During each of the hypercarbic periods in the three series described above, the significant increase in $P_{w CO_2}$ triggered a statistically significant increase in $P_{a CO_2}$. Although $P_{a O_2}$ appeared to increase during hypercapnia in each of the three treatments, it was statistically significant only in the control.
Cardiovascular effects of hypercarbia in trout

There was no change in \( P_w O_2 \) within each treatment (Table 1). Neither acetazolamide nor hyperoxia elicited a significant increase in resting/normocapnic plasma levels of noradrenaline and adrenaline (Fig. 7A,B). However, while hypercapnia elicited a slight, albeit statistically insignificant, increase in noradrenaline in each of the three treatments, adrenaline levels rose significantly for each group (i.e. control, acetazolamide-treated and hyperoxia-exposed).

Discussion

The results of the present study confirm that elevated levels of ambient \( CO_2 \) evoke significant increases in arterial blood pressure and systemic resistance in conjunction with a significant bradycardia in rainbow trout. Two important new findings are (i) that these effects cannot be attributed to any direct action of \( CO_2 \) on the systemic vasculature and (ii) that the cardiovascular effects are attributable to hypercarbia, not to internal hypercapnia. The results of this investigation

Table 1. The effects of an increase in external \( P_{wCO_2} \) on water/blood gases in rainbow trout (Oncorhynchus mykiss) in each of the three treatment groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ACTZ</th>
<th>Hyperoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normocarbia</td>
<td>Hypercarbia</td>
<td>Pre-ACTZ</td>
</tr>
<tr>
<td>( P_{wO_2} ) (mmHg)</td>
<td>153.7±2.2</td>
<td>151.2±2.4</td>
<td>162.7±7.8</td>
</tr>
<tr>
<td>( P_{wCO_2} ) (mmHg)</td>
<td>0.63±0.18</td>
<td>6.15±0.4*</td>
<td>0.33±0.01</td>
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<tr>
<td>( P_{aO_2} ) (mmHg)</td>
<td>121.4±3.2</td>
<td>138.0±2.8*</td>
<td>121.4±5.2</td>
</tr>
<tr>
<td>( P_{aCO_2} ) (mmHg)</td>
<td>1.96±0.32</td>
<td>6.19±0.70*</td>
<td>1.85±0.20</td>
</tr>
<tr>
<td>pHa</td>
<td>7.83±0.05</td>
<td>7.43±0.05*</td>
<td>7.82±0.03</td>
</tr>
</tbody>
</table>

* denotes a statistically significant difference from the pre-treatment value within that treatment; ‡ denotes a significant difference between post-acetazolamide/hyperoxia normocarbia and hypercarbia (\( P<0.05 \)).

Values are means ± s.e.m. (\( N=5–8 \)).

ACTZ, acetazolamide; pHa, arterial pH.

1 mmHg=0.133 kPa.

Fig. 5. Representative data-acquisition traces illustrating the typical effects of hyperoxia (dashed line) followed by hypercapnia (shaded area) on (A) water \( P_{wO_2} \), (B) water \( P_{wCO_2} \), (C) arterial \( P_{aCO_2} \), (D) systemic resistance (\( R_s \)) in rainbow trout Oncorhynchus mykiss. 1 mmHg=0.133 kPa.
provide the strongest support to date for the involvement of external CO₂ chemoreceptors linking the cardiovascular and autonomic nervous systems.

**Direct effects of CO₂ on the systemic vasculature**

*In situ* saline-perfused trunk perfusions have been used previously to investigate the direct effects of vasoactive agents on systemic vascular resistance (e.g. Wood and Shelton, 1975). It was recently suggested (Perry et al., 1999) that increases in systemic resistance observed during hypercarbia in rainbow trout were potentially the result of, or at least enhanced by, CO₂/H⁺ acting directly on the blood vessels. However, the three levels of CO₂/H⁺ examined in this study (0.6, 1.0 and 2.0%) did not elicit any vasoconstrictory effects in preparations devoid of neuronal or humoral tone. The suitability of the preparation and its ability to display substantial increases in systemic resistance was confirmed by a second series of *in situ* experiments (Fig. 1). Adrenaline (10⁻⁴ mol l⁻¹) evoked a profound vasoconstriction of the vasculature and was therefore used as a tool to pre-contract the preparations. Under such pre-contracted conditions, a clear vasodilatory effect of CO₂/H⁺ was observed. Because the systemic vasculature, *in vivo*, also displays vasomotor tone, hypercapnia alone would be expected to lower systemic resistance. Thus, the net increase in systemic resistance that occurs during hypercarbia *in vivo* must reflect the opposing influences of direct CO₂/H⁺-induced vasodilation and...
adrenergic (neuronal and/or humoral) vasoconstriction (Perry et al., 1999). Indeed, it is tempting to speculate that the reflex adrenergic vasoconstriction associated with hypercarbia (Perry et al., 1999) evolved as a mechanism to prevent potentially severe reductions in arterial blood pressure. The mammalian-like vasodilatory response of the systemic vasculature to elevated CO₂ levels is in marked contrast to the branchial vasculature of rainbow trout, which displays a significant vasodilatory response in saline-perfused gill preparations (Perry et al., 1984). However, because the study (Perry et al., 1984) used non-physiological levels of CO₂ (37.5 mmHg), additional studies are required before any meaningful comparisons can be drawn between the CO₂-sensitivity of the gill vasculature and the pulmonary vasculature of mammals.

In vivo response to hypercarbia: internal versus external chemoreceptors

In this study, exposing rainbow trout to hypercarbia caused cardiovascular adjustments similar to those observed by Perry et al. (Perry et al., 1999). Both studies reported significant bradycardia and increases in arterial blood pressures (both \( P_{VA} \) and \( P_{DA} \)) and systemic resistance, with no effect on cardiac output or gill vascular resistance. The present series of experiments not only confirmed the cardiovascular potency of hypercarbia but also, more importantly, provided a basis for comparing the cardiovascular effects elicited by hypercarbia with those elicited by hypercarbia after treatment with acetazolamide or exposure to hyperoxia.

The enzyme carbonic anhydrase is responsible for the production of molecular CO₂ via the rapidly catalyzed dehydration of \( H_2CO_3 \) in the red blood cell (for a review, see Henry and Heming, 1998). The carbonic anhydrase inhibitor acetazolamide, administered in the same concentrations as in this study (30 mg kg⁻¹), has previously been shown to slow the dehydration reaction to its uncatalysed rate, thereby increasing \( P_{ACO_2} \) (e.g. Gilmour et al., 1994). As \( P_{ACO_2} \) gradually rises, the increased \( P_{CO_2} \) gradient across the gill is able to reset CO₂ excretion to normal levels. Thus, 5–7 h after the injection of acetazolamide, a new elevated stable baseline \( P_{ACO_2} \) was established (\( P_{ACO_2} \) increased from 1.9±0.2 to 8.9±0.7 mmHg). Despite this large increase in \( P_{ACO_2} \), \( R_S \) and \( P_{DA} \) were unaffected. These results do not, therefore, support a role for internal CO₂/H⁺ chemoreceptors in the cardiovascular responses associated with hypercarbia. In some individual fish (e.g. see Fig. 4), the injection of acetazolamide caused a small rapid increase in \( R_S \). It is unlikely that this was related to inhibition of carbonic anhydrase per se because it preceded any increase in \( P_{ACO_2} \). Moreover, \( R_S \) remained relatively constant for the ensuing 7 h despite the continuing elevation of \( P_{ACO_2} \). Similarly, in accordance with previous data obtained using channel catfish (\textit{Ictalurus punctatus}; Henry et al., 1988), the bradycardia initiated by injection of acetazolamide also preceded the rise in \( P_{ACO_2} \). However, unlike the transient bradycardia observed in catfish, the decrease in \( f_V \) persisted after acetazolamide treatment in the present study.

Because carbonic anhydrase is known to play a role in mammalian carotid body CO₂ chemoreception (for a review, see Iturriaga, 1993), it is conceivable that the inability of fish to respond to hypercapnia reflected an impairment of chemoreceptor function. This seems unlikely, however, because hypercarbia in the carbonic-anhydrase-inhibited fish elicited cardiovascular responses similar to those observed in the untreated group of trout (i.e. significant increases in \( P_{DA} \) and \( R_S \)). These responses occurred despite the likelihood of inhibition of carbonic anhydrase within any CO₂/H⁺ chemoreceptor (internally or externally oriented) as a result of the high degree of permeability of acetazolamide across biological membranes (e.g. Swenson and Maren, 1987). Furthermore, it is unlikely that CO₂ chemoreception would be fully impaired by carbonic anhydrase inhibition unless the rate of intracellular pH change were the key factor determining the sensitivity to CO₂. In any case, because of the possibility of chemoreceptor impairment and the non-specific side-effects of acetazolamide (e.g. bradycardia), exposure to hyperoxic water was used as an additional mechanism to elevate endogenous levels of CO₂.

Hyperoxia has previously been shown to cause a significant hyperventilatory response in rainbow trout (e.g. Wood and Jackson, 1980). The hypoventilation impedes CO₂ excretion and causes an increase in \( P_{ACO_2} \) (Wood and Jackson, 1980). As during acetazolamide treatment, \( P_{ACO_2} \) eventually (4–6 h) reached levels great enough to increase the outward driving force for CO₂ diffusion, allowing a new elevated stable baseline to be established (\( P_{ACO_2} \) increased from 3.2±0.6 to 7.8±1.1 mmHg). Once again, despite elevated levels of endogenous CO₂, there was no increase in \( P_{DA} \) or \( R_S \). These results are consistent with those of a previous study (Wilkes et al., 1981) in which hyperoxia-induced endogenous hypercapnia failed to increase \( P_{DA} \) in the white sucker \textit{Catostomus commersoni}.

Recent branchial denervation studies with a tropical fish, the traira \textit{Hoplias malabaricus}, suggest that the hypercarbic bradycardia and increase in ventilation frequency arise from receptors exclusively within the gills but present on more than one gill arch (Reid et al., 2000). Furthermore, these CO₂/pH chemoreceptors seem to be more sensitive to changes in CO₂ than they are to changes in pH. Sundin et al. (Sundin et al., 2000) performed a similar investigation using the tambaqui \textit{Colossoma macropomum} and suggest that the hypercarbic bradycardia reflex is elicited, at least in that species, by branchial receptors confined to the first gill arch. However, selective branchial denervation of these tambaqui demonstrated that chemoreceptors mediating the hypercarbia-induced increase in ventilation frequency were confined exclusively to the gills, but not only to the first gill arch. The authors of these recent studies point out the wide array of combinations for the distribution of cardiorespiratory chemoreceptors and suggest that many more species will need to be examined before any reliable trends emerge.

To ensure that the absence of a response to internal CO₂ did not reflect an inhibitory influence of elevated \( P_{O_2} \), hyperoxic fish were exposed to hypercarbia. Reassuringly, hypercarbia...
during hyperoxia elicited cardiovascular responses similar to those observed during normoxic hypercapnia. Thus, in the present study, significant relationships between $P_{\text{aCO}_2}$ and cardiovascular variables ($R_S$ and $P_{\text{DA}}$) were observed exclusively in the presence of elevated external $P_{\text{CO}_2}$. Clearly, these results do not support a role for internally oriented CO$_2$/H$^+$ chemoreceptors, but instead support the involvement of externally directed receptors that probably reside within the gill (Burleson and Smatresk, 2000).

The increase in $R_S$ during hypercarbia in trout results from the stimulation of $\alpha$-adrenoceptors and can be blocked using the antagonist yohimbine (Perry et al., 1999). The stimulation of $\alpha$-adrenoceptors can result from increased discharge of sympathetic adrenergic neurons and/or by increased levels of circulating catecholamines. In the present study, plasma adrenaline levels increased during hypercarbia in all three treatments (i.e., untreated, acetazolamide-treated and hyperoxia-exposed), while circulating noradrenaline levels were not significantly elevated. Previous studies have also noted an increase in circulating catecholamine levels during hypercapnia in trout (Perry and Kinkead, 1989; Kinkead et al., 1993; Thomas et al., 1994; Perry and Gilmour, 1996) and a similar ratio of [adrenaline]:[noradrenaline] (approximately 2:1; Reid et al., 1998). However, the role of circulating catecholamines in the cardiovascular responses to hypercapnia is probably secondary to the role of the neuronal component of the autonomic nervous system.

In a recent study, Perry et al. (Perry et al., 1999) reported stable levels of plasma catecholamines during similar hypercarbic exposure. However, even in the absence of elevated plasma catecholamine levels, fish still displayed the characteristic significant increases in $P_{\text{DA}}$ and $R_S$ and a decrease in $fH$. Previous studies also have reported stable catecholamine levels during mild to moderate hypercapnia (Kinkead and Perry, 1991; Julio et al., 1998). It has been suggested that increases in circulating plasma catecholamine levels may be indicative of the severity of the hypercarbic assault and may reflect the extent of hypoxaemia elicited by the ensuing respiratory acidosis (Julio et al., 1998). In this regard, it is puzzling that fish experiencing hyperoxia displayed a significant elevation of plasma adrenaline concentration during hypercarbia. Previous studies demonstrated that hyperoxia is capable of preventing catecholamine secretion during hypercarbia (Perry et al., 1989) or metabolic acidosis (Aota et al., 1990). Indeed, it was argued that hyperoxia was able to offset the effects of acidosis on lowering blood O$_2$ content and thereby prevent the requirement for catecholamine secretion (Perry et al., 1989).

In summary, this study has demonstrated that elevated CO$_2$/H$^+$ has a vasodilatory effect on the systemic vasculature of rainbow trout. This direct effect probably reduces the net extent of the hypercarbia-induced vasoconstriction observed in vivo or indeed may be the cause of reflex vasoconstriction. While the physiological benefits of the cardiovascular responses to hypercarbia are unclear, this study finds that these changes are triggered by increased external CO$_2$/H$^+$ and are probably mediated via externally located CO$_2$/H$^+$ chemoreceptors linked to the neuronal and humoral components of the autonomic nervous system. Further studies should focus on determining whether the responses observed in rainbow trout are indeed representative of teleosts in general. In addition, differentiating between CO$_2$- and H$^+$-induced effects may help clarify the mechanisms involved in CO$_2$/H$^+$ chemoreception in fish.

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References


