

Cardiorespiratory adjustments during hypercarbia in rainbow trout *Oncorhynchus mykiss* are initiated by external CO₂ receptors on the first gill arch

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Summary

Experiments were performed to test the hypothesis that the marked ventilatory and cardiovascular responses to hypercarbia in rainbow trout *Oncorhynchus mykiss* arise from specific stimulation of chemoreceptors localised to the first gill arch. This was accomplished by measuring cardiorespiratory variables during acute hypercarbia (20 min at P_{CO_2} =8 mmHg; 1 mmHg=0.133 kPa) in fish subjected to selective bilateral extirpation of the first gill arch. The cardiovascular responses to hypercarbia in the intact fish included a significant bradycardia (from 75.0 ± 1.6 to 69.0 ± 2.0 beats min^{-1} ; means \pm S.E.M.; $N=16$), an increase in dorsal aortic blood pressure (from 30.8 ± 1.5 to 41.9 ± 2.5 mmHg; $N=16$) and a rise in systemic vascular resistance (from 1.1 ± 0.1 to 1.4 ± 0.1 mmHg $\text{ml}^{-1} \text{kg}^{-1} \text{min}^{-1}$; $N=16$). Removal of the first gill arch or pre-treatment with the muscarinic receptor antagonist atropine prevented the hypercarbic bradycardia without affecting the pressure or resistance responses. Correlation analysis, however, revealed shallow but significant inverse relationships between water P_{CO_2} and cardiac frequency in both atropinised ($r^2=0.75$) and gill-extirpated ($r^2=0.90$) fish, suggesting a direct mild effect of CO₂ on cardiac function. The ventilatory response to hypercarbia in the intact fish consisted of an increase in ventilation amplitude (from 0.62 ± 0.06 to 1.0 ± 0.13 cm; $N=16$) with no change in breathing frequency. Removal of the first gill arch lowered resting breathing frequency and prevented the statistically significant elevation of breathing amplitude. Gill extirpation, however, did not totally abolish the positive

correlation between water P_{CO_2} and ventilation amplitude ($r^2=0.84$), suggesting the presence of additional (although less important) chemoreceptive sites that are not confined to the first gill arch. Plasma catecholamine levels were elevated during hypercarbia, and this response was unaffected by prior gill extirpation.

To assess whether the CO₂ chemoreceptors of the first gill arch were sensing water and/or blood P_{CO_2} , bolus injections of CO₂-enriched water or saline were made into the buccal cavity or caudal vein, respectively. Injections of CO₂-enriched water to preferentially stimulate external receptors evoked catecholamine release and cardiorespiratory responses that closely resembled the responses to hypercarbia. As in hypercarbia, extirpation of the first gill arch prevented the bradycardia and the increase in ventilation amplitude associated with externally injected CO₂-enriched water. Except for a slight decrease in cardiac frequency (from 73.0 ± 2.8 to 70.3 ± 3.5 beats min^{-1} ; $N=11$), injection of CO₂-enriched saline to preferentially stimulate internal chemoreceptors did not affect any measured variable. Taken together, these data indicate that, in rainbow trout, the bradycardia and hyperventilation associated with hypercarbia are triggered largely by external CO₂ chemoreceptors confined to the first gill arch.

Key words: hypercarbia, catecholamine, chemoreceptor, chromaffin cell, gill, fish, rainbow trout, *Oncorhynchus mykiss*, cardiovascular, respiration, bradycardia.

Introduction

In response to an elevation of ambient CO₂ levels (hypercarbia), fish typically exhibit a marked hyperventilation (Dejours, 1973; Janssen and Randall, 1975; for a review, see Gilmour, 2001). Though less well studied, there is mounting evidence that hypercarbia also promotes distinct cardiovascular responses, including bradycardia, in numerous species (Kent and Peirce, 1978; Perry et al., 1999; Sundin et al., 2000; Reid et al., 2000; McKendry and Perry, 2001; McKendry et al., 2001) and increased systemic vascular resistance in salmonids (Perry et al., 1999; McKendry and

Perry, 2001; Perry and McKendry, 2001) and dogfish (Perry and McKendry, 2001). While previous studies (e.g. Randall, 1982; Smith and Jones, 1982) attributed the physiological effects of hypercarbia to impairment of blood O₂ transport (owing to Bohr and Root effects), it is now largely accepted that fish also respond directly to elevated CO₂ tensions independently of blood O₂ status (Milsom, 1995a,b; Gilmour, 2001). Thus, in recent years, there has been renewed interest surrounding the origin of the cardiorespiratory responses of fish to hypercarbia. In spiny dogfish (*Squalus acanthias*;

McKendry et al., 2001) and channel catfish (*Ictalurus punctatus*; Burleson and Smatresk, 2000), the cardiorespiratory effects of hypercarbia were prevented by bilateral denervation of the gills, thereby indicating an exclusively branchial location for CO₂/pH chemoreceptors in these species. In Atlantic salmon (*Salmo salar*), a population of branchial CO₂/pH receptors was identified by infusing CO₂-enriched water into the buccal cavity (Perry and McKendry, 2001). In tambaqui (*Colossoma macropomum*), the reflex responses to hypercarbia appear to be initiated solely by branchial chemoreceptors (Sundin et al., 2000), whereas in another tropical species, the traíra (*Hoplias malabaricus*), the responses are mediated by both branchial and extrabranchial receptors (Reid et al., 2000). Thus, in all fish that have been examined, there are branchial chemoreceptors that are responsive to CO₂.

The predominant site of O₂ chemoreception in fish is the first gill arch (Burleson et al., 1992; Fritsche and Nilsson, 1993). Current models of cardiorespiratory control (Burleson, 1995; Milsom et al., 1999) contend that the cardiovascular responses to altered environmental O₂ levels are initiated by externally oriented receptors whereas the ventilatory responses are triggered by external and internal receptors. Therefore, by analogy with the situation in mammals, in which the peripheral O₂ and CO₂ chemoreceptors share a common location (O'Regan and Majcherczyk, 1982), the present study was designed to test the hypothesis that the CO₂ chemoreceptors in trout are predominantly localised to the first gill arch. Additional experiments were performed to assess the potential involvement of externally and internally oriented receptors.

Materials and methods

Experimental animals

Rainbow trout *Oncorhynchus mykiss* (Walbaum) were obtained from a local supplier and transported to the University of Ottawa under hyperoxic conditions. Fish were maintained in fibreglass aquaria supplied with aerated and dechlorinated City of Ottawa tapwater. Water temperature was maintained at 13°C, and the light cycle was kept at 12 h:12 h L:D. Fish were fed *ad libitum* every second day; feeding was stopped 48 h prior to experimentation.

Animal preparation

All experimental protocols (including surgical procedures; see below) were previously approved by the University of Ottawa Animal Care Committee in accordance with guidelines provided by The Canadian Council for Animal Care. Fish were anaesthetised in a solution of benzocaine (0.1 g l⁻¹ ethyl-*p*-aminobenzoate; Sigma) and placed onto an operating table where the gills were ventilated with a solution of the anaesthetic. To permit measurement of arterial blood pressure (Pa), a polyethylene cannula (Clay Adams, PE 50) was implanted into the dorsal aorta *via* percutaneous puncture of the roof of the buccal cavity (Olson et al., 1997). To sample arterial blood or to inject drugs or CO₂-enriched saline into the

pre-branchial circulation, cannulae (PE 50) were implanted into the caudal artery and vein, respectively, using standard surgical procedures (Axelsson and Fritsche, 1994). Access to the caudal vessels was achieved by an incision lateral to the spinal cord slightly caudal to the anal fin. In those fish to which CO₂-enriched water was administered across the gills (see below), a delivery cannula (PE 160) was placed into the mouth through a hole in the snout.

To enable measurement of cardiac output, a small (1.5 cm) midline ventral incision was made to expose the pericardial cavity, and the pericardium was dissected away to expose the bulbus arteriosus. A 3S or 4S ultrasonic flow probe (Transonic Systems, Ithaca, NY, USA) was placed non-occlusively around the bulbus. Lubricating jelly (K-Y Personal Lubricant; Johnson and Johnson Inc.) was used with the perivascular flow probe as an acoustic couplant. Silk sutures were used to close the ventral incision and to anchor the cardiac output probe lead to the skin. Small brass plates (1 cm²) were sutured to the external surface of each operculum to allow the measurement of ventilation parameters by means of an impedance converter (Peyraud and Ferret-Bouin, 1960).

In those experiments in which the first gill arches were removed, the arches were initially ligated dorsally and ventrally and then removed with scissors. This procedure removed the entire respiratory surface of the gill arch; all that remained was a tiny stump at either end that was subsequently cauterised.

Following surgery, fish were placed into individual black Plexiglas boxes supplied with flowing, aerated water and were allowed to recover for 24 h prior to experimentation.

Experimental protocol

Series 1: effects of hypercarbia

These experiments were performed on intact ($N=16$), gill-extirpated ($N=12$) and atropine-treated (1 μmol kg⁻¹; $N=7$) fish; different fish were used in each group. Once blood pressures and water/cardiovascular values had stabilized, an initial blood sample (0.5 ml) was withdrawn from the caudal artery cannula. 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). The desired target water P_{CO_2} (P_{wCO_2}) of 8 mmHg (1 mmHg = 0.133 kPa) was controlled by adjusting the rates of water and/or gas flow thorough the equilibrium column. A second blood sample (0.5 ml) was withdrawn at maximum hypercarbic exposure, immediately prior to switching the inflow water back to normocarbic normoxia; a final blood sample was taken 20 min later.

Series 2: effects of external CO₂

These protocols were performed on intact ($N=22$) and gill-extirpated ($N=11$) fish. Experiments commenced with a 10 min recording period under normoxic, normocarbic resting conditions. After this pre-treatment period, air-equilibrated (controls) or CO₂-enriched (5% CO₂) water was injected (50 ml kg⁻¹) into the buccal cannula to deliver a bolus of water

to the gills. The injections were delivered over a 20 s period; fish continued to breathe during the injection period. Blood samples were removed *via* the caudal artery cannula immediately prior to injection of CO₂-enriched water and 2 min thereafter. As discussed by Perry and McKendry (2001), it was estimated that the P_{CO_2} of the water flowing over the lamellae would be approximately 10 mmHg after mixing and dilution by inspired water ($P_{\text{wCO}_2} \approx 0.5$ mmHg).

Series 3: effects of internal CO₂

Intact trout ($N=8$) were injected *via* the caudal vein cannula with saline (140 mmol l⁻¹ NaCl; 2 ml kg⁻¹) pre-equilibrated with 5% CO₂. Assuming negligible interconversion between CO₂, HCO₃ and H⁺ within the brief period of transit to the gill, mixing of the injected saline with the venous blood was estimated to yield a final P_{CO_2} of 9.0 mmHg (for further details, see Perry and McKendry, 2001). Blood samples were taken prior to and 2 min after injection of saline. Control fish ($N=8$) were injected with 2 ml kg⁻¹ of air-equilibrated saline. Owing to the absence of any marked effects of internal injections on cardiorespiratory function or plasma catecholamine levels, these experiments were not repeated on gill-extirpated fish.

Analytical procedures

Measurement of plasma catecholamine concentrations

All blood samples collected for measurements of catecholamine concentrations were centrifuged immediately at 12 000 g for 1 min and flash-frozen in liquid N₂ before being placed in storage at -80°C. Plasma noradrenaline and adrenaline levels were determined on alumina-extracted samples (200 µl) using high-pressure liquid chromatography (HPLC) with electrochemical detection (Woodward, 1982). 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-C₁₈ 5 µm column (HPLC Technology Ltd), and the separated amines were integrated with 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.

Measurement of water gas tensions

A pump-driven loop continuously withdrew inflowing water and passed it over P_{O_2} and P_{CO_2} electrodes (Radiometer) housed in temperature-controlled cuvettes (13°C) and connected to a Radiometer blood gas analyser. The P_{O_2} electrode was calibrated by pumping a zero solution (2 g l⁻¹ sodium sulphite) or air-saturated water continuously through the electrode sample compartments until stable readings were recorded. The P_{CO_2} electrode was calibrated in a similar manner using mixtures of 0.5% and 1.0% CO₂ in air provided

by a gas-mixing flowmeter (Cameron Instruments). The electrodes were calibrated prior to each individual experiment.

Measurement of cardiorespiratory variables

The dorsal aortic cannula was flushed with heparinised saline (100 i.u. ml⁻¹) to prevent clotting and then connected to a pressure transducer (Bell and Howell) precalibrated against a static column of water. Analog blood pressure signals were measured using Harvard Biopac amplifiers (DA 100). Cardiac output was determined by connecting the ultrasonic flow probe to a small animal blood flow meter (T106, Transonic Systems, Ithaca, NY, USA). All flow probes were pre-calibrated in the factory using diluted mammalian blood (haematocrit 25%) at 13°C. The frequency and amplitude of opercular displacements were assessed as indices of ventilation using a custom-built impedance converter that detected and quantified the changes in impedance between the brass plates attached to the opercula (Peyraud and Ferret-Bouin, 1960). All analog signals (water gas tensions, impedance values and cardiac output, \dot{V}_b) were converted to digital data and stored by interfacing with a data-acquisition system (Biopac Systems Inc.) using Acknowledge data-acquisition software (sampling rate 30 Hz) and a Pentium personal computer. Thus, continuous data recordings were obtained for mass-specific \dot{V}_b , cardiac frequency (f_H ; automatic rate calculation from the pulsatile \dot{V}_b trace), cardiac stroke volume (\dot{V}_s ; \dot{V}_b/f_H), ventilation frequency (f_G ; automatic rate calculation from the raw ventilation impedance traces), ventilation amplitude (V_{AMP} ; the difference between maximum and minimum impedance values), mean blood pressure (arithmetic mean) and systemic vascular resistance (R_S ; mean Pa/\dot{V}_b).

Statistical analyses

The data are reported as means \pm 1 standard error of the mean (S.E.M.). All data were statistically analysed by a two-way repeated-measures analysis of variance followed by a *post-hoc* multiple-comparison test (Bonferroni *t*-test). The limit of statistical significance was 5%.

Results

Series 1: effects of hypercarbia

Bilateral extirpation of the first gill arch caused a lowering of f_H (Fig. 1B) and a decrease in f_G (Fig. 2C) in normocarbic fish. In response to hypercarbia (see Fig. 1), two of the cardiorespiratory responses differed markedly in the intact and gill-extirpated fish. Specifically, the pronounced hypercarbic bradycardia (from 75.0 ± 1.6 to 69.0 ± 2.0 beats min⁻¹; Fig. 1B) and the increase in breathing amplitude (from 0.62 ± 0.06 to 1.0 ± 0.13 cm; Fig. 2B) were absent from fish that had been subjected to bilateral extirpation of the first gill arch. All other significant responses to hypercarbia, including elevated Pa (Fig. 1C), increased R_S (Fig. 1D) and raised plasma catecholamine levels (Table 1), were unaffected by extirpation of the first gill arch. Ventilation frequency (Fig. 2C) and

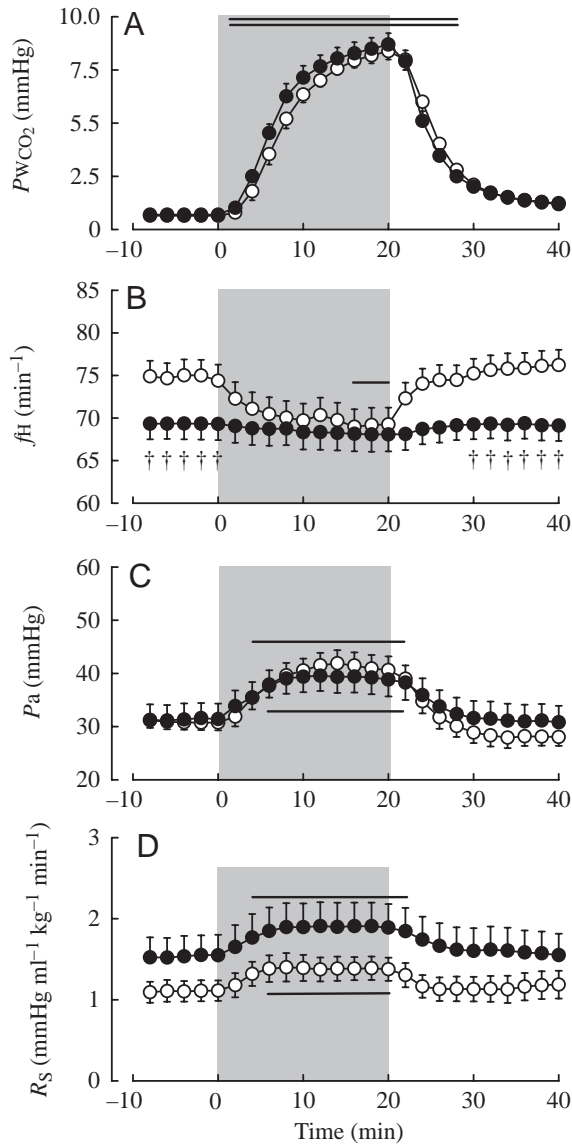


Fig. 1. The effects of acute hypercarbia (indicated by the shaded areas) on (A) water P_{CO_2} (P_{wCO_2}), (B) cardiac frequency (f_H), (C) arterial blood pressure (P_a) and (D) systemic vascular resistance (R_S) in rainbow trout (*Oncorhynchus mykiss*) previously subjected to sham extirpation (controls; open circles; $N=16$) or bilateral extirpation (filled symbols; $N=12$) of the first gill arch. Values are means \pm 1 S.E.M.; significant differences ($P<0.05$) from pre-hypercarbic values (time=0) are denoted by horizontal lines, whereas significant differences ($P<0.05$) between control and extirpated fish are indicated by daggers. 1 mmHg=0.133 kPa.

cardiac output (data not shown) were not statistically altered by hypercarbia in either treatment group. Despite the bradycardia, cardiac output was maintained in the intact fish during hypercarbia owing to an approximately 20% reduction in cardiac stroke volume (from 0.36 ± 0.02 to 0.42 ± 0.03 ml kg^{-1}). Pre-treatment of fish with the muscarinic receptor antagonist atropine prevented a significant decline in f_H during hypercarbia (Fig. 3A) without affecting any other

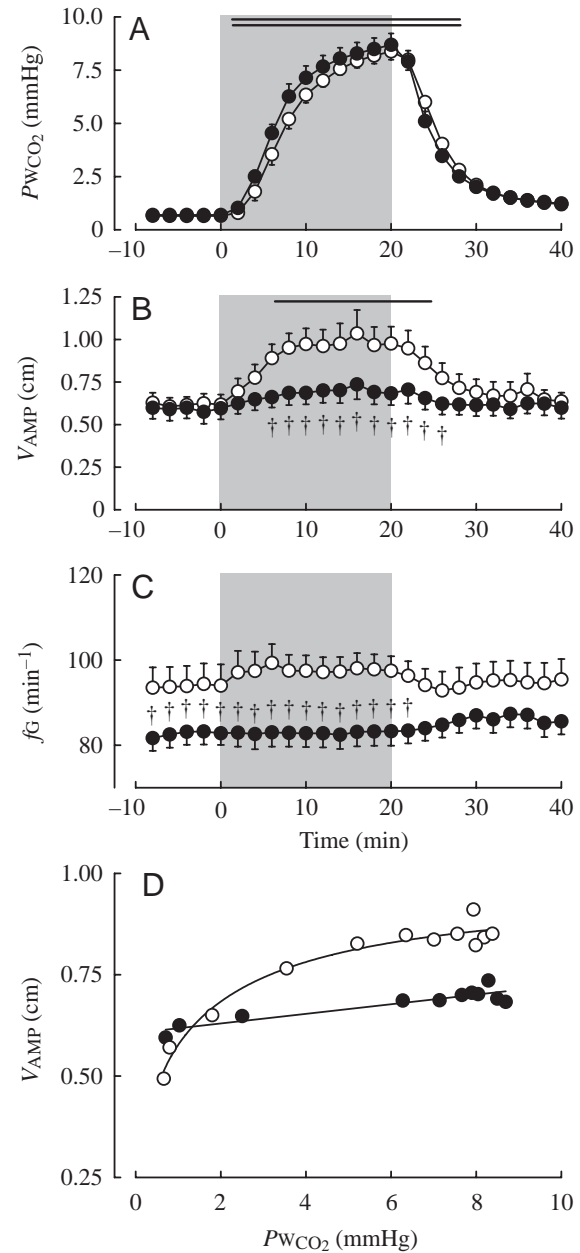


Fig. 2. (A–C) The effects of acute hypercarbia (indicated by the shaded areas) on (A) water P_{CO_2} (P_{wCO_2} ; same data as Fig. 1A), (B) ventilation amplitude (V_{AMP}) and (C) ventilation frequency (f_G) in rainbow trout *Oncorhynchus mykiss* previously subjected to sham extirpation (controls; open circles; $N=16$) or bilateral extirpation (filled symbols; $N=12$) of the first gill arch. Values are means \pm 1 S.E.M.; significant differences ($P<0.05$) from pre-hypercarbic values (time=0) are denoted by horizontal lines, whereas significant differences ($P<0.05$) between control and extirpated fish are indicated by daggers. (D) Relationships between water P_{CO_2} (P_{wCO_2}) and V_{AMP} in intact (open circles; $r^2=0.97$) and gill-extirpated (filled circles; $r^2=0.84$) rainbow trout. The data represent mean values for 2 min intervals immediately prior to, and during, the 20 min period of hypercarbia. The non-linear regression (double rectangular four-parameter hyperbola) for the intact fish was generated using iterative curve-fitting software (Sigmaplot 2001; SPSS Inc.). 1 mmHg=0.133 kPa.

Table 1. The effects of acute hypercarbia on plasma catecholamine levels in rainbow trout (*Oncorhynchus mykiss*)

	Intact fish			Extirpated fish		
	[NA]	[A]	Total concentration	[NA]	[A]	Total concentration
Pre	2.0±0.3	8.6±1.2	10.6±1.4	0.2±0.1	3.2±0.9	3.3±0.9
Hypercarbia	5.4±2.2*	17.4±3.1*	22.9±3.6*	6.4±2.0*	23.0±7.2*	29.4±8.8*
Post	1.7±0.3	10.3±1.6	11.9±1.9	2.2±0.4	9.7±3.9	12.9±4.3

A, adrenaline; NA, noradrenaline; Post, post-hypercarbia.

Data shown are means ± 1 S.E.M. (N=7); an asterisk indicates a significant difference from the pre-hypercarbia (Pre) value.

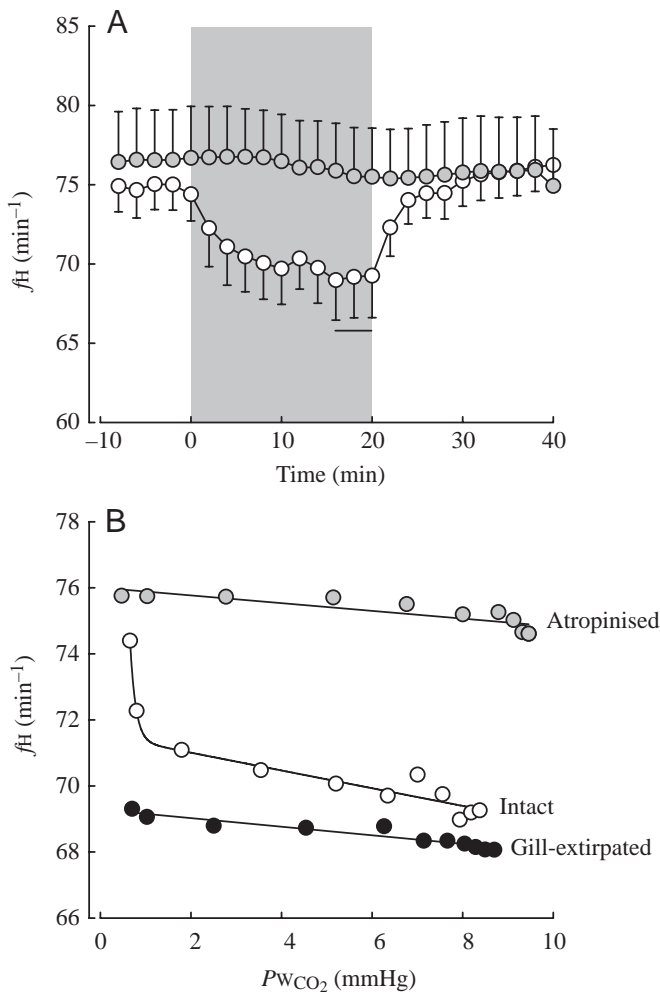


Fig. 3. (A) The effects of acute hypercarbia (indicated by the shaded area) on cardiac frequency (f_H) in control (open circles; $N=16$) and atropinised (shaded symbols; $N=7$) rainbow trout (*Oncorhynchus mykiss*). The data are presented as means ± 1 S.E.M.; significant differences ($P<0.05$) from pre-hypercarbic values (time=0) are denoted by a horizontal line. (B) Relationships between water P_{CO_2} (P_{wCO_2}) and f_H in intact (open circles; $r^2=0.97$), atropinised (shaded circles; $r^2=0.75$) and gill-extirpated (filled circles; $r^2=0.90$) rainbow trout. The data represent mean values for 2 min intervals immediately prior to, and during, the 20 min period of hypercarbia. The non-linear regression (four-parameter exponential decay) for the intact fish was generated using iterative curve-fitting software (Sigmaplot 2001; SPSS Inc.). 1 mmHg=0.133 kPa.

these results closely resembled the cardiorespiratory responses to hypercarbia (see above). Extirpation of the first gill arch prevented the bradycardia, the decline in \dot{V}_b and the increase in V_{AMP} associated with externally injected CO_2 -enriched water; all other responses were unaffected by gill extirpation.

Series 2: effects of internal CO_2

The injection of CO_2 -enriched saline into the caudal vein (Fig. 5) caused only minor changes in the measured cardiorespiratory variables: an increase in P_a and a slight, but significant, bradycardia (from 73.0 ± 2.8 to 70.3 ± 3.5 beats min^{-1}). The increase in P_a was also observed in fish injected with air-equilibrated saline, suggesting that this response (as well as an increase in \dot{V}_b) was a consequence of vascular volume loading. To ensure that the administered saline was reaching putative internal chemoreceptive sites, a bolus of sodium cyanide (0.1 mg kg^{-1}) was injected into the caudal vein. This procedure resulted in a marked increase in plasma catecholamine levels (Table 3) as well as initiating marked cardiorespiratory reflexes (f_H declined from 74.4 ± 2.7 to 38.3 ± 6.0 beats min^{-1} and V_{AMP} increased from 0.53 ± 0.07 to 0.72 ± 0.05 cm; S. G. Reid and S. F. Perry, unpublished data). These data indicate that the injected saline was at least reaching sites of O_2 chemoreception.

measured variable (data not shown). Correlation analysis, however, revealed shallow but significant relationships between water P_{CO_2} and ventilation amplitude (Fig. 2D) and cardiac frequency (Fig. 3B) in both atropinised and extirpated fish.

Series 2: effects of external CO_2

The effects of injecting CO_2 -enriched water into the buccal cavity are depicted in Fig. 4. In the intact fish, externally applied CO_2 caused a reduction in f_H and \dot{V}_b as well as increases in P_a , R_S , V_{AMP} and plasma catecholamine levels (Table 2); f_G was unaffected. Except for the reduction in \dot{V}_b ,

Discussion

Various techniques have been used to localise branchial chemoreceptors in fish, including selective irrigation of gill arches (Smith and Jones, 1978; Daxboeck and Holeton, 1978),

Table 2. The effects of a bolus injection of CO₂-enriched water into the buccal cavity on plasma catecholamine levels in rainbow trout (*Oncorhynchus mykiss*)

	Intact fish			Extirpated fish		
	[NA]	[A]	Total concentration	[NA]	[A]	Total concentration
Pre-CO ₂	3.8±2.2	6.7±0.8	10.5±1.9	1.2±0.3	6.4±0.9	7.8±1.2
External CO ₂	9.0±6.0	9.6±1.0	18.6±6.2*	2.5±1.2	10.7±2.3	14.2±3.2*

A, adrenaline; NA, noradrenaline.
Data shown are means ± 1 S.E.M. (N=7); an asterisk indicates a significant difference from the pre-CO₂ value.

physical removal of gill arches (Smith and Jones, 1978) and partial or total branchial denervation (Smith and Jones, 1978; Smith and Davie, 1984; Bursleson and Smatresk, 1990; McKendry et al., 2001). Despite reports of successful denervation of the first gill arch in salmonids (Smith and Jones, 1978; Smith and Davie, 1984), we were unable to section the required nerves (cranial nerves IX and X) in trout without severely impairing their ability to breathe (amplitude and frequency were reduced) and to tolerate hypercarbia. Thus, in the present study, we opted to assess the involvement of the first gill arch in CO₂ chemoreception by its ligation and removal, a procedure not previously used in studies of CO₂ chemoreception in fish. According to Davis (1971), extirpation of the first pair of gill arches would cause an approximately 30% reduction in gill surface area. Although a 30% reduction in gill surface area, *per se*, has little impact on levels of arterial blood gases in resting fish (Davis, 1971; Julio et al., 2000), specific bilateral extirpation of the first gill arch was previously shown to cause a significant lowering of arterial P_{O₂} in trout (Davis, 1971). This effect was attributed to destruction of the pseudobranch following disruption of its blood supply rather than to loss of gill surface area. Thus, while not specifically assessed in the present study, the experimental design used probably contributed to arterial hypoxaemia and loss of pseudobranch function. Although we cannot exclude altered blood respiratory status as a factor contributing to the present results, a significant contribution appears unlikely given (i) that

the responses to CO₂ were mediated almost exclusively by external receptors (see below) and (ii) that the cardiorespiratory responses to hypercarbia in trout appear to be insensitive to initial P_aCO₂ (Perry et al., 1999) or P_aO₂ within the physiological range (K. Gill and S. F. Perry, unpublished observations). To confirm that the pseudobranch was not a site of CO₂ chemoreception, experiments were performed on fish experiencing only denervation of the pseudobranch (Randall and Jones, 1973); their response to hypercarbia was unaffected (S. F. Perry and S. G. Reid, unpublished observations).

There was no attempt in the present study to discern between the specific effects of CO₂ *versus* H⁺ in the initiation of cardiorespiratory responses to hypercarbia. However, despite some earlier indirect evidence for H⁺ reception in fish (Heisler

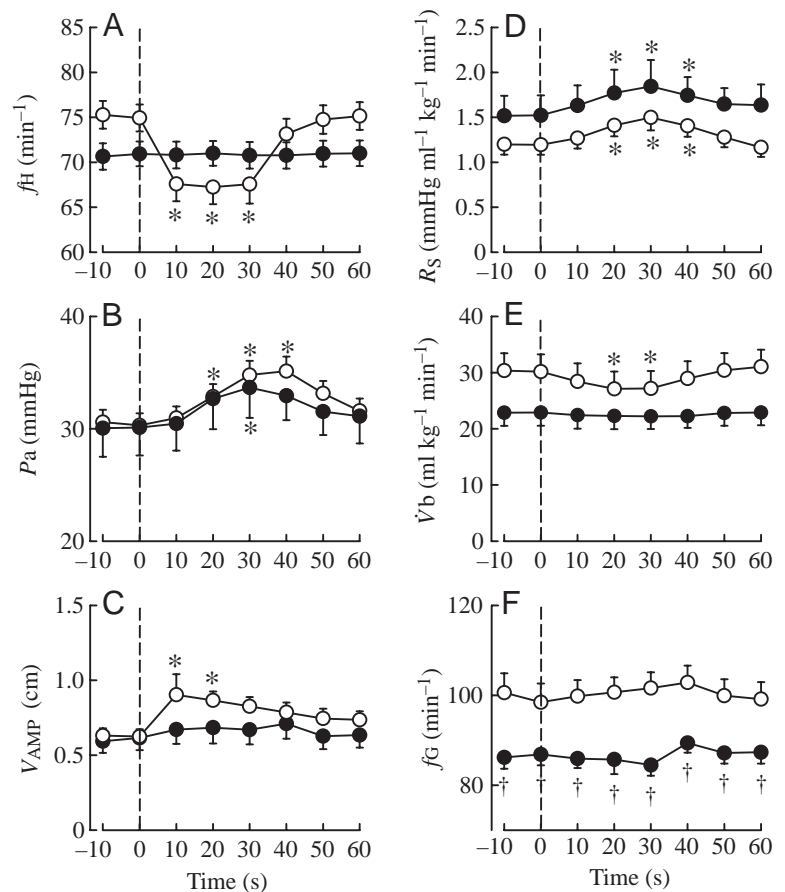


Fig. 4. The effects of a bolus injection of CO₂-enriched water (5% CO₂) into the buccal cavity on (A) cardiac frequency (*f_H*), (B) arterial blood pressure (*P_a*), (C) ventilation amplitude (*V_{AMP}*), (D) systemic vascular resistance (*R_s*), (E) cardiac output (*V_b*) and (F) ventilation frequency (*f_G*) in rainbow trout *Oncorhynchus mykiss* previously subjected to sham extirpation (controls; open circles; N=22) or bilateral extirpation (filled symbols; N=11) of the first gill arch. Values are means ± 1 S.E.M.; significant differences (*P* < 0.05) from pre-injection values (time=0) are denoted by asterisks, whereas significant differences (*P* < 0.05) between control and extirpated fish are indicated by daggers. 1 mmHg=0.133 kPa.

Table 3. The effects of a bolus injection of CO₂-enriched saline or cyanide into the caudal vein on plasma catecholamine levels in rainbow trout (*Oncorhynchus mykiss*)

	Intact fish		
	[NA]	[A]	Total concentration
Pre-CO ₂	2.1±0.5	6.9±1.9	9.0±2.0
Internal CO ₂	1.8±0.8	7.3±2.3	9.2±2.8
Pre-cyanide	1.7±0.5	6.0±2.5	7.7±2.7
Internal cyanide	22.8±5.4*	54.4±18.3*	77.2±19.6*

A, adrenaline; NA, noradrenaline.

Data shown are means ± 1 S.E.M. (N=6); an asterisk indicates a significant difference from the pre-treatment value.

et al., 1988; Graham et al., 1990; Wood et al., 1990), the results of more recent studies that have attempted to distinguish between CO₂ and H⁺ receptors are consistent with receptors that are predominantly activated by changes in extracellular P_{CO₂} (Sundin et al., 2000; Reid et al., 2000; Perry and McKendry, 2001). Regardless, as in mammals (Gonzalez et al., 1992), the proximate stimulus for CO₂ chemoreception is likely to be a decrease in the intracellular pH of the chemosensory cells (Gilmour, 2001).

In the presence of elevated ambient CO₂ tensions, trout exhibit bradycardia, increased arterial blood pressure (caused by an increase in systemic resistance) and an elevation of ventilation amplitude. This pattern of cardiorespiratory adjustment in response to CO₂, while consistently observed in salmonids (Perry et al., 1999; McKendry and Perry, 2001; for a review, see Perry and Gilmour, 2002), varies markedly among the other species that have been examined. For example, bradycardia, while exhibited by several species, is not displayed by channel catfish (*Ictalurus punctatus*; Burleson and Smatresk, 2000), white sturgeon (*Acipenser transmontanus*; Crocker et al., 2000) or American eel (*Anguilla rostrata*; see Table 1 in Perry and Gilmour, 2002) and thus cannot be considered a universal response to hypercarbia. Similarly, there is marked inter-specific variation in the blood pressure responses to hypercarbia spanning almost all possible

response patterns (see Table 2 in Perry and Gilmour, 2002). Although hyperventilation is a common response to elevated ambient P_{CO₂} (Gilmour, 2001), the sensitivity of the response varies amongst species, with some fish (e.g. trout) exhibiting a high degree of sensitivity (Thomas, 1983) and others (e.g. carp *Cyprinus carpio*) displaying relative insensitivity (Soncini and Glass, 2000).

Two previous studies have attempted to localise CO₂ chemoreceptors to the first gill arch. Sundin et al. (2000) demonstrated that hypercarbic bradycardia in tambaqui was mediated exclusively by first-gill-arch receptors whereas ventilatory responses were probably caused by stimulation of chemoreceptors on all arches. In contrast, the elevation of blood pressure during hypercarbia in tambaqui appeared to involve stimulation of extrabranchial receptors because the response persisted in fish experiencing total gill denervation (Sundin et al., 2000). Reid et al. (2000), however, were unable to provide any evidence for specific first-gill-arch CO₂ receptors in traíra and concluded that the hyperventilation and bradycardia that accompany hypercarbia in this species arise from branchial receptors that are present on more than just the first gill arch. In the only other studies to utilise denervation techniques to identify branchial CO₂ chemoreceptors (Burleson and Smatresk, 2000; McKendry et al., 2001), the gills were totally denervated and thus it was not possible to ascribe any preferential role to the first gill arch.

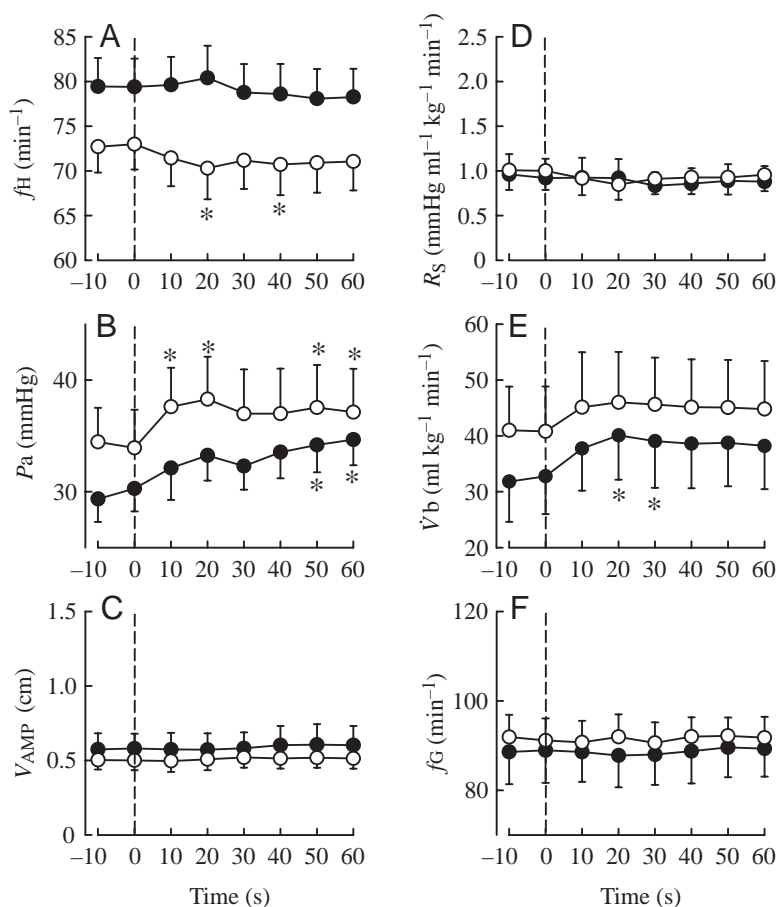


Fig. 5. The effects of a bolus injection of CO₂-enriched saline (5% CO₂; open circles; N=8) or air-equilibrated saline (controls; filled circles; N=8) into the caudal vein on (A) cardiac frequency (f_H), (B) arterial blood pressure (P_a), (C) ventilation amplitude (V_{AMP}), (D) systemic vascular resistance (R_S), (E) cardiac output (\dot{V}_b) and (F) ventilation frequency (f_G) in intact rainbow trout (*Oncorhynchus mykiss*). Values are means ± 1 S.E.M.; significant differences ($P < 0.05$) from pre-injection values (time=0) are denoted by asterisks. There were no significant differences between the control and treatment groups at any measurement time. 1 mmHg=0.133 kPa.

In the present study, both the bradycardia and the increase in ventilation amplitude caused by hypercarbia were initiated predominantly by receptors confined to the first gill arch. A shallow, yet statistically significant, positive correlation between P_{wCO_2} and ventilation amplitude was observed in fish previously subjected to extirpation of the first gill arch. This finding indicates the involvement, albeit limited, of a population of CO_2 receptors that are not specifically confined to the first arch. Upon examining the correlations between P_{wCO_2} and ventilation amplitude (Fig. 2), it would appear that the first-gill-arch receptors are particularly important in mediating the ventilatory adjustments at relatively low levels of P_{wCO_2} (e.g. <3 mmHg). Indeed, the ventilatory responses to higher levels of CO_2 (>3 mmHg) appear to be similar in the intact and extirpated fish. Similarly, a shallow negative correlation was observed between P_{wCO_2} and f_H in extirpated fish (Fig. 3). Because this response was unaltered by atropine, it probably reflects a direct effect of CO_2 on the heart rather than the presence of additional chemosensory sites for CO_2 . Alternatively, the bradycardia could reflect a loss of sympathetic neuronal tone to the heart that could not be overcome by increased humoral sympathetic stimulation (a consequence of the elevated plasma catecholamine levels).

Unlike in previous studies (Sundin et al., 2000; Reid et al., 2000), the present study included additional experiments designed to discern between external and internal orientation of the branchial CO_2 receptors. To preferentially stimulate presumptive external receptors, a bolus of CO_2 -enriched water was injected into the buccal cavity, whereas preferential stimulation of presumptive internal receptors was achieved by injecting CO_2 -enriched saline into the caudal vein. The interpretation of data from such experiments, however, is not totally straightforward because the injection of CO_2 -enriched water over the external surface of the gills would lead to some entry of CO_2 into the fish and thus cause the possible stimulation of both external and internal chemoreceptors. Similarly, the injection of CO_2 -enriched saline into the circulation could potentially activate both internal and external receptors because of additional excretion of CO_2 into the ventilatory water. Despite the improbability of exclusively stimulating a single population of receptors (external or internal), it is likely that preferential stimulation of receptor populations did occur in the present study. In other words, the extent of activation of putative internal receptors would be trivial during external injections in comparison with internal injections and *vice versa*.

The results clearly revealed the presence of external receptors linked to bradycardia, increased systemic resistance, elevated ventilation amplitude and catecholamine release. The external receptors linked to the cardiac and ventilation responses were confined to the first gill arch, whereas the receptors linked to resistance/pressure changes and catecholamine secretion were more widely distributed amongst all the gill arches and/or present on other external surfaces within the orobranchial cavity. The link between activation of external CO_2 chemoreceptors and elevation of plasma catecholamine levels is particularly interesting considering

current models for catecholamine secretion in fish contend that the effects of CO_2 (at least in part) are indirectly mediated by an impairment of blood O_2 transport (Reid et al., 1998). While we cannot resolve the relative importance of the direct and indirect actions of CO_2 on catecholamine secretion during hypercarbia, the potential for external CO_2 receptors to contribute directly to catecholamine secretion should be incorporated into future models. The evidence for the notion that CO_2 cannot directly stimulate catecholamine secretion was obtained in experiments that compared responses of trout to hypercarbia under normoxic or hyperoxic conditions (Perry et al., 1989). Because hyperoxia prevented both the lowering of arterial O_2 content and catecholamine release during hypercarbia, it was argued that hypercarbia, itself, could not be a specific stimulus. In the light of the present results, however, an alternative explanation is that hyperoxia caused a blunting of the response of the CO_2 chemoreceptors to hypercarbia.

Apart from a slight bradycardia, there were no specific effects of injecting CO_2 internally on cardiorespiratory function or plasma catecholamine levels. These results reaffirm the conclusions of previous studies that externally oriented chemoreceptors responding to changes in the P_{CO_2} of the ambient water are largely responsible for initiating the reflex responses to hypercarbia in trout (McKendry and Perry, 2001) and dogfish (Perry and McKendry, 2001). This differs from the situation for O_2 chemoreceptors, where separate populations of external and internal receptors are thought to play specific roles in mediating ventilatory and cardiac responses to altered ambient O_2 levels. For example, it is believed that external receptors preferentially confined to the first gill arch are linked to cardiovascular and ventilatory reflexes, whereas more broadly distributed internal receptors are linked to ventilatory reflexes (Burlinson et al., 1992).

Removal of the first gill arch, in the absence of any other treatment, resulted in bradycardia and a decrease in breathing frequency. These results indicate that there may be tonic neuronal output originating from the first gill arch that serves to elevate heart rate and breathing frequency under resting conditions.

The branchial O_2 chemoreceptors are believed to be phylogenetic antecedents of the mammalian carotid body chemoreceptors (Fritsche and Nilsson, 1993). While it is known that the same carotid body chemoreceptors respond to alterations in both P_{O_2} and P_{CO_2} (O'Regan and Majcherczyk, 1982), the response modalities of the branchial chemoreceptors are uncertain. Clearly, there are receptors associated with the gill that respond to changes in P_{O_2} and P_{CO_2} , but whether the same receptor responds to both stimuli has not yet been established. While we cannot exclude the presence of a class of receptor that responds to both O_2 and CO_2 , a comparison of the results of the present study (focusing on CO_2) with previous studies (focusing on O_2) does provide evidence for separate populations of O_2 - and CO_2 -sensing receptors. For example, the internal gill receptors (which are not confined to the first gill arch) linked to hypoxic hyperventilation (Fritsche and Nilsson, 1993) appear to be insensitive to CO_2 given (i) that

extirpation of the first gill arch eliminated the hyperventilation response to hypercarbia and (ii) that selective elevation of internal P_{CO_2} did not evoke a ventilatory response. Recent studies on traira (*Hoplias malabaricus*) that compared O_2 -mediated (Sundin et al., 1999) and CO_2 -mediated (Reid et al., 2000) reflexes also provided evidence for unique populations of branchial O_2 - and CO_2 -sensing receptors. Future research should be directed at determining whether, by analogy to the mammalian carotid body, there is a class of branchial receptor able to respond to changes in both P_{O_2} and P_{CO_2} .

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