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INFLUENCE OF HYPOXIA AND ADRENALINE ADMINISTRATION ON CORONARY BLOOD FLOW AND CARDIAC PERFORMANCE IN SEAWATER RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Summary

To investigate the relationship between cardiac performance and coronary perfusion, cardiovascular variables ($\dot{Q}$, V_S, f_H, P_{DA}) and coronary blood flow ($\dot{q}_{cor}$) were measured in rainbow trout (*Oncorhynchus mykiss*) (1.2–1.6 kg) before and after adrenergic stimulation (1.0 μg kg^{-1} adrenaline) under conditions of (1) normoxia, (2) hypoxia (approximate $P_{W_{O_2}}$ 12 kPa) and (3) 2.5 h after returning to normoxia. $\dot{q}_{cor}$ for resting fish under normoxic conditions was 0.14±0.02 ml min^{-1} kg^{-1} (approximately 0.85 % of $\dot{Q}$). When exposed to hypoxia, although both resting $\dot{Q}$ and $\dot{q}_{cor}$ increased, $\dot{q}_{cor}$ increased to a greater degree ($\dot{Q}$ by 17 % and $\dot{q}_{cor}$ by 36 %). During hypoxia, maximum adrenaline-stimulated $\dot{Q}$ was comparable to that observed for normoxic fish. However, because $\dot{Q}$ was elevated in resting hypoxic fish, the capacity of hypoxic fish to increase $\dot{Q}$ above resting levels was 50 % lower than that measured in normoxic fish. Although maximum $\dot{q}_{cor}$ in adrenaline-injected hypoxic trout was greater than that measured in normoxic trout, post-injection increases in $\dot{q}_{cor}$ (above resting levels) were not different between the two groups. Two and a half hours after hypoxic exposure, resting $\dot{Q}$ was still elevated (11 %) above normoxic levels, and the ability to increase $\dot{Q}$ when adrenergically stimulated was not fully restored. These results suggest (1) that resting $\dot{q}_{cor}$ in salmonids is approximately 1 % of $\dot{Q}$, (2) that increases in $\dot{q}_{cor}$ may be important in maintaining cardiovascular performance during hypoxic conditions, (3) that interactions between α-adrenergic constriction and metabolically related vasodilation of the coronary vasculature are important in determining $\dot{q}_{cor}$ in fish, (4) that exposure of fish to moderate environmental hypoxia reduces the scope for adrenergically mediated increases in $\dot{Q}$, and (5) that periods of recovery in excess of several hours are required before cardiovascular performance returns to pre-hypoxic levels.

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Key words: trout, *Oncorhynchus mykiss*, heart, adrenaline, hypoxia, coronary, cardiac performance, catecholamines.

Introduction

In fish, unlike mammals, the presence of a coronary circulation is species-dependent and closely related to activity level (Davie and Farrell, 1991*a*). This relationship implies that a coronary blood supply is only essential for maintaining cardiac function in fish capable of high cardiac work (i.e. high myocardial oxygen demand). Early experimental evidence for this generalization comes from the survival of coronary-ablated fish (Daxboeck, 1982; Farrell and Steffensen, 1987) and from the 35 % reduction in the maximum swimming speed of coronary-ablated trout (Farrell and Steffensen, 1987).

In recent years, the role of the coronary circulation in determining fish cardiac performance has been defined further. Gamperl *et al.* (1994) have shown that adrenaline-stimulated cardiac performance in trout during normoxic conditions is not affected by coronary ablation. In addition, Davie and Farrell (1991*b*) and Davie *et al.* (1992) have shown that perfusion of the coronary circulation with oxygenated red cell suspensions only restored the cardiac power output of hypoxic dogfish (*Squalus acanthias*) and eel (*Anguilla dieffenbechii*) hearts to 50–75 % of levels measured during normoxia. These experiments suggest that oxygen delivery from the luminal blood to the myocardium is not diffusion-limited during normoxic conditions in fish with a small percentage of compact myocardium and that increases in coronary oxygen delivery are unlikely to provide for near-maximal cardiac performance when venous oxygen content is diminished. Axelsson and Farrell (1993) measured coronary blood flow in the coho salmon (*Oncorhynchus mykiss*) during hypoxia, following the injection of adrenaline and during spontaneous activity. However, while these authors were able to show that resting coronary blood flow was approximately 1 % of cardiac output and that coronary blood flow increased upon exposure to all three experimental conditions, their study did not address the question of whether elevations in coronary blood flow are capable of supporting near-maximal cardiac performance during hypoxia.

Farrell (1987) using an *in vitro* perfused rainbow trout heart revealed that arterial pressure, adrenoreceptors, extravascular compression and cardiac metabolism are involved in the determination of coronary blood flow in fish. In addition, *in vivo* studies on coho salmon (Axelsson and Farrell, 1993) showed that α-adrenergic constriction, β-adrenergic vasodilation, dorsal aortic pressure and hypoxic exposure can all affect coronary blood flow. However, no studies have investigated the interactive effects of these variables on coronary flow *in vivo*.

We measured *in vivo* coronary blood flow, cardiac output and dorsal aortic pressure in trout (*Oncorhynchus mykiss*) exposed to adrenergic stimulation under conditions of normoxia and hypoxia. The goals of this study were twofold: (1) to investigate whether cardiac performance is depressed under the combined conditions of hypoxaemia and high output pressures, irrespective of alterations in coronary blood flow; and (2) to determine whether hypoxia (hypoxaemia) alters adrenoreceptor-mediated control of coronary blood flow. Adrenergic stimulation is an appropriate model for studying the *in vivo* relationship between cardiac performance and coronary blood flow because adrenaline increases both cardiac output and systemic vascular resistance; the concomitant increase in cardiac power output increases oxygen demand by the myocardium (Graham and Farrell, 1990).

Materials and methods

Fish

Seawater-adapted rainbow trout [*Oncorhynchus mykiss* (Walbaum)] (900–1500 g) were obtained from Merlin Fish Farms (Wentworth, Nova Scotia) and held in tanks (1 m×1 m×1.5 m) for at least 1 month prior to experimentation. Fish were fed daily, to satiation, on a diet of commercially available feed pellets, but were fasted for 48 h prior to surgery. Photoperiod was 12 h:12 h light:dark. Experiments were conducted between 1 May and 1 August 1992.

Blood P_{O_2} measurements during graded hypoxia

Surgical procedures

Dorsal and ventral aortic cannulae were prepared from PE 50 polyethylene tubing (Clay Adams Intramedic), total length 0.8 m. Bubbles were made 5.0 cm from the tip of the dorsal cannula and 2.5 and 5.0 cm from the tip of the ventral aortic cannula. Trout (1260±102 g) were anaesthetized (0.1 g l^{-1} tricaine methane sulphonate, MS 222) and placed supine in a wetted chamois leather sling. The fish were fitted with a dorsal aortic cannula (Smith and Bell, 1964), after which intermittent retrograde irrigation with sea water containing anaesthetic was begun. Ventral aortic cannulation was subsequently performed using a new technique (Fig. 1). For ventral aortic cannulation, the trout was held upright in the sling, a hole was made at the side of the mouth with a 13 gauge steel needle and a short piece of heated-flared PE 160 tubing was exteriorized through the

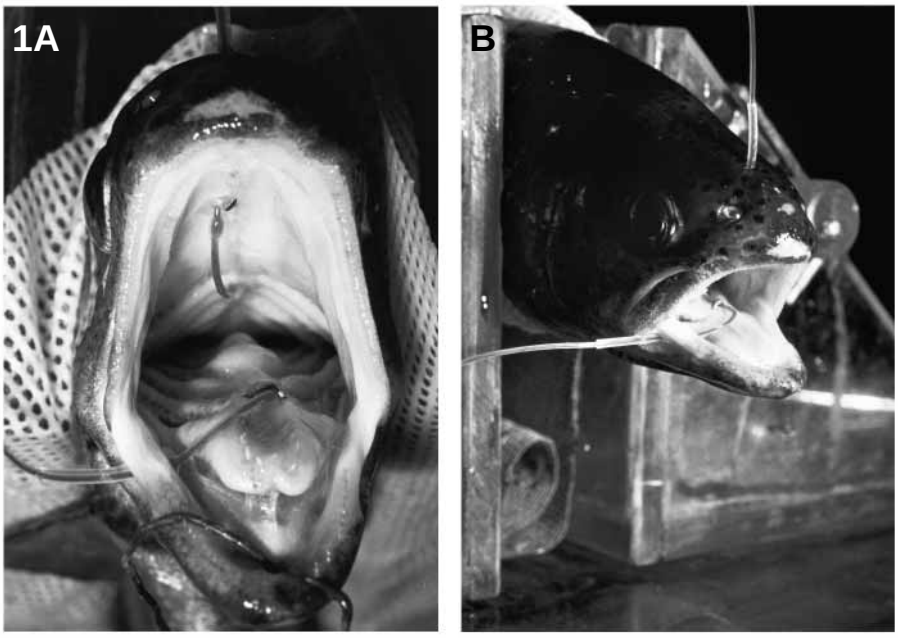

Fig. 1. Photographs of the rainbow buccal cavity (A) and head (B) showing the position of dorsal and ventral aortic cannulae.

resultant hole. A 21 gauge needle was inserted into the cartilage of the tongue (while slowly rotating the needle), just prior to the junction of the second gill arches at an angle of between 30 and 40 °. Once resistance ceased, the needle was removed and a PE 50 cannula, with indwelling stainless-steel wire, was advanced fully into the resultant hole. The steel wire was then withdrawn and the position of the PE 50 tubing was adjusted until blood flowed freely into the cannula. After the cannula had been positioned to maximize blood flow, a wire staple (0.75 cm long, 0.4 cm wide; fashioned from a paperclip) was used to secure the cannula to the tongue at a position just posterior to the first bubble. Finally, the remaining length of the cannula was threaded through the side of the mouth until the second bubble rested firmly against the flared PE 160 tubing, and a constricting knot was tied around the PE 160 tubing to prevent cannula movement.

The main advantages of this technique are: (1) a double cannulation can be completed in 5–7 min and therefore only limited irrigation of the gills is necessary; (2) there is no leakage from the ventral aorta because the cartilaginous tissue of the tongue closes tightly around the cannula; and (3) pressure records and blood samples can be obtained for periods in excess of a week. Although blood can be withdrawn from all ventral aortic cannulae, satisfactory pulsatile pressure records can only be obtained from approximately 50 % of fish cannulated using the above technique. Once surgery had been completed, fish were placed into black Perspex boxes (11 cm×16 cm×75 cm) to recover. Boxes were supplied with aerated sea water (8.6±0.5 °C) at a flow rate of 4 l min^{-1}.

Experimental protocol

After 24–48 h of recovery, to determine a point where fish were experiencing hypoxaemia but not bradycardia, venous P_{O_2} ($P\bar{v}_{O_2}$), arterial P_{O_2} (Pa_{O_2}), heart rate (f_H) and dorsal aortic pressure (P_{DA}) were monitored in trout (N=7) as the sea water P_{O_2} (Pw_{O_2}) was gradually reduced from 19.3 to 8.7 kPa. Pa_{O_2}, $P\bar{v}_{O_2}$, f_H and P_{DA} were measured in trout after every 2 kPa drop in Pw_{O_2}; time between blood samples (0.25 ml) ranged from 25 to 30 min. Seawater oxygen content was manipulated by bubbling a controlled mixture of air and nitrogen through a gas exchange column (8 cm in diameter, 45 cm in length) and was monitored with a YSI oxygen meter (model 50). Water oxygen content (mg l^{-1}) was converted to partial pressure (Pw_{O_2}) on the basis of calibrations obtained with a thermostatted Radiometer O_2 electrode (model 5046-0). If fish struggled during the experiment, a 10 min 'recovery' period was allowed before any further samples were taken. Trout that struggled repeatedly were discarded.

Measurements of cardiovascular performance during hypoxia and adrenergic stimulation

Surgical procedures

Trout (1400±47 g) (N=9) were anaesthetized (0.1 g l^{-1} MS 222) and placed supine in a wetted chamois leather sling. The fish were quickly fitted (approximately 45 s) with a dorsal aortic cannula (PE 50), after which retrograde irrigation with sea water containing anaesthetic (0.05 g l^{-1} MS 222) was begun. A 3–4 cm incision was made through the skin and muscle at a position overlying the ventral aorta and anterior aspect of the

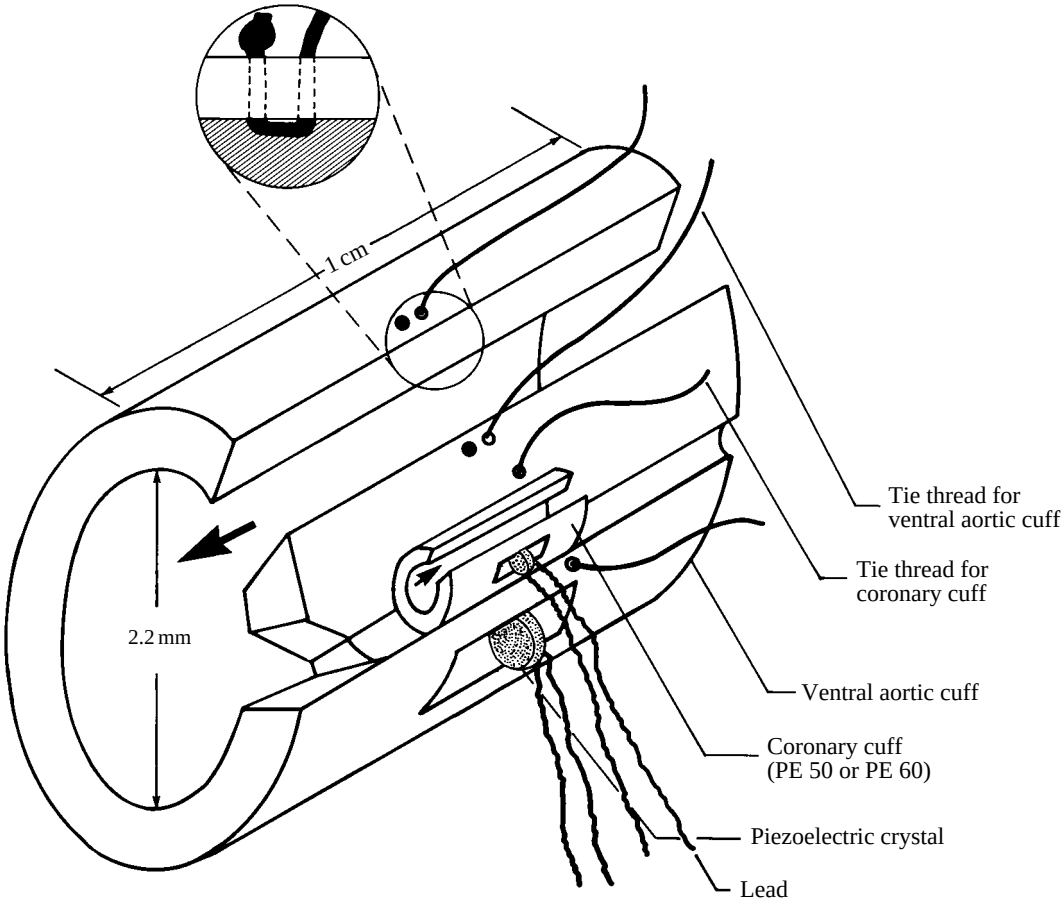

Fig. 2. Schematic diagram of the Doppler flow probes used to measure ventral aortic flow (cardiac output) and coronary flow in intact rainbow trout. Arrows indicate the direction of blood flow. The drawing is 21 times actual size.

pericardium. After cutting through the pectoral girdle and expanding the resultant cavity with tissue spreaders, the anterior portion of the pericardium was cut to expose the ventral aorta and coronary artery. The connective tissue from the anterior portion of the ventral aorta, and that attaching the coronary artery to the ventral aorta and the anterior bulbus, was subsequently removed to facilitate the placement of the Doppler flow probes (Fig. 2). Once the flow probes were in place, the musculature and skin were closed using continuous silk sutures. The Doppler probe leads were secured to the skin at the anterior apex of the incision, and at a position just posterior to the pectoral fins. The operation generally took between 1 and 2 h, and the bleeding was usually minimal. The integrity of the pericardium was not restored by suturing because of time constraints. Once surgery had been completed, the fish were placed into black Perspex boxes to recover. During recovery and subsequent experiments, the boxes were supplied with aerated sea water (10.9±0.9 °C) at a flow rate of 4 l min^{-1}.

Probe design

Flow probes (Fig. 2) were constructed by implanting piezoelectric crystals with 80 cm leads (Crystal Biotech, Hopkinton MA) into a 1 cm length of Tygon tubing (i.d. 2.2 mm) and into a 3–4 mm length of PE 50 or PE 60 tubing; these probes were utilised for the measurement of ventral aortic flow (cardiac output, $\dot{Q}$) and coronary flow ($\dot{q}_{cor}$), respectively. Both sections of tubing were split to facilitate placement on their respective vessels and had small notches to allow for crystal attachment. Piezoelectric crystals were secured in the notches with cyanoacrylate cement after thoroughly roughening the area with a scalpel blade. A notch at the anterior end of the ventral aortic cuff, and a shallow groove along the length of the ventral aortic cuff (for coronary cuff placement), ensured that the coronary artery was not bent or deformed as it passed over the ventral aortic cuff and through the coronary artery cuff. Probes were fitted with tie-strings to prevent tube diameter from increasing at elevated blood pressures.

Experimental protocol

After a 24–48 h recovery period, cardiovascular variables ($\dot{Q}$, f_H, P_{DA} and $\dot{q}_{cor}$) were measured in trout before and after the injection of 1.0 μg kg^{-1} adrenaline (Sigma Chemical Co., St Louis, MO) under three conditions: (1) normoxia; (2) following 30 min of graded hypoxia (final $P_{W_{O_2}}$ 12 kPa); and (3) after recovery from hypoxia. Treatments were separated by 2.5 h. During the normoxic treatments, 'resting' cardiovascular variables and $P_{a_{O_2}}$ were measured 5 min prior to epinephrine injection. In the hypoxic treatment, cardiovascular variables were continuously measured during the graded hypoxia, and 'resting' $\dot{Q}$, f_H, P_{DA}, $\dot{q}_{cor}$ and $P_{a_{O_2}}$ were measured 7–10 min after $P_{W_{O_2}}$ had stabilized at 12 kPa. In all treatments, $\dot{Q}$, f_H, P_{DA} and $\dot{q}_{cor}$ were recorded for 20 min following adrenaline injection. Adrenaline was administered through the dorsal cannula in 0.2–0.4 ml of carrier of saline. The second normoxic period was used to provide some preliminary information on the duration of the recovery period required to restore the scope for adrenergically stimulated cardiovascular performance and to ensure that hypoxia-induced differences in adrenaline-stimulated cardiovascular performance were not solely due to repeated adrenaline injection.

Measurement techniques

P_{DA} was measured by attaching the dorsal aortic cannula to a Gould Statham (model P23-10) pressure transducer connected to a amplifier recorder (Asea Brown and Boveri; model SE-120). Pressure was calibrated daily against a static water column.

Mean ventral aortic flow ($\dot{Q}$) was measured by connecting a pulsed Doppler flowmeter (model 545c-4; Bioengineering, University of Iowa) to an RC integrator and an amplifier recorder. Coronary flow ($\dot{q}_{cor}$) was measured by connecting the Doppler flowmeter to an amplifier recorder. In order to determine absolute flow rates (ml min^{-1}) an *in situ post-mortem* calibration of the ventral aortic flow probe and an *in vitro* calibration of the coronary flow probe were performed using a peristaltic pump (Gilson, MinipulsII) and human blood (approximate haematocrit 20 %). To calibrate the ventral aortic flow probe, the sinus venosus and atrium were removed, the ventricle was bisected laterally and the

peristaltic pump outflow tubing (PE 160) was tied into the ventricular lumen (see Gamperl *et al.* 1994, for further details). To calibrate the coronary flow probe, the coronary artery was cut and the flow probes (with coronary artery in place) were placed in a Petri dish filled with teleost saline (Hoar and Hickman, 1983). After tying a piece of 27 gauge needle, with an attached length of PE 10 tubing, into each end of the segments of coronary artery, the coronary flow probe was calibrated at flow rates between 0.05 and 0.55 ml min^{-1}.

P_{aO_2}, P_{vO_2} and P_{wO_2} were measured using a thermostatted Radiometer O_2 electrode (E 5046-0, Denmark) connected to an amplifier recorder. The P_{O_2} electrode was calibrated with humidified N_2 and air prior to each experiment, and the calibration was rechecked with air prior to each sample.

Analysis

Resting f_H, or f_H at a particular time post-injection, was determined by measuring the number of systolic peaks on the P_{DA} record during a 30 s interval, the interval being 15 s on either side of the desired time. Mean P_{DA} was calculated as [systolic pressure + 2(diastolic pressure)]/3 (Burton, 1972; Wood *et al.* 1979). Stroke volume (V_S) was calculated from $\dot{Q}/f_H$. Coronary vascular resistance (R_{cor}) was calculated as $P_{DA}/\dot{q}_{cor}$ (Axelsson and Farrell, 1993). Statistical differences ($P<0.05$) between treatments (normoxia, hypoxia, normoxia II) for post-injection cardiovascular variables were determined using a repeated-measures analysis of variance (Proc GLM; SAS Institute Inc.) with multiple contrasts. Comparisons between cardiovascular variables at normoxia (P_{wO_2} 19.3 kPa) and at various levels of hypoxia (P_{wO_2} 17.3–12 kPa) were made using the same analysis. Tests for between-subject effects (i.e. type effects, see Results) indicated that response type had no effect on resting or post-injection cardiovascular variables.

Results

Blood P_{O_2} *during graded hypoxia*

As P_{wO_2} was gradually lowered from 19.3 to 8.7 kPa, both arterial and venous P_{O_2} decreased (Fig. 3). In all fish, P_{aO_2} fell linearly ($0.92<r^2<0.99$), while the P_{vO_2} decline appeared to be curvilinear. As P_{wO_2} fell, P_{aO_2} decreased more (by 10.7–12 kPa) than did P_{vO_2} (1.5–3.3 kPa). P_{DA} and f_H remained at the normoxic value until P_{wO_2} was reduced below approximately 10.7–11.3 kPa. However, a severe bradycardia was seen in five of nine trout as P_{wO_2} dropped below 10.7 kPa. Because severe bradycardia would have complicated the comparison of cardiovascular performance and $\dot{q}_{cor}$ between hypoxic and normoxic fish, a P_{wO_2} of 12 kPa was chosen for subsequent experiments. At this P_{wO_2}, P_{aO_2} and P_{vO_2} were 4.7–6.0 kPa and 2.0–3.3 kPa, respectively; levels approximately 40–50 % of those seen during normoxia.

Cardiovascular performance during hypoxia and adrenergic stimulation

Resting cardiovascular variables

Cardiac output, $\dot{q}_{cor}$, P_{DA}, f_H, V_S and R_{cor} during the initial normoxic period averaged

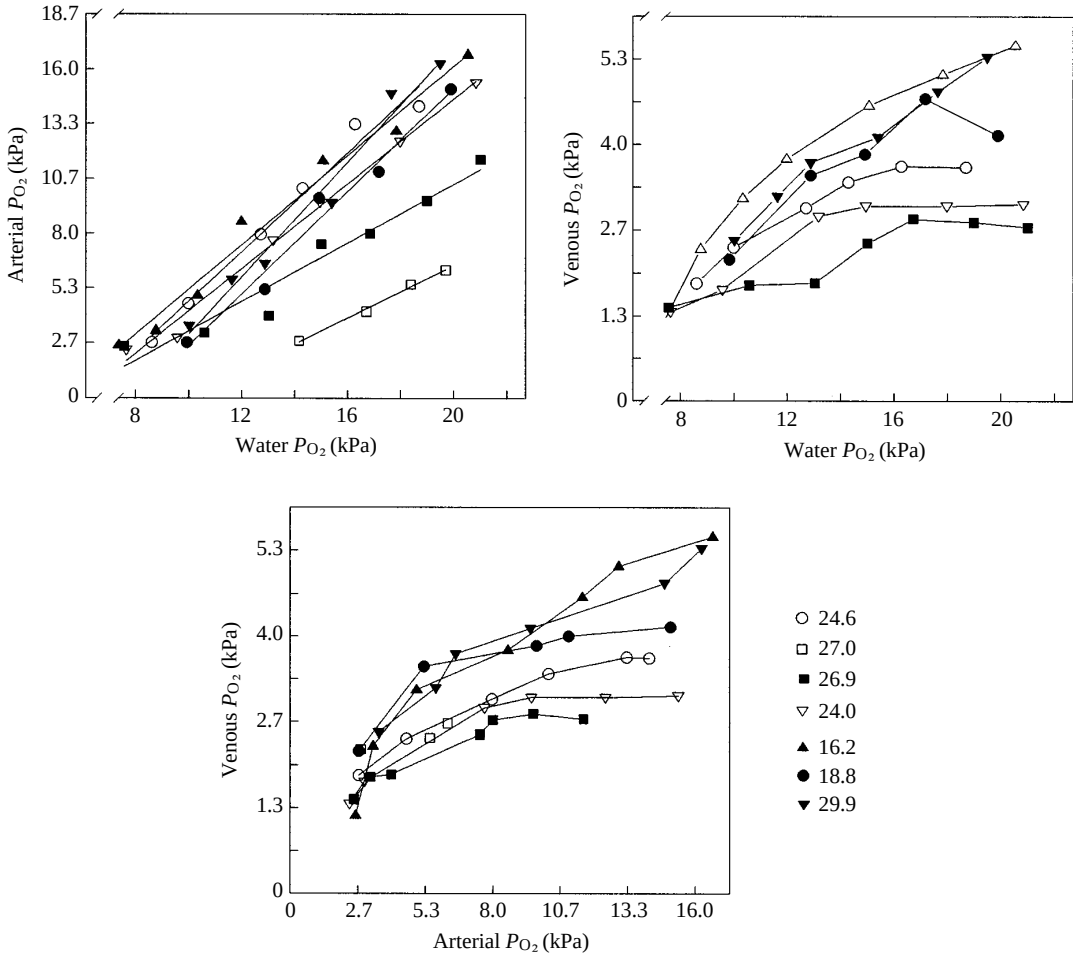

Fig. 3. The relationship between environmental oxygen tension (P_{WO_2}), arterial oxygen tension (P_{aO_2}) and venous oxygen tension (P_{VO_2}) in seven seawater-acclimated rainbow trout as P_{WO_2} was lowered from approximately 20 kPa to 8 kPa over a 3 h period. Numbers beside the symbols (beside C) indicate the haematocrit of individual fish.

18 ml min^{-1} kg^{-1}, 0.14 ml min^{-1} kg^{-1}, 2.8 kPa, 64 beats min^{-1}, 0.29 ml kg^{-1} and 22.5 kPa min kg ml^{-1}, respectively. During graded hypoxia, the P_{WO_2} threshold for hypoxia-induced alterations in cardiovascular variables ($\dot{q}_{cor}$, $\dot{Q}$, f_H, V_S) appeared to be between 13 and 14.5 kPa (Fig. 4). After 30 min of exposure to graded hypoxia and at a P_{WO_2} of 12 kPa, changes in $\dot{Q}$, $\dot{q}_{cor}$, V_S and R_{cor}, but not P_{DA} and f_H, were significant (P<0.05) (Table 1). $\dot{Q}$ and V_S increased by 3.1 ml min^{-1} kg^{-1} (17 %) and 0.07 ml kg^{-1} (25 %), respectively, while R_{cor} decreased by 6.3 kPa min kg ml^{-1} (28 %). Absolute $\dot{q}_{cor}$ increased significantly (0.052 ml min^{-1} kg^{-1}; 36 %) following exposure to graded hypoxia. Although the percentage increase in $\dot{q}_{cor}$ was greater than that for $\dot{Q}$, the percentage of $\dot{Q}$ delivered to the coronary circulation ($\dot{q}_{cor}/\dot{Q}\times100$) was not increased

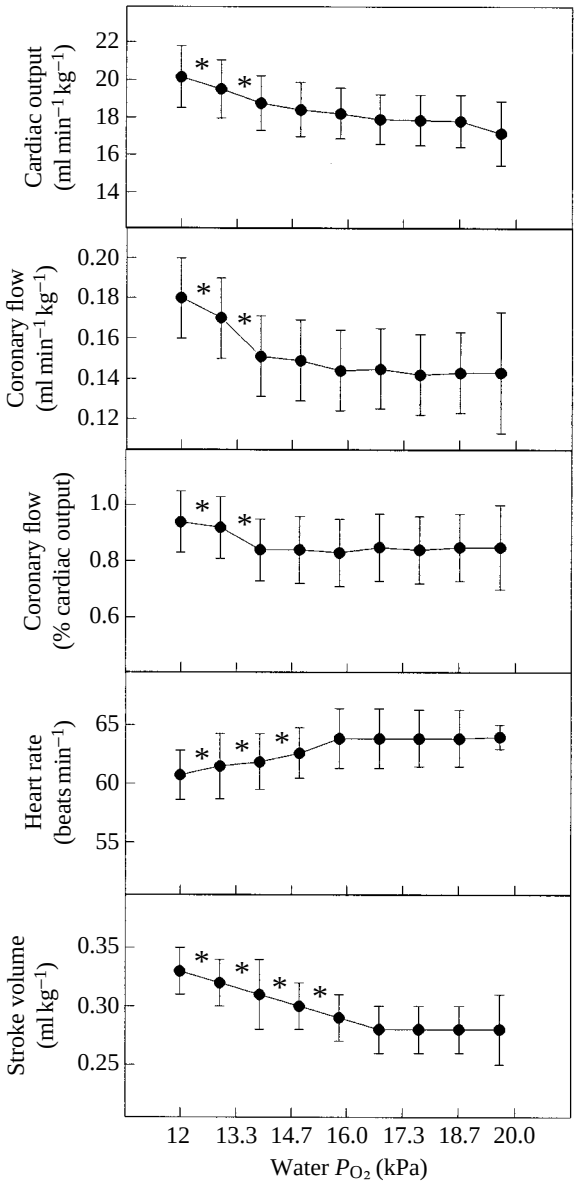

Fig. 4. A summary of the cardiovascular responses to 30 min of graded hypoxia in seawater-adapted rainbow trout at 10–12 °C (N=8). Asterisks indicate statistically significant differences from normoxic conditions ($P_{W O_2}$=20 kPa). Vertical bars indicate the S.E.M. for each mean value.

significantly (0.10>P>0.05). $P_{a O_2}$ fell from 11.7 to 5.1 kPa during the 30 min hypoxic exposure.

Two and a half hours after the trout had been returned to normoxia ($P_{W O_2}$≈20 kPa), values for most resting variables ($\dot{Q}$, $\dot{q}_{cor}$, f_H, V_S and R_{cor}) were intermediate between

Table 1. *Cardiovascular variables in resting rainbow trout (*Oncorhynchus mykiss*) (*N=*8) under conditions of normoxia and hypoxia*

	Normoxia	Hypoxia	Normoxia II
Cardiac output, $\dot{Q}$ (ml min^{-1} kg^{-1})	18.0±1.4^{a}	21.1±1.8^{b}*	20.0±1.5^{ab}
Coronary flow, $\dot{q}_{cor}$ (ml min^{-1} kg^{-1})	0.143±0.020^{a}	0.195±0.019^{b}*	0.148±0.018^{a}
Coronary flow (% cardiac output)	0.837±0.13^{a}†	0.937±0.11^{b}	0.791±0.12^{a}*
Heart rate, f_H (beats min^{-1})	63.6±2.8^{a}†	58.9±2.7^{b}	62.3±2.6^{ab}
P_{DA} (kPa)	2.81±0.17^{a}†	3.02±0.21^{a}*	2.64±0.19^{b}
Stroke volume, V_S (ml kg^{-1})	0.287±0.020^{a}*	0.358±0.02^{b}*	0.322±0.02^{c}*
Pa_{O_2} (kPa)	11.7±1.1^{a}*	5.1±0.5^{b}*	10.7±0.71^{c}*
Coronary resistance, R_{cor} (kPa kg min ml^{-1})	22.5±3.0^{a}*	16.2±1.6^{b}	18.9±2.1^{c}†

Values with dissimilar letters are significantly different at $P<0.05$ (*) and $P<0.10$ (†).

those measured during the initial normoxic period and those recorded during the hypoxic exposure (Table 1). A notable exception was P_{DA}, which was marginally lower than those of the normoxic and hypoxic groups ($P=0.07$). Pa_{O_2} during the second normoxic period was 10.7 kPa, a level significantly lower than that measured during the initial normoxic period (Table 1).

Variations in the response to adrenaline injection

Response type

Adrenergic stimulation during normoxia or hypoxia resulted in two types of cardiovascular responses (Figs 5, 6). Type 1 was characterised by an initial post-injection increase in $\dot{Q}$, which reached a maximum value within 4–6 min. Type 2 was characterised by an initial drop in $\dot{Q}$, followed by a steady increase until maximum values were reached at approximately 10–14 min. As shown in Gamperl *et al.* (1994), these two types of response to adrenaline injection were mediated by type-specific differences in the temporal pattern of f_H and V_S responses. Type 2 trout showed a greater post-injection bradycardia and a 2–4 min delay in the increase in V_S.

Differences in the temporal pattern of changes in coronary flow were also evident between fish exhibiting type 1 and type 2 responses. In type 1 fish, $\dot{q}_{cor}$ increased immediately following adrenaline injection and reached a maximum within 6–8 min (Figs 5, 7). In type 2 fish, increases in $\dot{q}_{cor}$ were delayed by approximately 4 min and maximum $\dot{q}_{cor}$ values were not reached until 8–12 min post-injection (Figs 6, 8). In type 2 fish, $\dot{q}_{cor}$, measured as a percentage of $\dot{Q}$, increased immediately following adrenaline

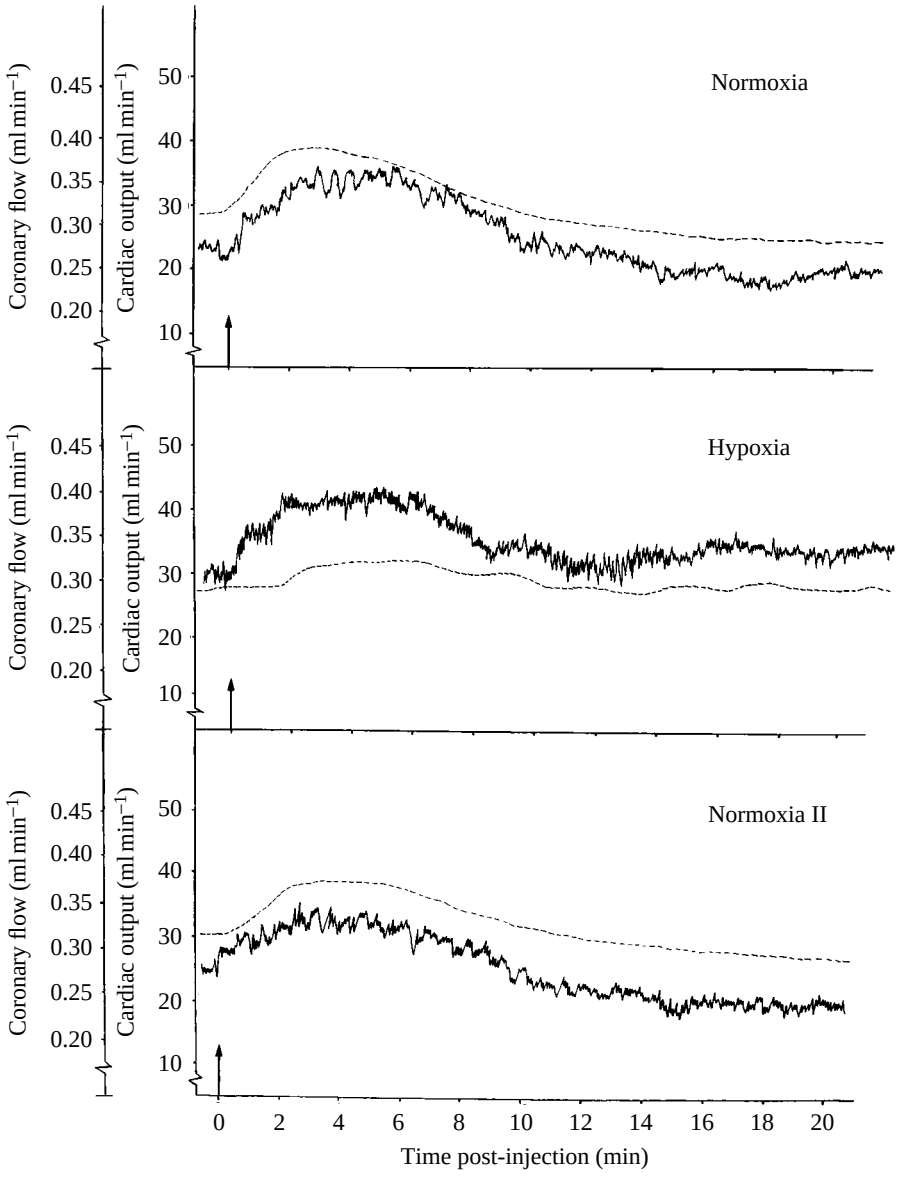

Fig. 5. An original recording of cardiac output (dashed line) and coronary blood flow (solid line) in a rainbow trout exhibiting a type 1 response to the injection of 1.0 μg kg^{-1} adrenaline, under conditions of normoxia (approximate P_{WO_2} 20 kPa), hypoxia (approximate P_{WO_2} 12 kPa) and after 2.5 h of recovery from hypoxia (Normoxia II). Arrows indicate the point of adrenaline injection.

injection and remained elevated until 6 min post-injection. In contrast, type 1 fish displayed no post-injection alterations in the percentage of $\dot{Q}$ delivered to the coronary circulation (see Fig. 10).

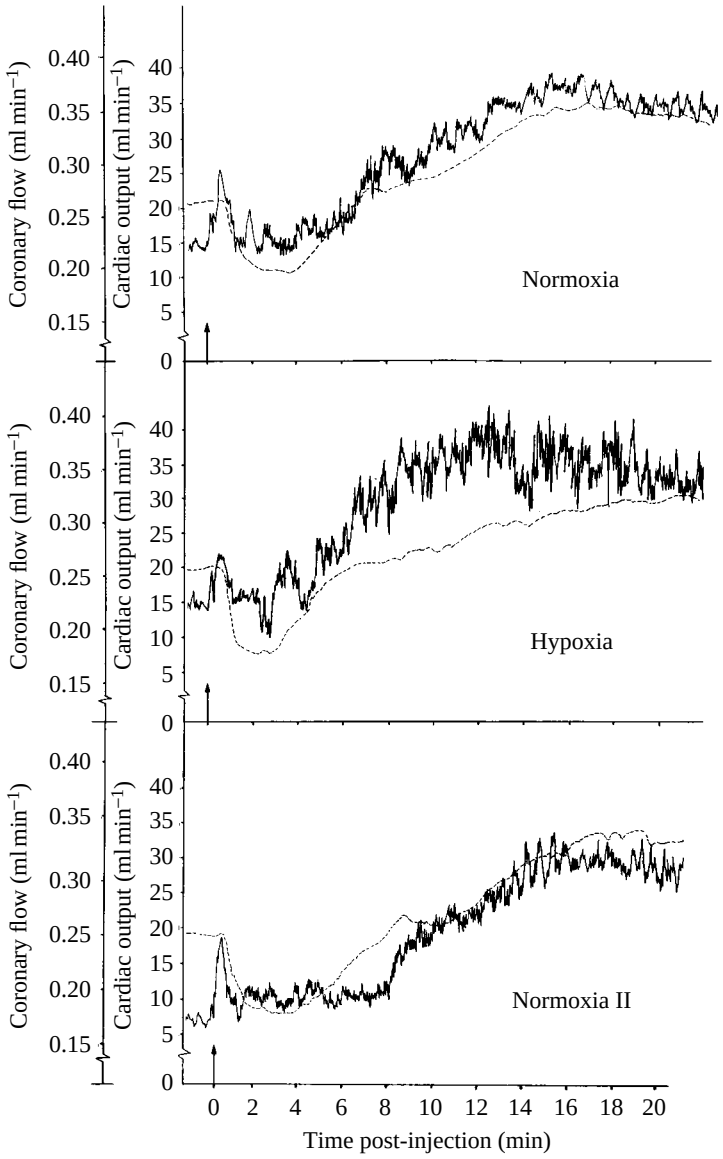

Fig. 6. An original recording of cardiac output (dashed line) and coronary blood flow (solid line) in a rainbow trout exhibiting a type 2 respose to the injection of 1.0 μg kg^{-1} adrenaline, under conditions of normoxia (approximate $P_{W_{O_2}}$ 20 kPa), hypoxia (approximate $P_{W_{O_2}}$ 12 kPa) and after 2.5 h of recovery from hypoxia (Normoxia II). Arrows indicate the point of adrenaline injection.

Response magnitude

The degree to which hypoxic exposure affected adrenergically stimulated cardiovascular variables and $\dot{q}_{cor}$ was quantitatively similar in both response types

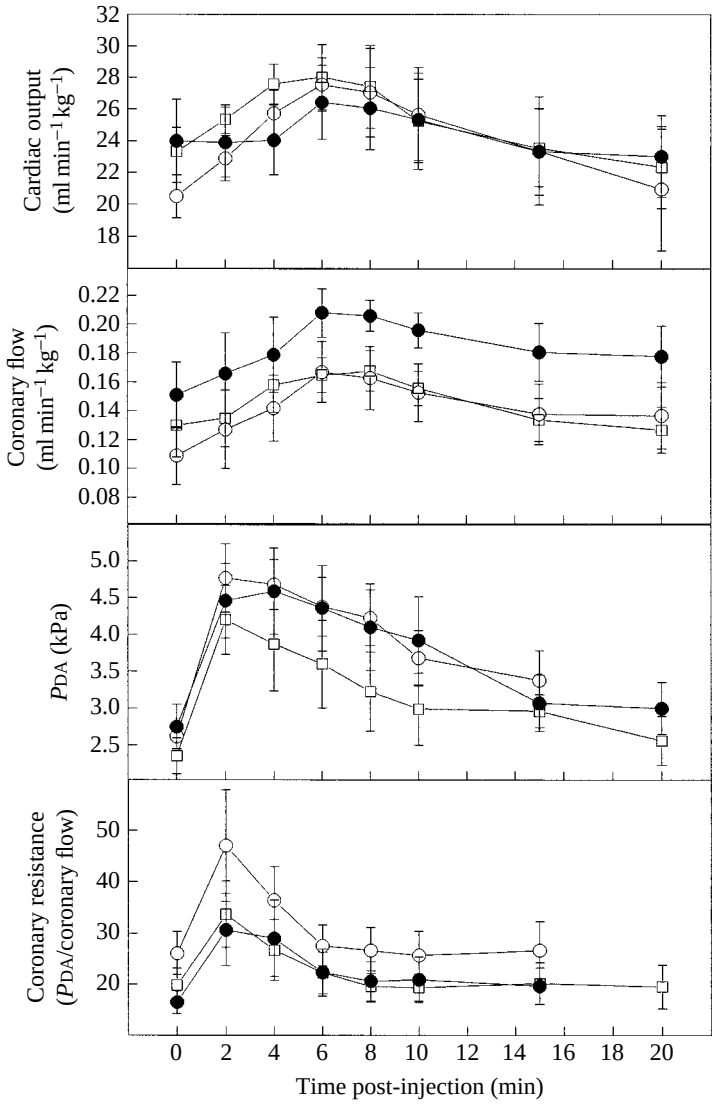

Fig. 7. Effect of adrenaline injection (1.0 μg kg^{-1}) on cardiac output ($\dot{Q}$), coronary blood flow ($\dot{q}_{cor}$), dorsal aortic pressure (P_{DA}) and coronary artery resistance (R_{cor}) in a type 1 rainbow trout (N=4) under conditions of normoxia (○; approximate P_{WO_2} 20 kPa), hypoxia (●; approximate P_{WO_2} 12 kPa) and after 2.5 h of recovery from hypoxia (□). Values are missing when N<4. Vertical bars indicate the S.E.M. for each mean value.

(Figs 5, 6). Adrenaline injection into normoxic trout increased $\dot{Q}$ by 45 %, $\dot{q}_{cor}$ by 66 % and P_{DA} by 100 % (Figs 7, 8; Table 2). However, adrenaline injection into hypoxic fish resulted in a 50 % smaller increase in $\dot{Q}$ compared with that in fish during the initial normoxic treatment, an effect that was evident despite similar increases in $\dot{q}_{cor}$ and P_{DA} (Figs 5–8; Table 2). The smaller increase in adrenaline-stimulated $\dot{Q}$ during hypoxia,

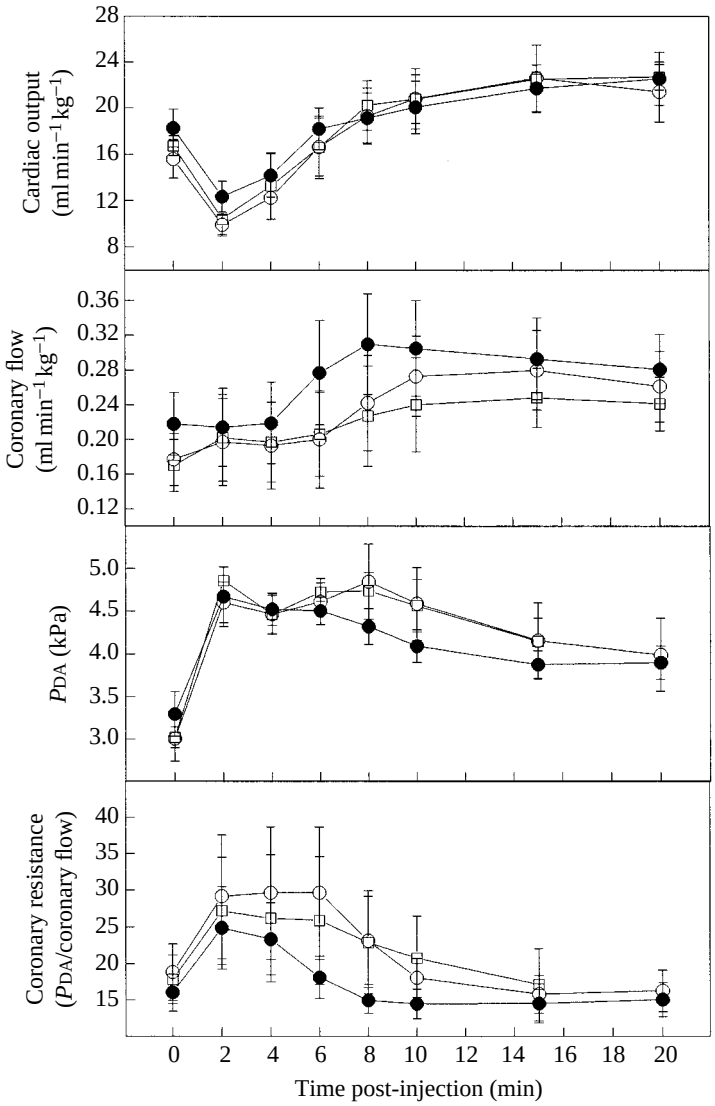

Fig. 8. Effect of adrenaline injection (1.0 μg kg^{-1}) on cardiac output ($\dot{Q}$), coronary blood flow ($\dot{q}_{cor}$), dorsal aortic pressure (P_{DA}) and coronary artery resistance (R_{cor}) in a type 2 rainbow trout (N=4) under conditions of normoxia (○; approximate $P_{W_{O_2}}$ 20 kPa), hypoxia (●; approximate $P_{W_{O_2}}$ 12 kPa) and after 2.5 h of recovery from hypoxia (□). Values are missing when N<4. Vertical bars indicate the S.E.M. for each mean value.

compared with that during normoxia, was due to differences in both V_S and f_H. For example, in type 1 normoxic fish, V_S increased by approximately 0.12 ml kg^{-1} while f_H returned to pre-injection levels by 4–6 min. However, in hypoxic fish, V_S only increased by approximately 0.08 ml kg^{-1} and f_H remained below pre-injection levels for at least 15 min (Fig. 9). Although hypoxic fish had a 50 % smaller increase in $\dot{Q}$, post-injection

Table 2. *Cardiovascular variables in adrenergically stimulated (1.0 μg kg^{-1} adrenaline) rainbow trout (*Oncorhynchus mykiss*) during normoxia and hypoxia*

	Normoxia	Hypoxia	Normoxia II
Maximum increase			
Cardiac output (ml min^{-1} kg^{-1})	8.17±1.1^{a}*	3.91±0.9^{b}*	6.33±0.9^{c}*
Coronary flow (ml min^{-1} kg^{-1})	0.095±0.020	0.074±0.016	0.079±0.015
P_{DA} (kPa)	2.84±0.17	2.46±0.29	2.86±0.24
Absolute levels at maximum			
Cardiac output (ml min^{-1} kg^{-1})	26.2±1.3	25.05±1.7	26.30±1.4
Coronary flow (ml min^{-1} kg^{-1})	0.221±0.02^{a}	0.270±0.03^{b}*	0.226±0.03^{a}
Cardiovascular variables at maximum coronary flow			
Coronary flow (% cardiac output)	0.88±0.12^{a}	1.13±0.15^{b}*	0.88±0.13^{a}
Coronary resistance (kPa kg min ml^{-1})	21.7±2.8^{a}	17.1±2.3^{b}*	18.5±1.8^{ab}

Values with dissimilar letters are significantly different at $P<0.05$ (*).

maximum $\dot{Q}$ in hypoxic fish (25.05±1.72 ml min^{-1} kg^{-1}) was not significantly different from that measured in normoxic fish (26.17±1.3 ml min^{-1} kg^{-1}) because hypoxic fish had a higher pre-injection $\dot{Q}$.

Although maximum post-injection $\dot{q}_{cor}$ (measured as % $\dot{Q}$ or ml min^{-1} kg^{-1}) was significantly greater during hypoxia than during normoxia (Table 2; Fig. 10), this difference was related to discrepancies that existed prior to adrenaline injection. Post-injection increases in $\dot{q}_{cor}$ were not significantly different between hypoxic and normoxic fish (Table 2). Although R_{cor} increased following adrenaline injection, differences in R_{cor} between resting hypoxic and normoxic fish were maintained (Figs 7 and 8). R_{cor} was 39 % higher at rest and 27 % higher after adrenaline injection in normoxic fish compared with values in hypoxic fish (Tables 1, 2).

Post-injection changes in $\dot{q}_{cor}$ (as %$\dot{Q}$ or ml min^{-1} kg^{-1}) and P_{DA} during the second normoxic period were comparable to those observed during the initial normoxic period (Table 2; Figs 5–8, 10). In contrast, although the maximum increase in $\dot{Q}$ (6.3 ml min^{-1} kg^{-1}) was higher than that measured during hypoxia (3.9 ml min^{-1} kg^{-1}), it was significantly lower than that recorded during the initial normoxic treatment (8.2 ml min^{-1} kg^{-1}). Post-injection maximum $\dot{Q}$ during the second normoxic period was not significantly different from that measured in either of the other two treatments. The magnitudes of changes in R_{cor}, measured during the second normoxic period,

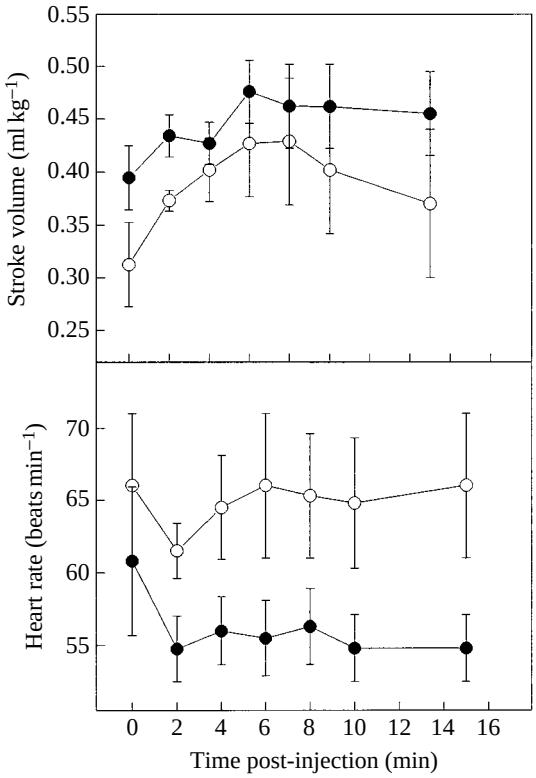

Fig. 9. Effect of adrenaline injection (1.0 μg kg^{-1}) on heart rate (f_H) and stroke volume (V_S) in a type 1 rainbow trout (N=4) under conditions of normoxia (○; approximate P_{WO_2} 20 kPa) and hypoxia (●; approximate P_{WO_2} 12 kPa). Vertical bars indicate the S.E.M. for each mean value.

were intermediate between those obtained in the initial normoxic and the hypoxic treatment.

Discussion

Cardiac performance

Effects of hypoxia

During the 30 min of graded hypoxia, V_S and $\dot{Q}$ increased, while f_H decreased (Fig. 4). The drop in f_H that was concomitant with hypoxia is thought to be caused by chemoreceptor-mediated inhibitory activity in the efferent cholinergic fibres of the vagus nerve (Randall and Smith, 1967; Wood and Shelton, 1980*b*; Smith and Davie, 1984; Fritsche and Nilsson, 1989). However, although an increase in V_S which results in the maintenance or elevation of $\dot{Q}$ is often observed during hypoxic bradycardia (Holeton and Randall, 1967; Cech *et al.* 1977; Wood and Shelton, 1980*a*; Fritshe and Nilsson, 1989), the mechanisms which mediate this effect are unclear. Numerous factors are known to increase V_S *in vivo* or *in vitro*, including elevations in the levels of circulating

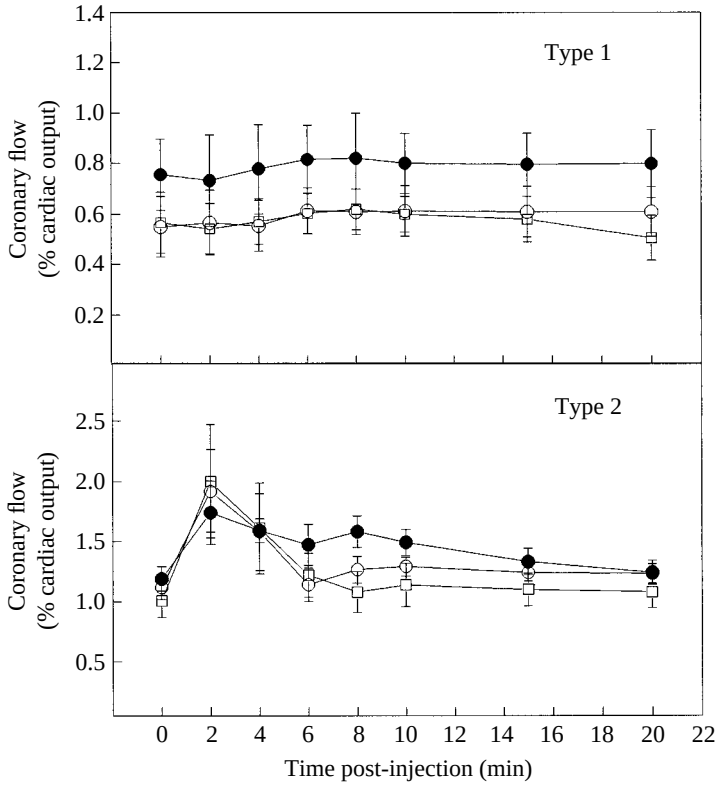

Fig. 10. Effect of adrenaline injection (1.0 μg kg^{-1}) on coronary blood flow (% $\dot{Q}$) in a type 1 (N=4) and a type 2 (N=4) rainbow trout under conditions of normoxia (○; approximate $P_{W_{O_2}}$ 20 kPa), hypoxia (●; approximate $P_{W_{O_2}}$ 12 kPa) and 2.5 h after recovery from hypoxia (□). Vertical bars indicate the S.E.M. for each mean value.

catecholamines (Wood and Shelton, 1980*a*; Farrell, 1984; Gamperl *et al.* 1994), increases in cardiac filling time (i.e. decreases in f_H; Farrell *et al.* 1989) and increases in cardiac filling pressure (venous pressure) (Farrell, 1984; Farrell and Jones, 1992). In rainbow trout exposed to gradual hypoxia, increases in plasma adrenaline and noradrenaline levels are not detected until $P_{W_{O_2}}$ falls below 6.7 kPa (approximate $P_{a_{O_2}}$ 2.8 kPa) (Perry and Reid, 1992). Because increases in V_S were detected at $P_{W_{O_2}}$ levels between 12 and 13.3 kPa (approximate $P_{a_{O_2}}$ 4.7–6.0 kPa), humoral adrenergic stimulation of the heart is highly unlikely. While a decrease in f_H did occur during hypoxic exposure, the magnitude of this change was relatively minor (Fig. 4). At a $P_{W_{O_2}}$ of approximately 13.3 kPa, f_H had only fallen by 2–3 beats min^{-1} (5 %), while V_S had increased by about 15 % (from 0.15 to 0.17 ml kg^{-1}). In addition, at the final $P_{W_{O_2}}$ of 12 kPa, f_H had decreased by approximately 5 beats min^{-1} (8 %) while V_S had increased by 25 %. Although a decrease in f_H will increase the time available for atrial and ventricular filling (Farrell, 1984), it is probable that some other factor, in combination with the small change in f_H, mediated the observed change in V_S. Data from Farrell *et al.* (1982) and Farrell (1984) for *in situ* perfused hearts

have shown that preloads (venous pressures) of 0.03–0.05 kPa (0.3–0.5 cmH_2O) are sufficient to generate $\dot{Q}$ levels comparable to those seen in resting fish, and that small increases in preload can substantially increase V_S and $\dot{Q}$. Hypoxic exposure in fish causes ventilation volume to increase (Holeton and Randall, 1967; Nonnette *et al.* 1993) and results in a synchrony between f_H and breathing (Randall and Smith, 1967). Because both of these adjustments have been suggested as possible mechanisms that increase venous return (Farrell, 1984), it is feasible that a hypoxia-induced increase in venous return partially mediated the elevated V_S that was concomitant with hypoxia. Although this is an attractive hypothesis for explaining the hypoxia-induced increase in V_S, Cech *et al.* (1977) found that V_S increased in the flounder despite non-significant changes in f_H or venous pressure. It is clear that the mechanisms mediating increases in V_S during hypoxia require further investigation.

Effects of adrenaline injection during normoxia

Adrenaline injection into normoxic trout resulted in two distinct types of cardiovascular response; a type 1 response characterised by a gradual increase in post-injection $\dot{Q}$, and a type 2 response characterised by an initial fall in $\dot{Q}$, followed by a gradual increase to a similar peak $\dot{Q}$ as for type 1 fish (see Figs 5–8). This confirms the results of Gamperl *et al.* (1994), who identified two response types in rainbow trout acclimated to 5 °C. Hypoxic exposure failed to alter the response type of any individual fish (see Figs 5–8), a result suggesting that response type is not influenced by blood oxygen tension and/or content.

Adrenaline injection (1.0 $\mu g\,kg^{-1}$) during normoxia increased $\dot{Q}$ and P_{DA} by 45 % and 100 %, respectively. Because these values are comparable to those obtained by Gamperl *et al.* (1994) for coronary-ablated trout ($\dot{Q}$ 48 %; P_{DA} 100 %), the results of the present study provide evidence that oxygen supplied by the venous (luminal) blood is sufficient to support elevated cardiac work during normoxia even though $\dot{q}_{cor}$ normally increases. As in the study of Gamperl *et al.* (1994), post-injection increases in $\dot{Q}$ were mediated by elevations in V_S that more than compensated for the effects of the pressor-stimulated bradycardia. Although the decrease in f_H associated with adrenaline injection may have increased the time available for atrial filling by *vis-a-tergo* mechanisms (Farrell *et al.* 1989), it is probable that direct adrenergic stimulation of the heart was the predominant factor mediating increases in V_S. This conclusion is based on (1) the observation that relatively small decreases in f_H (2–4 beats min^{-1}) were concordant with maximum post-injection $\dot{Q}$ (e.g. Fig. 9); and (2) evidence showing that adrenergic stimulation increases myocardial contractility and the heart's sensitivity to filling pressure (preload) (Farrell, 1984; Farrell *et al.* 1986; Franklin and Davie, 1992).

Effects of adrenaline injection during hypoxia

Maximum post-injection $\dot{Q}$ was the same in hypoxic and normoxic trout. However, hypoxic trout had a diminished capacity to increase $\dot{Q}$ over pre-injection levels compared with normoxic trout (Table 2), despite similar increases in $\dot{q}_{cor}$ ($ml\,min^{-1}\,kg^{-1}$). One possible explanation for the failure of hypoxic hearts to increase $\dot{Q}$ above levels measured in normoxic trout is that post-injection V_S during normoxia was already approaching a

maximum limit, and that the inability to increase V_S further was related to a reduction in cardiac emptying in the face of elevated output pressures (P_{DA}, and possibly P_{VA}). Kiceniuk and Jones (1977) showed that swimming rainbow trout can still elevate $\dot{Q}$ and V_S at values of P_{VA} greater than 6.7 kPa. Because our estimated value of P_{VA} at $\dot{Q}_{max}$ (5.3 kPa) is less than those in Kiceniuk and Jones (1977), these data suggest that the elevated blood pressures associated with adrenaline injection did not limit V_S in hypoxic trout. In contrast, Gamperl *et al.* (1994) suggested that most of the scope for adrenaline-stimulated increases in V_S ($\dot{Q}$) occurs below 0.5 μg kg^{-1} and that the large increases in systemic vascular resistance (output pressure) that accompany doses greater than 1.0 μg kg^{-1} limit the ability of adrenaline to increase V_S (systolic emptying).

A second explanation for the inability of hypoxic fish to increase $\dot{Q}$ above levels achieved during normoxia is that increases in $\dot{q}_{cor}$ (coronary oxygen delivery) during hypoxia were not sufficient to support further increases in myocardial power output. *In vitro*, the maximum tetanic force developed by cardiac muscle is reduced by hypoxia (Gesser *et al.* 1982; Gesser, 1985) and this effect is seen as a reduction in the ability of hypoxic perfused preparations to generate pressure (Farrell *et al.* 1989). In addition, while perfusion of the coronary circulation with air-saturated red cell suspensions (10 % haematocrit) partially restored the power output of severely hypoxic dogfish (Davie and Farrell, 1991*b*) and eel (Davie *et al.* 1992) hearts (P_{O_2} 1.1–1.6 kPa), the levels of cardiac power output achieved were 25–45 % lower than those measured during normoxia.

In our study, myocardial oxygen delivery through the coronary artery is estimated to be 10 % lower in hypoxic (P_{aO_2} 5.1 kPa) trout than in normoxic (P_{aO_2} 11.7 kPa) trout, at maximum cardiac output. This estimate is based on (1) the reported $\dot{q}_{cor}$ measurements, (2) the assumption that adrenergic contraction of the spleen leads to similar post-injection levels of haematocrit in hypoxic and normoxic fish (Pearson and Stevens, 1991), and (3) the *in vivo* oxygen dissociation curves for rainbow trout blood at comparable temperatures (Perry and Reid, 1992). Although this deficit in coronary O_2 delivery seems unlikely to explain the 50 % decrease in the scope for $\dot{Q}$ increases, it must be remembered that the coronary O_2 supply in salmonids supplements rather than replaces the luminal O_2 supply (Farrell and Jones, 1992).

Although the existence of a maximum adrenaline-stimulated V_S and a limitation in the ability of hypoxic trout to increase $\dot{q}_{cor}$ are the two most probable explanations for the failure of hypoxic trout to increase $\dot{Q}$ to the same extent as normoxic trout, other possible explanations do exist. These include constraints on V_S imposed by the lack of *vis-a-fronte* filling (i.e. the absence of an intact pericardium), hypoxia-related differences in the regulation of venous return to the heart, differences in adrenergic tone and a hypoxia-induced decrease in myocardial adrenoreceptor density and/or affinity.

Trout that had been allowed to recover from hypoxia for 2.5 h also had a maximum $\dot{Q}$ similar to that of normoxic trout and a diminished scope for adrenaline-stimulated increases in $\dot{Q}$. However, because it is clear that trout during the second normoxic period had not fully recovered from the hypoxic exposure, this result does not assist in resolving why hypoxic trout had a reduced increase in adrenaline-stimulated $\dot{Q}$. Resting trout, in the second normoxic period, had an elevated $\dot{Q}$ (V_S), a lower P_{aO_2}, and a diminished P_{DA} compared with trout during the initial normoxic period. Because the lower P_{aO_2} probably

indicates that $P_{\mathrm{V}\dot{\mathrm{O}}_2}$ was also depressed during the second normoxic period, and because Steffensen and Farrell (1994) found that the homeometric lowering of P_{VA} by coronary-ablated coho salmon was concomitant with a diminished ability of the heart to maintain cardiac power output, it is probable that the power-generating ability of the hearts in our 'recovered' trout was compromised prior to adrenaline injection.

Coronary blood flow

Resting $\dot{q}_{\mathrm{cor}}$, during normoxia, was $0.143\,\mathrm{ml\,min^{-1}\,kg^{-1}}$ or 0.84 % of $\dot{Q}$, an estimate very comparable to the value of 1.1 % $\dot{Q}$ measured by Axelsson and Farrell (1993) in coho salmon (*Oncorhynchus kisutch*). Taken together, these results indicate that $\dot{q}_{\mathrm{cor}}$ in salmonids is approximately 1 % of resting $\dot{Q}$. The only other direct measurement of $\dot{q}_{\mathrm{cor}}$ in fish was recently provided by Davie and Franklin (1993). Mean $\dot{q}_{\mathrm{cor}}$ in one of the paired coronary arteries of an anaesthetized school shark (*Galeorhinus australis*) was estimated to be $0.103\,\mathrm{ml\,min^{-1}\,kg^{-1}}$.

Effects of hypoxia

$\dot{q}_{\mathrm{cor}}$ in our trout increased as the $P_{\mathrm{W}\dot{\mathrm{O}}_2}$ fell below 13.3 kPa and was 36 % ($0.052\,\mathrm{ml\,min^{-1}\,kg^{-1}}$) greater in hypoxic trout than in normoxic trout prior to adrenaline injection (Table 1). Although an increase in $\dot{q}_{\mathrm{cor}}$ was also observed in hypoxic coho salmon (Axelsson and Farrell, 1993), the magnitude of the increase (60 %) was greater than that reported here. The difference between the two studies may be due to three factors. First, Axelsson and Farrell (1993) only used two fish to investigate the effect of hypoxia on coronary flow. Second, the increase in P_{DA} (60 %), and presumably P_{VA}, associated with hypoxia in the coho salmon would have increased coronary driving pressure and myocardial power output (i.e. oxygen demand) (Graham and Farrell, 1990). In our study, no change in P_{DA} was associated with hypoxic exposure. Third, the level of hypoxia used by Axelsson and Farrell (1993) (8–10 kPa) very probably lowered $P_{\mathrm{V}\dot{\mathrm{O}}_2}$ to a greater degree than in the present study (see Fig. 3). Because oxygen delivered to the heart by the venous blood appears to be limited by the partial pressure of oxygen ($P_{\mathrm{V}\dot{\mathrm{O}}_2}$) (Davie and Farrell, 1991*a*), it is likely that $\dot{Q}$ and V_{S} in the coho salmon were more dependent upon $\mathrm{O_2}$ supplied by the coronary artery. Taken together, the results of Axelsson and Farrell (1993) and those in the present study suggest that elevations in $\dot{q}_{\mathrm{cor}}$ are important for determining cardiac performance during environmental hypoxia. For example, in our study a 36 % increase in $\dot{q}_{\mathrm{cor}}$ was associated with the 25 % increase in myocardial power output (estimated from $\dot{Q}\times P_{\mathrm{DA}}$) that was concomitant with hypoxic exposure.

Increases in $\dot{q}_{\mathrm{cor}}$ during experimental hypoxia were associated with a significant decrease in coronary resistance (28 %) but no significant increase in P_{DA} (Table 1), indicating that the observed increase in $\dot{q}_{\mathrm{cor}}$ was mediated by coronary vasodilation and/or the decrease in f_{H}. This finding confirms the results of Axelsson and Farrell (1993), who found that an important component of the increase in $\dot{q}_{\mathrm{cor}}$ was not dependent upon α-adrenergic, cholinergic or physical (arterial pressure) mechanisms. Although changes in P_{DA} were not important for determining $\dot{q}_{\mathrm{cor}}$ in resting hypoxic fish, it is clear that increases in P_{DA} associated with adrenergic stimulation (see below) or more

severe hypoxia (Axelsson and Farrell, 1993) may mediate elevations in coronary flow.

Axelsson and Farrell (1993) indicated that β-adrenergic mechanisms and/or increases in cardiac metabolism can potentially alter $\dot{q}_{cor}$ to the myocardium. However, β-adrenergic control of $\dot{q}_{cor}$ was probably not the predominant factor mediating the increased $\dot{q}_{cor}$ in hypoxic trout since (1) Perry and Reid (1992) have shown that endogenous catecholamine release does not occur in rainbow trout until $P_{W_{O_2}}$ falls below 6.7 kPa; and (2) perfusion of the coronary circulation (coronary artery and associated arterioles) with adrenaline *in vitro*, and injection of adrenaline *in vivo*, indicates that α-adrenoreceptor effects dominate β-adrenoreceptor effects, i.e. that vasoconstriction predominates (Farrell and Graham, 1986; Farrell, 1987; Axelsson and Farrell, 1993). Results from Farrell (1987) and Axelsson and Farrell (1993) suggest that there is a small tonic α-adrenergic constriction of the coronary vasculature and that metabolism-related vasodilation of the coronary circulation, as occurs in mammals (Feigl, 1983), could override direct sympathetic vasoconstriction. Therefore, it is possible that the increase in $\dot{q}_{cor}$ and decrease in R_{cor} during hypoxia were due to a metabolically related coronary vasodilation.

f_H decreased by approximately 5 beats min^{-1} during hypoxia, an effect which would have increased the heart's diastolic period and reduced the fraction of the cardiac cycle occupied by systole. Because an increase in the diastolic period would reduce vascular compression, and thus R_{cor}, the decrease in f_H that was associated with hypoxia may have contributed to the increase in $\dot{q}_{cor}$. However, because the reduction in f_H was only 10 % (5 beats min^{-1}), it is doubtful that the decrease in f_H was the main factor mediating the 37 % increase in $\dot{q}_{cor}$. Although vasodilation of the coronary vasculature due to local changes in metabolism is the most plausible explanation for the decrease in R_{cor} associated with hypoxia, other mechanisms cannot be ruled out. These include neural vasodilatory reserve, alterations in α-adrenergic tone and the release of vasoactive substances from the myocardium.

Control of coronary blood flow following adrenaline injection

Injection of adrenaline into the dorsal aorta during normoxia increased $\dot{q}_{cor}$, P_{DA}, $\dot{Q}$ and R_{cor}. It is difficult, in an *in vivo* model, to determine to what extent a particular cardiovascular variable contributes to the observed elevations in $\dot{q}_{cor}$. However, the existence of two response types with different patterns of R_{cor}, $\dot{Q}$ and P_{DA} elevation (Figs 7, 8) provides indirect evidence that increases in R_{cor} (α-vasoconstriction of the coronary circulation), P_{DA} and myocardial oxygen demand (metabolic coronary vasodilation) can all mediate changes in $\dot{q}_{cor}$ in fish. This information complements the results of Axelsson and Farrell (1993), who used pharmacological agents to investigate the control of $\dot{q}_{cor}$ in coho salmon *in vivo*.

In type 2 fish, $\dot{Q}$ (f_H) fell by 40 %, R_{cor} increased by 55 %, P_{DA} increased by 55 % and $\dot{q}_{cor}$ increased by 5–10 % shortly (2 min) after adrenaline injection. Because the resultant cardiac power output ($P_{DA} \times \dot{Q}$) was slightly lower than the value recorded before drug injection, it is probable that the increase in $\dot{q}_{cor}$ was mediated by the increase in P_{DA} (Axelsson and Farrell, 1993) or the lowered f_H (increased diastolic blood flow) (Farrell, 1987), and not by increased myocardial oxygen demand. Farrell (1987) using perfused

in vitro hearts concluded that, in swimming trout, an increase in P_{DA} of 18 % would increase $\dot{q}_{cor}$ by approximately 30 %. In our study, because an increase in P_{DA} of 55 % only increased $\dot{q}_{cor}$ by 5–10 %, it must be concluded that the adrenergically mediated vasoconstriction of the coronary vascular bed (Farrell, 1987) severely limited, but did not preclude, increases in $\dot{q}_{cor}$ associated with elevations in P_{DA}. Axelsson and Farrell (1993) found that adrenaline injection (1.8 μg kg^{-1}) resulted in an increase in P_{DA} of 60 % and an increase in $\dot{q}_{cor}$ of 60 %. Although the reason for the difference between the two studies with regard to the relationship between $\dot{q}_{cor}$ and P_{DA} is not known, the results of Axelsson and Farrell (1993) support the conclusion that increases in R_{cor}, associated with constriction of the coronary circulation, can reduce but not obviate the effect of P_{DA} on $\dot{q}_{cor}$.

In type 1 fish, where myocardial power output ($P_{DA}\times\dot{Q}$) constantly increased during the first minutes post-injection and reached a maximum value at approximately 6 min post-injection, elevations in $\dot{q}_{cor}$ mirrored alterations in $\dot{Q}$ and increases in R_{cor} were short-lived (<6 min). However, in type 2 fish, where myocardial power output was decreased initially (through a severe drop in $\dot{Q}$) and failed to reach a maximum value until 10–15 min post-injection, initial increases in $\dot{q}_{cor}$ were minimal and the increase in R_{cor} was prolonged (8–10 min) (Figs 7, 8). These results suggest that increases in myocardial oxygen demand, correlated with increases in cardiac power output (Graham and Farrell, 1990), mediated changes in $\dot{q}_{cor}$ and R_{cor} following adrenaline injection. Farrell (1987) suggested that sympathetic stimulation of the heart could increase $\dot{q}_{cor}$ *via* metabolically related vasodilation, and Axelsson and Farrell (1993) suggested that increases in $\dot{q}_{cor}$ associated with activity may be achieved *via* a metabolically mediated vasodilation. When the results of Farrell (1987) and Axelsson and Farrell (1993) are considered, it appears that a metabolically related dilation of the coronary vasculature (i.e. decrease in R_{cor}) is the most probable explanation for the apparent relationship between myocardial power output and $\dot{q}_{cor}$ during normoxia.

In our trout, the coronary vasodilatory reserve [measured as (maximum post-injection hypoxic $\dot{q}_{cor}$ minus resting normoxic $\dot{q}_{cor}$)/resting normoxic $\dot{q}_{cor}$] was 88 %. Because Axelsson and Farrell (1993) calculated that the coronary vasodilatory reserve in coho salmon is at least 200 %, the results of the present study suggest that adrenergically mediated vasoconstriction of the coronary circulation (Farrell, 1987) may have limited the ability of the heart maximally to dilate the coronary vasculature (i.e. to increase myocardial oxygen delivery).

In summary, the *in vivo* estimate of resting coronary blood flow in this study (0.85 % $\dot{Q}$) compares well with that obtained by Axelsson and Farrell (1993) (1.1 % $\dot{Q}$) and indicates that coronary blood flow in salmonids is approximately 1 % of resting $\dot{Q}$. Increased $\dot{q}_{cor}$, probably mediated by metabolically related coronary vasodilation, is associated with hypoxia-induced alterations in resting $\dot{Q}$. Maximal dilation of the coronary vasculature (i.e. increase myocardial oxygen delivery) may be limited under conditions where an adrenergically mediated vasoconstriction is also present. Exposure of fish to moderate environmental hypoxia reduces the scope for adrenergically mediated increases in $\dot{Q}$ by approximately 50 %. A recovery period greater than 2.5 h is required to restore the cardiovascular performance of trout to levels seen during normoxia.

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