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EFFECT OF CORONARY ABLATION AND ADRENERGIC STIMULATION ON *IN VIVO* CARDIAC PERFORMANCE IN TROUT (*ONCORHYNCHUS MYKISS*)

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Summary

In fish, catecholamine-induced changes in cardiac performance *in vivo* are the result of complex interactions between the direct adrenergic effects on the heart and peripheral circulation and the reflex responses to increased blood pressure. In addition, coronary artery transport of catecholamines and oxygen to the compact myocardium may be essential for maximal *in vivo* cardiac performance during adrenergic stimulation. Cardiac output ($\dot{Q}$), heart rate (f_H), stroke volume (V_S) and dorsal aortic pressure (P_{DA}) were measured in trout with intact or ablated coronary arteries at rest and following intra-arterial administration of 0.2, 0.5, 1.0 and 2.0 $\mu g\,kg^{-1}$ adrenaline. Resting $\dot{Q}$, f_H, V_S and P_{DA} were the same in fish with intact and ablated coronaries at 48 h post-surgery, averaging approximately 18 $ml\,min^{-1}\,kg^{-1}$, 42 $beats\,min^{-1}$, 0.42 $ml\,kg^{-1}$ and 2.2 kPa, respectively. All cardiovascular variables showed a strong relationship between response magnitude and adrenaline dose. However, our results indicate that adrenaline doses above 0.5 $\mu g\,kg^{-1}$ may have a limited ability to increase $\dot{Q}$ (ED_{50} 0.22 $\mu g\,kg^{-1}$). Coronary artery ablation had little effect on post-injection $\dot{Q}$, V_S, P_{DA} or f_H at any dose of adrenaline. In both intact and ablated groups, two types of responses in $\dot{Q}$ were observed following adrenaline injection. In the 'type 1' response, $\dot{Q}$ increased shortly (15–30 s) after adrenaline administration, as increases in V_S more than compensated for a pressor-stimulated reflex bradycardia. In the 'type 2' response, alterations in $\dot{Q}$ were biphasic. In the initial minutes post-injection, $\dot{Q}$ fell and reached a minimum level at 1–2 min, the result of an immediate drop in f_H and a delayed post-injection increase in V_S. Thereafter, $\dot{Q}$ gradually increased as a result of concordant increases in f_H and V_S. Although time to maximum $\dot{Q}$ was 5–6 min longer for fish exhibiting type 2 responses, there was no difference in maximum $\dot{Q}$ increase or in the time courses for changes in f_H and P_{DA} between response types. Our results suggest (1) that during normoxic conditions, cardiac performance does not depend highly on coronary blood flow; (2) that the capacity of

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adrenaline to increase $\dot{Q}$ may be limited by elevations in output pressure and/or by the low dose (concentration) of adrenaline required to achieve near maximal adrenergic stimulation of the heart; and (3) that fish exhibiting type 2 responses have an increased barostatic gain (%Δf_H per unit P_{DA}) compared with those with type 1 responses.

Introduction

The chronotropic and inotropic effects of adrenaline on fish cardiac performance have been studied *in vitro* (Holmgren, 1977; Graham and Farrell, 1989), *in situ* (Farrell *et al.* 1982, 1986; Stuart *et al.* 1983) and *in vivo* (Pettersson and Nilsson, 1980; Wood and Shelton, 1980*a*; Farrell, 1981; Hipkins, 1985). *In vivo* studies proved especially valuable in elucidating the complex interactions between direct adrenergic effects (observed *in vitro* and *in situ*), pressor-stimulated vagal cardioinhibitory tone and pressor-mediated decreases in stroke volume (systolic emptying). However, these *in vivo* studies have provided only limited information on dose-dependent cardiovascular changes. Investigators usually report dose–response relationships for dorsal aortic pressure (P_{DA}) (Randall and Stevens, 1967; Wood and Shelton, 1980*a*) and, when data are included for other variables ($\dot{Q}$, V_S, f_H), only peak values are shown (e.g. Pettersson and Nilsson, 1980). In the present study, detailed information on the magnitude and time course of adrenaline-stimulated dose-dependent changes in cardiac performance have been collected. This information will be essential in future studies for predicting the effect of elevated circulating catecholamine levels on *in vivo* cardiovascular performance and for selecting adrenaline doses to achieve a desired change in cardiovascular function.

The role of the coronary circulation in salmonid fishes is unclear. However, experimental evidence from *in vitro* heart perfusions (Davie and Farrell, 1991; Davie *et al.* 1992) and from measurements of swimming performance in trout with intact or ablated coronary arteries (J. F. Steffenson and A. P. Farrell, in preparation) suggests that the coronary blood supply is essential only for maintaining cardiac output when the heart must work against elevated output pressures. To test this hypothesis further, *in vivo* measurements of dose-dependent changes in cardiovascular performance were made on rainbow trout (*Oncorhynchus mykiss*) with intact or ablated coronary arteries. Adrenergic stimulation is an appropriate model for studying the role of the coronary circulation in the maintenance of *in vivo* cardiac performance because adrenaline increases both cardiac output (through positive chronotropic and inotropic effects) and arterial blood pressure. The concomitant elevation in cardiac power output increases oxygen demand by the myocardium (Graham and Farrell, 1990) and may accentuate the reliance of the heart upon oxygen delivered by the coronary circulation.

Materials and methods

Rainbow trout, *Oncorhynchus mykiss* (Walbaum) (480–760 g), were obtained from Merlin Fish Farms (Wentworth, Nova Scotia) and held in tanks (1 m×1 m×1.5 m) supplied with sea water at 4–6 °C. Fish were fed daily, to satiation, on a diet of

commercially prepared feed pellets, but were fasted for 48 h prior to surgery. Photoperiod was 12 h:12 h light:dark. All experiments were conducted between March 15 and June 15.

Surgical procedures

Trout were anaesthetized (0.1 g l^{-1} tricaine methane sulphonate, 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, Clay Adams) (Smith and Bell, 1964), after which retrograde irrigation with sea water containing anaesthetic (0.05 g l^{-1} MS 222) was begun. A 3–4 cm midventral incision was made through the skin and muscle at a position overlying the ventral aorta and anterior aspect of the 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 (hypobranchial) artery. The connective tissue from the anterior portion of the ventral aorta was subsequently removed to aid in placement of a Doppler flow probe (see later description). In fish where the coronary artery had been ligated and electrocauterized, the Doppler flow probe was placed around the ventral aorta without further surgical procedures. However, when the coronary artery was left intact, placement of the Doppler flow probe necessitated that the connective tissue which adheres the coronary artery to the ventral aorta and the anterior bulbus arteriosus be removed. This allowed the flow probe to be placed underneath the freed coronary artery, thus maintaining normal coronary perfusion. Once the flow probe was in place, the musculature and skin were closed using continuous silk sutures. The integrity of the pericardium was not restored by suturing because of time constraints. The Doppler probe leads were secured to the body wall at the anterior apex of the incision and at a position just posterior to the pectoral fins. The operation generally took between 45 and 60 min, and bleeding was usually minimal. Some fish were fitted only with a dorsal aortic cannula (N=9). Comparisons between this group and those that underwent surgery (‘operated’ fish) allowed for the evaluation of flow probe placement on cardiovascular responses.

Once surgery had been completed, fish were given an intraperitoneal injection of tetracycline (5 mg kg^{-1}) and placed into black Perspex boxes (45 cm×8 cm×5 cm) to recover. Boxes were supplied with aerated sea water (4.5–6.2 °C) at a flow rate of 1 l min^{-1}.

Probe design

Flow probes were constructed by implanting a piezoelectric crystal with 80 cm leads (Crystal Biotech, Hopkinton MA) into a 5 mm length of PE 200 (i.d. 1.4 mm) or PE 240 (i.d. 1.67 mm) Intramedic tubing (Clay Adams). The tubing was split to facilitate placement on the ventral aorta and had a small (0.5 mm×1.0 mm) notch to allow for crystal attachment. The piezoelectric crystal was secured in the notch with cyanoacrylate cement after the area had been thoroughly roughened with a scalpel blade. Probes were not fitted with tie-string sutures to prevent tube diameter from increasing at elevated ventral aortic pressures because PE tubing is extremely rigid at 5 °C.

Experimental protocol

After 48 h of recovery, fish were sequentially injected with 0.2, 0.5, 1.0 and 2.0 μg kg^{-1} adrenaline at 1.5 h intervals. The inter-injection period allowed cardiovascular variables to return to 'resting' levels for approximately 1 h (results from preliminary experiment). All doses of adrenaline (Sigma Chemical Co., St Louis, MO) were injected slowly (over approximately 15 s) through the dorsal aortic cannula in a concentrated form using a 0.2–0.4 ml carrier volume of saline. Heart rate (f_H), dorsal aortic pressure (P_{DA}) and ventral aortic flow (cardiac output; $\dot{Q}$) were continuously recorded from 5 min prior to adrenaline injection ('resting' levels) to 20 min post-injection. Trout with irregular heart rates, low haematocrits (<10 %) or which continued to struggle periodically within the black Perspex boxes were not used for data collection.

Recording systems

P_{DA} was measured by attaching the dorsal aortic cannula to a Gould Statham (model P23-10) pressure transducer, connected to a Molytec amplifier-recorder (model 3501-MS). Mean P_{DA} was calculated as: [systolic pressure+2(diastolic pressure)]/3 (Burton, 1972; Wood *et al.* 1979). Pressure calibrations were performed daily against a static water column. f_H at rest, or at a particular time post-injection, was determined by measuring the number of systolic peaks during a 30 s interval; the interval being 15 s on either side of the desired time.

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 (Asea Brown and Boveri; model SE-120). In order to determine absolute flow rates (ml min^{-1}), an *in situ post-mortem* calibration of each flow probe was performed at physiologically relevant pressures using a peristaltic pump (Gilson, Minipuls II) and human blood (approximate haematocrit 20 %). To accomplish this, after removal of the sinus venosus and atrium, the ventricle was bisected laterally and the peristaltic pump outflow tubing (PE 160) was tied into the ventricular lumen. Because of the high flow rates required (>35 ml min^{-1}), two pieces of Viton tubing (4 mm i.d.) were attached to the peristaltic pump and PE 160 tubing connected the peristaltic pump to the ventricle. Doppler flow probes were calibrated over the range of flows of 5–38 ml min^{-1}, 5 ml min^{-1} being the minimal flow at which the ventral aorta adequately filled the probe lumen. Blood pressure was monitored through a side-arm in the peristaltic pump outflow tubing and was found to increase linearly (approximate range 2–7 kPa) with elevations in blood flow (pressure$=-0.44+0.54$flow, $r^2=0.995$).

Although coronary blood flow was not measured in animals with intact vessels, coronary patency was verified by visual inspection and by following the progression of Methylene Blue up the coronary artery. Methylene Blue was injected into the dorsal aorta of anaesthetized fish, just before they were killed for flow probe calibration.

Analysis

Stroke volume (V_S) was calculated from $\dot{Q}/f_H$. Barostatic gain (normalized gain, percentage change in f_H per unit change in mean arterial pressure, Smith *et al.* 1981) was

determined from pre-injection and 2 min post-injection values of f_H and P_{DA}. ED_{50} (the dose of adrenaline required to elicit 50 % of the maximal effect) values for maximum P_{DA} and $\dot{Q}$, for each group (type 1, type 2, fish with only cannulae), were calculated from Eadie–Hofstee plots of mean values.

Statistical differences ($P<0.05$) between means for resting and post-injection cardiovascular variables were determined using a 2('type') × 2(intact/ablated) factorial with repeated measures, followed by multiple contrasts (Proc GLM; SAS Institute Inc.). To facilitate the determination of whether surgical procedures (installation of flow probe) affected resting f_H and P_{DA}, means for fish with only cannulae were compared with those for 'operated' fish using a one-way analysis of variance (Proc GLM). Previous statistical analysis (2×2 factorial) had shown that the 'type' of cardiovascular response pattern and the presence/absence of a coronary artery had no effect on resting f_H and P_{DA}. Post-injection increases in P_{DA} were compared between fish with only a cannula and 'operated' fish using a one-way analysis of variance (ANOVA, Proc GLM) with repeated measures, followed by multiple contrasts. Again previous statistical analysis had shown that the presence/absence of the coronary artery and response type had no effect on the magnitude of post-injection P_{DA} increases.

Results

Resting cardiovascular variables

Cardiac output, f_H, P_{DA} and V_S were the same in trout with intact or ablated coronary arteries at 48 h post-surgery, averaging 18 ml min^{-1} kg^{-1}, 42 beats min^{-1}, 2.2 kPa and 0.42 ml kg^{-1}, respectively (Table 1). In addition, no significant differences in resting cardiovascular variables were observed between fish exhibiting 'type 1' and 'type 2' (see below) cardiac adrenergic responses. 'Operated' trout had a significantly higher f_H and a significantly lower P_{DA} compared with individuals fitted only with a dorsal aortic cannula. Mean haematocrit levels were not significantly different between any of the groups and ranged from 16.8 to 21.3 % (Table 1). Although it appears from Table 1 that differences in P_{DA} between groups may be correlated with haematocrit (Hct), analysis of the relationship between vascular resistance ($P_{DA}/\dot{Q}$) and haematocrit, for 'operated' fish, failed to reveal a significant trend (vascular resistance=0.102+0.001Hct; r^2=0.022).

Variations in the response to adrenaline injection

Injection of adrenaline into fish with intact or ablated coronary arteries resulted in two 'types' of responses in cardiac output (Fig. 1). In the type 1 response, $\dot{Q}$ began to increase shortly (<30 s) after adrenaline injection and reached maximum levels at 4–10 min post-injection (the time required to reach $\dot{Q}_{max}$ increasing with adrenaline dose). In the type 2 response, alterations in $\dot{Q}$ were biphasic. In the initial minutes post-injection, $\dot{Q}$ fell gradually and reached a minimum at 1–2 min. Thereafter, $\dot{Q}$ gradually increased, reaching pre-injection levels at approximately 4–6 min and maximum levels at approximately 10–12 min. Both trout with ablated coronary arteries and those with intact coronary arteries showed the two response types. The ratio (type 1:type 2) was 9:4 in coronary-

Table 1. *Haematocrit and resting cardiovascular variables in trout fitted with both a ventral aortic flow probe and a dorsal aortic cannula ('operated' fish) and trout possessing only a dorsal aortic cannula*

	Haematocrit (%)	$\dot{Q}$ (ml min^{-1} kg^{-1})	f_H (beats min^{-1})	V_S (ml kg^{-1})	P_{DA} (kPa)
Type 1					
Coronaries ablated (9)	19.8±1.7	19.0±2.2	42.9±0.8	0.44±0.05	2.21±0.09
Coronaries intact (4)	16.8±1.2	16.0±1.5	40.3±4.2	0.42±0.07	1.97±0.21
Type 2					
Groups combined (7)	19.2±0.8	18.4±1.1	43.3±1.1	0.41±0.03	2.33±0.20
Fish with only dorsal cannulae (8)	21.3±1.9	–	38.6±2.6*	–	2.84±0.17*

'Type' refers to the cardiovascular response to adrenaline injection (see text).

*Indicates a significant difference (P<0.05) between 'operated' trout and those only fitted with a dorsal aortic cannula.

Values are mean ± S.E.M., numbers in brackets indicate sample size.

$\dot{Q}$, cardiac output; f_H, heart rate; V_S, stroke volume; P_{DA}, dorsal aortic pressure.

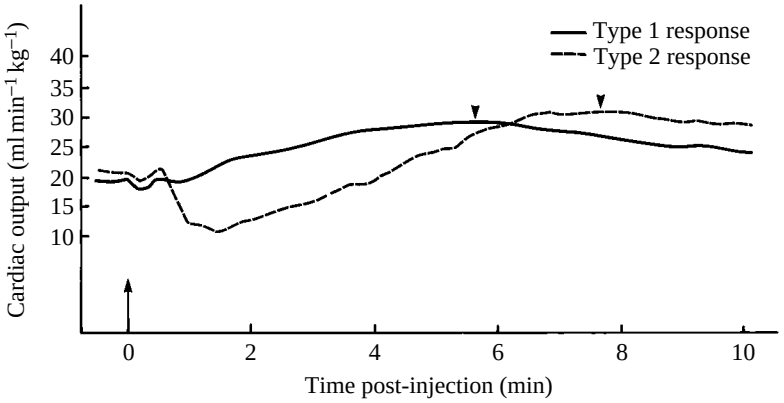

Fig. 1. Continuous recordings of cardiac output ($\dot{Q}$) in trout showing the two different responses, type 1 and type 2, to the injection of 0.5 μg kg^{-1} adrenaline. Arrowheads indicate the point of maximum cardiac output ($\dot{Q}_{max}$). The point of injection of adrenaline is marked with an arrow.

ablated fish and 4:3 in fish with intact coronaries. Response type, for individuals, was conserved across dose levels (Fig. 2); e.g. fish exhibiting type 1 alterations in $\dot{Q}$ at the lowest dose (0.2 μg kg^{-1}) also displayed type 1 responses at 0.5, 1.0 and 2.0 μg kg^{-1}. Although some trout with type 1 response patterns did show a transition to a type 2 response at high adrenaline doses (1.0 and 2.0 μg kg^{-1}) (Fig. 2), the decrease in $\dot{Q}$ was small compared with that in fish displaying the more typical type 2 responses.

The form of the pressor response varied slightly between fish, but remained consistent for individual fish. Wood and Shelton (1980*a*) separated P_{DA} responses into four basic configurations. However, we view these alterations to be part of a

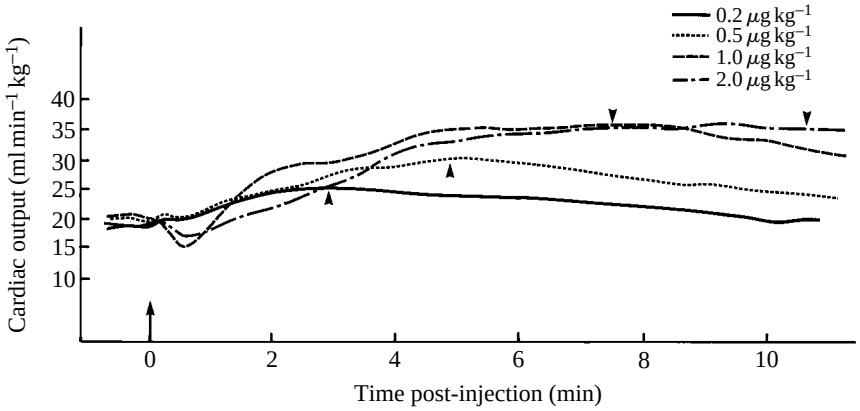

Fig. 2. Recordings of cardiac output ($\dot{Q}$) in a type 1 trout following the injection of various doses of adrenaline. Arrowheads indicate the point of maximum cardiac output ($\dot{Q}_{max}$). The point of injection of adrenaline is marked with an arrow.

Table 2. *Barostatic gain values (percentage change in* f_H *per unit change in* P_{DA}*) from heart rate and dorsal aortic blood pressures at pre-injection and at 2 min post-injection*

	Adrenaline dose (μg kg^{-1})			
	0.2	0.5	1.0	2.0
Type 1 (*N*=12)	5.9±2.2*	13.5±3.3	10.2±2.2	9.4±2.1
Type 2 (*N*=7)	30.3±7.1	28.0±6.0	21.6±4.7	15.7±3.4

Data are from trout with intact and ablated coronary arteries combined.
*Indicates a significant difference (P<0.05) between type 1 and type 2 trout.

continuum, with variations being of minimal physiological importance. In both type 1 and type 2 cardiac responses, P_{DA} always rose quickly following injection and reached maximum levels within 1–4 min (generally less than 2 min); for example, at 0.2 μg kg^{-1} the time required to reach peak P_{DA} was 123±29 s for type 2 fish and 99±13 s for type 1 fish. No obvious relationship between $\dot{Q}$ response pattern and P_{DA} response pattern was evident.

In both type 1 and type 2 fish, f_H decreased immediately post-injection (Figs 3, 4, 5). However, statistical analysis (2×2 factorial, repeated measures) of the drop in f_H between pre-injection and 2 min post-injection revealed that the degree of bradycardia was significantly greater in trout displaying type 2 responses at 0.2, 0.5 and 1.0 μg kg^{-1} (P<0.05), but not at 2.0 μg kg^{-1} (P=0.07). Because maximum P_{DA} was not significantly different between type 1 and type 2 fish at any dose (see next section), differences in barostatic gain (percentage change in f_H per unit P_{DA}) must have mediated discrepancies in the degree of bradycardia between response types. Indeed, barostatic gain was higher in type 2 fish at all dose levels; the difference was statistically significant at 0.2 μg kg^{-1} (Table 2). Although type 1 fish with intact coronaries (Fig. 4) appear to have a greater

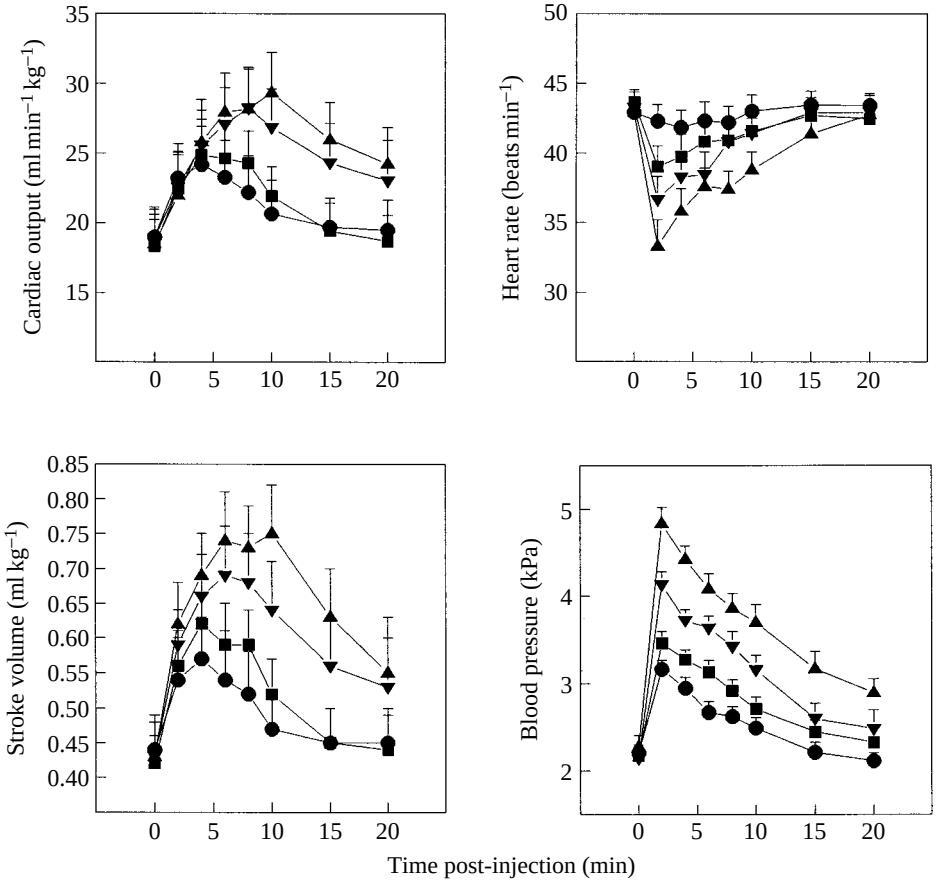

Fig. 3. Type 1 cardiovascular responses for trout with ablated coronary arteries to increasing doses of adrenaline. Each line represents a different dose: (●) 0.2 μg kg^{-1}; (■) 0.5 μg kg^{-1}; (▼) 1.0 μg kg^{-1}; (▲) 2.0 μg kg^{-1}. Vertical bars indicate the S.E.M. for each mean value (N=9).

reduction in f_H, compared with type 1 fish with ablated coronaries (Fig. 3), the difference was not significant.

Maximum V_S was reached at approximately 4–6 min at 0.2 μg kg^{-1} and at approximately 8–10 min at 2.0 μg kg^{-1} (Figs 3, 4, 5). In type 1 fish, V_S was elevated at 2 min post-injection at all dose levels. In type 2 fish, however, the initial response of V_S to adrenaline injection appeared to be dose-dependent. Increases in V_S were observed at 2 min post-injection at 1.0 and 2.0 μg kg^{-1}, but not at 0.2 or 0.5 μg kg^{-1}.

Maximum P_{DA} and minimum f_H were generally reached within 1–2 min post-injection, regardless of adrenaline dose. However, the time required to reach $\dot{Q}_{max}$ increased steadily with adrenaline dose (Figs 2, 6B). Trout with both type 1 and type 2 cardiac responses required significantly longer to reach $\dot{Q}_{max}$ at 1.0 and 2.0 μg kg^{-1}, compared with 0.2 and 0.5 μg kg^{-1} adrenaline. The presence or absence of a coronary artery had no effect on the time required to reach $\dot{Q}_{max}$. However, type 2 fish required substantially (170–270 %) longer to reach $\dot{Q}_{max}$ than did type 1 fish (Fig. 6B).

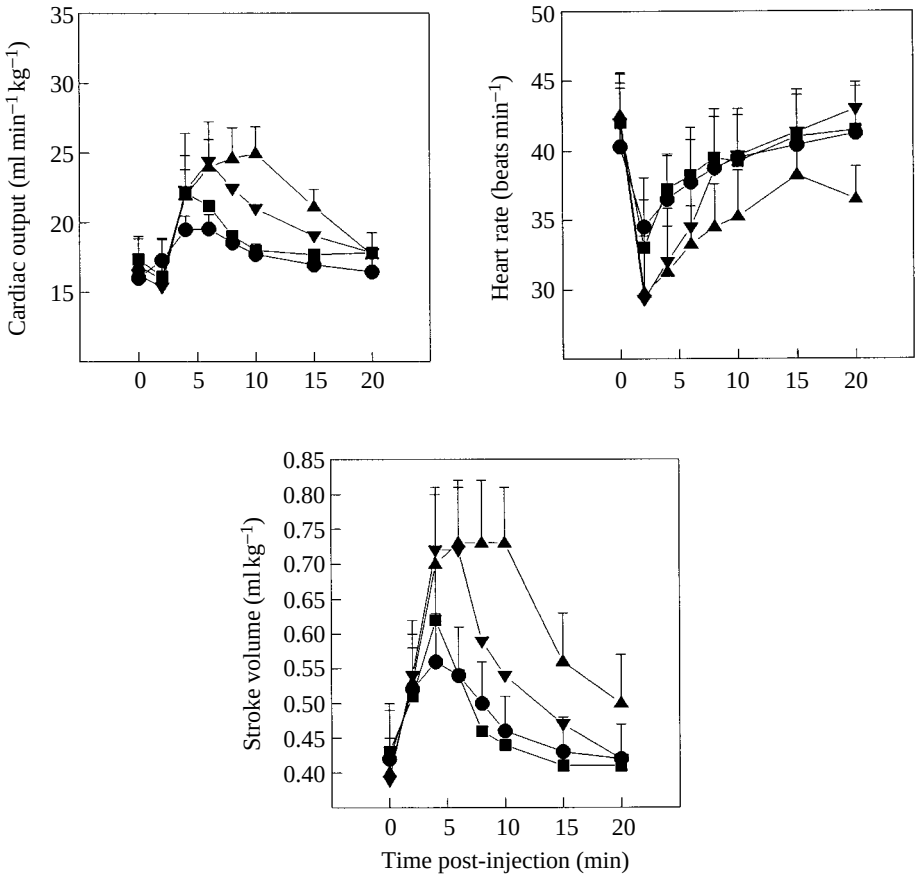

Fig. 4. Type 1 cardiovascular responses for trout with functional coronary arteries to increasing doses of adrenaline. Each line represents a different dose: (●) 0.2 μg kg^{-1}; (■) 0.5 μg kg^{-1}; (▼) 1.0 μg kg^{-1}; (▲) 2.0 μg kg^{-1}. Vertical bars indicate the S.E.M. for each mean value (N=4). Points without vertical bars, N=3. Response curves for P_{DA} are not shown because only two trout gave reliable recordings at all dose levels.

Magnitude of cardiovascular responses to adrenaline injection

A strong dose–response relationship was evident for all cardiovascular variables measured (Figs 3, 4, 5). The relationship was positive for $\dot{Q}$, P_{DA} and V_S, but negative for f_H. The maximum increase in $\dot{Q}$ ($\dot{Q}_{max}$), following adrenaline injection, was approximately 5.5 ml min^{-1} kg^{-1} at 0.2 μg kg^{-1} (30 % increase above resting values) and 10.3 ml min^{-1} kg^{-1} at 2.0 μg kg^{-1} (57 % increase above resting values). Although the adrenaline-stimulated increase in $\dot{Q}$ was significantly greater at 0.5 μg kg^{-1} than at 0.2 μg kg^{-1}, for all groups, only type 1 trout with intact coronary arteries showed a significant elevation in $\dot{Q}_{max}$ between 0.5 μg kg^{-1} and 2.0 μg kg^{-1}. At all dose levels, there was no significant difference in $\dot{Q}_{max}$ between fish displaying type 1 or type 2 responses (Fig. 6A). The mean ED_{50} value for the relationship between adrenaline dose and $\dot{Q}_{max}$, for all groups, was 0.22±0.07 μg kg^{-1} (range 0.17–0.34 μg kg^{-1}). Although the use of

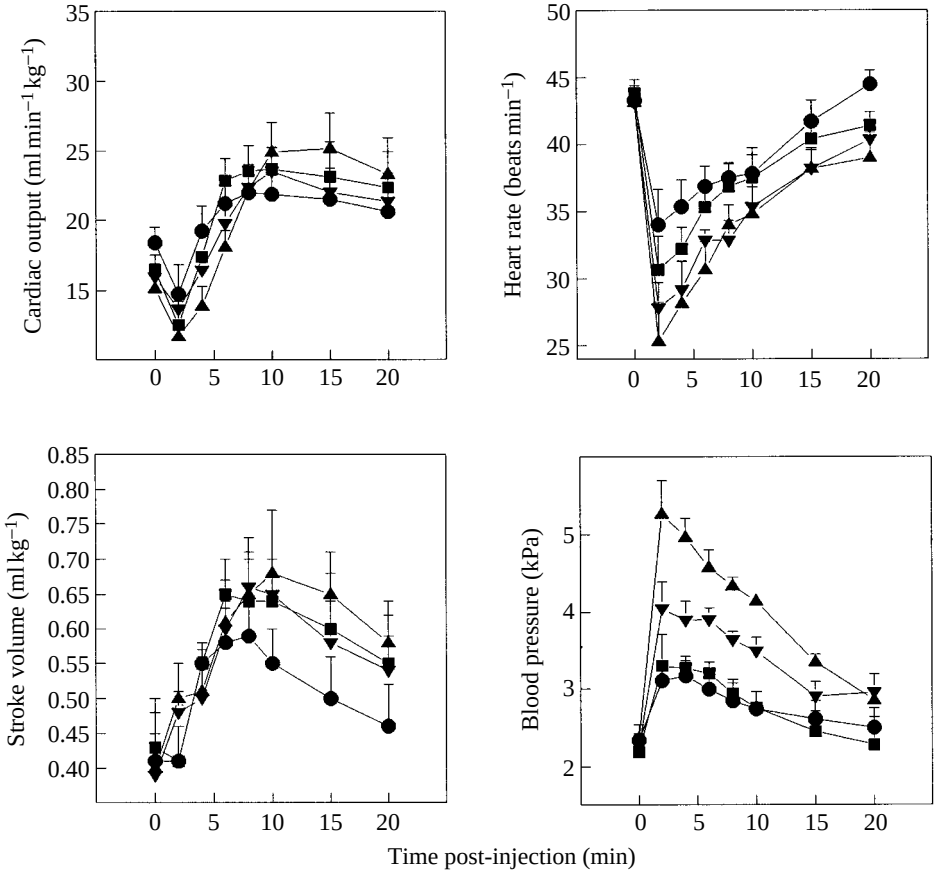

Fig. 5. Type 2 cardiovascular responses to increasing doses of adrenaline. Each line represents a different dose: (●) 0.2 μg kg^{-1}; (■) 0.5 μg kg^{-1}; (▼) 1.0 μg kg^{-1}; (▲) 2.0 μg kg^{-1}. Data are for trout with ablated (N=4) and functional coronary arteries (N=3) combined. Statistical analysis (2×2 factorial ANOVA, repeated measures) showed that the presence or absence of the coronary artery did not affect type-dependent alterations in cardiovascular performance. Vertical bars indicate the S.E.M. for each mean value.

chart recorders for measurement of $\dot{Q}$ and f_H precluded the determination of maximum Vs, analysis of post-injection Vs patterns indicates that elevations in Vs were also dose-dependent (Figs 3, 4, 5). Stroke volume increased, over pre-injection levels, by approximately 0.15 ml kg^{-1} (35 %) at 0.2 μg kg^{-1} and 0.31 ml kg^{-1} (74 %) at 2.0 μg kg^{-1}. Saline injection had negligible effects on any cardiovascular variable.

Post-injection elevations in P_{DA} were significantly different at all dose levels (Fig. 7). The mean increase for 'operated' fish was 1.03±0.01 kPa (48 %) at 0.2 μg kg^{-1} and 2.81±0.3 kPa (122 %) at 2.0 μg kg^{-1} adrenaline. No significant differences in P_{DA} elevation were detected between trout displaying dissimilar response types. However, fish possessing only a dorsal aortic cannula had a greater post-injection P_{DA} increase than did 'operated' fish. The increase in P_{DA}, compared with that in 'operated' fish, was approximately 30 % greater at 0.2, 0.5 and 2.0 μg kg^{-1}, and 38 % greater at 1.0 μg kg^{-1}.

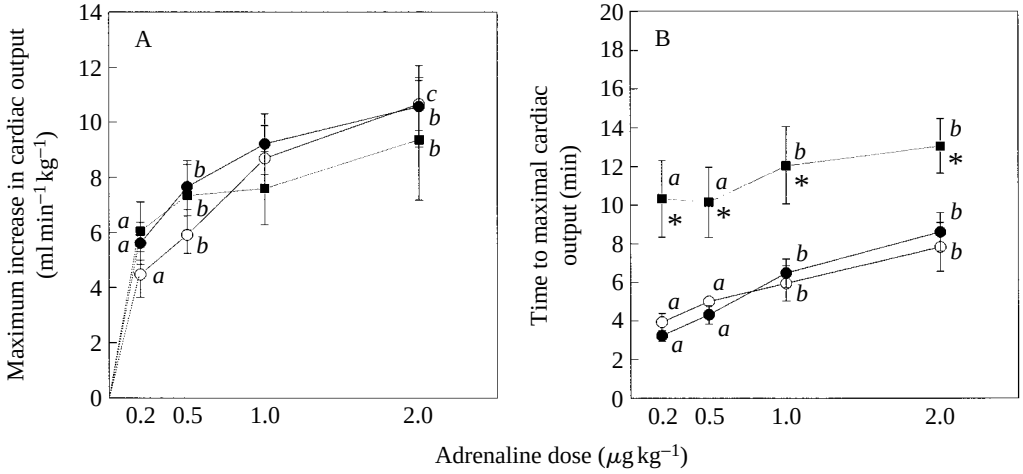

Fig. 6. Effect of increasing adrenaline dosage on the magnitude (A) and timing (B) of maximum cardiac output: (●) type 1 trout with ablated coronary arteries (N=9); (○) type 1 trout with functional coronary arteries (N=4); (■) type 2 trout, data from fish with ablated and functional coronary arteries combined (N=7). Vertical bars indicate S.E.M. for each mean value. Dissimilar letters indicate significant differences (P<0.05) between doses, within each group. * Indicates a significant difference (P<0.05) between type 1 and type 2 trout within a particular dose. Statistical analysis was performed using a 2×2 factorial (ANOVA) with repeated measures. Comparisons across doses were made using multiple contrasts. Saline injection had negligible effects on cardiac output. Each fish acted as its own control.

The ED_{50} values for only cannulated fish, type 1 fish, and type 2 fish, were 0.48, 0.39 and 0.56 μg kg^{-1}, respectively (mean 0.48 μg kg^{-1}).

Coronary ablation had negligible effects on adrenaline-stimulated cardiovascular performance. At all dose levels, no significant differences in P_{DA} or $\dot{Q}_{max}$ were identified between trout with intact and ablated arteries (Figs 3, 4, 6A).

Discussion

Cardiac performance in resting trout

Although resting f_H and P_{DA} in trout with dorsal aortic cannulae (39 beats min^{-1}; 2.8 kPa) were comparable to those obtained at 5 °C (Wood, 1968; 39 beats min^{-1}) and at 12 °C (Stevens and Randall, 1967; 47 beats min^{-1}, 3.51 kPa), the f_H of our trout was 10 beats min^{-1} higher than those reported by Wood *et al.* (1979) at 5 °C (29 beats min^{-1}; 2.95 kPa). In addition, while the values of resting $\dot{Q}$ (18 ml min^{-1} kg^{-1}) and V_S (0.42 ml kg^{-1}) are similar to those reported for resting trout (Kiceniuk and Jones, 1977; Cameron and Davis, 1970) and cod (Jones *et al.* 1974; Axelsson, 1988) at 10 °C, the values for $\dot{Q}$ are decidedly lower than those obtained by Wood and Shelton (1980*a*; 36 ml min^{-1} kg^{-1}) and Neumann *et al.* (1983; 46 ml min^{-1} kg^{-1}) for trout. Differences in experimental temperature (Q_{10} for cardiovascular variables of approximately 2.0, Farrell and Jones, 1992), in the time allowed for recovery from surgical procedures, in the method of $\dot{Q}$ measurement (Fick principle *versus* direct measurement) and in the

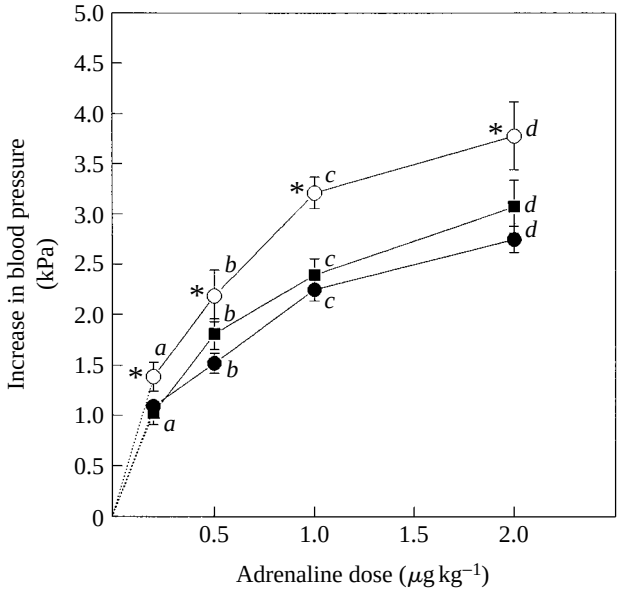

Fig. 7. Effect of increasing adrenaline dosage on peak dorsal aortic pressure (P_{DA}): (●) type 1 trout, data from fish with ablated and functional coronary arteries combined (N=13); (■) type 2 trout, data from fish with ablated and functional coronary arteries combined (N=7); (○) data from fish fitted only with a dorsal aortic (DA) cannula (N=9). Vertical bars indicate S.E.M. for each mean value. Dissimilar letters indicate significant differences (P<0.05) between doses within each group. * Indicates a significant difference (P<0.05) between 'operated' trout and trout possessing only a dorsal aortic cannula, within a particular dose. Statistical comparisons were performed using analysis of variance, followed by multiple contrasts. Saline injection had negligible effects on P_{DA}. Each fish acted as its own control.

magnitude of 'post-operative stress' make comparisons of resting cardiovascular variables between studies difficult. However, it appears that the values for resting f_H, P_{DA}, $\dot{Q}$ and V_S in our fish are comparable to those reported by other authors.

Coronary artery ablation did not affect resting cardiovascular variables. This result is consistent with the findings of Davie and Farrell (1991) and Davie *et al.* (1992), who showed that $\dot{Q}$ is only dependent upon coronary perfusion during periods of elevated output pressure and hypoxaemia. Trout exhibiting type 1 and type 2 cardiac responses to adrenaline injection had similar values for resting $\dot{Q}$, f_H, V_S and P_{DA}. This result indicates that resting cardiovascular variables cannot be used to predict whether a trout will show a type 1 or a type 2 response upon adrenergic stimulation.

Resting 'operated' trout had a significantly lower P_{DA} and a higher f_H compared with trout fitted only with a dorsal aortic cannula. Farrell *et al.* (1988) showed that cutting the pericardium of an *in situ* perfused heart (with constant cardiac filling pressure and f_H) reduced 'control' cardiac output, stroke volume and cardiac power output by 44–46%. Rainbow trout *in vivo* can clearly compensate for this effect, since cutting the pericardium does not appear to affect resting cardiac output; our values for $\dot{Q}$ are comparable with those of Kiceniuk and Jones (1977) and Cameron and Davis (1970). However, the

observed alterations in f_H and P_{DA} suggest possible mechanisms by which cardiac output could be maintained. Homeometric regulation (Farrell, 1984) of preload (increase) and afterload (decrease) may have maintained $\dot{Q}$ through increased V_S, without a concomitant increase in cardiac power output ($\dot{Q}\times$afterload). In addition, a decrease in cholinergic tonus and/or an increase in adrenergic tone could have increased f_H (Wood *et al.* 1979; Axelsson, 1988) and therefore $\dot{Q}$. Although increased levels of catecholamines caused by surgery/confinement could have enhanced $\dot{Q}$ through positive inotropic and chronotropic effects (Farrell, 1984), the lower P_{DA} in 'operated' fish compared with cannulated fish is inconsistent with this hypothesis. Elevated catecholamine titres would have increased systemic vascular resistance, and therefore P_{DA}, through α-adrenoreceptor-mediated vasoconstriction (Wood and Shelton, 1980*b*); these effects increase the requirement for cardiac power output.

Cardiovascular response types

Adrenaline injection resulted in two distinct types of cardiac 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. Different cardiac response patterns within a fish population have been demonstrated previously. Hughes *et al.* (1988) reported that rainbow trout displayed two different *in vitro* relationships between systolic pressure and f_H, and that fish that were unable to maintain systolic pressure at high heart rates ($>50\,\text{beats}\,\text{min}^{-1}$) had reduced maximum swimming speeds. In addition, Wood and Shelton (1980*a*) identified four patterns of cardiac response to adrenaline administration; two variations on a type 1 response, a type 2 response and a response in which there was no post-injection elevation of $\dot{Q}$. The results of Farrell *et al.* (1986) indicate that trout displaying type 2 responses may have 'failing hearts'. However, the lack of type-specific differences in resting cardiovascular variables and the dose-dependency of cardiac responses ($\dot{Q}$, P_{DA}, f_H) in type 2 fish suggest that an alternative mechanism determined the expression of response type.

Changes in $\dot{Q}$ following adrenaline injection are mediated through an atropine-sensitive reflex bradycardia, direct adrenergic stimulation of the heart (positive inotropic and chronotropic effects) and reduced systolic emptying (V_S) in the face of elevated output pressures (Helgasson and Nilsson, 1973; Wood and Shelton, 1980*a*; Farrell, 1984; Hipkins, 1985; Davie *et al.* 1992). Trout exhibiting type 2 responses had a greater post-injection decrease in f_H and an increased barostatic gain compared with type 1 trout. These results suggest that an increased vagal cardioinhibitory tone probably mediated the greater decrease in post-injection f_H ($\dot{Q}$) in type 2 fish. In addition, evidence suggests that an increased barostatic gain may have reduced post-injection V_S. Holmgren (1977) showed that acetylcholine inhibits the contraction force of paced atrial strips in the cod. Because ventricular filling is dependent upon atrial contraction (Jones and Randall, 1978), an increased vagal (cholinergic) reflex may have resulted in the diminished V_S increases observed for type 2 trout. Wood and Shelton (1980*a*) found that atropine treatment (blockade of cholinergic input to the heart) did not affect the pattern of *in vivo* $\dot{Q}$ alterations associated with adrenaline injection. Although this result contrasts with the

proposed barostatic (cholinergic) mediation of type 1 and type 2 cardiac responses, evidence suggests that the trout of Wood and Shelton (1980*a*) were severely stressed. First, resting cardiac output in their trout was 36 ml min^{-1} kg^{-1} and, second, propranolol (a β-adrenergic antagonist) severely reduced resting f_H, V_S and $\dot{Q}$ in some fish, indicating that a considerable adrenergic tone was required to maintain cardiac function. Direct adrenergic stimulation (chronotropic and inotropic) of the heart was evident post-injection (Figs 3, 4, 5). However, it is unlikely that differences in cardiac adrenergic sensitivity resulted in the presence of two distinct cardiovascular (f_H, V_S, $\dot{Q}$) response patterns. Farrell (1981) found that cardiovascular changes occurring less than 100 s following a pre-gill drug infusion are unlikely to be caused by direct agonist effects on the heart. In our study, although adrenaline was injected into the dorsal aorta, it is highly improbable that the injected adrenaline could have reached the heart in less than 40 s; there was less than 40 s between adrenaline injection and the start of the fall in $\dot{Q}$ in type 2 trout (Fig. 1). Wood and Shelton (1980*a*) suggest that adrenaline-stimulated increases in blood pressure limit V_S in individuals where heart contractility is close to maximal. However, the ability of type 2 fish to elevate V_S at 1.0 and 2.0 μg kg^{-1} (Fig. 5, where peak P_{DA}, and presumably P_{VA}, were highest) precludes this interpretation of our results. While an increased vagal (cholinergic) reflex is the most plausible explanation for the observed differences in V_S and f_H between type 1 and type 2 trout, other explanations cannot be ruled out. Discrepancies in the degree of damage to adrenergic nerves present in the bulbus arteriosus, in the degree of cardiac stimulation through spinal and vagal nerve sympathetic activity and in the homeometric regulation of preload (venous pressure) may have also contributed to the presence of type-specific $\dot{Q}$ response patterns.

The maximum increase in post-injection $\dot{Q}$ for type 1 and type 2 fish was not different at any dose. However, the time required to reach $\dot{Q}_{max}$ was 4–6 min greater at all dose levels for type 2 trout (Fig. 6B). Because the time course of P_{DA} and V_S did not differ greatly between type 1 and type 2 responses (Figs 3, 5), our data suggests that vagally (cholinergically) mediated increases in f_H depression were also responsible for the delayed $\dot{Q}_{max}$ observed in type 2 fish.

Dose-dependent alterations in cardiac performance

In both response types, there was a significant relationship between adrenaline dose and time to $\dot{Q}_{max}$ (Figs 2, 6B). Because increased afterload reduces the extent of systolic emptying (Wood and Shelton, 1980*a*; Davie *et al.* 1992), the dose-dependency of P_{DA} (and presumably P_{VA}) elevation could have resulted in a delayed maximum V_S at high adrenaline doses. However, it appears that temporal alterations in V_S can only partially explain the dose-dependent increase in time to $\dot{Q}_{max}$. In type 1 fish (Fig. 3), for example, it appears that maximum V_S was achieved at 4 min at 0.2 and 0.5 μg kg^{-1} and at 6 min at 1.0 and 2.0 μg kg^{-1}. This small difference in time to maximum V_S (2 min) only accounts for about half of the discrepancy in time required to reach $\dot{Q}_{max}$ between 0.2 and 2.0 μg kg^{-1}. Cardiac output is the product of f_H and V_S. Since the degree of f_H depression and the time required for f_H to return to pre-injection values were also dose-dependent (e.g. Fig. 3), the relationship between adrenaline dose and time to $\dot{Q}_{max}$ was probably determined by the interaction of changes in V_S and f_H.

From Fig. 6A, it appears that most of the scope for adrenaline-stimulated increases in $\dot{Q}_{max}$ occurs between 0 μg kg^{-1} adrenaline (saline) and 0.5 μg kg^{-1} adrenaline; injection of 0.5 μg kg^{-1} caused an increase in $\dot{Q}$ of 6–8 ml min^{-1} kg^{-1}, while increasing the dose to 1.0 or 2.0 μg kg^{-1} only increased $\dot{Q}$ a further 2–4 ml min^{-1} kg^{-1}. In contrast to $\dot{Q}$, maximum P_{DA} was significantly higher at all dose levels. This information on the dose-dependency of $\dot{Q}_{max}$ (ED_{50} 0.22 μg kg^{-1}) and P_{DA} (ED_{50} 0.48 μg kg^{-1}), when combined, suggests that the large increases in systemic vascular resistance (P_{DA}) and output pressure which accompany doses greater than 1.0 μg kg^{-1} limit the ability of adrenaline to increase V_S (systolic emptying). Farrell *et al.* (1986) found that adrenaline exerted its maximal effect on the perfused trout heart at 10 nmol l^{-1}. A dose of 4.0 μg kg^{-1} adrenaline results in maximal circulating adrenaline concentrations of 107 nmol l^{-1} (A. K. Gamperl, M. M. Vijayan and R. G. Boutilier, in preparation). If we assume a 1:1 relationship between injected adrenaline dose and realised plasma levels, a dose of 0.5 μg kg^{-1} could elevate circulating adrenaline levels to 14 nmol l^{-1}. According to Farrell *et al.* (1986), this plasma adrenaline titre would lead to near maximal stimulation of cardiac performance (V_S or cardiac power output). Therefore, the diminished ability of higher adrenaline doses (1.0 and 2.0 μg kg^{-1}) to elevate $\dot{Q}$ significantly may also have occurred because circulating adrenaline titres were above those required to achieve maximal adrenergic stimulation of the heart.

The dose–response curves for dorsal aortic pressure show that 'operated' fish have a diminished ability to elevate P_{DA} compared with trout having only a dorsal aortic cannula. Because implantation of the Doppler flow probe is unlikely to affect α-adrenoreceptor-mediated systemic constriction, the decrease in P_{DA} (approximately 30 %) must have resulted from a decrease in adrenaline-stimulated $\dot{Q}$. Farrell *et al.* (1988) showed that cutting the pericardium of *in situ* perfused trout hearts reduced maximum cardiac output and cardiac power output by 8 % and 18 %, respectively. These results indicate that the observed increases in $\dot{Q}$ (Fig. 6) may underestimate those in 'intact' fish by 10–30 %, providing indirect support for the hypothesis that the pericardium is important for determining maximum cardiac performance *in vivo*.

Ablation of the coronary artery in trout, under normoxic conditions, appears to have little effect on adrenaline-stimulated cardiac performance. Maximum $\dot{Q}$, maximum P_{DA} and the time required to achieve $\dot{Q}_{max}$ were not significantly different between fish with ablated and intact coronary arteries. These results support recent *in vitro* experiments on eel (*Anguilla australis*; Davie *et al.* 1992) and dogfish shark hearts (*Squalus acanthias*; Davie and Farrell, 1991), which concluded that maximum cardiac output, during normoxaemia, is not dependent upon coronary perfusion; i.e. the oxygen supplied by the venous (luminal) blood is sufficient to support cardiac performance in most fish. The time courses for alterations in stroke volume, following adrenaline injection, were similar in trout with intact and ablated coronaries (e.g. Figs 3, 4). This result also suggests (1) that during normoxaemia, coronary blood flow is not essential for cardiac pressure development and/or systolic emptying; and (2) that the presence of a coronary circulation is not a prerequisite for adrenergic stimulation of the compact myocardium by circulating catecholamines.

In type 1 trout, an adrenaline injection of $2.0\,\mu g\,kg^{-1}$ increased $\dot{Q}$ from $18\,ml\,min^{-1}\,kg^{-1}$ to $28.3\,ml\,min^{-1}\,kg^{-1}$, P_{DA} from 2.2 kPa to 3.8 kPa (this value representing P_{DA} at $\dot{Q}_{max}$, 10 min post-injection) and cardiac power output by approximately 200 % (assuming that the post-injection change in ventral aortic pressure/change in P_{DA} was approximately 1.2; Wood and Shelton, 1980*a*). In most fish, cardiac power output can increase two- to fourfold with exercise or adrenergic stimulation (Farrell and Jones, 1992; Table 2). Therefore, although this study suggests that the coronary circulation is not necessary for determining adrenaline-stimulated cardiac performance, or cardiac power output during moderate swimming, it does not preclude the possibility that coronary blood flow is important for maintaining maximum cardiac performance: e.g. rainbow trout swimming at 90 % of critical swimming speed (U_{crit}) must increase cardiac power output, over resting levels, by 360 % (Kiceniuk and Jones, 1977).

In summary, differences in adrenaline-stimulated cardiac performance between fish exhibiting type 1 and type 2 cardiovascular responses are probably mediated by disparities in the degree of vagally (cholinergically) mediated cardiac inhibition (i.e. barostatic gain). The capacity of elevated adrenaline titres to increase $\dot{Q}$ may be limited by increases in output pressure and/or by the relatively low concentration of adrenaline ($10\,nmol\,l^{-1}$) required to cause near maximal adrenergic stimulation of the heart. Cardiac performance, during normoxic conditions, does not depend greatly on coronary blood flow. Differences in cardiovascular variables between 'operated' and 'cannulated' trout provide indirect support for the hypothesis that the pericardium enhances cardiac performance in trout *in vivo*.

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