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CORONARY FLOW IN A PERFUSED RAINBOW TROUT HEART

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SUMMARY

A preparation was developed to perfuse the coronary circulation in working hearts from rainbow trout (*Salmo gairdneri* Richardson). The preparation was used to examine pressure–flow relationships for the coronary circulation as the heart generated physiological and subphysiological work loads. Coronary vascular resistance increased exponentially as coronary flow rate decreased. Coronary resistance was also influenced by cardiac metabolism and acclimation temperature. When heart rate was increased, extravascular compression increased in coronary resistance. Direct vasoconstriction of the coronary vessels, produced by injections of adrenaline into the coronary circulation, was temperature-dependent.

INTRODUCTION

Fish occupy an interesting position among vertebrates because the development of the coronary circulation in a given species is closely related to its activity level (McWilliam, 1885, as cited by Santer, 1985; Hesse, 1921; Grant & Regnier, 1926). The ventricle of benthic fish, such as the catfish (*Siluris glanis*) and the sea raven (*Hemitripterus americanus*), is composed of a spongy myocardium, which lacks a coronary circulation and which relies on venous blood being pumped through the heart for an adequate oxygen supply (Cameron, 1975; Jones & Randall, 1978; Farrell, Wood, Hart & Driedzic, 1985). In contrast, fish that are capable of a higher level of sustained activity, such as salmonids, *Anguilla anguilla*, *Scomber scombrus*, *Scomber colias*, *Esox lucius* and *Thymallus articus*, have a ventricle which consists of two types of myocardium; an outer, compact layer which receives an arterial oxygen supply from a coronary circulation, and an inner, spongy myocardium which receives oxygen from venous blood. The compact layer commonly accounts for 25–45 % of the ventricle mass in such species (Cameron, 1975; Santer & Greer Walker, 1980). Some fish, such as anchovy (*Engraulis encrasicolus*) and certain species of tuna (e.g. *Thunnus obesus*) possess a well-developed coronary system and the compact myocardium accounts for up to 76 % of the ventricle mass (Santer & Greer Walker, 1980). Furthermore, the development of the coronary circulation is generally associated with a relatively larger ventricle (Hesse, 1921); a factor which

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presumably relates to the higher blood pressure and cardiac output found in, for example, tuna compared to the sea raven (Farrell & Driedzic, 1981; D. R. Jones, personal communication; Farrell, 1985).

These relationships among fishes imply that the high cardiac work associated with high levels of activity requires a distinct coronary network to support aerobic metabolism (Cameron, 1975). The relative importance of the coronary circulation in this regard was recently demonstrated for chinook salmon (*Oncorhynchus tshawytscha*). Acute coronary ligation, which restricted arterial blood flow to the outer 30 % of the ventricle, reduced maximum aerobic swimming speed by 35 % (Farrell & Steffensen, 1986). While Daxboeck (1982) performed similar experiments and did not find a reduction in maximum swimming speed in rainbow trout (*Salmo gairdneri*), the importance of the coronary circulation was nevertheless implied by a rapid vessel regrowth which apparently restored coronary flow around the chronic coronary ablation site.

In fish information on coronary physiology, such as flow rate and mechanisms for its control, is scant. Observations on intact fish are limited by difficulty of access to the coronary vessels. However, Cameron (1975) used microspheres to measure coronary flow in the sucker (*Catostomus catostomus*) and burbot (*Lota lota*) as 0·65 % and 0·56 % of cardiac output, respectively. Farrell & Graham (1986) used coronary perfusion studies to estimate coronary flow as 0·6 % to 2·4 % of cardiac output in Atlantic salmon (*Salmo salar*). Other coronary perfusion studies with the conger eel (*Conger conger*, Belaud & Peyraud, 1971) and marlin (*Makaira nigricans*, Davie & Daxboeck, 1984) arbitrarily used a coronary flow of about 1 % of cardiac output. These studies indicate that coronary flow in fish is appreciably lower than the 4–5 % of cardiac output reported for mammals (Berne & Rubio, 1979; Feigl, 1983). However, the accuracy of the estimates for fish may be questioned on either methodological grounds or the fact that the perfused hearts were either performing at a subphysiological work load (i.e. only the coronary drainage; Farrell & Graham, 1986; Belaud & Peyraud, 1971) or were not working (Davie & Daxboeck, 1984). In terms of the control of the coronary circulation in fish, coronary vascular smooth muscle (VSM) contains adrenoceptors that are similar to those found in mammals (Davie & Daxboeck, 1984; Farrell & Graham, 1986). However, little is known about other major control mechanisms which are well-documented for mammals, namely aortic blood pressure, myocardial extravascular compression, and local factors relating to metabolism (Feigl, 1983).

The first objective of the present study was to develop a reliable heart preparation in which the coronary artery was perfused while the heart performed a physiological work load. The second objective was to examine pressure–flow relationships for the coronary circulation. The influence of myocardial work load, heart rate and acclimation temperature on this relationship were studied.

MATERIALS AND METHODS

Salmo gairdneri were obtained from local suppliers and held at Simon Fraser University in 2000 l tanks supplied with dechlorinated tap water. The fish were

exposed to a photoperiod simulating 49° N and acclimated to a water temperature of either 5, 10 or 15°C for at least 2 weeks. The acclimation temperature roughly followed the seasonal variation in water temperature at the hatchery. The fish were fed commercial trout chow *ad libitum*.

Saline

The composition of the basic saline was a modified version of Cortland saline (Wolf, 1963) and contained (in $g l^{-1}$): NaCl, 7·25; KCl, 0·23; $CaCl_2$, 0·22; $MgSO_4.7H_2O$, 0·24; $NaH_2PO_4.H_2O$, 0·014; Na_2HPO_4 0·35; $NaHCO_3$, 0·95; dextrose, 1·0. The saline was gassed with 99·5 % O_2: 0·5 % CO_2 and it had a pH of 7·9 at 10°C. Either 10 $g l^{-1}$ PVP (polyvinylpyrrolidone, M_r 40 000) or 10 $g l^{-1}$ albumin (fraction V, Sigma Chemicals) was added to the basic saline as a colloid substitute in series I and II. 1 % PVP was added to the perfusates in series III and IV. The perfusate for the coronary circulation was filtered (8 μm, Nucleopore) before use.

Heart preparation

The heart preparation was similar to that developed for the Atlantic salmon (Farrell & Graham, 1986). The heart was removed from the animal and placed in an ice-chilled dish of oxygenated saline for the cannulation procedures, following a caudal vein/artery injection of 75 i.u. of sodium heparin in 0·5 ml saline and a sharp cranial blow. Stainless steel or polyethylene (PE, Clay Adams) cannulae were secured in the ventral aorta and atrium (at the junction with the sinus venosus). A 0·8-cm long saline-filled PE 10 cannula was inserted into the coronary artery *via* a puncture hole made with either a 25 gauge or 23 gauge hypodermic needle. The coronary cannula was secured to the ventral aorta with 4–0 silk so that the tip of the cannula lay upstream of any branch point in the coronary artery. Coronary perfusion was started from a saline reservoir as soon as the cannula was in place and rapidly cleared the blood from the coronary circulation. Spontaneous beating of the heart cleared blood from the lumen of the heart by drawing saline from the operating dish into the atrium. Preparation time was 5–15 min.

Coronary perfusion

The heart was transferred to an organ bath for the experiment and was submerged in either basic saline (series I and II) or mineral oil (series III and IV). A water jacket around the organ bath maintained the experimental temperature the same as the acclimation temperature (5, 10 or 15°C). The coronary circulation was perfused with a peristaltic pump (Haake Buchler MCP2500, Saddlebrook, NJ) at an initial rate of 0·33 ml $min^{-1} kg^{-1}$ BM (BM = body wet mass). This flow rate represented about 2 % of the resting cardiac output in trout at 10°C (17 ml min^{-1} kg^{-1} BM, Kiceniuk & Jones, 1977). The ventral aortic cannula was connected to a pressure head at 45–50 cmH_2O (1 cmH_2O = 98·1 Pa) which simulated a physiological afterload (50 cmH_2O; Kiceniuk & Jones, 1977). The coronary flow drained into the atrium *via* the coronary veins and was ejected *via* the ventral aorta with each

heart beat. The integrity of the coronary perfusion system under representative pressure–flow regimes was established by comparing the known inflow into the coronary circulation with the measured outflow from the ventral aortic cannula at various inflow rates (series I and II). Since the atrial cannula was open to the atmosphere, the only fluid entering the atrium was coronary drainage, and therefore an intact coronary circulation was indicated by comparable inflow and outflow values. Hearts set up in this manner generated a subphysiological power output (i.e. the product of coronary flow and output pressure) and were used for series I and II.

Cardiac perfusion

In experiments where the heart generated a physiological power output (series III and IV), the atrial input cannula was connected to a saline reservoir *via* a constant-pressure head device (Fig. 1; Farrell, MacLeod & Driedzic, 1982). This reservoir could be adjusted to vary stroke volume of the heart and set cardiac output. Electrical pacing *via* electrodes attached to the atrial and ventral aortic cannulae overrode the spontaneous heart beat. The voltage (1·1–1·2 V) and duration (80 ms) of the electrical stimulation were just sufficient to override spontaneous contractions and did not appear to disturb the direction of flow through the heart. If the direction of flow had been disturbed, it is unlikely that high cardiac outputs (see below) could have been generated. Furthermore, higher voltages were observed to disrupt flow.

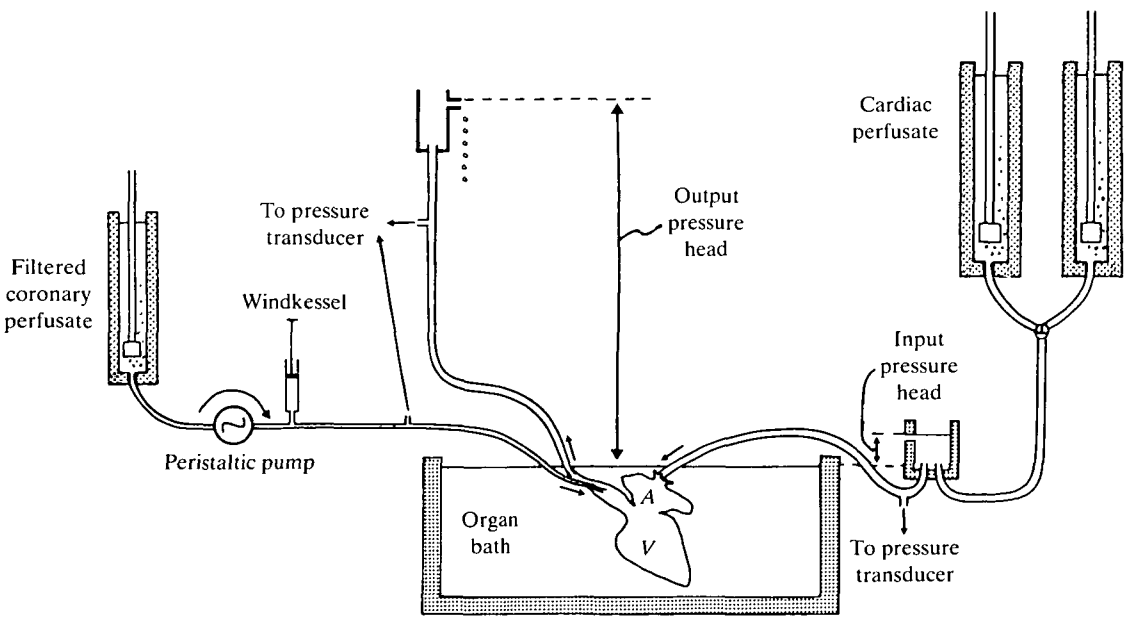

Fig. 1. A diagram of the apparatus for perfusing the coronary artery in working trout hearts. The coronary perfusate was delivered at a constant flow. The cardiac perfusate was delivered to the atrium at a constant input pressure, which was adjusted to set the stroke volume of the heart. Cardiac output was pumped against a physiological output pressure. The experimental temperature was maintained by water jackets (stippled area) around the organ bath and reservoirs. *A*, atrium; *V*, ventricle.

Protocols

Four experimental protocols were used. Series I and II provided pressure–flow curves for hearts performing at subphysiological work loads. They were also used to evaluate the usefulness of a colloid substitute in the perfusate and to confirm that adrenergic responses were retained. In series III and IV, pressure–flow characteristics of the coronary circulation were re-examined for hearts generating a physiological cardiac output at various heart rates.

Hearts performing a subphysiological work load

Series I

These experiments were performed at 5 °C with the heart immersed in oxygenated saline ($N = 7$ fish). The flow from the ventral aorta represented the coronary drainage into the atrium and was measured with a drop counter. Heart rate was a spontaneous rhythm.

The experimental protocol began after a 5–10 min equilibration to the experimental temperature and the initial flow rate. The coronary perfusion pressure was stable after this time. Basic saline was used as the perfusate when examining pressure–flow relationships as coronary flow was varied from 0·17 to 0·67 ml min^{-1} kg^{-1} BM in a stepwise fashion. Coronary pressure was stable for at least 5 min at each new flow rate before the value was recorded.

Coronary flow was returned to 0·33 ml min^{-1} kg^{-1} BM to evaluate the activity of coronary VSM adrenoceptors. Saline injections (50 μl containing 0, 0·01, 0·1 and 1·0 μmol l^{-1} L-adrenaline-HCl) were made into the coronary cannula. The maximum concentration of the adrenaline injection exceeded the maximum plasma level found in trout following stressful exercise (0·05 μmol l^{-1}; Primmett, Randall, Mazeaud & Boutilier, 1986) in order to ensure stimulation of alpha-adrenoceptors.

To establish whether vascular resistance or adrenergic vasoactivity changed with time, the above protocol was repeated with the basic saline for each preparation. In this way, each heart acted as its own control.

Series II

Series II examined coronary pressure–flow relationships using the same protocol as in series I, but at an experimental temperature of 15 °C ($N = 7$ fish). The usefulness of a colloid substitute in the perfusate (see Ellis & Smith, 1983) was examined by determining the first pressure–flow relationship with basic saline and the second with basic saline containing a colloid substitute. Again, each heart acted as its own control.

Hearts performing a physiological work load

Series III

The objective of this series was to examine pressure–flow relationships with the heart generating a constant cardiac output (10 ml min^{-1} kg^{-1} BM) and output pressure (45–50 cmH_2O) ($N = 5$ fish). The experimental temperature was 5 °C.

Coronary flow was varied between 0·17 and 0·67 ml min^{-1} kg^{-1} BM, and at each coronary flow heart rate was varied (15–55 beats min^{-1}) to examine the effect of heart rate on coronary vascular resistance. The following protocol was used. Initially, heart rate was 45 beats min^{-1} and coronary flow was 0·33 ml min^{-1} kg^{-1} BM. Coronary flow was then increased to 0·67 ml min^{-1} kg^{-1} BM and the heart was paced sequentially at 55, 45, 30 and 15 beats min^{-1}. Coronary input pressure stabilized within 1 min at each new heart rate. Heart rate was restored to 45 beats min^{-1} before repeating the protocol at lower coronary flow rates (0·50, 0·33 and 0·17 ml min^{-1} kg^{-1} BM). Each preparation acted as its own control for the effect of heart rate on coronary flow.

Series IV

The protocol for series IV was the same as for series III, except that the experimental temperature was 10°C and the resting cardiac output was accordingly set at about 17 ml min^{-1} kg^{-1} BM ($N = 6$ fish). The maximum cardiac performance of the perfused heart was assessed at the end of the 1·5- to 2-h routine protocol in five of the six preparations. Atrial input pressure was increased to generate the maximum cardiac output at 45 beats min^{-1} and output pressure was increased to determine the work done at maximum pressure, before cardiac output was compromised appreciably.

Instrumentation

Coronary input pressure, ventral aortic output pressure and atrial input pressure were measured *via* saline-filled tubes connected to Micron pressure transducers (Narco Life Sciences, Houston, TX). The transducers were calibrated before each experiment and regularly referenced to the fluid level in the organ bath during the experiment. All pressure measurements were corrected for the cannula resistance. The resistance of the coronary cannula, with the securing thread in place, was measured after each experiment. The pressure signals were suitably amplified and displayed on a chart recorder (Gould, Cleveland, OH). Cardiac output (averaged over 10 consecutive heart beats) was measured either with a drop counter (Narco Life Sciences) in series I and II, or gravimetrically with a top-loading balance accurate to 0·01 g in series III and IV.

Calculations

Pressures were measured in cmH_2O (1 cmH_2O = 98·1 Pa). Cardiac output (ml min^{-1}) was determined from the product of heart rate and stroke volume. Myocardial power output (mW) was calculated from [cardiac output/60 (ml s^{-1})] × [afterload − preload (cmH_2O)] × [980 (cm s^{-2})/10 000 (mW erg^{-1}) (1 erg = 10^{-7} J)]. The blotted wet mass of the ventricle was determined after each experiment. Myocardial power output and coronary flow were initially based on the

Table 1. *Fish and ventricle masses in rainbow trout*

	Series I	Series II	Series III	Series IV
Temperature (°C)	5	15	5	10
Fish mass (g)	1330 (60)	1620 (60)	1450 (60)	1500 (70)
Ventricle mass (g)	1·84 (0·14)	1·98 (0·12)	1·75 (0·17)	1·18 (0·09)
Relative ventricle mass (%)	0·138	0·122	0·121	0·078
N	7	7	5	6

Mean values (S.E.M.) are presented.

body mass of the fish, since this was known before the experiment, but were normalized per gram ventricle mass (VM) for data presentation. Fish and ventricle masses are presented in Table 1.

Vascular resistance of the coronary circulation was calculated from [coronary input pressure (cmH_2O)]/[coronary flow ($ml\,min^{-1}\,g^{-1}$ VM)]. Vascular resistance calculated in this manner reflects differences in the viscosity of the various perfusates due to temperature and/or the presence of a colloid substitute. Therefore, to compare vascular resistances under the different experimental conditions for the four experimental series a representative vascular resistance for a common flow rate ($0{\cdot}33\,ml\,min^{-1}\,g^{-1}$ VM) was normalized. The measured vascular resistance was then normalized to 10°C and 1 % PVP in the saline (i.e. the conditions used in series IV), using appropriate correction factors for the effects of temperature and PVP on the viscosity of the perfusate (see Table 2). A similar conversion was used for data given in Fig. 5 to account for the higher viscosity of blood compared with perfusate. These conversions are similar to those performed by Wood (1974) for the gill circulation.

Nine additional preparations were terminated when air bubbles entered the coronary circulation during the experiment. These incomplete data sets were not used in the results presented below because of the paired experimental design. Even so, the data were not at variance with the conclusions drawn from preparations with complete data sets.

Values are presented as mean ± S.E.M., and the Student's paired and unpaired *t*-tests were used, where appropriate, to determine statistically significant differences ($P < 0{\cdot}05$). Each preparation served primarily as its own control which permitted paired statistical analysis and minimized the influence of biological variability. Statistical statements pertaining to paired analyses are often complemented by a statement to indicate the number of preparations showing a particular response. Certain figures, nevertheless, present absolute values for the variables.

RESULTS

Pressure–flow relationships for hearts performing a subphysiological work load

The integrity of the coronary perfusion was demonstrated in series I and II by the fact that the measured outflow always exceeded 90 % of the inflow. The drop counter

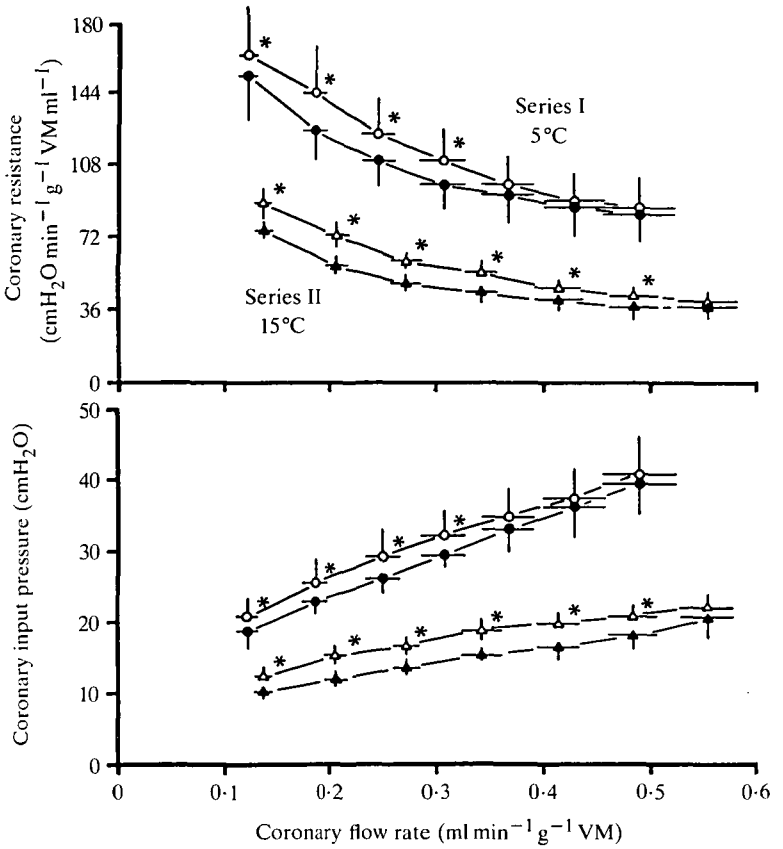

Fig. 2. Pressure–flow relationships for the coronary circulation in spontaneously beating hearts which were performing at a subphysiological work load at 5 °C (circles, series I) and 15 °C (triangles, series II). The initial relationship was determined with basic saline for the coronary perfusate (solid symbols). A second pressure–flow relationship was determined for each preparation (open symbols) with basic saline in series I and saline plus either 1 % polyvinylpyrrolidone (PVP) or albumin in series II. The asterisks denote statistically significant differences ($P < 0{\cdot}05$; paired analysis) between replicate measurements of input pressure or vascular resistance. The bars indicate the S.E. for each mean value ($N = 7$ fish for both series). VM, ventricle mass.

also resolved the lag between a step increase in coronary inflow and the corresponding increase in outflow from the ventricle.

The intrinsic heart rate was arrhythmic and slower at 5 °C (21 ± 3 beats min^{-1}) in series I than at 15 °C (43 ± 4 beats min^{-1}) in series II. An increase of several beats min^{-1} occurred whenever coronary flow was increased. Afterload of the heart was physiological but, because of the low cardiac output, myocardial power output was subphysiological (0·01 to 0·05 mW g^{-1} VM).

A reasonably linear pressure–flow relationship was evident for the coronary circulation using basic saline as the perfusate (Fig. 2). Coronary input pressure always increased when coronary flow was increased. Vascular resistance decreased

exponentially as coronary flow increased, especially at flow rates below about $0{\cdot}25\,ml\,min^{-1}\,g^{-1}$ VM.

Effect of perfusate composition

In series I the pressure–flow relationship was re-examined with basic saline, and vascular resistance was significantly higher ($P<0{\cdot}05$) at the lower coronary flow rates (Fig. 2). At flows up to $0{\cdot}32\,ml\,min^{-1}\,g^{-1}$ VM there was always a higher coronary input pressure for the second determination. No attempt was made to determine what caused the increase in vascular resistance with time.

In series II basic salines with and without a colloid substitute were compared for each preparation. Statistically similar pressure–flow relationships were obtained when PVP ($N=4$) and albumin ($N=3$) were present in the perfusate, and data sets were combined for comparison with the curve for basic saline. In every preparation coronary input pressure was greater at the six lowest flow rates when a colloid substitute was present in the perfusate (Fig. 2). Thus, the measured vascular resistance was significantly higher ($P<0{\cdot}05$) in the presence of a colloid substitute. However, some of the difference in the measured vascular resistance was due to the different viscosities of the two perfusates, a factor which is eliminated when normalized vascular resistances are compared. Normalized vascular resistance tended to be lower when the colloid substitute was present (series II), rather than higher, as was the situation when the pressure–flow curve was repeated without a

Table 2. *Measured and normalized coronary vascular resistance*

		Coronary resistance ($cmH_2O\,min^{-1}\,g^{-1}\,VM\,ml^{-1}$)	
		measured value*	normalized value†
Series I at 5 °C	basic saline	97·2	111·6
	basic saline repeated	104·4	122·4
Series II at 15 °C	basic saline	46·8	72·0
	saline plus colloid	54·0	61·2
Series III at 5 °C	saline plus colloid	68·4	57·6
Series IV at 10 °C	saline plus colloid	57·1	57·1

* The measured value for coronary resistance at a coronary flow of $0{\cdot}33\,ml\,min^{-1}\,g^{-1}$ VM was interpolated from Figs 2 and 4; VM, ventricle mass.

† The measured vascular resistance was normalized to permit comparison between experiments performed at different temperatures and with different perfusates. Values were normalized to the conditions found in series IV, where the viscosity of the perfusate was $1{\cdot}76\times10^{-3}$ Pa·s at 10 °C and with 1 % polyvinylpyrrolidone (PVP) in the perfusate. A viscosity of $1{\cdot}35\times10^{-3}$ Pa·s at 10 °C was used for basic saline without PVP. Temperature-related differences in viscosity were normalized, assuming that the perfusate viscosity was 15 % lower at 15 °C compared to 10 °C and 14 % higher at 5 °C compared to 10 °C. Viscosity values were derived from Perry (1941) and Graham (1985).

colloid substitute (series I, Fig. 2). Thus it appears that a colloid substitute was beneficial in preventing a time-dependent increase in vascular resistance, and it was decided to incorporate a colloid substitute for series III and IV. PVP was preferred over albumin because of the excessive foaming when albumin solutions are aerated.

Temperature effect

Coronary input pressure and vascular resistance were significantly higher ($P < 0{\cdot}05$) at 5 °C (series I) compared to 15 °C (series II, Fig. 2), but this was not a result of a higher viscosity of the saline at the lower temperature (Table 2). Normalized vascular resistance was 55 % higher at 5 °C compared to 15 °C in experiments performed with basic saline.

Vascular smooth muscle adrenoceptors

Adrenaline infusion into the coronary artery produced a statistically significant increase ($P < 0{\cdot}05$) in coronary input pressure with all three concentrations in series I

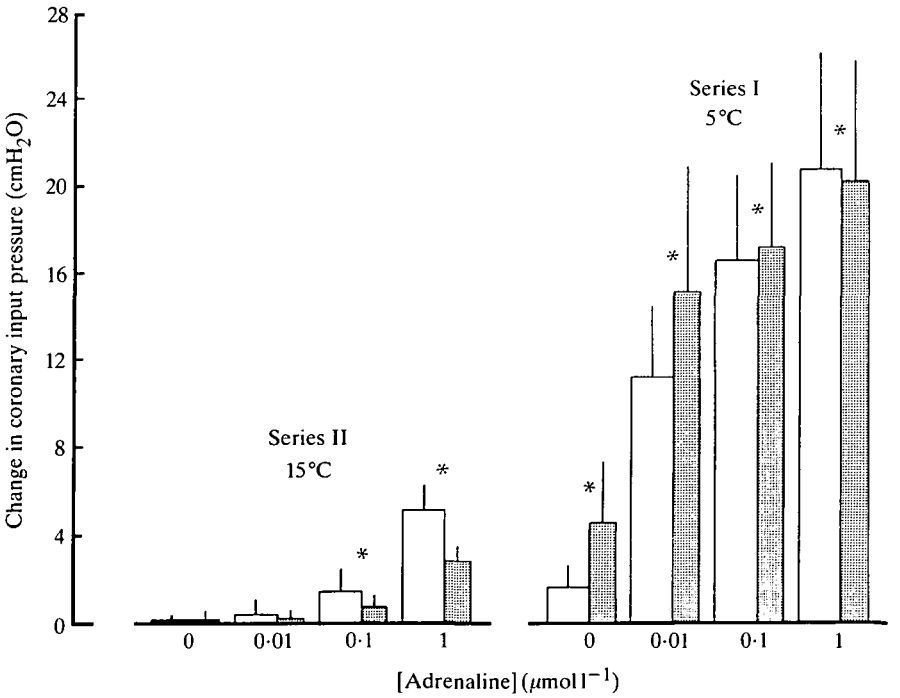

Fig. 3. The peak change in coronary input pressure following an adrenaline injection into the coronary artery in series I and II. An asterisk denotes a statistically significant increase ($P < 0{\cdot}05$) in input pressure. Basic saline was used as the initial perfusate (open bars) in both series and the second perfusate (stippled bars) was basic saline in series I and saline plus a colloid substitute in series II. The first and second adrenergic responses were not significantly different. A significant vasoconstriction in response to a saline injection (series I) occurred at least 30 s before the adrenergic effect and was easily distinguishable. Mean values and S.E.M. are indicated for seven fish in each series.

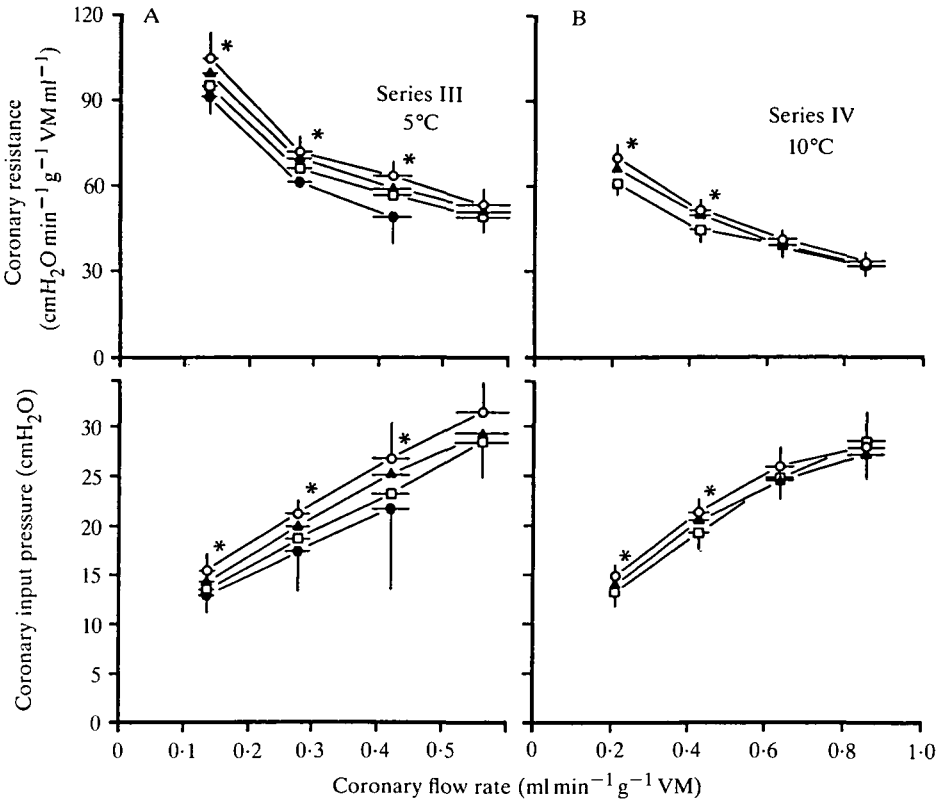

Fig. 4. (A) Series III. Pressure–flow relationships for the coronary circulation in electrically paced hearts which were performing a physiological work load at 5°C. The relationship was determined at different pacing frequencies to quantify extravascular compression due to changes in heart rate. Each line represents a different heart rate [symbols: ● = 15 beats min^{-1}; □ = 30 beats min^{-1}; ▲ = 45 beats min^{-1}; ○ = 55 beats min^{-1} (60 beats min^{-1} in series IV)]. An asterisk denotes a consistent (for each decrease in heart rate and in all six preparations) and a significant ($P < 0{\cdot}05$; paired analysis) decrease in vascular resistance or input pressure. One point is omitted for 15 beats min^{-1} because data were obtained in only three preparations. The bars indicate the S.E. for each mean value ($N = 5$ fish). (B) Series IV. As for A, except the experiments were performed at 10°C ($N = 6$ fish). VM, ventricle mass.

at 5°C (Fig. 3). This vasoconstriction is most easily explained as the result of stimulation of alpha-adrenoceptors which are known to be present in the coronary circulation of fish (Davie & Daxboeck, 1984; Farrell & Graham, 1986). In series I at 5°C, the control saline injection also produced a statistically significant vasoconstriction that occurred 30 s before the peak response to the adrenaline infusion. This response probably reflected a myogenic response of the VSM to the increase in coronary pressure as the injection was made.

Adrenergic vasoconstriction was clearly affected by temperature since only the two highest concentrations of adrenaline produced a statistically significant ($P < 0{\cdot}05$) vasoconstriction at 15°C (Fig. 3). Furthermore, the increase in coronary input

pressure produced by an injection of $0{\cdot}01\,\mu mol\,l^{-1}$ adrenaline at 5°C was significantly greater ($P<0{\cdot}05$) than that produced by an injection of a 100-fold higher concentration at 15°C.

Adrenergic vasoconstriction was not significantly different for the second trial with or without the presence of a colloid substitute.

Pressure–flow relationships for hearts performing a physiological work load

Series III

Experiments were performed at 5°C with the heart delivering a cardiac output of $10\,ml\,min^{-1}\,kg^{-1}$ BM. Myocardial power output was $0{\cdot}67\pm0{\cdot}08\,mW\,g^{-1}$ VM ($N=5$).

A reasonably linear pressure–flow relationship existed at all heart rates (Fig. 4A). Coronary input pressure and vascular resistance were significantly lower compared to hearts performing at a subphysiological work load and at the same temperature (series I, Fig. 2). The normalized vascular resistance for series III was half of that for series I (Table 2).

Each heart beat produced a small oscillation in coronary input pressure as a result of extravascular compression. However, this effect was not examined in detail because the oscillations in input pressure were damped by the windkessel (see Fig. 1). Extravascular compression was also demonstrated by statistically significant changes in mean input pressure and vascular resistance when heart rate was varied (Fig. 4A). In all preparations, input pressure always decreased for each stepwise decrease in heart rate, except at the highest coronary flow rate where there was either no change or a decrease in pressure. Given that an almost four-fold change in heart rate produced on average only a 20–25 % change in vascular resistance, extravascular compression was small over a reasonably physiological range for heart rate.

Cardiac output and myocardial power output were constant when the heart was paced between 15 and 55 beats min^{-1}. The ability of fish hearts to maintain cardiac output while heart rate varied has been noted before for unpaced hearts (Farrell, 1984). A pacing rate greater than 55 beats min^{-1} reduced cardiac output at this temperature.

Series IV

Experiments were performed at 10°C with the heart delivering a cardiac output of $17\,ml\,min^{-1}\,kg^{-1}$ BM. Myocardial power output was $1{\cdot}50\pm0{\cdot}15\,mW\,g^{-1}$ VM ($N=6$). The relative ventricle mass was lower in these fish (Table 1), which resulted in coronary flow being set at a relatively higher rate compared to the other series. Over this broader range of coronary flows there was a curvilinear pressure–flow relationship (Fig. 4B). Therefore, the effect of a further increase in coronary flow was examined in five preparations, after the normal protocol had been completed. Coronary resistance ($28{\cdot}8\pm5{\cdot}4\,cmH_2O\,min^{-1}\,g^{-1}\,VM\,ml^{-1}$) at a coronary flow of $1{\cdot}26\pm0{\cdot}083\,ml\,min^{-1}\,g^{-1}$ VM was not significantly lower than the resistance ($32{\cdot}4\pm2{\cdot}9\,cmH_2O\,min^{-1}\,g^{-1}\,VM\,ml^{-1}$) at a coronary flow of $0{\cdot}85\pm0{\cdot}047$ ml

$min^{-1} g^{-1}$ VM. This minimum resistance value suggests that the coronary circulation was at or near maximal dilatation as coronary flow approached 1 ml $min^{-1} g^{-1}$ VM.

Normalized vascular resistance was similar in series III and IV (Table 2).

Extravascular compression was increased at higher heart rates, but this effect was statistically significant only at coronary flows below about 0·5 ml $min^{-1} g^{-1}$ VM (Fig. 4B).

Cardiac output was constant at heart rates between 30 and 60 beats min^{-1}. Regular pacing was not possible below 30 beats min^{-1} at this temperature, because spontaneous contractions interrupted the regular electrical pacing.

After the routine protocol was completed, cardiac output could be increased to 30–40 ml $min^{-1} kg^{-1}$ BM by raising atrial input pressure (heart rate = 45 beats min^{-1}; $N = 5$). Also, afterload could be increased to 70–80 cmH_2O without compromising cardiac output appreciably. Adding adrenaline (0·1 μmol l^{-1}) to the cardiac perfusate improved the maximum cardiac output by about 20 % ($N = 3$ fish). When cardiac output was increased maximally, coronary resistance always decreased modestly (up to 4·7 $cmH_2O\ min^{-1} g^{-1}$ VM ml^{-1}). When afterload was increased, coronary resistance always increased (up to 18·0 $cmH_2O\ min^{-1} g^{-1}$ VM ml^{-1}).

DISCUSSION

Heart preparation

Previous studies of cardiac physiology with working perfused hearts have used either fish without a coronary circulation (e.g. Farrell *et al.* 1982; Farrell, MacLeod, Driedzic & Wood, 1983; Driedzic, Scott & Farrell, 1983; Stuart, Hedtke & Weber, 1983) or small trout where oxygenated saline raised the O_2 gradient across the myocardium to circumvent the absence of coronary perfusion (Bennion, 1968; Farrell, MacLeod & Chancey, 1986). The preparation described here provides a new and more physiological avenue to examine coronary and cardiac physiology in fish.

The preparation was robust and reliable. The present experiments regularly lasted 1–2 h, but it is apparent that longer experiments are possible. Maximum cardiac output (30–40 ml $min^{-1} kg^{-1}$ BM) and output pressure (70–80 cmH_2O), determined at the end of series IV, compare well with cardiac output (52·6 ml $min^{-1} kg^{-1}$ BM) and ventral aortic pressure (83 cmH_2O) for intact trout near their critical swimming speed (Kiceniuk & Jones, 1977). Adrenergic stimulation would improve maximum cardiac performance of the preparation given the present (series IV) and previous (Bennion, 1968; Farrell *et al.* 1986) observations. Furthermore, maximum cardiac output might be even higher if a mechanical one-way valve were fitted to the atrial input cannula to prevent backflow from the atrium. The sino-atrial valve is ineffective in the preparation because of the cannula placement, but its functional importance was clearly revealed at high stroke volumes when backflow from the atrium could be observed.

The size of the coronary artery limits the minimum fish size which can be used for this preparation. Trout weighing at least 1·5 kg were generally preferred. Attempts

were made to cannulate the coronary artery in trout as small as 1·0 kg, but the PE 10 cannula was often too large for the vessel. Smaller specimens of other species such as tuna could be used for the preparation since the coronary artery is relatively larger.

The adrenoceptor pharmacology of the coronary VSM was not examined extensively in the present study. However, the adrenaline infusions did confirm that alpha-adrenoceptors were functional in the preparation. Therefore, the preparation may be useful in the future for examinations of other aspects of coronary vasoactivity.

Factors influencing coronary flow in trout

Perfusion pressure

In mammals arterial pressure is a major determinant of coronary flow, and coronary artery pressure is directly related to the pressure work performed by the heart (Feigl, 1983). The pressure–flow relationships that have been established for the trout coronary circulation permit an evaluation of the relative importance of arterial blood pressure in regulating coronary flow to meet the oxygen demands of the working heart. Blood pressure in the coronary artery has not been measured, but it is likely to be similar to the dorsal aortic pressure near the point of origin of the hypobranchial artery (Fig. 5). During sustained exercise, dorsal aortic blood pressure increases by 7 cmH_2O in trout at 10°C (Kiceniuk & Jones, 1977). An increase in coronary input pressure of 7 cmH_2O would produce a 30 % increase in coronary flow (Fig. 5). However, during exercise myocardial power output – and presumably demand – increase about four-fold, while venous oxygen supply to the spongy myocardium is perhaps compromised by the 50 % reduction in venous P_{O_2}. A 30 % increase in coronary flow is unlikely to satisfy this increase in myocardial oxygen demand associated with swimming. Thus, it is possible that increases in coronary blood flow in trout may not be as closely matched to increases in cardiac work as they are in mammals, where a 4- to 5-fold increase in coronary flow accompanies a 5- to 6-fold increase in myocardial oxygen consumption (Berne & Rubio, 1979; Feigl, 1983). However, it is more probable that mechanisms other than arterial pressure play a more significant regulatory role in fish, given that fish hearts are primarily aerobic (Driedzic *et al.* 1983; Farrell *et al.* 1985). The apparent difference betwen trout and mammals with respect to the role of arterial perfusion pressure undoubtedly reflects the remote branchial origin of the coronary circulation compared with the situation in mammals (Grant & Regnier, 1926) and the dislocation of coronary perfusion pressure from the pressure developed by the heart. This may represent an evolutionary limitation on cardiac performance in fish.

Before discussing the evidence for other regulatory mechanisms, absolute coronary blood flow in intact trout can be estimated by correcting perfusion pressure for the viscosity of blood (Fig. 5). Coronary blood flow is estimated as 0·22–0·38 ml min^{-1} g^{-1} VM and it is clearly dependent on blood viscosity. This estimate of resting coronary blood flow is below the level where there is near maximal vasodilatation (1 ml min^{-1} g^{-1} VM; series IV), but it does not consider the possibility of a tonic coronary vasoconstriction in resting fish (see below). The present

estimate for resting coronary flow represents 1·6–2·9 % of resting cardiac output, which is greater than values measured in *Catostomus* and *Lota* (0·56 % and 0·65 %, respectively; Cameron, 1975). Coronary blood flow supplies only the compact myocardium, and so the tissue-specific flow will be about three times higher (0·66–1·14 ml min^{-1} g^{-1} compact myocardium). Coronary blood flow in mammals is 0·8 ml min^{-1} g^{-1} VM and represents 4–5 % of resting cardiac output (Berne & Rubio, 1979; Feigl, 1983).

Adrenergic controls

Alpha-adrenergic vasoconstriction dominates beta-adrenergic vasodilatation in coronary vessels of fish (Davie & Daxboeck, 1984; Farrell & Graham, 1986) and mammals (Berne & Rubio, 1979; Feigl, 1983). It is possible that in resting fish there is a tonic sympathetic vasoconstriction which is released or overridden during exercise. If this were the case, coronary flow in resting trout would be somewhat lower than the estimate made above. Also, tonic vasoconstriction would be reduced at higher water temperatures, given the apparent temperature sensitivity of the coronary adrenoceptors (Fig. 3).

Metabolic-related vasodilatation could override direct sympathetic vasoconstriction in trout, as occurs in mammals (Berne & Rubio, 1979; Feigl, 1983). Metabolic

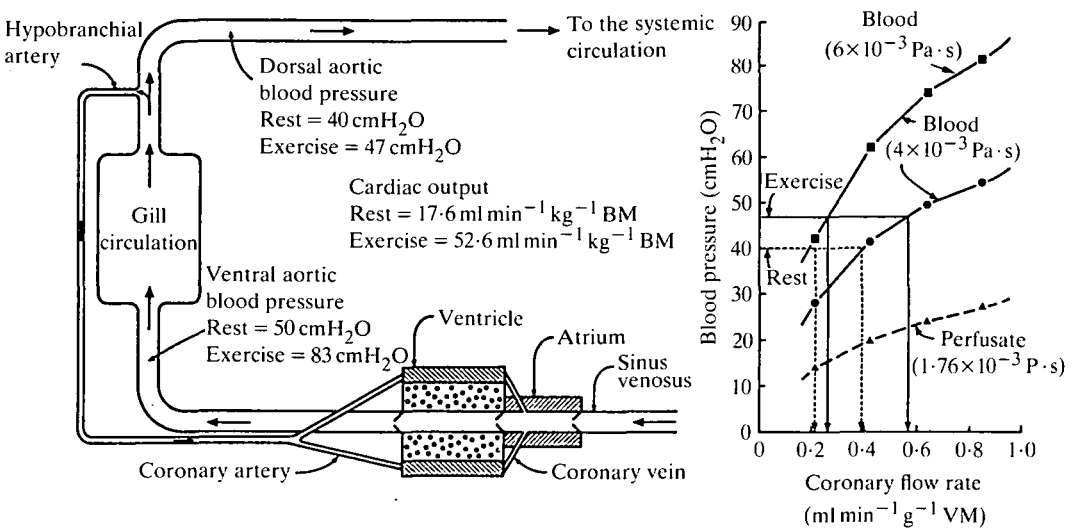

Fig. 5. A theoretical analysis of coronary blood flow in rainbow trout at 10°C. A schematic diagram is used to outline the coronary circulation to the outer, compact layer of the myocardium. The pressure–flow relationship for the coronary circulation was taken from series IV at a heart rate of 45 beats min^{-1}. A blood pressure–flow relationship was derived by multiplying perfusion pressures by the ratio of the viscosity for perfusate (perfusate = $1·76\times10^{-3}$ Pa·s) and blood (blood viscosity = $4–6\times10^{-3}$ Pa·s; Wood, 1974; Milligan & Wood, 1982; Graham, 1985). Blood pressures and cardiac output at rest and during exercise are taken from Kiceniuk & Jones (1977). Dorsal aortic blood pressure is used to estimate coronary blood flow at rest and during exercise. BM, body mass; VM, ventricle mass.

autoregulation of coronary flow was not directly studied here, but two observations [the significantly higher vascular resistance in hearts performing a subphysiological (series I) compared to a physiological (series III) work load at the same temperature, and the decrease in vascular resistance at maximum cardiac output] indicate that metabolic autoregulation is worth further investigation as a possible control mechanism. Sympathetic stimulation of cardiac metabolism, heart rate and contractility could, therefore, increase coronary blood flow *via* metabolic-related vasodilatation.

Vascular compression

Extravascular compression produced by myocardial contractions resulted in beat-by-beat changes in coronary input pressure and increased coronary vascular resistance when the heart was beating faster. In mammals, increased heart rate produces an increase in total coronary resistance in preparations where the coronary circulation is fully dilated. An increase in heart rate of 100 beats min^{-1} (100 to 200 beats min^{-1} or 150 to 250 beats min^{-1}) produces a 6–14 % increase in total coronary resistance (Feigl, 1983), which compares to a 20–25 % increase in coronary resistance in trout for a four-fold increase in heart rate (15 to 60 beats min^{-1}). Extravascular compression also produces a marked redistribution of flow across the wall of the mammalian left ventricle. Distribution of coronary flow in the compact myocardium of fish has not been examined.

In summary, a reliable preparation was developed to investigate coronary physiology in fish. Pressure–flow relationships were developed for hearts working at normal and subphysiological work loads and revealed that arterial pressure, adrenoceptors, extravascular compression and metabolism are involved in regulating coronary flow in trout. Acclimation temperature of the fish affected coronary vascular resistance and vasoconstriction mediated by coronary alpha-adrenoceptors.

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