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Coronary flow–pressure relationship in the working isolated fish heart: trout (*Oncorhynchus mykiss*) versus torpedo (*Torpedo marmorata*)

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SUMMARY

Isolated hearts of rainbow trout and torpedo were perfused via the atrium and coronary artery under conditions of low and high work and with two different levels of oxygen to determine the effects on coronary flow–pressure relationships and to estimate coronary resistance. In all cases, an increase in input pressure to the coronary artery resulted in an increase in coronary flow through a reduction in coronary resistance. The relationship between flow and pressure was linear but the resistance became less pressure dependent at higher input pressures. When the trout heart was perfused with oxygenated saline an increase in the atrial filling pressure (volume loading, high work condition) reduced coronary resistance and increased flow for a given coronary artery input pressure. The opposite effects were seen when atrial filling pressure was reduced (volume loading, low work condition). Increasing ventricular output pressure (pressure loading) resulted in an increased coronary resistance. In torpedo, changes in preload and afterload did not affect coronary perfusion. In both preparations, reduced levels of oxygen in the coronary perfusion fluid reduced coronary resistance. It is concluded that in trout the coronary resistance is intrinsically sensitive to input pressure and oxygen demand of the ventricle; these susceptibilities assist in the maintenance of the oxygen supply to the compact layer of ventricular muscle. In contrast, the coronary resistance of the torpedo heart appears to be insensitive to load conditions when steady-state perfusion pressure is used. The difference between the two species is discussed in terms of morphofunctional differences in the coronary system.

1. INTRODUCTION

In fish there is a remarkable variety both in the architecture and the extent of vascularization of the ventricular wall (Tota *et al.* 1983; Santer 1985; Tota 1989). In most teleosts the ventricle, which is entirely trabeculated and described as spongiosa, is supplied by the venous blood circulating through the intertrabecular lacunary spaces. In the most active teleosts and in all elasmobranchs a mixed type of ventricle is found, i.e. the spongiosa is covered by an outer layer of myocardial fibers packed in orderly arranged bundles described as compacta which is supplied by a hypobranchial arterial supply. In many cases this arterial supply consists of coronary arteries which approach the heart along the ventral aorta and the bulbus cordis (Grant & Regnier 1926; De Andres *et al.* 1990).

A difference exists between elasmobranchs and teleosts in the muscle fibre geometry in the compact myocardium (Sanchez-Quintana & Hurle 1987), as well as in the intracardiac coronary pattern (Tota *et al.* 1983). The impact of this different myoangioarchitecture on the cardiac performance under different loading conditions has yet to be analysed in these two groups of fishes. A large amount of information in mammals has shown that coronary flow depends upon, among other factors, the geometry, structure and functional properties of coronary blood vessels and the structural arrangement and properties of the surrounding myocardial network (Anderson & Anderson 1980; Stoker *et al.* 1982). Accordingly, it may be expected that coronary flow will differ significantly in elasmobranchs and teleosts.

The present study investigates the coronary function in the trout and torpedo. The hearts of these species have both an outer compact myocardium and a well developed coronary system. Apart from the fundamental differences in heart design, ventricular shape and myocardial architecture (Sanchez-Quintana & Hurle 1987; Lai *et al.* 1990), a significant difference exists between these species. In torpedo the

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trabeculae of the spongiosa are vascularized and the arterial oxygenated circuit is connected with the venous lacunary system by extensive arterio-luminal vessels (thebesian vessels, Tota 1989). In the trout heart the coronary circulation seems to be strictly limited to the compacta (as inferred in the review of Davie & Farrell (1991)). The vascularization of the trabeculae found in torpedo and in all elasmobranch species so far examined (Tota 1989; De Andres *et al.* 1990) implies that the nourishment of the spongiosa is not only through the venous blood but also by the oxygenated coronary blood.

In the present work the coronary circulation has been studied in isolated working heart preparations of trout and torpedo. As one of the most useful descriptions of the functional characteristics of the coronary circulation is in terms of the relationship between steady-state perfusion pressure and the amount of blood flow (Dole 1987), the dependence of the coronary flow–pressure relationship with volume loading, pressure loading and changes of pO_2 of perfusate has been evaluated. The purpose has not been to extrapolate directly the results to the *in vivo* behaviour of coronary flow, but to use this *in vitro* preparation to test to what extent a different vascular pattern can affect the hydraulic characteristics of coronary circulation in the two species.

2. MATERIALS AND METHODS

(*a*) *Animals*

Rainbow trout (*Oncorhynchus mykiss*) were obtained from local fish suppliers and held at Aberdeen University in flowing fresh water (10–15°C). The fish were fed a commercial fish diet *ad libitum* (Ewos-Baker Ltd) four times a day. The mean mass (±s.e.) of the animals was 0.64 ± 0.03 kg ($n = 41$). The smallest animal in which it was possible to perfuse the coronary artery weighed 0.32 kg, while the biggest animal used was 1.22 kg.

The experiments on torpedo (*Torpedo marmorata*) were carried out at the Stazione Zoologica 'A. Dohrn', Naples. Twenty specimens of both sexes (mean mass 0.52 ± 0.06 kg, range 0.26–0.96 kg) were obtained from local fish suppliers and maintained in a circulating sea water aquarium (15–20°C).

(*b*) *Perfusates*

The basic saline for trout heart perfusion was a modification of Cortland saline (Wolf 1963). Its composition was (in g l^{-1}): NaCl, 7.25; KCl, 0.23; $CaCl_2$, 0.23; $MgSO_4.7H_2O$, 0.23; $NaH_2PO_4.2H_2O$, 0.016; $Na_2HPO_4.2H_2O$, 0.41; glucose, 1.0. Polyvinylpyrrolidone (PVP, molecular mass 40 000, Sigma) was added as a colloidal substitute at a concentration of 10.0 g l^{-1}. The saline was gassed with 99.5% O_2: 0.5% CO_2, or with 99.5% air: 0.5% CO_2. In both cases the pH was adjusted to 7.9 at 10°C, with $NaHCO_3$.

The saline for the perfusion of the elasmobranch heart was prepared according to Forster *et al.* (1972). Its composition was as follows (in g l^{-1}): NaCl, 16.36; KCl, 0.45; $CaCl_2$, 0.55; $MgCl_2$, 0.61; Na_2SO_4, 0.071; $NaH_2PO_4.2H_2O$, 0.14; $NaHCO_3$, 0.67; urea, 21; glucose, 0.9; pH 7.6.The saline was gassed with 99.5% O_2: 0.5% CO_2, or with 99.5% air: 0.5% CO_2.

(*c*) *Heart preparation*

(i) *Isolated trout heart*

The heart preparation and perfusion was as described by Houlihan *et al.* (1988) with some modifications. The atrium was cannulated using a double cannula secured at the junction with the sinus venosus; one cannula provided the input for the atrium, the other was used to measure the intra-atrial pressure. The coronary artery was cannulated with a polyethylene tube 6 mm long, 0.35 mm diameter. The perfusion apparatus is shown in figure 1. A perfusion chamber with a lid, as described by Acierno *et al.* (1990), was used to obtain extracardiac pressure values similar to those recorded *in vivo* in the pericardium (Farrell *et al.* 1988). The perfusion chamber and reservoirs were maintained at 10°C with a temperature controlled water bath and circulating heater/cooler. Input and output pressures and input pressure to the coronary circulation were determined from the height of the pressure heads. Hearts infected with the digenean parasite *Apotemon gracilis* were rejected as performance is impaired (Tort *et al.* 1987).

(ii) *Isolated torpedo heart*

The animals were anaesthetized by cold (Acierno *et al.* 1990), and a caudal vein/artery injection of heparin solution was given (0.7 ml kg^{-1} body mass). The animal was pithed and opened ventrally, the pericardium cut and the ventral aorta cannulated. Care was taken to avoid the cannula entering the conus arteriosus. As the ventral aorta branches shortly after the conus arteriosus, the fourth and fifth afferent branchial arteries were ligatured at their bases. The heart was excised and the atrium cannulated using a double cannula as for the trout heart (see above). To cannulate the coronary artery, a polyethylene cannula 4 mm long, 0.3 mm diameter was used. Only one of the two coronary arteries was cannulated, while the other was ligatured. To ensure that complete perfusion of the coronary system was possible through only one of the coronary arteries, preliminary experiments were carried out using toluidine blue dissolved in saline. The perfusion apparatus was similar to that used for the trout heart (figure 1). All the experiments were performed at room temperature (18–20°C).

(*d*) *Protocols*

(i) *Determination of flow–pressure curves in trout*

This protocol involved the shift, through an intermediate step, of coronary perfusion from a physiological resting flow value to a physiological resting pressure value. Because of the low viscosity of the perfusate, the coronary flow was close to *in vivo* resting values at much lower input pressures than normal *in vivo*. In each condition, the coronary head pressure was adjusted to obtain a coronary flow of between 1.5

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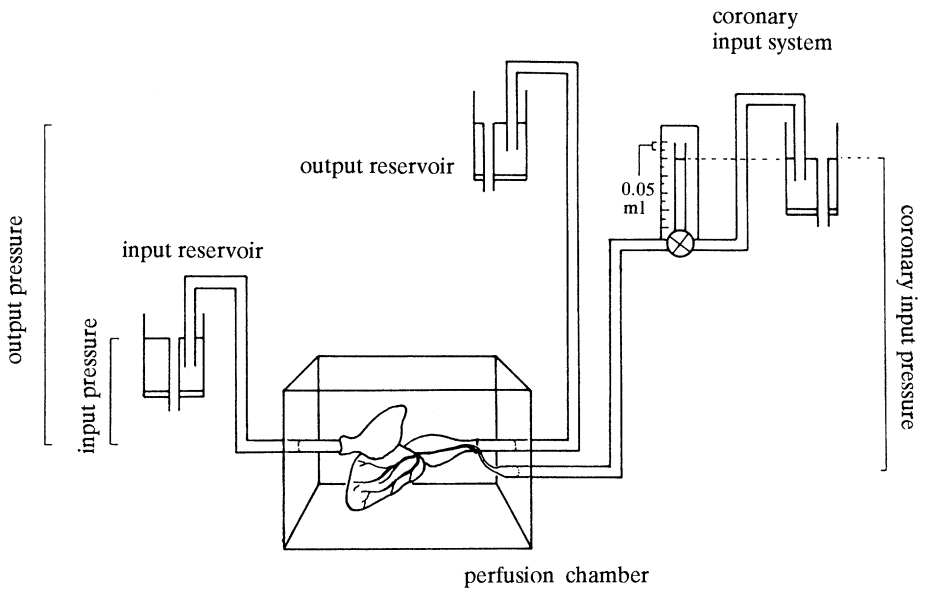

Figure 1. The perfusion apparatus, a modification of that described by Houlihan *et al.* (1988). It is characterized by the presence of an independent coronary pressure head and of a system for the measurement of coronary flow (see text for details).

and 3.0% of the cardiac output (according Farrell 1987). Typical values of the pressure heads were 1.8–2.2 kPa. The coronary pressure was then increased through two steps to about 3.9 kPa (resting dorsal aortic blood pressure; Kiceniuk & Jones 1977). Finally, the pressure was lowered to the initial value. This final step resulted in the return of flow and resistance to the initial values. At each step, coronary pressure and flow and all the basic cardiac performance parameters were measured. A complete curve determination took from 2 to 3 min.

(ii) *Determination of flow–pressure curves in torpedo*

This protocol was similar to that used for trout heart. As there are no measurements or estimates of physiological coronary flow in elasmobranch, the initial level of flow was chosen according to the teleost values (Farrell 1987). In each condition, the coronary head pressure was initially adjusted to obtain a coronary flow of about 1.5% of the cardiac output. A complete curve was determined by doubling the coronary pressure through three steps. The maximum coronary pressure was about 3.0 kPa, a value which is likely higher than the resting physiological coronary pressure in torpedo (see below).

(iii) *Control conditions*

In all the experiments the heart rate was the intrinsic rhythm of the heart and varied between preparations. The control perfusate was oxygenated saline (mean ± s.e. input $pO_2 = 533.7 \pm 10.6$ Torr†).

In trout, after 15 to 30 min of perfusion necessary to reach steady-state conditions, the output pressure was set at 4.5 kPa to simulate the physiological diastolic pressure in the ventral aorta (Kiceniuk & Jones 1977) and the input pressure was adjusted to obtain a cardiac output (V_b) of 15 ml min^{-1} kg^{-1} (Houlihan *et al.* 1988). As the hearts perfused with aerated saline were not stable at cardiac output values higher then 12 ml min^{-1} kg^{-1}, this value was used as control cardiac output for the lowered pO_2 protocol in trout.

In vivo values of systemic pressure in *Torpedo* have not been reported. We have measured the mean aortic pressure in three unrestrained living resting animals and the values ranged from 1.2 to 2.0 kPa. Cardiac output determined in two other animals was slightly lower than the values reported for dogfish (Short *et al.* 1977; Davie & Farrell 1991). On this basis, we have chosen for control ('resting') conditions for the torpedo heart the following protocol: after 15 min of perfusion necessary for reaching a steady-state condition, the output pressure was set at 1.5 kPa, while the input pressure was adjusted to obtain a V_b of 10 ml min^{-1} kg^{-1}.

(iv) *Effects of volume and pressure loading on coronary perfusion*

Each heart acted as its own control. Control perfusion conditions were set up as above. The perfusate was oxygenated saline. Each experiment lasted not more than 1 h.

To determine the effect of volume loading on the coronary flow–pressure relationship in the trout heart, two sets of experiments were done. In the first, starting from control conditions, the input pressure was increased to double the V_b (volume loading, high work conditions). In the second, after setting control conditions, the input pressure was decreased to half the V_b (volume loading, low work conditions). Only the volume loading, high work condition, was tested on the torpedo heart.

To determine in both the trout and torpedo hearts the effect of pressure loading on the coronary flow–pressure relationship, after setting the control condi-

† 1 Torr = 133.32 Pa.

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tions as above, the output pressure was increased as much as possible without changing V_b (pressure loading, high work conditions).

(v) *Effects of input* pO_2

To evaluate the effect of lowering perfusate pO_2 both the heart (trout and torpedo) and its coronary circuit were perfused with aerated saline (mean ± s.e. input $pO_2 = 142.0 \pm 3.7$ torr). A different set of animals, of similar heart size, perfused with oxygenated saline, were used as controls.

(*e*) *Measurements and calculations*

In the trout experiments, the atrial pressure and the input coronary pressure were continuously measured through saline-filled sidearms connected to a Statham P23B pressure transducer. The output pressure was recorded through a saline-filled sidearm connected to a Devices pressure transducer. Both transducers were connected to a pen recorder.

In the elasmobranch experiments, atrial pressure, input coronary pressure and output pressure were continuously measured through saline-filled sidearms connected to Elcomatic EM-751 pressure transducers. The signals were recorded with a pen recorder and analysed with a Computerized Acquisition Data System (Snapshot Storage Scope, HEM Data Corp., Michigan, U.S.A.).

The pressure measurements were referenced to the level of saline in the unsealed chamber and expressed in kPa.

Preload (= mean diastolic atrial pressure) and mean atrial pressure were determined from the atrial pressure records. Corrections were made for cannulae resistances to obtain the afterload (= mean bulbar pressure or mean conus pressure) and the coronary input pressures. Cardiac output was determined volumetrically as reported by Houlihan *et al.* (1988). The heart rate was obtained from the pressure records. The power output of the ventricle was calculated as (mW g^{-1}) = (afterload − mean atrial pressure) (kPa) × cardiac output (ml min^{-1})/60/(ventricular mass) (g).

The coronary flow was determined by measuring the time it took for 0.05 ml of perfusate to pass through the coronary artery, using the measuring system shown in figure 1. The constant error due to the fall of the coronary input level of a static value of 0.5 cm determines an effective reduction of coronary pressure that is less than 1%. Ten trials to measure with this system the efflux of 0.05 ml of distilled water (measured by mass) gave a mean value of 0.0496 ± 0.001 ml. Ten trials to measure a known flow (0.05 ml min^{-1}, measured volumetrically) gave a s.d. that was less than 5% of flow.

Flows were expressed per kilogram of animal mass, whereas the power output was expressed per gram of ventricular mass. The ventricular mass (blotted wet mass determined at the end of each experiment, Y,g) was related to the animal size (wet body mass measured before the experiment, X,kg) by the following relationships: trout, $Y = 0.81 \times X^{0.91}$ ($n = 41$, $r = 0.796$, $p < 0.01$); torpedo, $Y = 0.59 \times X^{0.90}$ ($n = 20$, $r = 0.920$, $p < 0.01$).

The coronary resistance (GPa s m^{-3}) was determined as: mean coronary pressure (kPa) × 60/coronary flow (ml min^{-1}).

Student's t-test was used to compare means (level of confidence 5%). Two way analysis of variance (ANOVA) test with replication was used to compare the effects of pressure and power output on coronary flow.

3. RESULTS

(*a*) *Control conditions and coronary perfusion*

Control values of cardiac parameters in all conditions used are reported in table 1. There were no significant differences between different control sets for each species, apart from the lower cardiac output in the low pO_2 experiments on trout hearts (table 1, see below).

In both preparations the preload was generally slightly subambient in control conditions and comparable with the values reported *in vitro* by Acierno *et al.* (1990) for the heart of the conger eel and *in situ* by Farrell *et al.* (1988) for the heart of the trout.

In terms of isolated heart performance, torpedo seems to differ from the trout mainly in its ability to produce pressure: not very different cardiac outputs (not statistically significant in the lowered pO_2 control experiments, see table 1) were produced by the two hearts at similar preload and heart rate values but at very different afterloads (about three times lower in torpedo than in trout, see table 1).

Figure 2 shows typical records of atrial, bulbar and coronary pressures in isolated and perfused trout heart. Coronary pressure usually displayed a pulse which was synchronous with ventricular contraction. The bulbus pulse pressure (0.81 ± 0.05 kPa, mean from all the experiments) was comparable with the *in vivo* pulse pressure recorded in the ventral aorta (Kiceniuk & Jones 1977). The mean bulbus pulse pressure in torpedo heart was 0.62 ± 0.06 kPa. There was no significant variation in this parameter in all conditions.

Both in trout and torpedo hearts coronary pressure, coronary flow and coronary resistance are related in such a way that the increase of coronary input pressure causes a decrease of coronary resistance and an increase of coronary flow, when all the other cardiac parameters are constant (figures 3–5). In our experimental conditions, the increase of coronary flow did not result in a significant increase in cardiac output. Although the flow–pressure relationships were generally linear, the resistance tended to become less pressure dependent at higher coronary input pressures. In trout, the coronary flow, when expressed as percentage of cardiac output, increased from the control value of 2.6 ± 0.2% to 8.6 ± 0.6% when physiological values of coronary input pressure were used (means from all the experiments; significantly different: $p < 0.05$). In torpedo these percentages were respectively 1.1 ± 0.2% and 11.3 ± 1.8 for the lowest (1.23 ± 0.06 kPa) and the highest (3.18 ± 0.08 kPa) pressure (means from all the experiments; significantly

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Table 1. *Cardiac parameters of the isolated trout and torpedo hearts perfused in volume loading, pressure loading and with oxygenated saline (controls, $pO_2 = 534$ torr) or aerated saline ($pO_2 = 142$ torr)*

(LW = low work; HW = high work; n = number of animals.)

	n	preload kPa	afterload kPa	heart rate (beats min^{-1})	cardiac output (ml min^{-1} kg^{-1})	power output (mW g^{-1})
volume loading						
O. mykiss						
LW controls	5	-0.02 ± 0.03	5.12 ± 0.06	35.5 ± 3.5	15.06 ± 0.57	1.23 ± 0.05
LW		-0.05 ± 0.02*	4.99 ± 0.07	32.1 ± 3.3	7.52 ± 0.34*	0.73 ± 0.12*
HW controls	6	-0.02 ± 0.03	5.29 ± 0.04	35.2 ± 3.2	14.84 ± 0.29	1.35 ± 0.04
HW		0.17 ± 0.08*	5.61 ± 0.10	37.9 ± 7.2	27.82 ± 2.26*	2.61 ± 0.23*
T. marmorata						
controls	8	-0.06 ± 0.02	1.80 ± 0.15	30.6 ± 2.2	10.65 ± 2.21	0.54 ± 0.14
HW		-0.03 ± 0.02	1.94 ± 0.15	33.2 ± 2.1	20.05 ± 3.26*	1.06 ± 0.19
pressure loading						
O. mykiss						
controls	4	-0.04 ± 0.01	4.92 ± 0.82	34.8 ± 7.1	15.66 ± 0.03	1.56 ± 0.14
HW		0.00 ± 0.03	7.96 ± 0.32*	39.5 ± 0.1	15.09 ± 3.71	2.33 ± 0.35*
T. marmorata						
controls	8	-0.01 ± 0.02	1.54 ± 0.33	31.0 ± 1.8	12.78 ± 3.75	0.48 ± 0.20
HW		-0.03 ± 0.03	2.34 ± 0.21	30.3 ± 1.7	12.28 ± 3.56	0.98 ± 0.36*
effect of pO_2 of perfusate						
O. mykiss						
controls	5	-0.02 ± 0.04	5.22 ± 0.12	34.2 ± 6.5	11.85 ± 1.05	1.42 ± 0.25
aerated saline		-0.01 ± 0.03	4.84 ± 0.16*	23.8 ± 2.6*	11.72 ± 1.31	1.19 ± 0.19
T. marmorata						
controls	7	-0.06 ± 0.02	1.81 ± 0.13	30.7 ± 1.8	11.61 ± 2.10	0.44 ± 0.09
aerated saline		-0.02 ± 0.01	1.75 ± 0.09	32.4 ± 2.0	12.24 ± 2.33	0.44 ± 0.06

* Significant variation with respect to control ($p < 0.05$).

different: $p < 0.01$). The coronary pressure necessary to obtain a physiological resting value of coronary flow (using teleost values) was generally lower in torpedo than in trout. At the lowest level of coronary pressure used, the coronary resistance in the isolated working torpedo heart was up to two times higher then in the trout heart (figures 3–5). However, the pressure dependence of coronary resistance was much higher in torpedo then in trout: a two fold increase of coronary pressure determines a 1.6-fold decrease of resistance in trout and a sixfold decrease in torpedo (mean variations from all control experiments). It should be noted that the coronary pressure range used in torpedo was relatively high with respect to the ventral aorta pressure observed *in vivo* (see §2). This suggests that the physiological resting level of coronary flow in torpedo is a value lower than 1.6% of cardiac output.

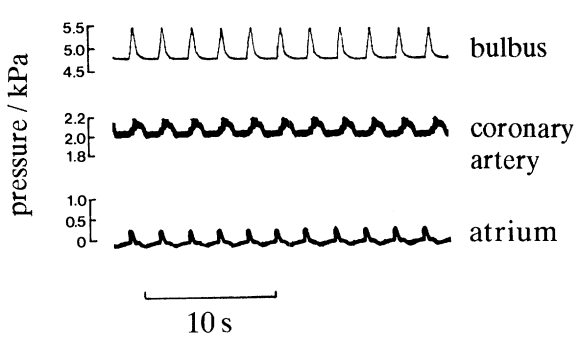

Figure 2. Simultaneous recordings of bulbus, coronary artery and atrial pressures from the isolated and perfused heart of trout, *Oncorhynchus mykiss*. Note the phasic increase of coronary pressure coincident with the late part of the ventricular systole.

In all the experiments the coronary flows measured at the same pressure at the beginning and the end of each trial were not significantly different (data not reported in the figures).

(*b*) *Coronary flow–pressure relation in volume and pressure loading conditions*

No significant change in heart rate was observed both in volume and pressure loading conditions.

An increase in input pressure to the isolated heart increased the cardiac output but scarcely affected the mean aortic pressure (afterload) (table 1). This is in accordance with previous observations (Farrell *et al.* 1985; Houlihan *et al.* 1988). Thus the observed increase in power output in this set of experiments was directly related to the increase of cardiac output (from 7.52 to 27.82 ml min^{-1} kg^{-1} in trout; from 10.65 to 20.05 ml min^{-1} kg^{-1} in torpedo).

We have evaluated the effect of doubling (high work in volume loading conditions, analysed both in trout and torpedo) or halving (low work in volume loading conditions, analysed only in trout) power output, consequent on changes in preload, on coronary perfusion. The coronary flow–pressure and coronary resistance–pressure relationships obtained in these conditions are shown in figure 3. A substantial

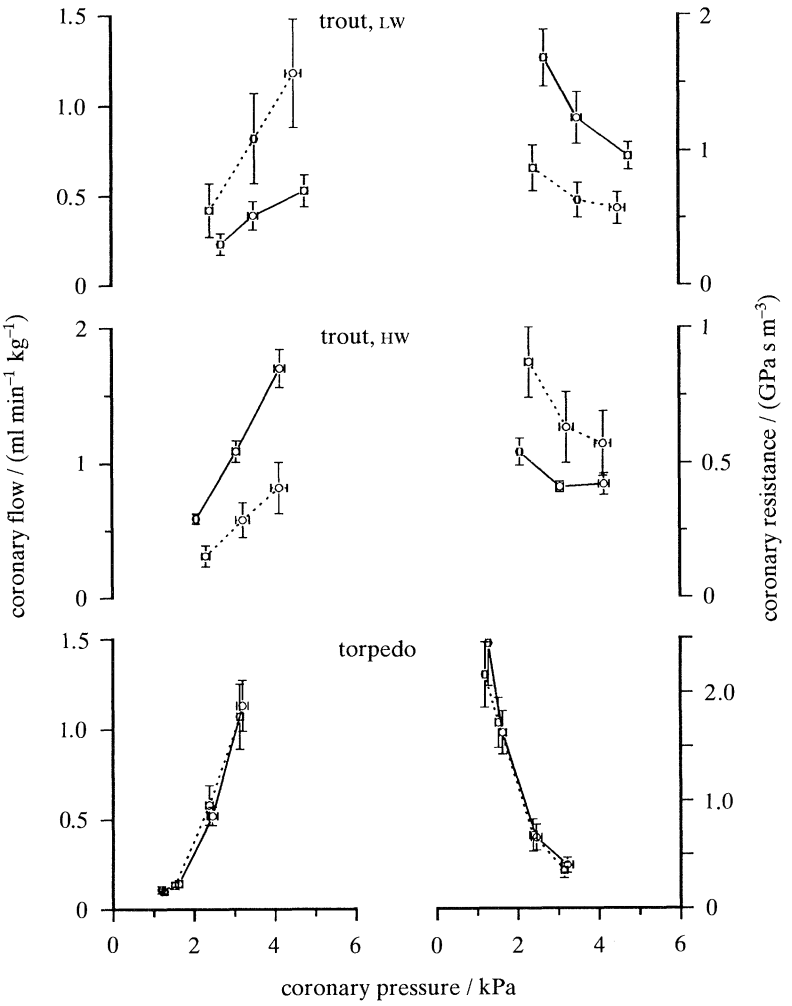

Figure 3. Flow–pressure and resistance–pressure relationships in isolated and perfused hearts of trout, *Oncorhynchus mykiss*, and torpedo, *Torpedo marmorata*, in control conditions (dotted lines) and submitted to volume loading (solid lines). LW = low work; HW = high work. Each heart acted as its own control. The mean ventricle masses were: trout, LW, 0.58 ± 0.08 g ($n = 5$); trout, HW, 0.50 ± 0.06 g ($n = 6$); torpedo, 0.31 ± 0.05 g ($n = 8$). The haemodynamic parameters are reported in table 1. The ANOVA analysis gave significant dependence of coronary flow from both coronary pressure and power output ($p < 0.05$) for the trout hearts only.

difference has been found between trout and torpedo. In trout the flow–pressure relationship significantly shifts down and the resistance–pressure relationship significantly shifts up when the preload is decreased (low work, figure 3, upper panel), while the opposite behaviour was found when the preload was increased (high work, figure 3, middle panel). In contrast, in torpedo, these relationships were not affected by volume loading (figure 3, lower panel). It should be noted that the torpedo heart is quite sensitive to preload changes: an increase of input pressure head sufficient to double cardiac output resulted in a very small (not significant) increase of preload, without significant changes of afterload (table 1).

In high work conditions on the trout heart (figure 3, middle panel), the coronary pressure necessary to maintain a physiological percentage of flow (2% of cardiac output) was significantly lower than in low work conditions ($p < 0.01$). Increasing the coronary pressure up to 4.5 kPa resulted in an increase in the coronary flow up to 7% of the cardiac output. Values of coronary flow up to 2 ml min^{-1} kg^{-1} were found in high work conditions. In these conditions coronary resistance became constant at pressures higher than 3 kPa.

The coronary responses in the hearts of the two species were also different in pressure loading conditions. In trout, an increase of afterload from 5.0 kPa to about 8 kPa without changes in preload or cardiac output (table 1), resulted in a downward shift of the flow–pressure relationship, indicating an increased coronary resistance. A halving of coronary flow was observed between control and high work conditions across the range of coronary pressure used (figure 4, upper panel). On the other hand, the coronary flow–pressure relationship of the torpedo hearts was unaffected by pressure loading (figure 4, lower panel).

In two experiments (not included with the data reported in the figure 4, upper panel) on the trout hearts, the increase of afterload was accompanied by a decrease of cardiac output so that power output was unchanged (1.04 and 1.68 mW g^{-1} in control conditions and 0.99 and 1.62 mW g^{-1} in high afterload conditions). Despite this constant power output, a threefold increase of coronary resistance was observed when afterload was increased (from 0.37 GPa s m^{-3} to

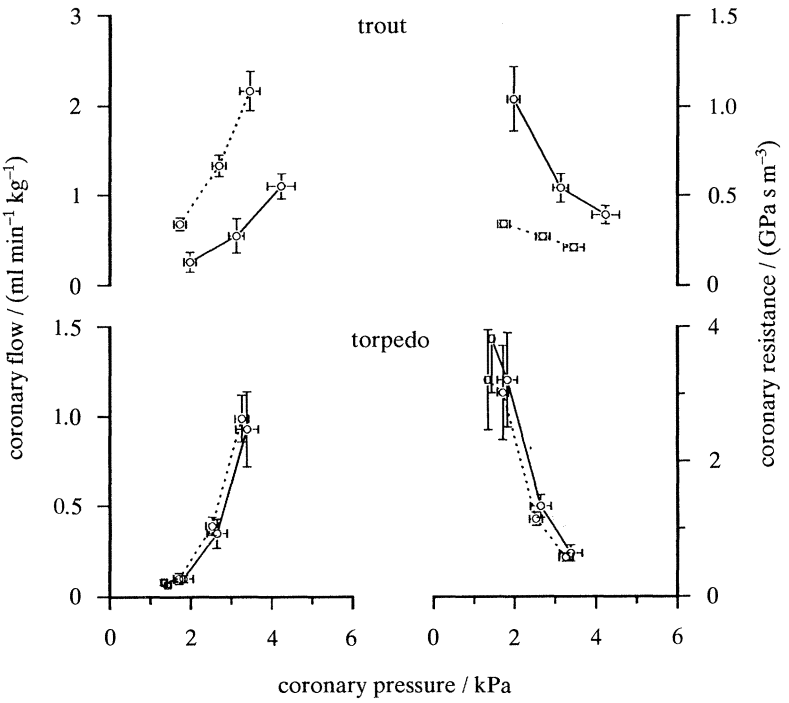

Figure 4. Flow–pressure and resistance–pressure relationships in isolated and perfused hearts of trout, *Oncorhynchus mykiss*, and torpedo, *Torpedo marmorata*, in control conditions (dotted lines) and submitted to pressure loading (solid lines). Each heart acted as its own control. The mean ventricle masses were: trout, 0.49 ± 0.08 g ($n=4$); torpedo, 0.34 ± 0.06 g ($n=8$). The haemodynamic parameters are reported in table 1. The ANOVA analysis gave significant dependence of coronary flow from both coronary pressure and power output ($p<0.05$) for the trout hearts only.

1.27 GPa s m^{-3} at a coronary pressure of 1.9 kPa, means of two experiments).

Statistical analysis by ANOVA of trout data revealed significant relationship between coronary flow and both pressure and power output ($p<0.05$) without interaction between these factors, both under pressure and volume loading.

(*c*) *Oxygen content of the perfusate and coronary perfusion of the heart*

Trout hearts perfused with aerated saline displayed poor performance compared with those perfused with oxygenated saline (table 1). In particular heart rate and afterload (i.e. the mean aortic pressure) produced at the same static output pressure were significantly lower. The controls in these experiments are not directly comparable with those of the other experiments, owing to the significantly lower cardiac output. In contrast, the performance of torpedo hearts perfused with aerated saline was comparable with that of oxygenated saline perfused hearts (table 1).

The hearts of the two species displayed similar responses to perfusion with lowered pO_2 saline. The coronary flow–pressure relationship was shifted up in hearts perfused with aerated perfusate compared with hearts from animals of the same size range perfused with oxygenated saline at conditions that gave comparable cardiac outputs (figure 5). As a corollary, the resistance–pressure relationship was shifted down. In trout, a significant reduction of pressure necessary to obtain a fixed flow (2% of cardiac output) was observed in aerated saline perfused preparations (1.85 ± 0.07 kPa against 2.30 ± 0.10 kPa in the control experiments, $p<0.01$).

Statistical analysis by ANOVA of data from both kinds of heart revealed in the experiments with lowered pO_2 significant relationship between coronary flow and both pressure and Po_2 ($p<0.05$) without interaction between these factors.

4. DISCUSSION

All the mechanisms by which coronary flow is altered act through the hydraulic relationship between pressure, flow and resistance on one hand, and the myocardial oxygen consumption on the other hand (Feigl *et al.* 1990). These mechanisms are woven together in a complex interrelationship. For example, the resistance is determined largely by the small and terminal arterioles, whose caliber is significantly affected by three factors: (i) the coronary artery pressure; (ii) the constricting action of the myocardium during systole (extravascular compression); and (iii) the tone or contractile state of the vessels, significantly affected by pO_2 via a number of humoral and metabolic factors.

The isolated and perfused heart preparation used here allows a separate evaluation of some important factors affecting cardiac power output and coronary response such as volume and pressure loads. Under volume loading the Starling mechanism enables the heart to increase its stroke volume with a concomitant rise in oxygen requirement. Under pressure loading any increase in ventral aorta pressure must increase the

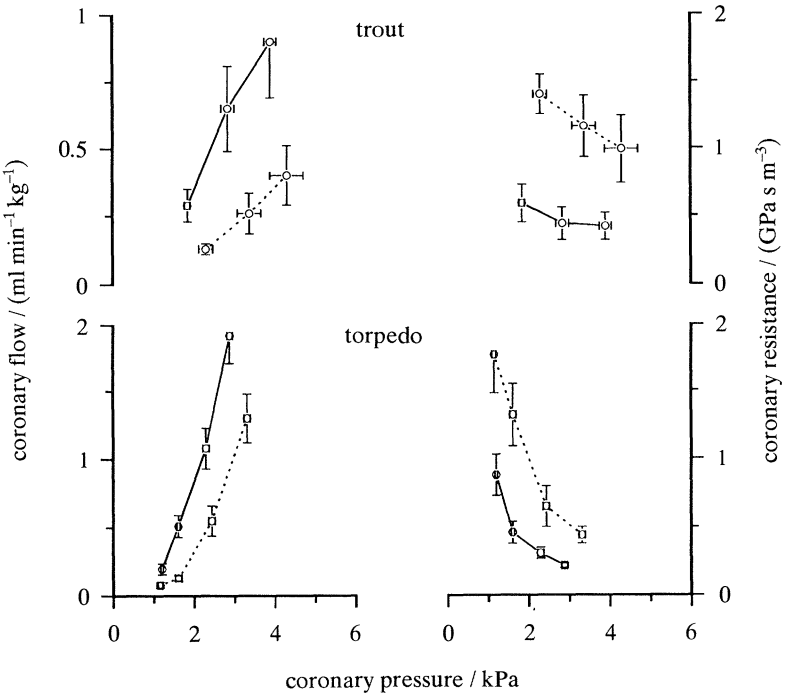

Figure 5. Effect of changing pO_2 of saline on the flow–pressure and resistance–pressure relationships in isolated and perfused hearts of trout, *Oncorhynchus mykiss*, and torpedo, *Torpedo marmorata*. Dotted lines: controls; solid lines: aerated saline. The mean ventricle masses were: trout, controls, 0.65 ± 0.10 g ($n = 5$); trout, aerated saline, 0.62 ± 0.05 g ($n = 5$); torpedo, controls, 0.36 ± 0.06 g ($n = 7$); torpedo, aerated saline, 0.41 ± 0.05 g ($n = 7$). The haemodynamic parameters are reported in table 1. The ANOVA analysis gave significant dependence of coronary flow from both coronary pressure and power output ($p < 0.05$) for both trout and torpedo hearts.

myocardial oxygen demands: as the heart ejects against a greater resistance, it must work harder. Hence, both conditions will increase myocardial oxygen consumption. In fish, volume loading increases the oxygen supply to the lumen, while in pressure loading this supply is constant.

The main difference observed between trout and torpedo hearts in our experimental conditions concerns the effects of loading conditions on the coronary flow and resistance. Although in trout these parameters change both in volume and pressure loadings, in torpedo they are independent from power output. Although differences in the characteristics of the cardiac cycle as well as in the metabolic or paracrine control cannot be excluded, the basic differences in the design of coronary circulation in the two species must be considered. As reported by Davie & Farrell (1991), indirect evidence (McWilliam 1885; Yamauchi & Burnstock, 1968) indicates that the trout heart belongs to type II (Tota 1983, 1989), i.e. the coronary arterial tree is limited to the compact outer layer where it branches into a capillary network from which the blood flows back to the atrium. The adrenergic innervation of the ventricle is confined to the compact region (Gannon & Burnstock 1969). Unpublished data produced in our laboratory have shown that vascular casts are obtained only in the outer compact layer when neoprene is injected into the coronary artery. This may indicate that an anatomical and/or functional separation between coronary and lacunary circuits exists in the trout heart. A similar situation has been reported in hearts of other poikilotherms (i.e turtle; Brady & Dubkin 1964). In trout, then, the driving force for coronary flow is the difference between the coronary artery pressure (closely linked to hypobranchial arterial pressure but less to ventricular pressure) and atrial pressure. In this anatomical condition the resistance is the mean of all the resistances to flow of the vessels embedded in the compact layer. The torpedo heart, on the other hand, belongs to heart type III, where both the spongy and compact myocardium are supplied with coronary vessels (Tota 1989; Farrell & Jones 1992). In this fish, as in other elasmobranchs (Tota *et al.* 1983), only part of the arterial tree forms capillaries in the compact layer. Instead, most of the small coronary arteries branch into vessels of similar caliber into the spongy layer where they either form capillaries into a trabecula or ends directly into the ventricular lumen via an extensive number of arterio-lacunary connections of different calibre (thebesian vessels). In this case, the coronary resistance reflects the instantaneous balance between two components: the subepicardial vascularization of the compacta draining into the atrium and the spongiosa vascularization, draining into the lumen. The coronary driving pressure in this case is directly affected by ventricular pressure. The existence of an extensive number of low-resistance thebesian vessels could affect the hydraulic behaviour of the coronary system. For instance, the driving force for flow in these vessels should be highly dependent from cardiac cycle; moreover, any increase of resistance in the compacta should increase the flow towards the lumen. These morphofunctional cosiderations could

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be sufficient to account for the maintenance of a constant coronary flow during both volume and pressure loadings.

In summary, the different coronary responses revealed in trout and torpedo are consistent with their different microvascular arrangements of the ventricular wall. This emphasizes that in fishes each coronary microvascular pattern has a specific set of structural characteristics that probably contribute in a specific manner to the overall control of coronary blood flow.

A phenomenon observed in the hearts from both species is the vasodilatory effect upon reducing the perfusate pO_2, suggesting a common metabolic control of the coronary resistance. It should be noted that, although the power production of the torpedo heart was not different at the two levels of pO_2 used, in trout the lower afterload and the higher preload produce a significantly lower power output in the hearts perfused with aerated saline compared with oxygenated controls. This observation suggests a greater degree of hypoxic resistance of the torpedo compared with the trout and agrees with the higher resistance to anoxia observed in some elasmobranchs (Helle *et al.* 1983). In trout the 0.4 kPa difference in afterload is not enough to explain the difference in coronary resistance. In the experiments with pressure loading an increase in afterload of 3 kPa was necessary to obtain an increase of resistance similar to that elicited by lowered pO_2. Thus the difference between the two preparations must be due to the different oxygen content of perfusate. Compared with the torpedo hearts, the trout hearts have a lower heart rate when exposed to aerated saline. This could account for at least part of the increased coronary flow, as the coronary flow in the trout heart would occur mainly during diastole as in mammals (Axelsson & Farrell 1993).

In the isolated trout heart, volume loading is clearly accompanied by a decreased resistance of the coronary vessels at constant coronary perfusion pressure. We have no specific evidence to explain the resistance change. A metabolic mechanism linked to the increased oxygen demand could exist. However, redistribution of coronary flow during the cardiac cycle in volume loading could also in part contribute to the observed increased flow. In contrast to volume loading, pressure loading is accompanied by an increased resistance at constant coronary perfusion pressure. This result confirms a previous preliminary observation reported by Farrell (1987), and can be related to the extra-vascular compression, a phenomenon which is also the basis of the oscillations in the coronary pressure reported in figure 2. The extra-vascular compression in trout seems to prevail *in vitro* on other intrinsic controls and could strongly limit the mechanical performance of compacta.

In the isolated working hearts of both species, perfused at constant loads and oxygen content, the increase of coronary pressure brought about a reduction in resistance. The simplest explanation of this behaviour is that a rise in the main intracoronary artery pressure can cause more coronary arterioles embedded in the compacta to be recruited as the critical pressures of the 'terminal arterial bed' is exceeded. Some indications of the existence of more complex mechanisms (possibly involving the myogenic response; Johnson 1986) in the coronary resistance–pressure relation comes from the demonstration that the flow–pressure relationship, determined in a wide range of pressures in the isolated non-working trout heart, is S-shaped (Agnisola 1992).

The large quantitative difference in the pressure dependence of coronary resistance observed between trout and torpedo seems to support the idea of a higher coronary reserve in torpedo. The presence in the heart of this species of low resistance shunts (thebesian vessels) could easily account for the high flow at high pressures.

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