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OXYGEN SUPPLY AND *IN VITRO* PERFORMANCE OF THE SYSTEMIC HEART OF *OCTOPUS VULGARIS*: EFFECTS OF HAEMOCYANIN

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Summary

The effect of increasing oxygen supply on the perfused systemic heart of *Octopus vulgaris* (Lam.) by using oxygenated or haemocyanin-containing perfusates was investigated. Providing aerated blood or seawater solutions of haemocyanin that were comparable with blood in oxygen-carrying capacity improved the performance of the isolated heart compared with that of hearts perfused with aerated sea water. Aortic outputs were similar to *in vivo* values ($44\,ml\,min^{-1}\,g^{-1}$) at close to *in vivo* values of preload and afterload owing to an increase in both heart rate (from 24.0 to $38.4\,beats\,min^{-1}$) and stroke volume (from 0.69 to $1.10\,ml\,g^{-1}$). Coronary flow fell in these conditions, becoming 2.5 % of the aortic output (against 24 % with aerated sea water). A parallel increase in coronary resistance was found. Oxygenated sea water also improved the performance of the heart, mainly by improving the stroke volume. Both with haemocyanin solutions or blood and with oxygenated sea water, the isolated heart was able to do more work at lower preloads compared with the hearts perfused with aerated sea water. Power output was linearly related to total oxygen consumption and carbon dioxide production. The major site of oxygen consumption was the coronary bed. Haemocyanin released about 70 % of the bound oxygen as it passed through the ventricular wall.

Introduction

Although isolated hearts of octopods have been used in a number of investigations, it has generally been recognized that their physiological performance is poor compared with the cardiac performance *in vivo* (Smith, 1981; Foti *et al.* 1985; Houlihan *et al.* 1987; Wells and Smith, 1987; Agnisola *et al.* 1989). Isolated hearts of *Octopus* perfused with aerated sea water generate an aortic output only 50–60 % of the likely *in vivo* value and this performance was achieved with input pressures 2–4 times higher than those likely to occur *in vivo* (Smith, 1985; Wells

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and Smith, 1987; Houlihan *et al.* 1987; Agnisola *et al.* 1989). Isolated *Octopus* hearts are also characterized by slow heart rates, low power output, poor endurance at physiological work rates and an unphysiological susceptibility to low P_{O_2} (Houlihan *et al.* 1987). However, they are unusual among *in vitro* preparations in that they are self-perfused: during each contraction a proportion of the perfusate in the heart's lumen flows through the muscular wall of the heart and can be collected as a coronary flow (Agnisola, 1990).

The poor performance of the *in vitro* octopod ventricle may be improved by perfusion with haemocyanin dissolved in sea water (Agnisola and Houlihan, 1989). This result implies that the oxygen supply to the heart is of critical importance and raises the question of the role of the coronary supply to the contracting ventricular muscle.

The aim of the research reported here was to analyze the relationships between *in vitro* heart performance and the oxygen content of the perfusate. Isolated hearts were provided with oxygenated sea water and a variety of haemocyanin concentrations in the perfusion saline as well as blood. The performance of the heart was monitored in terms of aortic and coronary output, heart rate, oxygen consumption, carbon dioxide output, stroke work and power output. The route and role of the coronary perfusion in supplying oxygen has been investigated through a study of the relationships between intraventricular pressure and coronary resistance in the isolated heart.

Materials and methods

Animals

The experiments were carried out in the Stazione Zoologica 'A. Dohrn', Naples, in May and September. All the animals were kept in the laboratory, without food, in flowing sea water at 20°C for a maximum of 7 days before use. The total number of animals was 45; they were of both sexes and their live masses varied between 300 and 1140 g (mean 742.56±40.48 g, N=45).

Isolated heart preparation

The isolated heart was prepared as described previously with perfusion occurring through the two cannulated atria and the aortic output was collected from the cannulated dorsal aorta (Foti *et al.* 1985; Houlihan *et al.* 1987). In addition, a cannula was inserted through the abdominal aorta inside the ventricular chamber to record intraventricular pressure (Agnisola *et al.* 1989). The heart was mounted in a chamber and the coronary flow was collected on the chamber's floor. In the haemocyanin and blood perfusion experiments in low work conditions, the input and output reservoirs were positioned 5 and 25 cm above the input and output cannulae, respectively. When necessary, the height of the input pressure heads was varied to change the input pressure and preload. The temperature of the perfusion fluid and hearts was 18–22°C (mean 20±0.5°C).

Perfusates

The seawater-perfused hearts received aerated (P_{O_2} 20±0.2 kPa, N=25) or oxygenated sea water (P_{O_2} 99.4±1.7 kPa, N=13) containing 0.05 % glucose. Blood was collected from large octopuses (>2 kg) by cannulation of the dorsal aorta. The blood was either used directly after a low-speed centrifugation to remove cells or the haemocyanin was precipitated by centrifugation at 100 000 *g* for 12 h at 0°C. Haemocyanin was dissolved in filtered sea water (similar in composition to *Octopus* blood, D'Aniello *et al.* 1986), glucose was added to a final concentration of 0.05 % and the oxygen content (C_{O_2}) of the aerated perfusate was determined. The pH of the haemocyanin solution was adjusted, if necessary, to 7.5 at 20°C.

Protocols

Series 1. The effects of oxygenated sea water, haemocyanin solutions and blood on the in vitro *heart performance*

Each heart experienced only one condition and was perfused with a fluid of known oxygen content. Aortic and coronary flow rates, heart rate, input and output P_{O_2}, total oxygen content (C_{O_2}), total carbon dioxide content (C_{CO_2}) and intraventricular pressures were measured over 10 min or until they had stabilized. If this had not occurred within 15 min, the heart was discarded. Mineral oil was then added to the heart chamber so that the coronary output collected at the bottom of the bath was out of contact with air. The chamber was not sealed. Aortic and coronary flow rates were measured for a further 10 min during which time aortic and coronary outputs were sampled anaerobically for the determination of P_{O_2}, C_{O_2}, C_{CO_2} and pH. To avoid mixing problems, the coronary output samples were collected as follows: the perfusate in the chamber was completely removed and the next 1 ml of perfusate flowing out of the coronary veins was collected by syringe. Measurements were made of the surface oxygen consumption of the heart by Houlihan *et al.* (1987) and were not found to be significant. The hearts were almost completely surrounded with oil and were out of contact with the coronary flow collecting at the bottom of the bath. Cardiac performance in terms of maximum intraventricular pressure and aortic output were not significantly different 10 min before and 10 min after the addition of the oil. Repeated sampling of a haemocyanin solution covered with oil and with an initial P_{O_2} of 0.93 kPa revealed that the P_{O_2} did not change significantly for up to 60 min.

Series 2. The effects of increased input pressure on the in vitro *heart performance using normal levels of haemocyanin or oxygenated perfusate*

The heart was perfused with haemocyanin solutions, blood or oxygenated sea water at different levels of static input pressure (=input pressure at zero flow). Each heart experienced one input pressure. Input reservoir heights of 5, 10, 15 and 10, 15, 20 cm were used for oxygenated sea water and haemocyanin or blood perfusion experiments, respectively. In each experiment the actual value of

preload depended upon the pressure head height, the type of perfusate and the total cardiac flow. Values of up to 1.5 kPa of preload were obtained. Heart rate, aortic output and intraventricular pressure were determined within 10 min of perfusion. Stroke work was calculated as a function of preload.

Measurements and calculations

The blotted wet mass of the ventricle was determined at the end of each experiment, and all results (except for coronary resistance) are expressed per gram of ventricular mass.

Throughout the isolated heart experiments the aortic and coronary outputs were collected over 1 min, weighed, and expressed as a volume measurement after correcting for temperature and density. Intraventricular pressures were continuously measured from the cannulated abdominal artery with an Elcomatic pressure transducer coupled to a Harvard chart recorder. From these pressure records, preload (=mean intraventricular pressure during diastole) and maximum ventricular pressure were determined directly. The simultaneous aortic pressure recording allowed a distinction to be made between the diastolic and systolic phases of intraventricular pressure changes. Pressures were referenced to the level of the input cannula and were expressed in kPa. Heart rates (beats min^{-1}) were determined from the pressure records. Stroke volume was calculated from the total of aortic and coronary output (ml g^{-1} min^{-1}) and heart rate and expressed in ml g^{-1} $beat^{-1}$.

Power output of the heart was calculated from the sum of the work per second done by the heart to move perfusate in the aorta (aortic power output) and that necessary to move perfusate in the coronary system (coronary power output). Aortic power output was calculated as (mW g^{-1})=(afterload−preload) (kPa)×aortic output (ml min^{-1} g^{-1})/60. Afterload (=mean output pressure) was calculated as 0.25 times maximum ventricular pressure plus 0.75 times diastolic aortic pressure. Coronary power output was calculated as (mW g^{-1})=(mean ventricular pressure−preload) (kPa)×coronary output (ml min^{-1} g^{-1})/60. Mean ventricular pressure was used to calculate coronary power output and coronary resistance (see below), as it can be considered to be the driving pressure for the coronary flow (Agnisola, 1990). Mean ventricular pressure was calculated as one-third of maximum ventricular pressure. These estimates of mean output pressure and of mean ventricular pressure were determined from comparison with the integrated pressure records, and were found to be the best approximation to the mean values. In the experiments of series 1, although the input reservoir was set 5 cm higher than the input cannulae, the preload changed with different perfusates, owing to the flow-related fall in pressure along the input cannula and the change in the viscosity of different perfusates. Pressure records over 1 min were analyzed to calculate pressures.

Stroke work was calculated as (mJ g^{-1} $beat^{-1}$)=[(afterload−preload) (kPa)×aortic output (ml min^{-1} g^{-1})/heart rate (beats min^{-1})]+[(mean ventricular

pressure−preload) (kPa)×coronary output ($ml\,min^{-1}\,g^{-1}$)/heart rate (beats min^{-1})].

Coronary resistance ($\times 10^9\,Pa\,s\,m^{-3}$) was calculated as mean ventricular pressure (kPa)×60/coronary output ($ml\,min^{-1}$), since the mean ventricular pressure was the driving pressure for the coronary flow and the coronary vein back pressure was zero.

Separate input, aortic and coronary output samples were taken anaerobically by syringe for the measurement of P_{O_2}, C_{O_2}, C_{CO_2} and pH. Oxygen tensions (in mmHg, converted to kPa) were measured with thermostatted Radiometer microelectrodes and Radiometer PHM73 and Strathkelvin analyzers. All samples were analyzed within 3 min. Oxygen concentrations were determined by the modification of the Tucker (1967) method described by Bridges *et al.* (1979). Protein concentrations of haemocyanin solutions were determined according to Konings *et al.* (1961) and the relationship between protein concentration (x, $\times 10^2\,g\,ml^{-1}$) and oxygen content of air-saturated solutions (y, $mmol\,O_2\,l^{-1}$) is described by the equation: $y=0.116+0.195x$ ($N=10$, $r^2=0.968$, $P<0.01$). Total CO_2 concentrations ($mmol\,l^{-1}$) were determined by the method of Cameron (1971) using 50 μl samples, unknowns being bracketted by sodium bicarbonate standards. pH was determined with an Orion pH electrode and meter.

The viscosity of the perfusion fluid was measured with a KPG-Ubbelohde Viskosimeter and referenced to distilled water at 20°C. The relationship between protein concentration (x, $\times 10^2\,g\,ml^{-1}$) and viscosity (y, $\times 10^9\,N\,s\,m^{-1}$) can be described by the relationship: $y=0.85\,e^{0.118x}$ ($N=10$, $r^2=0.958$, $P<0.01$).

Oxygen consumption ($\mu mol\,O_2\,g^{-1}\,min^{-1}$) of the hearts was determined as described by Houlihan *et al.* (1987) and CO_2 production ($\mu mol\,CO_2\,g^{-1}\,min^{-1}$) was calculated from the difference between input and coronary output total CO_2 levels. Oxygen bound to haemocyanin was calculated from the oxygen content of air-saturated haemocyanin solutions or blood, as described by Wood *et al.* (1979). Gross mechanical efficiency of the heart was calculated as described previously (Houlihan *et al.* 1987); net efficiency was calculated by subtracting the calculated oxygen consumption at zero power output from the measured oxygen consumption (Little, 1985).

The results were compared using Student's t-test and means±standard errors (S.E.) are given. The 5% level of confidence has been used throughout.

Results

Cardiac performance, coronary flow and coronary resistance in relation to the oxygen content of perfusion fluid

Increasing the oxygen content of the perfusion fluid by the addition of haemocyanin improved aortic output in a linear fashion up to an oxygen content of 1.44 $mmol\,O_2\,l^{-1}$ (Fig. 1A); thereafter, aortic output did not improve significantly. The isolated hearts, perfused with haemocyanin at concentrations comparable with those commonly found *in vivo* [3.4 vol% corresponding to 1.5 $mmol\,O_2\,l^{-1}$

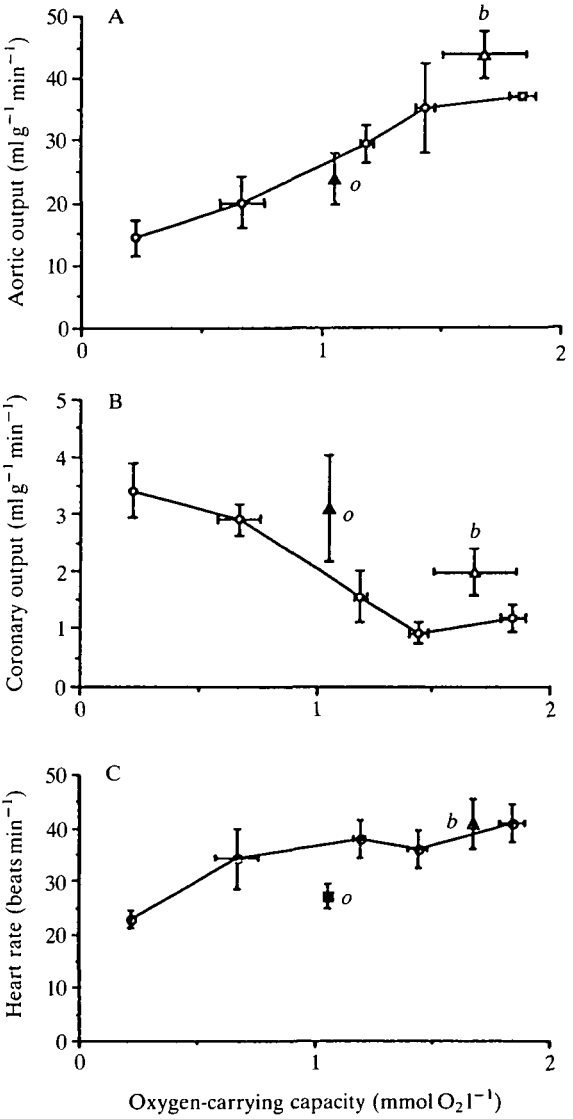

Fig. 1

(Houlihan *et al.* 1982)], have achieved aortic outputs similar to those of resting animals [44 ml g^{-1} min^{-1} (Houlihan *et al.* 1986; Wells, 1979)]. Perfusion of hearts with oxygenated sea water also significantly improved their aortic output compared with values for aerated sea water ($P<0.05$) (Fig. 1A). Perfusion of hearts with aerated centrifuged blood (oxygen content 1.7 ± 0.17 mmol O_2 l^{-1}) did not significantly improve aortic output compared with haemocyanin solutions in sea water of similar oxygen content (Fig. 1A).

Preload pressures during perfusion with aerated sea water were higher than those used for the highest haemocyanin concentrations and blood (see legend to Fig. 1 and Materials and methods), but in general preload pressures were all very

Fig. 1. Relationships between aortic output (A), coronary output (B), heart rate (C) and oxygen-carrying capacity of the perfusion medium. The perfusion media consisted of aerated sea water and haemocyanin dissolved in sea water (○), oxygenated sea water (*o*, ▲) and blood (*b*, △). The mean±S.E. heart masses for each experimental condition were: 0.61±0.06 g (aerated sea water, N=22); 0.66±0.08 g (oxygenated sea water, N=6); 0.66±0.13 g (haemocyanin, 0.67 mmol $O_2 l^{-1}$, N=3); 0.54±0.06 g (haemocyanin, 1.19 mmol $O_2 l^{-1}$, N=4); 0.66±0.12 g (haemocyanin, 1.44 mmol $O_2 l^{-1}$, N=4); 0.72±0.30 g (haemocyanin, 1.84 mmol $O_2 l^{-1}$, N=2); 0.54±0.08 g (blood, 1.7 mmol $O_2 l^{-1}$, N=4). In aerated and oxygenated sea water the preload varied between 0.20±0.01 kPa and 0.14±0.02 kPa, respectively (significantly different, P<0.01). The preload was 0.21±0.02 kPa with the haemocyanin solution at a carrying capacity of 0.67 mmol $O_2 l^{-1}$ and was lowest at the highest haemocyanin concentrations (0.13±0.01 kPa) and with blood (0.11±0.02 kPa). The afterload varied between 2.61±0.04 kPa for aerated sea water and 3.59±0.12 kPa for blood. This increase in afterload is linked to the increase in maximum intraventricular pressure (see Fig. 3), while the static output pressure was kept constant at 2.49 kPa.

low in these experiments (mean 0.16±0.01 kPa), even when compared with the lowest pressures normally applied to octopod hearts *in vitro* (0.49 kPa, Smith, 1981; Houlihan *et al.* 1987; 0.23 kPa, Agnisola *et al.* 1989). The hearts were therefore generating *in vivo* levels of aortic output at close to *in vivo* levels of input pressures (0.05–0.25 kPa in the efferent branchial vessel, Wells and Smith, 1987).

The increased aortic outputs described in Fig. 1A are due to changes in both of the variables that control aortic output, heart rate and stroke volume. Increasing the oxygen content of the perfusate with haemocyanin, at the lowest concentration used, increased the heart rate (Fig. 1C) compared with aerated sea water. The small further increase in heart rate at higher concentrations of haemocyanin and with blood was not significant. The stimulation of heart rate was not accompanied by a change in stroke volume between aerated sea water and the two lowest haemocyanin concentrations (Fig. 2A). With the plateau in heart rate, the increased aortic output was due to an increase in stroke volume which rose to 1.02 ml g^{-1} $beat^{-1}$ with a perfusion fluid with an oxygen content of 1.44 mmol $O_2 l^{-1}$ and was not significantly different at higher oxygen concentrations or with blood. The maximum intraventricular pressure only increased significantly at the highest haemocyanin concentrations and with blood (Fig. 2B).

The increased aortic flow found with oxygenated sea water is associated with stroke volume and intraventricular pressure significantly higher than those with aerated sea water (P<0.05 and P<0.01, respectively). No significant effect on the heart rate was found. When compared with the hearts perfused with haemocyanin solution of comparable oxygen content, those perfused with oxygenated sea water have a lower heart rate and higher stroke volume and intraventricular pressure (P<0.01). This results in a cardiac output not significantly different from that with comparable haemocyanin solutions.

Previous experiments using aerated sea water with glucose were not continued for more than 40 min as aortic output was found to fall appreciably after this time (Houlihan *et al.* 1987). In the experiments reported here, it was noted that the

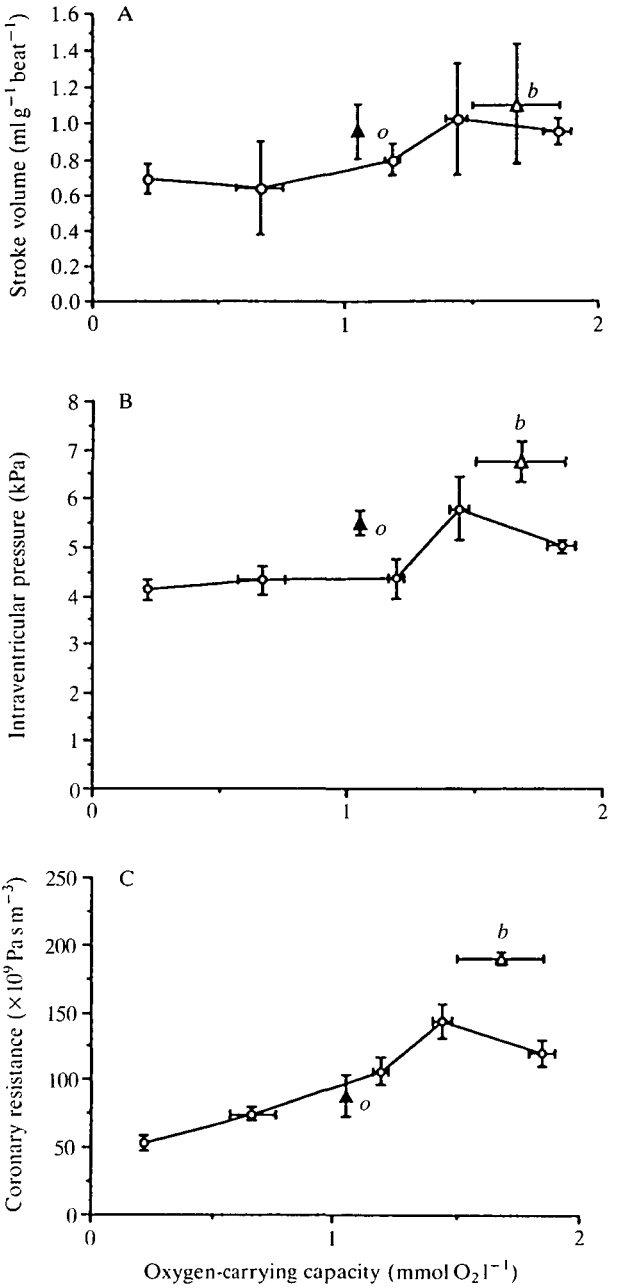

Fig. 2. Relationships between stroke volume (A), maximum intraventricular pressure (B), coronary resistance (C) and oxygen-carrying capacity of the perfusion medium. The perfusion media consisted of aerated sea water and haemocyanin dissolved in sea water (○), oxygenated sea water (*o*, ▲) and blood (*b*, △). Other details are as in Fig. 1.

higher aortic output when oxygenated sea water was used as a perfusate was sustainable for relatively short periods (10–15 min). The high performance with haemocyanin solutions was maintained for longer and blood-perfused hearts could maintain high aortic output for more that 40 min.

Coronary flow was significantly reduced when the hearts were perfused with haemocyanin solutions carrying more than 1 mmol O_2 l^{-1} (Fig. 1B). At high concentrations of haemocyanin, coronary output amounted to only 2.5 % of the aortic output, whereas with sea-water perfusion the former was 24 % of the latter (42 % in Houlihan *et al.* 1987). The increased aortic flow consequent on perfusion with oxygenated sea water was accompanied by a coronary flow not significantly different from that necessary in aerated sea water (Fig. 1B). However, because of the increased aortic flow with oxygenated sea water, the coronary flow amounted to 13.1 % of the aortic flow. The coronary flow with blood was significantly higher than with haemocyanin solutions of comparable oxygen content ($P<0.05$).

The coronary resistance increased significantly at higher levels of haemocyanin and with blood (Fig. 2C), thus accounting for the fall in coronary flow (Fig. 1B). This increase in resistance accompanied the almost 2.5-fold increase in viscosity of the perfusate (see Materials and methods). There was a slight increase in coronary resistance in the hearts perfused with oxygenated sea water. It should be noted that the coronary resistance was 5–30 times the *in vivo* systemic resistance found in another octopus, *Octopus dofleini* (5.65×10^9–7.77×10^9 Pa s m^{-3}, Shadwick *et al.* 1987).

Power output, oxygen consumption and CO_2 production of the heart

As a result of the provision of haemocyanin solutions with varying oxygen content, there was an improvement in oxygen supply which resulted in an increase in power output through an enhancement of volume flow. Oxygen consumption of the heart was increased over fivefold with increasing oxygen content of the perfusion fluid and this was accompanied by a fivefold increase of total power output (Fig. 3A). Combining all the data from hearts perfused with oxygenated sea water, haemocyanin solutions and blood gives a linear relationship between total oxygen consumption (y, μmol O_2 g^{-1} min^{-1}) and total power output (x, mW g^{-1}) which can be described by the equation: $y=0.604+0.982x$, $N=18$, $r^2=0.806$, $P<0.01$. There were no significant differences in the regression equations obtained from the individual sets from oxygenated sea water or haemocyanin solutions and blood experiments or between these equations and the above regression. Comparison with the previously published regression equations for increasing power output and oxygen consumption by volume loading when perfusing with sea water (Houlihan *et al.* 1987) is not possible as power output was calculated differently.

The total power output is the sum of the aortic and coronary power outputs (see Materials and methods). With increasing haemocyanin concentrations the fall in the coronary flow and the increasing aortic flow resulted in a fall in the percentage of total power used for the coronary perfusion (Fig. 4).

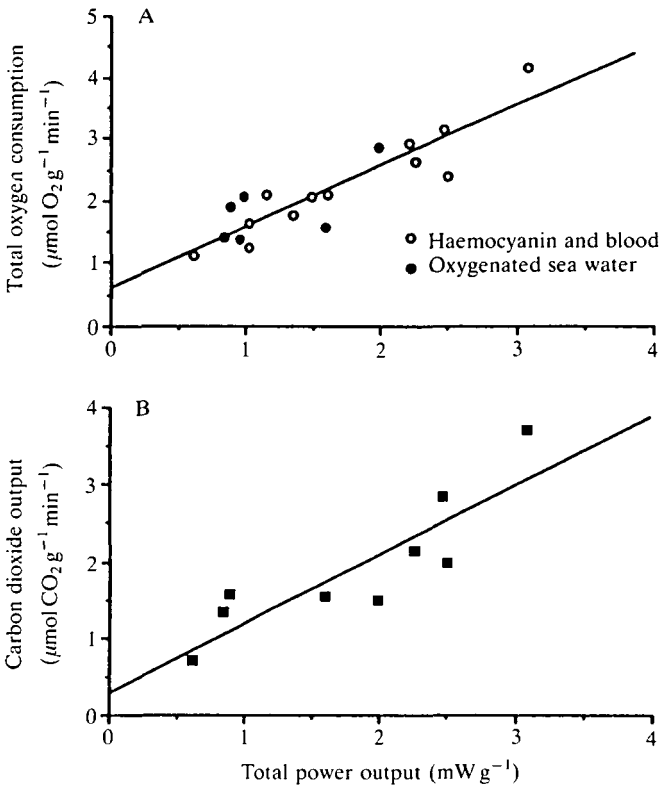

Fig. 3. Relationships between total oxygen consumption (A), carbon dioxide output (B) and total myocardial power output from hearts perfused with oxygenated sea water, haemocyanin or blood. In A a distinction is made between the hearts perfused with oxygenated sea water (●) or haemocyanin solutions and blood (○). In B this distinction is not made. The lines are from the regression equations are given in the text.

Gross mechanical efficiency was lower in hearts perfused with oxygenated sea water (8.6±1.5%) than in hearts perfused with haemocyanin or blood (10±0.5%). Net mechanical efficiency was 17.25±2.31% in hearts perfused with haemocyanin and blood.

Carbon dioxide production was only measurable through the coronary route; there were no significant differences between the CO_2 content of the input and aortic output. The CO_2 production (y, $\mu mol\,CO_2\,g^{-1}\,min^{-1}$) was related to total power output (x, $mW\,g^{-1}$) by the equation $y=0.329+0.891x$, $N=9$, $r^2=0.752$, $P<0.05$ (Fig. 3B). This line has a lower slope and intercept than that for oxygen uptake and this is in accord with the mean respiratory quotient (RQ) of 0.86±0.04 ($N=9$).

There are two routes for oxygen supply to the heart: removal of oxygen from fluid passing through the lumen (aortic flow) and removal of oxygen from fluid passing through the ventricular wall (coronary flow). In aerated conditions the

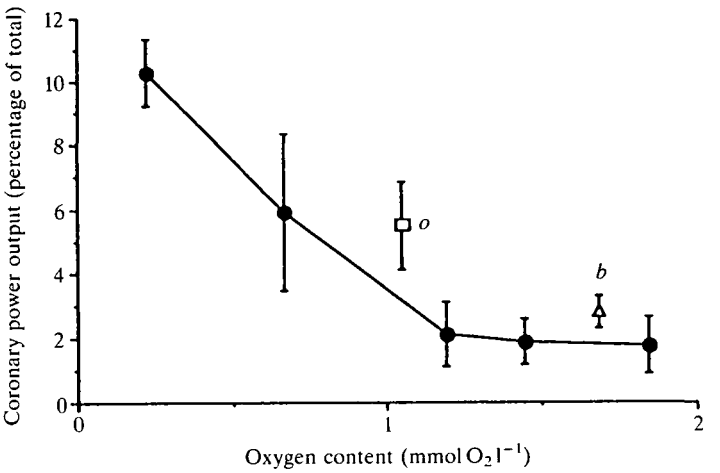

Fig. 4. Coronary power output, as a percentage of total power output, in relation to the oxygen content of the perfusate. The perfusion media consisted of aerated sea water and haemocyanin dissolved in sea water (●), oxygenated sea water (*o*, □) and blood (*b*, △). Other details are as in Fig. 1.

aortic route was characterized by a constant, small P_{O_2} difference between input and output (0.60±0.15 kPa) which did not change significantly with increasing haemocyanin concentration or with blood (Fig. 5A). As aortic flow increased with increasing haemocyanin concentrations the oxygen consumption through this route therefore increased (Fig. 5C, difference between total and coronary oxygen consumption). These small changes in aortic P_{O_2} were well in excess of the 100 % saturation of the haemocyanin and the oxygen delivered was coming from oxygen in solution.

The P_{O_2} of the coronary output was always lower than that of the aortic output and fell markedly when haemocyanin was added to the perfusion medium; the effect was independent of concentration (Fig. 5A) and was presumably due to the higher oxygen consumption/performance. The result of this stability of output P_{O_2} and increasing oxygen content of the perfusion medium was an increase in the oxygen unloading, with the maximum at the input oxygen concentration of 1.44 mmol O_2 l^{-1} (Fig. 5B). The flow through the coronary vessels was clearly the major site of oxygen consumption under all conditions (Fig. 5C). With increasing haemocyanin concentration, the reduced coronary flow (Fig. 1B) accompanied by increased oxygen removal resulted in an increased oxygen consumption which reached a plateau between 1.44 and 1.8 mmol O_2 l^{-1} (Fig. 5C).

When the hearts were perfused with oxygenated saline the P_{O_2} difference between input and aortic output was 2.67±0.15 kPa (significantly higher than in aerated hearts, $P<0.01$) and the aortic flow accounted for 30 % of total oxygen consumption (Fig. 5C). It should be noted that the P_{O_2} fall in the aortic output is linked to the input P_{O_2}.

With blood as the perfusion fluid the coronary output was increased. This was

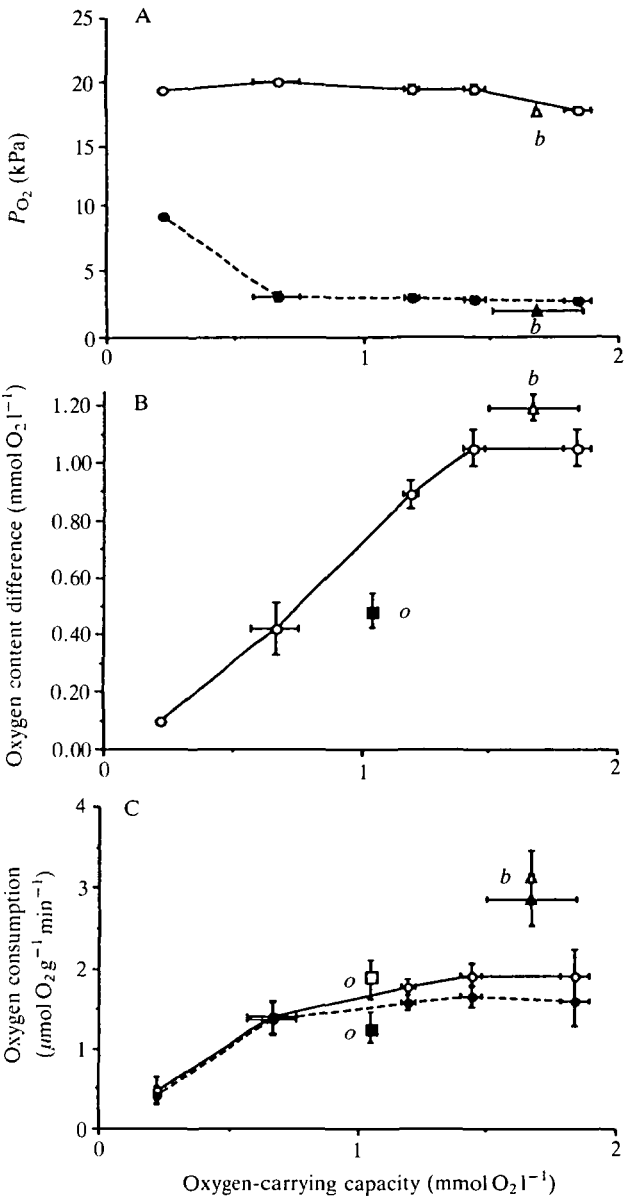

Fig. 5. Relationships between P_{O_2} of aortic and coronary outputs (A), oxygen content difference of the coronary output (B), total and coronary oxygen consumption of the isolated heart (C) and oxygen-carrying capacity of the perfusion medium. The perfusion media consisted of aerated sea water and haemocyanin dissolved in sea water (○, ●), oxygenated sea water (*o*, □, ■) and blood (*b*, △, ▲). In A and C open symbols represent the aortic output P_{O_2} and the total oxygen consumption, respectively, while closed symbols represent coronary output P_{O_2} and coronary oxygen consumption, respectively. The input P_{O_2} in the oxygenated seawater perfusion experiments was 97.52±1.31 kPa (N=6), while the drop in P_{O_2} between the input and aortic output was 2.67±1.47 kPa (N=6). Other details are as in Fig. 1.

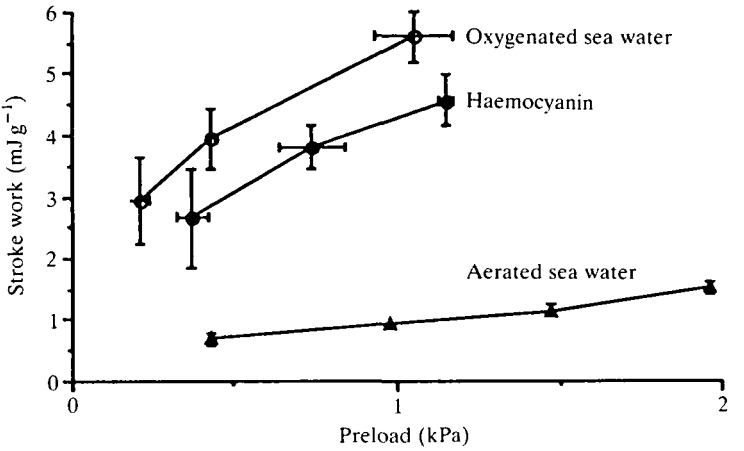

Fig. 6. The effect of increased oxygen content of the perfusion medium on the relationship between stroke work and preload. The perfusion media were oxygenated sea water (○, oxygen content 1.12±0.02 mmol l^{-1}, N=6) and haemocyanin (●, oxygen content 1.20±0.10 mmol l^{-1}, N=11). Static output pressure was always kept 2.0 kPa higher than preload. The afterload varied between 0.2 and 1.5 kPa. Data for aerated sea water (▲, oxygen content 0.23±0.01 mmol l^{-1}) calculated from Houlihan *et al.* (1987) are also reported.

accompanied by a significant fall in the coronary output P_{O_2} and an increase in the oxygen unloading, which resulted in higher oxygen consumption.

Effect of perfusion with haemocyanin, blood or oxygenated sea water on the preload–stroke work relationship

Increasing preload increases the ventricular stroke work in an almost linear fashion within the range 0.2–1.5 kPa. In Fig. 6, the curves obtained for oxyenated seawater perfusion and haemocyanin or blood perfusion are significantly higher than the curve for aerated seawater perfusion calculated from the data reported by Houlihan *et al.* (1987). Thus, the oxygen content of perfusate directly affects the heart contractility as defined by the work–preload curve. When the oxygen content was improved, the heart was able to do more work at a lower preload. There was no significant difference in stroke work between oxygenated sea water and haemocyanin or blood when comparable preloads were used.

Haemocyanin transport and unloading

At the lowest haemocyanin concentration the haemocyanin was 37.7% saturated in the coronary outflow. At other haemocyanin concentrations and with blood perfusion the mean saturation was 28.1±2.7% (N=14). There was some variability in haemocyanin saturation and some of the haemocyanin saturation values were notably higher than the *in vivo* values (14.3±3.2%, Houlihan *et al.* 1986). Experiments with higher power outputs gave no clear indication that this

apparent venous reserve could be called upon. There was a mean pH decline of 0.13 ± 0.04 units ($N=6$) in the coronary output from the input pH of 7.44 ± 0.01.

In the hearts perfused with physiological levels of haemocyanin, the perfusate takes about half a second to pass through the coronary bed, during which time approximately 1 mmol $O_2\,l^{-1}$ is extracted and approximately 0.86 mmol $CO_2\,l^{-1}$ is added with a 0.13 unit change in pH. These changes are very similar to the changes described from *in vivo* measurements and pH/CO_2 changes from *in vitro* measurements by Houlihan *et al.* (1986). Thus, the isolated, haemocyanin-perfused heart appears to be replicating the haemocyanin oxygen unloading and carbon dioxide production for the whole animal determined from *in vivo* sampling of arterial and venous blood.

The results reported here show that the isolated *Octopus* systemic heart is capable of functioning at close to *in vivo* power output with haemocyanin concentrations of 6.5×10^{-2}–$7\times10^{-2}\,g\,ml^{-1}$, which are not very different from those considered normal ($9\times10^{-2}\,g\,ml^{-1}$, Wells, 1983; D'Aniello *et al.* 1986). This has some relevance to the natural situation, as wide variations in haemocyanin concentration in the blood have been reported for *Octopus vulgaris* (Senozan *et al.* 1988). We found a mean haemocyanin concentration of $8.6\pm0.5\times10^{-2}\,g\,ml^{-1}$ from 23 animals, but individual values ranged from 3.5×10^{-2} to $13.3\times10^{-2}\,g\,ml^{-1}$. Indeed, the heart can compensate for reduced oxygen content by increasing the coronary flow.

Discussion

Cardiac performance

The systemic heart of the Octopoda has a complex myoarchitecture (Kling and Schipp, 1987; Agnisola *et al.* 1990), a highly aerobic myocardium (Houlihan *et al.* 1987) and a specific vascularized system for blood supply (Houlihan *et al.* 1987; Agnisola *et al.* 1990; Agnisola, 1990). The *in vivo* cardiac output of resting *Octopus* similar in mass to those used here, calculated from the Fick equation (allowing for cutaneous oxygen consumption), has been estimated to be $43.6\pm4.9\,ml\,kg^{-1}\,min^{-1}$ (Houlihan *et al.* 1986) when the oxygen capacity of the blood was 1.7 mmol $O_2\,l^{-1}$. Measurements of blood flow through the major aorta with flow probes have given values between 25 and $50\,ml\,kg^{-1}\,min^{-1}$ for resting animals (Wells and Wells, 1986). As the aortic flow in that study represented 70% of the total flow, this gives a total cardiac output of 35–$70\,ml\,kg^{-1}\,min^{-1}$. Thus, the cardiac outputs reported here are comparable to the reported *in vivo* values. When perfused with blood or a high concentration of haemocyanin, the heart performed at preload and afterload values comparable with *in vivo* values (Wells *et al.* 1987; Wells and Smith, 1987). The power outputs obtained exceed the resting *in vivo* values ($2\,mW\,g^{-1}$, Wells *et al.* 1987), while the stroke work range obtained in the work–preload curves (2.5–$6\,mJ\,g^{-1}$, Fig. 6) is comparable with the *in vivo* range (about $2.5\,mJ\,g^{-1}$ at rest; about $7\,mJ\,g^{-1}$ in exercise; Wells *et al.* 1987)

Thus, the haemocyanin seems to be a major factor in determining the resting heart performance *in vivo*.

There could be a number of reasons for the stimulatory effects of blood/haemocyanin perfusion. The addition of haemocyanin to the perfusate results not only in an increase in the oxygen content but also in an increase in the buffering capacity of the perfusion medium. Miller and Mangum (1988) have shown that the buffering capacity of any haemocyanin-containing blood depends much more on the protein concentration than on differences between haemocyanins. Given the CO_2 buffering capacity of haemocyanin solutions reported by these authors, the pH change in the aerated seawater experiments should not be very different from the pH change in the high-haemocyanin-concentration experiments when the CO_2 production is increased by about eightfold. Moreover, as demonstrated by the improved performance with oxygenated sea water, the heart seems to be oxygen-limited when perfused with aerated sea water. To produce a resting power output comparable with *in vivo* values, the heart needs the perfusate to release about $1.0\,\text{mmol}\,O_2\,l^{-1}$ of perfusate (see Fig. 5). This quantity of oxygen is much higher than the total oxygen content in aerated sea water and would require the total removal of the oxygen from the oxygenated sea water. Therefore, it is likely that the main effect of haemocyanin is to increase the oxygen availability.

The addition of haemocyanin to the perfusate also improves the long-term performance of the heart. The best long-term performance was obtained with blood perfusion, suggesting that there may be components in the blood, other than oxygen, that influence performance. These components may be fuels or cardioregulatory substances (Wells and Smith, 1987). The glucose concentration in the blood of freshly collected animals was $9.9\pm2.1\times10^2\,\text{mg}\,\text{ml}^{-1}$ ($N=17$), which is about one-fifth of the concentration used in the present experiments. The mean RQ of 0.86 suggests that the hearts do not have a solely carbohydrate-based metabolism. It has been reported that, in contrast to mammalian and fish hearts, cephalopod hearts rely largely upon amino acids as substrates for their oxidative metabolism (Hoeger and Mommsen, 1985). The absence of amino acids in the perfusion medium could explain the relatively rapid fatigue of the hearts perfused with oxygenated sea water (with added glucose) and the prolonged performance with blood (in which amino acids are present, D'Aniello *et al.* 1986). In addition, the release of end products of anaerobiosis from the myocardium which is receiving insufficient oxygen could explain the short performance of the hearts perfused with oxygenated sea water. The relatively prolonged performance reported for other *Octopus* heart preparations (Smith, 1981; Foti *et al.* 1985) may be explained by the markedly lower power output of these preparations; once the oxygen supply has been improved, the length of performance may be simply a product of the endogenous fuel supply and power output of the hearts.

Coronary flow and resistance

There are two main hypotheses concerning the source of the coronary vessels in the Octopoda. Isgrove (1909) described in *Eledone* a coronary supply through one

artery originating from the right branchial artery, whereas Wells and Wells (1986) mention that in *Octopus* there are two coronary arteries originating from the dorsal aorta. The existence of one coronary artery originating directly from the abdominal aorta is implied in the schema of the *Octopus* circulatory system reported by Wells and Smith (1987). A completely different arrangement is the proposal that the coronary system is a drainage system from the ventricular lumen (Grimpe, 1913; Kling and Schipp, 1987). We have not found any evidence for the existence of coronary arteries in *Octopus vulgaris*, while evidence for direct communication between coronary vessels and ventricular lumen has been reported by Agnisola *et al.* (1990). Moreover, in the isolated heart preparation the ventricle lumen seems to be the only possible source of a coronary supply. Finally, it has been demonstrated in the *in situ* perfused heart of *Octopus vulgaris* that the increases in the coronary vein pressure and the coronary flow in the veins occur during ventricular systole (Agnisola *et al.* 1990). This is the rationale for considering that the mean ventricular pressure is the driving force for coronary flow in the heart *in vitro*.

At the lowest haemocyanin concentrations, the increase in coronary resistance in haemocyanin-perfused hearts is mainly due to the increased viscosity. However, at the highest haemocyanin concentrations and with blood, the 2.4-fold increase in viscosity is not enough to explain the almost threefold increase in coronary resistance. The coronary resistance was significantly affected in hearts perfused with oxygenated sea water, when no change in viscosity occurred. Moreover, a significant increase in coronary flow has been reported when the hearts were perfused with solutions of decreasing P_{O_2} (20–6.67 kPa; Houlihan *et al.* 1987). These results raise the question of the nature of the coronary resistance and the mechanisms for its regulation. As there is no evidence for resistance vessels like arterioles in the coronary bed (Agnisola, 1990), it is possible that the extravascular resistance plays a role in the regulation of the coronary resistance. The extravascular resistance may change as a result of the compressive stresses within the myocardium associated with the development of pressure in the ventricular lumen during contraction (Downey, 1988).

Mechanisms that improve cardiac performance

A scheme of the relationship between oxygen content of the perfusate and aortic output or coronary flow of the isolated systemic heart of *O. vulgaris* is presented in Fig. 7, which could help to explain the link between oxygen supply to the heart and its performance.

The changes in oxygen content of the perfusate affect heart rate, cardiac contractility and coronary resistance. During perfusion with blood or high concentrations of haemocyanin, heart rate (Fig. 1C), heart contractility (Fig. 6) and coronary resistance (Fig. 2C) are all increased. The improvement in heart contractility causes the increase in intraventricular pressure (Fig. 2B) and hence that in stroke volume. The result is a large increase in aortic output. The increase in coronary resistance (about threefold, Fig. 2C), which is also linked to the

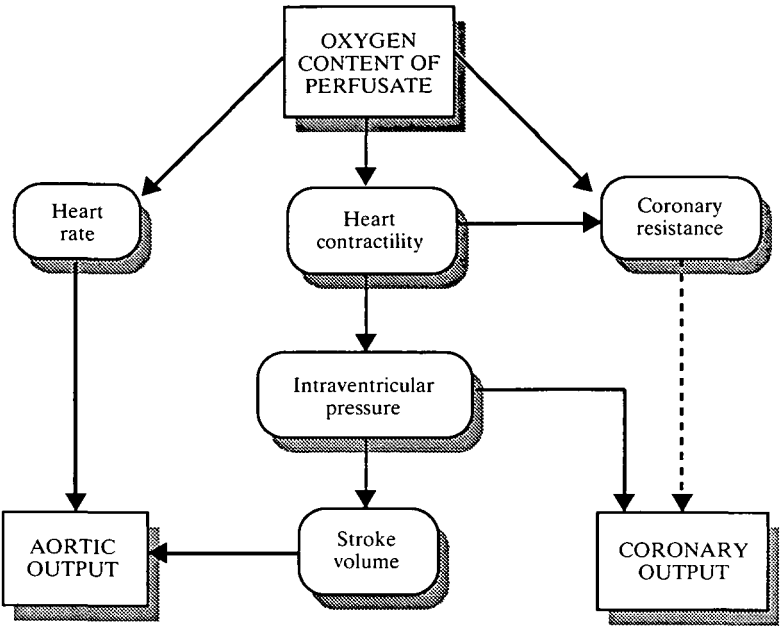

Fig. 7. Schematic diagram showing the relationship between the oxygen content of the perfusate and aortic and coronary outputs of the isolated and perfused systemic heart of *Octopus vulgaris*. —— positive relationship; --- negative relationship.

increased heart contractility, exceeds that of the intraventricular pressure (about 1.5 times, Fig. 2B), so that the coronary flow is reduced. However, blood and high concentrations of haemocyanin do not produce exactly the same effects, possibly because blood contains humoral factors as well as fuels (discussed above) which could affect both heart performance and coronary resistance.

At low concentration of haemocyanin, the heart rate is increased whilst heart contractility and intraventricular pressure are unaffected. Thus, the increase in aortic output is proportional to the increase in heart rate, while the coronary flow is reduced by an increase in coronary resistance (Fig. 2C).

With oxygenated seawater perfusion the heart rate is hardly affected but there is a large increase in heart contractility (Fig. 6). This is reflected in the higher maximum intraventricular pressure (Fig. 2B). Thus, the increase in aortic output is mainly due to an increase in stroke volume. The small increase in coronary resistance is not enough to overcome the increase in maximum intraventricular pressure and this results in a high coronary flow. The higher P_{O_2} in oxygenated sea water seems to be sufficient to stimulate heart contractility but not heart rate. It is possible that in conditions of oxygenated seawater perfusion the oxygen consumption of the inner myocardium is considerably improved (because of the higher P_{O_2} gradient), while the majority of the myocardium that is supplied by the coronary vessels is still relatively poorly provided with oxygen. This may explain the

increase in heart contractility, whereas heart rate could be dependent upon the whole of the myocardium receiving an adequate oxygen supply.

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