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PERFUSION-INDEPENDENT OXYGEN EXTRACTION IN MYOGLOBIN-RICH HEARTS

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SUMMARY

Cardiac myoglobin plays a role in oxygen consumption and has a protective effect during periods of hypoxia, but little is known about the role of myoglobin during periods of ischaemia. Myoglobin-rich sea raven hearts and myoglobin-poor ocean pout hearts were isolated and perfused at varying flow rates and under conditions of low and high oxygen demand to assess the role of myoglobin in oxygen extraction. In the myoglobin-rich hearts, oxygen extraction remained constant over the flow range. In the myoglobin-poor hearts, oxygen extraction was significantly elevated, relative to controls, at the lower flow rates but decreased as the flow rate increased. In hearts where myoglobin was inactivated by an oxidizing agent, oxygen extraction was similar to that observed in myoglobin-poor hearts. Under conditions of high oxygen demand, myoglobin-rich hearts again showed a constant oxygen extraction over the flow range. Myoglobin-inactivated hearts had a significantly elevated oxygen extraction at low flows, and this decreased as flow rate increased. These data suggest that myoglobin renders oxygen extraction by fish hearts independent of the rate of perfusion.

INTRODUCTION

The intracellular protein myoglobin is found in high concentrations in red skeletal muscle, where it binds reversibly with oxygen and facilitates oxygen consumption under conditions of hypoxia and exercise (Cole, 1982, 1983). In skeletal muscle, myoglobin concentrations correlate well with the activities of enzymes involved in aerobic energy metabolism (Holloszy & Booth, 1976). However, the function of myoglobin in cardiac muscle is equivocal. In view of both the biochemical properties of myoglobin and the abundant evidence for a function of myoglobin in skeletal muscle (Gayeski & Honig, 1986; Gonzalez-Fernandez & Atta, 1986), it would be surprising if the protein did not play a role in heart oxygen consumption. Although present in the hearts of all mammals, myoglobin content does not correlate with factors such as size, breath-hold capacity or sustainable levels of activity. As well, factors such as hypoxia or endurance training (Hagler *et al.* 1980; Van Bui &

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Banchero, 1980; Pietschmann & Bartells, 1985) apparently do not affect, or have only a marginal influence upon, cardiac myoglobin content. Myoglobin may play a role in oxygen consumption by the heart during steady-state conditions, but studies designed to test this hypothesis have not demonstrated any functional significance. Both oxygen consumption and mechanical performance were maintained at control levels by perfused dog hearts, despite nitrite treatment which oxidizes the ferrous ion of myoglobin to the non-functional ferric state (Cole, Wittenberg & Caldwell, 1978). In addition, respiration of isolated rat myocytes, even at extracellular P_{O_2} levels approaching 0·5 mmHg (1 mmHg = 133·3 Pa), was unimpaired by treatment with hydrogen peroxide, a myoglobin inhibitor (Jones & Kennedy, 1982). In contrast, Bailey & Driedzic (1986) demonstrated that in fish hearts myoglobin plays a role in oxygen uptake from the extracellular medium under both normoxic and hypoxic conditions.

The fish heart provides a simple model for the study of cardiac myoglobin (Driedzic, 1983). Depending on the species, the myoglobin content ranges from zero to close to mammalian levels, but the interspecific metabolic profiles are remarkably similar despite large differences in myoglobin content (Driedzic & Stewart, 1982; Sidell, Driedzic, Stowe & Johnston, 1987). Flow through the two-chambered (one atrium and one ventricle) fish heart is unidirectional to the ventral aorta and the subsequent site of blood oxygenation, the gills. Generally, the ventricle consists of two layers, an external compact layer, supplied by coronary arteries arising from the oxygenated side of the gill, and a spongy internal layer which makes up the bulk of the ventricle. The spongy layer receives all oxygen and nutrients directly from the blood in the lumen of the ventricle. Both the external compact layer and coronary arteries are poorly developed or non-existent in the majority of slow-moving fishes (Cameron, 1975; Santer & Greer Walker, 1980). Therefore, these hearts must receive all of their oxygen supply from mixed venous (deoxygenated) blood. Concomitant measurements of O_2 consumption and lactate production rates by perfused and isolated sea raven and ocean pout hearts show that 98% of ATP production is accomplished by oxidative means when hearts are perfused with media gassed with 22% O_2 (Driedzic, 1983). As well, the efficiency of these isolated heart preparations (Bailey & Driedzic, 1986) was comparable to values calculated for mammalian hearts (Guyton, 1981) and to *in situ* perfused fish hearts (Farrell, Wood, Hart & Driedzic, 1985). Thus, these hearts are ideal for perfusion experiments.

Myoglobin has been shown to play a role in the maintenance of performance and oxygen consumption of isolated fish hearts under hypoxic conditions (Driedzic, Stewart & Scott, 1982; Bailey & Driedzic, 1986). The previous work illustrates the importance of myoglobin under diffusion-limiting conditions. The objective of the present study was to assess the contribution of myoglobin to oxygen consumption under conditions of variable perfusion and oxygen demand. Hearts from the sea raven and ocean pout, with myoglobin contents of 75 and <5 nmol g^{-1} wet mass, respectively, were used as model systems. Hydroxylamine or sodium nitrite, which both convert myoglobin to a non-functional state, was used in some cases. The data

show that myoglobin plays a role in maintaining oxygen extraction over a wide range of oxygen delivery rates.

MATERIALS AND METHODS

Animals

Adult sea raven *Hemitripterus americanus* (Gmelin), weighing 800–1400 g, and ocean pout *Macrozoarces americanus* (Bloch & Schneider), weighing 1000–1400 g, were captured by otter trawl in Passamaquoddy Bay off St Andrews, New Brunswick and transported to Mount Allison University. Animals were held in filtered, recirculating sea water at 10 °C and fed pieces of fish at regular intervals. Photoperiod was 12 h light: 12 h dark.

Perfusate composition

The perfusate contained 150 mmol l^{-1} NaCl, 2 mmol l^{-1} $MgSO_4$, 5 mmol l^{-1} KCl and 3 mmol l^{-1} $CaCl_2$. Just before use 2·3 mmol l^{-1} Na_2HPO_4, 0·2 mmol l^{-1} NaH_2PO_4 and 2 mmol l^{-1} pyruvate were added. The perfusate was vigorously gassed with 0·5 % CO_2, 22 % O_2, balance N_2 for 30 min and pH was adjusted to 7·8 at 10 °C with approximately 11 mmol l^{-1} $NaHCO_3$. Vigorous gassing with the same mixture was maintained during the experimental period. In some cases either hydroxylamine (1 mmol l^{-1}) or $NaNO_2$ (10 mmol l^{-1}) was added to the perfusate as a myoglobin blocking agent.

Isolated heart preparation

Fish were removed from the holding tank, stunned with a blow to the head and the heart quickly exposed, excised and placed in a beaker of cold perfusion medium. The heart was then perfused in a manner previously described (Driedzic *et al.* 1982) with some modifications. These involved forced-filling of the heart rather than gravity-feeding, the addition of oxygen electrodes to the input and output lines and the addition of a pressure transducer to the atrial cannula to monitor input pressure. A large increase in input pressure and the visual observation of a ballooning atrium indicated that the heart preparation was failing. Hearts were forced to work at low and high afterloads and over a wide flow range. Hearts were perfused at 10 °C, the temperature to which the animals were acclimated.

Hearts were electrically paced at 30 beats min^{-1} for sea raven and 24 beats min^{-1} for ocean pout. Preliminary work had shown that the heart preparations were most efficient at these rates. Threshold voltage for each heart was determined at the start of each experiment. This voltage was then increased threefold, an arbitrarily chosen factor, and the resultant voltage used throughout the experiment. Hearts responded in an all-or-none fashion and voltage increases did not cause an increase in force of contraction.

Instrumentation and apparatus

Input and output P_{O_2} values were measured with a YSI model 16582 oxygen meter equipped with Clark-type oxygen electrodes which were incorporated in the perfusion apparatus. The calibration procedure was as follows. The input and output cannulae were connected with a 3 cm length of polyethylene tubing. N_2-saturated perfusate was run through the apparatus to zero the oxygen meter. To ensure that this perfusate was oxygen-free, a small quantity (approx. $0{\cdot}1\,g\,l^{-1}$) of sodium hydrosulphite was added. The apparatus was then flushed with distilled water and aerated perfusate. The system was subsequently refilled with aerated perfusate which was run through the apparatus at the highest experimental flow rate and the meter set to maximum sensitivity. Since Clark-type electrodes are flow-sensitive, the meter output in response to the aerated perfusate was read at each experimental flow rate and a standard curve constructed for each electrode. These curves remained valid until the electrode membranes were changed, when the calibration procedure was repeated. Electrode membrane condition was checked prior to each experiment by running aerated perfusate through the apparatus at the highest flow rate. If the observed percent oxygen saturation was either unstable or off the standard curve, the electrode membrane was replaced. Hearts were force-filled with a Cole Palmer Masterflex pump system (Model WZIR057) equipped with either a number 16 or number 13 head depending on heart size. In each case the pump was calibrated over a flow range of 5·0–30·0 ml min^{-1}. Pressure measurements were taken with a Biotronix BL630 meter and transducer with the signal displayed on a Biotronix BL882 strip chart recorder.

Experimental protocol

Sea raven hearts were placed at random in one of three experimental groups as follows: Group I, low afterload; group II, low afterload with perfusate containing hydroxylamine; group III, high afterload with sequential treatments with control perfusate, perfusate with nitrite and control perfusate. Ocean pout hearts were similarly randomized into group I and III experiments. Time control studies have shown that these perfused heart preparations remain viable with only a slight non-significant loss of function for at least 120 min under similar conditions (Bailey & Driedzic, 1986).

The experimental protocol used for groups I and II was as follows. The heart was perfused until all the blood had been washed out. It was then allowed to stabilize for 15 min at a constant moderate flow rate of 10–15 ml min^{-1}, depending on heart size. The set afterload was approximately 1–2 cmH_2O (1 cmH_2O = 98·1 Pa). Following the stabilization period, flow was reduced to 5·0 ml min^{-1}, the initial flow of the experimental range. This flow was chosen as the starting point, as it is approximately equivalent to half the resting cardiac output (Farrell, MacLeod, Driedzic & Wood, 1983) for these hearts. Flow rates were increased in increments of 5·0 ml min^{-1} at 5-min intervals until the maximum experimental flow rate of 25·0 ml min^{-1} was reached. This flow was chosen as it is approximately double the resting cardiac

output (Farrell *et al.* 1983). Input and output P_{O_2} and input and output pressures were recorded at the end of each flow period. Hearts were in steady-state conditions as evidenced by pressure traces. In the case of the group II hearts, the myoglobin-oxidizing agent hydroxylamine was added after the 15 min stabilization. The protocol used for the group III hearts was longer and more complex. Following the stabilization period, the heart was subjected to varying flows as described above except that the set afterload was 30 cmH_2O. After the 25 ml min^{-1} challenge, the flow rate was decreased to 10–15 ml min^{-1}, depending on heart size, sodium nitrite was added to the perfusate, and the heart was allowed to equilibrate with the nitrite for 15 min. Following the equilibration period, the heart was then subjected to the varying flow-rate regime. After the 25 ml min^{-1} challenge in the presence of nitrite the flow rate was again reduced to 10–15 ml min^{-1} and the nitrite-rich perfusate was replaced with nitrite-free perfusate. This was allowed to wash out the system for 15 min. After the washout period, the heart was again subjected to the varying flow rate regime.

Myoglobin content of tissue homogenates (Fig. 1A) was assessed with a direct spectrophotometric wavelength scan (Sidell, 1980). A nitrite concentration of 10 mmol l^{-1} was sufficient to oxidize 80–90 % of the cardiac myoglobin to a non-functional state (Fig. 1B). Darkening of the red heart was regarded as evidence that the myoglobin had reacted with the nitrite. Perfusing the heart with nitrite-free perfusate was sufficient to restore the majority of the myoglobin to a functional state (Fig. 1C).

Data analysis

Oxygen extraction was calculated by subtracting output P_{O_2} from input P_{O_2}. Since the size range of the hearts was large (0·8 g minimum to 1·5 g maximum) oxygen extraction is expressed as ml O_2 l^{-1} perfusate g^{-1} heart. Oxygen consumption was the product of oxygen extraction times and flow rate. Peak systolic pressures were calculated as cmH_2O (1 cmH_2O = 98·1 Pa). Mean pressure was calculated from the total area under the curve of the pressure trace. Pressure trace areas were determined with either an Apple graphics tablet and computer or a Houston Instruments HIPAD digitizer and IBM computer. All data are expressed as mean ± S.E.M. Differences between means of different groups or within groups were examined using either the Student's *t*-test or the Mann–Whitney *U*-test. Linear regression analysis was used to determine correlations between parameters. In all cases a P value of less than 0·05 was regarded as significant.

RESULTS

Low afterload conditions

Oxygen extraction (ΔO_2) was independent of perfusate flow rate in the isolated perfused myoglobin-rich sea raven heart (Fig. 2, left). Oxygen consumption showed a positive correlation ($y = 0{\cdot}14+0{\cdot}81x$, $N = 45$, $r = 0{\cdot}41$, $P < 0{\cdot}005$) with flow rate as

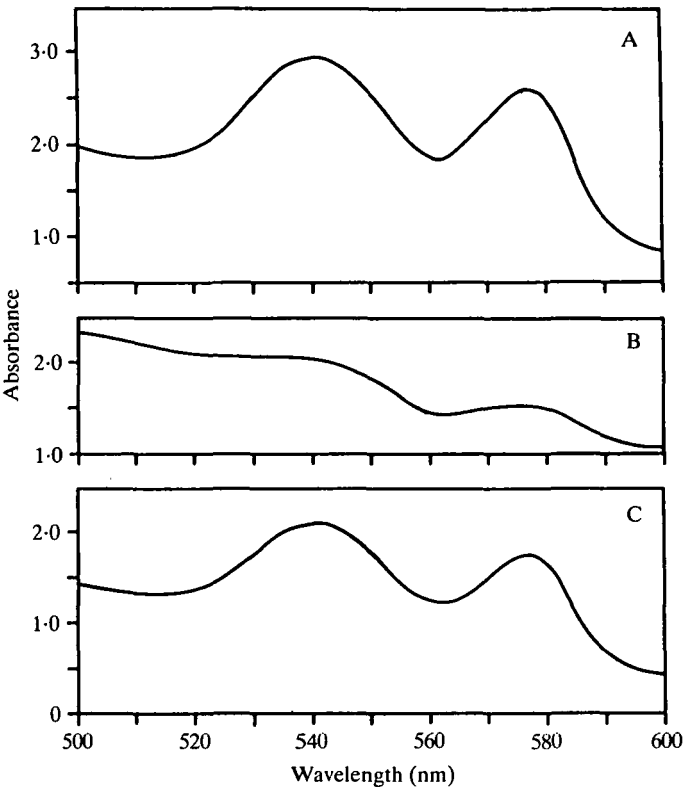

Fig. 1. (A) Absorption spectrum of sea raven heart homogenate. (B) Absorption spectrum of sea raven heart homogenate after the heart had been perfused for 15 min with 10 mmol l^{-1} sodium nitrite. (C) Absorption spectrum of sea raven heart homogenate after 10 mmol l^{-1} nitrite-treated hearts had been perfused for 15 min with nitrite-free perfusate.

did peak systolic pressure ($y = 5{\cdot}32+0{\cdot}62x$, $N = 45$, $r = 0{\cdot}66$, $P < 0{\cdot}005$) and mean pressure ($y = 1{\cdot}87+0{\cdot}22x$, $N = 45$, $r = 0{\cdot}54$, $P < 0{\cdot}005$).

Oxygen extraction by isolated perfused myoglobin-poor ocean pout hearts showed a significant negative correlation ($y = 2{\cdot}82-0{\cdot}09x$, $N = 38$, $r = -0{\cdot}79$, $P < 0{\cdot}005$) with flow rate. Oxygen consumption ($y = 12{\cdot}58+0{\cdot}30x$, $N = 38$, $r = 0{\cdot}35$, $P < 0{\cdot}005$) and mean pressure ($y = 7{\cdot}07+0{\cdot}14x$, $N = 34$, $r = 0{\cdot}59$, $P < 0{\cdot}005$) increased as flow through the heart increased. Peak systolic pressure remained relatively constant under these conditions (Fig. 2, right).

Hydroxylamine treatment of sea raven hearts resulted in a considerable change in ΔO_2 which was much higher than under control conditions at low flow rates and declined ($y = 1{\cdot}89-0{\cdot}07x$, $N = 40$, $r = -0{\cdot}72$, $P < 0{\cdot}005$) as perfusate flow rate increased (Fig. 2, middle). Oxygen consumption remained relatively constant while peak systolic pressure ($y = 5{\cdot}34+1{\cdot}05x$, $N = 40$, $r = 0{\cdot}85$, $P < 0{\cdot}001$) and mean pressure ($y = 3{\cdot}14+0{\cdot}23x$, $N = 40$, $r = 0{\cdot}71$, $P < 0{\cdot}005$) correlated positively with flow rate.

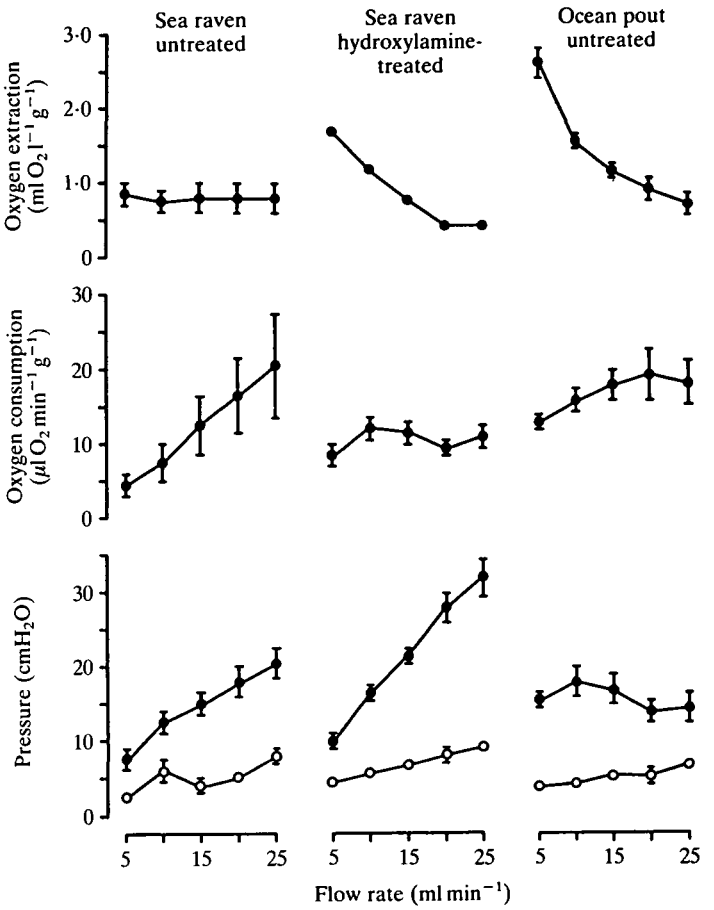

Fig. 2. Oxygen extraction (top), oxygen consumption (middle) and pressure development (bottom) in isolated perfused untreated sea raven hearts ($N = 9$), hydroxylamine-treated sea raven hearts ($N = 8$) and untreated ocean pout hearts ($N = 9$). Hearts were perfused under conditions of low afterload (1–2 cmH_2O) and varying flow rates. In the bottom panel and closed circles represent peak systolic pressure and the open circles represent mean pressure.

When the hydroxylamine-treated hearts are compared with the untreated sea raven hearts, it may be seen that ΔO_2 is significantly ($P < 0{\cdot}05$) higher at the 5 and 10 ml min^{-1} flow rates. There is no difference at the higher flow rates. Peak systolic pressure is significantly ($P < 0{\cdot}05$) higher at all flow rates, and mean pressure at the 5, 15 and 20 ml min^{-1} flow rates ($P < 0{\cdot}05$) is higher in hydroxylamine-treated than in control hearts.

High afterload conditions

Under high (30 cmH_2O) afterload conditions, sea raven hearts displayed a similar ΔO_2 pattern to that found under low (approx. 2 cmH_2O) afterload conditions, in that

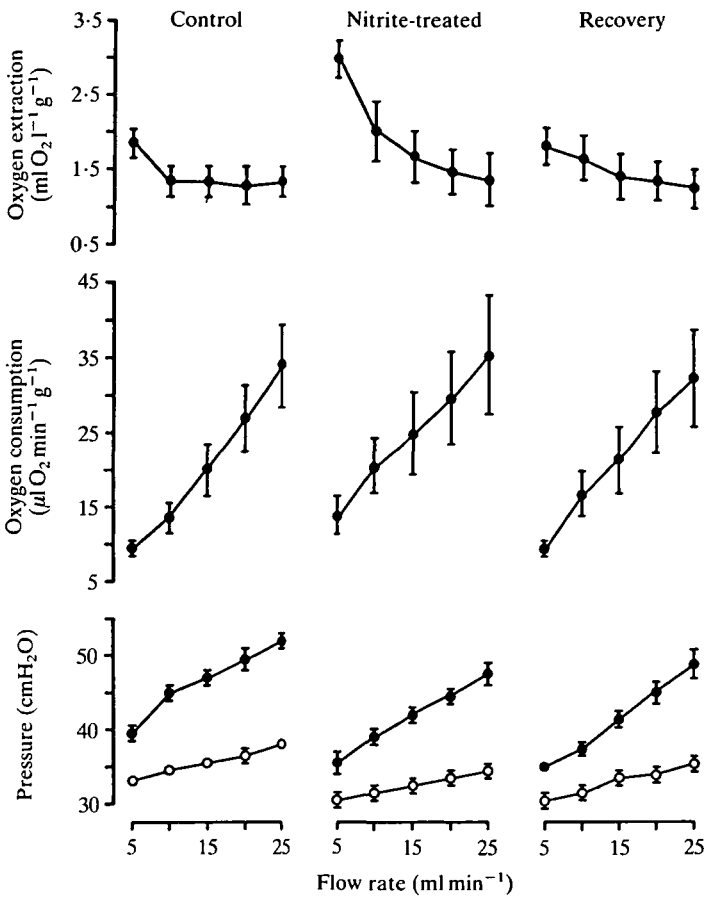

Fig. 3. Oxygen extraction (top), oxygen consumption (middle) and pressure development (bottom) in isolated perfused untreated sea raven hearts, 10 mmol l^{-1} nitrite-treated sea raven hearts and nitrite-treated sea raven hearts washed with nitrite-free perfusate. Hearts were perfused under conditions of high (30 cmH_2O) afterload and varying flow rates. In all cases $N = 10$. In the bottom panel the closed circles represent peak systolic pressure and the open circles represent mean pressure.

ΔO_2 stayed constant over the 10–25 ml min^{-1} flow range (Fig. 3). At the lowest flow rate ΔO_2 was significantly ($P < 0{\cdot}05$) elevated relative to the higher flow rates. Oxygen consumption ($y = 3{\cdot}20 + 1{\cdot}15x$, $N = 45$, $r = 0{\cdot}48$, $P < 0{\cdot}05$), peak systolic pressure ($y = 37{\cdot}89 + 0{\cdot}58x$, $N = 45$, $r = 0{\cdot}69$, $P < 0{\cdot}005$) and mean pressure ($y = 31{\cdot}29 + 0{\cdot}27x$, $N = 45$, $r = 0{\cdot}52$, $P < 0{\cdot}005$) increased as flow rate increased. Following treatment with 10 mmol l^{-1} $NaNO_2$ sea raven hearts showed a significant negative correlation ($y = 2{\cdot}90 - 0{\cdot}067x$, $N = 45$, $r = -0{\cdot}40$, $P < 0{\cdot}005$) between ΔO_2 and perfusate flow rate. Oxygen consumption ($y = 9{\cdot}68 + 1{\cdot}03x$, $N = 45$, $r = 0{\cdot}42$, $P < 0{\cdot}005$), peak systolic pressure ($y = 32{\cdot}84 + 0{\cdot}59x$, $N = 45$, $r = 0{\cdot}78$, $P < 0{\cdot}005$) and mean pressure ($y = 28{\cdot}90 + 0{\cdot}26x$, $N = 45$, $r = 0{\cdot}61$, $P < 0{\cdot}005$) increased as flow

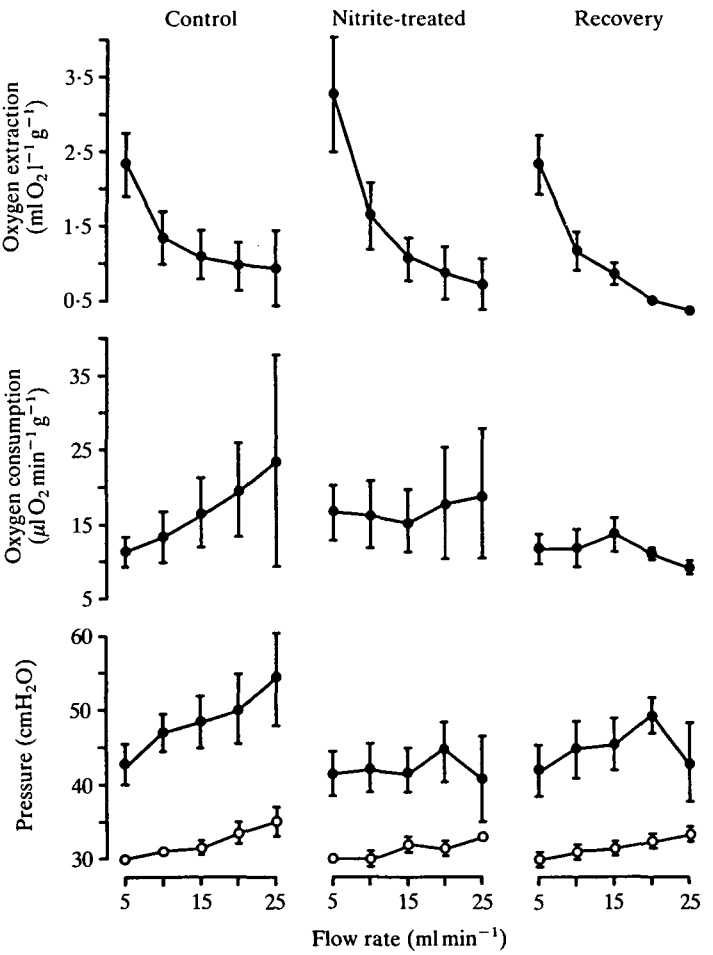

Fig. 4. Oxygen extraction (top), oxygen consumption (middle) and pressure development (bottom) in isolated perfused untreated ocean pout hearts, 10 mmol l^{-1} nitrite-treated ocean pout hearts and nitrite-treated ocean pout hearts washed with nitrite-free perfusate. Hearts were perfused under conditions of high afterload (30 cmH_2O) and varying flow rates. In the bottom panel the closed circles represent peak systolic pressure and the open circles represent mean pressure. In all cases $N = 10$.

rate increased. After the nitrite-treated hearts had been washed with nitrite-free perfusate, no correlation was found between ΔO_2 and flow rate. Oxygen consumption ($y = 4{\cdot}35 + 1{\cdot}15x$, $N = 40$, $r = 0{\cdot}56$, $P < 0{\cdot}005$), peak systolic pressure ($y = 35{\cdot}38 + 0{\cdot}38x$, $N = 40$, $r = 0{\cdot}42$, $P < 0{\cdot}005$) and mean pressure ($y = 29{\cdot}14 + 0{\cdot}26x$, $N = 40$, $r = 0{\cdot}63$, $P < 0{\cdot}005$) all showed a significant positive correlation with perfusate flow rates.

Under the initial control conditions, ocean pout hearts showed a significant negative correlation ($y = 1{\cdot}97 - 0{\cdot}045x$, $N = 33$, $r = -0{\cdot}33$, $P < 0{\cdot}05$) between ΔO_2 and flow rate (Fig. 4). Peak systolic pressure ($y = 40{\cdot}72 + 0{\cdot}53x$, $N = 33$, $r = 0{\cdot}36$,

$P<0{\cdot}025$), mean pressure ($y = 27{\cdot}92+0{\cdot}30x$, $N = 33$, $r = 0{\cdot}67$, $P<0{\cdot}005$) and oxygen consumption ($y = 7{\cdot}95+0{\cdot}60x$, $N = 33$, $r = 0{\cdot}44$, $P<0{\cdot}01$) showed a significant positive correlation with flow rate. The nitrite-treated ocean pout hearts also showed a significant negative correlation ($y = 3{\cdot}39-0{\cdot}13x$, $N = 31$, $r = -0{\cdot}58$, $P<0{\cdot}005$) between ΔO_2 and perfusate flow rate. Mean pressure showed a positive correlation with flow rate ($y = 27{\cdot}50+0{\cdot}23x$, $N = 31$, $r = 0{\cdot}62$, $P<0{\cdot}005$). Unlike in the treated sea raven hearts, oxygen consumption and peak systolic pressure remained relatively constant across the experimental flow range. Following nitrite washout, the ocean pout hearts still displayed a significant negative ($y = 1{\cdot}85-0{\cdot}06x$, $N = 22$, $r = -0{\cdot}50$, $P<0{\cdot}01$) correlation between ΔO_2 and perfusate flow rate. Oxygen consumption and peak systolic pressure remained relatively constant across the flow range but mean pressure showed a significant ($y = 28{\cdot}62+0{\cdot}19x$, $N = 22$, $r = 0{\cdot}70$, $P<0{\cdot}005$) correlation with flow rate.

When sea raven hearts were washed with the nitrite-free perfusate, oxygen extraction decreased at the low flow rates, relative to the nitrite-treated condition and approached that observed during the initial (control) perfusion period. Oxygen consumption returned to the control level. Peak systolic pressure and mean pressure were generally lower under nitrite treatment and did not recover following nitrite washout. The nitrite-treated hearts showed a significantly higher ($P<0{\cdot}05$) ΔO_2 at both the $5\,ml\,min^{-1}$ ($2{\cdot}79\pm0{\cdot}47$ *vs* $1{\cdot}92\pm0{\cdot}21\,ml\,l^{-1}\,g^{-1}$) and the $10\,ml\,min^{-1}$ ($2{\cdot}08\pm0{\cdot}39$ *vs* $1{\cdot}33\pm0{\cdot}23\,ml\,l^{-1}\,g^{-1}$) flow rates compared with the initial untreated hearts. Consequently, oxygen consumption was also significantly ($P<0{\cdot}05$) higher at these flow rates in the treated hearts. At the higher flow rates there was no significant difference in ΔO_2 and oxygen consumption between treated and untreated hearts. Peak systolic pressure and mean pressure were significantly lower ($P<0{\cdot}05$) in the nitrite-treated sea raven hearts than in the untreated hearts at all flow rates.

Treatment of ocean pout hearts with nitrite did not have any apparent effect. There was no significant difference in ΔO_2, oxygen consumption or pressure development between treated hearts and untreated hearts. When the hearts were perfused with nitrite-free perfusate no significant change in any of the examined parameters was noted.

DISCUSSION

The model system

The use of the isolated perfused fish heart offers certain advantages in the study of the function of cardiac myoglobin. The lack of coronary arteries coupled with the ability of myoglobin-rich hearts to withstand prolonged periods of hypoxia (Driedzic, 1983) means that these hearts are ideal for studies which involve wide ranges of oxygen tensions. Also, these hearts depend on the luminal blood for both oxygen and nutrient supply (Randall, 1970), which makes them very useful for studies involving

perfusion-limited oxygen transport. In addition, the lack of myoglobin in some species provides a good control for the model system.

Low afterload conditions

Under the low afterload conditions, oxygen extraction by the untreated myoglobin-rich sea raven hearts remains relatively constant over a wide range of flows. Oxygen consumption necessarily showed a significant positive correlation with flow rate. Both peak systolic and mean pressure showed a positive correlation with flow rate, indicating a direct matching of oxygen supply to mechanical performance under these conditions. The increased pressure was a Starling's law phenomenon, i.e. increasing perfusate flow rate resulted in an increase in atrial and ventricular stretch with a consequent increase in force of contraction.

The situation with hydroxylamine-treated sea raven hearts is somewhat more complex. In these preparations, oxygen consumption did not correlate with flow rate owing to the elevated oxygen extraction at low flow rates and possibly to an impairment of maximal oxygen consumption by hydroxylamine, as this agent may alter the redox state of cytochromes as well as myoglobin. As in the control group, both peak systolic and mean pressure increased in response to an increase in filling pressure; however, the Starling response was accentuated. Sensitivity to filling pressure of perfused sea raven hearts depends on the thermal history of the animal (Graham & Farrell, 1985). Although this does not explain the present observations, it does indicate that the Starling response is subject to control. It is possible that hydroxylamine is altering the set point of this mechanism. In these preparations there is a mismatching of oxygen consumption and mechanical performance, such that the hearts appear to be more efficient at the high flow rates. Presumably, the tissue is making up the energy shortfall through anaerobic metabolism. The single most important and unequivocal finding is that at the lowest flow rate through perfused sea raven hearts, the inclusion of hydroxylamine in the medium results in an enhancement of oxygen extraction at levels of mechanical performance similar to that of control hearts.

Oxygen extraction by ocean pout hearts was negatively correlated with flow rate, whereas oxygen consumption increased with increases in flow. Peak systolic pressure remained relatively stable. The reason for the mismatch between peak systolic pressure development and oxygen consumption in these preparations is unclear. Estimates of pressure measured in the ventral aortic cannula may be underestimates of the overall value because of the tendency of the ventricle to pump perfusate retrogradely. This does not occur in sea raven hearts. Retrograde pressure development, however, would not influence oxygen consumption measurements, as the pump sets the rate of fluid flow through the tissue. This interpretation is supported by the experiments conducted at the high afterload.

The most significant feature of the experiments conducted in the low afterload conditions is the relationship between oxygen extraction and flow; in ocean pout hearts there is a negative correlation between these two parameters, whereas in untreated sea raven hearts these variables are independent. Treatment of sea raven

hearts with hydroxylamine, which chemically ablates myoglobin function, alters the relationship between oxygen extraction and flow such that the correlation becomes similar to that observed in myoglobin-poor ocean pout hearts.

High-afterload conditions

There are a number of features of the first set of experiments which, when viewed independently, limit the interpretation. These include the low afterload, which sets end diastolic volume to a subphysiological level, the oxidation of myoglobin with hydroxylamine, which may induce secondary effects, and comparisons between two populations of sea raven. In the second set of experiments the afterload, and hence end diastolic pressure, was set to the physiological level of 30 cmH_2O, nitrite was utilized as the myoglobin-blocking agent allowing comparisons to be made within the same animals since the effects of nitrite are reversible and, finally, both sea raven and ocean pout hearts were exposed to nitrite to assess secondary effects of this agent.

Similar, that is relatively constant, patterns of oxygen extraction are observed when control sea raven hearts are subjected to high afterload and low afterload conditions. Untreated sea raven hearts have a much higher oxygen extraction at 30 cmH_2O afterload than under low afterload conditions. The increased afterload means an increase in work load and thus an elevated oxygen demand resulting in a greater oxygen extraction. But even with an increased work load and oxygen demand, oxygen extraction by myoglobin-rich sea raven hearts remains independent of flow. When myoglobin is oxidized into an inactive state, by nitrite, the pattern of oxygen use by sea raven heart changes. Under high afterload conditions, there is increased oxygen extraction at low flow rates in the treated hearts, but at the moderate and high flow rates there is no difference between the treated and untreated hearts. In the 30 cmH_2O afterload condition, pressure developed by untreated sea raven hearts is significantly higher than that developed by nitrite-treated sea raven hearts. Oxygen consumption is higher at the lower flow rates in treated sea raven hearts and the same at the moderate and high flow rates in both treated and untreated hearts. If nitrite-treated sea raven hearts are allowed to recover, by perfusion with nitrite-free perfusate, oxygen extraction returns to the untreated level at all flow rates. Two critical features emerge from these data. First, when myoglobin is in the reduced form, oxygen extraction by the heart is relatively independent of the rate of perfusion. Second, at low perfusion rates and comparable levels of mechanical performance, oxygen extraction is higher in hearts which have had myoglobin oxidized by nitrite than under either the initial control conditions or following nitrite washout.

In 30 cmH_2O afterload conditions, oxygen extraction by ocean pout hearts is negatively correlated with perfusate flow rate under the initial control conditions, nitrite treatment, and following nitrite washout. The critical finding is that inclusion of nitrite in the perfusion medium does not alter the oxygen extraction pattern in any substantial fashion in these myoglobin-poor hearts. In these experiments, oxygen

consumption patterns generally tracked peak systolic pressure development. However, oxygen consumption by ocean pout hearts performing against a 30 cmH_2O afterload was similar to that observed under the low afterload conditions. We believe the reason for this is an inability accurately to measure the overall component of pressure work by perfused ocean pout hearts, especially at low afterload conditions. Nevertheless, this potential limitation in the data set does not obfuscate the very clear patterns in oxygen extraction.

General considerations

Since diffusion depends on residence time, it is expected that decreasing residence time by increasing perfusate flow rate would result in a decrease in oxygen diffusion (extraction). This was observed in the ocean pout hearts and myoglobin-inhibited sea raven hearts but not in control sea raven hearts. A possible explanation is that myoglobin is affecting oxygen transport in such a fashion as to render oxygen extraction in myoglobin-rich hearts independent of flow through the heart, at least at elevated flows. The unanticipated finding is that in the absence of myoglobin the oxygen extraction *vs* flow relationship is shifted upwards. Myoglobin is not important in oxygen extraction at high flows, as there is no difference between untreated myoglobin-rich hearts and hearts with inactivated myoglobin. But, at low flow rates myoglobin may be critical in decreasing the energetic cost of maintaining the heart. Although evidence for a myoglobin-mediated intracellular gradient is equivocal (Wittenberg & Wittenberg, 1985; Kennedy & Jones, 1986; Taylor, Matthews & Radda, 1986), myoglobin may have a significant role in facilitating diffusion of oxygen across the cell membrane (Bailey & Driedzic, 1986). It is possible that the presence of myoglobin may allow greater diffusion distances from the extracellular space to mitochondria, so that at low flow rates in myoglobin-rich hearts less surface area is required for oxygen uptake. In myoglobin-poor hearts it may be necessary for an internal shift in the morphometry of the spongy layer to occur at low flow rates (i.e. low ventricle stretch) to maximize surface area for oxygen diffusion. Such a shift would require energy, thus further increasing oxygen demand. Myoglobin could therefore render the myoglobin-rich hearts less dependent on surface area and thus decrease the tissue oxygen requirements. This is largely speculative and there is no evidence for such a shift in internal morphology. In conclusion, myoglobin apparently reduces oxygen consumption under conditions of low flow, although when flow through the heart is high, myoglobin is apparently less essential.

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