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MYOCARDIAL OXYGEN CONSUMPTION AND LACTATE RELEASE BY THE HYPOXIC HAGFISH HEART

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Summary

Myocardial oxygen consumption ($\dot{M}_{O_2}$) and lactic acid release were measured in the isolated heart of a hagfish (*Eptatretus cirrhatus* Forster) perfused *in vitro*. Two different ranges of partial pressures of oxygen were employed (P_{IO_2} 3.87–5.87 and 1.60–2.67 kPa). All hearts released lactate into the perfusate, but the rate of release was greater and $\dot{M}_{O_2}$ was depressed at the lower P_{IO_2}.

When energy production through the glycolytic pathway to lactate is converted to oxygen equivalents and added to measured oxygen consumption rates, over a wide range of power outputs and different values of P_{O_2}, the data can be fitted to a single linear regression line. The rate of oxygen consumption of the hagfish myocardium, so obtained, is similar to values reported for teleost fish. The unusual ability of the hagfish myocardium to support perhaps up to 50% of its maximal power output through anaerobic metabolism is related to its extremely low cardiac energy demand.

Introduction

The hagfish is at one end of a spectrum of vertebrate cardiac energy demands (Driedzic *et al.* 1987). The heart operates with the lowest afterload of any vertebrate animal (Davie *et al.* 1987; Forster *et al.* 1988; Forster, 1989). There is no coronary blood supply. Even at maximal cardiac output, the mean circulation time must exceed 5 min, as the circulating blood volume is high (Forster *et al.* 1989). Following exercise, oxygen tensions in venous blood are low (P_{VO_2} 0.47±0.2 kPa, Wells *et al.* 1986) and rise only slowly on recovery. This information suggests that after periods of swimming the hagfish myocardium must be capable of extracting oxygen at extremely low partial pressures or of functioning anaerobically or a combination of the two. By contrast, experimental evidence indicates that the hearts of teleost fish function aerobically (Driedzic, 1983; Driedzic *et al.* 1983; Farrell *et al.* 1985).

The anoxic tolerance of the heart of the Atlantic hagfish *Myxine glutinosa* has been demonstrated *in vitro* (Rybak and Boivinet, 1959), together with its dependence upon glycolysis for the supply of energy (Rybak, 1959; Hansen and Sidell, 1983). Recently it has been shown that *M. glutinosa* can maintain cardiac

Key words: hagfish, heart, hypoxia, lactate, oxygen consumption, *Eptatretus cirrhatus*.

output *in vivo* even during severe environmental hypoxia (Axelsson *et al.* 1990). This paper provides the first measurements of myocardial oxygen consumption in a hagfish *Eptatretus cirrhatus* at known work rates, and quantifies the substantial contribution of anaerobic metabolism to cardiac pumping under hypoxic conditions.

Materials and methods

Experiments were performed on the isolated hearts of 14 hagfish *Eptatretus cirrhatus*, whose body mass ranged from 670 to 1332 g. The atrium and paired ventral aortas were cannulated, and the heart was enclosed in a Perspex box. The heart contracted spontaneously. The physiological solution used to fill the atrium had the following solute concentrations, expressed as mmol kg^{-1} H_2O: NaCl, 489; KCl, 8; $CaCl_2.2H_2O$, 5; $MgSO_4.7H_2O$, 3; $MgCl_2.6H_2O$, 9; NaH_2PO_4, 2.5; $NaHCO_3$, 31; glucose, 5.6. The same solution was drawn through the box using a peristaltic pump (Pharmacia, P1) to perfuse the epicardial surface.

The methods for determining cardiac output, for varying input pressure and afterload and for calculating power output have been described in Forster (1989). In the setting-up period preload was manipulated for each preparation to produce optimal flows. As the heart was enclosed in the fluid-filled box, a higher filling pressure had to be employed to distend the atrium than in previous work (Forster, 1989), though the transmural gradient was similar.

P_{O_2} of the physiological solution was adjusted within two ranges: P_{IO_2} 3.87–5.87 kPa (29–44 mmHg) and P_{IO_2} 1.60–2.67 kPa (12–20 mmHg). The composition of the gas mixture was controlled with a Wösthoff pump, and a short exchange column. P_{IO_2} varied somewhat with flow through the exchange column, as this was set by cardiac output. P_{CO_2} was 0.27±0.03 kPa and pH was 8.03±0.03. Experiments were carried out at 18 °C. The heart was perfused for periods of 30 min or more under a given set of conditions to achieve a steady state. First, myocardial oxygen consumption ($\dot{M}_{O_2}$) was measured at the higher P_{IO_2}. Starting with a moderate afterload, followed by high afterloads, produced initially high and then low cardiac outputs. The P_{IO_2} was then reduced to the lower value and after measuring $\dot{M}_{O_2}$ at low flow rates, the afterload was reduced to permit a smaller number of measurements at higher cardiac outputs. Thus, measurements of $\dot{M}_{O_2}$ and lactate production were made over a 2- to 3-h period. Hearts could maintain performance for at least 3 h, though prolonged exposure (>30 min) to low P_{O_2} could reduce cardiac output.

Oxygen consumption was calculated from the partial pressure difference of incoming and outgoing perfusate, at the measured flow rates. The oxygen capacitance was assumed to be identical to that of sea water at the same temperature. Both endocardial and epicardial uptake were measured, since the latter route has been shown to be a significant path for gas exchange in the mammalian heart *in vitro* (Loiselle, 1987). The 'plumbing' allowed the three thermostatted oxygen electrodes in the lines (IL 1302 electrodes and Strathkelvin

meters) to be perfused periodically with the same incoming solution to allow correction for drift, which was generally minimal.

As ventricular diastolic pressure is close to zero, the power output of the heart is expressed per gram wet mass of the ventricle (Farrell *et al.* 1985; Forster, 1989). However, the rate of oxygen consumption is expressed per gram of the combined wet masses of the ventricle and atrium, since both chambers are performing work. In *Eptatretus cirrhatus* the atrium is approximately one-third of the mass of the ventricle. Rybak and Boivinet (1959) reported that the atrium of *M. glutinosa* had a higher mass-specific oxygen consumption than the ventricle. The relatively small mass of the ventral aorta attached to the ventricle is ignored in the calculation of mass-specific $\dot{M}_{O_2}$, since it is not contractile.

We can assume that the complete oxidation of 1 mol of glucose by 6 mol of O_2 generates 36 mol of ATP, compared to 2 mol of ATP for each pair of lactate ions produced by fermentation (Driedzic, 1983; Storey, 1985). If all this lactate is exported, then it is possible to equate lactate production (anaerobic glycolysis) with oxygen usage (aerobic glycolysis) in terms of ATP supply. 6 μmol of lactate equate to 1 μmol of oxygen, or 1 μmol of lactate to 3.73 μl of O_2 (at STP). Lactate concentrations were measured in samples of perfusate passing both endo- and epicardial surfaces. Lactate, glucose and glycogen concentrations were measured in ventricular tissue at the conclusion of the experiment. The tissue was freeze-clamped in liquid nitrogen, then stored at −80°C to be later homogenised in 6% perchloric acid. As a control, ventricular tissue was also analysed from five animals that had been killed by rapid overanaesthesia in a benzocaine solution. Boehringer lactate and glucose kits and amyloglucosidase were used in the analyses. Results are given as means±the standard error of the mean.

Results

All hearts were able to maintain flow, even at low oxygen tensions. On average, oxygen uptake through the epicardial surface accounted for only about 8% of total oxygen uptake. When endocardial flow was low, the proportion of epicardial $\dot{M}_{O_2}$ relative to total $\dot{M}_{O_2}$ was increased, reaching 38.5% in one preparation. Proportionately less oxygen was taken up *via* the epicardial surface when the high P_{O_2} gas mixture was employed and at power outputs exceeding 0.2 mW. Lactate loss *via* the epicardial surface was too low to be detected.

For any given work rate, $\dot{M}_{O_2}$ was greater in hearts perfused at higher P_{O_2} than at lower tensions (Table 1). This suggests that O_2 supply was limiting at the lower P_{O_2} and that anaerobic metabolism was relatively more important in supporting cardiac work. This was confirmed by the measurements of lactate production in the two groups. All hearts released lactate, but the rate of release was higher in the low P_{IO_2} group (Table 1), despite the mean power output of the low P_{IO_2} group being only 78% of that of the high P_{IO_2} group. Mean power output was lower because fewer hearts were perfused at optimal afterloads in this group. Four

Table 1. *Equations for the regression lines for myocardial oxygen consumption and rates of lactate release in hypoxic hagfish hearts*

Equations for the regression lines:

High P_{O_2}, $\dot{M}_{O_2} = 3.39 + 44.3P\ \mu mol\ g^{-1}\ h^{-1}$ ($N = 25$, $r^2 = 0.891$)

Low P_{O_2}, $\dot{M}_{O_2} = 1.39 + 20.5P\ \mu mol\ g^{-1}\ h^{-1}$ ($N = 23$, $r^2 = 0.560$)

P is the power output in $mW\ g^{-1}$ ventricle wet mass.

Mean lactate release rate:

High P_{O_2}, $41.60 \pm 5.32\ \mu mol\ g^{-1}\ h^{-1}$ ($N = 25$)

Low P_{O_2}, $67.93 \pm 8.15\ \mu mol\ g^{-1}\ h^{-1}$ ($N = 23$)*

* Significantly different from the high P_{IO_2} group ($P<0.05$).

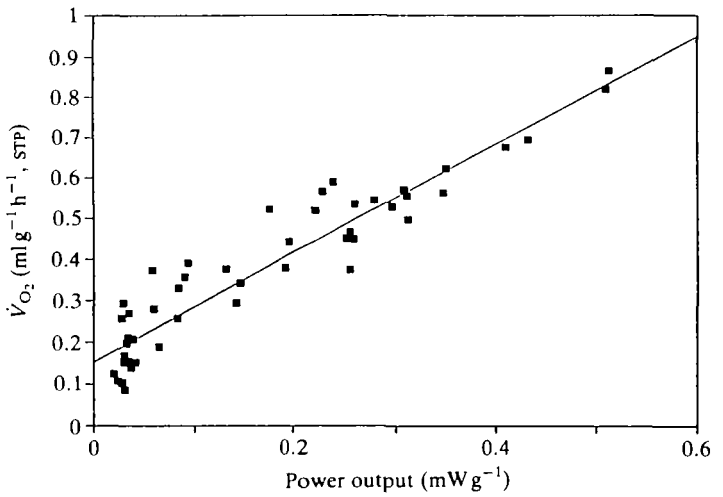

Fig. 1. Aerobic cost of power generation by the heart of *Eptatretus cirrhatus*, after converting lactate production to oxygen equivalents. The regression line is given by the equation: $\dot{V}_{O_2}=7.06+60.7P\ \mu mol\ g^{-1}\ h^{-1}$ ($N=48$, $r^2=0.884$), where $\dot{V}_{O_2}$ is expressed as O_2 equivalents and P is power in $mW\ g^{-1}$.

hearts perfused at a high P_{O_2} (>10.67 kPa) showed negligible lactate production, indicating that respiration was aerobic.

When energy usage is expressed in oxygen equivalents, by the method described above, and is then plotted against power output, a good linear relationship is found (Fig. 1). Oxygen extraction efficiency was greater at low flow rates (Fig. 2). When both the low and high cardiac output groups at the higher P_{O_2} are compared with the low P_{O_2} group, it is apparent that it is the lower oxygen tension that causes the switch to a greater reliance on anaerobic metabolism (Table 2). After exposure to the lower P_{IO_2}, the lactate concentration of the ventricular tissue was higher and the glycogen concentration lower than in hearts that had recently been perfused with blood *in vivo* (Table 3).

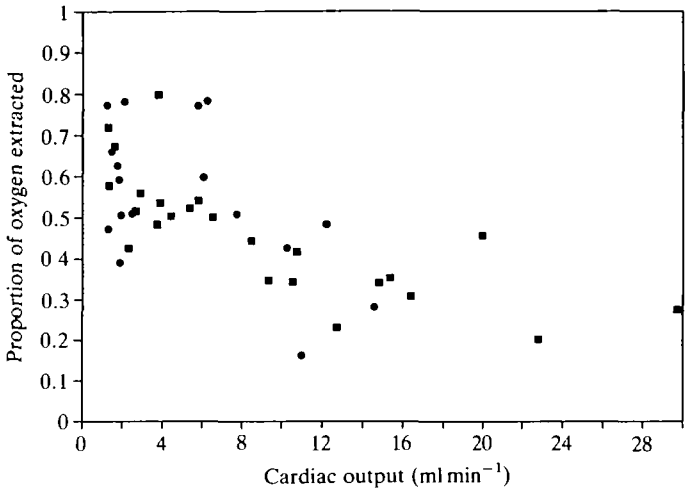

Fig. 2. Proportion of O_2 extracted from perfusate *via* the endocardial surface at different cardiac outputs. (■) P_{IO_2} 3.87–5.87 kPa, (●) P_{IO_2} 1.60–2.67 kPa.

Table 2. *The means and standard errors for the proportion of cardiac work performed aerobically under three different sets of conditions*

	Cardiac output (ml min^{-1})	Proportion of cardiac work performed aerobically
High P_{O_2}	13.11±1.96	0.657±0.043 (N=14)
High P_{O_2}	2.40±0.32	0.573±0.052 (N=11)
Low P_{O_2}	5.08±0.89	0.295±0.038 (N=23)

Mean for the low P_{O_2} group significantly different from the other two groups (P<0.01).

Discussion

In this preparation power output was low in some of the hearts, a probable consequence of the presence of an artificial 'pericardium', which restricted diastolic filling. However, the range of power outputs spanned those previously reported (Forster, 1989) and is comparable with those described for *M. glutinosa in vivo* (Axelsson *et al.* 1990). The experiments confirmed that the epicardial surface can be a route of oxygen uptake *in vitro*, particularly in hypoperfused hearts. *In vivo* it is unlikely that the route has such a significance.

It is clear that the well-developed anaerobic capability of the hagfish heart allows it to pump continuously at normal flows and pressures under conditions of low oxygen availability. Studies of the myocardial lactate dehydrogenase of *E. cirrhatus* (Baldwin *et al.* 1989) indicate that the specific activity of the enzyme would allow lactate to be generated at about four times the rate of its appearance in the perfusate. It may well be that it is the rate of clearance of lactate and/or protons from cardiac muscle that ultimately limits energy production through

Table 3. *Metabolite concentrations in the hagfish ventricle*

	Lactate	Glucose	Glycogen
Untreated	3.77±0.49 (5)	1.82±0.39 (5)	60.0±10.2 (5)
Hypoxia	15.22±1.20 (9)*	2.61±0.47 (5)	11.3±3.0 (5)†

Significantly different from control value: * $P<0.001$, † $P<0.05$.
Untreated tissue is compared with that exposed to hypoxia.
All concentrations are given as $\mu mol\,g^{-1}$ wet mass.
Glycogen is expressed as glucosyl equivalents.
Number of samples in group in parentheses.

anaerobic glycolysis. From this perspective, the role of cardiac perfusion is to supply glucose as substrate and remove lactic acid, rather than to function in gas exchange.

Two factors will reduce the reliability of the calculated contribution of anaerobic metabolism to total energy expenditure. Retention of lactate within the heart, though but a small quantity in relation to that released by the tissue, will lead to the oxygen equivalents being underestimated. Also, where endogenous glycogen is converted to lactate rather than exogenous glucose to lactate, then only a single ATP molecule is lost for each pair of lactate anions produced and the ratio for energy production will be 4 mol of lactate produced to 1 mol of O_2 consumed. Hansen and Sidell (1983) reported a $21\,\mu mol\,g^{-1}$ decrease in glycogen concentration in hypoxic *M. glutinosa* hearts. The decrease recorded here is more than twice this. These two sources of potential error in the calculation will partially offset each other, but it is likely that ATP generation by anaerobic metabolism will be underestimated. The concentration of metabolites in the ventricles at the end of the 3 h period of perfusion indicates that the fall in the molar concentration of glycogen (in glucosyl units) was fourfold greater than the rise in lactate.

The peak rate of lactate release in these experiments was $147.7\,\mu mol\,g^{-1}\,h^{-1}$ at a power output of $0.308\,mW\,g^{-1}$. If anaerobic glycolysis was the only energy source and supplies of glucose were not limiting, this rate of production theoretically could support a work rate of $0.281\,mW\,g^{-1}$, which is 52% of the peak power output recorded in isolated hearts (Forster, 1989). From their *in vivo* studies Axelsson *et al.* (1990) also deduce that resting cardiac output might be maintained anaerobically. However, the maximum recorded rate of lactate release measured at the lower $P_{I_{O_2}}$ may not be sustainable for more than relatively brief periods. Further, the heart *in vitro* was supplied with a saline that contained no lactate anions, but blood lactate can rise to $4.5\,mmol\,l^{-1}$ following exhausting exercise (Davison *et al.* 1990). A smaller concentration gradient between the myocytes and the blood would be expected to reduce the rate of lactate clearance.

Intuitively, one might expect that when functioning aerobically the hearts of all vertebrates would consume oxygen at a similar rate per unit work performed, once the cost of non-contractile, 'maintenance' metabolism is subtracted. These data indicate that a hagfish heart operating aerobically at a peak power output of

$0.536\,mW\,g^{-1}$ (Forster, 1989) consumes $36.2\,\mu mol\,O_2\,g^{-1}\,h^{-1}$. This is $67.8\,\mu mol\,O_2\,g^{-1}\,h^{-1}\,mW^{-1}$, 90% of which is the cost of performing mechanical work. Amongst the teleost fishes, in which a power output of $1\,mW\,g^{-1}$ would be submaximal, similar $\dot{M}_{O_2}$ values are reported for the sea raven heart ($64.3\,\mu mol\,O_2\,g^{-1}\,h^{-1}\,mW^{-1}$, Farrell *et al.* 1985) and the rainbow trout heart ($78.8\,\mu mol\,g^{-1}\,h^{-1}\,mW^{-1}$, Farrrell and Milligan, 1986, and $58.1\,\mu mol\,g^{-1}\,h^{-1}\,mW^{-1}$, Houlihan *et al.* 1988). It is possible that work on lower vertebrates, in particular, has ignored the potential contributions of anaerobic glycolysis and/or O_2 uptake through the epicardial surface. The relatively greater rate of energy production in the mammalian myocardium is assumed to be supported almost exclusively by oxidative metabolism (Suga, 1979). However, lactate can be discharged by the heart of the neonatal mammal in ischaemia and under certain other circumstances (Opie, 1980). It is the low power output of the hagfish heart that permits its sustained dependence upon anaerobic metabolism.

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