EFFECTS OF HYPOXIA AND DRUGS ON THE CARDIOVASCULAR DYNAMICS OF THE ATLANTIC HAGFISH *MYXINE GLUTINOSA*

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Summary

Cardiac output, ventral and dorsal aortic blood pressure and heart rate were recorded simultaneously in unanaesthetized hagfish, *Myxine glutinosa* L. Mean cardiac output was $8.7 \, \text{ml} \, \text{min}^{-1} \, \text{kg}^{-1}$ and mean heart rate $22.3 \, \text{beats} \, \text{min}^{-1}$. The absence of beat-to-beat oscillations in heart rate was consistent with the lack of an extrinsic cardiac innervation in hagfish. Mean blood pressures were 1.04 kPa (ventral aorta) and 0.77 kPa (dorsal aorta).

Injection of adrenaline into the caudal vein significantly increased cardiac output, stroke volume, heart rate and blood pressures of both aortas. Peak cardiac output was close to $25 \, \text{ml} \, \text{min}^{-1} \, \text{kg}^{-1}$. Injection of the β-adrenoreceptor antagonist sotalol decreased resting heart rate significantly. We conclude that endogenous catecholamines from chromaffin cells of the heart are instrumental in the regulation of cardiac function in *Myxine*.

Injection of acetylcholine had little direct effect on the recorded cardiac variables, but caused marked branchial vasoconstriction. Heart rate increased as the pressor response subsided. The cholinergic antagonist atropine had no effect on any of the resting parameters.

Injection of adenosine had no direct cardiac effects but reduced the systemic vascular resistance.

Severe hypoxia ($P\text{w}_{O_2}=1.5\text{--}2.2 \, \text{kPa}$ for 15–35 min) had very little effect on the cardiovascular variables recorded. The remarkable ability of the hagfish heart to maintain normal cardiac output during severe hypoxia is discussed with respect to the anaerobic pumping capacity of the heart.

Pump-perfused, *in situ* gill preparations were used to examine branchial vasoactivity. Acetylcholine and adrenaline produced dose-dependent vasoconstriction, whereas isoprenaline, noradrenaline and adenosine dilated the branchial vasculature.

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Introduction

The cardiovascular system of the myxinoid cyclostomes shows certain differences from that in the other fish groups. As in other fish, the systemic heart in the myxinoids is coupled in series with the branchial and systemic vascular circuits. However, in addition to the performance of the systemic heart, blood flow is enhanced by pumping action elicited by the striated muscle of the branchial baskets and the caudal venous sinus ('caudal heart'; Johansen, 1963; Johansen and Strahan, 1963; Bloom *et al.* 1963; Laurent *et al.* 1983; Satchell, 1984). A unique feature of the myxinoid circulation is the presence of a portal vein heart ('cor venosum portale'), which is composed of cardiac muscle and beats with an intrinsic rhythm set by a pacemaker. The portal heart collects blood from the right anterior cardinal vein and the supra-intestinal (portal) vein, and passes it into the common portal vein which runs to the liver (Fänge *et al.* 1963*a*).

A further and unusual feature of the systemic and portal hearts of myxinoids is the storage of large quantities of catecholamines in specialized myocardial cells (Bloom *et al.* 1961, 1963; Euler and Fänge, 1961; Fänge *et al.* 1963*a,b*). With the exception of the lungfish *Protopterus* and *Lepidosiren* (Abrahamsson *et al.* 1979; Scheuermann, 1979; Scheuermann *et al.* 1981; Axelsson *et al.* 1989*a*), such catecholamine-containing cells are not found in vertebrate hearts [a possible exception are the granulated endothelial cells of some teleosts, which are thought to be able to store catecholamines (Sætersdal *et al.* 1974; Leknes, 1980)].

Reports on the cardiovascular physiology of myxinoids are scarce, and studies of how blood pressure and cardiac performance are controlled *in vivo* are practically absent. The available studies show a relatively low blood pressure in both the ventral and dorsal aortas (Satchell, 1986; Davie *et al.* 1987).

The myxinoid heart receives no extrinsic innervation, and the isolated systemic heart of *Myxine* is remarkably insensitive to pharmacological agents (Fänge *et al.* 1963*b*). Thus acetylcholine, catecholamines and tyramine, all known to exert strong effects on the heart in other vertebrate species, have little or no effect on the heart of *Myxine*. However, depletion of the catecholamine stores with reserpine causes bradycardia or cardiac arrest (Bloom *et al.* 1961), suggesting a tonic influence on cardiac performance by the endogenous catecholamine stores. In hearts from reserpine-treated animals, positive inotropic and chronotropic effects of catecholamines can be obtained (Fänge and Östlund, 1954; Östlund, 1954; Augustinsson *et al.* 1956; Bloom *et al.* 1961).

Myxine glutinosa inhabits mounds (hillocks) made of soft mud, and it is reasonable to believe that the interior of these mounds may sometimes be hypoxic (Foss, 1963). The aim of the present study was to provide basic information about blood pressures and cardiac output in *Myxine*, and to investigate the effects of hypoxia and autonomic drugs on the cardiovascular system of this species.

Materials and methods

Female hagfish, *Myxine glutinosa*, with a body mass of 55–91 g and a body

length of 30–40 cm, were used in this study, which was performed during July. The hagfish were kept in well-aerated running sea water at 10–11 °C, and were used within a week of capture.

Surgical and preparative procedure

In vivo *experiments*

In this part of the study seven animals were used. The animals were anaesthetized in ice-cooled sea water and MS 222 (tricaine; 3-aminobenzoic acid ethylester methanesulphonate; $1-4\,\mathrm{g\,l^{-1}}$) for approximately 15 min. The animals were then transferred to a chilled rubber foam dam (0–5 °C) on the operating table. During surgery there was a continuous irrigation of the gills with cold (8–12 °C) sea water.

To measure cardiac output ($\dot{Q}$=ventral aortic blood flow), heart rate (f_H) and ventral aortic (prebranchial) blood pressure (P_{VA}), a midline incision was made just anterior to the common branchial and pharyngo-cutaneous aperture, and the anterior part of the heart exposed. The ventral aorta and the second afferent gill artery were carefully dissected free from the surrounding tissue, and a cannula (PE 25, drawn to a final tip size approximately corresponding to a PE 10) was occlusively implanted in the afferent artery of the second gill arch and advanced into the ventral aorta for recording of P_{VA}.

A cuff-type (1.3 mm) Doppler flow probe (single crystal, P. Pohl International Inc.) was placed around the ventral aorta, caudal to the first afferent gill artery for recording $\dot{Q}$ and f_H. The leads from the flow probe and the cannula were tunnelled caudally and secured by skin sutures. The Doppler probes were attached to a Doppler flow meter (Iowa University).

For recording of the dorsal aortic blood pressure (P_{DA}) and injection of different drugs, the dorsal aorta and caudal vein were exposed *via* an incision a few centimetres anterior to the tip of the tail. A cannula (PE 25) was occlusively inserted in the dorsal aorta and another cannula (PE 10) was inserted into the vein: both were secured *via* skin sutures posterior to the incision.

Cannulae were filled with 3.0 % NaCl containing heparin (approx. $50\,\mathrm{i.u.\,ml^{-1}}$) and attached to Statham P23 pressure transducers. Calibration of both pressure transducers was made simultaneously against a static water column. Signals were suitably amplified and displayed on a Grass Polygraph recorder system model 7D. f_H was derived from the phasic blood flow signals *via* a Grass 7P44 tachograph, and expressed as beats min^{-1}. $\dot{Q}$ and stroke volume (V_S) are expressed as ml min^{-1} kg^{-1} and ml beat^{-1} kg^{-1}, respectively.

After surgery, the hagfish was transferred to the experimental tube (25 mm in diameter and 470 mm long). A steady flow of well-aerated sea water (approx. $0.2\,\mathrm{l\,min^{-1}}$) at 10–11 °C was fed through the tube, which was immersed in a larger tank connected to the sea-water system.

The hagfish was allowed to recover in the tube for at least 24 h before the start of

experiments, to let the effects of surgery and anaesthesia wear off and the cardiovascular parameters stabilize (see also Smith *et al.* 1985).

Calibration of the Doppler flow probes

After each experiment, the Doppler flow probe was calibrated *in situ* after securing an inflow cannula in the ventricle and an outflow cannula in the ventral aorta (near the exit of the fourth pair of gill arteries) and tying off the afferent gill arteries. Diluted, heparinized hagfish blood was used to calibrate the probe over a range of flows from the lowest mean flow value to the highest peak pulsatile value recorded for that animal. The data were then analyzed by linear regression analysis to provide calibration values.

In vivo *protocols*

Control values

Resting values for all parameters (see Table 1) were recorded after at least 24 h in the experimental chamber and before any experimentation.

Drug injection experiments

In these experiments the responses to different pharmacologically active agonists and antagonists were investigated. Injections were made through the caudal vein cannula in volumes of 0.1 ml, followed by 0.2 ml of 3.0 % NaCl to flush the cannula.

Increased venous return is known to increase stroke volume and heart rate in hagfish (Reite, 1969). To investigate the possibility of volume effects, all drug injection experiments started with a sham injection of saline (0.3 ml). No less than 30–40 min was allowed between injections. Prior to each injection the cardiovascular variables were stable. The total volume of fluid injected into the animal over 8–9 h in these experiments was 1.5–2.0 ml.

Adrenaline and acetylcholine were injected at two different doses (10 or 100 nmol kg^{-1}). The order of injection of these two drugs was varied between the different animals. Adenosine, a known vasoactive agent produced when the ATP pool is depleted, e.g. during hypoxia, was injected at a dose of 1 μmol kg^{-1}. The β-adrenoreceptor antagonist sotalol was injected in steps starting with half the dose used for complete β-adrenoreceptor blockade in the Atlantic cod (*Gadus morhua*) (1.35 mg kg^{-1}), and subsequent injections were made until no further change in heart rate was seen (total dose 2.7–4 mg kg^{-1}). Atropine was injected at a dose of 1.2 mg kg^{-1}.

Chemicals used

The following drugs were used: L-adrenaline bitartrate (Sigma), atropine sulphate (Sigma), adenosine free base (Sigma), acetylcholine chloride (Sigma), L-isoprenaline hydrochloride (Sigma), L-noradrenaline bitartrate (Sigma) and sotalol hydrochloride (Hässle AB). The drugs were dissolved in 3.0 % NaCl or Ringer's solution.

Hypoxic exposure

To investigate the response to hypoxia in *Myxine*, a tank (12 l) with sea water at the same temperature as in the main system was bubbled with nitrogen to a final oxygen tension of 1–1.6 kPa. Hypoxia was induced in the experimental chamber by switching to the hypoxic water supply. The oxygen tension was continuously measured in the front end of the chamber (a few centimetres in front of the head of the animal) with a Clark-type oxygen electrode (Radiometer) connected to a Radiometer system (PHM 73). The final oxygen tension in the tube was reached after 1–2 min and ranged from 1.5 to 2.2 kPa. Normoxia was restored after 15–35 min and the recovery of the animal was monitored for a further 20 min.

In situ gill perfusion

Because the drug injections were likely to have vascular as well as cardiac effects, a perfused gill preparation was used to investigate gill vasoactivity. The hagfish ($N=7$; mass 45–73 g) were killed by immersion in ice-cooled MS 222 solution for approximately 30 min, and decapitated. The animals were severed just anterior to the heart and the ventral aorta was cannulated (PE 90). Another cannula for collecting the perfusate and adjusting the outflow counter-pressure was inserted and secured in the dorsal aorta. The preparation was transferred to a thermostatically controlled (10–11°C) organ bath, and the ventral aortic catheter connected to a pneumatically driven peristaltic perfusion pump providing a pulsatile constant flow (30 pulses min^{-1}). Perfusion counter-pressure was recorded using a three-way stopcock, with one arm attached to a Statham pressure transducer model P23 connected to a Grass polygraph recorder system model 7D. The pressure transducer was calibrated against a static column of water and referenced to the fluid in the organ bath. The flow (approx. 6–9 ml min^{-1} kg^{-1}) and the inflow pressure (1.0–2.0 kPa) were in keeping with the *in vivo* measurements of these parameters. A *Myxine* Ringer's solution of the following composition was used (g l^{-1}): NaCl, 27.7; KCl, 0.6; CaCl$_2$.2H$_2$O, 0.73; NaHCO$_3$, 1.26; NaH$_2$PO$_4$, 0.08; glucose, 1.00 (Holmgren and Fänge, 1981). The solution was continuously bubbled with a mixture of 97 % O$_2$ and 3 % CO$_2$. This solution has been shown to keep tissues from *Myxine* viable for at least several hours (Holmgren and Fänge, 1981).

Bolus injections of drugs (0.1 ml) were made through a side-arm in the perfusion line into the ventral aortic cannula. Sham injections of Ringer's solution did not produce any lasting effects.

In vitro protocol

Dose–response curves were established for up to four agonist doses of each drug. No more than four different drugs were tested on one preparation. In some preparations, the maximum agonist dose was repeated following the addition of a selective antagonist to the Ringer's solution. The peak response was used to

express the percentage change in gill resistance, and pD_2 values ($pD_2 = -\log ED_{50}$) were calculated.

Data acquisition, presentation and statistics

In addition to the polygraph tracings, all data were fed into an IBM PPC computer, allowing continuous sampling at 10 samples s^{-1} and on-line mean value calculation for 1-min periods. Data from individual *in vivo* experiments were superimposed and are presented in graphs as means±s.e.m. at 1- (agonists) or 2- (hypoxia) min intervals (Fritsche and Nilsson, 1989).

Stroke volume was calculated from $\dot{Q}$ divided by f_H, and vascular resistance as pressure drop over the branchial (R_G) or systemic (R_S) vascular bed divided by cardiac output ($\dot{Q}$). In either case, calculations were performed for each animal, and means then calculated from the individual values.

Means±s.e.m. are presented (N=number of animals). The non-parametric Wilcoxon's sign rank test for·paired samples was used (two-tailed) to determine the statistical significance of observed effects of drug injections or hypoxic exposure *in vivo*. Comparisons were made between the values before injection of the agonist and the values 5 min after the injection, at which time the effect (if any) was stabilizing. The development of effects of hypoxia was slower, and the statistical evaluations were made after 15 min of hypoxia and again after 20 min of recovery from the hypoxia. The level of significance was set to $P \leqslant 0.05$.

Results

The control parameters are presented in Table 1 for the seven hagfish used in the *in vivo* experiments. The pulsatile characteristics of the cardiovascular variables are presented in Fig. 1. Under control conditions the mean f_H was 22.3 beats min^{-1}. Slow oscillations of 1–2 beats min^{-1} around the mean resting value were common. The absence of beat-to-beat oscillations in f_H is consistent with the aneural control of the heart.

Table 1. *A summary of simultaneously recorded and calculated cardiovascular parameters from hagfish* Myxine glutinosa

P_{VA} (kPa)	1.04 ± 0.10
P_{DA} (kPa)	0.77 ± 0.12
f_H (beats min^{-1})	22.3 ± 1.0
$\dot{Q}$ (ml min^{-1} kg^{-1})	8.7 ± 1.7
V_S (ml beat^{-1} kg^{-1})	0.41 ± 0.08
R_G (Pa·min·kg ml^{-1})	49 ± 22
R_S (Pa·min·kg ml^{-1})	113 ± 27

The figures show ventral aortic pressure (P_{VA}), dorsal aortic pressure (P_{DA}), heart rate (f_H), cardiac output ($\dot{Q}$), stroke volume (V_S), branchial vascular resistance (R_G) and systemic vascular resistance (R_S).

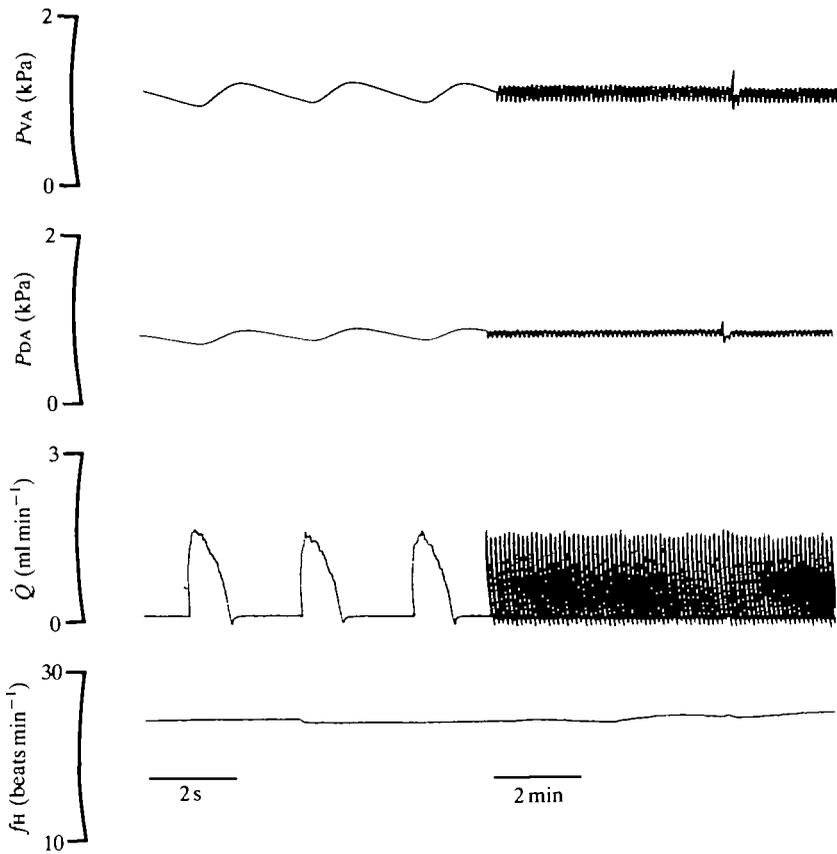The values are presented as means±s.e.m., N=7.

Fig. 1. Simultaneously recorded ventral aortic pressure (P_{VA}), dorsal aortic pressure (P_{DA}), phasic ventral aortic blood flow ($\dot{Q}$) and heart rate (f_H) in untreated *Myxine*. Note the zero flow during the diastolic phase of the cardiac cycle, and the notches in both P_{VA} and P_{DA} traces during a cough (right part of figure).

The mean $\dot{Q}$ was $8.7\,\mathrm{ml\,min^{-1}\,kg^{-1}}$, and there was a significant period of zero flow during diastole (Fig. 1).

Gill resistance accounted for 30 % of the total vascular resistance. Coughing was indicated by a peak in the blood pressure recording (Figs 1,2) and corroborated by visual observations (Steffensen *et al.* 1984). The pressure peak in the ventral aortic recording could be attributed to an artefact of cannula movement because of the proximity of the cannula to the gill aperture. This reasoning does not hold for the corresponding peak in the P_{DA} trace (Figs 1,2), since this cannula was at the tail of the animal. Thus, it seems likely that coughing can significantly affect systemic blood pressures.

Control injections

A sham injection of $0.3\,\mathrm{ml}$ of $3.0\,\%$ NaCl produced no marked changes in f_H or
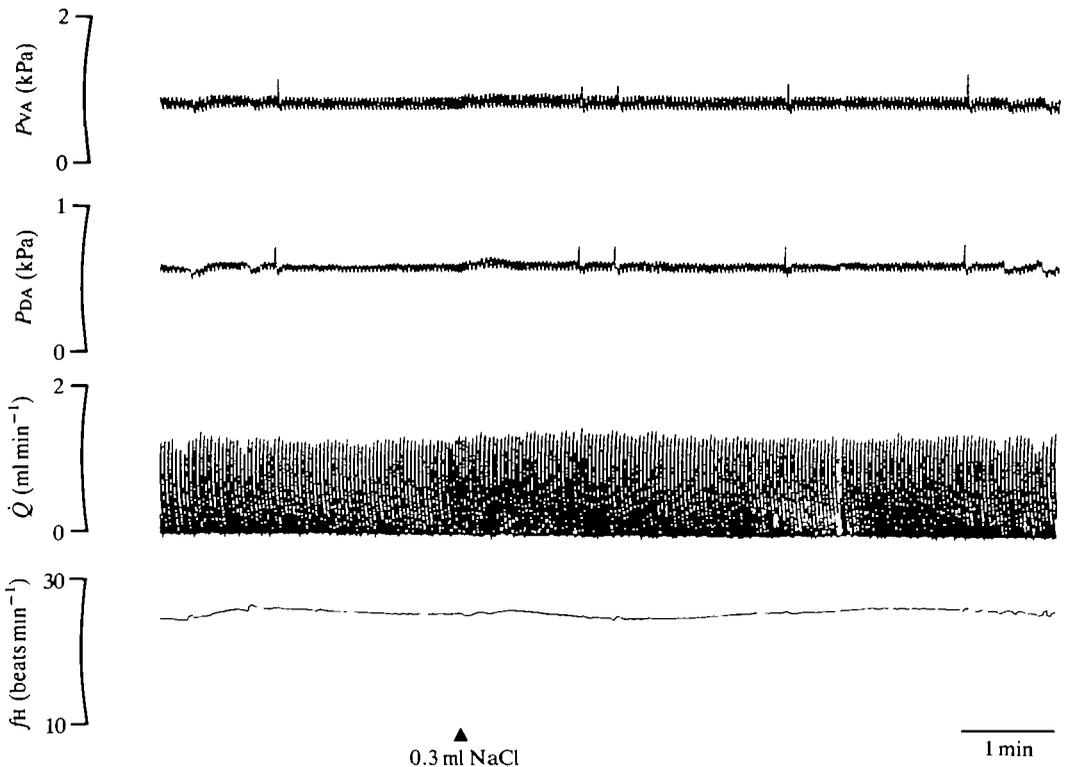
Fig. 2. Simultaneously recorded ventral aortic pressure (P_{VA}), dorsal aortic pressure (P_{DA}), phasic ventral aortic blood flow ($\dot{Q}$) and heart rate (f_H) in *Myxine* before and after injection of 0.3 ml of 3 % NaCl. Note absence of visible effects on the recorded parameters.

$\dot{Q}$ (Fig. 2). Therefore, we are confident that the cardiovascular effects observed are due directly to the drugs injected.

Adrenaline

Both doses of adrenaline produced an immediate increase in V_s and f_H, which elevated $\dot{Q}$ significantly (Fig. 3). The maximum value of $\dot{Q}$ approached 25 ml min^{-1} kg^{-1}, and was reached after 3–4 min. Recovery was not complete even 15 min after the injection. With the high dose of adrenaline (100 nmol kg^{-1}), f_H increased by approximately 20 % (20 to 24 beats min^{-1}), and V_s increased by 30 % (0.7 to 1.0 ml kg^{-1}). The changes in V_s, f_H and $\dot{Q}$ were quantitatively the same for both doses injected.

No significant effect on R_G was observed with adrenaline, but a significant decrease in R_S occurred, at least with the low dose of adrenaline (10 nmol kg^{-1}). The decrease in vascular resistance was proportionately less than the increase in $\dot{Q}$ and therefore there was a significant increase in both P_{VA} and P_{DA}.
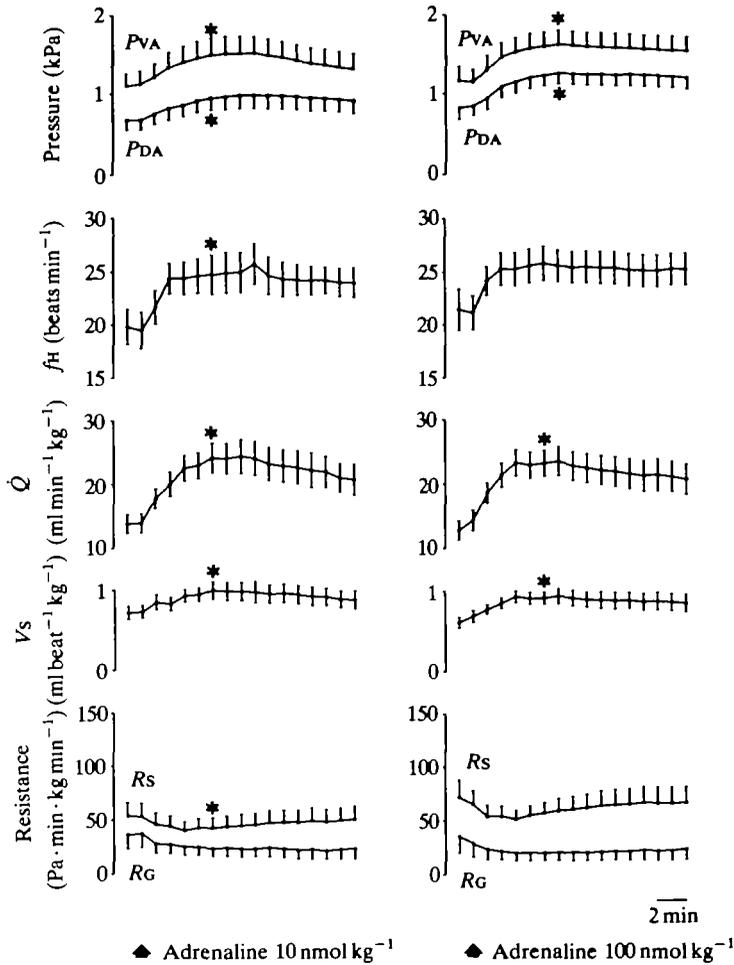
Fig. 3. Mean values for the simultaneously recorded and calculated parameters [ventral and dorsal aortic blood pressure (P_{VA}, P_{DA}), ventral aortic blood flow ($\dot{Q}$), heart rate (f_H), stroke volume (V_S) and systemic and branchial vascular resistance (R_S, R_G)] in *Myxine* prior to and after a bolus injection of adrenaline. Left part of figure: 10 nmol kg^{-1} adrenaline injected into the caudal vein in 0.1 ml kg^{-1} fish and then flushed with 0.2 ml of NaCl. Right part of figure: same procedure but with 100 nmol kg^{-1} adrenaline instead. Means±s.e.m., $N=7$. Statistical analysis (value before injection *vs* value 5 min after injection) shows significant effects ($P<0.05$) indicated by asterisks.

Acetylcholine

The low dose of acetylcholine (10 nmol kg^{-1}) produced a modest decrease in V_S, and a small increase in $\dot{Q}$, which must be explained in terms of an increase in f_H (although no statistically significant change could be demonstrated 5 min after the acetylcholine injection) (Fig. 4). There were also minor increases of P_{VA} and P_{DA} (Fig. 4). The high dose of acetylcholine (100 nmol kg^{-1}) produced a pronounced
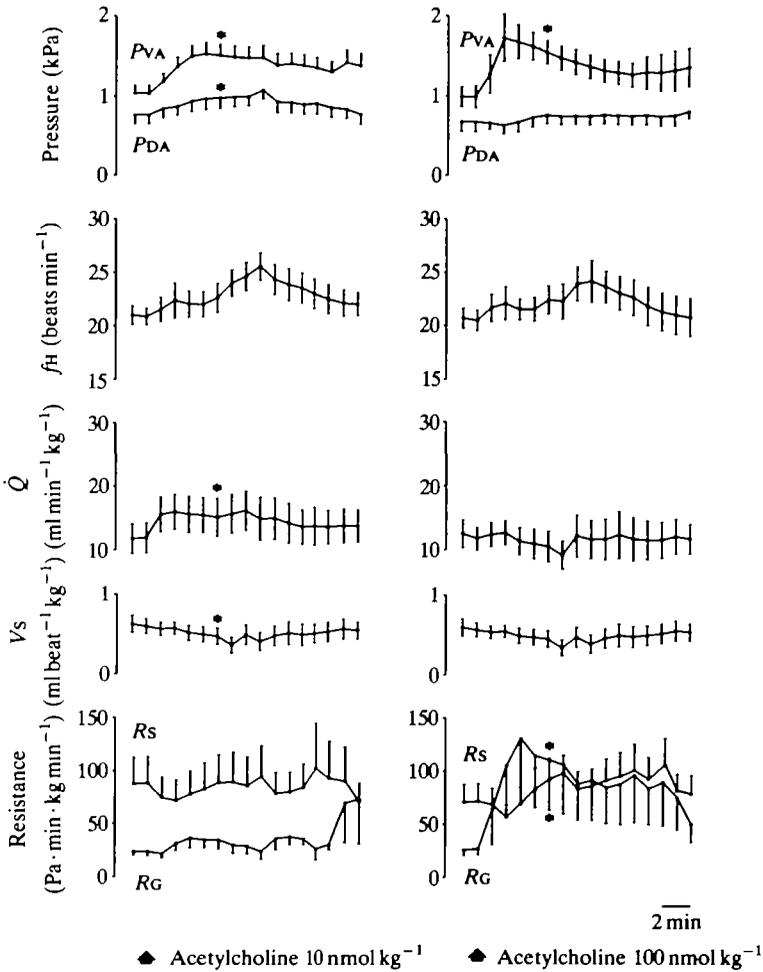
Fig. 4. Mean values for the simultaneously recorded and calculated parameters prior to and after a bolus injection of acetylcholine. Left part of figure: $10 \, \text{nmol} \, \text{kg}^{-1}$ acetylcholine injected into the caudal vein in $0.1 \, \text{ml} \, \text{kg}^{-1}$ fish and then flushed with $0.2 \, \text{ml}$ of NaCl. Right part of figure: same procedure but with $100 \, \text{nmol} \, \text{kg}^{-1}$ acetylcholine instead. Means±S.E.M., $N=7$. Statistical analysis (value before injection vs value 5 min after injection) shows significant effects indicated by asterisks.

increase in R_G and P_{VA}, while the change in R_S was smaller and P_{DA} remained unchanged. As P_{VA} began to decrease from the peak value of $1.5 \, \text{kPa}$ there was a noticeable increase in f_H.

Adenosine

The dose of $1 \, \mu\text{mol} \, \text{kg}^{-1}$ adenosine affected V_S and $\dot{Q}$, while f_H remained unchanged (Fig. 5). There was an immediate and marked systemic vasodilatation, with accompanying hypotension; R_G was not affected.
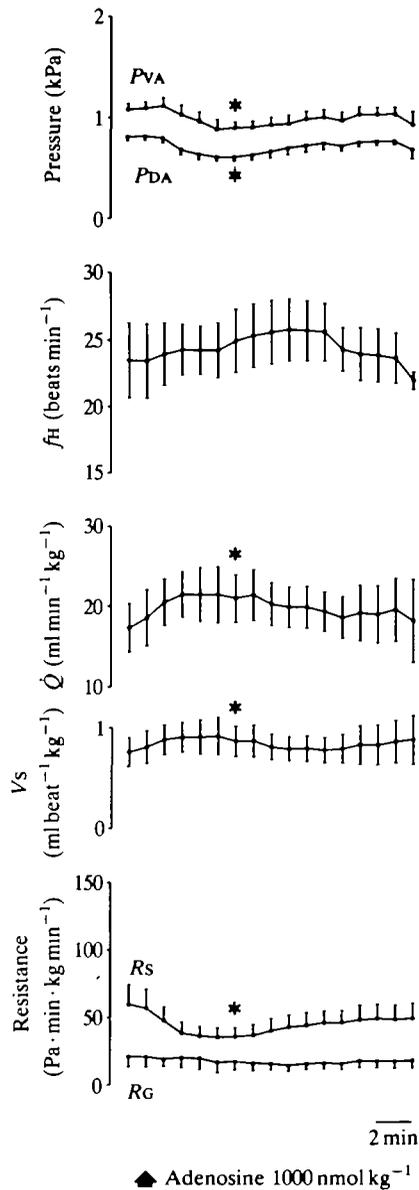
Fig. 5. Mean values for the simultaneously recorded and calculated parameters prior to and after a bolus injection of $1000\,nmol\,kg^{-1}$ adenosine into the caudal vein in $0.1\,ml\,kg^{-1}$ fish and then flushed with 0.2 ml of NaCl. Means±s.E.M., $N=7$. Statistical analysis (value before injection *vs* value 5 min after injection) shows significant effects indicated by asterisks.

Hypoxia

The hypoxic tolerance of the *Myxine* heart is well known, but before the present investigation it was not clear what level of pumping ability was retained during
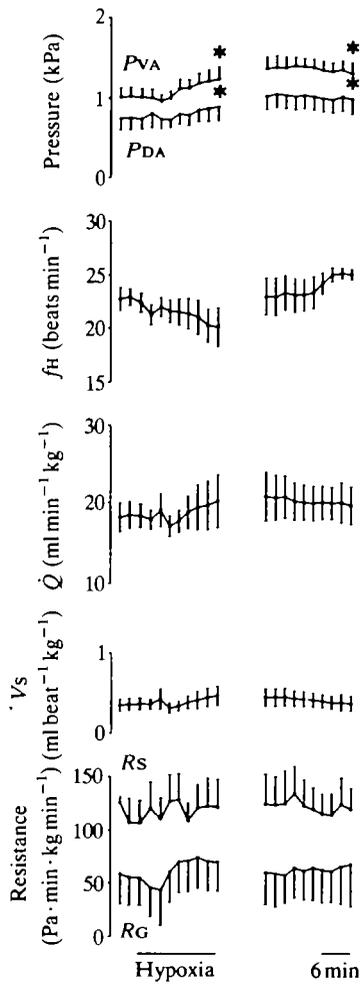
Fig. 6. Mean values for the simultaneously recorded and calculated parameters prior to, during and after a period of hypoxia (Pw_{O_2}=1.5–2.2 kPa). Statistical analysis (value pre-hypoxia *vs* value at 15 min of hypoxia; means±S.E.M., N=7 and value pre-hypoxia *vs* 20 min post-hypoxia; means±S.E.M., N=5) shows significant effects indicated by asterisks.

hypoxia. Preliminary experiments exposing two hagfish to a water P_{O_2} of 4.0 kPa had no effect on cardiovascular variables. The present level of hypoxic exposure (1.5–2.2 kPa for 15–35 min) is considered severe by teleost standards and the fact that the hagfish were able to maintain $\dot{Q}$ at resting levels is quite remarkable (Fig. 6).

There were statistically significant increases in P_{VA} and P_{DA} both during the hypoxic exposure and for as long as 20 min into the recovery from hypoxia (Fig. 6).
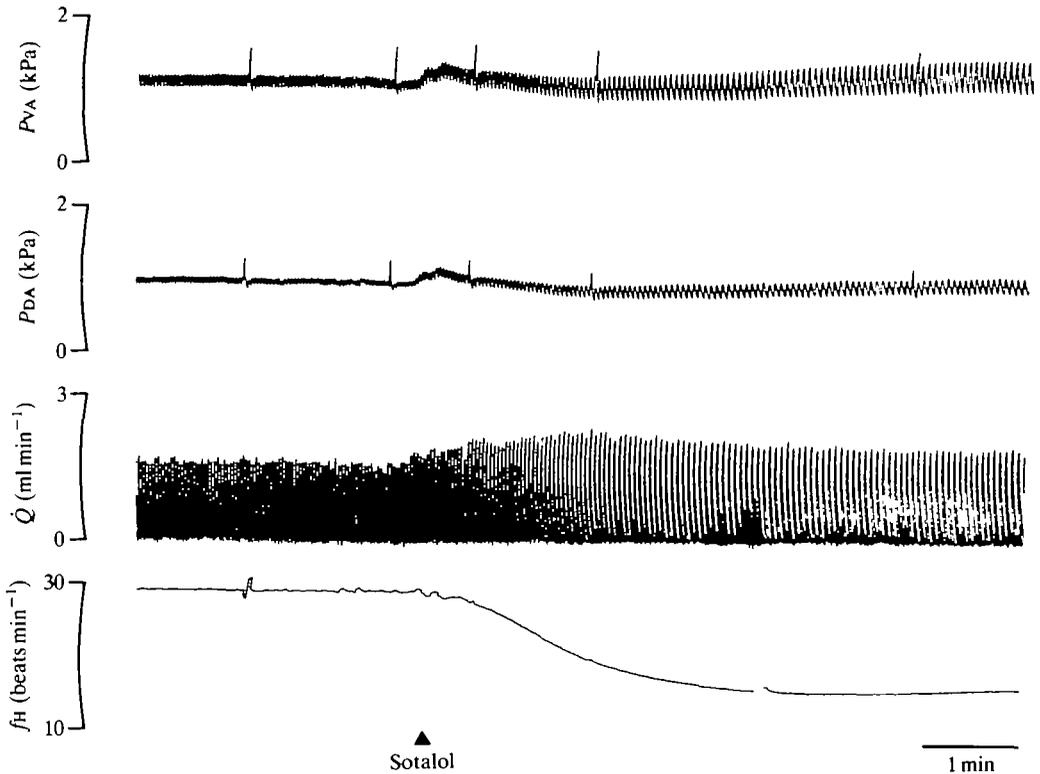
Fig. 7. Simultaneously recorded ventral aortic pressure (P_{VA}), dorsal aortic pressure (P_{DA}), phasic ventral aortic blood flow ($\dot{Q}$) and heart rate (f_H) prior to and after an intravenous injection of 1.35 mg kg^{-1} of the β-adrenoreceptor antagonist sotalol. Note the rapid and massive fall in f_H.

Sotalol and atropine

Injection of sotalol produced an immediate and pronounced decrease in f_H (Fig. 7), followed by a progressive decline in V_S.

Atropine had no significant effects on any of the measured parameters.

Gill perfusions

Dose-dependent vasoconstriction of perfused gills was observed with acetylcholine ($pD_2=6.64\pm0.35$; $N=4$) and adrenaline ($pD_2=7.06\pm0.30$; $N=6$). Vasodilatation was observed with the β-adrenoreceptor agonist isoprenaline ($pD_2=5.12\pm0.26$; $N=4$), adenosine ($pD_2=5.91\pm0.29$; $N=7$) and noradrenaline ($pD_2=5.92\pm0.31$; $N=5$) (Fig. 8).

Discussion

The present investigation is the first to measure $\dot{Q}$ along with other key cardiovascular variables in *Myxine*. The study demonstrates a period of zero flow
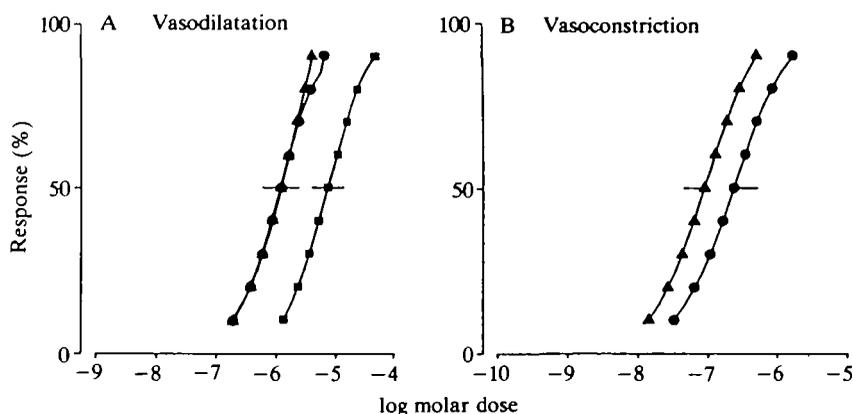
Fig. 8. Dose–response curves showing effects of bolus injections of increasing doses of agonists on the perfusion pressure in the perfused head preparations. (A) Vasodilatation produced by isoprenaline (▲, N=4), noradrenaline (●, N=5) and adenosine (■, N=7). (B) Vasoconstriction produced by acetylcholine (▲, N=4) and adrenaline (●, N=7). Means±S.E.M. (for the pD_2 values).

during diastole, which suggests that the windkessel properties of the hagfish ventral aorta and bulbus cordis are limited. This conclusion was supported by visual observations of the relatively thin ventral aorta. Furthermore, studies of the distribution of elastin have shown that elastin is absent in cyclostomes (Sage and Gray, 1979, 1980).

The resting $\dot{Q}$ value of close to 9 ml min^{-1} kg^{-1} is somewhat lower than resting $\dot{Q}$ in elasmobranchs and teleosts that do not have a very active lifestyle. For example, the following $\dot{Q}$ values are reported for water temperatures at or near 10°C: Atlantic cod (*Gadus morhua*) 17.3 ml min^{-1} kg^{-1} (Axelsson and Nilsson, 1986); lingcod (*Ophiodon elongatus*) 11.2 ml min^{-1} kg^{-1} (Farrell, 1982); sea raven (*Hemitripterus americanus*) 14.6 ml min^{-1} kg^{-1} (Farrell, 1986); long nose skate (*Raja rhina*) 21.1 ml min^{-1} kg^{-1} (Satchell *et al.* 1970).

In contrast, *Myxine* can increase $\dot{Q}$ substantially. The peak $\dot{Q}$ of 24 ml min^{-1} kg^{-1}, which was recorded after an adrenaline injection and may not be the maximum $\dot{Q}$ possible, is impressive compared with $\dot{Q}$ values reported for elasmobranchs and teleosts during brief exercise (long nose skate 23.3 ml min^{-1} kg^{-1}, Satchell *et al.* 1970; Atlantic cod 25.4 ml min^{-1} kg^{-1}, Axelsson and Nilsson, 1986; sea raven 30.9 ml min^{-1} kg^{-1}, Axelsson *et al.* 1989b). Studies with isolated perfused hearts indicate that a $\dot{Q}$ of 22 ml min^{-1} kg^{-1} is possible in the larger New Zealand hagfish, *Eptatretus* (Forster, 1989). Thus, even though hagfish have a non-innervated heart, their ability to pump relatively large volumes, and indeed vary Vs significantly, is not apparently restricted by the absence of a cardiac innervation. One can presume that this is appropriate for a fish with a large blood volume.

The present study provided quantitative information on some of the mechanisms which influence cardiac pumping in *Myxine*. Previous studies have focused on

the regulation of f_H and have stressed the importance of the pressure-sensitive pacemaker (Jensen, 1961; Chapman *et al.* 1963; Johansen, 1963). Interestingly, in hagfish not otherwise used for the present analysis, larger injections of saline (0.6–1.0 ml) produced increases in V_S and f_H, confirming early reports about the sensitivity of V_S and f_H to venous filling pressure. An interesting observation was that apparently dying hagfish typically had a very low V_S and blood had pooled in their subcutaneous sinuses. Infusion of saline immediately restored $\dot{Q}$. Since loss of blood volume through bleeding was not apparent, it seems that the inability to maintain venous return may contribute to the death spiral in the hagfish.

Under both aerobic conditions and severe hypoxia, f_H was typically about 22 beats min^{-1}. The maximum f_H we observed in our animals was 26 beats min^{-1}. Thus, increases in f_H produce at best a 20 % increase in $\dot{Q}$. If we assume that increased venous return could produce a similar increase in f_H, then the pressure-sensitive pacemaker could play a modest role in regulating $\dot{Q}$ under aerobic and hypoxic conditions.

Clearly, the physiological role of the pressure-sensitive pacemaker needs further study. Perhaps, in the absence of a cardiac innervation, the mechanism is important after prolonged periods of anoxia in the reactivation of cardiac activity, when vigorous sinusoidal tail movements, under neural control, increase venous return. Our observation that venous volume loading significantly increased f_H and V_S in apparently dying *Myxine* is consistent with this idea.

Jensen (1961, 1965, 1969) suggested that a pressure-sensitive pacemaker was important in the regulation of f_H in lampreys, elasmobranchs and teleosts. However, there is now some question about the physiological significance of this mechanism in ocean pout, sea raven, Atlantic salmon, rainbow trout, long-finned eel, rough skate and dogfish (Farrell *et al.* 1983, 1986; Farrell, 1984; P. S. Davie, A. P. Farrell and C. Franklin, unpublished observations): perfused hearts performing a physiological work load under aerobic and hypoxic conditions do not show major changes in f_H in response to filling pressure, although filling pressure markedly affects V_S. The only situation in which increases in f_H are observed in response to increases in filling pressure is when the perfused heart shows atrio-ventricular desynchrony and beats with a ventricular rhythm. The physiological significance of such a mechanism is not immediately obvious. Clearly, the pressure-sensitive pacemaker has a physiological significance in hagfish. However, even though the mechanism is retained in other aquatic vertebrates, its importance for controlling f_H has been superseded by other mechanisms.

Previous studies provide equivocal evidence for catecholamine involvement in the regulation of f_H in hagfish. The studies of Fänge and Östlund (1954) demonstrate that the insensitivity of the hagfish heart to catecholamine treatment was altered by dihydroergotamine, such that positive chronotropic and inotropic responses were elicited. It was suggested that the high catecholamine content of the heart might provide an endogenous cardiac tonus, against which further adrenergic stimulation could not be detected. Indeed, Bloom *et al.* (1961) found that reserpine treatment impaired cardiac performance and reduced the catechol-

amine content of the heart, an observation that is consistent with the idea that the granulated catecholamine-storing cells in the hagfish heart are related to chromaffin cells.

Our observations on intact *Myxine* support these ideas. Adrenaline injections into the caudal vein produced immediate increases in f_H and V_S, which we conclude were a result of direct adrenergic stimulation of the heart. Perhaps more significant was the clear evidence for a tonic β-adrenergic stimulation of f_H, as indicated by the pronounced decrease in f_H with sotalol treatment. Whether this adrenergic tonus to the *Myxine* heart is endogenous was not established and we are still left with the question: what is the selective advantage of catecholamine granules in the hagfish heart? The answer may, again, relate to recovery from anoxic metabolic depression. Interestingly, the only other vertebrate group with significant cardiac storage of catecholamines is the lungfish (Dipnoi), which also experience metabolic depression (although of another kind) during torpor (Abrahamsson *et al.* 1979; Scheuermann, 1979; Scheuermann *et al.* 1981; Nilsson, 1983). Perhaps release of endogenous catecholamines from the heart of hagfish and lungfish is pressure-sensitive.

We were unable to show any immediate and direct effects of acetylcholine, hypoxia or adenosine on f_H. The insensitivity to acetylcholine is consistent with earlier work on hagfish (see Johansen, 1963, for references). Hypoxia has no effect on the intrinsic f_H of perfused hearts from sea raven, rough skate and dogfish (Farrell *et al.* 1985; P. S. Davies and A. P. Farrell, unpublished observations).

Regulation of V_S in hagfish appeared at least as important (adrenergic stimulation, Fig. 3; recovery from hypoxia, Fig. 6) as f_H in modulating $\dot{Q}$. Also, increases in V_S occurred either when f_H decreased abruptly following sotalol treatment or when f_H increased following large volume injection. *In vitro* the hagfish heart responds to increased filling pressure by increasing V_S in accordance with the Frank–Starling mechanism (Chapman *et al.* 1963; Forster, 1989). Thus, it seems likely that the *in vivo* modulation of V_S observed here is, in part, explained by changes in filling pressure. Adrenergic stimulation increases the sensitivity of the perfused trout heart to filling pressure, so that a greater V_S is possible at the same filling pressure (Farrell *et al.* 1986). In the present study, adrenaline injection produced an increase in V_S (Fig. 3). A potential role for adrenergic modulation of the sensitivity of the *Myxine* heart to filling pressure was also apparent but needs to be quantified.

V_S in the hagfish also appeared to be sensitive to afterload pressure. V_S decreased 5–8 min after acetylcholine injection and, because of this time lag, the reversal in V_S is unlikely to be a direct cardiac effect of acetylcholine. Instead, it appears likely that the heart could not continue to pump against this elevated outflow resistance. This conclusion is supported by observations in other hagfish, where even higher doses of acetylcholine produced a complete cessation of ventral aortic flow. The maximum P_{VA} observed in these hagfish was 1.6 kPa.

Typically, fish and mammalian hearts are capable of homeometric regulation; that is, V_S is relatively independent of physiological afterload pressures. However,

as supraphysiological pressures are imposed on the heart, power output is initially constant, with a trade-off between pressure and flow development, and then declines with disproportionate decreases in flow. It is in the area of pressure development that the pumping ability of the *Myxine* heart undoubtedly differs most drastically from the situation in teleost fishes. Normal ventral aortic pressures for *Myxine* are one-third to one-quarter of those reported for teleost fish. A 50 % increase is possible without compromising $\dot{Q}$ in *Myxine* (Fig. 4) and teleosts (Farrell, 1984). Nevertheless, comparisons of myocardial power output reveal the low pumping capacity of the *Myxine* heart. The resting performance presented in Table 1 approximates to a power output of $0.154\,\mathrm{mW\,g^{-1}}$ ventricle mass, assuming a ventricle mass of $1\,\mathrm{g\,kg^{-1}}$ body mass and an ambient venous filling pressure. The peak response to adrenaline approximates to a power output of $0.62\,\mathrm{mW\,g^{-1}}$ ventricle mass and the peak response to acetylcholine approximates to $0.40\,\mathrm{mW\,g^{-1}}$ ventricle mass. These power output levels are about an order of magnitude lower than the maximum power output levels for perfused hearts from sea raven ($4\,\mathrm{mW\,g^{-1}}$; Farrell *et al.* 1985) and trout ($6\,\mathrm{mW\,g^{-1}}$; Farrell *et al.* 1989). Because the peak power output with adrenaline is greater than with acetylcholine, when $\dot{Q}$ was clearly compromised, it appears that adrenergic stimulation of the hagfish heart *in vivo* extends the range for homeometric regulation. This conclusion is consistent with the reported inotropic effect of adrenaline on perfused hearts from hagfish (Fänge and Östlund, 1954; Chapman *et al.* 1963) and teleosts (Graham and Farrell, 1989).

The ability of the hagfish heart to maintain resting cardiac performance during severe anoxia is remarkable. The venous P_{O_2} during the present hypoxia experiment was not measured but must have been well below the water P_{O_2} of 1.5–2.2 kPa. There are at least two reasons for this hypoxic tolerance: the high anaerobic capacity and the low aerobic requirement of the *Myxine* heart. Previous studies have clearly demonstrated that the hagfish heart can tolerate anoxia, but pumping capacity has not been assessed (Hansen and Sidell, 1983). Therefore, the present study is the first to estimate the pumping capacity in terms of power production during severe hypoxia. The aerobic requirement of the heart is low. If we assume a 15 % efficiency for mechanical work (Farrell *et al.* 1985), then $0.05\,\mu\mathrm{l\,O_2\,s^{-1}\,g^{-1}}$ ventricle mass is required to maintain a power output of $0.154\,\mathrm{mW\,g^{-1}}$. This O_2 requirement may be somewhat higher if the hagfish heart is less efficient at this low power output and the basal metabolic rate of the heart is a more significant component of the myocardial energy requirement. Using a more conservative estimate of mechanical efficiency (5 %), the O_2 requirement is $0.15\,\mu\mathrm{l\,O_2\,s^{-1}\,g^{-1}}$. These levels of O_2 consumption require an ATP turnover rate of $12.8\text{–}39.4\,\mathrm{nmol\,ATP\,s^{-1}\,g^{-1}}$ ventricle mass, depending on the mechanical efficiency of the heart. If the hagfish heart has a maximal anaerobic ATP production rate similar to that in the sea raven ($20\,\mathrm{nmol\,ATP\,s^{-1}\,g^{-1}}$; Turner and Driedzic, 1980), it appears that most, if not all, of the ATP requirements of the heart can be met anaerobically. Further studies would be useful to determine exactly how much of the range for cardiac pumping in the hagfish can be fuelled anaerobically.

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