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EFFECTS OF PRELOAD AND AFTERLOAD ON THE PERFORMANCE OF THE *IN SITU* PERFUSED PORTAL HEART OF THE NEW ZEALAND HAGFISH *EPTATRETUS CIRRHATUS*

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Summary

The portal heart of the New Zealand hagfish (*Eptatretus cirrhatus*) was perfused *in situ*. Stroke volume, cardiac output and power output increased in response to increased preload, in accordance with Starling's law of the heart. A positive chronotropic effect was found when the input pressure increased from 0.05 to 0.1 kPa. Increased afterload decreased stroke volume and cardiac output. Power output peaked at an output pressure of 0.22 kPa, after which it decreased. There was no change in heart rate in response to increased afterload. In unanaesthetized resting animals, the pressure in the supraintestinal vein, which supplies the portal heart, ranged from 0.025 to 0.07 kPa (mean 0.040±0.005 kPa).

The β-adrenoceptor antagonist sotalol did not affect the response to different input and output pressures. Sotalol produced a significant decrease in heart rate and abolished the pressure-sensitive increase in heart rate. Bolus injections of adrenaline produced a transient increase in portal heart rate. The negative chronotropic response to sotalol and the response to adrenaline indicate the presence of an endogenous β-adrenergic tonus on the portal heart.

Key words: *Eptatretus cirrhatus*, New Zealand hagfish, portal heart, perfusion, heart rate, stroke volume, cardiac output, power output.

Introduction

The systemic heart of the New Zealand hagfish delivers a cardiac output comparable with that of the hearts of other moderately performing fish species, but at unusually low blood pressures (Forster, 1989; Forster *et al.* 1991). Venous return in hagfishes is facilitated by the action of a number of accessory pumps. The portal vein heart (portal heart) is one such accessory pump, which forces blood through the liver and onto the systemic heart. Blood is supplied from the supraintestinal vein and the anterior cardinal vein (Fänge *et al.* 1963). The portal heart has valves at its inlets and outlet to prevent backflow and is composed of cardiac muscle, in contrast to the other venous propulsors whose action depends upon skeletal muscle (Johansen, 1960, 1963; Satchell, 1991).

Contraction of the portal heart, which is not synchronous with the systemic heart (Arlock, 1975; Davie *et al.* 1987), originates in the widened proximal region of the supraintestinal vein (sinus supraintestinalis) (Müller, 1845; Fänge *et al.* 1963). Davie *et al.* (1987) compared the sinus supraintestinalis with the sinus venosus of the systemic heart and argued that it is responsible for the small increase in pressure preceding systole. In teleost fish, atrial contraction fills the ventricle and thereby has a major influence on cardiac output. Mechanisms controlling cardiac function in hagfish are largely unknown. The hagfish systemic heart is notable for its apparent lack of neural regulation (Augustinsson *et al.* 1956; Jensen, 1965). The systemic and portal hearts of hagfish have internal stores of catecholamines (Östlund, 1954; von Euler and Fänge, 1961; Bloom *et al.* 1961; Östlund *et al.* 1961; Laurent *et al.* 1983; Forster *et al.* 1991), which may modulate heart function and/or act on structures downstream (Axelsson *et al.* 1990; Perry *et al.* 1993).

Hagfish lack a rigid pericardium and must depend upon positive venous pressures to fill the systemic and portal hearts. The only previous study of changes in rate and force development of the portal heart was made on *Eptatretus stoutii* using a perfusion technique (Chapman *et al.* 1963). In that study, input and output pressures were not recorded; instead, intracardiac pressure was recorded by insertion of a catheter into the lumen of the portal heart through its wall. No calculations of flow or power output for the portal heart were presented. Chronotropic effects of different filling pressures and volumes have been reported for the systemic heart of *Myxine glutinosa* and *E. stoutii* (Johansen, 1960; Jensen, 1961; Bloom *et al.* 1963; Chapman *et al.* 1963), although the rate of

the isolated perfused heart of *E. cirrhatus* did not change in response to increased filling pressure (Forster, 1989). The portal heart of *M. glutinosa* has been observed to accelerate when distended with blood (Johansen, 1960; Fänge *et al.* 1963).

Cardiac function curves published for fish hearts are the product of at least two muscular, valved chambers in series. The portal heart of the hagfish offers the opportunity to investigate the effects of filling pressure and afterload on the work performed by a heart that is effectively a single chamber, filled at low pressures (Davie *et al.* 1987).

Materials and methods

Animals

New Zealand hagfish, *Eptatretus cirrhatus* Forster, weighing between 810 and 1700 g were used in the experiments. The hagfish were captured in baited traps off Motunau, North Canterbury, New Zealand. They were kept indoors in aquaria supplied with circulating sea water at approximately 16 °C.

In situ *experiments*

Animals were anaesthetized in sea water containing MS222 ($0.4\,g\,l^{-1}$) and benzocaine ($0.4\,g\,l^{-1}$). The portal heart was exposed through a large ventral incision. The supraintestinal vein was cannulated with polyethylene tubing (PE200). This inflow cannula was attached to a Marriot bottle containing Ringer's solution, allowing continuous perfusion of the portal heart as the portal vein was cannulated (PE200). For the recording of input and output pressures close to the inlet and outlet of the heart, a piece of PE10 tubing was inserted into the PE200 cannulae. This short piece of PE10 tubing was connected *via* PE90 tubing to the pressure transducers (see below and Franklin and Axelsson, 1994).

The head and tail of the hagfish were cut off and the part of the hagfish containing the perfused heart (approximately 20 % of total body mass) was placed in a thermostatted (16–17 °C) organ bath filled with Ringer's solution. The inflow cannula was attached to a constant-pressure device (see Franklin and Axelsson, 1994) connected to a reservoir containing perfusion fluid. By raising and lowering the constant-pressure device, different input pressures could be achieved. In a similar way, the cannula inserted into the portal vein was connected to a tube which could be moved vertically, thus changing the afterload. The input and output pressures were recorded using a Devices Mx4 recorder and Bell and Howell type 4-327 pressure transducers. The pressure transducers were calibrated against a static water column, where zero pressure was set to the level of Ringer's solution in the organ bath.

The Ringer's solution had the following composition ($g\,l^{-1}$): 27.7 NaCl, 0.6 KCl, 0.75 $CaCl_2{\cdot}2H_2O$, 0.75 $MgSO_4{\cdot}7H_2O$, 1.83 $MgCl_2{\cdot}6H_2O$, 0.405 NaH_2PO_4, 3.45 $NaHCO_3$, 1 glucose. Noradrenaline ($3\,nmol\,l^{-1}$) and adrenaline ($1\,nmol\,l^{-1}$) were added to provide physiological catecholamine levels (see Perry *et al.* 1993) in the perfusion fluid. The saline was bubbled with a gas mixture of nitrogen (93.5 %), oxygen (6 %) and carbon dioxide (0.5 %) (see Sundin *et al.* 1994).

Starling curves were constructed by increasing the preload gradually while maintaining the output pressure at 0.2 kPa. In a similar way, power curves were established by raising the output pressure incrementally until the cardiac output reached a plateau. The input pressure was kept constant at a physiological level (0.04 kPa). Cardiac output was recorded gravimetrically when it had stabilized (1–3 min).

Bolus injections of adrenaline (0.1 ml, $10^{-3}\,mol\,l^{-1}$) into the inflow tube provided information on chronotropic effects in response to adrenaline. To study the chronotropic effect of the β-adrenoceptor antagonist sotalol, the drug was added to the Ringer's solution and the effect was recorded 30 min later.

Starling and power curves were also determined following the addition of sotalol. Each preparation acted as its own control for investigating differences in performance after the addition of sotalol.

In vivo *experiments*

A ventral incision was made in anaesthetized animals to expose and allow cannulation of one of the small superficial veins situated on the intestine and emptying into the supraintestinal vein. The cannula was advanced into the supraintestinal vein, with its opening towards the portal heart, and firmly secured. After 24 h of recovery, recordings of the supraintestinal blood pressure at rest were made using a Devices Mx4 recorder and Bell and Howell type 4-327 pressure transducers. Calibration of the transducers was performed against a static water column.

Calculations

Cardiac output, stroke volume and power output were normalised per gram portal heart mass (PHM). Separate curves, in which the different parameters were plotted against filling pressure (preload graphs) and output pressure (afterload graphs), were fitted to the data using a third-order polynomial (see Franklin and Axelsson, 1994). As measured pressures were slightly different in each preparation, this method made it possible to construct composite graphs for which data had been generated at set pressure intervals. Data were calculated and plotted in steps of 0.01 kPa for the preload curves and in steps of 0.02 kPa for the afterload curves.

Data are presented as means ± S.E.M. Wilcoxon matched-pairs signed-ranks test were used to determine statistically significant differences ($P<0.05$).

Results

Data obtained from the portal heart preparation are summarised in Table 1. The portal heart mass was 0.0104±0.0004 % of body mass (N=11). The supraintestinal vein pressure varied between 0.025 and 0.07 kPa (N=8) (Fig. 1), with a mean pressure of 0.040±0.005 kPa (N=8). Portal heart rate at physiological input and output pressures was 40.8±1.1 beats min^{-1} (N=10). It was observed during the

Table 1. *Mean and maximum values of cardiovascular variables*

Body mass (BM) (kg)	1.31±0.07 (12)
Portal heart mass (PHM) (g)	0.137±0.008 (11)
Relative portal heart mass (%)	0.0104±0.0004 (11)
Venous pressure (kPa)	0.040±0.005 (8)
Heart rate (beats min^{-1})	40.8±1.1 (10)
Maximal cardiac output (ml min^{-1} g^{-1} PHM)	87.5±16.7 (10)
Maximal stroke volume (ml $beat^{-1}$ g^{-1} PHM)	1.84±0.19 (10)
Maximal power output (mW g^{-1} PHM)	0.20±0.03 (10)
Maximal cardiac output (ml min^{-1} kg^{-1} BM)	9.34±1.85 (10)
Maximal stroke volume (ml $beat^{-1}$ kg^{-1} BM)	0.23±0.05 (10)

Data are presented as means ± S.E.M. (*N*).

Relative portal heart mass is expressed as a percentage of body mass.

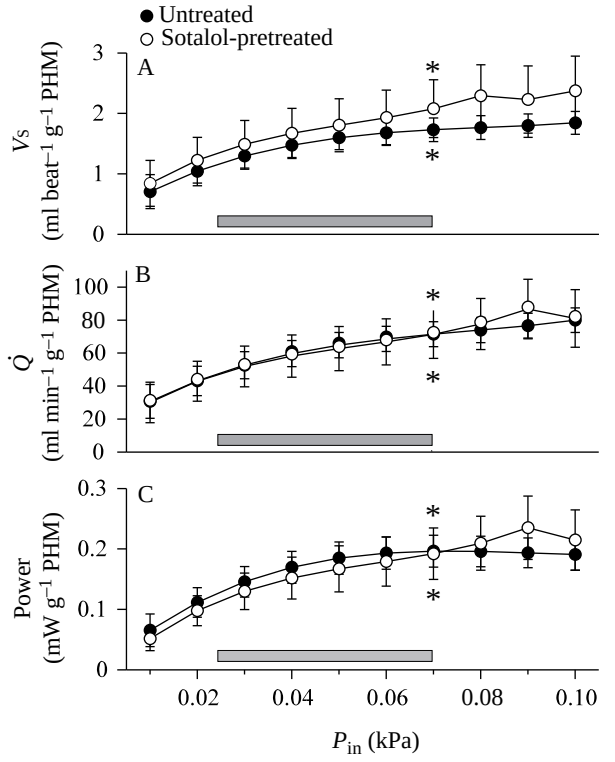

Fig. 1. Effects of increasing preload (P_{in}) on portal heart performance. (A) Stroke volume (*V*S), (B) cardiac output ($\dot{Q}$) and (C) power output. The shaded bar marks the range of venous pressures recorded *in vivo*. * indicates a significant difference between the value at an input pressure of 0.02 kPa and the value at 0.07 kPa (the point where power output plateaued) (P<0.05, N=10 for control curve, N=9 for post-sotalol curve). Values are means ± S.E.M. PHM, portal heart mass.

cannulation that when the membrane covering the heart was cut, the heart rate immediately increased and averaged 7.3±1.8 beats min^{-1} higher than before cutting (32.0±4.3 beats min^{-1}, N=8).

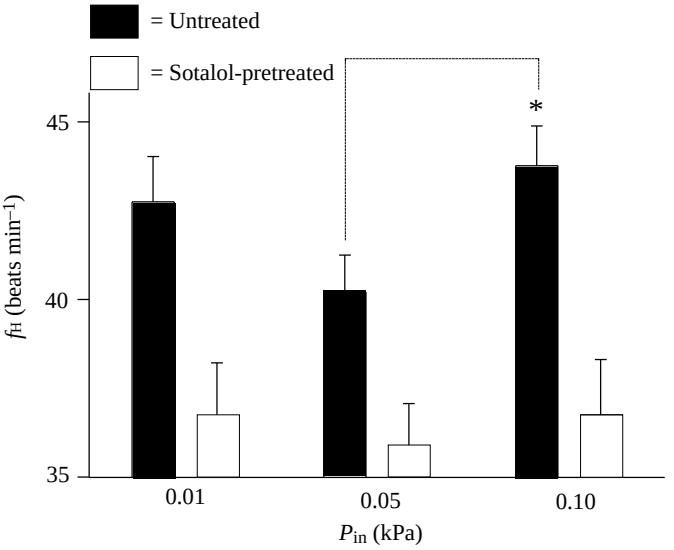

Fig. 2. Portal heart rate (f_H) before (solid bars) and after (open bars) sotalol treatment at three different preset input pressures (P_{in}). * indicates a significant difference between values at 0.05 kPa and 0.1 kPa (P<0.05, N=10). Values are means + S.E.M.

Starling curves

Increased filling pressure caused a marked increase in cardiac output ($\dot{Q}$), stroke volume (*V*S) and power output (Fig. 1). At an input pressure of 0.04 kPa, which was the mean venous pressure observed *in vivo*, *V*S was 1.5±0.2 ml $beat^{-1}$ g^{-1} PHM (N=10). Maximum *V*S was reached at 0.1 kPa and was 1.8±0.2 ml $beat^{-1}$ g^{-1} PHM (N=10). $\dot{Q}$ was 59.7±7.9 ml min^{-1} g^{-1} PHM (N=10) at 0.04 kPa and peaked at 0.1 kPa, measuring 87.5±16.7 ml min^{-1} g^{-1} PHM (N=10). Power output was 0.17±0.03 mW g^{-1} PHM (N=10) at 0.04 kPa and reached a maximum of 0.20±0.03 mW g^{-1} PHM (N=10) at 0.07 kPa. Heart rate (f_H) increased between an input pressure of 0.05 kPa (40.8±1.1 beats min^{-1}) and 0.1 kPa (44.7±1.2 beats min^{-1}) (Fig. 2). No pressure-induced rate changes could be detected after sotalol treatment. Sotalol decreased heart rate significantly, but did not change the responses in *V*S and power output to increased filling pressure (Fig. 1).

Response to afterload

Portal heart rate did not change significantly in response to increased output pressure. *V*S and $\dot{Q}$ decreased significantly when afterload increased above 0.22 kPa, while power output peaked (0.16±0.02 mW g^{-1} PHM) at an output pressure of 0.22 kPa and then decreased. Sotalol pretreatment did not alter any of the responses to increased afterload (Fig. 3).

Effects of adrenaline and sotalol

Adrenaline, administered as a bolus injection (0.1 ml, 10^{-3} mol l^{-1}), accelerated the portal heart. Mean heart rate was 42.0±1.5 beats min^{-1} before adrenaline injection and 45.5±1.6 beats min^{-1} (N=9; P<0.05) after treatment. Input and output pressures were kept constant at physiological levels,

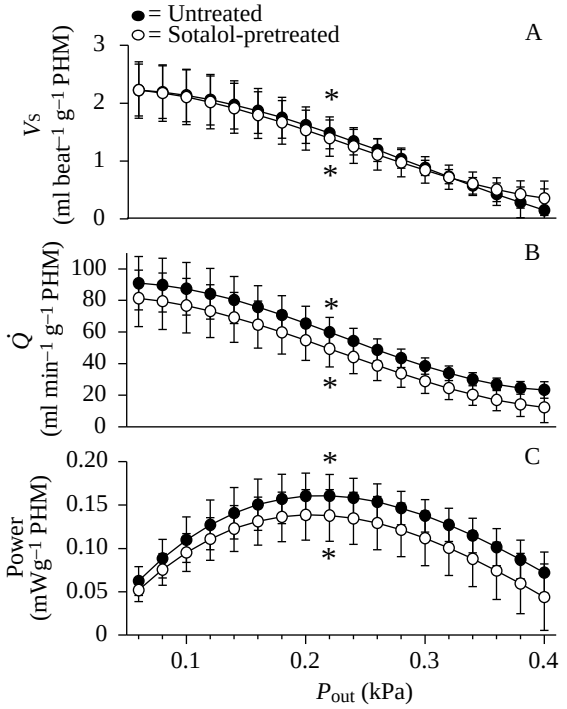

Fig. 3. Effects of increasing afterload (P_{out}) on portal heart performance. (A) Stroke volume (V_S), (B) cardiac output ($\dot{Q}$) and (C) power output. * indicates a significant difference between the value at an output pressure of 0.06 kPa and the value at 0.22 kPa (the point where power output peaked) (P<0.05, N=10). Values are means ± S.E.M. PHM, portal heart mass.

mean input pressure was 0.037±0.004 kPa and mean output pressure was 0.15±0.01 kPa (N=9). The heart was slowed down by addition of the β-adrenoceptor antagonist sotalol to the perfusate (10^{-5} mol l^{-1}). Post-sotalol mean heart rate was 36.6±1.6 beats min^{-1} (N=9).

Discussion

The Starling curve for the portal heart (Fig. 1) indicates that, for the range of venous filling pressures measured, output is increased by 36 % owing to an increased stroke volume. Thus, if an increased venous return elevates central venous pressures, the portal heart is capable of responding with an increased output. However, its function is compromised at output pressures exceeding 0.22 kPa (Fig. 3). In the systemic heart of *E. cirrhatus*, the power output is maximal in the range 1.7–2.0 kPa (Forster, 1989). On a mass-specific basis, the peak power output of the portal heart (approximately 0.2 mW g^{-1} PHM) is not very different from that of the systemic heart (0.37 mW g^{-1} ventricle mass, Forster, 1989), although it operates at about a tenth of the afterload.

The maximum stroke volume of the portal heart, approximately 0.2 ml kg^{-1} body mass, is about 30 % of the maximum stroke volume of the systemic heart in this species (Forster, 1989). It is interesting to note that gut blood flow in teleosts represents approximately 30–40 % of the cardiac output (Axelsson *et al.* 1989; Axelsson and Fritsche, 1991). The hagfish is the only vertebrate with a specific venous pump supplying its liver. The modest fivefold increase in venous pressure downstream from the portal heart must provide the necessary conditions for adequate flow through the liver and back to the systemic heart. In elasmobranch and teleost fish, the blood pressure is high enough to drive the blood back to the heart without the assistance of a venous pump.

The portal heart rate increased by 10 % when the input pressure was raised from 0.05 to 0.1 kPa (Fig. 2). Swimming has been shown to result in an increase in systemic heart rate in *E. cirrhatus*, possibly by an augmented venous return (Forster *et al.* 1988). The venous pressures presented in this study were measured at rest (Table 1). The highest value recorded was 0.07 kPa, and it is possible that swimming can increase venous pressure to well above 0.1 kPa. The present results and observations by Johansen (1960) and Fänge *et al.* (1963) indicate that the portal heart is sensitive to preload. Intrinsic rate regulation has been shown for elasmobranchs (Jensen, 1970), but information concerning teleosts is limited (Farrell, 1984). Several investigations have indicated the existence of a pressure-sensitive pacemaker in hagfish heart (Johansen, 1960; Jensen, 1961; Bloom *et al.* 1963; Chapman *et al.* 1963; Axelsson *et al.* 1990). Jensen (1961) concluded that diastolic volume governs acceleration in the systemic heart ventricle of *E. stoutii*. In contrast, the rate of the isolated perfused systemic heart ventricle of *E. cirrhatus* was not sensitive to changes in preload (Forster, 1989). A possible explanation for these contradictory results is that the preparation used by Forster did not include the sinus venosus, which is the pacemaker region *in vivo* (Davie *et al.* 1987). Considering the relatively low venous pressure of the hagfish, it is likely that earlier methods did not allow the monitoring of rate changes in response to small elevations in input pressure within the physiological range.

There was an increase in f_H of about 7 beats min^{-1} when a layer of tough membrane covering the heart was cut and the portal heart could distend without the restraint of the tissue. This is further evidence for the existence of a pressure-sensitive pacemaker. Cutting the membrane possibly allowed the heart to distend more than it would *in vivo*, emphasizing the response to a rise in preload.

Portal heart rate decreased by 13 % when sotalol was added to the perfusion fluid (Fig. 2). Axelsson *et al.* (1990) showed that the systemic heart of *M. glutinosa* responded to sotalol with a decrease in heart rate and concluded that endogenous catecholamines are important for the normal activity of the heart. That conclusion was further strengthened by the observation that adrenaline caused an increase in systemic heart rate and stroke volume *in vivo*. In the present experiment, injection of 0.1 ml of 10^{-3} mol l^{-1} adrenaline prior to sotalol treatment increased portal heart rate by 8 %. Our results show that portal heart rate in hagfish is tonically stimulated by release/leakage of endogenous catecholamines that act on β-adrenoceptors, but is still able to respond to stimulation. A differential distribution of adrenaline and noradrenaline in the

walls of the portal heart and systemic heart atrium and ventricle (von Euler and Fänge, 1961) indicates that control of the two hearts may be complex (Forster *et al.* 1991).

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