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CONTROL OF THE SYSTEMIC HEART AND THE PORTAL HEART OF *MYXINE GLUTINOSA*

MARIANNE JOHNSSON AND MICHAEL AXELSSON
Department of Zoophysiology, University of Göteborg, Medicinaregatan 18, S-413 90 Göteborg, Sweden

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Summary

The effects of preload and afterload on the performance of the systemic heart of the hagfish *Myxine glutinosa* were investigated before and during sotalol treatment using an *in situ* perfusion technique. Elevation of input pressure (preload) increased flow by means of increased stroke volume and heart rate in accordance with Starling's law of the heart, while increased output pressure (afterload) decreased flow mainly because of decreased stroke volume. Treatment with the β-adrenoceptor antagonist sotalol did not change the quality of the responses to increased preload or afterload, although power output decreased by 40 % and flow rate was reduced by 35 % mainly due to a decrease in heart rate.

Isolated preparations of the systemic heart and the portal heart provided information on the chronotropic effects of different agonists and antagonists. Both the systemic heart and the portal heart were insensitive to adrenergic and cholinergic agonists, adrenocorticotropic hormone and the cholinoceptor antagonist atropine. Sotalol treatment lowered the rate of spontaneous contractions by 30 % in the systemic heart preparation and by 21 % in the portal heart preparation.

This study has given further evidence for the existence of a tonic β-adrenoceptor stimulation of the hagfish systemic heart and portal heart, and demonstrated the importance of that stimulation in maintaining systemic heart performance.

Key words: catecholamines, hagfish, *Myxine glutinosa*, perfusion, portal heart, systemic heart, venous blood pressure.

Introduction

Several investigations in the past have shown that the systemic heart of hagfish behaves as if it is aneural (Augustinsson *et al.* 1956; Jensen, 1965), even though nerves in the heart and adjacent to it have been reported for the Pacific hagfish *Eptatretus stoutii* (Hirsch *et al.* 1964). Experiments performed on the isolated systemic heart of the Atlantic hagfish *Myxine glutinosa* have shown that drugs known to have strong effects on the hearts of other vertebrates (catecholamines, acetylcholine) have almost no effect (Östlund, 1954; Fänge and Östlund, 1954). However, *in vivo* adrenaline application increased systemic heart rate, stroke volume and blood pressure and application of the β-adrenoceptor antagonist sotalol decreased heart rate, which suggests the presence of a tonic β-adrenergic stimulation of the systemic heart (Axelsson *et al.* 1990).

In addition to the systemic heart, hagfish have a portal heart, an accessory heart situated on the hepatic portal vein (Fänge *et al.* 1963) and unique among the vertebrates. Both this and the systemic heart contain high levels of catecholamines (Östlund, 1954; Östlund *et al.* 1961; Bloom *et al.* 1961; von Euler and Fänge, 1961). Jensen (1961) suggested that the hagfish systemic heart may have an endocrine function. The basis for this suggestion was the finding that the systemic heart of *E. stoutii* was insensitive to an extract made from hagfish systemic hearts and thus containing catecholamines.

The insensitivity to catecholamines and the effect of sotalol can be explained if there is a continuous saturated stimulation of the β-adrenoceptors which makes the heart unresponsive to further stimulation although still sensitive to the antagonistic action of sotalol (Axelsson *et al.* 1990). This would be consistent with the observation that reserpine, which depletes the catecholamine content of the heart, had a negative chronotropic effect on the perfused systemic heart of *Myxine glutinosa*, but subsequent addition of catecholamines to the perfusion fluid caused the heart to resume beating (Bloom *et al.* 1963). Release or leakage of catecholamines could constitute a means of regulating or maintaining heart function and/or could act on structures downstream (Forster *et al.* 1991). Perry *et al.* (1993) have shown that the cholinoceptor agonist carbachol can elicit catecholamine release in an *in situ* saline-perfused hagfish heart, although some other mechanism, possibly involving pituitary hormones, may operate *in vivo*.

Increased filling pressure/volume has been observed to accelerate the systemic heart of *M. glutinosa* and *E. stoutii* (Johansen, 1960; Jensen, 1961; Bloom *et al.* 1963; Chapman *et al.* 1963), although information on pressure is limited. In a

study of the isolated perfused systemic heart of *E. cirrhatus*, heart rate remained unchanged as either preload or afterload was varied (Forster, 1989).

Some aspects of possible control mechanisms of the systemic heart and the portal heart were investigated in the present study. The effects of preload and afterload on the performance of the systemic heart were studied using an *in situ* perfusion technique which allowed accurate pressure measurements and calculations of power generation. The blood pressure in the posterior cardinal vein, which supplies the systemic heart, was measured in resting animals, and the chronotropic effects of different agonists and antagonists were investigated in isolated systemic and portal hearts.

Materials and methods

Experimental animals

Hagfish (*Myxine glutinosa* L.) were caught in the Gullmars Fiord on the Swedish west coast and were transported to Göteborg. Animals of either sex, weighing between 20 and 100 g, were used in the experiments. Prior to use, animals were kept in aquaria with circulating sea water at 10–11 °C. The animals were killed by decapitation.

In situ *perfusions*

A large ventral incision revealed the systemic heart and connecting vessels of freshly killed animals. Saline-filled cannulae (PE200) were advanced into the ventral aorta and the posterior cardinal vein (PCV) and firmly secured. The cannulae were of the same construction as those used by Franklin and Axelsson (1994), making pressure measurements close to the heart possible. This is an advantage compared with methods in which the pressure is measured in a connecting tube some distance from the preparation and where the actual pressure must be calculated by taking into consideration factors such as the resistance of the connecting tubes. In this study, pressure was measured using a PE90 catheter tipped with a short piece of PE10 tubing inserted into the PE200 cannulae. The PE90 tubing was connected to a Honeywell pressure transducer (model 156PC06GW2).

The inflow cannula was connected to a reservoir containing Ringer's solution (perfusion fluid) and a constant-pressure device which could be raised or lowered to set the input pressure. The Ringer's solution had the following composition ($g\,l^{-1}$): 27.7 NaCl, 0.6 KCl, 0.73 $CaCl_2{\cdot}2H_2O$, 0.72 $MgSO_4{\cdot}7H_2O$, 1.82 $MgCl_2{\cdot}6H_2O$, 0.08 NaH_2PO_4, 1.26 $NaHCO_3$, 1 glucose. Noradrenaline ($3\,nmol\,l^{-1}$) and adrenaline ($1\,nmol\,l^{-1}$) were added to the perfusion fluid to provide physiological catecholamine levels (see Perry *et al.* 1993). The Ringer was bubbled with 0.3 % CO_2 in air and kept at 10 °C. The outflow cannula was connected to a tube which could be adjusted vertically to alter the afterload. Input and output pressures and heart rate were displayed on a chart recorder (Grass Polygraph model 79). Heart rate was derived from the pulsatile output pressure signals using a Grass 7P44 tachograph unit. AD/DATA software (P. Thorén, Hässle AB, Sweden) was used to sample and store the data on a personal computer. The digital display of the computer allowed accurate calibration and pressure measurements. The pressure transducers were calibrated against a static water column, where zero was set to the level of Ringer's solution in the organ bath in which the preparation was immersed.

Starling curves were constructed by gradually increasing the inflow pressure until no further increase in flow rate was seen. Flow rate was determined gravimetrically. The output pressure was kept constant close to physiological values for ventral aortic pressure (see Axelsson *et al.* 1990). Power curves could be determined by keeping the inflow pressure constant at a nearly physiological level and gradually increasing the output pressure. The β-adrenoceptor antagonist sotalol ($10^{-5}\,mol\,l^{-1}$) was added to the perfusing Ringer's solution and the effects of preload and afterload were investigated during sotalol treatment, i.e. each preparation acted as its own control.

Individual curves where cardiac variables (stroke volume, flow rate and power output) were plotted against input or output pressures, and fitted to the data using a third-order polynomial, made it possible to construct composite graphs with data generated at set pressure intervals (see Franklin and Axelsson, 1994). Stroke volume and flow rate were normalised per kilogram body mass (BM) and power output was normalised per gram ventricle mass (VM).

The Wilcoxon signed-ranks test was used to evaluate statistically significant differences ($P<0.05$). Comparisons were made between the value at an input pressure of 0.02 kPa and the value where the input pressure corresponded to the mean *in vivo* venous blood pressure (preload curves) or the value where the output pressure corresponded to the peak of the power output curve (afterload curves). Comparisons between untreated and sotalol-treated preparations were made at the same points. Data are presented as means ± S.E.M.

Venous blood pressure

Hagfish were anaesthetised in a mixture of $0.4\,g\,l^{-1}$ benzocaine and $0.4\,g\,l^{-1}$ MS222. A small ventral incision close to the tail exposed the PCV underneath the intestine. The vein was cannulated with PE50 tubing filled with 3 % NaCl containing 50 i.u. of heparin. Animals were allowed to recover for 24 h prior to venous pressure measurement. The animals were transferred to an experimental tank and the cannula was attached to a Honeywell pressure transducer (model 156PC06GW2) calibrated against a static water column, where zero was set to the water level in the experimental tank. Pressures were recorded on a Grass recorder. Data are presented as means ± S.E.M.

In vitro *preparations*

The systemic heart and the portal heart were removed from freshly killed animals and suspended in organ baths containing the Ringer's solution described above. The isolated hearts were attached to Grass isometric force transducers (FT03) connected to a Grass Polygraph recorder system (model 79). Calibration of the system was made using a 1 g weight. The rate of

contraction was obtained from the spontaneously contracting hearts using a Grass 7P44 tachograph unit. AD/DATA software was used to sample data.

Chronotropic effects of adrenaline and acetylcholine before and after sotalol (10^{-5} mol l^{-1}) treatment were investigated by cumulative addition of these agonists (10^{-9} to 10^{-5} mol l^{-1}) to the organ baths, except for the addition of acetylcholine after sotalol treatment (10^{-5} mol l^{-1}), when only one concentration was tested (10^{-5} mol l^{-1}). Chronotropic effects of carbachol before and after atropine (10^{-5} mol l^{-1}) treatment were investigated by cumulative addition of carbachol (10^{-9} to 10^{-3} mol l^{-1}). Chronotropic effects of adrenocorticotropic hormone (ACTH) were investigated by cumulative addition of ACTH (10^{-8} to 10^{-6} mol l^{-1}) to the organ baths. The Wilcoxon signed-ranks test was used to evaluate statistically significant differences (P<0.05). Data are presented as means ± S.E.M.

Drugs

The following drugs were used: adrenaline bitartrate (Sigma), arterenol bitartrate (Sigma), sotalol hydrochloride (Bristol Laboratories, Bristol-Myers Squibb), carbamylcholine chloride (Sigma), atropine sulphate (Sigma) and porcine adrenocorticotropic hormone (Sigma). The drugs were dissolved in distilled water to the appropriate concentrations.

Results

In situ *perfusions*

Increased input pressure (P_{in}) produced an increased flow rate ($\dot{Q}$) through the systemic heart by an increase in both stroke volume (V_S) (Fig. 1) and heart rate (f_H) (Fig. 2). $\dot{Q}$ was 29.4±3.0 ml min^{-1} kg^{-1} BM and V_S was 1.3±0.2 ml beat^{-1} kg^{-1} BM in untreated preparations at a P_{in} of 0.1 kPa, which corresponds to the mean *in vivo* venous blood pressure (0.10±0.02 kPa). Table 1 summarizes the results from venous pressure measurements and gives mean values for cardiovascular variables at physiological pressures. Treatment with the β-adrenoceptor sotalol (10^{-5} mol l^{-1}) resulted in a significant decrease in $\dot{Q}$ to 19.1±2.4 ml min^{-1} kg^{-1} BM.

Comparison of minimum and maximum values of f_H occurring during the course of P_{in} elevation for each preparation shows that both the untreated and sotalol-treated preparations are pressure-sensitive at relatively low pressures. The lowest f_H values recorded were 23.0±1.0 beats min^{-1} at a P_{in} of 0.020±0.002 kPa for the untreated preparations and 20.5±1.5 beats min^{-1} at a P_{in} of 0.020±0.002 kPa for sotalol-treated hearts. The highest f_H values were 26.7±1.2 beats min^{-1} at a P_{in} of 0.10±0.02 kPa and 24.4±1.5 beats min^{-1} at a P_{in} of 0.15±0.02 kPa, respectively, for untreated and sotalol-treated preparations. Sotalol treatment significantly decreased the f_H by 2.8±1.3 beats min^{-1}. The P_{in} ranges where minimum and maximum f_H were observed were not very different between untreated and sotalol-treated heart preparations (Fig. 2).

Power output increased in response to elevated P_{in} (Fig. 1) in both untreated and sotalol-treated preparations, although not to the same extent. There was a significant difference between the untreated preparations, in which the power output was 0.50±0.06 mW g^{-1} VM, and the sotalol-treated preparations, in which the power output was 0.30±0.04 mW g^{-1} VM, at an input pressure of 0.1 kPa.

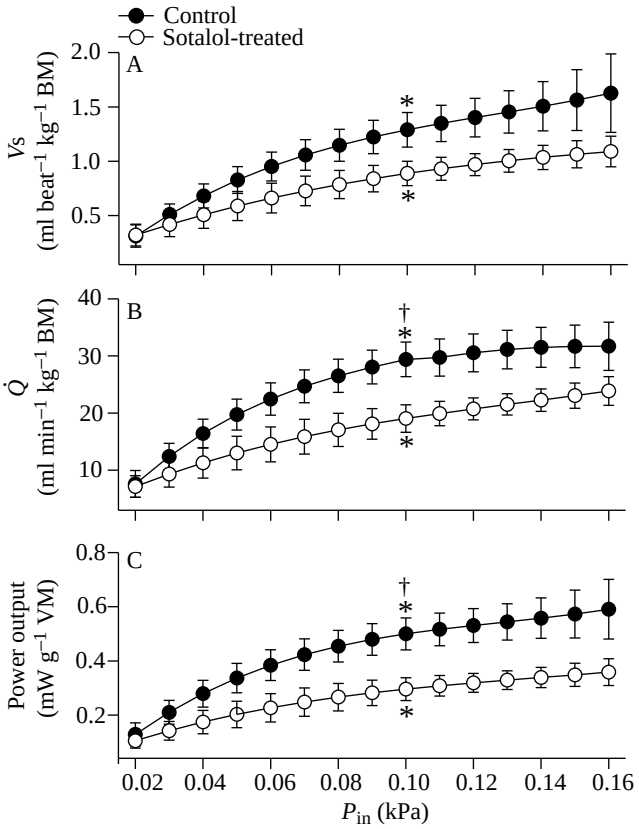

Fig. 1. Effects of increasing preload (P_{in}) on the performance of the systemic heart of *Myxine glutinosa*: (A) stroke volume (V_S), (B) flow ($\dot{Q}$) and (C) power output. Asterisks indicate statistically significant differences (P<0.05) from the point in the diagram where P_{in}=0.02 kPa. Daggers indicate statistically significant differences between untreated (filled circles, N=12–13) and sotalol-treated values (open circles, N=11). Values are means ± S.E.M.

$\dot{Q}$ decreased significantly as a result of a decrease in V_S when the output pressure (P_{out}) was raised (Fig. 3) and, in sotalol-treated hearts, a small decrease in f_H (not shown). At a P_{out} of 1.8 kPa, the value at which power output peaked, $\dot{Q}$ was 16.5±1.8 ml min^{-1} kg^{-1} BM and V_S was 0.68±0.09 ml beat^{-1} kg^{-1} BM in the untreated preparations. Sotalol treatment produced a significant decrease in $\dot{Q}$ (10.5±2.1 ml min^{-1} kg^{-1} BM) due to a decrease in f_H by 4.8±1.1 beats min^{-1} (N=11), while V_S remained unchanged. Power output was substantially lower in the sotalol-treated heart (0.31±0.07 mW g^{-1} VM) than in the untreated heart (0.54±0.07 mW g^{-1} VM).

In vitro *preparations*

f_H for the systemic heart and the portal heart were 19.3±1.5 beats min^{-1} (N=8) and 19.7±1.0 beats min^{-1} (N=9),

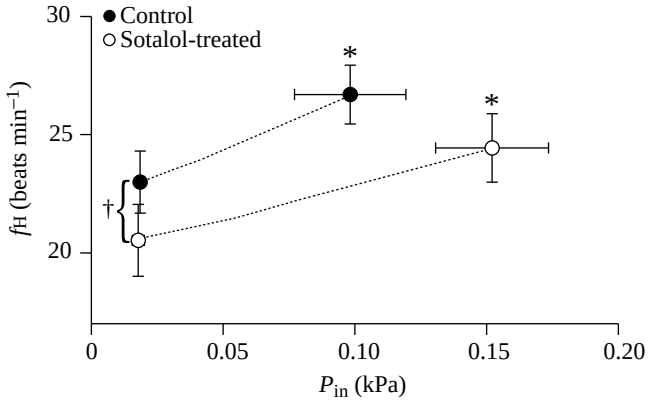

Fig. 2. Effects of increasing preload (P_{in}) on the heart rate (f_H) of the systemic heart of *Myxine glutinosa*. Asterisks and daggers indicate statistically significant differences (P<0.05) within and between treatments, respectively. Filled circles denote untreated preparations (N=12–13) and open circles denote sotalol-treated preparations (N=10). Values are means ± S.E.M.

respectively, before addition of adrenaline. Adrenaline had no effect on f_H while the β-adrenoceptor antagonist sotalol decreased systemic f_H to 13.5±0.8 beats min^{-1} (N=7) and portal f_H to 15.5±1.0 beats min^{-1} (N=8). Fig. 4 illustrates the effect of sotalol added to the Ringer's solution bathing the preparations expressed as mean values taken from individual preparations over time. Moderate concentrations (10^{-9} to 10^{-6} mol l^{-1}) of adrenaline after sotalol treatment did not elicit any response, although a small acceleration could be observed in both the systemic heart and the portal heart at adrenaline concentrations between 10^{-6} and 10^{-5} mol l^{-1}.

Acetylcholine application at 10^{-6} and 10^{-5} mol l^{-1} decreased portal f_H by 3.0±0.7 and 2.1±0.6 beats min^{-1}, respectively (N=8), and also decreased systemic f_H by 1.8±0.4 beats min^{-1} (N=8), but in the latter case the difference was significant only at an acetylcholine concentration of 10^{-6} mol l^{-1} (results not shown). Acetylcholine (10^{-5} mol l^{-1}) application after sotalol treatment had no significant effect on portal f_H, but produced a significant decrease (1.6±0.4 beats min^{-1}) in systemic f_H (N=8). Neither the cholinergic agonist carbachol nor the cholinergic antagonist atropine had any effect on the f_H of the systemic heart (N=12) or the portal heart (N=8). A small but significant negative chronotropic effect on the systemic heart was caused by carbachol (10^{-3} mol l^{-1}) after atropine treatment (N=10). ACTH (10^{-8} to 10^{-6} mol l^{-1}) had no effect on the f_H of either heart (N=8).

Table 1. *A summary of recorded and calculated cardiovascular variables at physiological preload (P_{in}=0.10 kPa) and afterload (P_{out}≈1.0 kPa)*

Body mass (kg)	0.048±0.006 (N=13)
Ventricle mass (g)	0.044±0.004 (N=13)
Relative ventricle mass (%)	0.096±0.005 (N=13)
Mean venous pressure (kPa)	0.10±0.02 (N=10)
Flow rate (ml min^{-1} kg^{-1} BM)	29.4±3.0 (N=13)
Stroke volume (ml beat^{-1} kg^{-1} BM)	1.3±0.2 (N=13)
Power output (mW g^{-1} VM)	0.50±0.06 (N=13)

Values are means ± S.E.M.
BM, body mass; VM, ventricle mass.

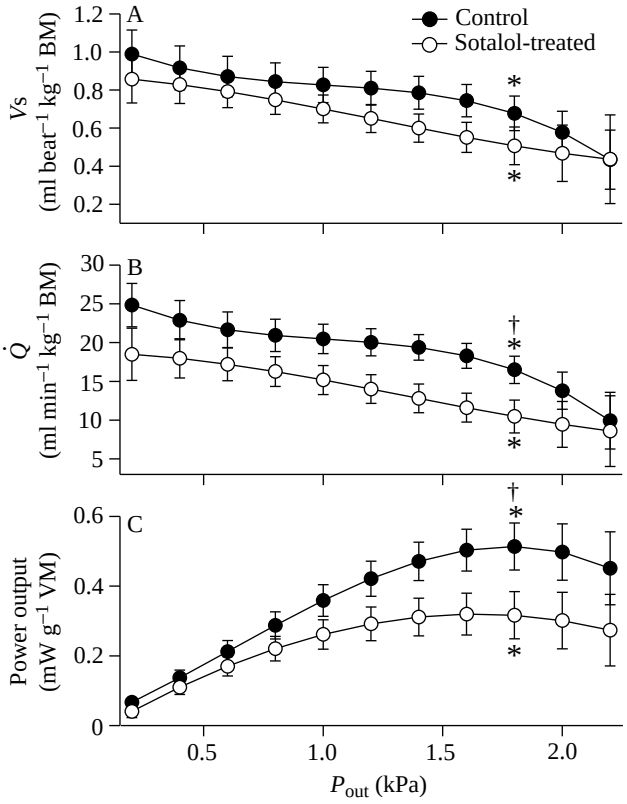

Fig. 3. Effects of increasing afterload (P_{out}) on the performance of the systemic heart of *Myxine glutinosa*: (A) stroke volume (V_S), (B) flow ($\dot{Q}$) and (C) power output. Asterisks indicate statistically significant differences (P<0.05) from the point in the diagram where P_{out}=0.2 kPa. Daggers indicate statistically significant differences between untreated (filled circles, N=11) and sotalol-treated values (open circles, N=11). Values are means ± S.E.M.

Discussion

This study is the first evaluation of the power-generating capacity of the *Myxine glutinosa* systemic heart. The experiments investigating the effects of different agonists and antagonists on the systemic heart are not without precedent, although previous reports are inconsistent and, in the case of the portal heart, scarce.

The systemic heart and the portal heart of *Myxine glutinosa* are comparatively insensitive to adrenergic and cholinergic agonists. It has been suggested that the chromaffin cells of the systemic heart are able to release catecholamines in response to cholinergic agonists, and possibly ACTH (Perry *et al.* 1993); however, it was not possible to detect any effects of endogenous catecholamines on the isolated heart preparations in the present study.

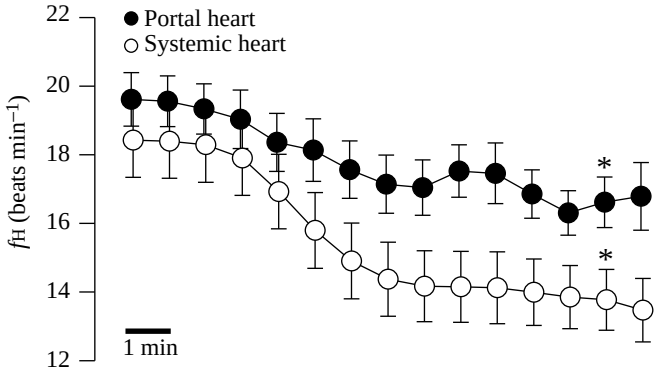

Fig. 4. Effect of sotalol (10^{-5} mol l^{-1}) treatment on the rate (f_H) of the systemic heart and the portal heart preparations (N=8). Asterisks indicate statistically significant differences from the value at time zero, when sotalol was added (P<0.05). Values are means ± S.E.M.

The effects of the cholinergic agonists are contradictory. Acetylcholine at high concentrations decreases f_H of the systemic heart by 9 % and of the portal heart by 15 %, while carbachol has no effect. The results of Östlund (1954) and Fänge and Östlund (1954) on the systemic heart showed no effect of acetylcholine, which is in agreement with the lack of innervation of the systemic heart (Augustinsson *et al.* 1956). The physiological relevance of the chronotropic effects of acetylcholine in this study and the finding that catecholamine release can be cholinoceptor-mediated remain unresolved.

In accordance with *in vivo* studies on the systemic heart (Axelsson *et al.* 1990), sotalol decreased systemic f_H by 30 % and portal f_H by 21 % in the isolated heart preparations and decreased f_H by 12 % in the *in situ* perfused systemic heart. This provides further evidence for a tonic β-adrenoceptor stimulation of the systemic heart and also demonstrates that β-adrenoceptors on the portal heart are tonically stimulated. Endogenous catecholamine stores appear to saturate cardiac β-adrenoceptors as no stimulatory effect of adrenaline could be detected on the isolated hearts.

The previously reported accelerating effect of adrenaline on the systemic heart *in vivo* (Axelsson *et al.* 1990) is not consistent with the insensitivity to adrenaline demonstrated in this study and with observations by Östlund (1954) and Fänge and Östlund (1954). Adrenaline may cause vasoconstriction and thereby augment venous return to the heart *in vivo* and, by means of a pressure-sensitive mechanism, increase f_H. Intrinsic rate regulation has been shown in elasmobranchs (Jensen, 1970), although information concerning teleosts is scarce (Farrell, 1984). Heart rate in *E. cirrhatus* increases during swimming, possibly caused by an increased venous return (Forster *et al.* 1988). In the present study, an increase in f_H of 14 % was found when P_{in} was raised within the range of venous blood pressures observed in resting hagfish. In the *in situ* perfused portal heart of *E. cirrhatus*, an increase in P_{in} caused a β-adrenoceptor-dependent increase in the rate of contraction (Johnsson *et al.* 1996). The systemic f_H of *E. cirrhatus* did not change in response to increased preload (Forster, 1989), possibly because the preparation did not include the sinus venosus, which is the pacemaker region *in vivo* (Davie *et al.* 1987).

The power output at a P_{in} of 0.1 kPa, which was the mean venous pressure in resting animals (Table 1), and at a P_{out} close to mean aortic pressure was 0.50±0.06 mW g^{-1} VM. This is considerably higher than the reported maximal power output achieved by the systemic heart of *E. cirrhatus* (0.37±0.03 mW g^{-1} VM) (Forster, 1989). Estimates of resting power output for *Myxine glutinosa* by Driedzic *et al.* (1987) (0.05 mW g^{-1} heart mass) and Axelsson *et al.* (1990) (0.15 mW g^{-1} VM) are lower than the present results, probably because of the different methods employed. Sotalol treatment reduced power output by 40 % and flow rate by 35 %, which again supports the suggestion that β-adrenoceptor stimulation is vital for the normal function of the heart (Axelsson *et al.* 1990).

There was a fourfold increase in $\dot{Q}$ for the systemic heart preparation when P_{in} was elevated from 0.02 to 0.1 kPa. This indicates that augmented venous pressure could modulate V_S as well as f_H. When afterload was increased from 0.2 to 1.8 kPa (P_{in}≈0.05 kPa), $\dot{Q}$ decreased by 33 % due to a 31 % decrease in V_S, although the systemic heart was still able to pump approximately 17 ml min^{-1} kg^{-1} BM and to develop a V_S of approximately 0.7 ml beat^{-1} kg^{-1} BM.

In conclusion, endogenous catecholamines are important for the normal activity of the systemic heart and the portal heart of *Myxine glutinosa*, as illustrated by the effect of sotalol on f_H, $\dot{Q}$ and power output. The previously reported increase in f_H and $\dot{Q}$ caused by adrenaline *in vivo* (Axelsson *et al.* 1990) was probably a secondary effect due to an increased venous return to the heart. The systemic heart responds to increased preload by increased $\dot{Q}$ in accordance with Starling's law of the heart. $\dot{Q}$ was augmented by means of an increased V_S and a β-adrenoceptor-independent pressure-sensitive acceleration of the heart.

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