

INTRINSIC MECHANICAL PROPERTIES OF THE PERFUSED RAINBOW TROUT HEART AND THE EFFECTS OF CATECHOLAMINES AND EXTRACELLULAR CALCIUM UNDER CONTROL AND ACIDOTIC CONDITIONS

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SUMMARY

A perfused rainbow trout heart was developed which generated its own intrinsic heart rate and a physiological power output. This preparation was used to examine the intrinsic mechanical properties of the trout heart, the dose-response effects of catecholamines and extracellular calcium on these properties, and the effects of catecholamines and extracellular calcium during exposure to acidotic conditions. The trout heart was relatively pressure-insensitive to a physiological range of ventral aortic pressures. Preload exerted an important control over cardiac output through the Starling response. Heart rate was independent of both these intrinsic mechanisms. The intrinsic mechanical capabilities of the trout heart were greater than those observed previously in less active, benthic teleosts. Physiological concentrations of catecholamines significantly improved cardiac contractility through positive inotropy and chronotropy. Adrenaline was more potent than noradrenaline, indicating that these effects were mediated by β_2 -adrenoceptors. Elevated extracellular calcium produced only a modest improvement of cardiac contractility compared to that produced by adrenaline. Positive inotropy and negative chronotropy were observed with elevated extracellular calcium. Extracellular acidosis always reduced cardiac contractility through negative chronotropy and inotropy. Extracellular calcium improved the inotropic state of the acidotic heart and restored contractility, but the overall improvement of cardiac performance was compromised by an accompanying negative chronotropy. Physiological levels of adrenaline improved cardiac performance during extracellular acidosis. The roles of catecholamines and extracellular calcium are discussed with respect to post-exercise cardiac performance in trout.

INTRODUCTION

Recent work with *in situ*, perfused hearts from two benthic teleosts (sea raven, *Hemirhamphus americanus*, and ocean pout, *Macrozoarces americanus*) demonstrated the heart's intrinsic ability to maintain stroke volume independent of

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physiological ventral aortic pressures, i.e. homeometric regulation (Farrell, MacLeod & Driedzic, 1982; Farrell, MacLeod, Driedzic & Wood, 1983). The work also confirmed the importance of the Starling response of the heart. Small changes in the filling pressure of the heart (preload) generated large changes in stroke volume (SVH) without significantly affecting heart rate (fh). Preload is therefore an important determinant of cardiac output (\dot{V}_b) through its effect on SVH.

While hearts from benthic teleosts have similar intrinsic mechanical properties to mammalian hearts (Farrell, 1984, 1985a), mammalian hearts are capable of generating a much higher power output (Gibbs & Chapman, 1979). This, of course, parallels the much higher flow and pressure work loads placed on the mammalian, compared with the benthic teleost, heart. More active teleosts should therefore have a greater intrinsic capacity for pressure and flow development compared with inactive species. Homeometric regulation has not been examined previously in an active teleost. The Starling response was examined in isolated, perfused hearts from rainbow trout (Bennion, 1968, as reported by Randall, 1970), but the preloads were supraphysiological. This led to the conclusion that the Starling response was probably of secondary importance to neurohumoral factors with respect to changing SVH (Jones & Randall, 1978). The intrinsic mechanical properties of the perfused heart from rainbow trout are the subject of the present study.

Extrinsic control of cardiac performance is also important in fish. The roles of extracellular calcium (Ca_e^{2+}) and catecholamines are not entirely clear. In isolated ventricular strips, catecholamines and Ca_e^{2+} both improve cardiac contractility (Gesser & Jorgensen, 1982; Gesser, Andresen, Brams & Sund-Laursen, 1982). In intact fish and isolated perfused trout hearts, catecholamines increase fh (Randall, 1970). Catecholamines also slightly improve the Starling response in isolated perfused trout hearts, but have equivocal effects on SVH when injected into intact fish (Farrell, 1984). In perfused sea raven hearts, catecholamines increase fh, but do little to modify the Starling response (Farrell *et al.* 1983). With respect to Ca_e^{2+} , the sea raven heart has an absolute requirement for Ca_e^{2+} such that sub-physiological levels (below 2 mmol l^{-1}) limit \dot{V}_b , but levels above 2 mmol l^{-1} only modestly improve \dot{V}_b (Farrell, Hart, Wood & Driedzic, 1984). In this study, we use the perfused trout heart to investigate and compare the dose-response effects of catecholamines and Ca_e^{2+} in an intact heart and resolve whether different effects are found in active and inactive teleosts.

During stress such as exhaustive exercise, extrinsic factors are apparently needed to maintain normal levels of cardiac contractility. The extracellular acidosis, which accompanies exhaustive activity, is largely respiratory in origin during the first 0.5 h and should severely debilitate cardiac performance (Gesser & Poupa, 1983; Farrell, 1984). A reduction of extracellular pH by 0.4 pH units reduced force development in ventricular strips to 60–70% of control (Gesser & Poupa, 1978; Gesser & Jorgensen, 1982; Gesser *et al.* 1982). A change in contractility of this magnitude in the intact trout heart would preclude the increase in \dot{V}_b that has been observed after exercise in trout (Neumann, Holeton & Heisler, 1983). The effect of extracellular acidosis on the intact, perfused trout heart has not been examined. However, the Starling

response to a perfused sea raven heart was severely attenuated by a decrease in extracellular pH from 7.9 to 7.4 (Farrell *et al.* 1983, 1984). In trout ventricular strips, Ca_e^{2+} restored force development more effectively than adrenaline during acidosis (pH 7.0 at 22°C) (Gesser *et al.* 1982; Nielsen & Gesser, 1984). In contrast, in a perfused sea raven heart, Ca_e^{2+} was relatively ineffective at restoring cardiac performance during acidosis compared to adrenaline (pH 7.4 at 10°C). The question of possible species differences between trout and sea raven, with respect to adrenaline and Ca_e^{2+} sensitivity during extracellular acidosis, is addressed by examining the effects of adrenaline and Ca_e^{2+} during extracellular acidosis in the perfused trout heart.

An *in situ* perfused trout heart, similar to that used previously with sea raven and ocean pout, was developed for the present study. This permitted direct comparisons between intact, perfused hearts from active and inactive teleosts. Furthermore, because the perfused heart preparation developed a myocardial power output comparable to that of the intact trout, extrapolations back to the intact animal could be made with a measure of confidence.

MATERIALS AND METHODS

Animals

Rainbow trout, weighing 300–700 g, were obtained from local hatcheries and held at Simon Fraser University in fibreglass tanks, supplied with dechlorinated tap water (7–12°C). The fish were fed commercial trout chow *ad libitum* three times a week.

Heart preparation

Fish were anaesthetized (a buffered solution of 0.2 g l⁻¹ ethyl *m*-amino benzoate, Sigma Chemicals, St Louis) and placed on an operating sling where the gills were irrigated with a buffered, aerated anaesthetic solution (0.07 g l⁻¹ ethyl *m*-amino benzoate) cooled to about 4°C. The fish were injected with 60 i.u. heparin in 0.4 ml of saline (Sigma Chemicals) *via* the caudal vessels. This prevented the formation of blood clots when the input cannula was placed in the sinus venosus. The procedure for preparing the heart followed that described for the sea raven (Farrell *et al.* 1982). There was minimal interruption of flow to the heart and no direct physical manipulation of the heart during the preparation. The input cannula was introduced into the sinus venosus through the hepatic vein and saline perfusion was begun immediately. Removal of the stomach and intestine from the fish resulted in consistent and reliable placement of the input cannula. (Partial occlusion of the tip of the input cannula by tissue was indicated by a preload ≥ 1 cmH₂O (1 cmH₂O = 98.1 Pa) under control conditions. The cannula was repositioned to remedy this.) The silk thread securing the input cannula also occluded the hepatic veins. Separate silk ligatures were tied tightly around each ductus Cuvier to occlude these veins and to crush the cardiac branches of the vagus nerve. Small veins to the sinus venosus were not individually tied off, as was done in the sea raven. There was no detectable loss of perfusate during perfusate recirculation for 25 min in subsequent

experiments. Therefore, backflow into small vessels was assumed to be negligible. The output cannula was placed in the ventral aorta. The compact myocardium, which normally receives blood from the coronary vessels, was not perfused. Preparation time was 10–15 min.

The fish were transferred and fully immersed in a Cortland saline bath maintained at 10°C for the experiments. The input cannula was switched from the temporary perfusate reservoir to a constant pressure head receiving perfusate from water-jacketed reservoirs also at 10°C. The output cannula was connected to the output pressure head. An in-line flow probe measured cardiac output. The heights of the input and output pressure heads were varied to change the preload and diastolic afterload, respectively. Input and output pressures were measured through saline-filled sidearms.

Perfusate

The perfusate was a modification of Cortland saline (Wolf, 1963) and its composition was (in g l^{-1}): 7.25 NaCl, 0.23 KCl; 0.367 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; 0.014 $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$; 0.33 Na_2HPO_4 ; 0.23 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 1.0 dextrose; 10 polyvinylpyrrolidone ($M_r = 40\,000$, added as a colloid substitute). The control perfusate was gassed with 0.5% CO_2 in 99.5% O_2 and the pH was adjusted to 7.9 (Kiceniuk & Jones, 1977) with NaHCO_3 (approx. 1 g l^{-1}). The pH of the perfusate was measured with an IL 113 acid–base analyser at 10°C.

Protocols

Control condition

Each heart was initially equilibrated to the saline bath and control perfusate for 5–15 min. During this period, \dot{V}_b became steady with \dot{V}_b approximately $15\text{ ml min}^{-1}\text{ kg}^{-1}$ and mean output pressure was set at $50\text{ cmH}_2\text{O}$. Heart rate was determined by the intrinsic rhythm of the pacemaker and varied between preparations. Therefore, the control \dot{V}_b was set by adjusting the preload to alter SVH. In intact trout, resting \dot{V}_b is $17.6\text{ ml min}^{-1}\text{ kg}^{-1}$ and ventral aortic blood pressure is $47\text{--}54\text{ cmH}_2\text{O}$ (Kiceniuk & Jones, 1977).

Series A: homeometric regulation

The independence of stroke volume with respect to pressure development was examined by increasing afterload with preload constant ($N = 8$). Control perfusion conditions were set up as above. Mean output pressure was lowered from 50 to $30\text{ cmH}_2\text{O}$. Diastolic afterload was then increased in a stepwise fashion to generate mean output pressures of approximately 40, 50, 55, 60 and $65\text{ cmH}_2\text{O}$. Stable cardiovascular responses were recorded after 2–4 min at each afterload.

Series B: time controls

In subsequent series, preload was raised to elicit the maximal increase in \dot{V}_b and thereby assess inotropic potential with each perfusate. A standardized protocol was

used which consisted of four steps: (a) a 4- to 5-min equilibration to the perfusate with a control \dot{V}_b , (b) an increase in preload of about 1 cmH₂O for 2–4 min, (c) a further increase in preload to produce the maximal stable \dot{V}_b , and (d) a return to control \dot{V}_b by reducing preload before a new perfusate was introduced. The observed change in cardiac performance *versus* preload, as presented in subsequent figures, is referred to as the Starling response. The maximum power output that was generated when preload evoked no further increase in \dot{V}_b is used as a relative measure of maximum power output.

Each heart acted as its own control, and the Starling response was repeated up to five times (series C, D) on the same heart. Series B acted as a control for this procedure. It examined whether cardiac performance changed significantly with time after five repetitions of the Starling response ($N=6$). Control perfusate was used throughout the time control experiments. The 4- to 5-min period between each repetition and the total duration of the trial (approximately 30 min) were similar to the longest experiments in series C and D.

Series C: catecholamines

Cumulative dose–response curves were established for DL-adrenaline HCl, DL-noradrenaline HCl, DL-isoproterenol HCl and DL-phenylephrine (Sigma Chemicals). One agonist drug was tested per preparation and six to eight preparations were used for each drug. The drugs were prepared immediately before use and were added directly to the perfusate reservoir. Separate perfusate reservoirs were used for each concentration. At each concentration (1 nmol l⁻¹, 10 nmol l⁻¹, 0.1 μ mol l⁻¹ and 1 μ mol l⁻¹), the heart was allowed to equilibrate for 4–5 min before the inotropic potential of the heart was assessed by raising preload. Blood catecholamine levels in salmon increase to approximately 50 nmol l⁻¹ following burst activity (Primmitt, Randall, Mazeaud & Boutilier, 1986).

Series D: extracellular calcium

Calcium dose–response curves were generated by varying the [Ca_e²⁺] of the perfusate ($N=17$). Calcium concentrations of 1.5, 2.0, 3.0, 4.0 and 5.0 mmol l⁻¹ were tested. Plasma calcium levels in salmonids are usually between 2 and 4 mmol l⁻¹ (Holmes & Donaldson, 1969). The equilibration time was 4–5 min. Separate reservoirs were used for each [Ca_e²⁺] and the inotropic potential assessed at each [Ca_e²⁺] by increasing preload.

Series E: extracellular acidosis

Blood pH in trout decreases from 7.88 to 7.37 immediately after burst exercise and is below 7.5 after 0.5 h (Turner, Wood & Höbe, 1983). This experimental series examined the effect of pH 7.4 perfusate on cardiac performance in the presence and absence of either adrenaline or additional Ca_e²⁺. The experimental protocol involved equilibration with control perfusate, determination of the Starling response, and return to resting \dot{V}_b before changing to the acidotic perfusate. The acidotic perfusate

was gassed with 1.8% CO₂ (balance O₂) and 1.2 g l⁻¹ NaHCO₃ was added to adjust the pH to 7.4. After a 5- to 6-min equilibration to the acidotic perfusate, the Starling response was redetermined. A third and final evaluation of the Starling response was made following a 5- to 6-min equilibration to acidotic perfusate containing either 4 mmol l⁻¹ calcium ($N=5$) or 10 nmol l⁻¹ adrenaline ($N=6$). DL-adrenaline was added to the perfusate reservoir immediately before use. Additional CaCl₂·2H₂O was used to increase the [Ca_e²⁺] from 2.5 to 4.0 mmol l⁻¹ and the different perfusates were kept in separate, water-jacketed reservoirs.

Instrumentation

Flow was measured with an electromagnetic flow meter (Zepeda Instruments, Seattle, WA) and pressures were monitored with Micron pressure transducers (Narco Life Sciences, Houston, TX). The pressure and flow signals were suitably amplified and displayed on a chart recorder (Gould, Cleveland, OH). The pressure and flow signals were also put into an APPLE II+ microcomputer *via* an analogue-to-digital interface for analysis (details of the hardware and software for collection and analysis of pulsatile physiological signals using the APPLE personal computer can be obtained from the author free of charge).

Analysis

All analyses were based on 10-s samples of the cardiovascular signals following suitable equilibration to the particular experimental conditions. Microcomputer analysis yielded average values for that time period. \dot{V}_b (ml min⁻¹) = [SVH(ml) × fh (beats min⁻¹)] and power output of the heart (mW) = [pressure development (cmH₂O)] × \dot{V}_b × [980/60] × 10⁻⁴, where pressure development = [mean output pressure - mean input pressure]. Pressures were referenced to the level of saline in the bath. Corrections were made for the pressure drops across the input and output cannulae. \dot{V}_b and SVH were normalized per kilogram fish body weight, and power output was normalized per gram ventricular wet weight. The wet weight of the fish (489 g, S.E.M. = 12, $N=71$) was measured before the experiment and the blotted wet weight of the ventricle was determined afterwards (0.371 g, S.E.M. = 0.014, $N=71$). The ventricle represented 0.077% (S.E.M. = 0.002, $N=71$) of body weight. Statistical differences ($P < 0.05$) were determined using a Wilcoxon paired-rank test.

RESULTS

Series A

Perfused hearts generated mean output pressures from 30 to 65 cmH₂O without a significant change in SVH (Fig. 1). At output pressures above this range SVH decreased markedly. These observations are consistent with homeometric regulation over a physiological range of ventral aortic blood pressures. Homeometric regulation was achieved without major changes in fh. Heart rate did decline by 1–5 beats min⁻¹ as afterload was increased from 30 to 65 cmH₂O (note a somewhat greater slope for

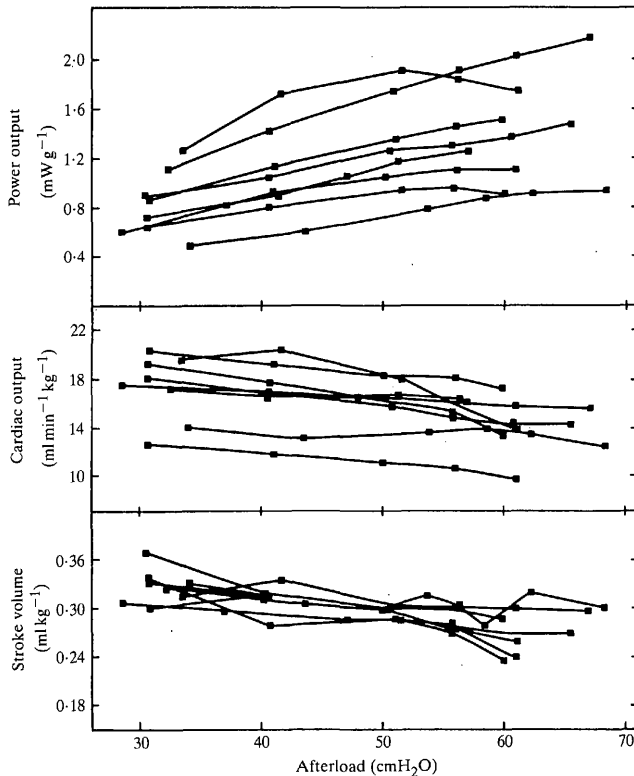


Fig. 1. The effect of afterload on cardiac performance in the perfused trout heart (series A). Each line represents a different preparation ($N = 8$). The variability in cardiac output and power output between preparations reflects different intrinsic heart rates and relative heart weights. Heart rate did not change significantly with afterload.

\dot{V}_b compared to \dot{V}_{H_1} ; Fig. 1), but this probably reflected a time-dependent decrease in \dot{V}_H (see series B) rather than a direct effect of afterload on \dot{V}_H .

Series B

The time series (Fig. 2) illustrated several important features of the Starling response of the heart. Control preloads were typically below 1 cmH₂O. Stroke volume was more sensitive to lower preloads. The maximum response to preload was usually produced at pressures between 3 and 5 cmH₂O. The increase in preload typically reduced \dot{V}_H by 0–3 beats min⁻¹.

During the course of the time series, the maximum power output response to preload did not change significantly. There was a 5–8 beats min^{-1} reduction in fH , which was offset by a compensatory increase in SVH . Most of the modest decrease in fH occurred during the first and second trials. This decrease in intrinsic fH differed

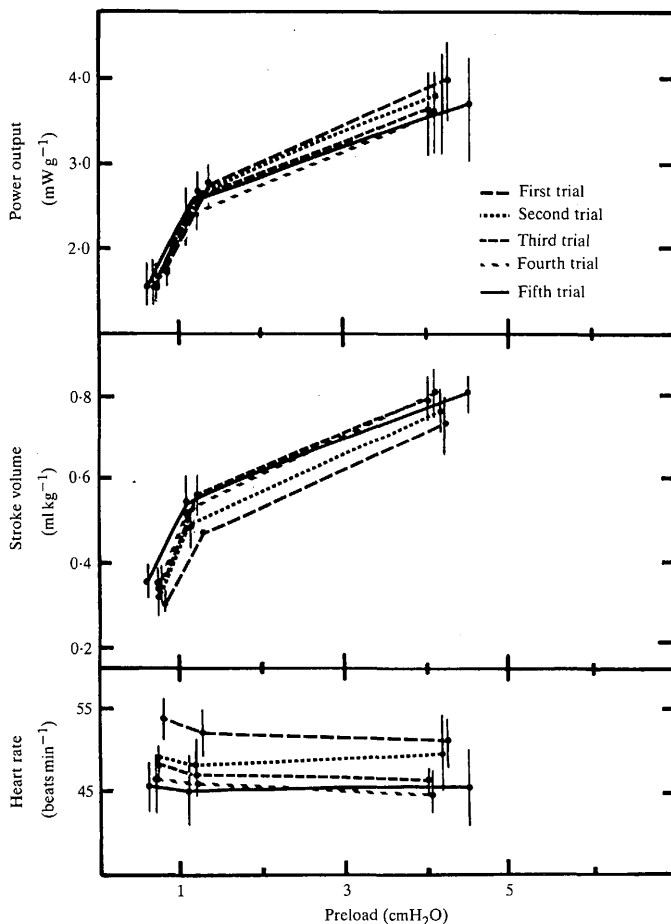


Fig. 2. The effect of five consecutive preload challenges on cardiac performance under control conditions (series B). Mean values ($N = 6$) are presented and standard error bars are also presented for reference. Each preparation acted as its own control. There was no statistically significant change in the Starling curve for power output after five repetitions of the Starling response.

considerably from that observed when a heart was failing, in that a failing heart would either miss heart beats before switching to a much lower intrinsic fh (20–30 beats min⁻¹), or display a rapidly declining fh, which stabilized somewhat at between 20 and 30 beats min⁻¹. Heart preparations that displayed these changes were discarded.

Series C

Adrenaline improved cardiac performance in a dose-dependent fashion (Fig. 3). The Starling curve for power output was shifted upwards and to the left by adrenaline. Positive inotropic and chronotropic effects were statistically significant at concentrations of 1 and 10 nmol l⁻¹, respectively. 1 μ mol l⁻¹ adrenaline did not significantly improve contractility beyond the level achieved with 0.1 μ mol l⁻¹ adrenaline.

Noradrenaline produced a dose-dependent shift in the Starling curve for power output (Fig. 4). However, noradrenaline was less potent than adrenaline and marked changes in power output were only evident with 0.1 μ mol l⁻¹ and 1.0 μ mol l⁻¹ noradrenaline. Positive inotropic and chronotropic effects were statistically significant with a concentration of 0.1 μ mol l⁻¹ noradrenaline. The maximum stimulation of contractility by noradrenaline was probably not achieved with 1 μ mol l⁻¹ noradrenaline.

The dose-dependent shift in the Starling curve for power output with isoproterenol, a synthetic β -adrenergic agonist (Fig. 5), and the absence of a response with phenylephrine, a synthetic α -adrenergic agonist (Fig. 6), illustrate that adrenergic stimulation of contractility in the perfused heart was mediated by β -adrenoceptors.

In addition to adrenergic stimulation of normal hearts, adrenaline also restored contractility in failing hearts. A dose-response curve for adrenaline was established for three failing hearts (Fig. 7). With control perfusate, these hearts exhibited an abnormally high preload and an extremely low fh. In addition, fh increased with preload, the Starling response to preload was limited, and a power output equivalent to resting fish was not achieved. Contractility was improved significantly with 10 nmol l⁻¹ adrenaline as a result of positive inotropy and chronotropy. The positive chronotropic effect was unusually large. Concentrations of 0.1 and 1 μ mol l⁻¹ adrenaline resulted in Starling curves for power output in the same range as those generated for normal hearts (cf. Fig. 3).

Series D

The dose-response characteristics for Ca_e²⁺ were complex because of simultaneous positive inotropic and negative chronotropic effects of Ca_e²⁺, plus the absolute requirement of the heart for Ca_e²⁺. Three protocols for varying Ca_e²⁺ were used to reveal these characteristics. The absolute requirement for Ca_e²⁺ was clearly revealed when Ca_e²⁺ was increased in a stepwise fashion starting with 1.5 mmol l⁻¹ (Fig. 8A). Here, the first Starling response with 1.5 mmol l⁻¹ apparently damaged the heart so that maximum \dot{V}_b and power output progressively decreased with subsequent

challenges at higher Ca_e^{2+} levels. Stroke volume did increase significantly with each increase in $[\text{Ca}_e^{2+}]$, but this was a passive response to the marked decrease in fH with Ca_e^{2+} , since \dot{V}_b did not increase. The apparently sub-physiological nature of $1.5 \text{ mmol l}^{-1} \text{ Ca}_e^{2+}$ under the present experimental conditions was supported by

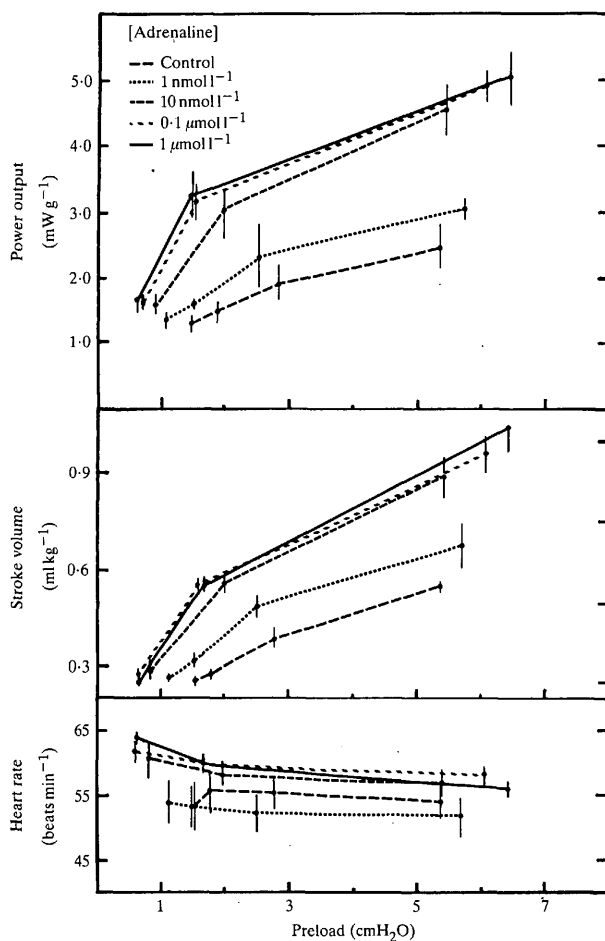


Fig. 3. The effect of adrenaline on cardiac performance and on the Starling response of the perfused trout heart (series C). Mean values ($N = 8$) are plotted and standard error bars are presented for reference. Each preparation acted as its own control.

preliminary experiments where an initial $[Ca_e^{2+}]$ of 1.0 mmol l^{-1} at a resting power output produced complete loss of cardiac contractility within 5–10 min.

The positive inotropic effect of Ca_e^{2+} was revealed by the two other protocols, in which either randomized changes in the $[Ca_e^{2+}]$, starting with 2.0 mmol l^{-1}

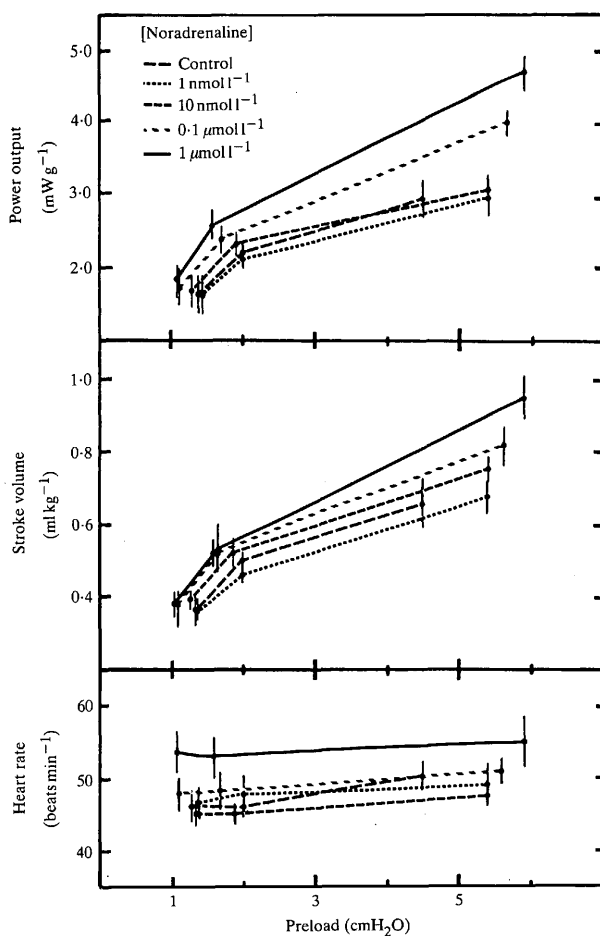


Fig. 4. The effect of noradrenaline on cardiac performance and on the Starling response of the perfused trout heart (series C). Mean values ($N = 6$) are plotted and standard error bars are presented for reference. Each preparation acted as its own control.

(Fig. 8B), or stepwise decreases in the $[Ca_c^{2+}]$, starting with 5.0 mmol l^{-1} (Fig. 9), were used. Together these protocols revealed that there was always (13 preparations) an increase in maximum power output with each increase in $[Ca_c^{2+}]$ and a decrease in maximum power output with each decrease in $[Ca_c^{2+}]$. In addition, the small, consistent decrease in the preload that was required to generate the resting Vb

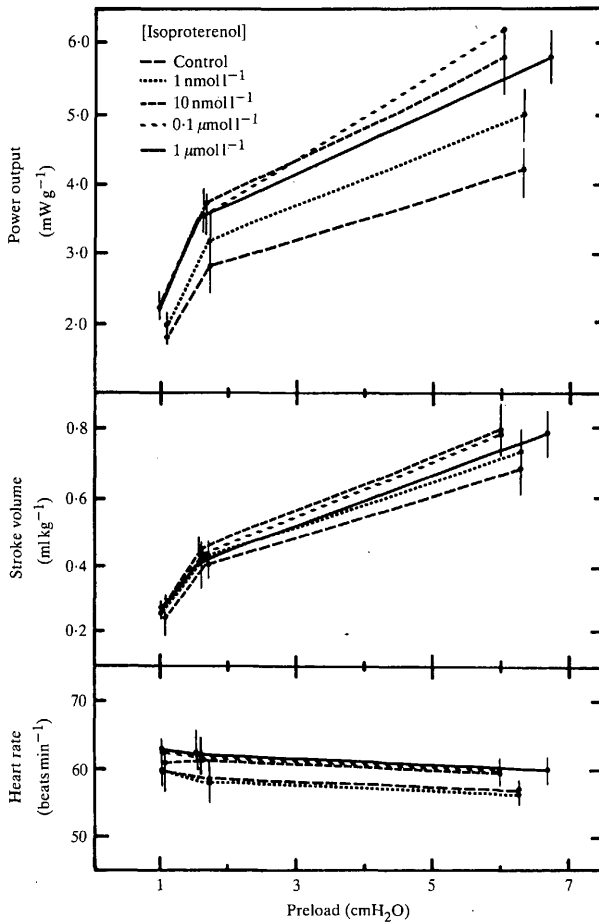


Fig. 5. The effect of isoproterenol on cardiac performance and on the Starling response of the perfused trout heart (series C). Mean values ($N = 6$) are plotted and standard error bars are presented for reference. Each preparation acted as its own control.

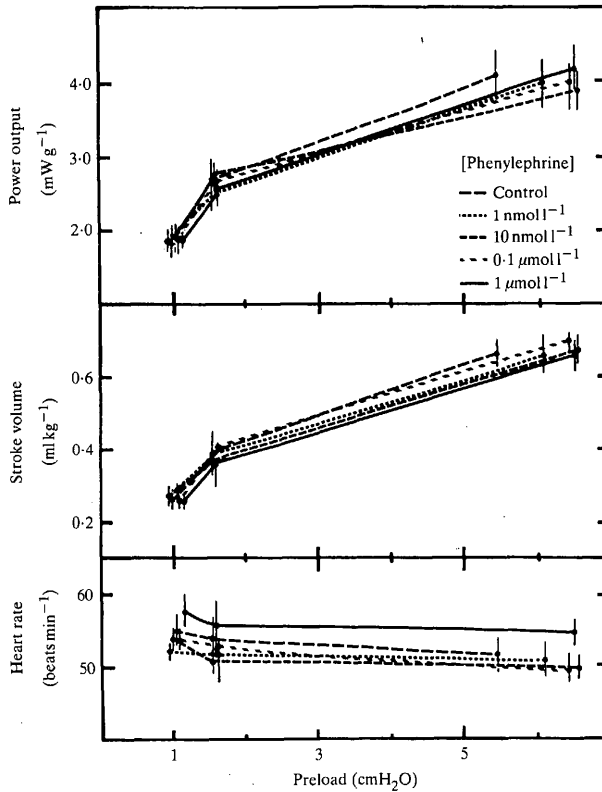


Fig. 6. The effect of phenylephrine on cardiac performance and on the Starling response of the perfused trout heart (series C). Mean values ($N = 6$) are plotted and standard error bars are presented for reference. Each preparation acted as its own control. Phenylephrine did not significantly affect the Starling curve for power output.

indicated that the increase in Ca_e^{2+} was exerting a positive inotropic effect. Although maximum power output was significantly increased at each level of Ca_e^{2+} in each of these protocols, the absolute change in maximum power output was modest. This was especially true over the physiological range for Ca_e^{2+} (2–4 mmol l⁻¹).

The positive inotropic effects of Ca_e^{2+} also resulted in significant increases in the maximum SVH and Vb generated by preload. Some of the increase in SVH also occurred passively because of the negative chronotropic effect of Ca_e^{2+} . Negative chronotropy was confirmed by the observation that a stepwise change in Ca_e^{2+} always produced a corresponding change in fH in all preparations, independent of the protocol.

Series E

Extracellular acidosis produced a significant negative chronotropy in all heart preparations under resting conditions. Resting \dot{V}_b was unaffected because of a compensatory increase in SVH (Figs 10, 11). However, the negative inotropic effect of acidosis reduced the maximum \dot{V}_b in all heart preparations. As a result of this and

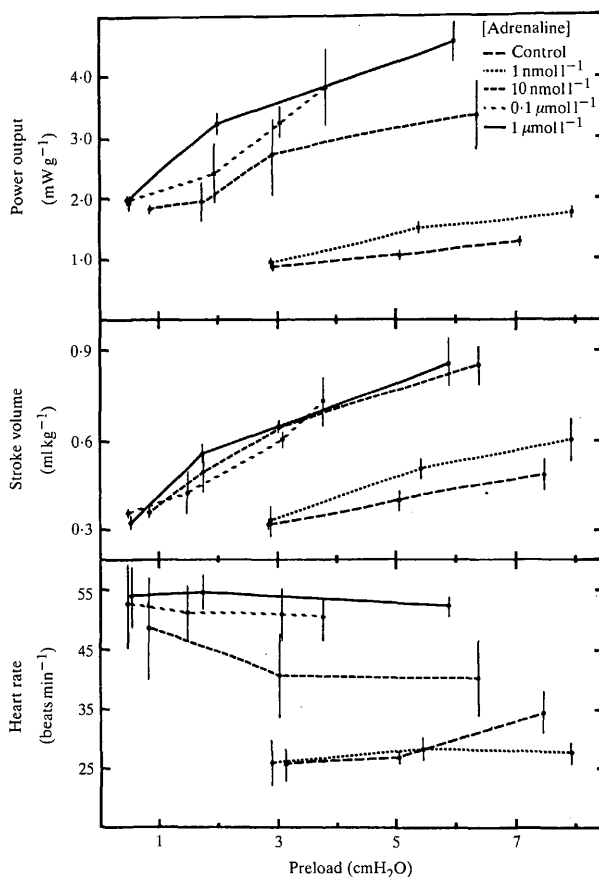


Fig. 7. The effect of adrenaline on three perfused trout heart preparations that were considered to be failing under control conditions. Mean values are plotted and standard error bars are presented for reference. Each preparation acted as its own control. Adrenaline restored contractility to the failing hearts through positive chronotropy and positive inotropy.

a reduction in systolic pressure, maximum power output was also reduced in all preparations and the Starling curve for power output was shifted significantly downwards by extracellular acidosis (Figs 10, 11). Nonetheless, the perfused trout heart could still double \dot{V}_b in response to preload during extracellular acidosis.

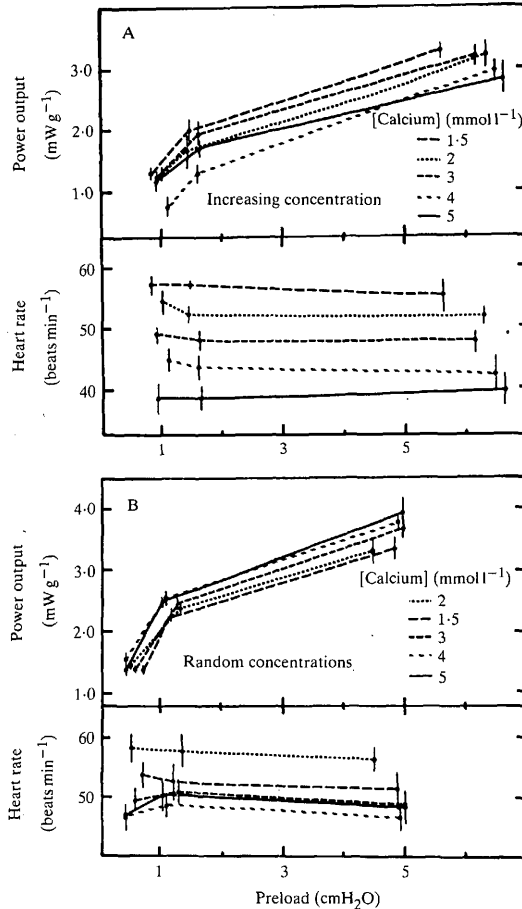


Fig. 8. The effect of extracellular calcium on cardiac performance and on the Starling response in the perfused trout heart (series D). Mean values are plotted and standard error bars are presented for reference. Each preparation acted as its own control. (A) The Ca_a^{2+} concentration was progressively increased from the initial level of 1.5 mmol l^{-1} ($N=4$). (B) The initial Ca_a^{2+} concentration was always 2.0 mmol l^{-1} and the order of the subsequent Ca_a^{2+} concentrations was randomized ($N=8$).

Increasing Ca_e^{2+} from 2.5 to 4.0 mmol l^{-1} during acidosis improved the inotropic state of the heart. As a result, the heart was capable of its normal maximum power output, but at a lower preload (Fig. 10). Maximum power output was not increased

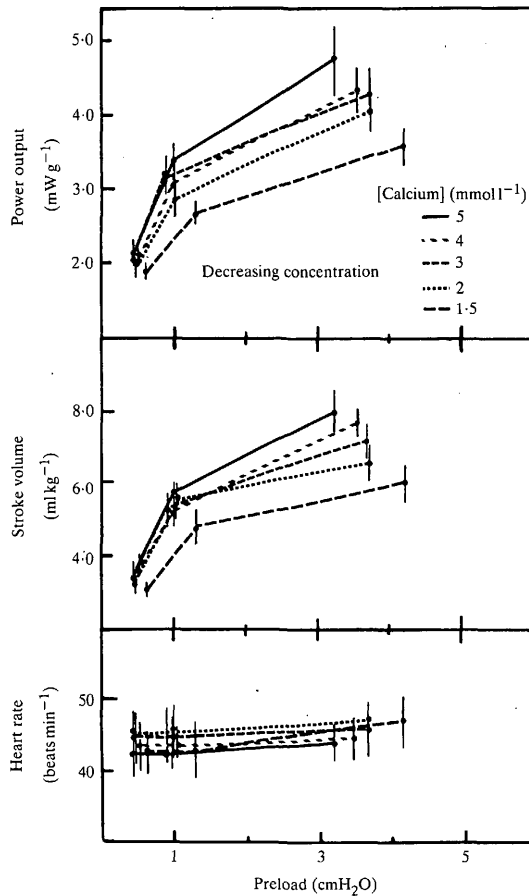


Fig. 9. The effect of extracellular calcium on cardiac performance and on the Starling response in the perfused trout heart (series D). Mean values ($N=5$) are plotted and standard error bars are presented for reference. Each preparation acted as its own control. With this protocol, the initial perfusate contained $5 \text{ mmol l}^{-1} \text{ Ca}_e^{2+}$ and, after the Starling response had been determined at this calcium concentration, the Ca_e^{2+} concentration was decreased and the Starling response was remeasured at each new level of Ca_e^{2+} .

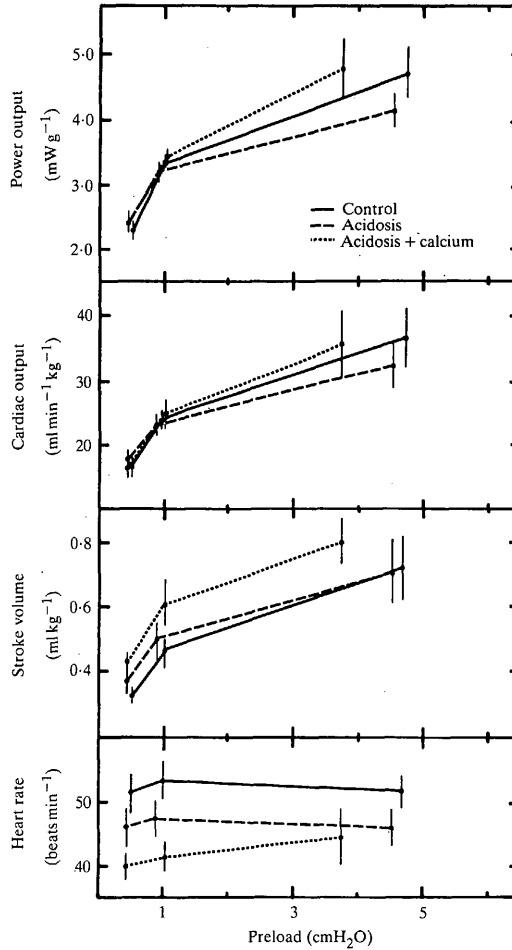


Fig. 10. The response of the perfused trout heart ($N = 5$) to an increase in preload with control perfusate (pH 7.9), acidotic perfusate (pH 7.4) and acidotic perfusate with an additional $1.5 \text{ mmol l}^{-1} \text{ Ca}_e^{2+}$. The point on the extreme right of each curve represents the maximum response to preload. Mean values are plotted and standard error bars are presented for reference. Each preparation acted as its own control. Acidosis produced a statistically significant reduction in the maximum power output and maximum power output was restored with additional Ca_e^{2+} .

significantly by additional Ca_e^{2+} . Overall cardiac performance was clearly compromised by the further reduction in fH produced by additional Ca_e^{2+} (Fig. 10).

The addition of 10 nmol l^{-1} adrenaline during extracellular acidosis improved the inotropic and chronotropic state of the heart (Fig. 11). Heart rate was almost fully restored to control levels and maximum SVH was significantly higher. Consequently, adrenaline shifted the Starling curve for power output upwards and to the left, and the heart became significantly more responsive to preload than under control conditions. The maximum power output was significantly increased by adrenaline. During acidosis, adrenaline clearly improved cardiac performance to a greater degree than did Ca_e^{2+} .

DISCUSSION

Perfused trout heart

The coronary arteries were not perfused in the present experiments. Thus, the compact myocardium, which constitutes up to 30% of the ventricle in trout (Santer & Greer Walker, 1980), was not oxygenated in its normal fashion. However, it is unlikely that the compact myocardium was completely anoxic. The oxygen partial pressure of the perfusate was considerably higher than venous blood, and the bath saline was equilibrated with air. Therefore, the compact myocardium could receive oxygen from both the perfusate and superfusate. Also it is unlikely that the low O_2 content of the perfusate, compared with that of blood, was a limiting factor, since the P_{O_2} of the perfusate leaving the heart far exceeded that of venous blood. The level of hypoxia in the compact myocardium and its effect on overall cardiac performance are difficult to ascertain. The perfused trout heart generated a maximum power output of $5\text{--}6.5 \text{ mW g}^{-1}$ when maximally stimulated by preload in the presence of catecholamines. This value is 13–33% less than the myocardial power output (7.4 mW g^{-1}) calculated for trout swimming at their maximum sustained speed (Farrell, 1985a; calculated from Kiceniuk & Jones, 1977). The difference may reflect either hypoxic compact myocardium in the perfused heart, a general limitation of contractility in perfused heart preparations, or that the maximum power output was not reached by merely increasing the preload. It is possible that the perfused heart may also be able to generate even higher output pressures when \dot{V}_b is maximal, but this was not tested. Certainly, maximum \dot{V}_b was not limited, since values of $50\text{--}60 \text{ ml min}^{-1} \text{ kg}^{-1}$ for the perfused heart compare well with $52.6 \text{ ml min}^{-1} \text{ kg}^{-1}$ measured in intact trout at their maximum sustained swimming speed (Kiceniuk & Jones, 1977). A definitive answer will have to await techniques to perfuse coronary arteries in working fish hearts. The maximum power output of the perfused trout heart was, nevertheless, double that for the perfused sea raven heart which lacks a compact myocardium (Farrell *et al.* 1982).

Notwithstanding the absence of coronary perfusion, the perfused trout heart proved to be durable as well as reliable, based on the fact that important intrinsic mechanisms were functional. Under control conditions, fH , \dot{V}_b , output pressure and thus power output were comparable to the values in intact, resting trout at 10°C

(Kiceniuk & Jones, 1977). The heart was relatively pressure-insensitive over a physiological range of ventral aortic pressures (series A) and preload exerted an important control over \dot{V}_b through the Starling response. Both of these intrinsic

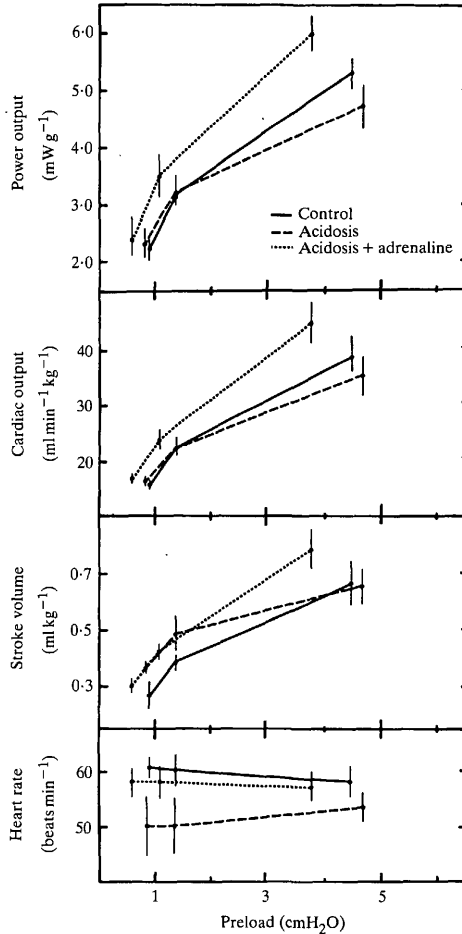


Fig. 11. The response of the perfused trout heart ($N = 6$) to an increase in preload with control perfusate (pH 7.9), acidotic perfusate (pH 7.4) and acidotic perfusate with 10 nmol l^{-1} adrenaline. Mean values are plotted and standard error bars are presented for reference. Each preparation acted as its own control. Adrenaline given during acidosis produced a statistically significant improvement in cardiac contractility beyond that found under control conditions.

mechanisms were independent of f_H , contrary to a previous suggestion for the trout heart (Jensen, 1969). The Starling response operated at physiological pressures, which were much lower than those previously used for isolated trout hearts (Bennion, 1968). The heart also tolerated several high work load challenges over a short time period with only a minor decrease in f_H , which was fully compensated by a passive increase in SV_H (series B). Series B also validated the approach of letting each heart act as its own control.

\dot{V}_b is independent of physiological output pressures in trout, sea raven and ocean pout. In the sea raven and ocean pout, \dot{V}_b begins to decline substantially at pressures above 50–55 cmH₂O (Farrell, 1985a). The trout heart is, therefore, capable of developing greater output pressures (60–70 cmH₂O) than the aforementioned benthic species before \dot{V}_b is compromised. The trout heart is also capable of a much greater increase in \dot{V}_b when preload is raised compared to the sea raven and ocean pout hearts (Farrell *et al.* 1982, 1983). Such differences in intrinsic mechanical properties are expected for active fish. Increases in \dot{V}_b and arterial pressure (Kiceniuk & Jones, 1977) during sustained exercise are required to increase blood supply to active muscle (Randall & Daxboeck, 1982). The higher intrinsic contractility of the trout heart seems appropriate to meet these demands.

The present work was conducted during spring months when the water temperature of the holding tanks increased from 7 to 12°C. The seasonal change in holding temperature noticeably affected cardiac performance, even though the experimental temperature was always 10°C. As the holding temperature increased, the perfused heart typically required a lower preload for the resting \dot{V}_b . In addition, the heart to body weight ratio decreased, which contributed to the higher maximum power output normalized to ventricular weight in fish held at higher temperatures. Lower control preloads and smaller hearts were also observed in sea ravens acclimated to high temperature (Graham & Farrell, 1985). These changes did not affect the general conclusions of each study since each heart acted as its own control, and each series was performed within a short time frame such that the holding temperature was constant for each series. However, experimental series that were performed several months apart do show variability (e.g. Figs 3, 8) and this precludes direct comparisons. The important point is that acclimation to a relatively small temperature change (4°C) significantly influenced *in vitro* cardiovascular performance. This observation serves to stress the importance of the acclimation temperature when dealing with cardiovascular responses (see for example Peyraud-Waitzenegger, Barthelemy & Peyraud, 1980).

The effects of catecholamines improved the Starling response of perfused hearts. This positive inotropy is consistent with other studies of the teleost heart (see Laurent, Holmgren & Nilsson, 1983; Farrell, 1984). In addition, the positive inotropic response was quantified in a meaningful manner. Our values for maximum increases in \dot{V}_b (60 ml min⁻¹ kg⁻¹; Fig. 3) compare well with estimates of cardiac output in trout during maximum aerobic swimming (52.6 ml min⁻¹ kg⁻¹; Kiceniuk & Jones, 1977).

A positive chronotropy also contributed to improved cardiac performance when catecholamines were present. Resting fh was increased by 10–15%, which is consistent with the 8–15% increase reported for the intact trout (Wood; Pieprzak & Trott, 1979) and the perfused sea raven heart (Farrell, 1984).

Positive chronotropy and inotropy were mediated by β -adrenoceptors in the trout heart. This is consistent with the general finding for teleost hearts (see reviews by Laurent *et al.* 1983; Farrell, 1984), although α -adrenoceptors were reported for the eel (Chan & Chow, 1976). With the perfused trout heart, the order for adrenergic potency was isoproterenol > adrenaline > noradrenaline. This indicates the presence of β_2 -adrenoceptors, which again is consistent with previous findings for trout (Ask, Stene-Larsen & Helle, 1980, 1981), plaice (Falk, Mecklenburg, Myhrberg & Persson, 1966) and Atlantic cod (Holmgren, 1977). Phenylephrine had no effect on cardiac performance in the trout heart, which suggests that α -adrenoceptors did not contribute significantly to adrenergic control of mechanical performance under the present conditions.

The dose-response curves for the β -adrenergic agonists revealed three additional points about adrenergic control of cardiac performance. First, adrenaline must dominate noradrenaline in terms of cardiac stimulation. It is the more potent of the two agonists, has a higher concentration in the blood (Wahlqvist & Nilsson, 1980; Primmitt *et al.* 1986) and is released from cardiac sympathetic nerve endings in addition to noradrenaline (see Ask, 1983). Second, adrenaline exerted its maximal effect on the perfused heart at 10 nmol l^{-1} . The plasma adrenaline concentration is $10\text{--}50 \text{ nmol l}^{-1}$ during sustained exercise (Primmitt *et al.* 1986). Thus, sustained swimming may well be accompanied by maximal adrenergic stimulation of the heart. Last, inotropic stimulation consistently occurred at a lower catecholamine concentration than chronotropic stimulation. The physiological importance of this is not clear at the present time.

Effects of Ca_e^{2+}

A positive inotropic effect of Ca_e^{2+} was observed with the perfused trout heart and this is consistent with previous observations using electrically paced ventricular strips (Gesser & Jorgensen, 1982; Nielsen & Gesser, 1984; Driedzic & Gesser, 1985). Unlike in studies with trout ventricular strips, where tension is typically doubled by an increase in Ca_e^{2+} from 1 to 5 mmol l^{-1} and frequency is not compromised, it was difficult precisely to quantify the inotropic effect of Ca_e^{2+} in the perfused trout heart. Increasing Ca_e^{2+} from 1.5 to 5 mmol l^{-1} only provided up to a 35% improvement in the maximum power output (as determined by the maximum response to preload). This increase underestimates the inotropic effect of Ca_e^{2+} because fh decreased whenever Ca_e^{2+} was increased. However, the change in fh was rarely more than 15% and so the underestimation is small. Changes in SVH, which show relatively larger increases with Ca_e^{2+} than power output, might be viewed as a better measure of inotropic stimulation of the perfused heart. Relative changes in SVH unfortunately overestimate the inotropic effects of Ca_e^{2+} , because SVH increases passively to some extent as fh decreases. Even though it is difficult accurately to

quantify the inotropic effect of Ca_e^{2+} in perfused hearts, there is no doubt about the overall effect of Ca_e^{2+} on cardiac performance of the intact perfused heart. The positive inotropic effect of Ca_e^{2+} is compromised to some degree by negative inotropy so that there is only a modest increase in cardiac output. This is true for the active trout and the inactive sea raven, where limited inotropic and negative chronotropic effects of Ca_e^{2+} have also been demonstrated (Farrell *et al.* 1984).

Plasma Ca_e^{2+} concentrations in teleosts are usually between 2 and 4 mmol l⁻¹, i.e. 73% of the 60 values reported by Holmes & Donaldson (1969). Furthermore, almost half of these values (48%) were in the 2–3 mmol l⁻¹ range. While Ca_e^{2+} concentrations in the range 1.5–5 mmol l⁻¹ modify cardiac performance, the role of Ca_e^{2+} in modulating cardiac contractility in the intact fish must be re-examined with these physiological plasma concentrations in mind. Regardless of the protocol used here, cardiac stimulation by Ca_e^{2+} within the range of 2–4 mmol l⁻¹ was very modest. Furthermore, the indications were that 1.5 and 5 mmol l⁻¹ Ca_e^{2+} were, respectively, sub- and supraphysiological, given their untoward effects on contractility (Fig. 8A) and pacemaker activity (Fig. 9). Thus changes in plasma calcium probably have a very limited role in modulating cardiac performance in the trout and sea raven under normal conditions. In fact, calcium homeostasis in the blood may be a more appropriate strategy in order to preclude unwanted negative inotropy (too little Ca_e^{2+}) and chronotropy (too much Ca_e^{2+}).

The sensitivity of the heart to Ca_e^{2+} does vary amongst teleosts (Driedzic & Gesser, 1985) and, therefore, modulation of cardiac performance by Ca_e^{2+} may be more important in other teleost species, or in situations such as post-exercise when plasma calcium is increased and the heart is faced with extracellular acidosis.

Extracellular acidosis

Extracellular acidosis with a constant $[\text{HCO}_3^-]$ in the perfusate had negative chronotropic and inotropic effects on the perfused trout heart. This finding is consistent with previous work with trout and other teleosts using either ventricular strips or perfused hearts (Poupa & Johansen, 1975; Gesser & Poupa, 1978; Gesser & Jorgensen, 1982; Gesser *et al.* 1982; Farrell *et al.* 1983; Nielsen & Gesser, 1984; Farrell, 1985b). The downward shift in the Starling curve for power output was much less pronounced than for the sea raven heart under comparable conditions (Farrell, 1985b). This was a surprising observation since negative inotropy reduced force development in trout ventricular strips 60–70% or more with a 0.4 pH unit decrease in extracellular pH and a constant $[\text{HCO}_3^-]$ (Gesser & Poupa, 1978; Gesser *et al.* 1982; Gesser & Jorgensen, 1982; Nielsen & Gesser, 1984). In contrast, a 0.5 pH unit decrease in extracellular pH with constant $[\text{HCO}_3^-]$ did not significantly alter *resting* cardiac output or power output in the perfused trout heart because a passive increase in SVH compensated for the negative chronotropy produced by the respiratory acidosis. The negative inotropic effect of extracellular acidosis was manifest only when the work load of the perfused trout heart was raised, and then maximum power output was reduced by only 10–15%. Why this difference exists

between perfused trout hearts and trout ventricular strips with respect to the negative inotropic effect of extracellular acidosis is not clear.

It was possible to increase \dot{V}_b by 60% or more in the intact perfused trout heart when extracellular pH was reduced from 7.9 to 7.4. After exercise in intact trout, \dot{V}_b is increased by 60% (Neumann *et al.* 1983) and arterial and venous blood pH are decreased, respectively, to 7.45 and 7.40 from their control levels of 7.91 and 7.89 (Milligan & Wood, 1986). In addition, the arterial and venous HCO_3^- concentrations are decreased from 8.0 and 8.5 to 6.0 and 4.5 mmol l^{-1} , respectively. Thus, the present study only simulated the extracellular pH change that occurs after exercise and did not establish whether the decrease in extracellular $[\text{HCO}_3^-]$ also influences cardiac performance during acidosis. Preliminary experiments with the perfused trout heart have indicated, however, that a reduction in extracellular $[\text{HCO}_3^-]$ may exacerbate the negative inotropy of acidosis (A. P. Farrell, unpublished results). In addition, ventricular strips from plaice, but not Atlantic cod, are also more sensitive to acidosis when $[\text{HCO}_3^-]$ is reduced from supraphysiological levels (30 mmol l^{-1}) to 12 mmol l^{-1} (Gesser & Poupa, 1983). The present findings indicate that the trout heart is fully capable of generating the levels of cardiac performance observed after exercise without any extrinsic influences, even though acidosis does reduce cardiac performance. However, if the decrease in extracellular $[\text{HCO}_3^-]$ proves to be as important a modifying factor during acidosis as preliminary studies indicate, then extrinsic factors such as calcium and adrenaline, which restore contractility during acidosis, may have a greater importance.

After exercise, the plasma $[\text{Ca}_e^{2+}]$ of teleosts increases by 0.5 mmol l^{-1} to 1.0 mmol l^{-1} (Graham, Wood & Turner, 1982; Turner *et al.* 1983; Milligan & Farrell, 1986; Milligan & Wood, 1986). In the present study, a somewhat greater increase in $[\text{Ca}_e^{2+}]$ (1.5 mmol l^{-1}) restored normal contractility during extracellular acidosis by improving the inotropic state of the heart. Thus, trans-sarcolemmal movements of calcium may be important in restoring contractility of the trout heart after exercise.

Adrenaline also must play an important role in restoring cardiac contractility in trout after exercise. The adrenaline concentration used here, which was within the observed range for blood catecholamine levels during exercise (Primmatt *et al.* 1986), improved cardiac contractility such that maximum power output during extracellular acidosis was even greater than that under control conditions. Thus, provided adrenaline was present, \dot{V}_b could be increased threefold during pH conditions simulating those after exercise in trout. A quantitatively similar effect of adrenaline was observed with acidotic perfused hearts from the sea raven (Farrell, 1985b).

The finding that physiological levels of adrenaline improved contractility of the acidotic perfused trout heart to a greater degree than a 1.5 mmol l^{-1} increase in $[\text{Ca}_e^{2+}]$ is at variance with previous observations on trout ventricular strips, where tension is restored to a greater degree with Ca_e^{2+} than adrenaline (Gesser *et al.* 1982; Gesser & Jorgensen, 1982). In fact a supraphysiological dose of adrenaline (1 $\mu\text{mol l}^{-1}$) was required just to restore the control tension during acidosis. The

difference between these two sets of observations probably lies with the chronotropic effects of adrenaline and Ca_e^{2+} . The positive chronotropic effect of adrenaline complements the recovery of cardiac contractility during acidosis, whereas the negative chronotropic effect of Ca_e^{2+} compromises the cardiac recovery. In electrically paced ventricular strips, chronotropic responses are not a factor. However, in terms of intact fish, chronotropic and inotropic responses must both be considered. Therefore, it seems that adrenergic stimulation of the heart probably plays a more important role than changes in Ca_e^{2+} in order that cardiac performance can be restored after exercise in trout.

Not all teleosts necessarily rely upon adrenaline to restore contractility to the acidotic myocardium. The flounder, for example, releases intracellular calcium stores during acidosis, and contractility is recovered intrinsically (Gesser & Poupa, 1983). However, adrenaline, but not Ca_e^{2+} , restores contractility in perfused sea raven hearts during extracellular acidosis with a constant $[\text{HCO}_3^-]$. In the case of the sea raven, plasma $[\text{HCO}_3^-]$ does not decrease significantly after exercise, even though there is the characteristic 0.5 pH unit decrease in blood pH (Milligan & Farrell, 1986).

Adrenaline also restored contractility to failing hearts under non-acidotic conditions. The reason for cardiac failure under control conditions was not established, but failure rarely occurred after the experimental techniques were refined. Therefore, it seems likely that cardiac failure may have reflected some stress which was introduced during preparation, even though great care was taken not to handle the heart directly and always to maintain perfusion of the heart with either venous blood or perfusate. In view of this finding, adrenaline may be an important factor in preserving cardiac contractility in trout under other stressful situations besides extracellular acidosis.

Another interesting aspect of the failing heart was its low fH , which was similar to that reported in earlier studies with isolated, perfused trout hearts (Jensen, 1969; Bennion, 1968 as reported by Randall, 1970). Furthermore, a tachycardia was associated with an increase in preload in these earlier studies and in the failing hearts from the present work. In normal hearts from trout, sea raven and ocean pout, fH is independent of preload. Thus, the tachycardia associated with increases in preload may be restricted to stressed or damaged hearts.

The mechanism underlying adrenergic recovery of contractility of the acidotic trout heart is unclear. β -Adrenoceptors mediate an increase in the inward calcium current across the sarcolemma in mammals and amphibia (Reuter, 1983). If a similar mechanism exists in fish, adrenergic stimulation of the heart would increase the calcium influx into the myocardium. This would help alleviate the intracellular calcium deficit that is thought to be detrimental to cardiac performance during acidosis (Gesser & Poupa, 1983). Adrenergic modulation of sarcolemmal Ca^{2+} channels would also provide Ca^{2+} -mediated positive inotropy without the compromising effect of negative chronotropy which occurs if $[\text{Ca}_e^{2+}]$ is increased. An alternative mechanism may involve intracellular pH regulation. β -Adrenoceptors mediate intracellular pH regulation during myocardial acidosis in mammals and in

fish erythrocytes (Gonzalez & Clancy, 1984; Nikinmaa, 1982). The accompanying study (Farrell & Milligan, 1986) investigates whether adrenergic pH regulation restores cardiac contractility during extracellular acidosis in the perfused trout heart.

In summary, the intrinsic mechanical properties of the trout heart permit a greater response to preload and a higher pressure development without compromising cardiac output when compared to less active, benthic teleosts. This higher scope for cardiac performance in trout is undoubtedly matched to the demands placed on the heart during sustained exercise. Catecholamines produce positive inotropy and chronotropy which are mediated by β_2 -adrenoceptors. Adrenaline increases the intrinsic fh by 10–15% and increases the sensitivity of the heart to preload such that there is a 60–100% improvement in maximum power output. Extracellular calcium also has a positive inotropic effect which is compromised in terms of overall cardiac performance by negative chronotropy, unlike the catecholamine effect. Thus under control conditions, physiological levels of catecholamines have a greater effect on \dot{V}_b and power output than physiological increases in $[Ca^{2+}]$. Extracellular acidosis (pH 7.4) with constant $[HCO_3^-]$ reduces cardiac contractility through negative chronotropy and inotropy. Elevated $[Ca^{2+}]$ improves the inotropic state of the heart to restore normal contractility, even though there is an accompanying negative chronotropy. Physiological levels of adrenaline improve cardiac performance during extracellular acidosis and it seems likely that catecholamine release after exercise contributes significantly to the recovery of cardiac performance during the associated respiratory acidosis.

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