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CARDIAC PERFORMANCE IN THE *IN SITU* PERFUSED FISH HEART DURING EXTRACELLULAR ACIDOSIS: INTERACTIVE EFFECTS OF ADRENALINE

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SUMMARY

1. The physiological integrity of the *in situ* perfused heart of the ocean pout was established by its ability to maintain cardiac output ($\dot{Q}$) over a range of work loads, and by the dependence of $\dot{Q}$ upon the filling pressure of the heart. Similar observations have been reported previously for the *in situ* perfused heart of the sea raven.

2. Physiological levels of extracellular acidosis (pH 7·6/1 % CO_2 and pH 7·4/2 % CO_2) significantly depressed cardiac performance in sea raven and ocean pout hearts *in situ*. Negative chronotropic and inotropic responses were observed.

3. Adrenaline (AD; 10^{-7} M) under control conditions (pH 7·9/0·5 % CO_2) produced a sustained tachycardia. The tachycardia reduced filling time of the ventricle and stroke volume was compromised because of the constant preload to the heart. Consequently, AD produced only an initial, transient increase in stroke volume and $\dot{Q}$. Thereafter, stroke volume was reduced in proportion with the increase in heart rate, and $\dot{Q}$ remained unchanged.

4. The combined challenge of extracellular acidosis and AD demonstrated interactive effects between AD and acidosis *in situ*. $\dot{Q}$ and power output were maintained in both species at both levels of extracellular acidosis during the combined challenge. Thus AD alone can maintain (but not improve upon) basal $\dot{Q}$ during extracellular acidosis.

5. The effects of extracellular acidosis, circulating catecholamines and venous return pressure to the heart are discussed in relation to the regulation of $\dot{Q}$ following exhaustive exercise.

INTRODUCTION

The ventricle of the teleost heart pumps venous blood, and, since many fish lack coronary arteries (Santer & Walker, 1980), the composition of the venous blood must influence myocardial contractility. In resting teleosts at 10 °C, venous blood is generally pH 7·8 to 8·0, but following exhaustive exercise blood pH falls precipitously.

Key words: Heart, acidosis, adrenaline.

Values of pH 7·4 and lower have been recorded within a few minutes after exercise, and restoration of blood pH may take up to 24 h (Wood, McMahon & McDonald 1977; Ruben & Bennett, 1981; Graham, Wood & Turner, 1982; Neumann, Holeton & Heisler, 1983). Clearly, the myocardium of teleost fish has to face a marked extracellular acidosis lasting several hours after exhaustive activity.

Extracellular hypercapnic acidosis depresses cardiac contractility in mammals through a reduction of intracellular pH (Williamson *et al.* 1976; Serur, Skeleton, Bodem & Sonnenblick, 1976; Fabiato & Fabiato, 1978; Schaffer *et al.* 1978). Similar effects are also found in fish, at least at extreme levels of hypercapnic acidosis. Poupa & Johansen (1975) demonstrated that an extracellular pH of 7·1 (15 % CO_2, 35·7 mM-HCO_3^-) produced a 45 % reduction in the force of contraction of isolated, electrically paced ventricular strips from *Gadus morhua*. This finding has been extended subsequently to a number of teleosts, both with and without coronary vessels, and over a range of quite severe acidosis treatments (pH 6·8 to 7·1 and 3 % to 15 % CO_2) (Poupa, Gesser & Johansen, 1978; Gesser & Poupa, 1978, 1979; Gesser, Andresen, Brams & Sund-Laursen, 1982). These changes in myocardial force development reduce cardiac output ($\dot{Q}$) as was demonstrated recently using an isolated, perfused sea raven heart in which there was an 80 % reduction in $\dot{Q}$ after a 40-min exposure to pH 7·1 (1 % CO_2) (Turner & Driedzic, 1980). Thus it appears that the less severe acidosis associated with exhaustive activity (i.e. pH 7·4) should reduce $\dot{Q}$ and thereby compromise metabolic recovery. Trout, however, increase $\dot{Q}$ following exhaustive activity even though the pH of the blood bathing the heart is 7·4 (Neumann *et al.* 1983). This observation can be explained if fish have evolved a mechanism to compensate for the effects of acidosis on cardiac performance. Limited evidence suggests that adrenaline (AD) may be involved in such a compensation (Gesser *et al.* 1982), but further work in this area is needed. Also, it must be unequivocally demonstrated that physiological levels of extracellular acidosis do indeed depress cardiac performance in fish.

The present work establishes to what degree physiological levels of extracellular acidosis (i.e. pH 7·6 and 7·4) affect $\dot{Q}$ and examines the interactive effects of AD and acidosis on cardiac performance. An *in situ* perfused heart preparation was preferred to muscle strip or isolated heart preparations, which have been used previously to examine more severe levels of acidosis. Two species of fish were used, the sea raven and ocean pout. Both fish lack a coronary circulation and this renders them ideal for heart perfusion studies. Previous work has demonstrated that the *in situ* sea raven heart retains its intrinsic mechanical properties and can generate a $\dot{Q}$ similar to that of resting fish (Farrell, MacLeod & Driedzic, 1982). The present investigation establishes that this is also true for the ocean pout heart *in situ*. The work also demonstrates that physiological levels of acidosis depress basal $\dot{Q}$ in both species. Moreover, the presence of 10^{-7} M-AD during acidosis restores basal $\dot{Q}$, but cannot markedly improve upon basal $\dot{Q}$ without the involvement of other mechanisms.

MATERIALS AND METHODS

Animals

The sea raven, *Hemitripterus americanus* (Gmelin), and the ocean pout, *Macrozoarces americanus* (Bloch & Schneider), are benthic species. They were caught

by otter trawl in Passamaquoddy Bay off St. Andrews, N. B. and held in flowing sea water at ambient temperature (10–12 °C). Animals were not fed during the 1–3 weeks in captivity.

Perfusate composition

A perfusate was developed in which ionic composition and pH values approximated to the physiological values in teleost fish. The perfusate composition was 150 mM-NaCl, 2 mM-$MgSO_4 \cdot 7H_2O$, 5 mM-KCl, 2·3 mM-$CaCl_2$, 2·3 mM-Na_2HPO_4, 0·2 mM-NaH_2PO_4, 16·7 mM-dextrose and 10 g l^{-1} polyvinylpyrrolidone (PVP, $M_r = 40\,000$). The phosphates, dextrose and PVP were added just before use. The control perfusate was gassed with 0·5 % CO_2: balance air and, after equilibration, the pH was adjusted to pH 7·9 with the addition of $NaHCO_3$ (approximately 10·7 mM). The acidotic perfusates were produced by changing the CO_2 composition in the gas and making small adjustments in the $NaHCO_3$ additions. A perfusate with pH 7·6 was generated by gassing with 1 % CO_2: balance air and adding about 10·7 mM-$NaHCO_3$. A perfusate with pH 7·4 was generated by gassing with 2 % CO_2: balance air and adding about 11·9 mM-$NaHCO_3$. The perfusates were gassed vigorously throughout the experiment with their respective gas mixture. The pH of the perfusates was measured at 10 °C using a Radiometer PHM 84 initially and later an acid base analyser (IL113, Boston, Mass.), plus their associated electrodes.

In situ *perfused heart preparation*

The details of the *in situ* heart preparation have been presented previously (Farrell *et al.* 1982). Stainless steel tubes were inserted into the hepatic vein and ventral aorta to act as inflow and outflow cannulae, respectively. All other veins entering the sinus venosus were ligated and the nerves to the heart were cut. Preparation time was 15–25 min, during which the fish was maintained in an anaesthetized state on an operating sling with the gills irrigated with sea water containing anaesthetic (0·5 g l^{-1} Tricaine methanesulphonate, Ayherst, New York). Throughout the operative procedures the heart received either venous blood or perfusate once the input cannula was in place. Following these procedures, the fish was transferred to and fully immersed in a constant temperature Cortland saline bath (10 °C) where the input cannula was connected immediately to a line delivering the control perfusate at a constant pressure (0–2 cmH_2O). The output cannula was connected to a pressure head of 36 cmH_2O to simulate vascular resistance (Fig. 1). The temperature of the water bath, the perfusate reservoirs and the perfusate lines were maintained at 10 °C with water jackets and a circulator/cooler (Lauda RM 3). The advantages of this preparation were that the chambers of the heart were physically undisturbed during preparation, and the heart retained intrinsic physiological mechanisms (Farrell *et al.* 1982; see below).

Instrumentation and apparatus

The input and output pressures to the heart were monitored with a saline filled Micron pressure transducer (Narco Life Sciences, Houston, Texas) *via* saline filled tubes connected to side arms on the input and output cannulae. Cardiac output was measured in the outflow line using a flowthrough electromagnetic flow probe and associated BL 610 Biotronix flowmeter. The signals from the flowmeter and pressure

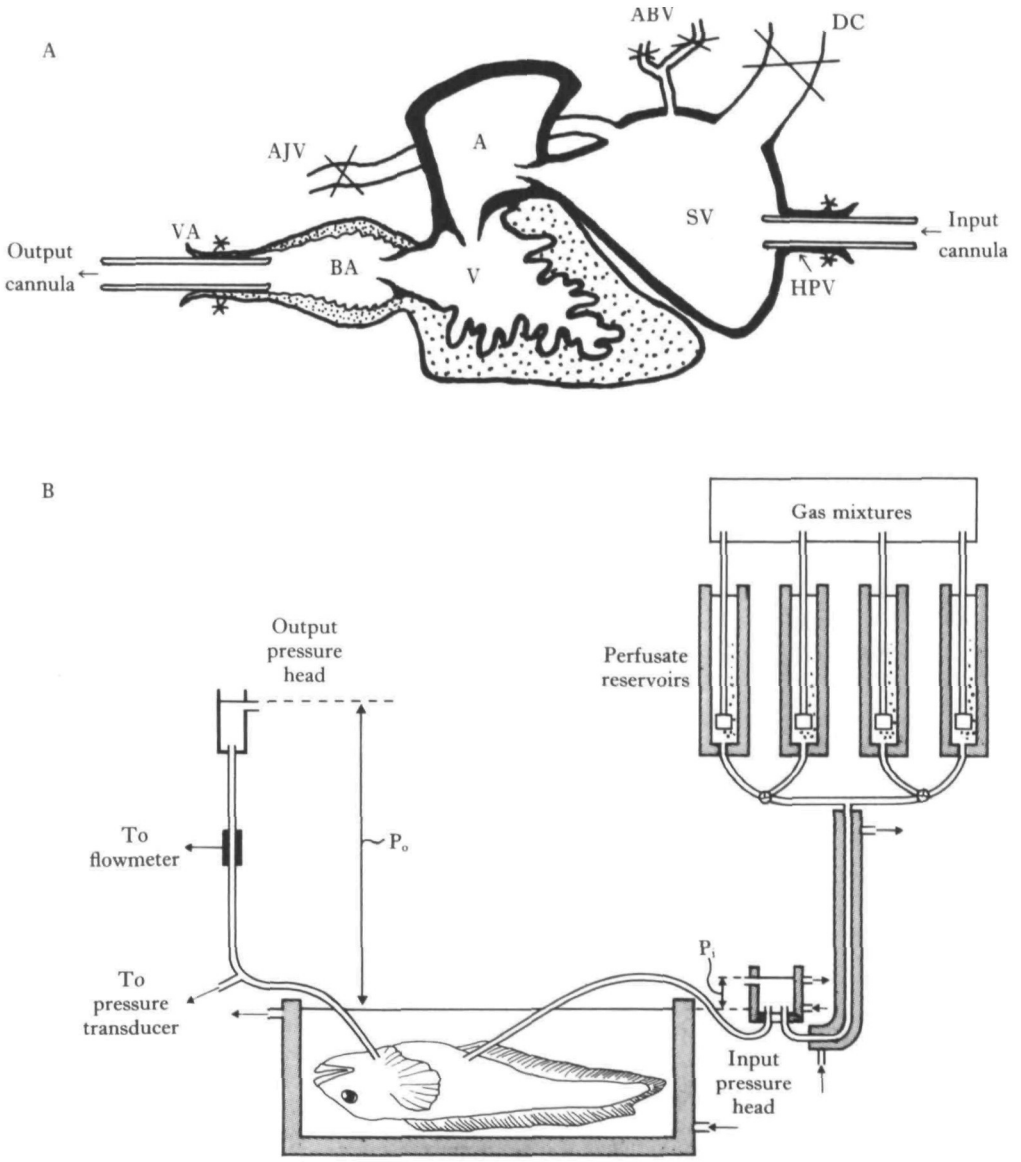

Fig. 1. (A) A schematic diagram of the *in situ* heart preparation. A = atrium, ABV = abdominal veins, AJV = anterior jugular vein, BA = bulbus arteriosus, DC = ductus Cuvier, HPV = hepatic vein, SV = sinus venosus, V = ventricle, VA = ventral aorta, • = ligatures securing the input and output cannulae into the hepatic vein and ventral aorta, respectively, and X = ligatures around veins (performed bilaterally). (B) A schematic diagram of the experimental set up. The stippled areas represent water jackets with the arrows representing the inflow and outflow of the coolant. The heart had a steady intrinsic rhythm and the input pressure head (P_i) determined the stroke volume. The output pressure head (P_o) simulated vascular resistance. The cardiac output and P_o were measured in the output line. The input pressure was monitored in the input line (not indicated). Descriptions of the gas mixtures are found in the text.

transducer were suitably amplified and displayed on a chart recorder (Biotronix BL 882, Kensington, Maryland).

Experimental protocols

Initially the output pressure head was set to produce an after-load (the mean output pressure in the ventral aorta) of approximately 36 cmH_2O. At this fixed after-load, the input pressure was adjusted to produce a basal $\dot{Q}$ of about 10–12 $ml\,min^{-1}\,kg^{-1}$ of body weight. Consequently, the pre-load varied between preparations (−0·3 to 2·0 cmH_2O, $\bar{x} = 0{\cdot}33$ for the sea raven; −0·2 to 1·7 cmH_2O, $\bar{x} = 0{\cdot}56$ for the ocean pout). The basal values were selected to simulate the *in vivo* values of resting fish. The *in situ* heart therefore performed with a basal power output of 6–7 $\times\ 10^{-4}\,J\,s^{-1}$, which is comparable to the *in vivo* resting situation for the ocean pout and sea raven (Farrell & Driedzic, 1980). The heart received the control (pH 7·9) perfusate for an initial 20–30 min to allow for post-operative recovery. This recovery period assured stability of the preparation. If $\dot{Q}$ began to decline during the recovery period the preparation was discarded (14 of 49 preparations). In the remaining 35 preparations $\dot{Q}$ remained unchanged, though there were small decreases (1–4 $beat\,min^{-1}$) in heart rate and compensatory increases in stroke volume in some preparations.

Responses to pre-load and after-load in the ocean pout heart

To ascertain whether the ocean pout heart retained its intrinsic mechanical properties *in situ*, the effects of changing pre-load and after-load were examined in eight fish (1·0–1·7 kg; $\bar{x} = 1{\cdot}3$ kg). The protocol was similar to that used previously for the sea raven (Farrell *et al.* 1982), where hearts were exposed to brief, sequential changes in (a) pre-load (after-load constant), and (b) after-load (pre-load constant). Each change lasted 2–5 min, which was sufficient time for the response to stabilize.

The effects of acidosis and adrenaline

Hearts from 14 sea ravens (0·8–1·5 kg; $\bar{x} = 1{\cdot}2$ kg) and 13 ocean pout (0·8–1·5 kg; $\bar{x} = 1{\cdot}2$ kg) were used. Each heart was tested with the following sequence of treatments: (a) pH 7·6 perfusate, (b) pH 7·4 perfusate, (c) control (pH 7·9) perfusate plus 10^{-7} M-L-adrenaline (AD), (d) pH 7·6 perfusate plus 10^{-7} M-AD, and (e) pH 7·4 perfusate plus 10^{-7} M-AD. The treatments lasted only 5 min and after each of the treatments the heart was rapidly returned to the control perfusate. Each heart was required to recover its basal $\dot{Q}$ before continuing with the sequence. The recovery time varied for each treatment and was between 3–12 min. The longer recovery times were associated with the slow return of basal heart rates following AD treatments. This presumably reflected a slow washout of AD from the cardiac tissue. An additional six ocean pout did not recover their basal $\dot{Q}$ following the pH 7·4 treatment and therefore only received the first two acidosis treatments. These hearts had shown a normal 20 min post-operative stability, but since they apparently became acidosis-damaged, the data were analysed separately.

Data analysis and presentation

Both pre-load and after-load were calculated from recorded input and output

Table 1. *Control values for the* in situ *perfused hearts of the sea raven and ocean pout used in the acidosis study*

	Sea raven ($N = 14$)	Ocean pout ($N = 13$)
Heart rate (beat min^{-1})	42·1 ± 1·9	56·2 ± 1·3
Stroke volume (ml kg^{-1})	0·29 ± 0·01	0·19 ± 0·01
Cardiac output (ml min^{-1} kg^{-1})	11·97 ± 0·28	10·47 ± 0·25
Power output ($\times 10^{-4}$ J s^{-1} kg^{-1})	6·95 ± 0·17	6·13 ± 0·16
Pre-load (cmH_2O)	0·33 ± 0·19	0·56 ± 0·15
After-load (cmH_2O)	35·8 ± 0·2	36·4 ± 1·0

Mean values ± standard error of N fish (in parentheses).

pressures. Mean values were obtained from pulsatile traces where mean = diastole + 1/3 pulse, and pulse = systole−diastole. Appropriate corrections were made for the pressure drops across the input and output cannulae. The cannulae resistances were calibrated over a range of $\dot{Q}$ values. All pressures are expressed in cmH_2O (1 cmH_2O = 0·098 kPa). Stroke volume (ml)/fish weight (kg) and heart rate (beat min^{-1}) were determined on a beat-by-beat basis from the area under the flow record and its periodicity, respectively. Cardiac output ($\dot{Q}$, ml min^{-1} kg^{-1}) = stroke volume × heart rate. Power output of the heart (J s^{-1} kg^{-1}) = (after-load−pre-load) × $\dot{Q}$ × (980/60) × 10^{-7}.

The control values for the cardiac variables at the basal work load are presented as mean values ±S.E.M. in Table 1. Data from the acidosis experiment are expressed as the percentage change from the control value immediately preceding each treatment. The graphs present the mean values of these changes. The significance of the changes was statistically analysed using a Wilcoxon signed-rank test for paired observations and $P \leq 0{\cdot}05$ was considered to be a significant change.

RESULTS

Mechanical properties of the in situ *ocean pout heart*

Cardiac output and power output were highly dependent upon the pre-load, which is in accordance with Starling's law of the heart. Increases in pre-load, especially in the 0–1·5 cmH_2O (above ambient) pressure range, produced marked increases in stroke volume without any effect on heart rate (Fig. 2). Up to a six-fold change in stroke volume could be produced by a small change in pre-load.

Changing after-load over the 25–46 cmH_2O pressure range had no effect on stroke volume or heart rate (Fig. 2). Thus the observed increase in power output was directly proportional to the increases in the mean pressure developed by the heart while performing at a constant $\dot{Q}$.

These experiments demonstrate that the ocean pout heart retains fundamental intrinsic mechanical properties *in situ*. This is also true for the *in situ* sea raven heart (Farrell *et al.* 1982).

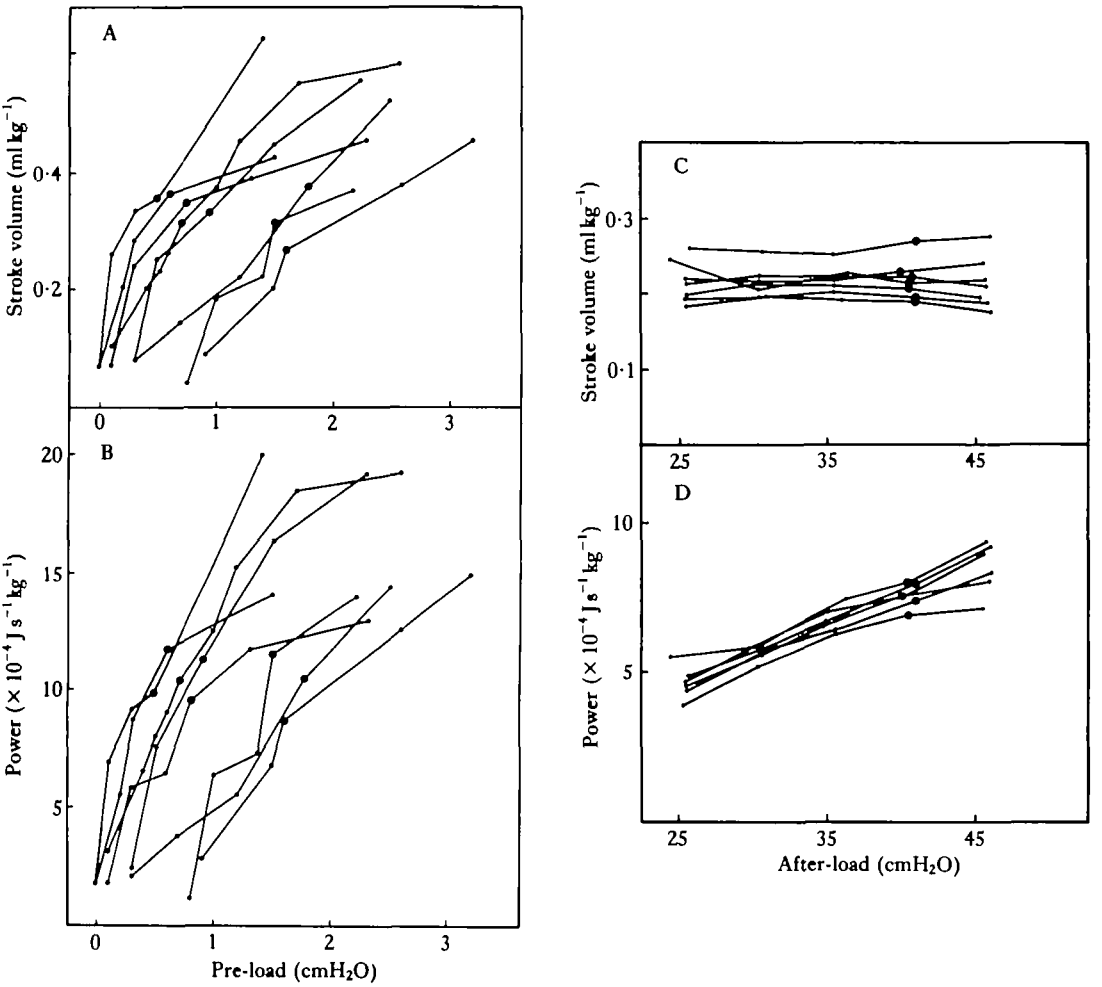

Fig. 2. Individual curves ($N = 8$) illustrating the changes in stroke volume and power output brought about by changing pre-load (A and B) and after-load (C and D) to the *in situ* heart of the ocean pout. The pre-load and after-load were measured relative to the ambient pressure (the level of the saline bath). Circled points indicate basal values.

Extracellular acidosis

The hearts of the sea raven and the ocean pout were sensitive to levels of extracellular acidosis common in other fish following exhaustive exercise. Exposure to pH 7·6 and pH 7·4 significantly depressed $\dot{Q}$ and power output in both fish by the end of the 5-min treatment (Fig. 3). The greatest effects were seen at the lower pH where $\dot{Q}$ was reduced by 12 % and 18 % in the sea raven and ocean pout, respectively. In individual fish, $\dot{Q}$ was reduced by as much as 26 % and 52 % in the sea raven and ocean pout, respectively, at pH 7·4.

Adrenaline (AD) at pH 7·9

The AD concentration of 10^{-7} M was considered to be within the physiological range for circulating catecholamines in fish (Wahlqvist & Nilsson, 1980). Continuous

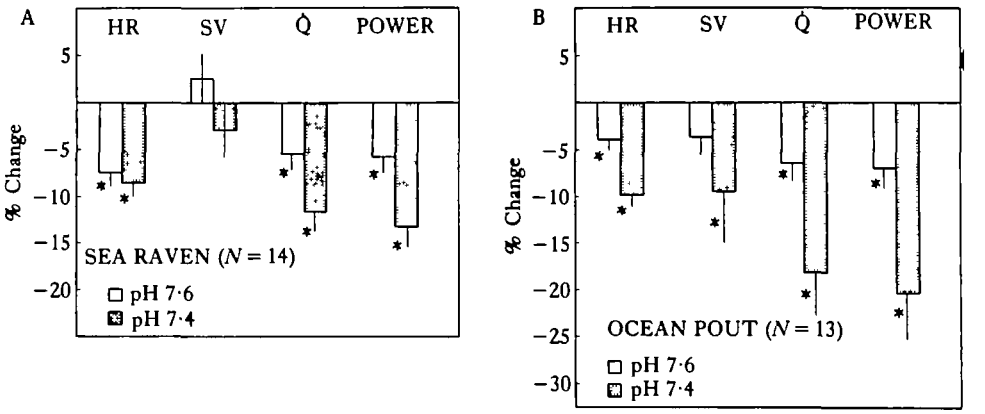

Fig. 3. The effects of hypercapnic acidosis (pH 7·6/1 % CO_2 in air, and pH 7·4/2 % CO_2 in air) on the *in situ*, perfused heart of (A) the sea raven, and (B) the ocean pout. This and all subsequent figures present the data as a percent change from the control value ±1 S.E.M. (vertical bars) for N fish (in parentheses). Between treatments the heart restored its basal cardiac output on control perfusate (pH 7·9/0·5 % CO_2 in air). HR = heart rate, SV = stroke volume, $\dot{Q}$ = cardiac output and POWER = power output, * = statistically significant change from basal level.

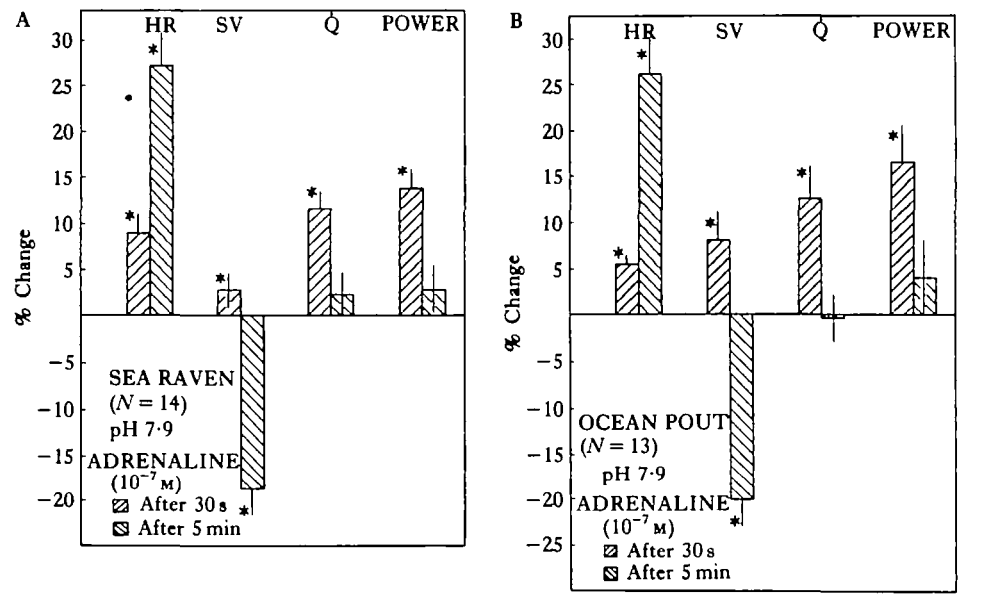

Fig. 4. The biphasic response of *in situ* hearts from (A) sea raven and (B) ocean pout to continuous perfusion of 10^{-7} M-adrenaline in the control perfusate (pH 7·9/0·5 % CO_2 in air). Legend and details as in Fig. 3.

perfusion of the heart at this concentration produced a similar response in both species (Fig. 4). Heart rate increased continuously to a more or less stable level by the end of the 5-min treatment. After only 30 s there was a consistent and statistically significant tachycardia in both species. Stroke volume displayed a biphasic response. There was an initial, transient increase in stroke volume, which peaked 30 s after perfusion had started (Fig. 4). Thereafter, stroke volume declined in proportion with the increase in heart rate.

These changes in heart rate and stroke volume resulted in a modest, but statistically significant, increase in $\dot{Q}$ and power output after 30 s. However, $\dot{Q}$ and power output quickly returned to and remained at their basal levels for the remainder of the perfusion period, emphasizing the fact that the changes in heart rate and stroke volume were quantitatively the same, but in the opposite directions.

Combined effects of extracellular acidosis and adrenaline

The combined treatment of AD plus acidosis at pH 7·6 produced a statistically significant increase in $\dot{Q}$ and power output after 30 s of perfusion (Fig. 5). These increases were not maintained. $\dot{Q}$ and power output quickly returned to and remained at their basal levels for the remainder of the perfusion. This was in contrast to the reduction of $\dot{Q}$ and power output seen with the pH 7·6 acidosis treatment in the absence of AD.

Similar changes were seen with the combined treatment of AD plus acidosis at pH 7·4 (Fig. 6). $\dot{Q}$ and power output initially increased significantly, but quickly returned to their basal level in the sea raven, or to a modestly elevated level (+3 %) in the ocean pout. This was in marked contrast to the significant reduction of these variables seen with the pH 7·4 acidosis treatment in the absence of AD. Clearly whatever negative effects extracellular acidosis (pH 7·6 and pH 7·4) has on overall cardiac performance, they were completely offset by the interactive effects of 10^{-7} M-AD. The interactive effects of AD and acidosis were well illustrated by the modifications in heart rate. Extracellular acidosis greatly attenuated the AD-induced tachycardia in both fish and at both acidosis levels.

Comparative sensitivity of the ocean pout and sea raven hearts to acidosis

The heart of the ocean pout was apparently slightly more sensitive to acidosis than

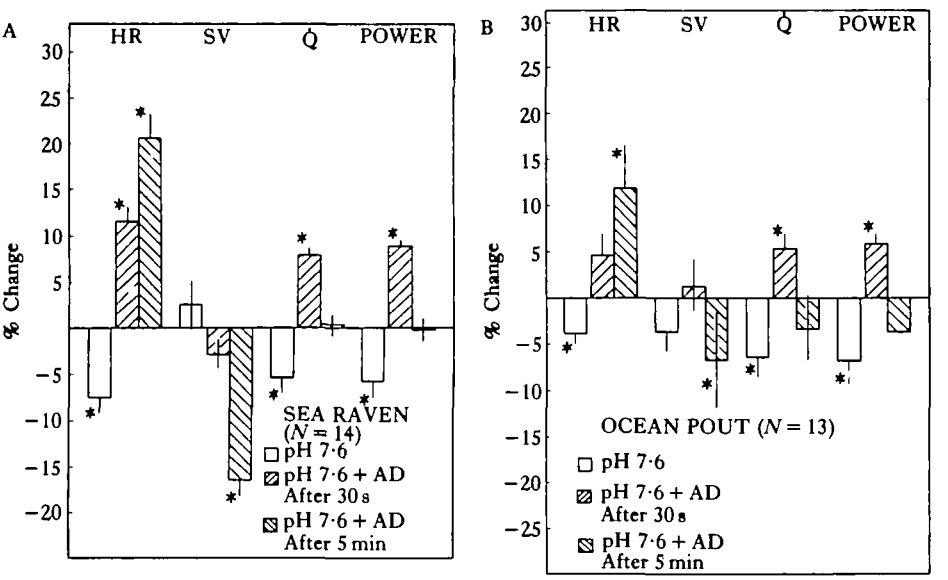

Fig. 5. The combined effect of continuous perfusion of 10^{-7} M-adrenaline (AD) during hypercapnic acidosis (pH 7·6/1 % CO_2 in air) in (A) the sea raven, and (B) the ocean pout. The effects of hypercapnic acidosis alone (from Fig. 3) are included for comparison. Legend and details as in Fig. 3.

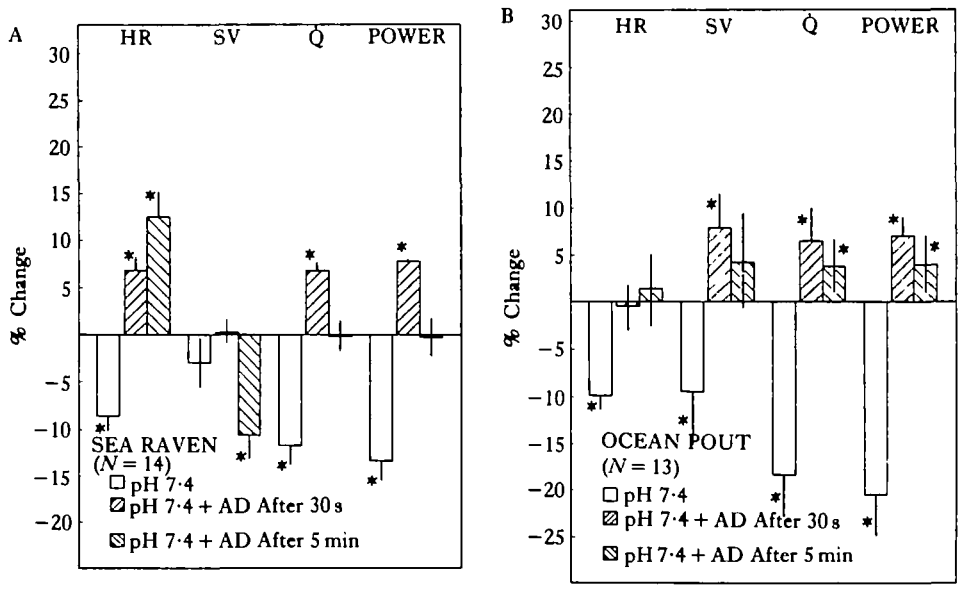

Fig. 6. The combined effect of continuous perfusion of 10^{-7} M-adrenaline (AD) during hypercapnic acidosis (pH 7·4/2 % CO_2 in air) in (A) the sea raven, and (B) the ocean pout. The effects of hypercapnic acidosis alone (from Fig. 6) are included for comparison. Legend and details as in Fig. 3.

the sea raven heart, as shown by a 21 % *versus* a 13 % reduction in power output at pH 7·4 (Fig. 3). This point is supported by the fact that six ocean pout hearts failed to recover fully from the pH 7·4 treatment. These six preparations are considered significant because they represent almost one-third of the 19 successful ocean pout preparations that displayed satisfactory post-operative stability. By comparison there were 14 successful sea raven preparations that displayed satisfactory post-operative stability, and all of these recovered fully from the same acidosis sequence. The data from these six additional ocean pout preparations were analysed separately and clearly exhibited a greater sensitivity to both levels of acidosis than the sea raven heart. Notably, there were statistically significant reductions in stroke volume and heart rate at both pH levels. As a result, $\dot{Q}$ was significantly reduced by −14 % and −26 % at pH 7·6 and pH 7·4, respectively.

DISCUSSION

In situ *heart preparation*

The *in situ* heart preparation, originally developed for the sea raven (Farrell *et al.* 1982), was applied successfully to the ocean pout. Both the sea raven and ocean pout *in situ* heart preparations were extremely stable over the initial 20–30 min when they were performing at the 'basal' work load, and were still capable of performing at 'basal' levels after 2 h. Such longterm stability was appropriate for the present physiological investigation. The complete absence of catecholamines from the control perfusate did not appear to cause problems, since basal cardiac outputs were generated at pre-loads similar to those found *in vivo*. Also, the addition of 10^{-9} M-AD has been found to have no significant effect on the sea raven heart *in situ* (Farrell *et al.* 1982). Oxygen delivery

to the myocardium appeared to be adequate, since myocardial oxygen consumption reduces the P_{O_2} of the perfusate by only 15–20 mmHg on its single passage through the ventricle (A. P. Farrell, unpublished observations).

Intrinsic properties of the in situ *heart preparation*

The present work on the ocean pout and the previous work on the sea raven (Farrell *et al.* 1982) provide concrete evidence that only small changes in venous return pressure are required to elicit large changes in $\dot{Q}$ through intrinsic mechanisms. Other authors (Randall, 1970; Jones & Randall, 1978) have suggested previously that $\dot{Q}$ in teleost fish is dependent on venous return and performs in accordance with Starling's law of the heart. Their suggestions were based, however, on marginal experimental data, since even the pioneer work in this field (Bennion, 1968) must be criticized because the input pressures of 2·6–15·6 cmH_2O were far from physiological. Thus it is highly significant that the marked changes in stroke volume observed in the *in situ* heart occur over the 0–1·5 cmH_2O pressure range, pressures which are consistent with the few measurements of central venous pressure in teleost fish (1·2 cmH_2O in the eel, Chan & Chow, 1976; 0·5–1 cmH_2O in the sea raven; A. P. Farrell, unpublished observations).

Another intrinsic property demonstrated by the *in situ* heart was the interdependence of stroke volume and heart rate. Changes in heart rate alter ventricular filling time and so stroke volume has to change, assuming the filling pressure is constant (as is the case here). Increases in heart rate, for example, should produce proportionate decreases in stroke volume in the absence of other influences. This intrinsic property was demonstrated during the stabilization period preceding the experiments when there were small decreases in heart rate but no change in $\dot{Q}$ because of the proportionate increase in stroke volume. An additional and more striking example was provided when AD was added to the control perfusate. In both fish, a significant increase in heart rate (+27 %) was accompanied by a significant and proportional decrease (−19 to −20 %) in stroke volume such that $\dot{Q}$ was unchanged. The sustained tachycardia is a typical response of the teleost heart to AD (Bennion, 1968; Gannon & Burnstock, 1969; Holmgren, 1977; Ask, Stene-Larsen & Helle, 1980; Forster, 1981; Cameron & Brown, 1981; Donald & Campbell, 1982; Farrell *et al.* 1982). AD is also reported to produce positive inotropic effects in the teleost heart* (Bennion, 1968; Holmgren, 1977; Ask *et al.* 1980; Forster, 1981). Clearly this inotropic effect of AD was not manifest in the *in situ* heart as a longterm increase in stroke volume, because of the compromising increases in heart rate. Instead the inotropic effect of AD was seen as an initial transient increase in stroke volume prior to any large change in heart rate. The modest increases in stroke volume after 30 s no doubt reflect the fact that heart rate had also increased significantly by this time, especially in the sea raven. In view of the above, it is necessary, in the following discussion of the effect of acidosis on contractility, to consider the interdependence of heart rate and stroke volume.

The effects of acidosis

The inability of the ocean pout and sea raven hearts to maintain their basal power

* The sustained inotropic effect of AD has been observed previously either in isolated hearts working at a low (if any) after-load, or in muscle strips that were electrically paced. Electrical pacing precludes any potential interference of the inotropic response, and a non-physiological after-load alters the inotropic state of the heart.

output and $\dot{Q}$ during extracellular acidosis is in keeping with the previous observations, where more extreme levels of hypercapnic acidosis were examined (Poupa & Johansen, 1975; Poupa *et al.* 1978; Gesser & Poupa, 1978, 1979). The pH conditions used here (pH 7·4 and pH 7·6) are well within the physiological range for venous blood following exhaustive activity in fish (Wood *et al.* 1977; Ruben & Bennett, 1981; Graham *et al.* 1982; Neumann *et al.* 1983) and are equivalent to a respiratory acidosis. Following exhaustive exercise the initial precipitous fall in pH and the lowest pH values are associated with a predominantly respiratory acidosis (Wood *et al.* 1977; Graham *et al.* 1982). This initial period lasts at least 5 min during which time the CO_2 partial pressure in arterial blood reaches 7–8 Torr on average, but as high as 11 Torr in individuals (M. S. Graham, personal communication). The remainder of the fish's recovery is spent in a mixed acidosis. The CO_2 partial pressures used in the present work were 7 and 14 Torr. The higher value is therefore a few Torr higher than the maximum found *in vivo*, given an allowance of 1–2 Torr for the arteriovenous difference. This situation came about largely because of the limitations of using saline perfusates and the desire to keep HCO_3^- relatively constant rather than an attempt to exceed *in vivo* limits. The CO_2 partial pressures used in the present work do nevertheless approach physiological conditions, unlike previous investigations where values of 21–125 Torr were used (see Introduction for references). Thus, given our desire to elicit rapid, short-term changes in intracellular pH and the proximity of the acidotic conditions to the *in vivo* situation, we feel justified in our experimental approach and in applying our findings to intact fish. Consequently, the present findings imply that the extracellular acidosis following exhaustive activity should depress $\dot{Q}$ in the absence of any compensatory mechanisms.

Cardiac output was reduced during extracellular acidosis as a result of two responses; a bradycardia and a reduction of (or negative effect on) stroke volume. The acidosis treatments decreased heart rate in every heart preparation examined. Bradycardia associated with hypercapnic acidosis has not been observed previously in fish, but a 20–30 % reduction in heart rate has been reported for dogs exposed to 15 % or 30 % CO_2 (Manley, Nash & Woodbury, 1964). The mechanism responsible for the bradycardia associated with hypercapnic acidosis is unknown.

Previous work has shown that myocardial acidosis exerts a negative inotropic effect through H^+/Ca^{2+} competition for binding sites on troponin (Williamson *et al.* 1976; Gesser & Jørgensen, 1982). A negative inotropic effect was clearly demonstrated by the significant reduction of stroke volume in the ocean pout heart. This response was evident at both pH levels, particularly in the six acidosis-damaged ocean pout hearts, and even with a concomitant bradycardia. In the sea raven there was no statistically significant change in stroke volume, although 10 of the 14 sea raven hearts from each acidosis treatment displayed a small decrease in stroke volume below their pretreatment control level. This observation is important in view of the interdependence of stroke volume and heart rate described above. Heart rate fell during both acidosis treatments and so stroke volume should have increased (with little change in $\dot{Q}$) if acidosis had no other effect than a slowing of the intrinsic heart rate. However, this was not the case since stroke volume decreased slightly in the majority of preparations in spite of the increased ventricular filling time favouring an increase in stroke volume. We view this as a negative effect on stroke volume, which may have been

a consequence of a negative inotropic effect brought about by the acidosis, as in the ocean pout.

Interactive effects of AD during acidosis

A physiological concentration of AD can fully compensate for the negative effects of acidosis, and for a brief period can actually increase $\dot{Q}$ and power output. This was found to be true in the sea raven and the ocean pout, and at both levels of acidosis. These observations are consistent with earlier work which demonstrated that AD protects contractile force development during hypercapnic acidosis in the trout and the eel (Gesser *et al.* 1982). An extreme level of acidosis (pH 7·0/13 % CO_2) was used in the earlier work, but even so the AD restored the force development of heart strips back to, or even slightly above, the basal level. The mechanism by which AD compensates for the effects of acidosis is not known for fish, but it is likely to be complex, as was pointed out by Gesser *et al.* (1982). In mammals, AD causes a redistribution of cellular Ca^{2+} and a stimulation of glycolysis (Williamson, 1964; Grossmann & Furchgott, 1964; Williamson *et al.* 1976; Niedergerke & Page, 1977).

Interactive effects of AD and acidosis on heart rate were apparent in the present work. The tachycardia associated with an AD addition to the control perfusate was either much attenuated or completely offset by acidosis. The mechanism responsible for this interaction is unknown.

Catecholamine levels in the blood increase during stressful exercise (Mazeaud, Mazeaud & Donaldson, 1977). Therefore, circulating catecholamines probably play an important role in maintaining $\dot{Q}$ under acidotic conditions. Based on the present findings and those of Gesser *et al.* (1982), AD by itself is unlikely to produce increases in $\dot{Q}$ much beyond the basal level during acidosis. Thus, how trout achieve a 60 % increase in $\dot{Q}$ while blood pH is 7·4 (Neumann *et al.* 1983) remains unclear. Trout do possess coronary arteries, but these vessels would still supply acidotic blood (albeit arterialized) to the myocardium. In addition, there appears to be no difference between fish possessing or lacking coronary vessels in their susceptibility to extreme extracellular acidosis (see Introduction for references). One way of increasing $\dot{Q}$ during acidosis might be to increase pre-load in addition to releasing AD into the venous blood from the chromaffin tissue. Stroke volume is very sensitive to pre-load, and the increase in pre-load would also compensate for the reduced ventricular filling time as heart rate is increased. Neural mechanisms and alterations in the availability of Ca^{2+} to the contractile proteins may also be involved and should be the focus of future work.

The sea raven and ocean pout are benthic species that lack a coronary circulation. The intrinsic mechanical properties and the biochemical make-up of their hearts (Driedzic & Stewart, 1982) are very similar. One major difference is the myocardial myoglobin level. The sea raven is typical of most teleost fish in possessing a myoglobin-rich ventricular tissue. The ocean pout, however, is atypical in that its myoglobin level is six times lower and the ventricular muscle has a white instead of red appearance if the blood is washed out of the lumen (Driedzic & Stewart, 1982). Another difference demonstrated here was the limited ability of the ocean pout heart to recover from the pH 7·4 acidosis treatment compared to the sea raven. Whether this is related to the lack of myoglobin is unknown. Myoglobin does act as an intracellular

buffer, but its relative contribution in the overall buffering capacity of the myocardium is minor (N. Heisler, personal communication). The buffering capacities of the sea raven and ocean pout hearts are presently being investigated in an attempt to explain the greater sensitivity of the ocean pout heart to extracellular acidosis.

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