SHORT COMMUNICATION

A PROTECTIVE EFFECT OF ADRENALIN ON THE ACIDOTIC TELEOST HEART

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In the teleost heart, catecholamines improve the tension development in isolated muscle strips and increase stroke volume (SVH) in perfused hearts working at sub-physiological levels (see Laurent, Holmgren & Nilsson, 1983 and Farrell, 1984 for recent reviews). Adrenalin (AD) can also restore or improve tension development during acidosis and anoxia (Gesser, Andresen, Brams & Sund-Laursen, 1982). However, physiological concentrations of AD do not increase SVH in any marked or consistent fashion in either intact fish or perfused hearts working at physiological levels (see Farrell, 1984), and only restore control levels of cardiac output (Vb) in perfused hearts exposed to pH 7.4 at constant preload (Farrell, MacLeod, Driedzic & Wood, 1983). These observations pose the question: what is the role of catecholamine-mediated inotropic stimulation in fish?

SVH is determined by the preload (Starling response) and by the inotropic state of the myocardium. Myocardial inotropy and the Starling response are severely curtailed by acidosis (Gesser et al. 1982; Farrell, Hart, Wood & Driedzic, 1984). It is possible, therefore, that an important action of circulating catecholamines is to protect the inotropic state of the myocardium under adverse conditions, such that changes in Vb are still possible through increases in preload. The present investigation examines the response of the perfused heart to preload during physiological levels of extracellular acidosis (pH 7.4) with and without a physiological level of AD (0.1 μmol l⁻¹) in the perfusate.

Sea ravens, Hemitripterus americanus (N = 14) were caught by otter trawl in Passamaquody Bay off St Andrews, NB and transported to Mt Allison University where they were held at 10 ± 1°C. The animals were not fed during the 1–4 weeks in captivity. The details of the in situ heart preparation have been presented previously (Farrell, McLeod & Driedzic, 1982; Farrell et al. 1983). 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. The fish was fully immersed in a constant temperature Cortland saline bath (10°C) and control perfusate was delivered at a constant preload.

Key words: Acidosis, fish myocardium, adrenalin.
so that control \( \dot{V}_b \) was 11 ml min\(^{-1}\) kg\(^{-1}\) wet weight of the fish. The output cannula was connected to a pressure head to simulate vascular resistance: the mean output pressure was about 40 cmH\(_2\)O. The heart of the sea raven has no coronary circulation serving the ventricle and so the ventricle normally derives its nutrition from venous blood passing through its chambers.

The perfusate composition was (in mmol l\(^{-1}\)) NaCl, 150; MgSO\(_4\).7H\(_2\)O, 2; KCl, 2; CaCl\(_2\), 2.3; Na\(_2\)HPO\(_4\), 2.3; NaH\(_2\)PO\(_4\), 0.2; dextrose, 16.7; and 10 g l\(^{-1}\) polyvinylpyrrolidone (PVP, \( M_r = 40,000 \)). Control perfusate was gassed with 0.5% CO\(_2\) in air and, after equilibration, the pH was adjusted to pH 7.9 with NaHCO\(_3\) (approximately 10-17 mmol l\(^{-1}\)). The acidotic perfusate was gassed with 1.87% CO\(_2\) in air and adjusted to pH 7.4 by adding about 11.9 mmol l\(^{-1}\) NaHCO\(_3\). The perfusate pH was measured at 10°C using an IL 113 acid-base analyser (Boston, MA) plus associated electrodes. The temperatures of the water bath, the perfusate reservoirs and the perfusate lines were maintained at 10°C with water jackets and a circulator/cooler (Lauda RM3).

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 BL610 Biotronix flowmeter. The signals from the flowmeter and pressure transducer were suitably amplified and displayed on a chart recorder (Biotronix BL882, Kensington, Maryland).

Heart rate (fH) was set by the intrinsic rate of the sino-atrial pacemaker and was extremely stable under control conditions during the initial perfusion period (~10–15 min) which preceded each experiment. Seven preparations were exposed to the following sequence of challenges.

(1) Using control perfusate, the preload to the heart was increased by about 1 cmH\(_2\)O and the response recorded. Preload was then increased to elicit the maximum increase in \( \dot{V}_b \), the response recorded, and control conditions were restored.

(2) The heart was switched to acidotic perfusate (pH 7.4) and the response was recorded after 5 min. The two preload changes were repeated during the acidosis challenge, and control conditions were restored with pH 7.9 perfusate.

(3) The acidosis challenge and preload changes were repeated with the acidotic perfusate containing 0.1 \( \mu \)mol l\(^{-1}\) AD (L-epinephrine bitartrate, Sigma).

Each heart acted as its own control and fully recovered the control level of cardiac performance between treatments.

A cumulative dose-response curve (1 nmol l\(^{-1}\), 10 nmol l\(^{-1}\), 0.1 \( \mu \)mol l\(^{-1}\), 1 \( \mu \)mol l\(^{-1}\) and 10 \( \mu \)mol l\(^{-1}\)) was developed for a synthetic \( \beta \)-agonist, isoprotenerol (ISO) (Sigma). The stable response to ISO at a constant preload was recorded after 5 min. In the sixth minute, preload was increased by about 1 cmH\(_2\)O to produce a stable change in \( \dot{V}_b \). Preload was returned to the control level before increasing the drug concentration in a stepwise fashion.

The mean preload and afterload were determined from the input and output pressure records and are expressed in cmH\(_2\)O (= 0.098 kPa). Stroke volume (ml)/
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fish weight (kg) and heart rate (beat min⁻¹) were determined, beat-by-beat, from the flow record. \( \dot{V}_b (\text{ml min}^{-1} \text{kg}^{-1}) = SVH \times fH \). Power output (mW g⁻¹) = (afterload-preload) \times \dot{V}_b \times (980/60)/\text{wet weight of ventricle}. The cardiac variables are presented as mean values ± S.E.M. Significant changes \((P \leq 0.05)\) were analysed using a Wilcoxon signed-rank test for paired observations.

Increasing preload by 1 cmH₂O almost doubled power output of the heart through an increase in \( \dot{V}_b \) and no change in fH (Fig. 1). Further increases in preload produced much smaller changes \( \dot{V}_b \).

Extracellular acidosis (pH 7·4) produced a small, statistically significant decrease in power output when preload was constant (Fig. 1). Increasing preload improved cardiac performance, but the maximum power output was significantly lower than that possible under control conditions.

When AD was added to the acidotic perfusate there was a small, statistically significant increase in power output with preload constant (Fig. 1). Increasing preload by 1 cmH₂O under these conditions, doubled power output to a level significantly higher than with the control perfusate.

ISO produced a dose-dependent increase in fH (Fig. 2), but \( \dot{V}_b \) and power output were unaffected since there was a concomitant decrease in SVH. ISO did not alter the response of the heart to a 1-cmH₂O increase in preload.

The present work supports previous findings that preload is a major determinant of SVH and that extracellular hypercapnic acidosis severely curtails myocardial inotropy.

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**Fig. 1.** The effects of acidosis (pH 7·4) with and without adrenalin (AD) (0·1 mmol l⁻¹). The broken line represents the response at a constant preload and the solid line indicates the effect of increasing preload. **Denotes a significant difference from the control for each treatment.**
even though there is only a small reduction in control power output at a constant preload (Poupa & Johansen, 1975; Gesser et al. 1982; Farrell et al. 1982, 1983, 1984). The most significant finding is that the Starling response of the acidotic heart is fully restored by a physiological concentration of AD, and therefore supports the idea that circulating catecholamines protect cardiac performance during extracellular acidosis. Consequently, when fish are stressed or swim in bursts the increase in circulating catecholamines may ameliorate the negative inotropic effects of the accompanying respiratory acidosis (e.g. Wood, McMahon & McDonald, 1977; C. L. Milligan & A. P. Farrell, unpublished results). This seems appropriate since additional demands

![Fig. 2. A dose-response curve to isoproterenol under control (V) conditions to illustrate the absence of major changes in Vb despite a statistically significant (★) tachycardia. Isoproterenol had no significant effect on the response to preload (▲).](image-url)
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are placed on the heart at this time (Neumann, Holeton & Heisler, 1983). Similarly, a recommended method for transporting fish stocks using 1:1 CO\textsubscript{2}:O\textsubscript{2} anaesthesia produces a severe respiratory acidosis (blood pH < 6.9; Itazawa & Takeda, 1982). Under such conditions, a sustained release of circulating catecholamines would seem essential for any level of cardiac integrity to be maintained.

The mechanism underlying the ameliorative effect of AD is not clear. Studies with mammalian myocardium reveal differences between \textit{in vitro} and \textit{in vivo} buffer curves resulting from $\beta$-adrenoceptor modulation of intracellular pH (Clancy, Gonzalez & Fenton, 1976; Strome, Clancy & Gonzalez, 1976). Comparable to the mammalian situation, the \textit{in vitro} buffer capacity of sea raven myocardium was approximately 1/7th of that calculated from \textit{in vivo} intracellular pH and HCO\textsubscript{3}\textsuperscript{-} values measured immediately after activity (C. L. Milligan & A. P. Farrell, unpublished results). Presently, however, there is no direct evidence that fish, like mammals, regulate myocardial pH through $\beta$-adrenoceptors, even though intracellular pH of fish red blood cells is regulated through an adrenergic mechanism (Nikinmaa, 1982).

ISO, a synthetic $\beta$-agonist, did not modify the response to a 1-cmH\textsubscript{2}O increase in preload at pH 7.9 (Fig. 2), yet AD, a natural $\alpha$- and $\beta$-agonist, improved the response to preload during extracellular acidosis. Whether this represents a confounding effect of the acidosis or a real difference between the actions of ISO and AD is unclear. If the latter is true, then $\alpha$-adrenoreceptors may also be involved in the inotropic stimulation of the sea raven heart, as was found for the eel heart (Chan & Chow, 1976).

In conclusion, the present work has clearly demonstrated that physiological levels of AD protect the inotropic state of the fish heart during respiratory acidosis and by doing so restore the heart’s ability to regulate Vb through the Starling response to preload.

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REFERENCES


