CONTRACTILE AND METABOLIC RESPONSE OF ISOLATED FISH HEARTS TO ANOXIA AND ACIDOSIS

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The contractile response of the isolated fish heart to oxygen deprivation and decreased extracellular pH has been the subject of numerous recent studies. Amongst fish species there occur vast differences in resistance to these insults. For instance, the time taken by isolated heart muscle strips to reduce force development after anoxia to 50% of pre-anoxic levels is 20-fold greater by plaice than by cod (Gesser and Poupa, Comp. Biochem. Physiol. 48A: 97-103, 1974). Furthermore, when exposed to a pH transition of 7.6 to 7.1, isolated preparations from the former species undergo a transient loss of force development but then recover to the initial level, whereas cod hearts continue to exhibit a marked decrease in force development (Poupa and Johansen, Am. J. Physiol. 208: 684-688, 1975). On the basis of enzymatic studies the plaice heart appears to have a greater anaerobic capability for energy generation than the cod heart (Gesser and Poupa 1974). It was therefore considered to be of interest to ascertain if hearts which are better able to sustain contractility during anoxic and acidotic challenges are also better able to maintain ATP levels.

In the present investigation this problem has been approached by perfusing isolated hearts under various conditions and subsequently determining the ATP concentration in the tissue. Two species with hearts of probable extreme biochemical differences were selected: the sea raven (*Hemitripterus*) which possesses a bright red heart in common with almost all vertebrate species, and the ocean pout (*Macrozoarces*) which has a white heart apparent only after blood washout. Preliminary results indicate that ATP generation mechanisms are not the primary locus of contractile failure due to either anoxia or acidosis.

Methods

Sea ravens and ocean pout weighing between 600-1500 gm were captured by otter trawl near Seal Cove, Maine. The fish were transported to M.D.I.B.L. and held in running water at ambient temperatures. Animals were sacrificed by severing the spinal cord, the hearts were exposed ventrally, excised and placed in cold perfusion media. The basic perfusion media consisted of NaCl 148 mM, KCl 5.1 mM, $CaCl_2$ 1.56 mM, $MgSO_4$ 0.93 mM, Na_2HPO_4 2.74 mM, NaH_2PO_4 0.26 mM, glucose 5mM, and bovine insulin 25 m units/ml. The perfusate was equilibrated with 100% O_2 and adjusted to pH 7.8.

Hearts were perfused by the method described by Driedzic (Physiol. Zool. 51: 42-50, 1978). Perfusate was delivered into the sinus venosus from a constant pressure head of 5 cm H_2O. The heart filled and emptied its contents by ventricular contraction through the cannulated ventral aorta which incorporated an inline flow meter probe. Hearts were required to pump against a pressure head of 15 cm H_2O. In addition a pressure transducer was connected via a side arm to the outflow cannula. All hearts were paced electrically at 20 beats per min and the temperature of the perfusate was maintained at $10 \pm 1^{\circ}C$.

Hearts were initially perfused for 5 min to allow washout of blood prior to cannulation of the ventral aorta. The ventral aorta was then cannulated and hearts were perfused for a further 5 = 10 min until a stable contractile response was attained. Thereafter perfusion was continued with buffer equilibrated with 100% O_2 (pH 7.8) or there was a switch over to perfusate equilibrated with 100% N_2 (pH 7.8) or 99% O_2:1% CO_2 (pH 6.8-7.0). Following a further 50 min perfusion period the hearts were freeze clamped between metal plates cooled to the temperature of liquid nitrogen. The tissue was analyzed for ATP and lactate content by standard enzymological techniques. Heart work per min was calculated from the product of flow (mls/min) X average systolic pressure (gm/cm^2). Average systolic pressure was considered to be (1/3 pulse pressure + diastolic) - filling pressure.

Results

The hearts of both the sea ravens and the ocean pout exhibited a decrease in performance relative to the 100% O_2 group when subjected to either 100% N_2 or 99% O_2:1% CO_2(Table 1).

Table 1. Percentage of initial work following 50 min of perfusion

	100% O_2	100% N_2	99% O_2:1% CO_2
Hemitripterus	60 ± 8	35 ± 9	16 ± 4
Macrozoarces	62 ± 20	< 1	< 1

However, the sea raven hearts were able to sustain a higher level of power output than the ocean pout hearts. When ocean pout hearts were challenged with buffer equilibrated with either N_2 or 99% O_2:1% CO_2 contractility had exxentially ceased by the end of the 50 min perfusion period. The sea raven hearts though still maintained 35% of their initial power output under the anoxic condition and 16% under the hypercapnic acidotic condition. Although there was a tendency for ATP levels to be lower in the anoxic than in the 100% O_2 groups the decreases were not statistically significant. As such, ATP levels remained relatively constant under all conditions. Lactate levels were elevated in the hearts subjected to anoxia relative to the 100% O_2 group (Table 2).

Table 2. Heart metabolite concentrations (μmoles/gm weight wet)

		100% O_2	100% N_2	99% P_2:1% O_2
Hemitripterus	ATP*	1.30 ± .24	1.19 ± .18	1.42 ± .35
	lactate	.60 ± .09	2.60 ± .50	0.44 ± .08
Macrozoarces	ATP*	1.74 ± 0.17	1.19 ± 0.22	1.49 ± 0.28
	lactate	1.25 ± 0.20	3.33 ± 0.43	2.12 ± 0.30

*All values represent the mean ± S.E. of 4-5 individuals

In addition, lactate output during the perfusion period was increased (data not shown). This increased level of anaerobic metabolism was probably instrumental in maintaining the ATP pools.

Discussion

The challenges of anoxia and hypercapnic acidosis resulted in a deterioration of cardiac function in both species of this study. The heart of the sea raven though is clearly more resistant to these stresses than the heart of the ocean pout. The content of ATP in the hearts of the anoxic and hypercapnic acidotic groups was not significantly lower than the content in the 100% O_2 groups. In the mammalian myocardium the state of irreversible tissue damage associated with ischemia appears to be associated with depletion of the ATP pool (Neely et al, Am. J. Physiol. 225: 651-658, 1973); however, initial contractile failure is possibly related to decreased intracellular pH (Williamson et al, Acta med. scand. Suppl. 587: 95-111, 1975). In the present study decreases in intracellular pH may be related to an increased rate of contractile failure. In the hearts perfused with 99% O_2:1% CO_2 the pH of the buffer was approximately 6.9. This most certainly must have resulted in a decrease in intracellular pH relative to normal values. Also, the increased

rate of lactic acid production in all hearts subjected to 100% N_2 would presumably be associated with a decrease in intracellular pH. It is probable that the sea raven hearts have a higher intracellular buffering capacity than the ocean pout hearts. Even after an extensive perfusion period the sea raven heart is bright red, whereas after only two or three contractions the ocean pout heart is creamy white in appearance. The red colouration is probably due to myoglobin which could function as an intracellular buffer. This could account for the greater resistance of the sea raven heart than the pout heart to the experimental perturbations. This work was supported in part by operating grants from New Brunswick Heart Foundation, Canadian National Sportsmen's Fund, N.S.E.R.C. of Canada and the NIH General Research Support Grant awarded to M.D.I.B.L.

ACTION OF cAMP AND THEOPHYLLINE ON ION MOVEMENT DURING OUABAIN INHIBITION OF Na-K-ATPase IN PERFUSED RECTAL GLAND OF SQUALUS acanthias

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Salt secretion by the rectal gland of Squalus acanthias is stimulated by cyclic adenosine monophosphate (cAMP), inhibited by furosemide and chemical analogs of that diuretic ompound, and depends on the presence of chloride, which is contransported with sodium across the basolateral membrane of rectal gland cells. These features are reminiscent of a transport system for cations, described in avian erythrocytes, which is also activated by cAMP, inhibited by furosemide and dependent on the presence of chloride. In avian erythrocytes, the carrier-mediated cotransport of sodium and potassium down their combined electrochemical gradients is enhanced by cAMP.

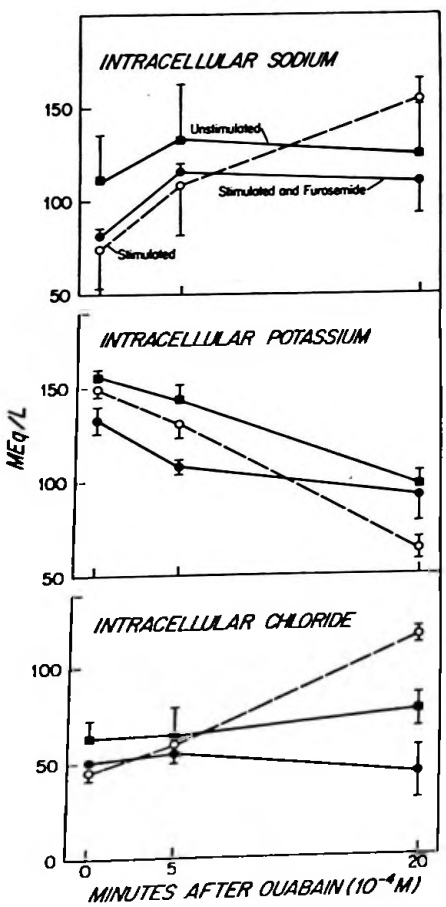

Figure 1. Calculated intracellular concentrations (mEq/L intracellular water) of Na^+, K^+ and Cl^- in perfused rectal glands after inhibiting active transport with ouabain. Values are mean ± S.E.; n=5.