

SHORT COMMUNICATION

CONTRACTILE PERFORMANCE OF CEPHALOPOD HEARTS UNDER ANOXIC CONDITIONS

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Accepted 29 January 1985

The cephalopod circulatory system includes an efferent systemic ventricle and two afferent branchial hearts. Branchial hearts lack coronary arteries and thus must be nourished by only venous blood. During routine metabolism in normoxic water, oxygen availability to branchial hearts is only a small percentage of that to the systemic heart; during hypoxic excursions the venous oxygen content may, at least transiently, approach zero (Johansen, Brix & Lykkeboe, 1982; Houlihan, Innes, Wells & Wells, 1982). The extreme difference in oxygen delivery to these tissues suggests that branchial hearts are better able to meet their energetic demands through anaerobic metabolism than are systemic hearts. This hypothesis was tested by following isometric force development of the cardiac tissue of the cuttlefish, *Sepia officinalis*, during cyanide poisoning. Experiments were also conducted with the systemic heart of *Octopus vulgaris*, in order to allow a comparison between a decapod and an octapod.

Specimens of *Sepia officinalis* Lam. (193 ± 29 g; $N = 7$) and *Octopus vulgaris* Cuvier (1550 ± 367 g; $N = 6$) were maintained in running sea water. Animals were anaesthetized in 3% ethanol, decapitated and the hearts excised. *Sepia* systemic ventricles were sectioned along the median line and each half utilized. *Octopus* ventricles were further sectioned transversely, allowing four preparations per animal. Each *Sepia* branchial heart was used for a single preparation. Tissues were spread flat to expose the interior surface, one end firmly fixed with a plastic clip, and the other end tied with surgical silk to a Harvard 363 isometric tension transducer interfaced with a Biotronex BL recorder. Hearts were forced to contract *via* field stimulation from two parallel silver electrodes, one on each side of the tissue. Square wave pulses of 20 ms duration with the minimum voltage necessary to produce the maximal mechanical response were generated from a Grass SD9 stimulator. After positioning, tissues were allowed a 30 min rest period and thereafter paced at 12 beats min^{-1} . Following 5 min of contraction, NaCN (1 mmol l^{-1} final concentration) was added to the bathing media in one half of the preparations and the recording of force continued for a further 25 min. Preliminary experiments showed that 2 mmol l^{-1} NaCN did not have any greater effect than 1 mmol l^{-1} NaCN, a concentration which inhibits

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Key words: Anoxia, cephalopod, heart.

respiration of cephalopod tissues (Ghiretti-Magaldi, Guiditta & Ghiretti, 1958). The bathing media was filtered sea water (37%) for *Octopus* hearts and sea water plus 10 mmol l^{-1} KCl for *Sepia* tissues (Robertson, 1953). Media were continuously aerated, maintained at 15°C , had a pH of 8.0 and contained 1 mmol l^{-1} glucose. Control and experimental preparations were run simultaneously. Seven paired preparations were obtained from *Sepia* hearts and eleven preparations from six *Octopus* under both control and experimental conditions. In cases of duplicated experiments, values were averaged prior to statistical analysis. Relative force development was calculated as a percentage of that exhibited at time zero. Data are presented as mean \pm s.e.m.. The effect of the cyanide treatment at the 30-min point was assessed within tissues and between tissues with a paired and independent *t*-test, respectively.

Sepia systemic ventricles were, on average, able to maintain 98 % of the initial tension development during the experimental period in the absence of cyanide (Fig. 1). Cyanide poisoning led to rapid contractile failure, and after 30 min of treatment, only 11 % of the initial force was developed. The difference in relative performance between paired preparations was $87 \pm 14\%$. *Sepia* branchial hearts failed both in the presence and absence of cyanide. At the end of the 30-min period, the control and experimental preparations developed only 74 and 37 % of the initial tension, respectively. The relative final performance was not significantly different between the two groups of branchial hearts. *Octopus* ventricles, even under control conditions, entered into failure and by the end of the experimental period could develop only 82 % of the initial force. Cyanide treatment accelerated the decline in performance and at the 30 min point led to a significant decrease in performance relative to controls of 38 % of the initial tension. Difference in relative performance between paired preparations

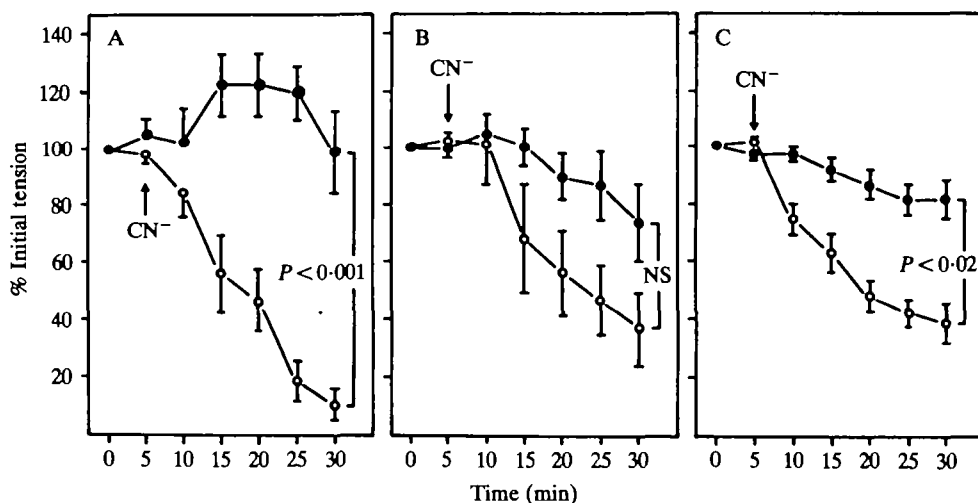


Fig. 1. Percentage of initial tension development by cardiac tissue in the presence and absence of cyanide. (A) *Sepia* systemic ventricle; (B) *Sepia* branchial heart; (C) *Octopus vulgaris* systemic ventricle. $N = 7$ for *Sepia* experiments; $N = 6$ for *Octopus* experiment. Closed circles represent control conditions and open circles cyanide treatment. Values represent mean \pm s.e.m. NS, not significant.

of *Octopus* ventricles was $43 \pm 12\%$. The treatment effect was significantly greater on *Sepia* than *Octopus* systemic ventricles ($P < 0.05$).

Both *Sepia* branchial hearts and *Octopus* systemic ventricles entered into contractile failure even under control conditions. This suggests that the bathing media were inadequate to support cellular integrity at the imposed level of energy demand. It is possible that cephalopod cardiac tissue has a necessary requirement for amino acids, which are known to be at extremely high levels in plasma (Hochachka *et al.* 1983). Another feature that may have been inappropriate was the level of CO_2 which is higher in *Sepia* and *Octopus* blood than in aerated sea water (Johansen *et al.* 1982; Houlihan *et al.* 1982). Carbon dioxide tension is known to be extremely critical in the performance of teleost hearts (Gesser, 1977; Farrell, MacLeod, Driedzic & Wood, 1983). The failure of *Sepia* branchial hearts and *Octopus* systemic ventricles, however, does not preclude an analysis of the effect of cyanide treatment.

The essentially complete failure of *Sepia* systemic ventricles under simulated anoxic conditions shows that this tissue is obligately aerobic. This finding is consistent with early manometric studies (Barron, Sights & Wilder, 1953) and the high mitochondria content of squid ventricles (Dyken & Mangum, 1979). As implied by blood gas data, *Sepia* branchial heart is better able to meet its energetic demands *via* anaerobic metabolism than the systemic ventricle. Maximal activity levels of enzymes of carbohydrate metabolism do not suggest that better performance of branchial hearts is due to an enhanced ATP generation capability (W. R. Driedzic & B. D. Sidell, unpublished observations). The mechanical work, and presumably the ATP demand, of the cephalopod branchial heart *in situ* is far lower than that of the systemic ventricle (Bourne, 1982; Wells & Wells, 1983). The present experiments show that anaerobic ATP production can more easily match ATP demands in the former.

The poisoning of oxidative metabolism in *Octopus* systemic ventricles resulted in an impairment of function but not in a complete collapse over the experimental period. A partial resistance to anoxia has been demonstrated for the ventricle of *Octopus dofleini* (Pritchard, Huston & Martin, 1963). *Octopus* ventricle is more resistant to simulated anoxia than is *Sepia* ventricle. The ventricle of the octopod, *Eledone cirrhosa*, has similar activity levels of enzymes associated with carbohydrate metabolism but a much lower level of total ATPase activity than *Sepia* ventricle (W. R. Driedzic & B. D. Sidell, unpublished observations). If *Octopus vulgaris* is similar to *Eledone cirrhosa* in this respect, then the difference in performance between *Octopus* and *Sepia* ventricles may be related to lower levels of ATP demand and hence a greater capability to match that demand through anaerobic metabolism alone.

In conclusion, the present experiments show that under conditions of impaired oxidative metabolism, both *Sepia* branchial heart and *Octopus* systemic ventricle are better able to maintain performance than *Sepia* systemic ventricle.

This work was supported by operating grants from N.S.E.R.C. of Canada and the New Brunswick Heart Foundation. Appreciation is extended to the members of the cephalopod group and the staff at the Laboratoire Arago, France for their considerable help.

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