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THE CONTRIBUTION OF THE BRANCHIAL HEART TO THE ACCESSORY BRANCHIAL PUMP IN THE OCTOPODA

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SUMMARY

Experimental and anatomical observations upon the Octopoda suggest that the branchial hearts are not the sole contributors to the increase in venous blood pressure between the anterior vena cava and the afferent branchial vessel.

The lateral venae cavae are proposed as an additional source of pressure generation, thus contributing to the octopod accessory branchial pump.

INTRODUCTION

The octopus circulatory system is atypical of the molluscs, with a medially situated ventricle generating high arterial blood pressures (Johansen & Martin, 1962; Wells, 1979). Using free-moving *Octopus dofleini*, Johansen & Martin (1962) demonstrated the presence of an accessory branchial pump, boosting the venous blood pressure, between the anterior vena cava and the afferent branchial vessel. Traditionally it has been assumed that the branchial hearts are responsible for this increase in the venous pressure, although there is no direct physiological evidence to support this idea. Within the published literature, however, there are several observations which indicate an additional source of pressure generation. The present paper draws these observations together and re-examines the possible locations of the accessory branchial pump.

MATERIALS AND METHODS

For most of this study, specimens of the octopod *Eledone cirrhosa* (Lam.) were acquired from the North Sea and maintained in a recirculating aquarium at the Department of Zoology, University of Aberdeen. Additional observations were made on this species and *Octopus vulgaris* Cuvier, at the Laboratoire Arago, Banyuls, France.

For both *in vivo* and *in vitro* preparations, animals were anaesthetized in 2% ethanol in sea water. All *in vivo* measurements were made 1 h after recovery from anaesthesia. Animals used for the *in vitro* observations were killed by the surgical destruction of the central nervous system.

The apparatus for maintaining an isolated and contracting branchial heart was as described by Smith (1981*a*) for work on the isolated ventricle. Perfusion pressures

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were originally set at levels measured in the anterior vena cava (0–17 cm of water) and the afferent branchial vessel (15–50 cm of water) by Johansen & Martin (1962). The input cannula (i.d. 3 or 5 mm) was tied into the lateral vena cava close to the branchial heart valve. The pallial vein was ligatured distal to the cardiac ganglion. The output cannula (i.d. 2 mm) was tied into the afferent branchial vessel 2 mm downstream from the heart. No valve was present at the junction of this vessel and the heart. To prevent backflow and allow the measurement of stroke volume, an excised auriculo-ventricular valve was tied to the end of the cannula and immersed in the output reservoir.

The contraction cycle of an isolated preparation was monitored using a CFP wire strain gauge, attached to the outer connective tissue layer of the heart via a thread and thin wire hook. Permanent records were made on a Devices pen recorder.

In vivo ventricular cardiograms were recorded by implanting trimel-coated stainless steel electrodes (Smith, 1981*b*). Similar electrodes were used for the impedance measurement of movement from the branchial heart, a technique used by Wells (1979). All electrodes were sewn through the dorsal surface of the mantle sac. Cardiogram signals were amplified using an Epil preamplifier. Impedance measurements were made with a Washington coupling unit FC108.

The preparation for measuring the pressure in the afferent branchial vessel was as described by Wells (1979), except that the cannula was passed through the opening of the mantle sac, instead of being threaded through the flesh of the posterior part of the mantle. Two pressure transducers were used, a Statham P23V or a Washington PT400. The signals were amplified using a Morgan transducer amplifier or a Washington coupling unit FC135. Permanent records were made on a Washington 400MD2 pen recorder.

RESULTS

The structure of the branchial heart

A thin muscular bi-layer encapsulates the heart, and muscle cords traverse the interior (Fig. 1). A large proportion of the heart is occupied by packing cells. All the muscle is innervated from the cardiac ganglion. Nerve bundles arising from this ganglion also innervate the branchial heart appendage, the efferent branchial vessel, and the muscular valves at the junction of the branchial heart and the lateral vena cava. A fuller description is given by Smith (1979) and P. J. S. Smith and P. R. Boyle (in preparation). Proximally, the cardiac ganglion is connected to the fusiform ganglion and distally to the branchial ganglia. The main vascular space runs directly from the lateral vena cava to the afferent branchial vessel. Several large lumen spaces diverge into and ramify throughout the packing cells.

Effect of perfusion pressure on output

In vitro the isolated branchial heart contracted regularly on perfusion. In no preparation was there any indication that the hearts could pump effectively at the pressure levels expected from the work of Johansen & Martin (1962). In the following experiments the pressure levels were set at lower values.

Two preparations were perfused at a constant pressure (4 and 5 cm of water). The

Ventral surface

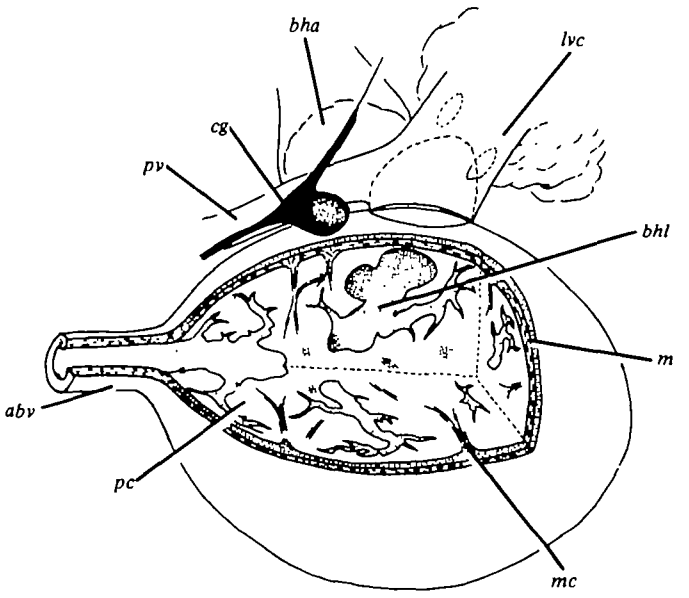

Fig. 1. Diagrammatic representation of the branchial heart, associated vessels and the cardiac ganglion. A section of the wall is cut away to illustrate the internal structure. Two of the renal appendages and a section of the lateral vena cava are also omitted.

abv, afferent branchial vessel; *bha*, branchial heart appendage; *bhl*, branchial heart lumen; *cg*, cardiac ganglion; *lvc*, lateral vena cava; *m*, outer muscle layer; *mc*, muscle cords; *pc*, packing cells; *pv*, pallial vein.

output back pressure was varied in steps of 2 or 1 cm of water. Both preparations showed a decrease in stroke volume as the difference between the input and output back pressures increased (Fig. 2). A mean stroke volume of 0·2–0·3 ml was pumped when the difference was zero. Neither preparation pumped a significant volume of fluid when the difference approached 4–6 cm of water.

Nine isolated branchial hearts were perfused at a constant output back pressure of 10 cm of water with a variable input pressure. Although low pressure levels were used, and all preparations contracted regularly, only one heart pumped fluid against this back pressure when the input pressure was altered. In this case the pressure difference was between 4 and 6 cm of water. The maximum stroke volume was 0·08 ml.

Out of three preparations with an output back pressure of 10–15 cm of water, two showed a significant relationship between heart rate and input pressure. Two preparations perfused at a constant input pressure and a variable output back pressure showed no clear alteration of contraction rate in response to changes in pressure difference.

Ablation of the cardiac ganglion

Experiments on the branchial heart, either *in vivo* or *in vitro*, were complicated by the close association of the heart with the cardiac ganglion. Ablation of the ganglion *in vitro* resulted in chaotic activity (n = 10). On no occasion did recovery occur. *In*

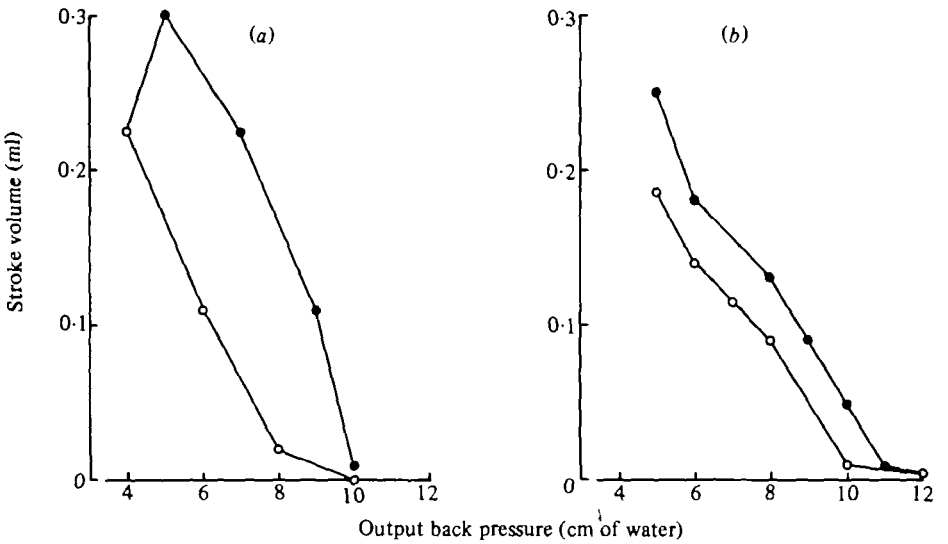

Fig. 2. (*a*, *b*) Variation of the branchial heart stroke volume with alteration of the output back pressure, for two preparations. In (*a*) the input pressure was held constant at 4 cm of water. In (*b*) this value was 5 cm of water. Mean stroke volumes for both descending (○) and ascending (●) values of output back pressure are presented.

vitro it appeared that the normal and rhythmic activity of the branchial heart was dependent upon the integrity of the ipsilateral cardiac ganglion.

In the free-moving *Eledone cirrhosa* the contraction of the branchial hearts was monitored using the impedance technique (n = 11). No relationship was observed between the branchial heart rate of contraction and the contractions of the mantle sac, although the former corresponded exactly with the ventricular contraction rate (n = 7). Where the ipsilateral cardiac ganglion was ablated, only irregular contractions were subsequently recorded from the branchial heart (n = 6). As with the isolated heart it appeared that, *in vivo*, the regular contraction of this organ was dependent upon the cardiac ganglion.

In the intact preparations the systolic pressure in the afferent branchial vessel ranged between 2 and 15 cm of water (Table 1), and often showed more than one peak (Fig. 3*a*). In preparation 3, where lower systolic pressures approximated in rate to the contractions of the mantle, the levels may be artifacts. After the ablation of the ipsilateral cardiac ganglion, three preparations continued to show high systolic pressures in the afferent branchial vessel (5–15 cm of water, Table 1 and Fig. 3*b*). Subsequent examination of preparation 4 showed that the cannula had lodged in the base of a vessel leading to the gill lamellae.

In the ablated preparations the pressure pulse in the afferent branchial vessel and the contractions of the mantle showed the same rate. This raised the possibility that the high pressures observed were caused by mantle-sac contractions. However, in preparation 2 contractions of the mantle sac continued when the pressure pulse in the afferent branchial vessel showed temporary arrest (Fig. 4). Also, there was no mantle contraction artifact of this magnitude superimposed on the pressure records in the intact preparations.

Table 1. *Examples of the blood pulse pressure in the afferent branchial vessel of* Eledone cirrhosa, *before and after the ablation of the ipsilateral cardiac ganglion*

The right vessel was cannulated in all cases. Mantle contraction rates are also presented for a number of preparations.

Condition	Intact				Right cardiac ganglion ablated			
Preparation	Systolic pulses per min.	Mantle contractions per min.	Diastolic pressure (cm of water)	Systolic pressure (cm of water)	Systolic pulses per min.	Mantle contractions per min.	Diastolic pressure (cm of water)	Systolic pressure (cm of water)
1 (North Sea)	24	20	0–4	10–15	14	16	2–3	10–15
2 (North Sea)	19	6	2–4	6–11	15	15	2–4	5–10
3 (North Sea)	9	10	0–1	2–5	5	5	0–1	6–12
4 (North Sea)	24	12	0–2	6–10	11	11	0–1	2–4
5 (North Sea)	21	11	3–4	8–10				

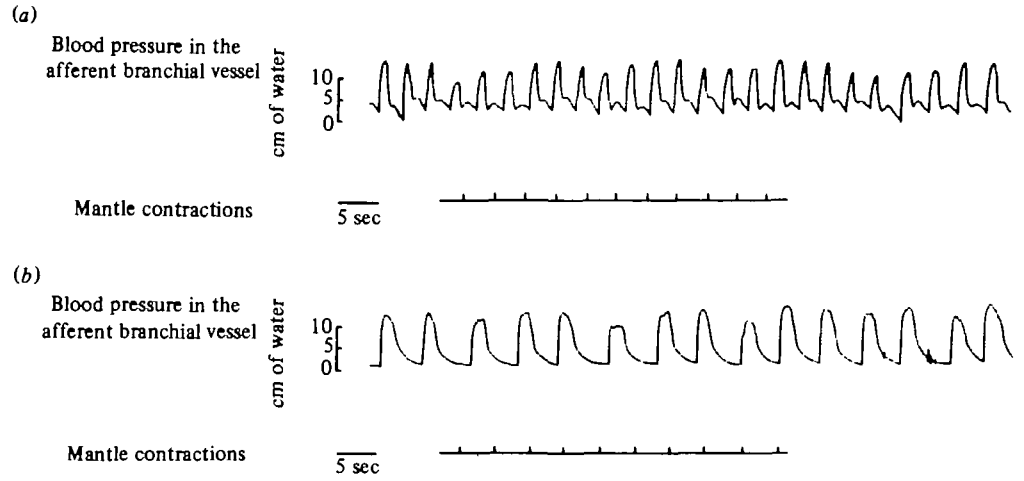

Fig. 3. (*a*) The *in vivo* pressure pulse recorded from the afferent branchial vessel of an intact *Eledone cirrhosa*. Note the magnitude and complex nature of the pressure pulse. This is the same preparation as in Table 1, preparation 1.

(*b*) The pressure pulse in the same animal and vessel as in (*a*) but after the ablation of the ipsilateral cardiac ganglion. The pressure pulse, although slower in rate, exhibits a similar amplitude as in the intact condition.

Fig. 4. A pressure record taken from the afferent branchial vessel of preparation 2, Table 1, after the ablation of the ipsilateral cardiac ganglion. During the recording the pressure pulse ceased temporarily. Mantle contractions were observed during this period.

If it is correct to assume that, on ablation of the cardiac ganglion, the branchial heart behaves *in vivo* as it does *in vitro*, then the high pressures recorded after the ablation of the ganglion cannot be attributed solely to the contraction of the branchial heart. An alternative would be that a pressure pulse is generated by structures contracting independently of the branchial heart.

In vivo, it was possible to implant electrodes in both the ventricle and the branchial hearts. In the preparation used to make Fig. 5 the left cardiac ganglion had been ablated. Where the heart was intact the record was distinguished by a double peak (Fig. 5*a*). The record from the left heart showed only one peak (Fig. 5*b*). Relative to the ventricular cardiogram the peak recorded from the denervated heart coincided with the first and smaller peak from the intact condition. The source of these peaks was therefore a synchronous, if not common, site of contraction.

The lateral venae cavae were the only structures observed to contract with the same frequency as the ventricle and the branchial hearts. As these vessels receive their innervation primarily from the ventricular nerves (Smith, 1979) they might not be

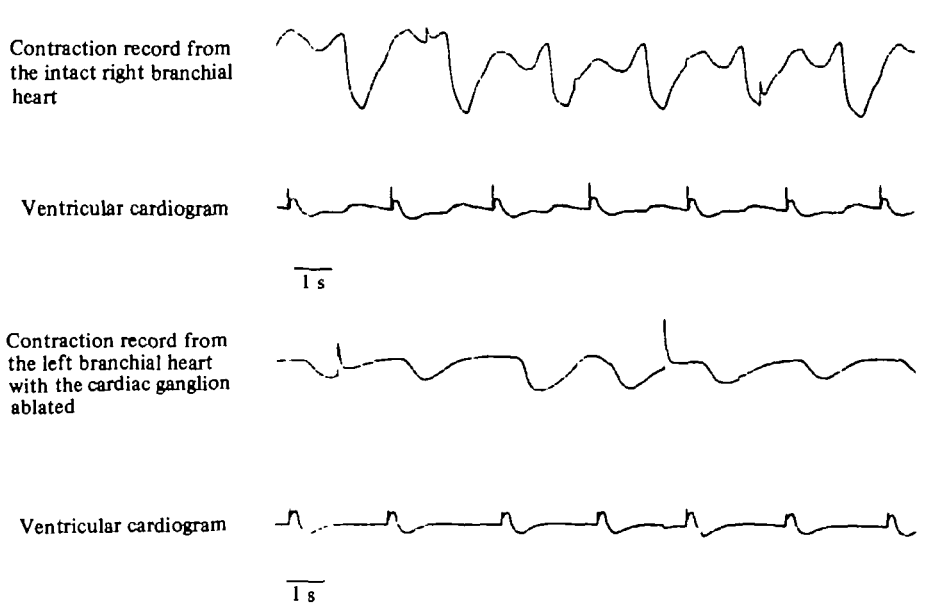

Fig. 5. (*a*) *In vivo* impedance recording from the right branchial heart and the simultaneous recording of the systemic ventricular cardiogram. Two distinct downward deflexions in the branchial heart record are evident within each cycle of the cardiogram.

(*b*) Consecutive record to the above, but from the left branchial heart with the ipsilateral cardiac ganglion ablated. Only one downward deflexion in the heart record per cycle is now evident. This deflexion occurs at the same time, relative to the ventricular cardiogram, as the smaller deflexion in the intact condition.

affected by the ablation of the cardiac ganglion. The common contraction illustrated in Fig. 5 and the pressure pulse remaining after ganglion ablation may be attributed to the activity of the lateral venae cavae.

DISCUSSION

A number of observations argue that the branchial hearts are not the sole site of the accessory branchial pumps.

Anatomically, the branchial heart is atypical of a pumping organ. The majority of the volume is occupied by packing cells. These cells contain a considerable quantity of iron, bound to the adenochrome molecule (Ghiretti-Magaldi, Guiditta & Ghiretti, 1958; Nardi & Steinberg, 1974). Cuenot, Gonet & Bruntz (1908) proposed that this tissue has an excretory role, a view supported by the ultrastructural studies of Witmer (1974). Schipp & Schäfer (1969) favoured either an endocrine or haemopoietic role. To the author's knowledge no other heart has such a large proportion of the volume occupied by a non-contractile tissue. Also, the absence of a valve at the junction of the branchial heart and the afferent branchial vessel would seem to allow backflow.

In the perfused isolated condition, the branchial heart did not pump effectively at the pressure levels expected from the work of Johansen & Martin (1962). Even at the lower pressure levels recorded in this study and by Wells (1979), the isolated organ

failed to respond either predictably or effectively. This is in contrast to the performance of the isolated ventricle (Smith, 1981*a*).

Ablation of the cardiac ganglion, *in vitro* or *in vivo*, disrupted the normal rhythmic contraction of the ipsilateral branchial heart. After this operation a high systolic pressure was still recorded from the *in vivo* afferent branchial vessel.

Wells (1979; his fig. 1) presented a record from *Octopus vulgaris* which illustrated that the main pressure pulse in the afferent branchial vessel preceded the movement of the branchial heart. As suggested by Wells, this may reflect a limitation of the impedance technique. Alternatively, the pressure pulse may have arisen from a structure contracting with the same frequency as the branchial heart.

The accessory branchial pump is located between the anterior vena cava and the afferent branchial vessel, as a mean pressure rise occurs between the two (Johansen & Martin, 1962). The inference of the observations listed above is that the branchial hearts are not solely responsible for this increase in pressure. The lateral venae cavae are potentially an additional site of pressure generation.

The lateral venae cavae possess aminergic innervation, to striated or pseudostriated muscle fibres (Smith, 1979). Sectioning of the innervation to these vessels *in vivo* can disrupt the systemic ventricular beat to the same extent as the ablation of the cardiac ganglion (Smith, 1981*a*).

Bourne, Redmond & Johansen (1978) recorded a pressure pulse of 9–10 cm of water in the afferent branchial vessel of *Nautilus pompilius*, which does not possess branchial hearts. These authors attribute pressure generation to the active contraction of the renal appendages and pericardial glands. The amplitude of the pressure pulse is similar to that in the afferent branchial vessel of *Octopus vulgaris* (8 cm of water; Wells, 1979), *Octopus dofleini* (4–14 cm of water; Potts and Todd, 1965), and *Eledone cirrhosa* (5–15 cm of water; this study). The difference between the values given for *Octopus dofleini* by Potts & Todd (1965) and Johansen & Martin (1962) will require further study of this species.

There must be some doubt whether the branchial hearts alone perform the function of the accessory branchial pumps in the octopods. On the basis of the material discussed above, it can be suggested that the lateral venae cavae contribute significantly to the increase in the returning venous blood pressure in the Octopoda, as in the Nautiloidea. The size of this contribution cannot be assessed on the basis of *in vitro* preparations, as the possibility exists for more complex interactions occurring *in vivo*. The relative contributions of the branchial hearts and lateral venae cavae, to the accessory branchial pumps, can only be assessed by the further study of *in vivo* blood pressures in the lateral venae cavae.

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