Neural and Neurosecretory Control of the Decapod Crustacean Auxiliary Heart

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SYNOPSIS. An auxiliary heart is found in many decapod crustaceans at the anterior end of the dorsal median artery. A brief description of the anatomy is given here, followed by an exploration of the reflex and neurosecretory control. The relationship between the activity of the main heart and auxiliary heart and the integration of the auxiliary heart with other blood pressure regulating systems of the body are discussed.

INTRODUCTION

The presence of an auxiliary heart in decapod crustaceans at the anterior end of the dorsal median artery was noted some time ago when it was termed the cor frontale (L. frontal heart) (Bouvier, 1891; Baumann, 1917; and Maynard, 1960, for review). However, only a brief description of the anatomy of the muscles was given in this early work. A more comprehensive study of the anatomy, including the neural elements was recently provided (Steinacker, 1978) but there has been, to date, no information on the physiology of the heart. The work presented below is intended to fill this gap by providing some basic electrophysiological data on the function of the auxiliary heart. The relationship of the activity of the auxiliary heart to that of the main heart is demonstrated and aspects of the reflex and neurosecretory control explored. It is concluded that the auxiliary heart acts as a "booster pump" to maintain adequate blood flow through the extensive capillary system of the supraesophageal ganglion and optic lobes.

The decapods used were, depending upon availability, the Californian spiny lobsters, _Panulirus interruptus_ or one of the marine crabs, _Callinectes sapidus_, _Cancer productus_, _Cancer antennarius_ or _Cancer borealis_. Electrophysiological recordings were made either from the intact or isolated perfused animal, as specified in the text. Recording from the intact animal was done by insertion of insulated tungsten electrodes through a small hole in the carapace using external carapace markings of the cor frontale (CF) tendon attachments as a guide. The preparation used for _in vitro_ recording was an isolated perfused section of the anterior cephalothorax (for details, see Steinacker, 1975a). The eyes, supraesophageal ganglion, circumesophageal ganglia and auxiliary heart with nerves, tendons and blood supply were intact and perfused via a dorsal median artery cannula at a rate of approximately 200 ml/hr. Both extracellular and intracellular recordings from the CF muscles were made with the sinus wall intact and intervening between the muscles and electrode to preserve the integrity of the vascular system. For intracellular recording from the CF muscle, long shanked beveled microelectrodes (3mm glass) were used which could penetrate the sinus wall and muscle fiber with minimal vascular leakage.

Anatomy

The anatomy of the auxiliary heart is quite complex. Figures 1 and 2 provide...
FIG. 1. Midsagittal section of the cephalothorax of the spiny lobster, Panulirus interruptus illustrating the location of the cor frontale (CF) in relation to other anatomical features. Blood flows from the main heart (MH) through the dorsal median artery (DMA) over the stomach (S) to the CF from which it exits to the eyecup via the ophthalmic artery (OA) and to the supraesophageal ganglion (SOG). Dorsal tendons of the CF (DT); single ventral tendon (VT); circumesophageal connectives (CC).

The muscles of the cor frontale consist of two compact strips attached to the heart wall only at the point where their tendons enter and exit the heart sinus (Fig. 2). This muscle does not resemble typical crustacean heart muscle; rather, it is identical to crustacean somatic muscle in its appearance and fine structure and may be somatic muscle secondarily adapted for cardiac function. Each muscle is supplied by four motoneurons whose somata are located in the supraesophageal ganglion. The motor axons exit this ganglion two from each side, first in the tegumentary nerve and then the smaller CF nerve. As the nerve approaches the muscles, each axon splits into two branches to innervate both ipsi- and contralateral muscles. When stimulating electrodes were placed under both CF nerves cut proximal to the supraesophageal ganglion, one or both axons in each nerve could be made to discharge by slight changes in stimulation voltage. Using intracellular recording and stimulating the right and then the left CF nerve at 80 msec intervals with varying stimulus voltages, the presence of four separate excitatory junction potentials (EJPs) in one muscle fiber is seen (Fig. 3). All the fibers which were penetrated showed such multiple innervation. Because of the similarity of rise time and amplitude of the EJPs, they do not appear to result from the electrotonic

FIG. 2. Transverse view of the cor frontale of Panulirus interruptus. Dorsal CF tendons (DCFT); anterior gastric muscles (AGM); CF nerve (CFN); alternate course of the CF nerve (AC); tegumentary nerve (TN); stomatogastric nerve (SGN); ventral tendon (VT); inferior ventricular nerve (IVN); posterior eyestalk muscles (PEM); supraesophageal ganglion (SOG); CF muscles (CFM); CF sinus wall (CFSW); ophthalmic artery (OA); tendon ganglion (TG); occasional separate tendon sensory supply (TSS); and nerve to main heart valve (MHVN); dorsal median artery (DMA).
coupling of several muscle fibers. No evidence of electrotonic coupling was seen in electron microscopy of the fibers or during electrophysiological testing for coupling. It appears that each muscle fiber is innervated by all four motoneurons whose axons have similar thresholds and conduction velocities. Active responses of the muscle membrane were never detected and even when all four axons were stimulated simultaneously at frequencies up to 100/sec only minimal facilitation was observed.

RELATION OF THE AUXILIARY HEART TO THE MAIN HEART FUNCTION

In order to determine the relationship between the activity of the auxiliary heart and the main heart, the EJPs of the two hearts were recorded extracellularly from the intact animal sitting in a shallow pan of salt water. Small tungsten electrodes were inserted through the carapace over the two hearts. To locate the auxiliary heart discharge, the characteristic high amplitude response of the posterior eyestalk muscles to pitch and roll (Steinacker, 1975b) were used as landmarks. The CF muscles are located between the two eyestalk muscles and can be distinguished from them by the lack of a pitch response. In addition, as an anatomical landmark, two small pits in the dorsal carapace which mark the attachment of the CF tendons can be used. The auxiliary heart appears not to beat continuously since the muscles have either a low or no tonic firing and in the intact animal, with correctly located electrodes, their discharge cannot always be found. The discharge, if recorded at its inception, starts out as a low amplitude, low frequency discharge of regular interpulse intervals whose frequency and amplitude increase when the frequency of the main heart decreases (Fig. 4). The amplitude and frequency of the auxiliary heart discharge is always invariably inversely related to the frequency of the main heart discharge. In the animal in Figure 4, the auxiliary heart comes into play when the main heart frequency falls below approximately one/second. It appears that the auxiliary heart does not begin to function until some critical level of main heart

FIG. 4. Simultaneous extracellular recording from the cor frontale (CF) and main heart (MH) in an intact animal. Examples one to five are discontinuous samples from a continuous recording covering a five minute recording period. This example illustrates the common inverse relationship between the frequency of the two hearts. Low amplitude of initial and final CF firing is believed to be due to “falling out” of individual motoneurons from a burst.
function is reached. In order to investigate the control of the auxiliary heart in greater detail, an isolated perfused preparation of the cephalothorax was used. With a perfusion rate of 200 ml/hr (which is adequate to maintain the responsiveness of the more anoxia sensitive visual and oculomotor systems) a large amplitude extracellular EJP can be recorded which is synchronous over the entire muscle surface (consistent with the muscle's quadruple innervation). This EJP has a frequency of 0.5 to five/sec with a regular interpulse interval. This EJP is a summed response which may represent synchronous firing of one to four motoneurons as can be demonstrated by simultaneously recording from the CF nerves. The frequency of this EJP is inversely proportional to the perfusion pressure. This can be most strikingly demonstrated when the perfusion is cut off entirely and the EJP recorded extracellularly with a suction electrode applied to the intact sinus wall. A characteristic response then ensues (Fig. 5). After an approximate one-minute latency, the EJP frequency begins to increase with a smooth decrease in the interpulse interval. No neural bursting or muscle "beating" is seen. Rather, the muscle contraction is a smooth maintained decrease in length. The EJP rate has been observed to increase steadily for up to 30 min after the cessation of perfusion. With reinstatement of perfusion, there is a gradual return to the control values. This response can be recorded in CF nerves even if CF nerves are cut proximal to the muscle and the cerebral artery ligated. Therefore, the necessary sensory elements must be endogenous to the supraesophageal ganglion. Perfusion with solutions of low pH or oxygen were not sufficient to trigger this response. The sensory receptors are thus likely to be baroreceptors. The discharge of the oculomotor and optic nerves, which were used as controls, showed gradual decreases in discharge during perfusion cessation.

The maintained contractile response of the CF muscle observed in response to decreased perfusion would not be effective in influencing blood pressure in the cerebral system. Various electrical stimulation patterns were therefore tried in order to see if a pulsatile beating of the heart could be produced which would influence blood pressure. It was found that simultaneous stimulation of both CF nerves in a burst-pause pattern resulted in a contraction sufficient to produce a pulsatile pressure in one of the ophthalmic arteries leaving the CF sinus (Fig. 6). Here a train of 300 μsec pulses at 50/sec for one second every five seconds produced a beat-like contraction of the muscles with an accompanying pulsatile pressure change in the artery. Longer interburst intervals also produced an effective contraction and appear closer to frequencies recorded in the intact animal. Lower interburst intervals resulted in fused contractions. It would appear that a high frequency burst is necessary for effective heart function. High frequency...
bursts have not been seen in the isolated perfused preparation, perhaps because of the absence of a necessary neurosecretory substance and/or necessary afferent input to rhythm generators.

**INPUT FROM THE CIRCUMESOPHAGEAL CONNECTIVES**

Stimulation of the circumesophageal connectives produces strong synaptic input to the motoneurons which can be either excitatory or inhibitory. Stimulation of the ipsilateral connective is most effective but stimulation of the contralateral connective can also increase the motor output. The effect of stimulation of the connective can have a long duration of action. In fact, high frequency stimulation (100/sec for 100 msec) can be used to produce an activation of several hours duration of a preparation which had previously been silent. Occasionally, when the initial firing rate is very high, stimulation of the connectives, even at a low frequency, inhibits ongoing firing of the motoneurons.

**THE SENSORY INNERVATION OF THE VENTRAL TENDON**

Methylene blue staining reveals a dense innervation of the tendons of the CF muscles. In both lobster and crab, the innervation of the dorsal tendons is diffuse and recording is not feasible. In the lobster, however, where the ventral tendon is very long, the inferior ventricular nerve runs through an opening in the tendon and is joined by a distinct sensory nerve from the tendon. This sensory innervation is quite well developed and runs a considerable length down the long ventral tendon. One tonic spike whose firing can be accelerated by stretch of the tendon originates from a sensory unit on the tendon. Other units which are phasic can be recruited by stretch. By recording from the inferior ventricular nerve on both sides of the tendon, the action potentials were found traveling not towards the supraesophageal ganglion but rather toward the circumesophageal, stomatogastric or esophageal ganglion, transmitting information on auxiliary heart function to a lower center.

**THE COR FRONTALE SYSTEM AND THE MAIN HEART VALVE**

In histological sections of the CF nerve, at least seven axons are seen. Two of these were shown to be motoneurons to the auxiliary heart by simultaneous muscle and nerve recording. A third axon is assumed to be a motoneuron which innervates the main heart valve. In methylene blue stained preparations a large axon can be traced from the supraesophageal ganglion into the CF nerve. It leaves the CF nerve near the auxiliary heart and travels embedded in the wall of the dorsal median artery all the way to the valve of the dorsal median artery at the main heart when it terminates in a dense innervation of the muscle of the valve (see Steinacker, 1978, for more detailed description of the main heart valve system). When recording from the isolated perfused preparation, one large action potential traveling away from the supraesophageal ganglion is seen in the CF nerve which does not have a corresponding EJP in the CF muscle. With normal perfusion rates, the unit fires with a regular interpulse interval discharge at about the same frequency as the CF motoneurons but does not maintain any phase relationship with them. This is assumed to be the motoneuron to the valve of the main heart. The function of this motoneuron in arterial valve control was not investigated. Since its origin is with the CF motoneurons, rather than with the main heart control system, it is likely that the two are functionally linked in the supraesophageal ganglion for cerebral pressure regulation.

**NEUROSECRETORY CONTROL**

On the dorsal tendon of each CF muscle, there is a ganglion which contains cells with dark vesicles which may be neurosecretory. Axons containing the same dark vesicles were seen in cross sections of the CF muscles. In addition to the possible neurosecretory role of the tendon gan-
glion, the auxiliary heart is exposed to secretory products from the main heart pericardial organ. It is arterially directly downstream from the site of release of the peptide, 5-hydroxytryptamine, dopamine, and octopamine of the pericardial system. For these reasons and because of the long latency of onset and decay of the response to perfusion cessation the effect of the pericardial system on the auxiliary heart was investigated. Test substances were introduced into the perfusion cannula as a single injection. The pericardial peptide was isolated from 50 crab pericardial organs in a standard peptide isolation using G 25 Sephadex columns. When microgram amounts of this peptide were added to the perfusion fluid, there was a delay of up to five minutes before the EJP frequency gradually began to change from a control low frequency beating to a high frequency bursting pattern. In response to a single injection of the peptide, with constant Ringer perfusion, the bursting activity continued for periods of up to four hours. Addition of lysine vasopressin (Sigma) to the perfusion saline (final concentration approximately $10^{-6}$ M) produced an identical response with the same latency of onset and long duration of action (Fig. 7).

This is similar to the response of a neurosecretory cell in Otala where lysine vasopressin can be substituted for an endogenous peptide (Baker and Gainer, 1974). The pericardial peptide was tested in high concentrations ($10^{-6}$ M) by radioimmunoassay (Robertson et al., 1973) which is sensitive to lysine vasopressin ($10^{-11}$ to $10^{-12}$ M concentrations) but no cross reactivity was found.

5-Hydroxytryptamine ($10^{-8}$ M) introduced into the perfusate in the same manner produced a short duration increased firing without pronounced bursting tendencies. The response to dopamine ($10^{-6}$ M) was extremely variable, with inhibition of ongoing firing sometimes occurring. The effects of octopamine on the motoneuron firing were not tested. Since the entire supraesophageal ganglion is exposed to the perfusate, the effects of any of the above substances may not be on the motoneurons directly but may act either on sensory receptors or interneurons of the auxiliary heart.

The pericardial system can also influence auxiliary heart function more directly by increasing muscle tension and pulsatile pressure in the arteries leaving the auxiliary heart (Fig. 8). When stimulating the CF nerves and recording muscle tension and ophthalmic arterial pressure, injection of an extract of a fresh pericardial organ into the perfusion tube will increase both parameters (Fig. 8). Unlike the effect on the motoneurons the peripheral effects of pericardial extract are short-lived. However, since there is no decrement of the response upon repeated application of pericardial extract, constant release by the pericardial system may produce effective long term control of aux-

![Image 1](https://academic.oup.com/icb/article-abstract/19/1/67/174662/167714662)

**FIG. 7.** Response of the auxiliary heart to pericardial peptide and lysine vasopressin. Intracellular recording CF EJP in isolated perfused preparation: A. control condition. B. 15 minutes after the injection of the pericardial peptide into the perfusate. C. 15 minutes after a similar addition of lysine vasopressin to the perfusate.

**FIG. 8.** Electrically elicited tension of the CF muscles and pressure in the ophthalmic artery in control, and following dotted lines, immediately after the injection of pericardial extract in the perfusate.
crustacean auxiliary heart function. The primary effect appears to be on the muscle fiber directly since no marked effect of the pericardial extract on the EJP was seen.

Since the pericardial organ and tendon ganglia are likely to release specific neurosecretory products for a specific purpose, the effects of the different substances of the pericardial system on muscle tension was tested (Fig. 9). All produced a short-lived increase in peak tension elicited by direct muscle or motor nerve stimulation, 5HT being the most and the peptide, the least, effective in this regard. The principle difference between the action of the various test substances on the muscle is in their effects on the muscle tension rise and decay times. The effects of dopamine in reducing tension decay time were striking. An opposite effect was produced by the pericardial peptide which greatly prolongs tension decay time with little, or no effect on peak tension.

The preceding data supplies some basic information on the crustacean auxiliary heart. Much more work is necessary before the understanding of its operation is complete. In particular, it is desirable to know why the auxiliary heart was never observed to beat in a functional manner without electrical stimulation of the nerves. The

The second system of integrated control of the various blood pressure regulating organs in the body exists in the pericardial and related neurosecretory organs. The location of the CF deep in the cephalothorax makes visual observation of its behavior in the intact animal difficult. An earlier paper (Baumann, 1917) reports observations of auxiliary heart pulsations but no methodology is given. Judging by the amplitude, the "beat" recorded extracellularly from the auxiliary heart in the intact animal is the result of a high frequency burst. A coordinated burst pattern was not seen in the isolated preparation, although bursting activity was seen in response to the pericardial peptide. It appears that the four motoneurons are coupled centrally and some sensory or interneuronal input may be necessary to convert their output into the correctly timed bursting pattern. Alternatively, or perhaps in addition, the tendon ganglion or pericardial system may release a neurosecretory substance necessary for production of the correct motor output.

With the aim of constructing a complete picture of blood pressure regulation in crustaceans, the data provided here on the auxiliary heart can be added to what is known about other systems of the crustacean body whose functions control blood pressure. A common integrating center(s) for these systems is necessary. The finding of regulatory fibers in the circumsophageal connectives for the auxiliary heart parallel those for the main heart (Wiersma and Novitski, 1942; Field and Larimer, 1975) and gills (Wilkens et al., 1974). In addition to these regulatory or "command" fibers in the connectives, the supraesophageal ganglion contains the control center for the auxiliary heart, the main heart valve motoneuron (evidence above) and command fibers to the stomatogastric ganglion (Dando and Selverston, 1972). Although the latter system is not usually linked with blood pressure regulation, in an animal with a predominately open venous system and a fixed carapace volume, expansions and contractions of the large capacity stomach have great influence on blood pressure.

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long term action of the pericardial peptide vs. conventional neurotransmitters makes it likely that the pericardial system provides a modulation of long time constant. The actions of this neurosecretory system on the main heart are well known; its action on the respiratory system has recently been reported (Berlind, 1976). It has now been shown here to influence auxiliary heart function. There is also a report of excitatory effects of a pericardial substance on the isolated gut (Parrot, 1941). The stomatogastric ganglion is directly exposed to the secretory products of the pericardial system as they are carried anteriorly from the main heart by the dorsal median artery. The stomatogastric ganglion is found either in the lumen of the dorsal median artery or in the CF sinus in decapods (see Steinacker, 1978). Since the ganglion survives quite well as an unperfused in vitro preparation (Mulloney and Selverston, 1974) it is unlikely that anoxia necessitates the location of the ganglion inside the blood vessel but rather the location of the ganglion makes possible exposure to pericardial secretions and coordination of stomatogastric activity with other pressure regulating systems.

Much work remains to be done before the picture of the function of the crustacean auxiliary heart is complete. Its anatomy and physiology show it to be a highly complex organ. In addition to the cor frontale, there appears to be another auxiliary heart in each eyecup just before the ophthalmic arteries enter the highly vascularized optic lobes (Steinacker, 1978). These putative hearts also appear to be somatic (oculomotor) muscle secondarily adapted for cardiac function. The CF and optic auxiliary hearts appear to be adaptations to solve the problem created by the increased peripheral resistance of a highly developed vascular bed in ganglia with high oxygen demands.

Although the peripheral ganglia of decapods survive quite well when isolated from their blood supply, the central supraesophageal ganglion and optic lobes soon cease functioning under such conditions and electrophysiological recording from them requires either an intact arterial system or proper perfusion (Taylor, 1974; Steinacker, 1975). The sensitivity of these ganglia to anoxia threatens their survival following blood loss from internecine warfare and during the reflex cessations of main heart function which are commonly observed. In addition, the supraesophageal ganglion and optic lobes have a very well developed intraganglionic capillary system between afferent and efferent blood vessels (Sandeman, 1967). Circulation through such a capillary system requires an elevated pressure on the arterial side which may be difficult to maintain when blood pressure or main heart function is inadequate. Although the high oxygen saturation of the blood should be sufficient to sustain tissue respiration during short periods of anoxia (Florey and Kriebel, 1974) longer periods of reduced blood flow place the cerebral respiration in jeopardy. From the preceding data it appears that the function of the auxiliary heart is that of a "booster pump" to maintain the necessary blood pressure levels for the vital crustacean CNS.

REFERENCES