THE CARDIOREGULATORY SYSTEM OF CRAYFISH: NEUROANATOMY AND PHYSIOLOGY

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SUMMARY

1. The anatomical arrangement of the cardioregulatory nerves and their physiological activity during cardiac modulation were analysed in *Procambarus clarkii*.

2. The bilaterally arranged pairs of cardioinhibitors and cardioaccelerator axons, in nerves SN II and SN III respectively, were physiologically identified by correlating spikes in SN II and SN III with the same spikes in the dorsal nerve, which innervates the heart.

3. The cardioinhibitor neurone fired tonically in varied sporadic bursts. During periods of cardiac inhibition, however, this neurone discharged in a long chain of spikes at a characteristic frequency of 40–50 Hz.

4. The cardioaccelerator neurone fired tonically at 2–3 Hz but on occasion its activity reached 12 Hz.

5. Three inhibitory cardiac reflexes were analysed. The sensory modalities for the reflexes included (*a*) stretch of the dorsal pericardial wall, (*b*) chemical stimulation of coxal hair sensilla with glucose and (*c*) tactile stimulation of hair sensilla in and below the gill chamber, on the antennae, the antennules and on the anterior cephalothorax.

6. The discharge of both cardioinhibitor neurones showed a weak temporal correlation suggesting a common presynaptic drive, while the pair of cardioaccelerators appeared to have a reciprocal relationship with the cardioinhibitors.

INTRODUCTION

The cardiac ganglion of crustacean hearts is controlled by extrinsic cardioregulator nerves, both accelerators and inhibitors, which arise from the suboesophageal ganglion (Maynard, 1960). The general locations and specific functions of these nerves were demonstrated for a variety of decapod crustaceans by Dogiel (1876), Jolyet & Viallanes (1893) and by Carlson (1909). Alexandrowicz (1932) examined heart innervation in detail, but, due to the complicated neuroanatomy of the lateral thoracic wall, he was unable to elucidate the complete pathway followed by the nerves between the suboesophageal ganglion and the heart. Although Maynard (1953) has detailed the cardioregulator nerves in *Panulirus*, an equivalent diagram is lacking for the crayfish.

Modern physiological studies of the cardioregulator nerves have been reported by Wiersma & Novitski (1942), Maynard (1953), Florey (1960), and reviewed by Maynard (1960). In these studies heart rate was monitored while the cardioinhibitor or cardio-accelerator nerve was stimulated, and the resulting changes were characterized.

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Detailed effects of such stimulation on individual cells of the cardiac ganglion have been reported by Hagiwara & Bullock (1957), Otani & Bullock (1957), Maynard (1960), and others.

To elucidate the overall function of the system, activity in the respective regulator nerves must be recorded during normal activity and during sensory-induced changes in the heart rate. Although a variety of external stimuli are known to affect the heart-beat (Larimer & Tindel, 1966), the underlying neural correlates are largely unknown. Maynard (1960) briefly reported discharge rates of tonic, spontaneous cardioregu-latory activity in *Homarus*, and Taylor (1970) recorded spontaneous activity in the cardioaccelerator nerve of *Astacus*. In *Astacus*, Taylor demonstrated an increase in the firing of one axon during an increase in heart rate, but the active neurone was not positively identified as the cardioaccelerator. In neither of the above studies were specific stimuli introduced to modify the heart rate or cardioregulator activity.

The initial purpose of the present work was to determine the anatomy of the cardio-regulator nerves in the crayfish *Procambarus clarkii* and to identify the inhibitor and accelerator axons in each. Following this, the aim was to characterize the activity of these axons during spontaneous behaviour and during sensory-induced modification of cardiac rate. In addition, an attempt was made to determine any neural connexions which might exist between the cardioregulator neurones.

METHODS

Adult *Procambarus clarkii* were obtained from a supplier (Tropical Fish Gardens, Smithville, Texas) and maintained in pans of well water at room temperature for as long as 3 months. Dried dog food was provided weekly and water was changed on the following day.

The anatomy of the thoracic nerves was examined by immersing freshly dissected crayfish in van Harreveld's saline (van Harreveld, 1936) to which a few drops of 0·5 % methylene blue were added, producing a sky-blue solution. The preparation was then transferred to a 10 °C refrigerator and inspected at regular intervals. Sketches of stained nerves were made with the aid of a binocular microscope. Histological sections of the cardioregulator nerves were made after fixation in Bouin's solution and embed-ding in paraffin. Sections 10 μm thick were cut and stained in Mallory's Triple stain (Humason, 1962).

In order to rigidly mount the animals for recording, a 12 mm diameter plastic (Lucite) rod was attached to the left branchiostegite with epoxy glue (Devcon, Danvers, Mass.). After removing the claws by autotomy, the thoracic cavity was opened dorsally, and the viscera, mandibular muscles and green glands were removed in a manner similar to that described by Wiersma & Novitski (1942). The cavity was washed with van Harreveld's solution and the animal was immersed in a 3 l oxygenated saline bath which was cooled to 15 °C with a water-jacket. In some experiments it was necessary to have access to the pericardial cavity and to the cardioregulator axons as they entered the heart via the paired dorsal nerves (Alexandrowicz, 1932). This was accomplished by making a rectangular cut through the carapace which overlies the pericardium. After the exoskeleton was carefully separated and removed from the hypodermis, a median longitudinal incision was then made to open the pericardium. The dorsal

pericardial wall was reflected laterally and pinned with *minutien nadeln* to expose the heart and dorsal nerves. In most preparations the medial and lateral arteries were ligated, to divert the blood flow to the ventral nerve cord (Bowerman & Larimer, 1974a). This procedure, along with cooling and enriching the saline with O_2, appeared to extend the viability of preparations for up to 6 h.

Recording and stimulation of the nerves was done with glass suction electrodes that were fire-polished at the tips. Heart beat was monitored with a pair of silver leads inserted through holes in the carapace above the heart (Larimer, 1962), except in those experiments in which the pericardium was exposed. The electrical signals were amplified and displayed by conventional means and stored on magnetic tape (Ampex FR 1300A recorder) for later analysis and photography.

<div align="center">RESULTS</div>

<div align="center">*Anatomy*</div>

The cardioregulator nerves in *P. clarkii* include one pair of inhibitors and one pair of accelerators, arising from the suboesophageal ganglion. They are the second and third superior nerves (inhibitors and accelerators, respectively) which are designated SN II and SN III in Fig. 1. The inhibitor emerges from a medial, rectangular foramen of the endophragmal skeleton, above the suboesophageal ganglion (Wiersma & Novit-ski, 1942) and follows a cuticular bar laterally to the thoracic wall, at which point it courses dorsally and caudally along the insertion of the epimeral attractor muscle. This nerve contains a prominent motor neurone to the epimeral attractor and appears to provide extensive innervation to other lateral and dorsal thoracic muscles. The histological section in Fig. 2 shows that the axons in SN II comprise three size classes: (1) the large epimeral attactor muscle motor neurone, 30–40 μm diam., (2) about ten axons 10–15 μm diam. and (3) at least forty axons less than 10 μm diam.

The cardioaccelerator nerve (SN III) passes laterally from the suboesophageal ganglion but lies beneath the ventral thoracico-abdominal flexor muscles. It emerges near the thoracic wall and extends dorso-caudally across the lateral thoracico-abdominal musculature, at which point it becomes loosely organized as its axons intermingle with a rather distributed network of thoracic nerves on the body wall. A few axons could be traced dorsally, where they join the loosely arranged SN II and continue caudally between the lateral thoracic muscle and the attractor epimeralis muscle. As they reach the caudal side of the lateral thoracic muscle the cardioinhibitor and cardioaccelerator axons join to form the fine dorsal nerve (D.N.) which passes through the pericardium and into the heart. The cardioaccelerator nerve, at the point of emergence from the ventral thoracico-abdominal muscles, contains seven axons ranging from 10 to 35 μm diam. (Fig. 2). Several axons less than 5 μm diam. appear to be present as well. This differs from the cardioaccelerator nerve in *Astacus pallipes*, which only has six large axons, between 10 μm and 20 μm diam. (Taylor, 1970).

<div align="center">*Physiology*</div>

<div align="center">1. *Characterization of the cardioinhibitor and cardioaccelerator activity*</div>

As noted by Alexandrowicz (1932), it is extremely difficult to actually trace the pathway of the individual cardioaccelerator and cardioinhibitor axons from the sub-
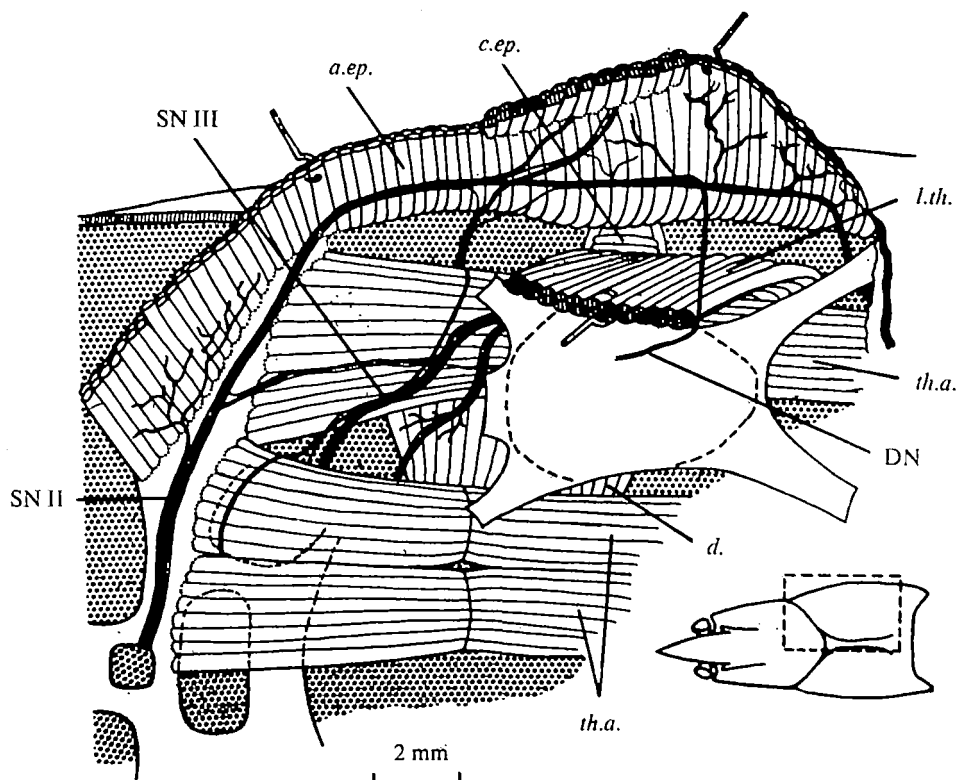
Fig. 1. Dordal view of the right half of the dissected thoracic cavity of the crayfish, as shown by the dashed line of the inset. The view is a projection so the heart (dashed outline) appears to be laterally displaced. The innervation was sketched from preparations stained with methylene blue. SN II is the cardioinhibitor nerve, SN III is the cardioaccelerator nerve and DN is the dorsal nerve. Muscle terminology: *a.ep.*, attractor epimeralis; *c.ep.*, contractor epimeralis; *d.*, depressor (of cheliped); *l.th.*, laterales thoracoabdominales; *th.a.*, thoracies anteriores. The lateral thoracic muscle and attractor epimeralis have been cut and reflected.
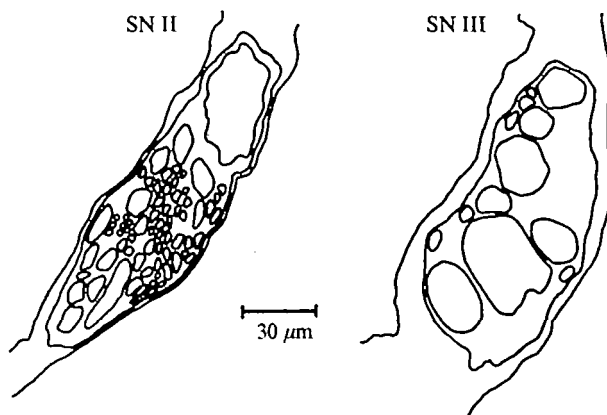

Fig. 2. Tracings of the axon profiles in histological sections (10 μm, paraffin) of the cardio-inhibitor nerve, SN II, and the cardioaccelerator nerve, SN III.
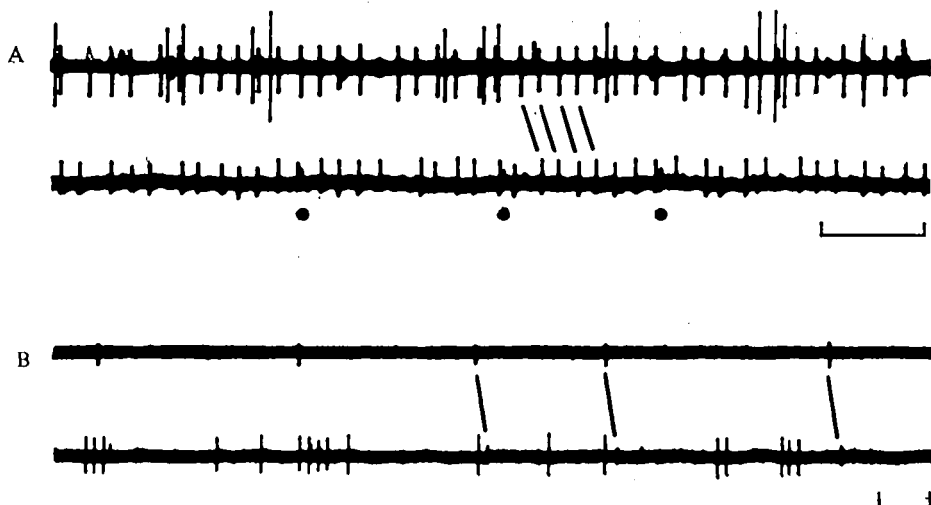
Fig. 3. Simultaneous records from (A) the cardioinhibitor nerve (upper trace) and the dorsal nerve (lower trace) and (B) the cardioaccelerator nerve (upper trace) and the dorsal nerve (lower trace). The slanted lines join cardioinhibitor (A) or cardioaccelerator (B) spikes recorded from the two different locations. The large spike in the dorsal nerve is the cardioinhibitor while the small spike (indicated by dots in A) is the cardioaccelerator. Time calibration for A and B: 100 msec.

oesophageal ganglion to the dorsal nerve. Positive identification of these individual axons can, however, be easily accomplished with electrical recording techniques. Fig. 3A shows simultaneous recordings from the cardioinhibitor nerve and from the dorsal nerve during a period of spontaneous inhibition of the heart. Two axons can be seen to fire in the dorsal nerve, the larger of which followed a spike in the cardioinhibitor nerve with a constant latency of 20 msec. The onset and termination of the burst of this intermediate-sized spike in the cardioinhibitor nerve was always coincident with onset and termination of cardiac inhibition. There is little doubt that this axon belongs to the cardioinhibitor neurone. The other, smaller spike seen in the dorsal nerve record of Fig. 3A (indicated by dots) represents the cardioaccelerator. This is clearly shown in Fig. 3B, in which a single spike firing in the cardioaccelerator nerve (upper trace) preceded the small spike in the dorsal nerve (lower trace) by a constant latency of 32 msec. The records in Fig. 3B were taken during normal heart activity while the animal was motionless and unstimulated.

Several properties characterize the cardioinhibitor discharge. First, the spike was always intermediate in amplitude, which suggests that the cardioinhibitor is one of the 10–15 μm diameter axons shown in Fig. 2A. Tonic activity of this neurone varied from essentially zero to sporadic bursts of two to five spikes which became more frequent as the preparation aged. The spike frequency within such bursts was typically 35–40 Hz (Fig. 3B). Spontaneous cardiac arrest, which also occurred more frequently in older preparations, was accompanied by a continuous discharge of both cardio-inhibitor neurones at a characteristic frequency of 40–50 Hz (Fig. 4, middle record). The burst character of these discharges is seen as the activity builds up to the maximum maintained frequency (top record, Fig. 4). It is interesting to note that there is an apparent temporal correlation in bursting between the left and right inhibitor neurones
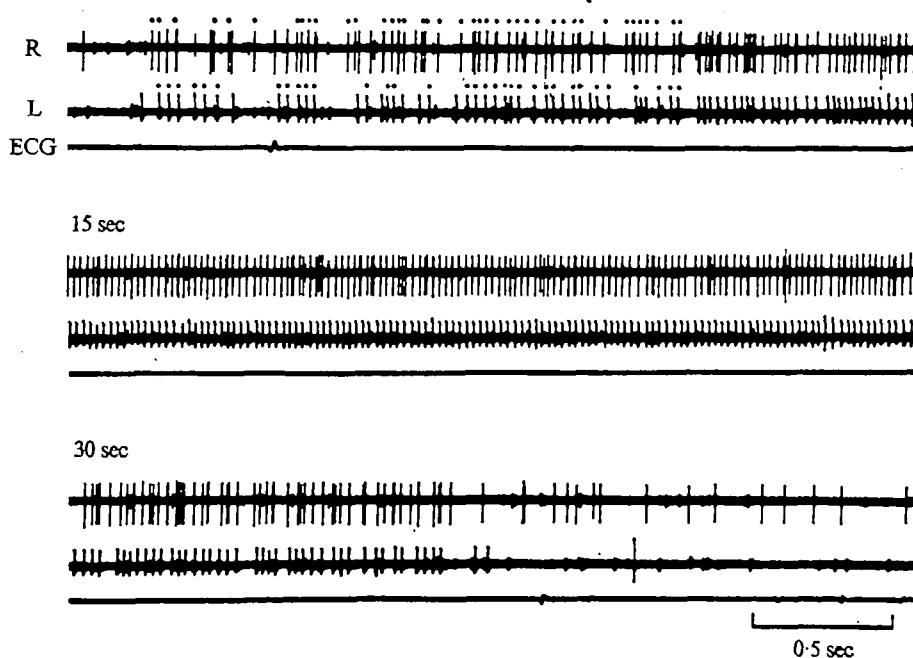
Fig. 4. Sections of a recording from the right cardioinhibitor nerve (top trace), the left cardioinhibitor nerve (middle trace) and electrocardiogram (ECG, bottom trace) during a period of spontaneous inhibition which resulted in cardiac arrest (note last ECG spike in top section). Records are shown for onset of inhibition (0–6 sec), maintained inhibition (15–21 sec), and termination (30–36 sec). The tiny deflexions on the last ECG trace are muscle potentials.

during this initial build-up. Evidence is presented below to suggest that this correlation is due to a common pre-synaptic drive rather than positive excitatory connexion between the two cardioinhibitor neurones. Fig. 4 (bottom set of traces) also shows that the inhibitory discharge terminates rather sharply, suggesting an active shut-off mechanism rather than a simple decay process.

The spike amplitude of the cardioaccelerator neurone was relatively small in size, which would suggest that it might be one of the 4–5 μm diam. axons of SN III (see Fig. 2B). In a quiescent preparation this neurone had a regular tonic discharge frequency of 2–3 Hz, as seen in Fig. 3B, but upon occasion the firing rate reached 12 Hz. There appeared to be a reciprocal relationship between the activity of the cardioinhibitor and cardioaccelerator, since the tonic frequency of the latter was depressed during cardiac inhibition (Fig. 6). This question is examined in more detail in the following paper (Field & Larimer, 1974b).

2. Inhibitory cardiac reflexes

Three sensory modalities were found to mediate reflex inhibition of the heart. The first case appeared to be a reflex activated by stretch of the dorsal and dorso-lateral wall of the pericardium. This conclusion was reached indirectly after noting that stimulation of one cardioinhibitor nerve evoked activity in the contralateral cardio-inhibitor (Fig. 5A–E). It was initially suspected that the contra-lateral excitation was due to positive coupling between left and right cardioinhibitor neurones, expecially since such interaction would help explain the coincident bursting and prolonged
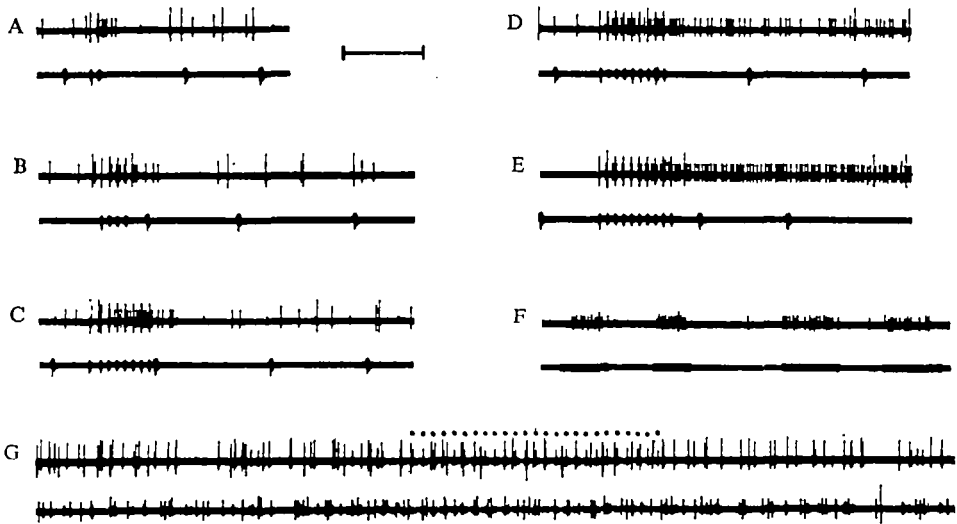
Fig. 5. Records from the left cardioinhibitor nerve (top trace) and ECG (bottom trace) during stimulation of the right cardioinhibitor nerve. Reflex activation of the contralateral (left) inhibitor axon is shown in A–E for 2, 4, 7, 9, 10 stimulus pulses respectively (stimulus artifact in ECG trace). The last two records in this series, D and E, illustrate the characteristic cascading burst property of the inhibitors which leads to cardiac arrest. (F) Record from the cut, peripheral stump of the right cardioinhibitor nerve showing afferent responses to gentle tactile stimulation of the dorsal surface of the exposed pericardium (see text). The lower trace indicates the duration of the mechanical stimulus. (G) Records from the left (top trace) and right (bottom trace) cardioinhibitor nerves during stimulation of the left dorsal nerve which innervates the heart. Evoked antidromic spikes of the left inhibitor are marked by dots. Time calibration: 1 sec for A–F; 0·5 sec for G.

character of the inhibitor discharge shown in Fig. 4. To test this hypothesis, one cardioinhibitor would have to be stimulated selectively, which is not possible with extracellular stimulation of the mixed nerve at the level of SN II. Selective antidromic stimulation is possible, however, at the level of the dorsal nerve, since the cardio-inhibitor is only one of two axons in that nerve. Fig. 5 G shows that no contralateral activity could be evoked when the left cardioinhibitor was antidromically stimulated at the dorsal nerve. The alternative possibility remained that the contra-lateral cardio-inhibitor was driven by afferent axons which were activated during electrical stimula-tion of the whole SN II root. A reasonable assumption was that SN II could contain sensory axons from some region of the pericardium. To test this possibility, the peri-cardium was exposed by removing the overlying carapace, a recording electrode was placed on the cut, peripheral end of the right SN II, and the pericardium was gently stimulated with a fine polished glass probe (mounted on a mechano-electrical trans-ducer). It was found that a well-defined area on the dorsal mid line and several small areas on the mid and anterior aspects of the dorso-lateral wall of the pericardium were sensitive to mechanical distortion by the probe. Small afferent spikes were recorded in the peripheral stump of the right SN II nerve during such stimulation (Fig. 5 F). In other preparations with intact SN II nerves, it was found that probing the same dorsal areas of the pericardium evoked long cardioinhibitor discharges and concomi-tant cardiac arrest. The tentative conclusion from this evidence is that stretch of the pericardium evoked an inhibitory cardiac reflex. The weak temporal correlation in the
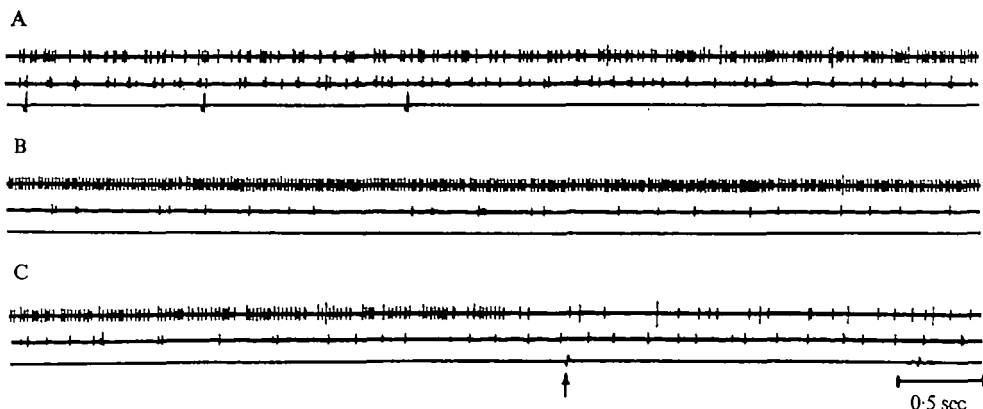
A

B

C

0·5 sec

Fig. 6. Records from the right cardioinhibitor nerve (top trace), right cardioaccelerator nerve (middle trace) and the ECG (bottom trace) during reflex inhibition of the heart evoked by the presence of glucose in the gill chamber. (A) Record begins 4 sec after 0·25 M glucose was pipetted into the right respiratory stream. Cardiac arrest occurred midway through this record. (B) Maintained cardiac arrest 25 sec later. Note reduced rate of tonic cardioaccelerator discharge. (C) Inhibition ceased 50 sec later and heart beat returned (arrow), together with an increase in cardioaccelerator activity.

onset of cardioinhibitor discharge must be due to pre-synaptic drive rather than direct positive coupling.

Staining of the pericardium and epimeral plates with methylene blue failed to reveal any likely sensory receptors in this area, although two groups of sensory cells, termed 'N cells' by Alexandrowicz (1952), invariably appeared on the inner, ventro-lateral surface of the epimeral plate near the epimeral contractor muscle. It is doubtful that these could have been stimulated during the reflex experiments. Possibly the thin cuticular covering of the dorso-lateral pericardium wall prevented the successful staining of the receptors in that region.

The second inhibitory reflex was evoked by adding a solution of glucose to the incurrent stream of the branchial cavity. This reflex was originally reported by Ashby & Larimer (1965) but was not described in neural terms. Fig. 6A shows simultaneous records from the right cardioinhibitor root (SN II), right cardioaccelerator root (SN III) and the electrocardiogram (ECG), commencing 4 sec after introduction of 0·25 M glucose into the posterior opening of the right branchiostegite. Cardiac arrest occurred with a latency of 6 sec and persisted for 50 sec. Activity in the cardioinhibitor neurone accompanied by the cardiac arrest and showed a slow increase in discharge rate which reached a characteristic frequency plateau of about 50 Hz. As the inhibition progressed, a corresponding decrease in firing frequency occurred in the cardioaccelerator nerve (compare Figs. 6A and B).

Localization of glucose receptors was made by applying glucose solution, with a fine glass pipette, to various areas of the gill chamber (exposed by removing the branchiostegite). The only sensitive area was a band of hair sensilla located just dorsal to the coxa of each pereiopod. The structure and physiology of the hair sensilla are presently under investigation.

The third type of reflex consisted of a bradycardia or cardiac arrest evoked by tactile stimulation of hair sensilla on various parts of the body, or by electrical stimulation of sensory nerves in the cephalothorax. The greatest sensitivity was found in

'clusters of hairs in and below the gill chamber (including coxal hairs), the antennae, the antennules and the region innervated by the tegumentary nerve. The reflex response to tactile stimulation was not always consistent or predictable, and occasionally a preparation showed little or no response. Furthermore, the response readily habituated, particularly when antennal, antennular and tegumentary nerves were stimulated repeatedly. Simultaneous records from both SN II nerves showed that phasic bursts of spikes were evoked in the cardioinhibitor axons during tactile or electrical stimulation. If the stimulus was sufficiently strong, the cardioinhibitors underwent the typical prolonged discharge that mediated cardiac arrest. If the inhibitor failed to burst for long periods, only bradycardia was produced, along with a decrease in ECG amplitude.

DISCUSSION

· Although a number of phyla are known to possess cardioregulatory nerves, the physiology of these systems is essentially limited to the demonstration, in a few arthropods and molluscs, that electrical stimulation produces cardiac inhibition or acceleration (Bullock & Horridge, 1965). The results of the present study suggest that the crayfish promises to be a good preparation for the neuronal analysis of cardioregulation by the central nervous system. Therefore it is useful to carefully delineate the neuroanatomy of the cardioregulators, not only to avoid confusion in location and terminology but also to aid future researchers in locating nerves which are difficult to find.

Keim (1915) showed that in *Astacus* the nerves which follow the pathways described above for the cardioregulators are the second and third superior nerves of the suboesophageal ganglion. The same anatomy holds true for *Procambarus*. Taylor (1970) described the cardioregulators of *Astacus pallipes* as the first and second superior nerves, which introduced some confusion as to which structures were under observation. His criterion for identification of the cardioaccelerator nerve was the appearance of tachycardia following stimulation. This criterion may not be totally satisfactory since Maynard (1961 *b*) has shown that a number of the superior nerves in crabs contain axons which are acceleratory in function by way of the pericardial organs. However, the similarity in histology between the nerve studies by Taylor and the cardioaccelerator on the present study suggests that they were both SN III. A useful review of the terminology applied to the anterior dorsolateral nerves of the decapod thorax is provided by Maynard (1961 *a*).

Tonic activity in cardioregulatory neurones has been the subject of continued debate. Maynard (1960) described spontaneous inhibitor activity of 1–16 Hz recorded in the dorsal nerve of *Homarus*. Accelerator activity in the same preparations reached a maximum of 36 Hz. The spiking was erratic at low frequencies and became rythmic and continuous at high frequencies. Nevertheless, Maynard (1961 *c*) believed that central nervous control of the heart is probably limited to brief acceleration or inhibition. Similarly, Wiersma (personal communication quoted in Bullock & Horridge, 1965, p. 996) suggested that tonic activity of the crayfish cardioregulator neurones is unlikely to be consequential because cutting the nerves does not influence the heart rate. Although this would apply to the cardioinhibitors, it would perhaps be difficult to assess the effects of cardioaccelerator (SN III) ablation unless all innervation to the pericardial organs was also cut. On the other hand, Larimer & Tindel (1966)

provided some indirect evidence for tonic acceleratory control of the crayfish heart by showing that sensory deprivation over a 150 min period produced a marked decline in heart rate. In support of this, Taylor (1970) postulated that the bursting neurone, which he described, represented tonic activity of the cardioaccelerator. The accelerator neurones observed in the present study discharged tonically at 2–12 Hz, typically not in bursts. No attempt was made in this investigation to separate the tonic effects of the pericardial organs from that of the cardioaccelerator nerves on the overall heart rate. At present the evidence suggests that either or both of these acceleratory systems provides a maintained heart tonus which is at least partially driven by a variety of external sensory pathways.

Cardiac stretch reflexes in crustaceans have been previously described, but they were always reported as acceleratory in nature (Bullock & Horridge, 1965). Passive stretch of the cardiac ganglion in *Limulus* (Carlson, 1940) or *Carcinus* (Mangold, 1924) produces an increase in heart rate. Maynard (1960) considered this reflex to be mediated. by dendritic arborizations within the ganglion. Other potential sensory elements within the pericardium have been described anatomically (Alexandrowicz, 1932), and postulated to mediate local excitatory stretch reflexes (Taylor, 1970). Small branches within the dorsal nerves end in plate-like bodies, termed 'apparatus nervi dorsalis' by Alexandrowicz. In addition, he observed fine axons in the dorsal nerves which extend out on to the heart surface, where they branched profusely, presumably to serve a stretch receptor function. None of these structures could be responsible for the inhibitory stretch reflex described in the present work, because the sensory field was confined to local areas on the dorsal pericardial wall. Alexandrowicz (1932) briefly mentioned some receptor-like elements (which were not 'N cells') on the epimeral plates that could possibly be involved in the reflex, but unfortunately he discussed them no further. Wiersma & Pilgrim (1961) recorded activity from 'N cells' in crayfish during imposed movements of the epimeral plate, but were unable to evoke responses by imposing fluid pressure increases in the pericardium. It is therefore unlikely that these receptors are involved in the pericardial stretch reflex. Acceleratory stretch reflexes in other invertebrates have been described only for gastropod molluscs (Zubkov, 1934). Inhibitory stretch reflexes have been described for pelecypod molluscs (Woortman, 1926), gastropod molluscs (S.-Rozsa & Salanki, 1973) and ascidians (Carlson, 1909)

Inhibition of crustacean hearts by tactile stimulation has been found repeatedly (see review of literature in Larimer & Tindel, 1966). The study of sensory modification of heart rate by Larimer and Tindel demonstrated that such changes are not an artifact of restraining the animals. Bradycardia or cardiac arrest occurred under two conditions which could involve stimulation of tactile hairs: contact with and swallowing of food, and execution of the tail-flip escape response. Direct tactile stimulation or walking usually produced cardiac acceleration rather than inhibition. Evidently the inhibitory reflex from the coxal hairs (present study) is centrally inhibited when the crayfish activates neuronal walking commands. It is interesting to note that in the present study it was difficult to evoke acceleratory reflexes. It is possible that cardio-inhibitor activity was abnormally high in the dissected preparations and therefore suppressed acceleratory reflexes. Inhibitory overriding of accelerator effects on the heart has been well established (Wiersma & Novitski, 1942; Florey, 1960)

The reflexive inhibition of the heart beat by carbohydrates still remains a functional enigma. Larimer & Tindel (1966) observed cardiac arrest when unrestrained crayfish encounter and swallow food; presumably both chemical and tactile receptors are activated during these events. The detection of sugars is unusual in crustaceans (Case, 1964; Ashby & Larimer, 1965) and for this reason the coxal receptor hairs are currently under investigation.

REFERENCES

ALEXANDROWICZ, J. S. (1932). The innervation of the heart of the Crustacea. I. Decapoda. *Q. Jl microsc. Sci.* **75**, 182–249.

ALEXANDROWICZ, J. S. (1952). Receptor elements in the thoracic muscles of *Homarus vulgaris* and *Palinurus vulgaris. Q. Jl microsc. Sci.* **93**, 315–46.

ASHBY, E. A. & LARIMER, J. L. (1965). Modifications of cardiac and respiratory rhythms in crayfish following carbohydrate chemoreception. *J. cell. comp. Physiol.* **65**, 373–9.

BOWERMAN, R. F. & LARIMER, J. K. (1974a). Command fibres in the circumesophageal connectives of crayfish. I. Tonic fibres. *J. exp. Biol.* **60**, 95–117.

BOWERMAN, R. F. & LARIMER, J. L. (1974b). Command fibres in the circumesophageal connectives of crayfish. II. Phasic fibres. *J. exp. Biol.* **60**, 119–34.

BULLOCK, T. H. & HORRIDGE, G. A. (1965). *Structure and Function of the Nervous Systems of Invertebrates* San Fransisco: W. H. Freeman and Co.

CASE, J. F. (1964). Properties of the dactyl chemoreceptors of *Cancer antennarius* Stimpson and *C. productus. Biol. Bull. mar. biol. Lab., Woods Hole* **127**, 428–46.

CARLSON, A. J. (1909). Vergleichende Physiologie der Herznerven und der Herzganglion bei den Wirbellosen. *Ergebn. Physiol.* **8**, 371–462.

CARLSON, A. J. (1940). The nervous origin of heartbeat in *Limulus* and the nervous nature of co-ordination or conduction in the heart. *Am. J. Physiol.* **12**, 67–74.

DOGIEL, J. (1876). Anatomie du cœur des crustaces. *C. r. hebd. Séanc. Acad. Sci., Paris* **82**, 1117–20.

FIELD, L. H. & LARIMER, J. L. (1974b). The cardioregulatory system of crayfish. The role of circumesophageal interneurones. *J. exp. Biol.* **62**, 531–543.

FLOREY, E. (1960). Studies on the nervous regulation of the heart beat in decapod crustacea. *J. gen. Physiol.* **43**, 1061–81.

HAGIWARA, S. & BULLOCK, T. H. (1957). Intracellular potentials in pacemaker and integrative neurons in the lobster cardiac ganglion. *J. Cell comp. Physiol.* **50**, 25–47.

HUMASON, F. L. (1962). *Animal Tissue Techniques.* San Fransisco: W. H. Freeman and Co.

JOLYET, F. & VIALLANES, H. (1893). Recherches physiologiques sur le système nerveux accélerateur et modérateur du coeur chez le crabe. *Ann. Sci. nat. (Zool.)* **14**, 387–404.

KEIM, W. (1915). Das Nervensystem von *Astacus fluviatilis* (*Potamobius astacus* L.) *Z. wiss. Zool.* **113**, 485–545.

LARIMER, J. L. (1962). Responses of the crayfish heart to respiratory stress. *Physiol. Zool.* **35**, 179–86.

LARIMER, J. L. & TINDEL, J. R. (1966). Sensory modifications of heart rate in crayfish. *Anim. Behav.* **14**, 239–45.

MANGOLD, E. (1924). Studien zur Physiologie des Krebsherzens, besonders über ein aktive Funktion des 'Pericard' bei *Cancer pagurus. Z. vergl. Physiol.* **2**, 184–208.

MAYNARD, D. M. (1953). Activity in a crustacean ganglion. I. Cardioinhibition and acceleration in *Panulirus argus. Biol. Bull. mar. biol. Lab., Woods Hole* **104**, 156–70.

MAYNARD, D. M. (1960). Circulation and heart function. Chapter V in *Physiology of Crustacea* (ed. T. Waterman), pp. 161–214. New York: Academic Press.

MAYNARD, D. M. (1961a). Thoracic neurosecretory structures in Brachyura. I. Gross anatomy. *Biol. Bull mar. biol. Lab. Woods Hole* **121**, 316–29.

MAYNARD, D. M. (1961b). Thoracic neurosecretory structures in Brachyura. II. Secretory neurons. *Gen. and comp. Endocr.* **1**, 237–63.

MAYNARD, D. M. (1961c). Cardiac inhibition in decapod Crustacea. In *Nervous Inhibitions* (ed. E. Florey), pp. 144–78. London: Pergamon Press.

OTANI, T. & BULLOCK, T. H. (1957). Responses to depolarizing currents across the membrane of some invertebrate ganglion cells. *Anat. Rec.* **128**, 599.

S.-ROZSA, K. & SALANKI, J. (1973). Single neuron responses to tactile stimulation of the heart in the snail *Helix pomatia* L. *J. comp. Physiol.* **84**, 267–79.

TAYLOR, E. W. (1970). Spontaneous activity in the cardioaccelerator nerves of the crayfish, *Astacus pallipes* Lereboullet. *Comp. Biochem. Physiol.* **33**, 859–69.

VAN HARREVELD, A. (1936). A physiological solution for freshwater crustaceans. *Proc. Soc. exp. Biol. Med. N.Y.* **34**, 428–32.

WIERSMA, C. A. G. & NOVIKSKI, E. (1942). The mechanism of the nervous regulation of the crayfish heart. *J. exp. Biol.* **19**, 255–65.

WIERSMA, C. A. G. & PILGRIM, R. L. C. (1961). Thoracic stretch receptors in crayfish and rock lobster. *Comp. Biochem. Physiol.* **2**, 51–64.

WOORTMANN, K. D. (1926). Beitrage zur Nervenphysiologie von *Mytilus edulis.* *Z. vergl. Physiol.* **4**, 488–527.

ZUBKOV, A. A. (1934). Materials for comparative physiology of the heart. (In Russian.) *Fiziol. Zh. SSSR.* **17**, 299–306.