# Both the Heart and Pericardium in the Chiton Acanthopleura japonica Receive Dual Innervation from the Central Nervous System

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# Both the Heart and Pericardium in the Chiton *Acanthopleura japonica* Receive Dual Innervation from the Central Nervous System

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**ABSTRACT**—The isolated heart or pericardium of the chiton *Acanthopleura japonica* each showed rhythmic automaticity, even after they were isolated from the body. Intracellular action potentials could be recorded from spontaneously active myocardial cells in the isolated heart and pericardium. They always appeared to be preceded by pacemaker potentials much as has been found for myocardial cells of other molluscs. These show that both heart and pericardial beats of *Acanthopleura* have a myogenic nature. Stimuli applied to each of the lateral and ventral nerve cords produced both excitatory and inhibitory effects on the heart and the pericardium. Both the heart and the pericardium in *Acanthopleura* may receive excitatory and inhibitory control from each of the lateral and ventral nerve cords. The dual innervation of the heart which is seen commonly in molluscs, at higher stages of evolution, may have been established in *Acanthopleura* at the stage of the phylogenetic beginning of the Mollusca. On the contrary, the dual innervation which is required for acceleratory and inhibitory control of the spontaneously rhythmically active pericardium is different from the innervation seen in molluscs at the higher stages.

## INTRODUCTION

Major molluscan taxa such as gastropods, bivalves and cephalopods have been used in investigations on the cardiovascular system itself and neural and humoral control of the system. There are, however, few papers in which physiology of the circulatory system has been mentioned for placophoran species (see review of Boyle, 1977). Greenberg (1962) reported that the ventricle, auricle and ventral pericardium of *Cryptochiton* were pulsatile, giving only descriptions are given without illustrations and electrophysiological data.

Carlson (1905a) observed that in the heart of *Cryptochiton*, one or, sometimes, two of the tiny nerves leading from the lateral nerve cord (which is one of two paired nerve cords) could be followed in the wall of the efferent branchial vessels and on to the auricle. He did not state whether or not the heart was innervated by the ventral nerve cord which is another nerve cord. He (Carlson, 1905b) also reported that electrical stimulation of the cerebral commissure, or the lateral nerve cord, produced a slight acceleration of the rhythm, but no slowing nor arrest of heart beat was observed. Økland (1980) showed by means of electron microscopy that there were neural processes in the auricle, ventricle and auriculo-ventricular valve of *Lepidopleurus* and *Tonicella*.

Greenberg (1962) noticed that, in *Cryptochiton*, the ventricle, auricle and the ventral pericardium beat independently, and their activity was increased by stimulation of the brain. However, it was reported by Greenberg *et al.* (1973) that the isolated pericardium hung in an organ bath was usually quiescent. The nerve processes in the pericardium, often more than 50 axons in a bundle, run free in the collagen matrix and make synaptic contacts with pericardial muscle fibers (Økland, 1981). The central origin and function of these nerves have not been shown.

In the present study we first show intracellular potentials as evidence for myogenic automaticity of the heart and the pericardium in the chiton, and then regulatory innervation of the heart and the pericardium from the central nervous system. A preliminary account has appeared elsewhere in an abstract form (Matsumura and Kuwasawa, 1987).

## MATERIAL AND METHODS

The placophoran mollusc, *Acanthopleura* (=*Liolophura*) *japonica* was used. Animals (5–8 cm in body length) were collected at the sea shore in the Tokai area of Japan and kept in a laboratory aquarium at 20°C for periods up to three months before use.

*Dissection for neuroanatomical observations*

Before dissection, animals were anesthetized by injection of 3 to 8 ml isotonic $MgCl_2$ solution. The body was pinned to the soft plastic sheet of a dissection dish by pins through the girdle around the *Acanthopleura* body. The head to 8th shell valves were severed using a pen-type grinder, a pair of scissors and a surgical knife. The mantle under the head to 5th shell valves was carefully torn off, and the digestive organ was resected. The cerebral commissure, the pair of lateral nerve cords and the pair of ventral nerve cords were exposed by making an incision at the body wall and pedal muscles (see Fig. 1). The heart and pericardium were exposed by means of removing

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the mantle under the 6th to 8th shell valves, leaving the median region of shell valves a few mm wide to keep the heart intact. Efferent branchial vessels on both sides were exposed at the 5th and 6th shell valves. A pair of cannulae were inserted into the efferent branchial vessels on the right and left sides in order to perfuse the heart. The preparations were stained with methylene blue dissolved in filtered natural sea water (SW). After bathing preparations in 4% ammonium molybdate SW over night, the preparation was repeatedly stained with methylene blue while dissection was carried out in tap water.

*Electrophysiology*

*Semi-intact preparations* The heart was exposed as described above for neuroanatomical observations. The heart was perfused with SW through two cannulae inserted into the efferent branchial vessels. At the same time, the whole specimen was superfused with SW. SW was supplied from a reservoir tank by means of gravity feed and while the experimental chamber was aspirated, to keep the water level steady. The middle of the ventricle on the dorsal side and the frontal end of the pericardium on the ventral side were connected to straingauge mechano-transducers *via* a fine suture for the heart, and a fine needle for the pericardium, to record the mechanogram.

*Isolated preparations* The heart or the pericardium was isolated and mounted on a Sylgard (Dow-Corning) lining at the bottom of the experimental chamber (8 ml volume), which was filled with SW. The dorsal wall of the ventricle was slit longitudinally from the posterior end to the anterior one. The dorsal wall of the auricle was slit between junctions of the auricle and efferent branchial vessel on both the left and right junctions. Then, the heart was opened and pinned to the bottom of the chamber. The pericardium on the ventral side was isolated. The isolated pericardial specimen was pinned inside out to the bottom of the chamber.

Glass capillary suction electrodes (tip diameter, 50–500 $\mu$m) were employed for extracellular recording of electrocardiogram (ECG) and electropericardiogram (EPG). Glass microelectrodes filled with 3 M KCl (tip resistance, 20 to 30 M$\Omega$) were used for intracellular recording of membrane potentials from cardiac and pericardial muscle cells. A Ag-AgCl reference electrode was positioned at the bottom of the chamber.

Electrical signals were displayed on an oscilloscope (Tektronix 5110) and a pen writing oscillograph (Nihon Kohden WI-641G). Some of them were recorded by a DAT recorder (TEAC RD-101T) and redisplayed on the recorders.

Glass capillary suction electrodes were used to apply stimuli (0.5 to 5 msec in duration) to the lateral and ventral nerve cords. These tip diameters were fitted to the size of the cut end of the nerves to be introduced into the electrode. The nerve cords were cut at a site beneath the 2nd to 5th shell valve, and the distal cut end was introduced into the electrode.

## RESULTS

*Anatomy*

The cardiovascular system and central nervous system of *Acanthopleura* are schematically illustrated in Figure 1.

In *Ischnochiton*, a pericardial cavity lies beneath the last two, 7th and 8th, shell valves and contains a median ventricle and a pair of lateral auricles (Hyman, 1967). The pericardial cavity of *Chiton sp.* lies beneath the 6th and 7th shell valves containing the heart (Haller, 1882). The location of the heart and the pericardium in *Acanthopleura* differs from both the above-mentioned species, but is essentially the same as in *Cryptochiton* as shown by Greenberg *et al.* (1973). The heart is enveloped in the pericardium located underneath the last three, 6th, 7th and 8th, shell valves. The dorsal side of the pericardium adheres to the mantle along the median line, while the ventral side is free from other tissues. The heart consists of a median ventricle and a "U" shaped auricle having a narrow connection at the hindmost, and encompassing the ventricle. In *Amicula*, the auricle makes junctions with the ventricle at a single site on either side (Okutani and Saito, 1987). In *Acanthopleura*, these junctions exist at two sites on either side. An auriculo-ventricular valve, which consists of a pair of flaps, is found at each of four junctions. The dorsal side of the ventricle adheres to the pericardium along the median line which adheres to the mantle, thus the ventricle is suspended along its length from the dorsal body wall. Five pairs of arterioles arise from the artery in its course, until it opens anteriorly into the diffuse body sinuses around the head and buccal apparatus, as shown in *Cryptochiton* by Heath (1950).

In placophorans, there are only two pairs of ganglion-like structures which are referred to as the buccal ganglion and subradular ganglion at the head (Heath, 1905; Bullock and Horridge, 1965). *Acanthopleura* also has these ganglionic structures. Two pairs of longitudinal nerve cords i.e. the lateral nerve cords and the ventral nerve cords run from the head to tail. These nerve cords unite at the cerebral commissure, as shown in *Chiton siculus* by Haller (1882). A pair of ventral nerve cords are embedded in foot musculature, run over the length of the foot and end blindly. They are connected with many fine commissures. The lateral nerve cords are located on the branchial vessels. The lateral nerve cords on either side are linked through the suprarectal commissure. In *Cryptochiton* and *Achantochiton*, part of the suprarectal commissure runs close by the auricle. In *Acanthopleura*, the suprarectal commissure runs in the body wall along the hindmost part of the auricle. There are many connectives linking the ventral nerve cord to the lateral nerve cord.

*Spontaneous activities of the heart and pericardium*

Extracellular recordings of activity of the heart and pericardium were made simultaneously in a semi-intact *Acanthopleura* preparation. The heart and pericardium beat with a constant delay between them (about 1 second in Fig. 2A), when the rates of their beats are relatively high (19 beats/minutes in Fig. 2A). Three examples of correlation between the heart and pericardium are shown in Figure 2B. The heart and pericardium beat regularly at their individual rates (Fig. 2B. top panel). The pericardium beat relatively regularly until the heart stopped beating (Fig. 2B. middle panel). The heart beat rhythmically, while the pericardium beat irregularly (Fig. 2B. bottom panel).

Intracellular potentials were recorded from cardiac and pericardial muscle cells (Fig. 3). In Figure 3, slow depolarizing potentials, i.e. pacemaker potentials were observed between maximum diastolic membrane potentials and spike potentials in cycles of the ventricle, auricle and pericardium. Averages of maximum diastolic membrane potentials were –68 ±5.5 mV (mean ±SD, n=67) at the auricle of 12 specimens, –60 ±4.5 mV (n=150) at the ventricle of 34 specimens and –56±4.5 mV

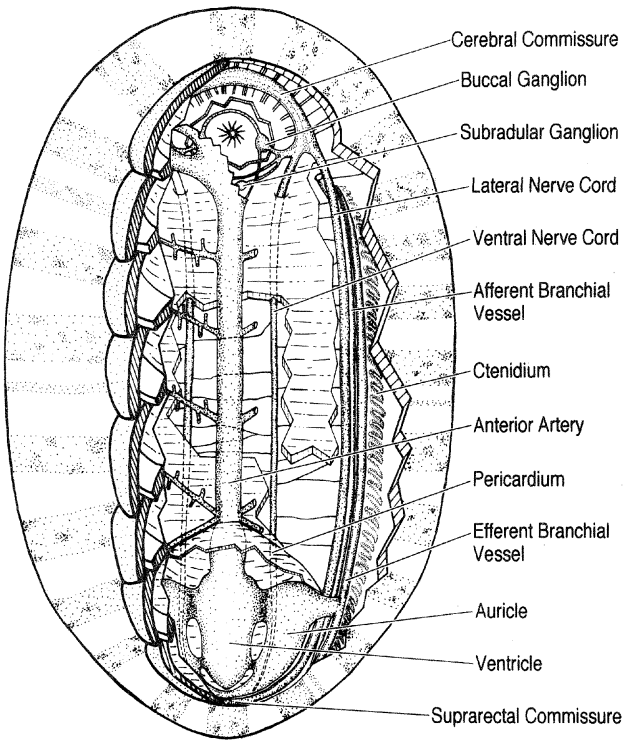


Fig. 1. A dorsal view of the circulatory system and the central nervous system of *Acanthopleura japonica*.

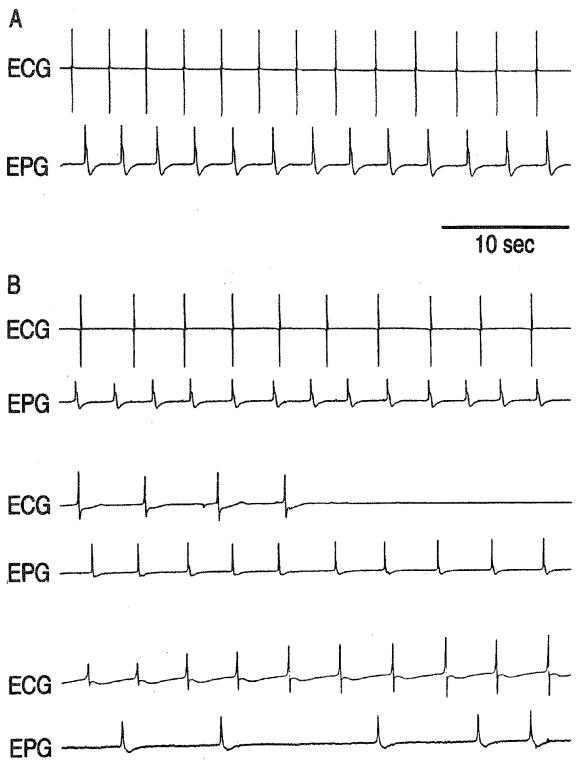


Fig. 2. Extracellular simultaneous records obtained from the heart and the pericardium in a semi-intact preparation. A. The heart and pericardium beat correspond to each other, with a constant delay. B. Three examples of different modes of correlation between the heart and the pericardium. ECG; electrocardiogram. EPG; electropericardiogram.

(n=131) at the pericardium of 16 specimens. These values of the heart are similar to those of other molluscan hearts reviewed by Jones (1983). Resting potentials of these cells in the quiescent state were –48.9 ±5.3 mV (n=72) at the auricle of 27 specimens, –49.2 ±5.3 mV (n=291) at the ventricle of 58 specimens, –49.7 ±6.1 mV (n=26) at the auriculo-ventricular valve of 5 specimens and –54.9 ±3.3 mV (n=56) at the pericardium of 35 specimens. These results may show that in the placophoran not only has the heart myogenic automaticity, as do other molluscan hearts, but also the pericardium has a myogenic nature.

*Cardiac and pericardial responses to stimulation of the lateral and ventral nerve cords*

In the preparation whose ventral nerve cords were resected, electrical stimuli applied to the lateral nerve cord elicited excitation of the heart (Fig. 4A1), or inhibition of the heart (Fig. 4A2). In the preparation whose lateral nerve cords and the suprarectal commissure were resected, electrical stimuli applied to the ventral nerve cord elicited excitation of the heart (Fig. 4B1), or inhibition of the heart (Fig. 4B2). These results show that the heart may receive both excitatory and inhibitory nerves from both the lateral and ventral nerve cords.

Electrical stimuli applied to the lateral nerve cord increased pericardial beating rate and contraction force (Fig. 5A1), or decreased these (Fig. 5A2). Electrical stimuli applied to the ventral nerve cord increased pericardial beating rate and contraction force (Fig. 5B1) or decreased these (Fig. 5B2). These results show that the pericardium may receive both excitatory and inhibitory nerves from both the lateral nerve cord and the ventral nerve cord, as well as the heart.

Electrical stimuli produce combined effects on the heart and pericardium, when applied to a semi-intact preparation (in which both the heart and pericardium remain intact). When repetitive stimuli were applied to the nerve cord, a combination of excitatory and inhibitory effects resulted, in which the heart and the pericardium often showed the same excitatory or inhibitory effects. In some cases, while cardiac inhibition was induced in response to stimuli to the lateral nerve cord, pericardial excitation increased beating rate and contraction force (Fig. 6A). While cardiac activity was not changed significantly by repetitive stimuli to the lateral nerve cord, pericardial arrest was elicited (Fig. 6B). When the heart was excited by stimuli applied to the nerve cord, excitatory or inhibitory effects appeared on the pericardium (not shown). The combination of the excitatory and inhibitory effects on the heart and pericardium, which were induced in response to repetitive stimuli applied to the ventral nerve cord, sometime coincided and sometimes was opposite to each other (not shown).

Both the heart and pericardium have an autonomous rhythmic nature (cf. Fig. 3). However, their beating correspond

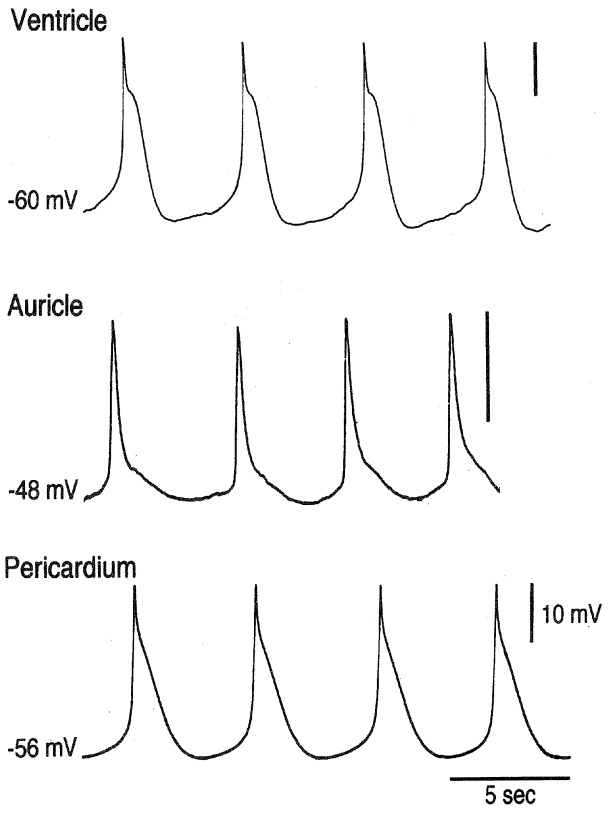


Fig. 3. Intracellular records of spontaneous activities of ventricular and auricular muscle cells in the isolated heart and pericardial muscle cells in the isolated pericardium. Numerals are membrane potentials at the beginning of each record.

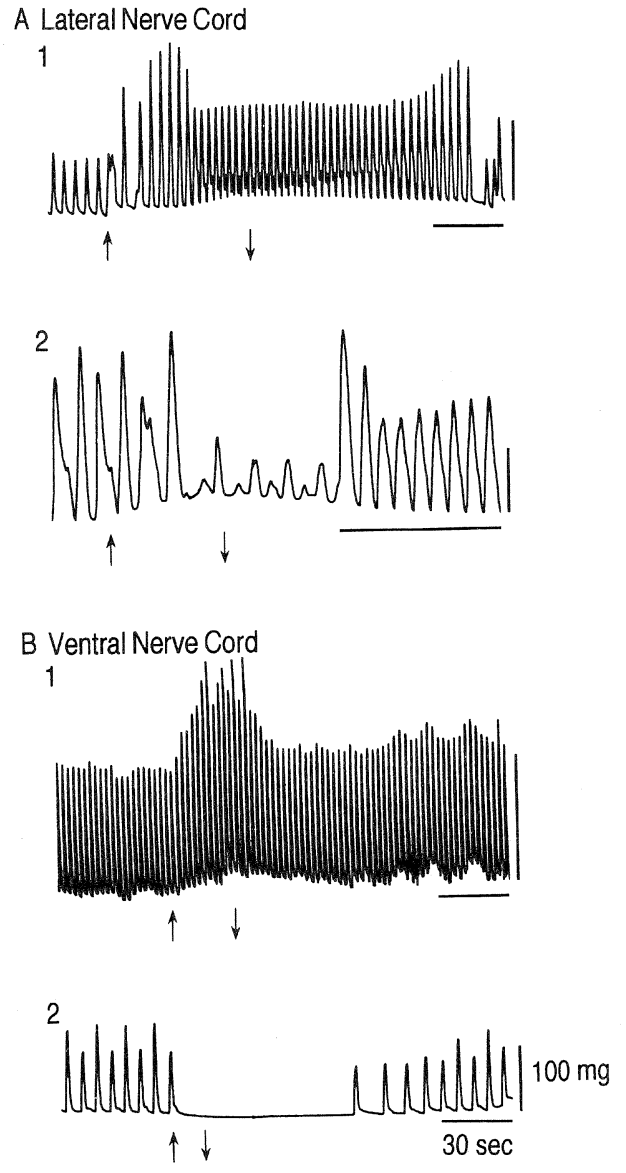


Fig. 4. Cardiac mechanical responses to stimuli applied to the lateral nerve cord or the ventral nerve cord. A1. A mechanical record of cardiac excitatory effects elicited by electrical stimuli at 5 Hz applied to the lateral nerve cord, between the arrows. A2. A mechanical record of cardiac inhibitory effects induced by electrical stimuli at 5 Hz applied to the lateral nerve cord. B. Cardiac excitatory (B1) and inhibitory (B2) effects were elicited by electrical stimuli at 10 Hz applied to the ventral nerve cord.

as seen in Figure 2A. To observe the relationship between the heart and pericardial beats, electrocardiograms from the ventricle and auricle, the electropericardiogram and the mechanocardiogram were recorded simultaneously from a semi-intact preparation which consisted of the intact heart and pericardium (Fig. 7). The mechanocardiogram shows that contractions of the heart were large when beats of the heart and the pericardium synchronized. When their beats were not synchronized, as seen in periods during and after repetitive stimuli applied to a nerve cord, contraction force was reduced. Contraction force gradually recovered when the activities of the heart and pericardium became synchronized again, after the stimulation. This may indicate the presence of some mechanism for co-ordinative interaction between them.

## DISCUSSION

*Spontaneous activity of the heart*

Intracellular electrical records have been obtained from myocardial cells in gastropods (Nomura, 1963; Kuwasawa, 1967, 1979; Kuwasawa *et al.*, 1987; Kiss and S-Rózsa, 1973; Kiss, 1980), in bivalves (Irisawa *et al.*, 1961a, b; Irisawa *et al.*, 1967; Shigeto, 1970; Ebara, 1964a, b; Wilkens, 1972a, b; Hill and Kuwasawa, 1990) and in a cephalopod (Hill and Kuwasawa, 1990). All these records from a variety of molluscan hearts showed that action potentials were preceded by pacemaker potentials of myogenic nature. Intracellular action potentials recorded from spontaneously active auricular and ventricular myocardial cells of *Acanthopleura* always appeared to be preceded by pacemaker potentials. These results in this study verify a diffuse myogenic nature for the heart of chitons, very much as in other species (see reviews of Krijgsman and Divaris, 1955; Hill and Welsh, 1966; Irisawa, 1978; Jones, 1983).

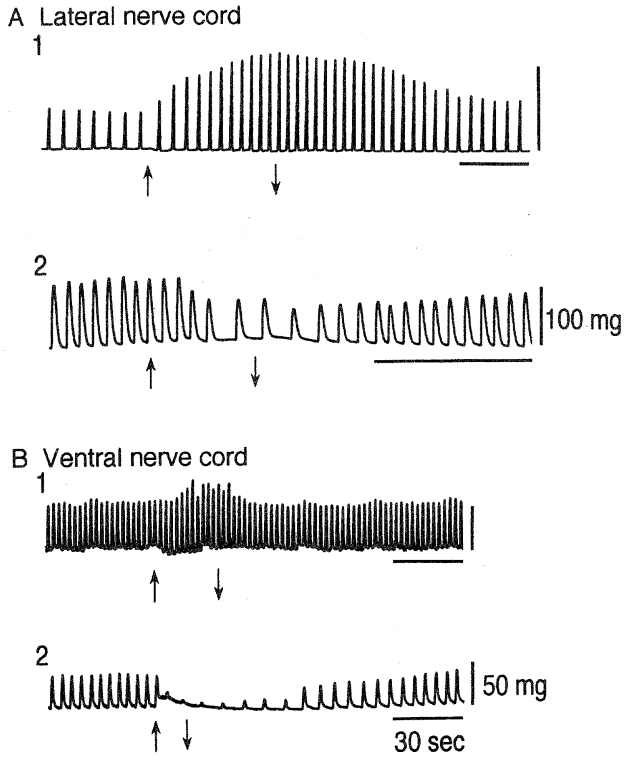


Fig. 5. Pericardial mechanical responses to stimuli applied to the lateral nerve cord or to the ventral nerve cord. A1. A mechanical record of pericardial excitatory effects elicited by electrical stimuli at 20 Hz applied to the lateral nerve cord, between the arrows. A2. A mechanical record of pericardial inhibitory effects induced by electrical stimuli at 5 Hz applied to the lateral nerve cord. B. Pericardial excitatory (B1) and inhibitory (B2) effects elicited by electrical stimuli at 10 Hz applied to the ventral nerve cord.

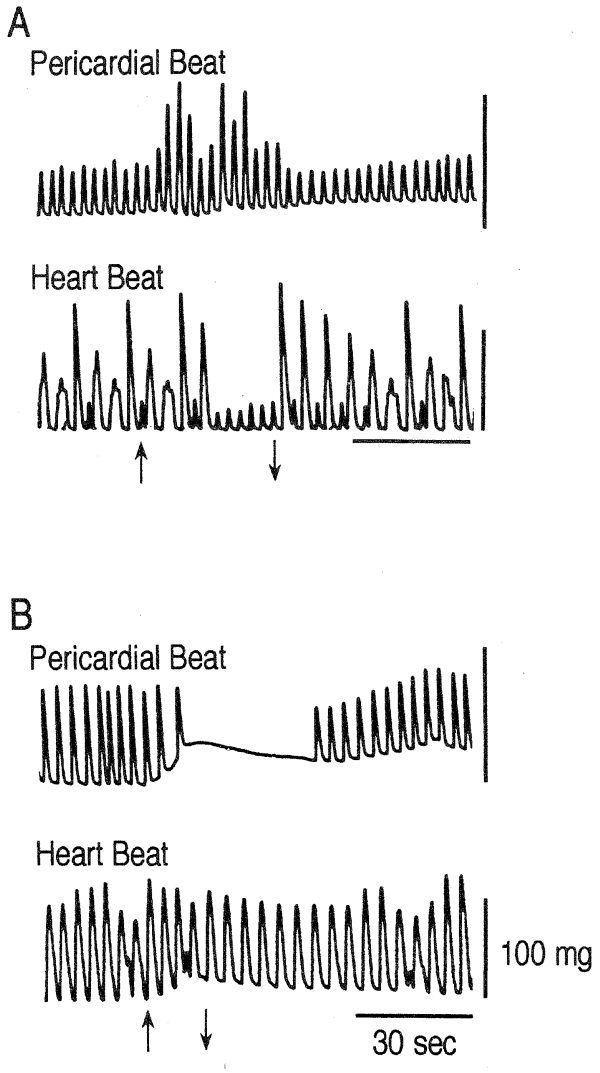


Fig. 6. Effects of stimulation of the lateral nerve cord on the heart and the pericardium. Simultaneous mechanical records from the heart and the pericardium. A. Electrical stimuli at 5 Hz applied to the lateral nerve cord between the arrows elicited excitatory effects on the pericardium (upper trace) and inhibitory effects on the heart (lower trace) at the same time. B. Electrical stimuli at 10 Hz applied to the lateral nerve cord between the arrows elicited inhibitory effects on the pericardium (upper trace), while no significant effects were elicited in the heart (lower trace).

*Spontaneous activity of the pericardium*

Haemolymph is filtered through the wall of the heart into the pericardial cavity and passes into the nephridia through the renopericardial ducts (Jones, 1983). In *Lepidopleurus asellus* and *Tonicella marmorea*, the basement membrane of the heart is a functional filter of the excretory system (Økland, 1981). Truman (1966) stated that, in the bivalves *Mya arenaria* and *Margaritifera margaritifera*, siphonal movement and contraction of the foot increase the pressure in the pericardium, which may help the circulation of fluid in the pericardial cavity. The pericardial rhythmic contraction in *Acanthopleura* may provide pulsating motive force to convey the pericardial fluid into the nephridial duct.

Civil and Thompson (1972) and Sommerville (1973), using *Helix* hearts, examined the relationship between cardiac performance and pericardial pressure in an artificial pericardial cavity where the pericardial pressure and the pressure of the heart and sinus were independently varied. Their results show that one of the important factors is the pressure difference between the pericardial cavity and the heart. An increase in heart rate could be induced by reducing the pericardial pressure. Jones (1983) stated that, in molluscs, hearts with a leaking or damaged pericardium do not beat regularly, and that tension in the pericardial wall would be as important as blood pressure in the heart. When both the pericardium and the heart beat synchronously, the contraction force which was recorded from the heart was larger (Fig. 7). This indicates that the synchronized heart and pericardial contractions might strengthen the efficacy of heart beat for cardiac output. When the heart and the pericardium beat asynchronously (Fig. 7) contraction force decreased or the duration of contraction appeared prolonged. It is likely that cardiac output is altered by changes not only in force of heart contraction but also in synchronization of the heart and the pericardium. This may suggest that the heart and the pericardium mechanically interact with each other since they are not electrically coupled,

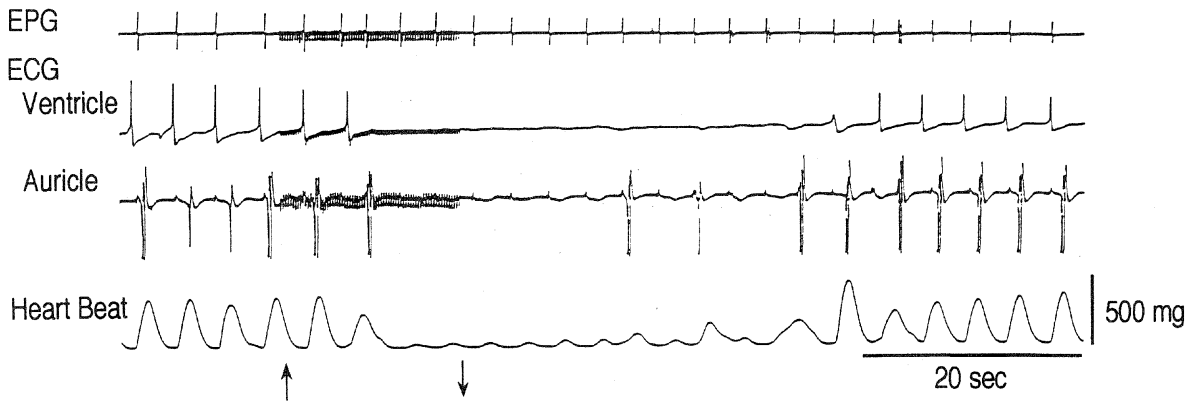


Fig. 7. Simultaneous records from the heart and the pericardium. The uppermost trace shows the EPG. The 2nd trace shows the ECG from the ventricle. The 3rd trace shows the ECG from the auricle. The lowest trace shows a mechanocardiogram. Electrical stimuli at 5 Hz applied between the arrows elicited cardiac inhibitory effects and pericardial excitatory effects.

as can be seen during a period of cessation of the ECG in Figure 7.

*Dual innervation of both the heart and the pericardium*

Haller (1882) described the presence of some heart nerves arising from the end of the lateral nerve cord in *Chiton squamosus*. Carlson (1905a) observed fine nerves running from the lateral nerve cords to the auricle of *Cryptochiton*. Electronmicroscopic investigation showed that nerve processes run free in the matrix or between the muscle fibres in bundles of the auricle or ventricle in *Tonicella marmorea* and *Lepidopleurus asellus* (Økland, 1980). In the present study, we have confirmed that electrical stimuli applied to the ventral and lateral nerve cords elicit excitatory effects on the heart in *Acanthopleura*. However, Greenberg (1962) suggested that increase of the heart activity induced by stimulation of the cerebral commissure might be attributed to a secondary effect resulting from the contraction of body musculature. This is not the case in *Acanthopleura*.

Neither Carlson (1905b) nor Greenberg (1962) mentioned whether or not electrical stimuli to the nerve cords elicited cardio-inhibitory effects in *Cryptochiton*. In the present study, in *Acanthopleura*, electrical stimuli to the nerve cords evidently elicited cardiac inhibition.

In the gastropod *Dolabella,* it has been suggested that the automaticity of the heart was inhibited effectively at the pacemaker region, since the auriculo-ventricular valve had a higher spontaneous activity than other regions, and inhibitory junctional potentials were recorded from the region of the auriculo-ventricular valve and its vicinity (Kuwasawa, 1979). In the gastropod *Busycon*, the bivalve *Mercenaria* and the cephalopod *Octopus*, sites where IJPs were recorded were expanded to the auricle and ventricle (Kuwasawa and Hill, 1972; Kuwasawa and Hill, 1973a, b; Hill and Kuwasawa, 1990). It is interest from a phylogenetic view point to be examined whether or not the inhibitory innervation is confined to any particular region in the chiton heart.

In *Aplysia californica*, Koester and Kandel (1977) identified a pericardial motor neuron in the abdominal ganglion that caused a lateral shortening of the pericardium. Rittenhouse and Price (1986) revealed that the axons of some R3-R14 neurons arborized in the pericardial region. Furgal and Brownell (1987) showed that, when the circulation through the abdominal ganglion was decreased, a transient increase occurred in the amplitude of pericardial contractions. Such contractions were not evoked after the pericardial nerve was severed. In *Lymnaea*, a pericardial excitatory neuron was identified which induced cardiac-inhibition through contraction of the pericardial wall (Buckett *et al*., 1990). In these animals, the pericardia, however, are not pulsatile, and only excitatory neural control is shown.

The results of stimuli applied to either the lateral or ventral nerve cords supported the existence of dual innervation of both the heart and pericardium. When excitatory and inhibitory effects combined, the effects, on the heart and the pericardium could coincide or oppose each other. These results show that the excitatory motor axons to the heart and to the pericardium originate from different neurons, as do the inhibitory motor axons.

*The physiological significance of pericardial beat*

In molluscan haemodynamics, the constant volume mechanism proposed by Ramsay (1952) and Krijgsman and Divaris (1955) explained the refilling of the heart as depending principally on the volume of the pericardial cavity remaining constant throughout the cardiac cycle. Support for that mechanism has been provided by studies of *Patella vulgata* (Jones, 1970), *Helix pomatia* (Jones, 1971), *Haliotis corrugata* (Bourne and Redmond, 1977) and *H. ruber* (Russell and Evans, 1989). In *Aplysia californica*, this mechanism is occasionally disturbed by contraction of the pericardium which induces transient surges in arterial pressure (Furgal and Brownell, 1987). In the *Lymnaea stagnalis* heart, neurally induced contraction of the pericardium produces a reduction in heart beat amplitude, with a concomitant elevation in diastolic tonus (Buckett *et al*., 1990). End-diastolic volume is

important in affecting cardiac output in molluscs (Koester *et al.*, 1974; Smith, 1985; Smith and Hill, 1986, 1987). In *Busycon canaliculatum,* pericardial contraction may be an important factor in cardioregulation, since base pressure and particularly end-diastolic volume of the heart are controlled by the contraction of the pericardium (Jones, 1988). In *Acanthopleura*, when the heart and the pericardium beat synchronously, with a constant delay, the heart contraction was augmented. When they did not beat synchronously, contraction force of the heart decreased considerably (Fig. 7). It seems to be that, in the chiton, the pericardium and heart beats cooperate or opposed in performance of refilling and cardiac outflow. Therefore, it is likely that the constant volume mechanism depending on the pericardial cavity does not operate in the chiton. With dual innervation of both the heart and pericardium, the chiton may show compound modes of cardiac performance rather than the direct mode seen in ascendant molluscan species.

## ACKNOWLEDGMENTS

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