Cardioacceleratory Neurons of the Isopod Crustacean, Ligia exotica: Visualization of Peripheral Projection onto the Heart Muscle

Authors: Sakurai, Akira, and Yamagishi, Hiroshi

Source: Zoological Science, 15(1) : 19-25

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.15.19

ZOOLOGICAL SCIENCE **15**: 19–25 (1998)

Cardioacceleratory Neurons of the Isopod Crustacean, *Ligia exotica*: Visualization of Peripheral Projection onto the Heart Muscle

Akira Sakurai and Hiroshi Yamagishi*

Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

ABSTRACT—Innervation of the heart muscle by the cardioacceleratory neurons was morphologically and electrophysiologically examined in the isopod crustacean, *Ligia exotica*. Intracellular injection of neurobiotin into the first and second cardioacceleratory neurons (CA1 and CA2) revealed their peripheral axonal projections. Inside the heart, the CA1 and CA2 axons ran along the trunk of the cardiac ganglion. Finely arborized branches with many varicosities arose from the axon and projected over the heart muscle. Stimulation of either the CA1 or CA2 axon caused an overall depolarization in the muscle of a quiescent heart. The amplitude of the depolarization increased with increasing stimulus frequency. During stimulation, the membrane resistance of the heart muscle decreased. In a beating heart, the cardioacceleratory nerve stimulation caused multiple effects on the heart muscle activity and the heartbeat. The results suggest that the cardioacceleratory neurons of *Ligia exotica* regulate the amplitude of the heartbeat (inotropic effect) and the heart tonus (tonotropic effect) via the synaptic contacts on the heart muscle, while the heartbeat frequency (chronotropic effect) is regulated via the synapses on the cardiac ganglion neurons.

INTRODUCTION

The neurogenic heartbeat of crustaceans is regulated by extrinsic acceleratory and inhibitory nerves from the central nervous system (CNS) (reviewed by Maynard, 1960; Hagiwara, 1961). Many investigators have shown that the impulses of these cardioregulatory nerves affect the heartbeat via excitatory and inhibitory synapses on the cardiac ganglion (CG) neurons (in decapods, Terzuolo and Bullock, 1958; Otani and Bullock, 1959; Shimahara, 1969a, b; Matsui *et al*., 1973; Yazawa and Kuwasawa, 1984a; in a stomatopod, Watanabe *et al*., 1968, 1969; in isopods, Kihara and Kuwasawa, 1984; Yamagishi *et al*., 1989; Sakurai and Yamagishi, 1997).

In addition to the innervation of CG neurons, the cardioregulatory nerves were suggested to innervate the heart muscle on the basis of anatomical observations in decapods with methylene blue (Alexandrowicz, 1932; Maynard, 1961). Electrophysiological recordings of the excitatory (in a decapod, Yazawa and Kuwasawa, 1984b) or the inhibitory (in isopods, Delaleu and Holley, 1976; Yamagishi *et al*., 1989) junctional potentials also suggest the innervation of the heart muscle by the cardioacceleratory or cardioinhibitory nerves. Recently, Tanaka and Kuwasawa (1991a, b) identified the cardioacceleratory and cardioinhibitory neurons in the CNS of an isopod *Bathynomus doederleini*. They showed morphologically central projections of these cardioregulatory neurons, but did not show their peripheral projections onto the heart muscle. Thus, the morphology and physiology of the axonal projections of individual cardioregulatory neurons into the heart have not been thoroughly described.

In the isopod *Ligia exotica*, we have recently identified the first and second cardioacceleratory (CA1, CA2) neurons in the CNS and described their central projections and synaptic effects on CG neurons (Sakurai and Yamagishi, 1997). In this study, we revealed morphologically their axonal projections onto the heart muscle by intracellular injection of neurobiotin. Innervation of the heart muscle by these neurons was also examined electrophysiologically. Some of the results have appeared in abstract form (Sakurai and Yamagishi, 1996).

MATERIALS AND METHODS

Animals and preparations

We used adult males and females of the littoral isopod *Ligia exotica* (Roux), 6 to 22 mm in body length. They were collected on Pacific coasts (Izu and Boso Peninsulas, Japan) and kept in the laboratory at ambient temperature. We used semi-intact heart preparations that contained the thoracic ganglia and the heart as described in earlier studies (Yamagishi *et al*., 1989; Sakurai and Yamagishi, 1997).

The experimental chamber was usually perfused with aerated physiological saline of the following composition (mM): NaCl 577, KCl 14, $CaCl_2$ 25, $MgCl_2$ 21, Na_2SO_4 4.5 and Tris 5 (Yamagishi and Ebara, 1985). pH was adjusted to 7.4 with HCl. In some experiments, saline containing a higher concentration of $CaCl_2$ (100 mM) and gamma-

* Corresponding author: Tel. +81-298-53-6670;
FAX. +81-298-53-6614.

aminobutyric acid (GABA, 0.05 mM; Wako) was used to suppress the spontaneous activities of CG neurons and the heart muscle membrane (Sakurai and Yamagishi, 1997). Experiments were performed at 22–26°C.

Histology

CA1 and CA2 neurons have their cell bodies in the second and third thoracic ganglia, respectively, sending the axons to the heart through the third roots of their own ganglia (Fig. 1) (Sakurai and Yamagishi, 1997). The axons run alongside the anterior aorta down to the heart. To label peripheral axons of the cardioacceleratory neurons, neurobiotin tracer [N-(2-aminoethyl) biotinamide hydrochloride, Vector labs.] was injected intracellularly (see Hidaka and Hashimoto, 1993; Sakurai and Yamagishi, 1997). Electrical current pulses (positive square pulses of 5–10 nA at 50% duty cycle) were injected into the cell body of CA1 or CA2 neuron through a microelectrode filled with neurobiotin solution (4% in 1 M KCl) at a frequency of 1–2 Hz for 30–60 min (cf., Fig. 1). In some experiments, neurobiotin was also injected into the cell body of a CG neuron after the injection into a cardioacceleratory neuron (cf., Fig. 1). The injection period for CG neuron was about 30 min. After the injection, the preparation was incubated in aerated physiological saline containing streptomycin (50 μg/ml) and penicillin (100 U/ml) for 18–24 hr at room temperature. After the incubation, the heart was isolated and fixed in 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS, pH 7.4) for 5 hr at 4°C. After rinsing with PBS several times, the preparations were treated with Triton-X 100 (2.0% in PBS) overnight, with H_2O_2 (0.3% in distilled water) for 1 hr, and then incubated in VECTASTAIN ABC Reagent (for 24–72 hr at 4°C). The preparation was rinsed with PBS several times and was reacted with 0.02% diaminobenzidine (Sigma) and 0.006% H_2O_2 in 0.05 M Tris-buffer (pH 7.4). The stained preparation was dehydrated in graded ethanol series, cleared by methyl salicylate, and mounted on a glass slide for light microscopy.

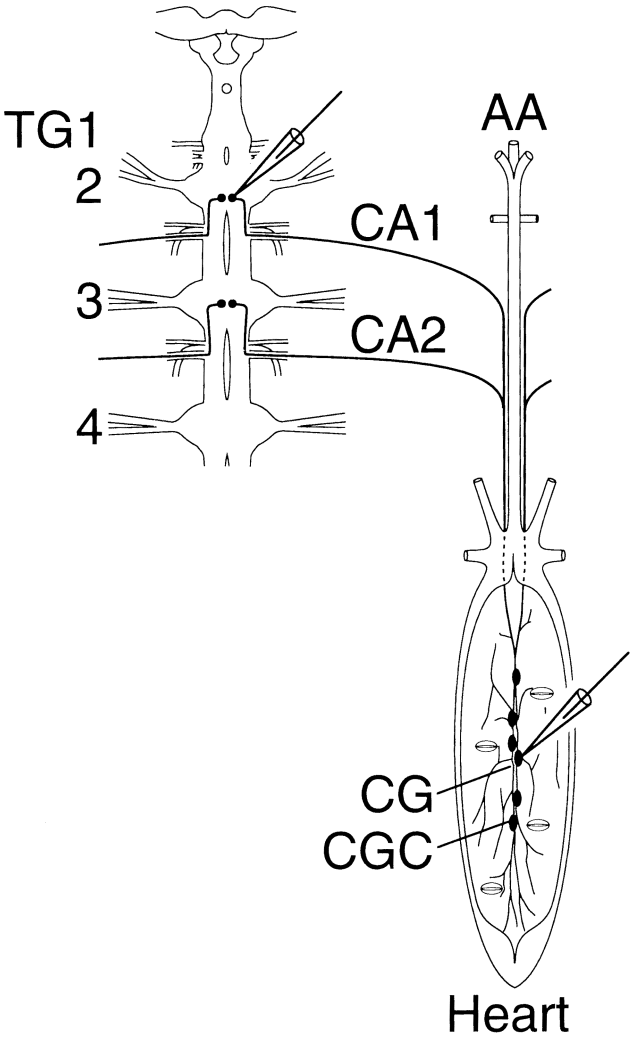

Fig. 1. Schematic drawing of the central nervous system (CNS, dorsal view) and the heart (ventral view). Microelectrodes for tracer injection into cell bodies of a cardioacceleratory neuron and a cardiac ganglion cell were also shown. The heart is opened by longitudinal incision of the ventral wall to expose the inner surface. The posterior part of the CNS is omitted. The cell bodies of the first cardioacceleratory (CA1) neurons are in the second thoracic ganglion (TG2), and those of the second cardioacceleratory (CA2) neurons in the third thoracic ganglion (TG3). Their peripheral axons, which are sent out through the third roots of their own ganglia, run alongside the anterior aorta down to the heart. *AA*, anterior aorta; *CA1*, the first cardioacceleratory neuron; *CA2*, the second cardioacceleratory neuron; *CG*, cardiac ganglion; *CGC*, cardiac ganglion cell; *TG1–4*, thoracic ganglia.

Electrophysiology

Electrophysiological equipment was conventional. Glass capillary microelectrodes filled with 3 M KCl (electric resistance, 15–40 MΩ) were used for recording intracellular electrical activity from the heart muscle and neurons, and for electrical current injection. Intracellular activity of the heart muscle was recorded from the central region of the ventral heart wall. To measure changes in membrane resistance of the heart muscle in response to the cardioacceleratory nerve stimulation, repetitive hyperpolarizing current pulses (100 ms, 2 Hz) were injected into the heart muscle via a second microelectrode. Extracellular recordings of ganglionic nerve impulses were made with a glass capillary suction electrode. For stimulation of the cardioacceleratory axons, the third root of the second or the third thoracic ganglion was transected at a site immediately proximal to the longitudinal connective. The distal cut end of the third root was sucked into a glass capillary suction electrode or hooked with an Ag-AgCl wire. The axon was stimulated by repetitive voltage pulses (1–5 V, 0.3 ms) at 10–60 Hz. Mechanogram of the contraction of the heart muscle was recorded by connecting a dorsal suspensory ligament of the heart to a mechano-electrical transducer (Nihon Kohden TB612T) with a nylon fiber.

RESULTS

Axonal branching of cardioacceleratory neurons inside the heart

Intracellular injection of neurobiotin into the cell bodies of CA1 and CA2 neurons revealed their peripheral axons inside the heart. Figure 2A shows the stained CA1 axon running onto the inner surface of the cut-opened heart. The thick CA1 axon ran along the trunk of CG, in which CG neurons lie longitudinally, down to the posterior end. Fine axons bearing many varicosities arose laterally from the thick axon and arborized over the heart muscle (Fig. 2B, C). Similar features were also observed in the axonal branching of the CA2 neuron (Fig. 3A–C). It seems that the projection patterns of the CA1 and CA2 axons inside the heart shown in Figs. 2 and 3 are different. However, since their projection patterns inside the heart varied from preparation to preparation, we could not detect any significant differences between CA1 and CA2 neurons. In the anterior region of the heart, the axons were deeply stained

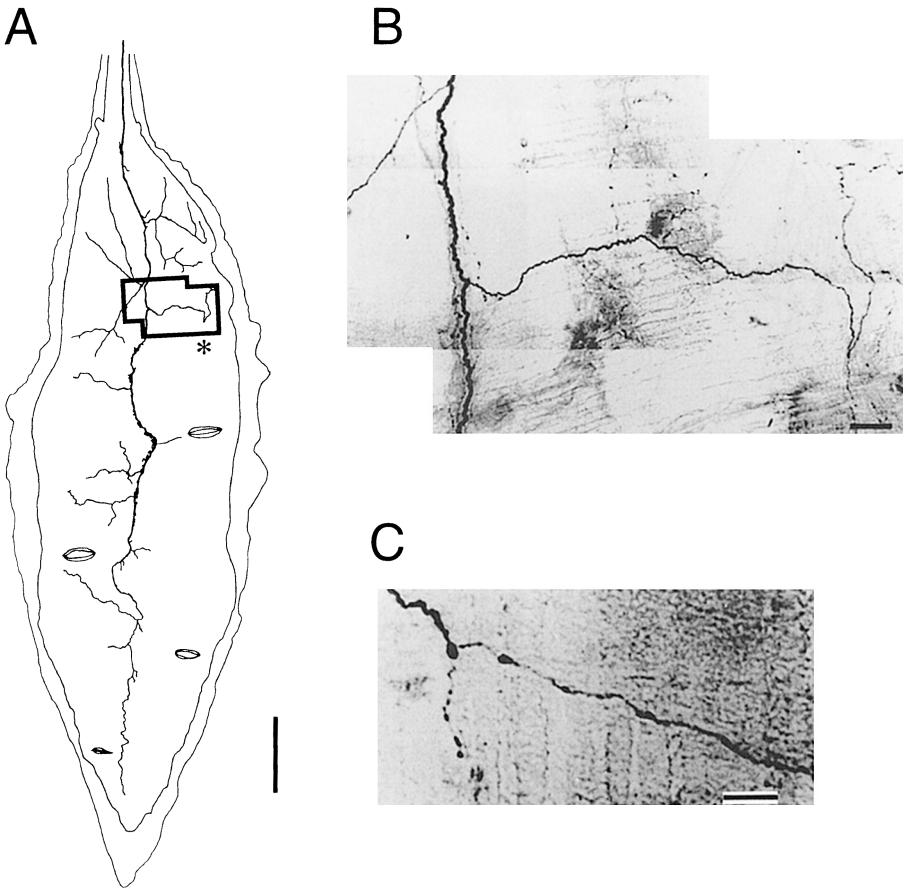

Fig. 2. Axonal branching of the CA1 neuron in the heart. (**A**) Line drawing of the CA1 axon inside the heart. The peripheral axon of the CA1 neuron was labeled by intracellular injection of neurobiotin into the cell body. The inner surface of the heart was exposed by longitudinal incision of the ventral heart wall. (**B**) Light micrograph of the CA1 axon in the designated area shown by the asterisk in **A**. (**C**) Varicosities of the CA1 axon. Scale bar: **A**, 500 μm; **B**, 40 μm; **C**, 20 μm.

and most of the fine branches were seen. The axons in the posterior region were faintly stained.

To examine the relationships between the peripheral axons of the cardioacceleratory neuron and those of CG neurons, double labeling of CG neurons and CA1 or CA2 neuron was performed. Since CG neurons are electrically coupled, the axons of all six CG neurons can be stained by intracellular injection of neurobiotin into one of them (unpublished observation). Figure 4A shows the stained axons of both CG neurons and CA1 neuron, projecting over the heart muscle. The CG axons bore many varicosities and distributed evenly over the heart muscle. As the CG axons appeared much thicker and could be traced from their cell bodies, they could be distinguished easily from the CA1 axon. The CA1 axon went laterally alongside the CG axon, and then separated from it at various sites, and projected onto the heart muscle with varicosities. A similar pattern of axonal pathways was observed in the CA2 axon (Fig. 4B).

Membrane potential responses of the heart muscle to cardioacceleratory nerve stimulation

To investigate the membrane potential responses of the heart muscle to cardioacceleratory nerve stimulation, the heart was arrested by perfusing saline containing a higher concentration of $CaCl_2$ (100 mM) and GABA (0.05 mM). Under these conditions, the resting potential of the heart muscle was in the range of –53 to –58 mV. Repetitive stimulation of the CA1 or the CA2 axon caused an overall depolarization of the heart muscle (Fig. 5A). The mean amplitude of the depolarization caused by CA1 stimulation at 10, 20, 30, and 40 Hz were 0.88 ± 0.05 (n = 6), 2.13 ± 0.35 (n = 8), 3.06 ± 0.37 (n = 8), and 4.13 ± 0.25 (n = 24), respectively (mV; mean ± SE). The mean amplitude of the depolarization evoked by CA2 stimulation at 10, 20, 30, and 40 Hz were 0.95 ± 0.14 (n = 6), 2.03 ± 0.20 (n = 8), 3.28 ± 0.45 (n = 9), and 4.04 ± 0.27 (n = 21), respectively (mV; mean ± SE). The amplitude of the depolarization increased with increasing the stimulus frequency (Fig. 5B). No noticeable differences were found between the depolarizing responses of the heart muscle to the CA1 and CA2 stimulation.

We next examined changes in membrane resistance of

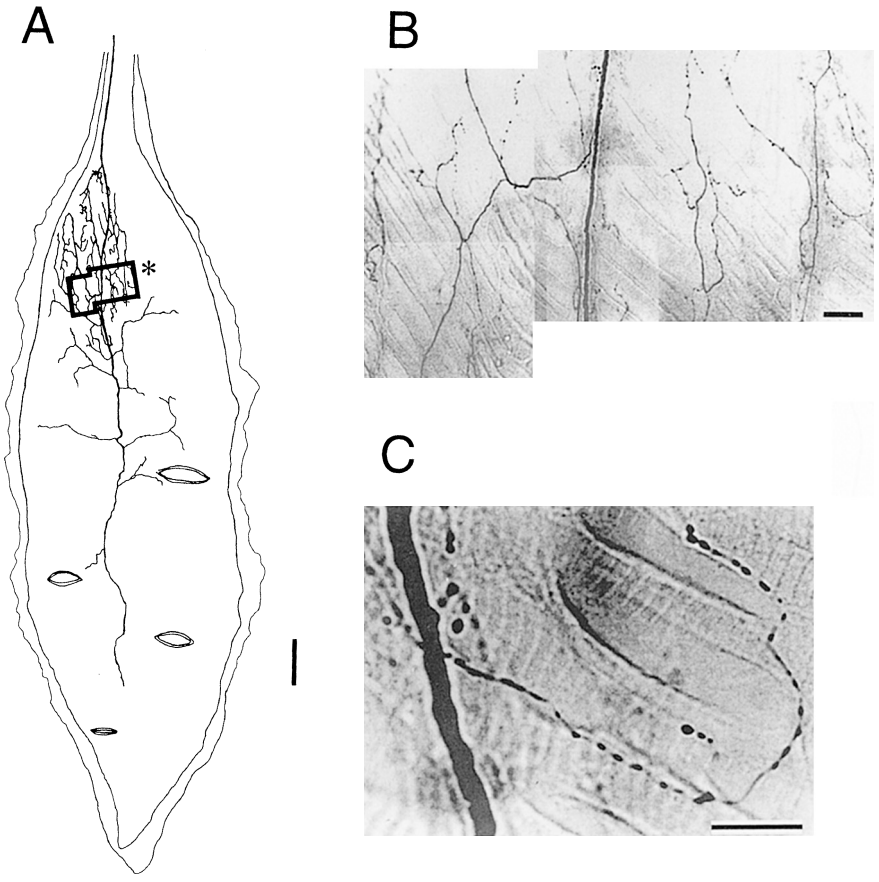

Fig. 3. Axonal branching of the CA2 neuron in the heart. (**A**) Line drawing of the CA2 axon inside the heart. The peripheral axon of CA2 neuron was labeled by intracellular injection of neurobiotin into the cell body. (**B**) Light micrograph of the CA2 axon in the designated area shown by the asterisk in **A**. (**C**) Varicosities of the CA2 axon. Scale bar: **A**, 500 μm; **B**, 40 μm; **C**, 20 μm.

the heart muscle caused by the cardioacceleratory nerve stimulation by injecting hyperpolarizing current pulses into the heart muscle. As shown in Fig. 6, either CA1 (A) or CA2 (B) stimulation caused a decrease of about 15% in the amplitude of the membrane potential change induced by the injected current.

Effects of cardioacceleratory nerve stimulation on electrical activity and contraction of the heart muscle

CG neurons exhibited spontaneous bursting activity that produces periodic bursts of excitatory junctional potentials (EJPs) on the heart muscle (Fig. 7A1). Each EJP burst is overlaid by a muscle action potential, which is composed of a few spikes and a plateau potential (Holley and Delaleu, 1972; Yamagishi and Hirose, 1997). The repetitive stimulation of the CA1 axon caused an increase in the frequency (about 11%) of the muscle action potential (Fig. 7A2), which was correlated with the increase in the ganglionic burst frequency (cf., Sakurai and Yamagishi, 1997). The stimulation also caused an increase in the amplitude of the plateau (about 15%) and a decrease in the maximum hyperpolarization (about 7 mV) (Fig. 7A2). The CA2 stimulation caused similar effects on the heart muscle; that is, the increase in the frequency (about 7%) and in the plateau amplitude of the muscle action potential (about 15%), and the decrease in the maximum hyperpolarization (about 6 mV) (Fig. 7A3).

We next examined the effects of the cardioacceleratory nerve stimulation on the heartbeat (Fig. 7B1). The CA1 stimulation caused an increase in the frequency (about 31%) and the amplitude (about 14%) of the heartbeat, and the heart tonus (about 8 mgw) (Fig. 7B2). The CA2 stimulation caused the similar effects, increasing the frequency (about 21%) and the amplitude (about 13%) of the heartbeat, and the heart tonus (about 7 mgw) (Fig. 7B3).

DISCUSSION

Peripheral projection of the cardioacceleratory neurons

In this study, we demonstrated peripheral projections of the cardioacceleratory neurons by intracellular injection of neurobiotin. Inside the heart, the CA1 and CA2 axons ran along the trunk of CG down to the posterior end, and many fine axon branches arose from them (Figs. 2 and 3). The double staining of both CG neurons and the cardioacceleratory neuron revealed that the fine axons of the cardioacceleratory neuron went along the CG axons and separated from them at vari-

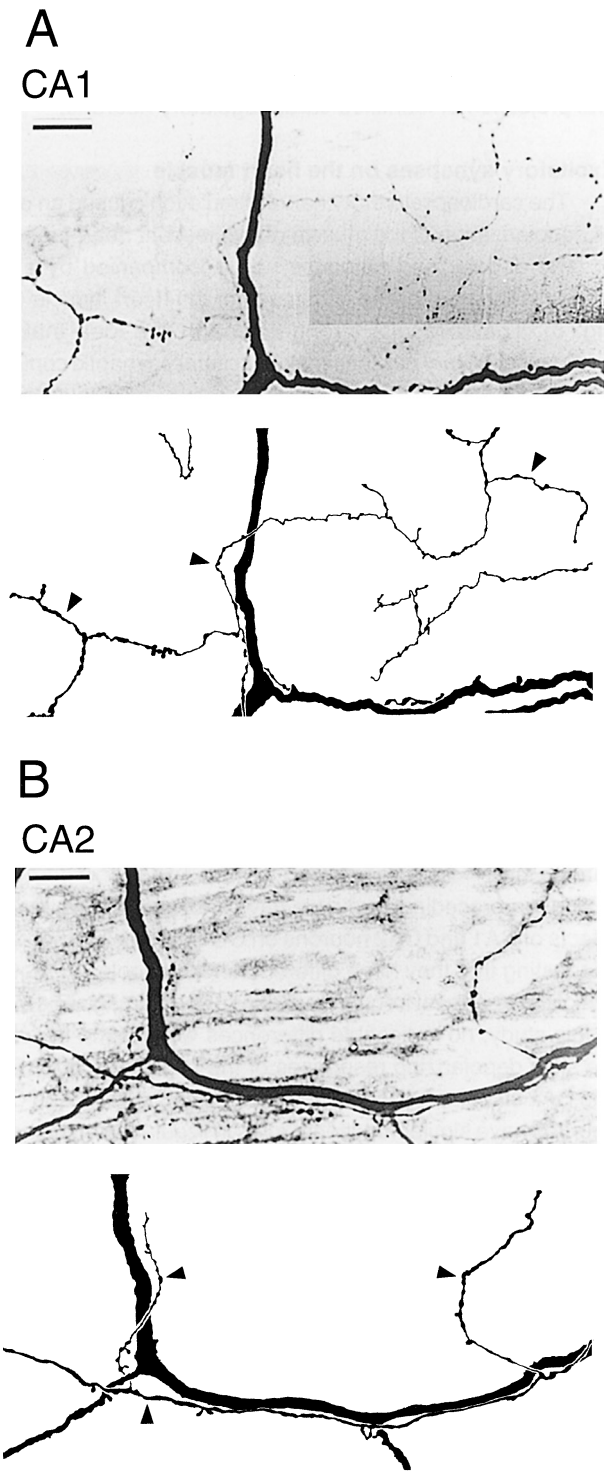

Fig. 4. Axonal branching of CG neurons and the cardioacceleratory neurons in the heart. Neurobiotin was injected into both CG neuron and the cardioacceleratory neuron (**A**, CA1; **B**, CA2). In each of **A** and **B**, a light micrograph (upper) and a camera lucida drawing (lower) of the stained axons are shown. The arrowheads in the lower drawings show the cardioacceleratory axons. Scale bar: 30 μm.

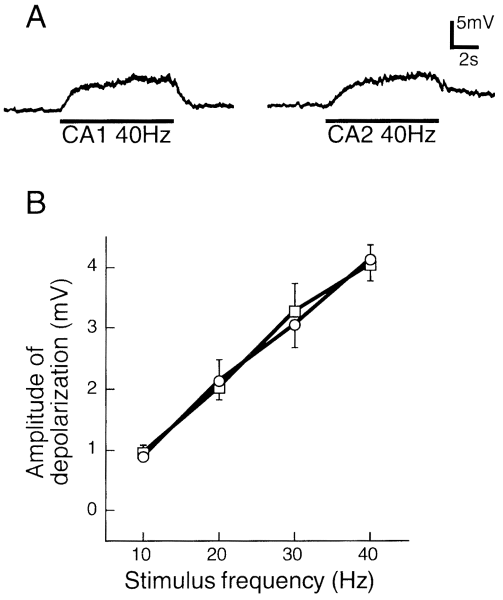

Fig. 5. Membrane potential responses of the heart muscle to cardioacceleratory nerve stimulation. Spontaneous activities of CG and the heart muscle were suppressed by perfusion of saline containing 100 mM calcium and 0.05 mM GABA. (**A**) Depolarizing responses of the heart muscle to the cardioacceleratory nerve stimulation. The CA1 (left trace) and CA2 (right trace) axons were individually stimulated at 40 Hz for the periods shown by the horizontal bars. The resting potential of the heart muscle was –57 mV. Records were obtained from the same preparation. (**B**) The relationships between the stimulus frequency (Hz) and the maximum amplitude of the depolarizing response (mV; mean ± SEM, n = 6–24) to the CA1 (open circle) and CA2 (open square) stimulation.

ous sites (see Fig. 4). The fine axons of the cardioacceleratory neuron bore a number of varicosities and arborized over the heart muscle. It is well known that the varicosities of the nerve terminals provide most of the synaptic input to the muscle fibers (Atwood and Cooper, 1995). Our observations strongly suggest that the cardioacceleratory neurons directly innervate the heart muscle in *Ligia exotica*.

Inside the heart, most of the fine axon branches of CA1 and CA2 neurons were stained locally in the anterior region (see Figs. 2 and 3). This localization was probably due to the scarce diffusion of the tracer molecules to the distal fine processes, since the distance from the CNS to the heart is very long (more than 10 mm). We showed previously that the patterns of central projection of CA1 and CA2 neurons were different (Sakurai and Yamagishi, 1997). However, we could not detect any difference between their projection patterns onto the heart muscle.

Some investigators have shown the pathways of the cardioregulatory nerves inside the hearts of crustaceans by the nerve staining method with methylene blue (Alexandrowicz, 1932, 1934, 1952, 1954; Maynard, 1961; Kihara and Kuwasawa, 1984; Yazawa and Kuwasawa, 1984a). However, the axonal pathways of individual cardioregulatory neurons cannot be determined by this method alone. Recently, Tanaka and Kuwasawa (1991a, b) identified the cardioregulatory neurons in the CNS of the isopod *Bathynomus doederleini* by intracellular injection of Lucifer yellow. They described the central projections of the cardioregulatory neurons, but failed to

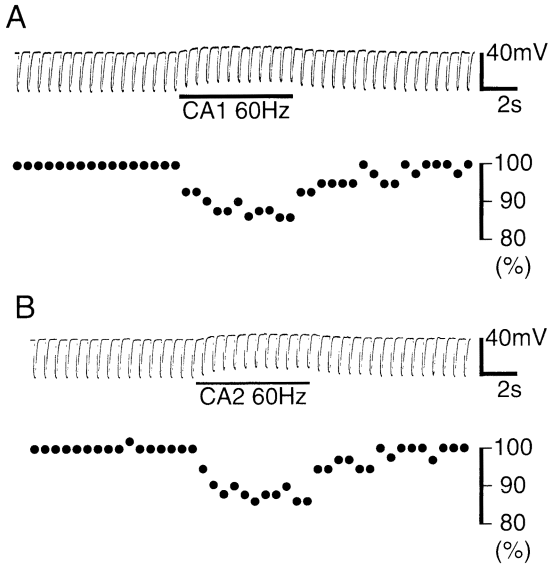

Fig. 6. Changes in the membrane resistance of the heart muscle induced by cardioacceleratory nerve stimulation. In each record, upper trace, intracellular recording from the heart muscle; lower trace, relative amplitude (%) of the membrane potential response of the heart muscle to the injected current. To measure changes in the membrane resistance of the heart muscle, repetitive square pulses of hyperpolarizing current were applied to the heart muscle via a second electrode. The CA1 (**A**) and CA2 (**B**) axons were individually stimulated at 60 Hz for the periods shown by the horizontal bars. Spontaneous activities of CG and the heart muscle were suppressed by perfusion of saline containing 100 mM calcium and 0.05 mM GABA.

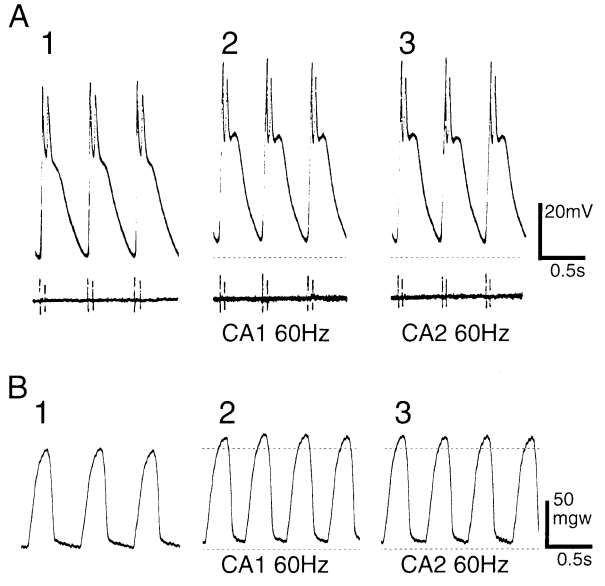

Fig. 7. Effects of the cardioacceleratory nerve stimulation on the activity and contraction of the heart muscle. (**A**) Intracellular activity of the heart muscle (upper trace) and impulse activity of CG (lower trace) recorded from the beating heart (1). The impulses were recorded extracellularly from an axon branch of CG. The CA1 (2) and CA2 (3) axons were individually stimulated at 60 Hz. Dotted lines show maximum hyperpolarization (–63 mV) of the control. (**B**) Mechanical recording of the heartbeat (1). The CA1 (2) and CA2 (3) axons were individually stimulated at 60 Hz. Dotted lines show the maximum systolic (upper) and diastolic (lower) levels of the control. The records in **A** and **B** were obtained from different preparations.

trace their peripheral projections onto the heart muscle. Visualization of the peripheral cardioacceleratory axons inside the heart is thus the first morphological evidence for the peripheral projection of identified cardioregulatory neurons.

Excitatory synapses on the heart muscle

The cardioacceleratory nerve stimulation caused an overall depolarization of the muscle of a quiescent heart (see Fig. 5). The depolarizing response was accompanied by a decrease in the membrane resistance of the heart muscle (see Fig. 6). These results strongly support the idea that the cardioacceleratory neurons make excitatory synaptic contacts on the heart muscle. Kihara and Kuwasawa (1984) suggested that, in the isopod *Bathynomus doederleini*, cardioacceleratory axons might induce a depolarization of the heart muscle. In hermit crabs, Yazawa and Kuwasawa (1984b) recorded discrete EJPs from the heart muscle in response to the cardioacceleratory nerve stimulation. In the present study, we failed to record the discrete EJPs corresponding one-to-one to the cardioacceleratory nerve stimuli. However, Yamagishi and Hirose (1992) did record discrete EJPs in response to the stimulation of the cardioregulatory nerve in juveniles of *Ligia exotica*. Since myocardial cells of adult animals are relatively larger than those of juveniles, it is more difficult to record the discrete EJPs evoked on the adult heart muscle. It is likely that the overall depolarization represents the electrical spread of the summated EJPs evoked at sites distant from the recording site.

In our preceding study, we have shown that the synaptic effects of CA1 and CA2 neurons on CG neurons are different, suggesting that they have different functional roles in regulation of the heartbeat frequency (Sakurai and Yamagishi, 1997). In this study, no noticeable differences were found between the slow depolarizing responses of the heart muscle to CA1 and CA2 stimulation (Fig. 5). This suggests that CA1 and CA2 neurons have similar functional roles in regulation of the heart muscle in adult *Ligia*.

Acceleratory effects on heart muscle activity

Repetitive stimulation of the cardioacceleratory axon caused an increase in heartbeat frequency (see Fig. 7B). In adult *Ligia*, CG acts as a pacemaker of the heartbeat (Yamagishi and Hirose, 1997). We showed previously that stimulation of the CA1 or CA2 axon causes an increase in heartbeat frequency via their excitatory synapses on CG neurons (Sakurai and Yamagishi, 1997). Therefore, the frequency of the muscle activity increased following the increase in the burst frequency of CG (see Fig. 7A).

The cardioacceleratory nerve stimulation also caused an increase in the plateau amplitude of the action potential and a decrease in the maximum hyperpolarization of the heart muscle (Fig. 7A). During the stimulation, the amplitude of the heartbeat and the heart tonus increased (Fig. 7B). The degree of mechanical tension of the skeletal and heart muscles of crustaceans is dependent on the absolute value of membrane potential (Orkand, 1962; Brown, 1964; Holley and

Delaleu, 1972). Therefore, the changes in the plateau amplitude of the action potential and in the maximum hyperpolarization of the heart muscle may result in the changes in the amplitude of the heartbeat and in the heart tonus.

In the hearts of decapods and stomatopods, the heart muscle is driven by the summated EJPs produced by bursts of the ganglionic impulses (reviewed by Prosser, 1973). In those animals, the amplitude and duration of the contraction of the heart muscle can be regulated by modulating the spike frequency and duration of ganglionic burst. In contrast, in the isopods, the EJPs evoked by ganglionic burst were overlaid by an active membrane response of the heart muscle (Holley and Delaleu, 1972; Yamagishi and Hirose, 1997). Little changes were seen in the number of intraburst spikes of CG in response to cardioacceleratory nerve stimulation (see Fig. 7A). Therefore, the amplitude of the heartbeat in *Ligia* must be regulated by the cardioacceleratory neurons via modulation of the action potential of the heart muscle. We conclude that the cardioacceleratory neurons of *Ligia exotica* regulate the heartbeat frequency (chronotropic effect) via the synaptic contacts on CG neurons, and the amplitude of the heartbeat (inotropic effect) and the heart tonus (tonotropic effect) via the neuromuscular junctions on the heart muscle.

ACKNOWLEDGMENTS

We thank Dr. K. Oami for valuable discussions and Dr. R. S. J. Weisburd for revising the manuscript. This work was supported in part by a Grant-in-Aid for Scientific Research (C) no. 08640857 from the Ministry of Education, Science, Sports and Culture of Japan to H. Y. Contribution no. 616 from Shimoda Marine Research Center, University of Tsukuba.

REFERENCES

Alexandrowicz JS (1932) The innervation of the heart of the crustacea. I. Decapoda. Q J Microsc Sci 75: 181–249

Alexandrowicz JS (1934) The innervation of the heart of the crustacea. II. Stomatopoda. Q J Microsc Sci 76: 511–548

Alexandrowicz JS (1952) Innervation of the heart of *Ligia oceanica*. J Mar Biol Assoc UK 31: 85–97

Alexandrowicz JS (1954) Innervation of an amphipod heart. J Mar Biol Assoc UK 33: 709–719

Atwood HL, Cooper RL (1995) Functional and structural parallels in crustacean and *Drosophila* neuromuscular systems. Amer Zool 35: 556–565

Brown HF (1964) Electrophysiological investigations of the heart of *Squilla mantis*. II. The heart muscle. J Exp Biol 41: 701–722

Delaleu JC, Holley A (1976) Neural regulation of the heart muscle in an isopod crustacean: acceleration and peripheral inhibition. J Exp Biol 64: 345–356

Hagiwara S (1961) Nervous activities of the heart in crustacea. Ergebn Biol 24: 288–311

Hidaka S, Hashimoto Y(1993) Intracellular labeling with injection of biotinylated compounds and its application for analysis of neuron network. J Physiol Soc Japan 55(6): 241–253

Holley A, Delaleu JC (1972) Electrophysiology of the heart of an isopod crustacean: *Porcellio dilatatus*. J Exp Biol 57: 589–608

Kihara A, Kuwasawa K (1984) A neuroanatomical and electrophysiological analysis of nervous regulation in the isopod crustacean, *Bathynomus doederleini*. J Comp Physiol 155A: 883–894

Matsui K, Ai N, Kuwasawa K (1973) Spontaneous inhibitory postsynaptic potentials in the cardiac ganglion preparation of the lobster *Panulirus japonicus*. Comp Biochem Physiol 44(A): 953–965

Maynard DM (1960) Circulation and heart function. In "The Physiology of Crustacea, Vol 1" Ed by Waterman TH, Academic Press, New York, pp 161–226

Maynard DM (1961) Cardiac inhibition in decapod Crustacea. In: Florey E (ed) Nervous inhibition. Pergamon, Oxford, pp 144–178

Orkand RK (1962) The relation between membrane potential and contraction in single crayfish muscle fibers. J Physiol 161: 143–159

Otani T, Bullock TH (1959) Effects of presetting the membrane potential of the soma of spontaneous and integrating ganglion cells. Physiol Zool 32: 104–114

Prosser CL (1973) Circulation of body fluids. In "Comparative Animal Physiology" Ed by DL Prosser, WB Saunders Co, Washington DC, pp 822–856

Sakurai A, Yamagishi H (1996) Central and peripheral projections of two cardio-acceleratory neurons in the isopod crustacean, *Ligia exotica*. J Soc Comp Physiol Biochem 13(4): 309

Sakurai A, Yamagishi H (1997) Identification of two cardioacceleratory neurons in the isopod crustacean, *Ligia exotica* and their effects on cardiac ganglion cells. J Comp Physiol A, in press

Shimahara T (1969a) The inhibitory post synaptic potential in the cardiac ganglion of the lobster, *Panulirus japonicus*. Sci Rep Tokyo Kyoiku Daigaku 205: 9–26

Shimahara T (1969b) The effect of the acceleratory nerve on the electrical activity of the lobster cardiac ganglion. Zool Mag 78: 351–355

Tanaka K, Kuwasawa K (1991a) Identification of cardio-acceleratory neurons in the thoracic ganglion of the isopod, *Bathynomus doederleini*. Brain Research 544: 311–324

Tanaka K, Kuwasawa K (1991b) Identification of cardio-inhibitory neurons in the thoracic ganglion of the isopod, *Bathynomus doederleini*. Brain Research 558: 339–342

Terzuolo CA, Bullock TH (1958) Acceleration and inhibition in crustacean ganglion cells. Arch Ita Biol 96: 117–134

Watanabe A, Obara S, Akiyama T (1968) Inhibitory synapses on pacemaker neurons in the heart ganglion of a stomatopod, *Squilla oratoria*. J Gen Physiol 52: 908–924

Watanabe A, Obara S, Akiyama T (1969) Acceleratory synapses on pacemaker neurons in the heart ganglion of a stomatopod, *Squilla oratoria*. J Gen Physiol 54: 212–231

Yamagishi H, Ebara A (1985) Spontaneous activity and pacemaker property of neurons in the cardiac ganglion of an isopod crustacean, *Ligia exotica*. Comp Biochem Physiol 81A: 55–62

Yamagishi H, Hirose E (1992) Nervous regulation of the myogenic heart in early juveniles of the isopod crustacean, *Ligia exotica*. In "Phylogenic Models in Functional Coupling of the CNS and the Cardiovascular System. Comp Physiol Vol 11" Ed by Hill RB, Kuwasawa K, Karger, Basel, pp 141–148

Yamagishi H, Hirose E (1997) Transfer of the heart pacemaker during juvenile development in the isopod crustacean *Ligia exotica*. J Exp Biol 200: 2393–2404

Yamagishi H, Uesaka H, Ebara A (1989) Inter- and motor-neuronal functions of the cardio-inhibitory nerve in the isopod crustacean, *Ligia exotica*. Comp Biochem Physiol 94A (3): 471–476

Yazawa T, Kuwasawa K (1984a) The cardio-regulatory nerves of the hermit crabs: anatomical and electrophysiological identification of their distribution inside the heart. J Comp Physiol 154A: 871–881

Yazawa T, Kuwasawa K (1984b) The cardio-regulator nerves of hermit crabs: Multimodal activation of the heart by the accelerator axons. J Comp Physiol 155A: 313–318

(Received September 24, 1997 / Accepted October 24, 1997)