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Cardioinhibitory Neurons in the Isopod Crustacean *Ligia exotica*

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ABSTRACT—We identified electrophysiologically cardioinhibitory neurons in the central nervous system of the isopod crustacean *Ligia exotica*. A pair of the cardioinhibitory neurons are located at the ventral side in the anterior region of the 1st thoracic ganglion. Intracellular injection of neurobiotin into the cardioinhibitory neurons revealed that the each neuron sends the peripheral axon to the heart *via* a contralateral nerve root of its own ganglion. In the central nervous system, each of the neurons has arborization in the 1st thoracic and subesophageal ganglia and sends the longitudinal processes through the ipsilateral connective toward the circumesophageal connective and down to the 7th thoracic ganglion.

INTRODUCTION

The heart of many crustaceans has a pair of cardioregulatory nerves from the central nervous system (CNS) (reviewed by Krijgsman, 1952). Many investigations on nervous regulation of the heart revealed that stimulation of the cardioregulatory nerve affects the frequency, force and tonus of the heartbeat, and that each of the paired cardioregulatory nerves generally contains one cardioinhibitory and two cardioacceleratory axons (reviewed by Maynard, 1960; Hagiwara, 1961; Yazawa and Kuwasawa, 1992; McMahon, 1995; Tanaka *et al.*, 1996). However, the cardioregulatory neurons in the CNS have long been unidentified. Using electrophysiological and intracellular dye injection methods, Tanaka and Kuwasawa (1991a, b) have first identified one pair of cardioinhibitory and two pairs of cardioacceleratory neurons in the CNS of the giant marine isopod *Bathynomus doederleini*. Then, Ando and Kuwasawa (1995) reported location of cardioinhibitory and cardioacceleratory neurons in the CNS of the stomatopod *Squilla oratoria* by axonal back-filling.

We showed previously in the isopod *Ligia exotica* that each of the cardioregulatory nerves contains one cardioinhibitory and two cardioacceleratory axons, and that the cardioinhibitory axon innervates both the cardiac ganglion and myocardium (Yamagishi *et al.*, 1989; Yamagishi and Hirose, 1992). Then, we identified two pairs of cardioacceleratory neurons in the CNS and showed their innervation on both the cardiac ganglion neurons and the myocardium (Sakurai and Yamagishi, 1998a, b). However, the cardioinhibitory neurons have remained unidentified. The aim of the present study is to

identify the cardioinhibitory neurons in the CNS of *L. exotica*.

MATERIALS AND METHODS

Adult males and females of the littoral isopod *L. exotica* Roux, 15 to 20 mm in body length, were used. They were collected at Pacific seashores (at Izu and Boso, Japan) and kept in the laboratory at room temperature. Over 50 specimens were used for the experiments.

The anatomy of the heart and CNS were detailed in a previous paper (Sakurai and Yamagishi, 1998a). The heart and CNS were isolated together keeping the nerves connecting them intact and were pinned in the experimental chamber. The chamber was perfused continuously with aerated physiological saline of the following composition (in mM): 577 NaCl, 14 KCl, 25 CaCl₂, 21 MgCl₂, 4.5 Na₂SO₄ and 5 Tris-HCl (pH 7.4) (Yamagishi and Ebara, 1985).

Intracellular activity of neurons in the CNS was recorded using a conventional glass capillary microelectrode (resistance, 20–40 M Ω) filled with 3M KCl. Electric current was injected into the neuron through the recording electrode using a bridge circuit. Extracellular recording of nerve impulses and stimulation of nerves were made using a glass capillary suction electrode. Mechanograms of the heartbeat were recorded by connecting a dorsal suspensory ligament of the heart to a mechano-electric transducer (Nihon Koden AT601G) with a nylon fiber. Signals were stored in an FM data-recorder and displayed on a chart recorder or a cathode ray tube and photographed.

The morphology of the cardioinhibitory neurons was examined with neurobiotin [N-(2-aminoethyl) biotinamide hydrochloride, Vector Labs] by means of axonal backfilling from the heart or intracellular injection into the cell bodies of the neurons. These methods are detailed in previous papers (Sakurai and Yamagishi, 1998a, b). The experiments were performed at room temperature (22–26°C).

RESULTS

To determine the pathways of the cardioinhibitory axons from the CNS to the heart, we examined the effects of electrical stimulation of nerve roots derived from the CNS on the heartbeat. Before application of electrical stimuli (duration 0.3 msec, frequency 40–60 Hz), all nerve roots were transected near the ganglia. When one of the paired nerve roots that

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derive laterally from the 1st thoracic ganglion (TG1) (Fig. 1A) was stimulated, the heartbeat was stopped (Fig. 1B). Single unit impulses that correspond one-to-one to stimuli with a constant latency were recorded from an anterior nerve branch of the cardiac ganglion system inside the heart (Fig. 1C). Antidromic impulses identical to the orthodromic ones were also recorded by exchanging the stimulating and recording sites (not shown). Stimulation of the other nerve roots derived from the CNS produced no inhibitory effects on the heartbeat. These results indicate that each of the paired nerve roots derived laterally from TG1 contains a cardioinhibitory axon. Vital staining with methylene blue revealed that a fine branch of the nerve root joined the ipsilateral anterior cardiac nerve which runs alongside the anterior aorta to the heart (cf., Fig. 2A). By axonal backfilling with neurobiotin from the cut end of the nerve trunk of the cardiac ganglion, a pair of cell bodies were stained in the anterior region of TG1, as well as two pairs of cardioacceleratory neurons (Sakurai and Yamagishi, 1998a, b) (not shown).

We next examined the electrophysiology of the neurons in the anterior region of TG1. Intracellular activity of the neuron and extracellular activity of a posterior nerve branch of the cardiac ganglion system were recorded simultaneously (Fig. 2A). The action potentials recorded from the cell body of a neuron located in the anterior region of TG1 corresponded one-to-one to the impulses recorded from the posterior nerve branch of the cardiac ganglion with a constant latency (Fig. 2Bi, C). Antidromic action potentials were recorded from the neuron with the same latency as the orthodromic one in re-

sponse to the stimulation of the nerve trunk (Fig. 2Bii). Repetitive action potential firing in the neuron induced by depolarizing current injection resulted in suppression of periodic burst discharges of the cardiac ganglion (Fig. 2C). Similar results were obtained from a neuron located in the other side of the anterior region of TG1 (data not shown). Thus, a pair of neurons located in the anterior region of TG1 were electrophysiologically identified as cardioinhibitory neurons. The resting potential of the cardioinhibitory neurons ($n = 9$) was in the range of -60 to -50 mV. In these experiments, no spontaneous firing activity were observed except injury firing after penetration of the electrode.

Intracellular injection of neurobiotin into the cardioinhibitory neuron revealed the cell body ($30\text{--}35\ \mu\text{m}$ in diameter), and its central and peripheral projections (Fig. 3A). The cardioinhibitory neuron had three major processes, central, ipsilateral, and contralateral processes with respect to the cell body. The central process projected anteriorly with arborization in TG1 and in the subesophageal ganglion (Fig. 3A). The ipsilateral process bifurcated into anterior and posterior processes; the anterior process reached the circumesophageal connective and the posterior process TG7 (Fig. 3B, C). Definite arborizations were not found along these processes. The contralateral process was the peripheral axon which went out of the ventral nerve cord through the contralateral nerve root of TG1 (Fig. 3A, C).

DISCUSSION

We showed previously in *L. exotica* that the cardioinhibitory axons in the ACNs join the cardiac ganglion system inside the heart and make inhibitory synaptic contacts on both the cardiac ganglion cells and myocardial cells (Yamagishi *et al.*, 1989). Stimulation of the nerve roots derived from the CNS revealed that the each cardioinhibitory axon comes ipsilaterally from a lateral nerve root of TG1 (Fig. 1). The action potentials recorded from a pair of neurons located in the anterior region of TG1 conducted into the cardiac ganglion system of the heart (Fig. 2B) and suppressed periodic burst discharges of the cardiac ganglion (Fig. 2C). Moreover, each of the neurons sends its peripheral axon contralaterally into a lateral nerve root of TG1 (Fig. 3). These results lead us to a conclusion that the pair of neurons recorded is cardioinhibitory.

Two pairs of cardioacceleratory neurons of *L. exotica* are located in TG2 and TG3, respectively, and each of these neurons sends the peripheral axon into the ipsilateral 3rd nerve root of its own ganglion (Sakurai and Yamagishi, 1998a). On the other hand, each of the paired cardioinhibitory neurons located in TG1 sends its peripheral axon contralaterally into the lateral nerve root of TG1 (Fig. 3). These locations of the cardioinhibitory neurons and the pathways of their peripheral axons were very similar to those reported in the isopod *B. doederleini* (Tanaka and Kuwasawa, 1991a,b). However, there are some differences in arrangement of the nerve roots in TG1 between *Bathynomus* and *Ligia*; for example, the cardioinhibitory neuron of *Bathynomus* sends the peripheral axon

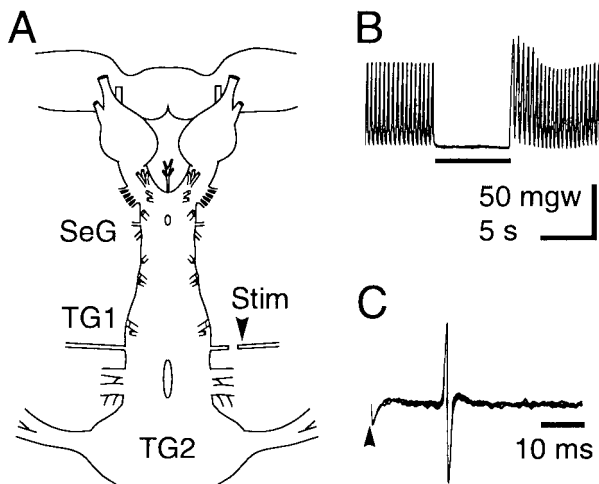


Fig. 1. Nerve roots that contain the cardioinhibitory axons. (A) Schematic drawing of the anterior part of the central nervous system of *Ligia exotica* (ventral view). SeG, subesophageal ganglion; TG1, the 1st thoracic ganglion. (B) The effects on the heartbeat of stimulation (60 Hz) of the cardioinhibitory axon. The bars under the records show the period of stimulation. (C) Impulses of the cardioinhibitory axon recorded extracellularly from an anterior nerve branch of the cardiac ganglion inside the heart. Electrical stimuli were applied to the distal cut ending of the nerve root shown by an arrowhead (Stim) in A. Each sweep was triggered by the stimulus pulse. Five sweeps were superimposed.

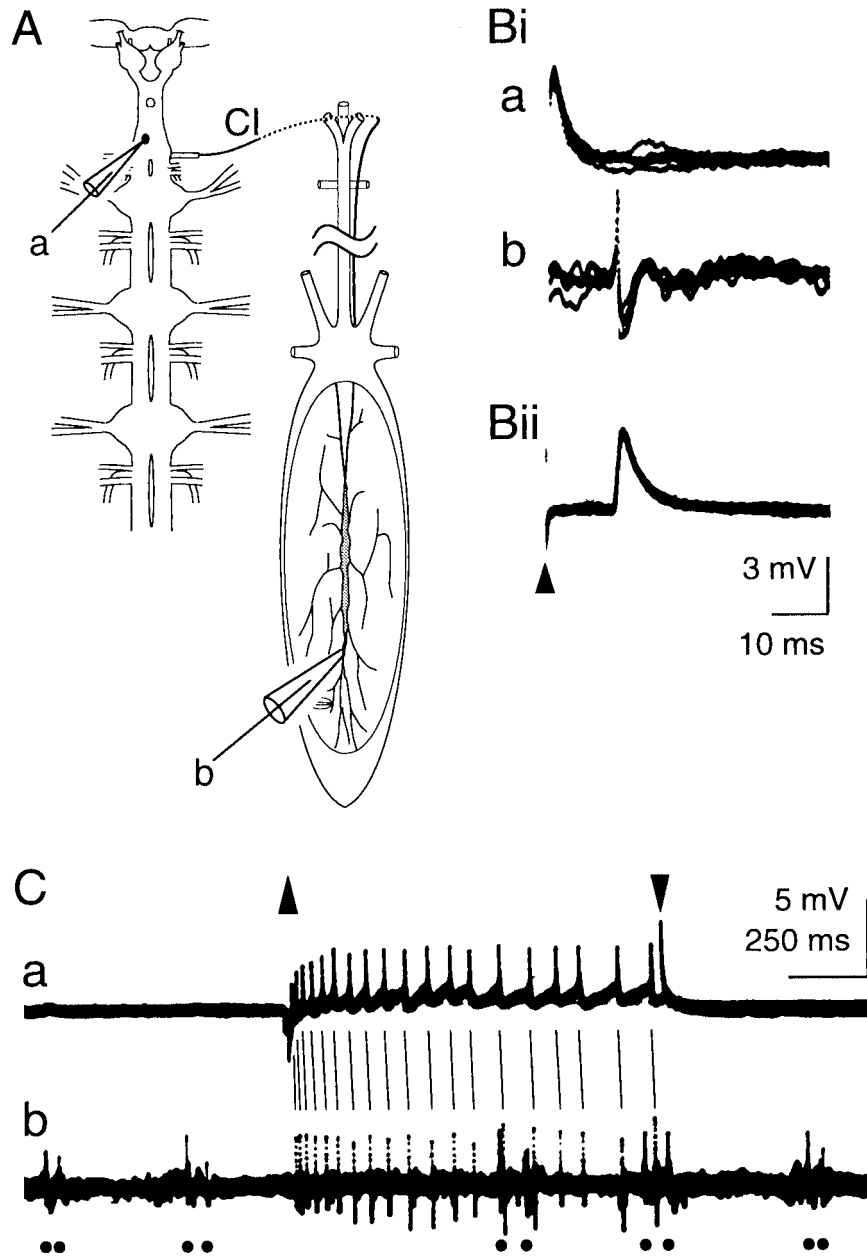


Fig. 2. Identification of the cardioinhibitory neuron in the CNS. **(A)** Schematic drawing of the anterior parts of the central nervous system (ventral view). The location of the cell body of the recorded neuron and the intracellular and extracellular recording electrodes (**a** and **b**) are shown. **(Bi)** Action potentials of the neuron recorded intracellularly from the cell body (**a**), and impulses recorded extracellularly from the cardiac ganglionic trunk inside the heart (**b**). Each sweep was triggered by an injury action potential recorded shortly after penetration of the microelectrode into the cell body. Five sweeps were superimposed. **(Bii)** Antidromic action potentials recorded from the cell body of the neuron in response to the stimuli applied to the nerve trunk of the cardiac ganglion. Each sweep was triggered by a stimulus pulse (arrowhead). Five sweeps were superimposed. The records in **Bi** and **Bii** were obtained from the same preparation. **(C)** Effects of repetitive firing of the neuron on bursting activity of the cardiac ganglion. Intracellular activity of the neuron (**a**) and impulse activity of the cardiac ganglionic trunk (**b**) were recorded simultaneously. Repetitive action potential firing of the neuron was induced by injecting a depolarizing current pulse during the period indicated by the upward and downward arrowheads. Correspondence between the evoked action potentials of the neuron and the impulses recorded from the ganglionic trunk are shown by the lines. The impulse bursts of the cardiac ganglion are indicated by dots.

into the contralateral 4th nerve root of TG1 (Tanaka and Kuwasawa, 1991b).

Some differences were also observed in central projections of the cardioinhibitory neurons between *Bathynomus* and

Ligia, as observed in the case of cardioacceleratory neurons (Sakurai and Yamagishi, 1998a). In *Bathynomus*, the cardioinhibitory neurons have only some fine processes in TG1 (Tanaka and Kuwasawa, 1991b), whereas in *Ligia* their pro-

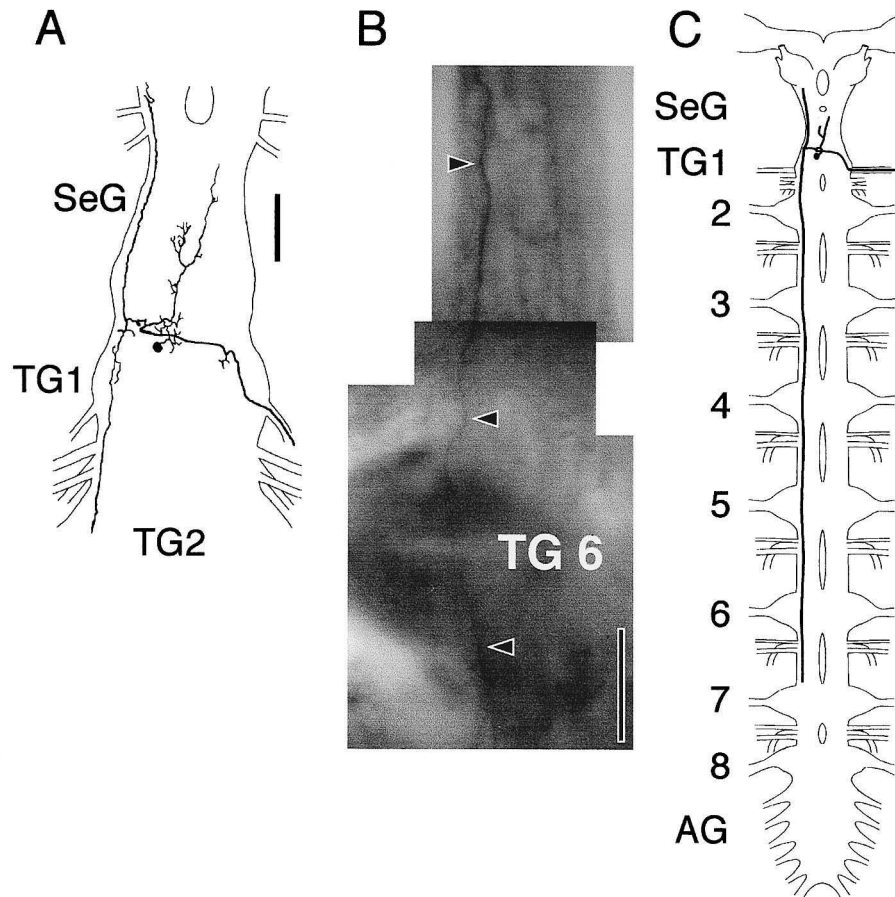


Fig. 3. Morphology of the cardioinhibitory neurons. **(A)** Camera lucida drawing of a cardioinhibitory neuron in the subesophageal ganglion (SeG) and the 1st and the 2nd thoracic ganglia (TG1, 2). Scale bar, 200 μ m. **(B)** A light micrograph of the posterior process of the cardioinhibitory neuron passing through TG6. The arrowheads show the axon. Scale bar, 200 μ m. **(C)** Schematic drawing of the cardioinhibitory neuron in the central nervous system. AG, abdominal ganglion; SeG, subesophageal ganglion; TG1–8, thoracic ganglia.

jection territories appeared to be wider (Fig. 3). These differences may have resulted from the differences in the size of the neurons and/or in the methods of tracer injection applied (eg. neurotracers and incubation time). Physiological roles of the widely projected processes of the cardioinhibitory neurons in *Ligia* are uncertain in the present study. Investigations on the functional roles of cardio-regulatory neurons of *Ligia* are needed as did in *Bathynomus* (see review, Tanaka *et al.*, 1996).

By the results of present and previous studies (Yamagishi *et al.*, 1989; Yamagishi and Hirose, 1992; Sakurai and Yamagishi, 1998a, b), all cardio-regulatory neurons of *L. exotica*, a pair of cardioinhibitory and two pairs of cardio-acceleratory neurons, have been identified in the CNS and their innervation inside the heart were determined. This will enable investigation of the cellular mechanisms of nervous cardiac regulation in various cardiac reflexes or under various behavioral conditions.

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REFERENCES

- Ando H, Kuwasawa K (1995) An analysis of cardio-regulatory nerves in the stomatopod crustacean *Squilla oratoria*. *Physiol Zool* 68 (Supple): 75
- Hagiwara S (1961) Nervous activities of the heart in crustacea. *Ergebn Biol* 24: 288–311
- Krijgsman BJ (1952) Contractile and pacemaker mechanisms of the heart of arthropods. *Biol Rev* 27: 320–346
- McMahon BR (1995) Integrated neural and neurohormonal control of respiratory and circulatory function in crustaceans: is there evidence for an “autonomic” control system? *Verh Dtsch Zool Ges* 88: 87–101
- Maynard DM (1960) Circulation and heart function. In: *The physiol-*

- ogy of crustacea Vol 1 Ed by Waterman TH, Academic Press, New York, pp 161–226
- Sakurai A, Yamagishi H (1998a) Identification of cardioacceleratory neurons in the isopod crustacean *Ligia exotica* and their effects on cardiac ganglion cells. *J Comp Physiol A* 182:145–152
- Sakurai A, Yamagishi H (1998b) Cardioacceleratory neurons of the isopod crustacean, *Ligia exotica*: Visualization of peripheral projection onto the heart muscle. *Zool Sci* 15: 19–25
- Tanaka K, Kuwasawa K (1991a) Identification of cardio-acceleratory neurons in the thoracic ganglion of the isopod, *Bathynomus doederleini*. *Brain Res* 544: 311–324
- Tanaka K, Kuwasawa K (1991b) Identification of cardio-inhibitory neurons in the thoracic ganglion of the isopod, *Bathynomus doederleini*. *Brain Res* 558: 339–342
- Tanaka K, Kuwasawa K, Okada J, F-Tsukamoto Y, Kihara A, Yazawa T, Kurokawa M (1996) Neural control of cardiac output in the isopod crustacean, *Bathynomus doederleini*. In "Basic Neuroscience in Invertebrates" Ed Koike K, Kidokoro Y, Takahashi K, Kanaseki T, Japan Scientific Societies Press, Tokyo, pp 341–354
- 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" Ed Hill RB, Kuwasawa K, *Comp Physiol* Vol 11, Karger, Basel, pp 141–148
- 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: 471–476
- Yazawa T, Kuwasawa K (1992) Intrinsic and extrinsic neural and neurohumoral control of the decapod heart. *Experientia* 48: 834–840

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