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Source: Zoological Science, 15(5) : 673-676

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.15.673

ZOOLOGICAL SCIENCE **15**: 673–676 (1998)

Isolation of Neurogenic and Myogenic Activities by Joro Spider Toxin in the Adult Heart of the Isopod Crustacean *Ligia exotica*

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ABSTRACT—The effects of Joro spider toxin (JSTX), a specific glutamate antagonist, on the adult heart of the isopod crustacean *Ligia exotica* were examined. By application of JSTX, excitatory junctional potentials (EJPs) caused by the cardiac ganglion activity in the myocardium were gradually abolished. Subsequently, the cardiac ganglion and myocardium exhibited independent activities with their respective rhythms. In saline containing JSTX, no changes were observed in the muscle activity when the ganglionic activity was changed by current injection into the cardiac ganglion neuron. These results indicate that two pacemaker sites, the cardiac ganglion and cardiac muscle, are present in the adult heart of *Ligia exotica* and suggest glutamatergic neuromuscular transmission between them.

INTRODUCTION

The heartbeat of many crustaceans is neurogenic and the cardiac ganglion acts as the dominant pacemaker (reviewed by Krijgsman, 1952; Maynard, 1960; Prosser, 1973). The myocardium has no endogenous automaticity and is driven by periodic burst activity of the cardiac ganglion *via* EJPs. Glutamate that induces a depolarizing membrane potential response in the cardiac muscle has been suggested to be the transmitter between the cardiac ganglion and myocardium in several crustaceans (Hallett, 1971; Holley and Delaleu, 1972; Benson, 1981; Yazawa *et al*., 1990). In addition, in the isopod *Bathynomus doederleini*, glutamate has been confirmed as the transmitter of the cardiac ganglion by pharmacological experiments and an analysis using a high performance liquid chromatography (Yazawa *et al*., 1997, 1998; Tanaka *et al*., 1996).

Recently, Yamagishi and Hirose (1997) showed a type of late-developing neurogenic heartbeat different from that of any other known crustaceans. They showed that, during the juvenile development of the isopod *Ligia exotica*, the heart pacemaker is transferred from the myocardium to the cardiac ganglion. They also suggested that, in the adult heart, the cardiac ganglion acts as a primary pacemaker to entrain the myogenic activity *via* EJPs.

In order to obtain direct evidence for the presence of the two pacemaker sites in the adult heart of *Ligia exotica*, we attempted to isolate the two pacemaker activities by blocking the neuromuscular transmission between them. We therefore examined the effects of JSTX known as a specific glutamate antagonist on the heart. The results show that the two pacemaker sites, the cardiac ganglion and myocardium, are present in the adult heart, and suggest that the neuromuscular transmission between them is glutamatergic.

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MATERIALS AND METHODS

Adult males and females of the littoral isopod *Ligia exotica* (Roux), 25 to 40 mm in body length, were used. Collection and maintenance of the animals were described in a previous paper (Yamagishi and Hirose, 1997).

Semi-isolated hearts were used for the experiments. The heart was isolated while keeping it attached to the dorsal carapace and fixing it with ventral side up in the experimental chamber by pinning the dorsal carapace. In some experiments, isolated and opened hearts were also used for recording intracellular activity of the cardiac ganglion neurons. The heart was isolated, opened by longitudinal incision of the ventral heart wall and fixed in the chamber with its inner surface up. The anatomy of the heart was detailed in a previous paper (Yamagishi and Ebara, 1985). The heart tube consists of a single layer of striated muscle. The cardiac ganglion which consists of six neurons lies longitudinally on the inner surface of the dorsal heart wall. The preparation was perfused with aerated physiological saline solution of the following composition (mM); NaCl 577, KCl 14, $CaCl_2$ 25, $MgCl_2$ 21, Na_2SO_4 4.5 and Tris 5 (pH 7.4 with HCl) (Yamagishi and Ebara, 1985). JSTX (Wako Pure Chemicals) was added in saline at a concentration of 10 μM prior to the experimentation. Intracellular activities of the cardiac muscle and the cardiac ganglion neuron were recorded with conventional glass capillary microelectrodes filled with 3 M KCl (electric resistance, 10-30 MΩ). Electric current was injected into the cardiac ganglion neuron through the recording electrode using a bridge circuit. Impulse activity of the cardiac ganglion was recorded from a ganglionic nerve branch with a suction electrode. Signals were stored in a FM data-recorder and displayed on a chart recorder or a cathode ray tube and photographed. All experiments were performed at a temperature of 20-24°C.

RESULTS

We first examined the effects of JSTX on the activities of the cardiac ganglion and cardiac muscle in semi-isolated beating hearts. Figure 1 shows typical results. Intracellular activity of the cardiac muscle (M, upper trace) and impulse activity of the cardiac ganglion (CG, lower trace) were recorded simultaneously. Each muscle activity which consists of a slow depolarizing potential and superimposed spike potentials followed each burst of impulses of the cardiac ganglion in a one to one relation (Fig. 1A). By application of JSTX (10 μM), the muscle activity failed gradually to follow the EJPs evoked by the ganglionic activity (Fig. 1B). Then the EJPs were abolished completely and the cardiac muscle exhibited periodic activity with a rhythm different from that of the ganglionic activity (Fig. 1C).

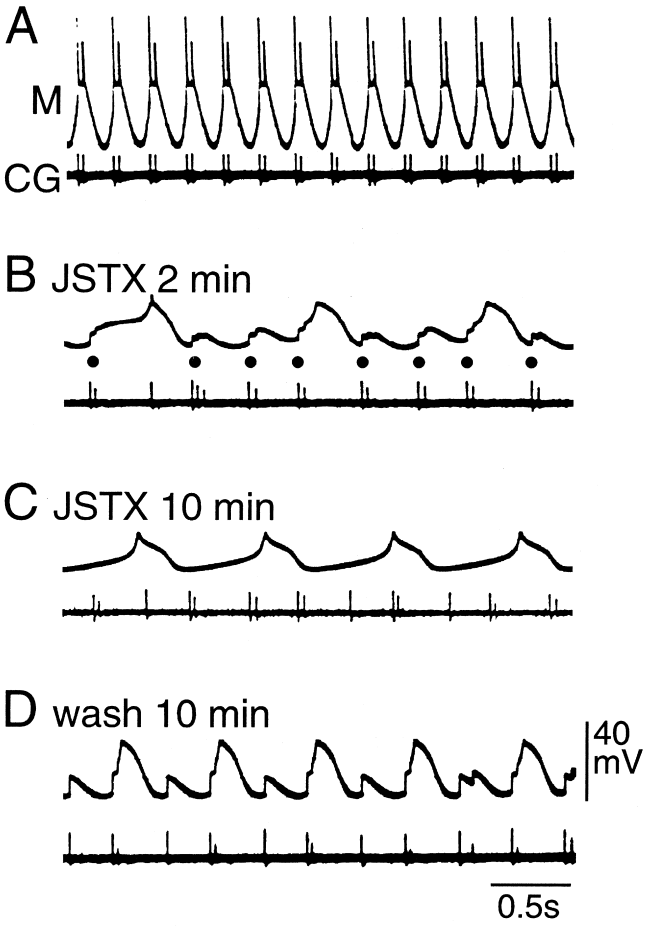

Fig. 1. Effects of JSTX on the heart of adult *Ligia exotica*. In each record, intracellular activity of the cardiac muscle (M, upper trace) and extracellular impulse activity of the cardiac ganglion (CG, lower trace) were recorded simultaneously in a semi-isolated beating heart. (**A**) Before application of JSTX. EJPs evoked by the ganglionic activity are overlapped by action potentials of the cardiac muscle. (**B**) 2 min after the onset of application of JSTX (10 μM). Closed circles indicate EJPs evoked by the ganglionic activity. (**C**) 10 min after the onset of application of JSTX (10 μM). EJPs vanish completely. (**D**) 10 min after washout of JSTX. EJPs recover partially.

After washout of JSTX, the EJPs evoked by the ganglionic activity were recovered partially and the muscle activity became to follow the ganglionic activity (Fig. 1D). Thus, the neurogenic and myogenic activities were isolated in the heart by application of JSTX.

The effects of JSTX were further examined in the opened hearts. As shown in Fig. 2, intracellular activity of the myocardium (upper trace) and that of the cardiac ganglion neuron (lower trace) were recorded simultaneously. The muscle activity followed the ganglion neuron activity in a one to one relation (Fig. 2A). When the frequency of the ganglion neuron activity was increased or decreased by depolarizing or hyperpolarizing current injection, the frequency of the muscle activity was increased or decreased keeping a one to one relation with the ganglion neuron activity (Fig. 2A). By application of JSTX (10 μM), the myocardium and the cardiac ganglion became to exhibit periodic activity with different rhythms (Fig. 2B). When the frequency of the ganglion neuron activity was changed by depolarizing or hyperpolarizing current injection, no changes were observed in the frequency of the muscle activity (Fig. 2B).

DISCUSSION

The EJPs caused by impulse activity of the cardiac ganglion in the myocardium were abolished by application of JSTX (Fig. 1C). In the saline containing JSTX, no changes were induced in the cardiac muscle activity by changing the frequency of the cardiac ganglion activity (Fig. 2B). JSTX is known as a specific blocker of the glutamatergic synaptic transmission (Kawai *et al.*, 1982). Therefore, the above results suggest that the neuromuscular transmission between the cardiac ganglion and myocardium in *Ligia exotica* is glutamatergic. Glutamate induces a depolarizing membrane potential response in the cardiac muscle and is suggested to be the transmitter between the cardiac ganglion and myocardium in decapods (Hallett, 1971; Benson, 1981) and isopods (Holley and Delaleu, 1972; Yazawa *et al.*, 1990). In addition, in the isopod *Bathynomus doederleini*, several glutamate antagonists block both the ganglionic EJP and the glutamate response in the cardiac muscle (Yazawa *et al.*, 1997, 1998).

By application of JSTX, the EJPs evoked in the myocardium by the cardiac ganglion activity were abolished and the cardiac ganglion and myocardium exhibit independent activities with their respective rhythms (Fig. 1D). In the neurogenic heart of many crustaceans, the myocardium has no inherent automaticity and the cardiac ganglion acts as a dominant pacemaker (reviewed by Krijgsman, 1952; Maynard, 1960; Prosser, 1973). However, it has been suggested in the heart of adult *Ligia exotica* that two pacemaker sites, the cardiac ganglion and myocardium, are present in the heart and the cardiac ganglion acts as a primary pacemaker to entrain the myogenic activity *via* EJPs (Yamagishi and Hirose, 1997). Isolation of the neurogenic and myogenic activities resulting from the suppression of EJPs provides direct evidence for the presence of two pacemaker sites in the heart. JSTX blocks irre-

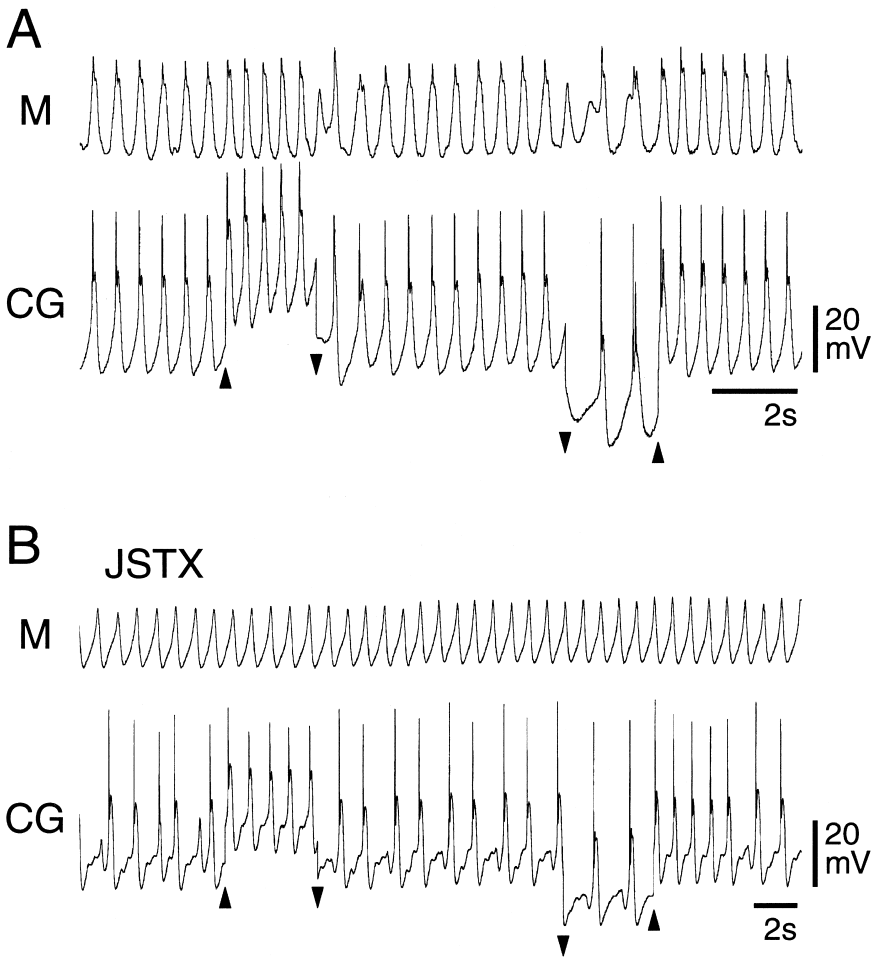

Fig. 2. Effects of JSTX. In each record, intracellular activity of the cardiac muscle (M, upper trace) and that of the cardiac ganglion neuron (CG, lower trace) were recorded simultaneously in an opened beating heart. Records obtained before (**A**) and 20 min after the onset of application of JSTX (10 μM) (**B**) are shown. Depolarizing or hyperpolarizing current was injected into the cardiac ganglion neuron during the periods indicated by the upward and downward arrowheads using a bridge circuit.

versibly the glutamatergic neuromuscular transmission in crustacean skeletal muscle (Kawai *et al*., 1982; Abe *et al*., 1983) and stomach muscle (Chiba and Tazaki, 1992; Tazaki and Tazaki, 1997). However, in the heart of *Ligia exotica*, the EJPs were recovered partially after washout JSTX. In association with the partial recover of the EJPs, the correlation between the neurogenic and myogenic activities also partially restored (Fig. 1D). This strongly supports the idea that the neurogenic activity entrains the myogenic activity *via* EJPs.

ACKNOWLEDGMENTS

We thank Dr. Darryl Macer for his critical reading of this paper. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan to HY. This is Contribution no. 623 from Shimoda Marine Research Center, University of Tsukuba.

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(Received April 24, 1998 / Accepted June 16, 1998)