0014-4754/96/060583-04$1.50 + 0.20

Endogenous oscillatory activity and TTX-sensitive spikes of the heart muscle in early juveniles of the isopod crustacean *Ligia exotica*

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Received 18 April 1995; accepted 26 September 1995

Abstract. The heart beat of early juveniles of the littoral isopod *Ligia exotica* occurred at a frequency of 250 to 350/min, associated with rhythmic activity of the heart muscle. Each burst was composed of a slow depolarizing potential with superimposed spike potentials. The spike potential was eliminated by perfusion with TTX-containing or Na^+-free saline. In TTX-saline, the slow potential was unchanged in frequency and amplitude. By current injection into the heart muscle, the rhythm of the slow potential was phase-shifted and its frequency was changed in a membrane potential-dependent manner. These results show that the heart of *Ligia* early juveniles acts as an endogenous muscle oscillator generating oscillatory slow potentials and Na^+-dependent spikes.
Key words. Crustacea; heart; muscle; oscillator; tetrodotoxin.

In the neurogenic heart of crustaceans, the cardiac ganglion acts as a pacemaker[1–3]. Rhythmic bursts of impulses generated spontaneously in the cardiac ganglion conduct peripherally and drive the heart muscle via excitatory neuro-muscular junctions[4,5]. As the heart muscle has no spontaneous activity, the heart beat stops completely if the cardiac ganglion is removed or following application of tetrodotoxin (TTX) which suppresses neuronal spiking in the cardiac ganglion[4].

In the isopod *Ligia*, the heart beat of adults is basically neurogenic[6] and each beat follows a spontaneous burst discharge of the cardiac ganglion[7,8]. However, the heart beat of early juveniles occurs in response to electrical events of the heart muscle in the absence of the cardiac ganglion activity[9]. The present paper deals with intracellular activity of the heart muscle in early juvenile *Ligia* and will provide the first description of the characteristics of crustacean heart muscle which acts as an endogenous oscillator.

Materials and methods

Juveniles of the littoral isopod *Ligia exotica* were obtained during its breeding period (April to September) from the colony maintained for many years in the laboratory. After copulation the female holds about 80 to 120 fertilized eggs in her brood pouch. The embryo undergoes direct development in the egg for about three weeks and hatches as a juvenile. The juvenile stage lasts for about three weeks. More than 100 early juveniles, about 3.0 mm in body length, were used for experiments. They were used within three days of hatching.

The anatomy of the heart and the methods of dissection are the same as reported previously[8]. The heart is tubular and is located in the dorsal side of the posterior half of the body. The heart wall consists of a single layer of striated muscle fibers forming a right-hand spiral. The cardiac ganglionic trunk contains six ganglion neurons and runs longitudinally in the midline of the inner surface of the dorsal heart wall. In early juveniles, the heart tube was about 1 mm in length and 0.1 mm in diameter. The heart was isolated intact together with the dorsal carapace and pinned, ventral side up, in the experimental chamber. The cardiac ganglion was thus left in place, while it exhibited no spontaneous activity[9]. The chamber was continuously perfused with aerated physiological saline solution throughout the experiments. The standard saline had the following composition based on that of the *Ligia* serum[10] (in mM); NaCl 586, KCl 14, $CaCl_2$ 25, $MgCl_2$ 16.5, Tris-HCl (pH. 7.4) 5. In some experiments, tetrodotoxin (TTX) (Sigma), $CoCl_2$ or $MnCl_2$ was added to the saline. Na^+-free saline was made by replacing NaCl with an equimolar amount of choline chloride.

Spontaneous activity of the heart muscle was intracellularly recorded from the intact heart preparation using a conventional microelectrode filled with 3 M KCl (resistance, 10–30 MΩ). As the heart muscle cells couple electrically with each other[9], the second microelectrode for current injection was inserted into the heart muscle at a distance from the recording electrode. The data were stored in a FM magnetic tape recorder and displayed on a cathode ray oscilloscope and a chart recorder. All the experiments were performed at a temperature of 20 to 23 °C.

Results

The heart beat of early juveniles was associated with rhythmic burst discharges of the heart muscle at a frequency of 250 to 350/min (fig.1, A1). Each burst was composed of 1 to 4 spike potentials followed by a

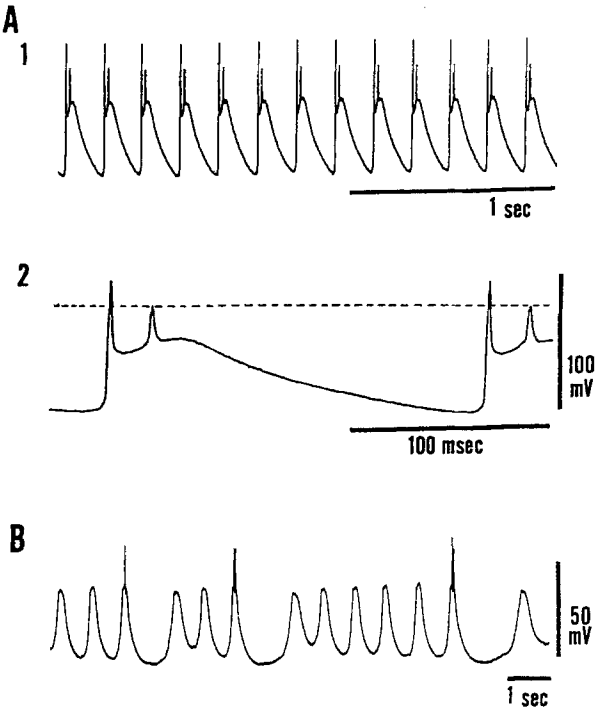

Figure 1. Muscle activity in the heart of *Ligia* early juveniles. *(A1)* Rhythmic burst activity recorded intracellularly from the heart muscle from an early juvenile 1 day after hatching. *(A2)* The same data recorded with faster sweep. Dotted line, reference level for the potential (0 mV). *B.* Intracellular activity recorded from the heart muscle shortly after dissection from an early juvenile 2 days after hatching.

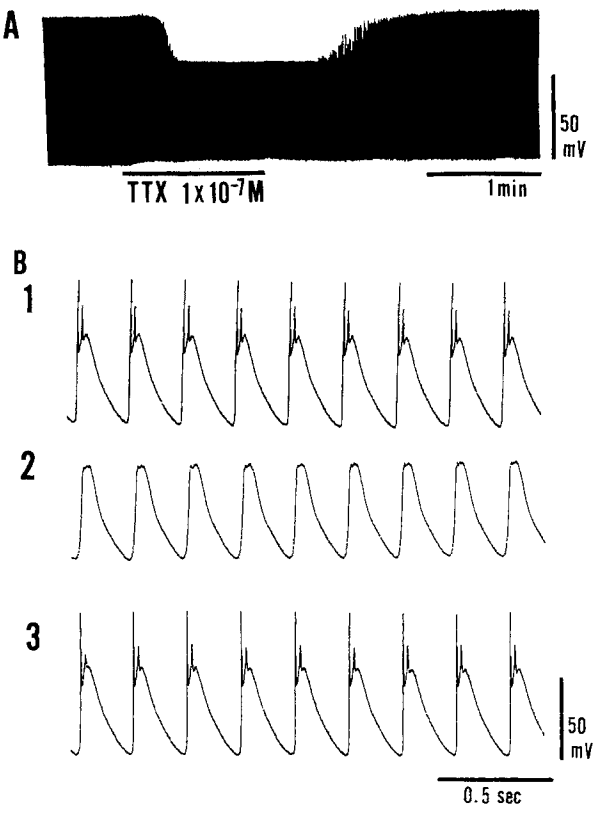

Figure 2. Effects of TTX on muscle activity in the heart of *Ligia* early juveniles. *A.* Intracellular activity recorded from the heart muscle from an early juvenile 1 day after hatching. TTX (1×10^{-7} M) was applied during the period indicated by the black bar. *B.* The same data as in A recorded with faster sweep. *(B1)* 1 min before application of TTX. *(B2)* During application of TTX (1 min after onset of TTX-saline). *(B3)* 2 min after returning to standard saline.

plateau-like slow potential (fig. 1, A2). The first spike of a burst usually overshot up to +20 mV. The plateau potential had an amplitude of 40 to 60 mV and a duration of 90 to 100 msec at the point of half amplitude. The level of maximum membrane potential during a burst cycle was -78.8 ± 5.6 mV (mean $\pm$ SD, n = 68). Under unstable conditions such as the state just after dissection, the heart muscle often exhibited only oscillatory slow potentials. The slow potential increased gradually in amplitude and spike potentials began to fire on top of the slow potential (fig. 1B). A threshold voltage of the spike potential was estimated about −40 mV.

The above observations suggested that the burst activity of the heart muscle is composed of oscillatory slow potentials and voltage-dependent spike potentials. To investigate the idea, the ionic dependence of the spike potential was examined. The action potential of crustacean striated muscle is known to be Ca^{2+}-dependent[11,12]. Therefore, the effects of Co^{2+} and Mn^{2+}, known as Ca^{2+} channel blockers[13], were examined. When perfusing saline containing Co^{2+} (6 mM) or Mn^{2+} (8 mM) the burst activity increased in frequency, whereas no definite changes were observed in the spike potential (data not shown). In contrast, perfusion with the saline containing TTX, known as a blocker of Na^+ channels[14] eliminated the spike potential within 1 min (fig. 2A). The slow potential, however, remained almost unchanged in frequency and amplitude in TTX-saline (fig. 2, B1 and B2). The effect of TTX was reversible and the spike potential was gradually restored when the preparation was returned to standard saline (fig. 2, B3). The threshold concentration of TTX for eliminating the spike potential was 3 to 5×10^{-8} M. With higher concentrations of TTX, there was later restoration of the spike potential.

The effect of TTX suggested that the spike potential of the heart muscle was Na^+-dependent. The effects of Na^+-free saline on the muscle activity were next examined. By perfusing Na^+-free saline, the spike potential could be rapidly (within 1 min) eliminated (fig. 3, A and B2), and it was restored soon after the preparation was returned to standard saline (fig. 3A and B3). During perfusion of Na^+-free saline, the membrane potential of the heart muscle depolarized gradually and a slow potential decreased successively in amplitude and frequency (fig. 3, A and B2). If the perfusion of Na^+-free saline continued, the slow potential vanished completely at a depolarization 20 to 30 mV from the

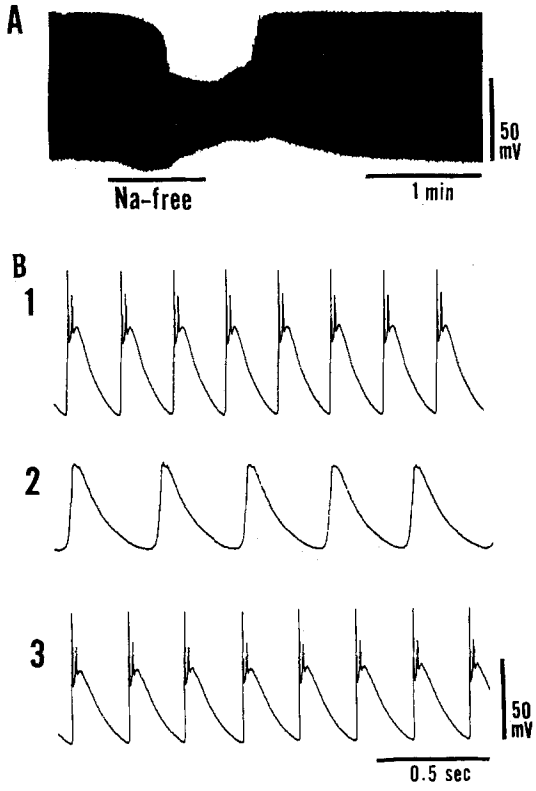

Figure 3. Effects of Na^+-free saline on muscle activity in the heart of *Ligia* early juveniles. *(A)* Intracellular activity recorded from the heart muscle of an early juvenile 1 day after hatching. Na^+-free saline was applied during the period indicated by the black bar. *(B)* The same data as in A recorded with faster sweep. *(B1)* 1 min before application of Na^+-free saline. *(B2)* During application of Na^+-free saline (50 sec after onset of Na^+-free saline). *(B3)* 1 min after returning to standard saline.

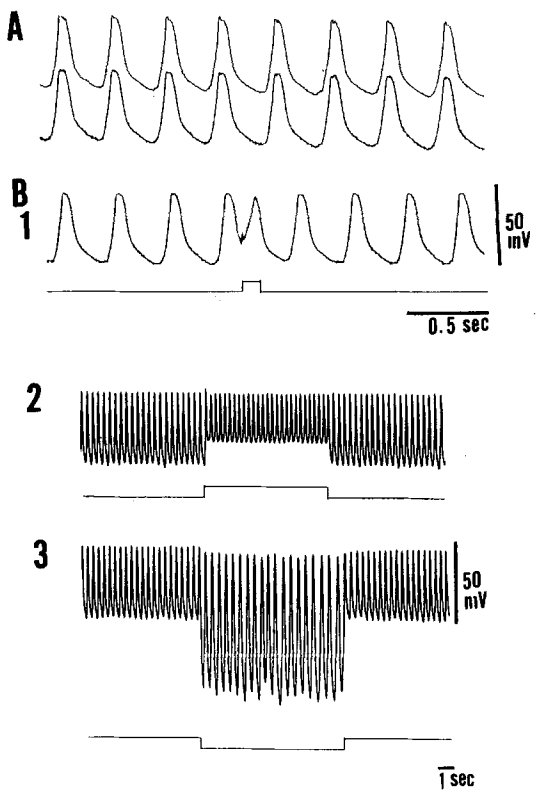

Figure 4. Effects of injected current on muscle activity in TTX-saline in the heart of *Ligia* early juveniles. *(A)* Intracellular muscle activity in TTX (1×10^{-7} M) saline recorded simultaneously from two sites, near anterior and posterior ends of the heart, from an early juvenile 1 day after hatching. *(B)* Effects of current injection into the heart muscle. Intracellular activity of the heart muscle (upper trace) and monitor of current injection (lower trace). *(B1)* Shift of the slow potential rhythm by a brief depolarizing current (100 msec, 10 nA). *(B2)* Increase in frequency of the slow potential by a long depolarizing current (8 sec, 20 nA). *(B3)* Decrease in frequency of the slow potential by a long hyperpolarizing current (9 sec, 20 nA).

maximum membrane potential under normal conditions (data not shown). The slow potential recovered gradually on return to standard saline (fig. 3, A and B3).

To test oscillatory properties of the slow potential, electric current was injected into the heart muscle. In early juveniles, the heart muscle cells couple electrically with each other and exhibit synchronous burst discharges[9]. In TTX-saline, the slow potentials were synchronously recorded from any two portions of the heart (fig. 4A). A brief depolarizing current injected into the heart muscle at any region of the heart resulted in earlier occurrence of the slow potential relative to the preceding spontaneous ones, and thus the rhythm of the slow potential was phase-shifted (fig. 4B). With longer depolarizing or hyperpolarizing currents, the slow potential increased or decreased significantly in frequency (fig. 4, C1 and C2). The change in frequency of the slow potential was larger with larger stimulating current.

Discussion

The results obtained show clearly that the rhythmic activity of the heart muscle in *Ligia* early juveniles is composed of oscillatory slow potentials with spike potentials superimposed on them. The action potential of crustacean striated muscle is Ca^{2+}-dependent and no evidence of TTX-sensitive action potential has been found[11–14]. However, the overshooting spike potential recorded in this study from the heart muscle was not blocked by Co^{2+} or Mn^{2+} which blocks Ca^{2+} action potential of crustacean striated muscle[12], and it was reversibly blocked by TTX of relatively low concentration (less than 5×10^{-8} M) (fig. 2). Moreover, the spike potential was rapidly eliminated in Na^+-free saline and restored soon on return to standard saline (fig. 3). The same type of spike potential was also recorded from the adult heart muscle (unpublished data). These results suggest that the *Ligia* heart muscle generates a TTX-sensitive Na^+ action potential which has never before been found in crustacean muscle.

The heart beat of *Ligia* early juveniles is thought to be myogenic because the rhythmic muscle activity occurs in the absence of the cardiac ganglion activity[9]. This

idea was further supported by the fact that the oscillatory slow potential remained unchanged in TTX-saline which suppresses neuronal spiking in the crustacean cardiac ganglion[15,16]. Moreover, by current injection into the heart muscle, the rhythm of the slow potential was phase-shifted and its frequency changed in a membrane potential-dependent manner (fig. 4). These results demonstrate that the heart muscle possesses properties which are characteristic of endogenous oscillators in general[17].

Though crustacean muscles generally lack myogenicity, endogenous oscillatory properties of the muscle induced by isolation and/or application of biogenic amines have been reported in the crustacean stomatogastric system[18–20]. However, a functional role of the myogenicity remains uncertain. The results of this study show clearly that, in early juveniles of *Ligia*, the whole heart acts as an endogenous muscle oscillator and produces myogenic heart beats, previously unknown in crustaceans except in some branchiopods, the primitive crustacean species[21–24].

Acknowledgments. I thank Dr Darryl R. J. Macer for his critical reading of this manuscript. This is contribution No. 584 from the Shimoda Marine Research Center, University of Tsukuba.

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