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Research Articles

Cardiac activation and inhibition involved in molting behavior of a spiny lobster

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Abstract. To study the molting behavior of the spiny lobster, electrical activity of the heart, stomach and skeletal muscles was recorded using in-dwelling electrodes. For 1–2 h before molting the heart rate gradually increases. At the same time shorter trains of stomach burst discharges frequently occur. The heart rate then declines and burst discharges of skeletal muscles begin. The skeletal bursts are regularly spaced (10–15 s intervals). A peristalsis pattern of short and long bursts continues for 10–20 min and is terminated by a few bursts corresponding to abdominal flips. The short skeletal burst is followed by a drop in heart rate. Bioassay using the isolated heart suggests that at the final stage of molting the blood contains some substance(s) which inhibit heart beat.
Key words. Lobster; ecdysis; molting behavior; heart; stomach; cardiac inhibitor.

Molting behavior, called ecdysis, is essential for arthropod growth and reproduction. Ecdysis is under CNS control, mediated by the endocrine system and neurosecretory complex[1–5]. Ecydsis of decapod crustaceans has been divided into two major physiological activities: intensive uptake of water and withdrawal from the old exoskeleton[1]. The water uptake swells the body, pushing apart the old carapace. Swelling of the thorax is due to changes in stomach volume[6,7]. In crab ecdysis, blood pressure falls in the cephalothorax while appendages become turgid[6]. Changes in electrical activity of the heart and stomach have not yet been reported. In this study, simultaneous recordings of the electrical activity of heart, stomach and skeletal muscles involved in ecdysis, have been obtained. The data suggest that the heart rate slowly rises and falls in parallel with swelling and shrinking of the new soft body. The slow changes in heart rate may be mediated by excitatory and inhibitory factors in the blood.

Materials and methods

Adult lobsters (*Panulirus japonicus*, wt 200 g, n = 10, both sexes) were used. Since their ecdysis started only in the dark (1 to 3 AM) and was prevented by illumination, they were reared under light conditions before use. The method of taking electrical recordings from the heart, stomach and skeletal muscles in vivo was substantially the same as reported previously[8]. The active electrode for the heart was 2 cm posterior to that for the stomach.

The skeletal muscle discharges were recorded synchronously with the discharges of heart and stomach. Continuous recordings throughout ecydsis were possible because the implanted electrodes remained in situ. Thus the electrical activity was observed for 2–3 weeks before and 1 h after ecdysis. The electrical signals and time were recorded simultaneously on videotape. The data were displayed by a thermal dot-array recorder (Graphtec WR7700, DC-1k Hz). The heart rate (beats/min) was displayed via an electronic counter measuring each interval between sequential pulses of electrocardiogram (ECG).

Blood (20–30 ml/animal) was collected by bleeding from cut ends of the legs and antennae of the lobster. Serum was prepared from the blood, which was boiled at 100 °C for 5 min and was centrifuged at 3000 cpm for 10 min. The fresh blood and heat-treated serum (0.5–1 ml) were injected by a micropipette into an ostium of the heart isolated from the normal lobster. Heart contractions recorded with a strain gauge were displayed on a pen recorder chart. (See our previous papers for responsiveness of the heart[9–12].)

Results

Along the coast of Izu Peninsula, adult lobsters (*P. japonicus*) molt frequently in summer (July to August). Based on direct observation, their ecdysis could be divided into three steps. First the animals, in a standing position, slowly shed the exoskeleton of the cephalothorax and then that of the abdomen. They then rapidly withdraw from the exuviae with repeated flips of the abdomen.

In intermolt stages, the heart periodically stops beating (intervals, 1–15 min)[8]. Such bradycardia disappeared 1–2 weeks before ecdysis. Burst discharges of the stomach (10 min or longer) could be elicited by feeding, until a few days before ecdysis. Although the feeding was stopped at least one day before ecdysis, a long train of stomach bursts was observed until 1–2 h before ecdysis. After that,

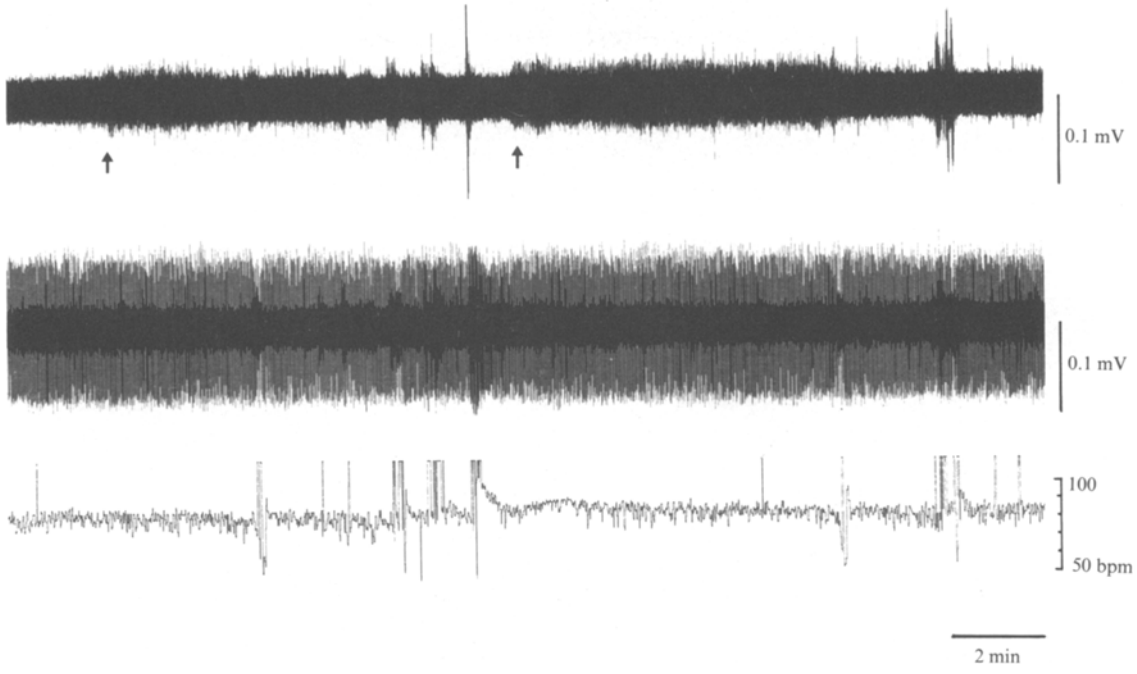

Figure 1. Electrical activity of heart and stomach 1 h before ecdysis of the Japanese spiny lobster. Top, stomach burst discharges. Arrows indicate the burst initiations. Middle, ECG. Bottom, heart rate. Skeletal muscle discharges are seen synchronously on both top and middle records, and often disturb correct counting of the heart rate, the values of which are scaled out in the bottom record.

shorter trains of stomach bursts appeared frequently for about 30 min (fig. 1). This change in stomach activity appeared to take place in parallel with the activation of the heart. The heart rate began to rise 1–2 h before ecdysis. After reaching a peak (80–120 bpm), the rate declined around 15 min before the start of ecdysis, during which time bursting of skeletal muscles began. Amplitude and frequency of the skeletal burst discharges increased with time and a steady burst pattern was attained. Figure 2 shows a typical example recorded during ecdysis. The skeletal bursts were divided into two types; large short and small long bursts (fig. 2B and C). Although several bursts at the start (fig. 2B, 1) and end (fig. 2C, 4) were solitary, a large one was usually followed by a small (fig. 2B, 2) or large one (fig. 2B, 3), or both (fig. 2C, 2). Each of the burst groups was regularly spaced (period 10–15 s). The short burst appeared to be followed by a drop in heart rate, and the rate dropped markedly with the increase in burst frequency (fig. 2A and B). The amplitude of the short burst increased while ECG amplitude decreased markedly, and was often diminished during cephalothoracic shedding (fig. 2A and C). The frequency of the skeletal bursting as a group rose as the abdominal shedding proceeded (from 2 to 6/min in fig. 2). Thus the shedding time was usually shorter in the abdomen than in the thorax. The electrical signals terminated with a few bursts corresponding to repeated flips of the abdomen, observed just before the new body swam out from the old exuvium. The characteristic discharges for ecdysis lasted for 10–20 min in the present study.

The electrical activity of the heart and skeletal muscles during ecdysis could also be recorded from the crayfish, *Promcambarus clarkii* (not shown in figure). An increase and decrease in heart rate similar to those of the lobster were observed during crayfish ecdysis.

For several minutes after ecdysis, the animals moved about with occasional abdominal flipping. This was followed by at least 30 min quiescence with the heart rate as low as 20–50 bpm. Unfortunately, the magnitude of the stomach activity following ecdysis was too small to be measured.

When blood of the lobster collected 1 h before ecdysis was applied to isolated lobster heart, both the rate and amplitude of the beat increased (fig. 3A). Serum alone also enhanced the heartbeat. In contrast, serum obtained just after ecdysis inhibited the heartbeat (fig. 3B). The heart inhibition continued for several minutes and was dose-dependent.

Discussion

Morphological and biochemical studies on the process of ecdysis have been extensively reported[1–4,13]. In contrast, electrophysiological studies on crustacean ecdysis have yet to be reported. This paper is the first report describing the electrical activity of the heart and skeletal muscles during ecdysis of decapod crustaceans. The peristalsis motor pattern of ecdysis has been analyzed physiologically in several insects[14–19]. The field potential of skeletal muscles recorded from the lobster cephalothorax (fig. 2) exhibits a peristalsis pattern of ecdysis similar to that

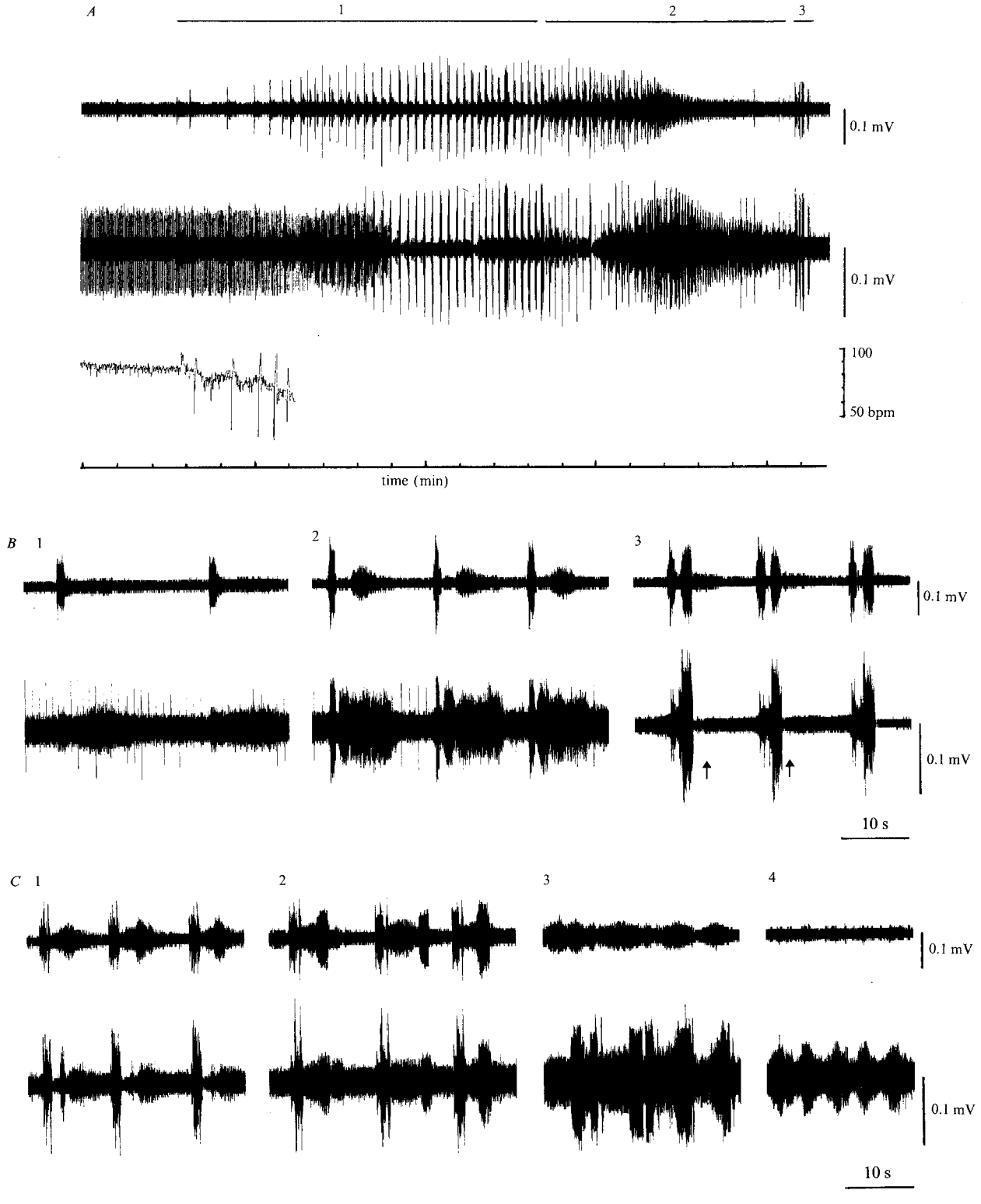

Figure 2. A typical, simultaneous recording from heart and skeletal muscles during ecdysis of the spiny lobster.
A A simultaneous display of skeletal muscle burst discharges (top), ECG (middle) and heart rate (bottom). Bars 1–3 indicate three steps: cephalothoracic shedding, abdominal shedding and swimming out. The heart rate is shown only for early times in shedding because later the skeletal discharges disturb the counting.
B Skeletal bursts (top) and ECG (bottom) in the cephalothoracic shedding shown over an enlarged time scale. 1, single short bursts in the initial period; 2, doublet bursts of short and long duration in the middle period; 3, doublets of large, short bursts in the late period. These patterned discharges of skeletal muscles are clear in top traces. In 1 and 2, each of the short bursts (top) is followed by a decrease in heart rate (bottom). In 3, only two small pulses of ECG (arrows) are seen.
C The burst patterns in abdominal shedding shown over an enlarged time scale. 1, doublet bursts of short and long duration are generated again; 2, short burst additionally joins the doublet burst after a delay; 3–4, the large bursts disappear and single small bursts continue until the end of the abdominal shedding.

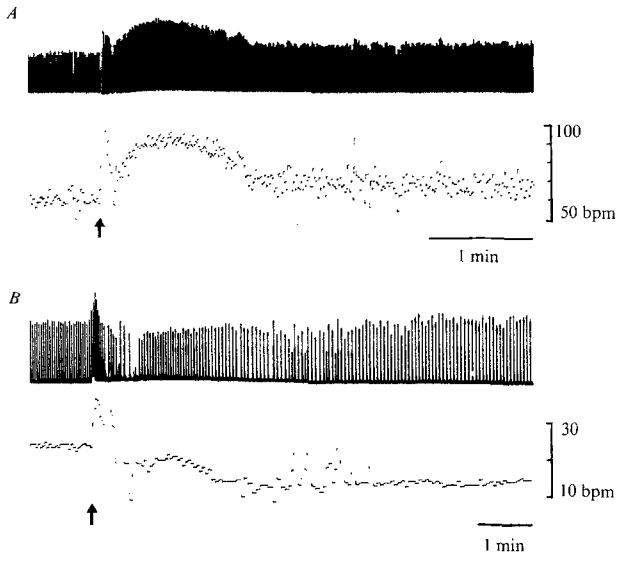

Figure 3. Assay of cardioactive factors in blood using the isolated heart of lobster.
A Heart beat (top) and rate (bottom) increased by a pulse application of blood (arrow, 0.5 ml), which was collected about 1 h before ecdysis.
B Heart beat (top) and rate (bottom) decreased by a pulse application of serum (arrow, 1 ml), which was obtained just after ecdysis.

exhibited by insect motor neurons[18]. Therefore, the lobster might shed its exoskeleton with peristaltic movements even though the segmental movements are not entirely obvious.

The change in heartbeat involved in insect ecdysis, called eclosion, has also been reported in the moth, *Manduca sexta*[20]. Three kinds of peptides are found to be released into the blood[20–22]. One of these is a cardioinhibitor (200–300 Da) obtained from whole blood at the pharate stage. The other two are cardioaccelerators (500 and 1000 Da) associated with wing-spreading just after eclosion. Such cardioaccelerators might not be released in decapod ecdysis because the heart rate did not rise markedly during or immediately after ecdysis in the lobster and crayfish. However, some cardioactivators might be present in blood of the decapods 1 h before ecdysis because the blood collected at that time activated the isolated heart (fig. 3A).

The heart inhibition which is clearly observable in the lobster is not so evident in the moth[20]. As mentioned above, stage-specific cardioaccelerating peptides have been found in lepidopteran insects[23]. The insects which show wing-spreading behavior may need such cardioactivators just after ecdysis, while crustaceans, living in water without wings, may not.

The inhibitory factor(s) in the lobster blood (fig. 3B) might inhibit the heartbeat during the actual shedding process. The excitatory and inhibitory factors found in this study were heat stable. Therefore, they might be small molecules like amines and peptides. Further analysis of the molecular characteristics of these substances is in process.

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1 Passano, L. M., in: The Physiology of Crustacea I, p. 473. Ed. T. H. Waterman. Academic Press, New York 1960.
2 Skinner, D. M., in: The Biology of Crustacea 9, p. 43. Eds D. E. Bliss and L. H. Mantel. Academic Press, Tokyo 1985.
3 Kleinholz, L. H., in: The Biology of Crustacea 9, p. 495. Eds D. E. Bliss and L. H. Mantel. Academic Press, Tokyo 1985.
4 Ohnishi, E., and Ishizaki, H., Molting and Metamorphosis. Japanese Science Society Press, Tokyo 1990.
5 Truman, J. W., in: Molting and Metamorphosis, p. 67. Eds E. Ohnishi and H. Ishizaki. Japanese Science Society Press, Tokyo 1990.
6 Drach, P., Ann. Inst. Oceanog. Paris, *19* (1939) 103.
7 Travis, D. F., Biol. Bull. Mar. biol. Lab. Woods Hole *107* (1954) 433.
8 Kuramoto, T., Physiol. Zool. *63* (1990) 182.
9 Kuramoto, T., and Ebara, A., J. exp. Biol. *109* (1984) 121.
10 Kuramoto, T., and Ebara, A., J. exp. Biol. *111* (1984) 123.
11 Kuramoto, T., and Ebara, A., J. comp. Physiol. *B148* (1988) 403.
12 Kuramoto, T., and Ebara, A., J. comp. Physiol. *B161* (1991) 339.
13 Travis, D. F., Biol. Bull. Mar. biol. Lab. Woods Hole *109* (1955) 484.
14 Provine, R. R., J. Insect Physiol. *22* (1976) 127.
15 Carlson, J. R., J. comp. Physiol. *115* (1977) 299.
16 Carlson, J. R., J. comp. Physiol. *115* (1977) 319.
17 Hughes, T. D., Physiol. Ent. *5* (1980) 55.
18 Week, J. C., and Truman, J. W., J. comp. Physiol. *A155* (1984) 407.
19 Week, J. C., and Truman, J. W., J. comp. Physiol. *A155* (1984) 423.
20 Tublitz, N. J., and Truman, J. W., J. exp. Biol. *114* (1985) 381.
21 Tublitz, N. J., and Truman, J. W., J. exp. Biol. *114* (1985) 365.
22 Tublitz, N. J., and Truman, J. W., Science *228* (1984) 1013.
23 Tublitz, N. J., Brink, D., Broadie, K. S., Loi, P. K., and Sylwester, A. W., Trends Neurosci. *14* (1991) 254.