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Developmental Changes in Dopamine Modulation of the Heart in the Isopod Crustacean *Ligia exotica*: Reversal of Chronotropic Effect

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ABSTRACT—Developmental changes in dopamine modulation of the heart were examined in the isopod crustacean *Ligia exotica*. The *Ligia* cardiac pacemaker is transferred from the myocardium to the cardiac ganglion during juvenile development and the heartbeat changes from myogenic to neurogenic. In the myogenic heart of early juveniles, dopamine affected the myocardium and caused a decrease in the frequency and an increase in the duration of the myocardial action potential, resulting in negative chronotropic (decrease in beat frequency) and positive inotropic (increase in contractile force) effects on the heart. Contrastingly, in the heart of immature adults just after juvenile development, dopamine caused effects of adult type, positive chronotropic and positive inotropic effects on the heart affecting the cardiac ganglion and myocardium. During the middle and late juvenile stages, dopamine caused individually a negative or a positive chronotropic effect on the heart. These results suggest that the chronotropic effect of dopamine on the *Ligia* heart is reversed from negative to positive in association with the cardiac pacemaker transfer from the myocardium to the cardiac ganglion during juvenile development.

Key words: heart, modulation, dopamine, development, crustacea

INTRODUCTION

The heart of many crustaceans is known to be neurogenic with the cardiac ganglion acting as the pacemaker; the myocardium has no inherent automaticity and is driven by periodic bursting activity of the cardiac ganglion (reviewed by Maynard, 1960; McMahon *et al*., 1997). However, in the isopod *Ligia exotica,* the cardiac pacemaker is transferred from the myocardium to the cardiac ganglion during juvenile development and the cardiac ganglion becomes the primary pacemaker with the myocardium having a latent pacemaker property (Yamagishi, 1996; Yamagishi and Hirose, 1997; reviewed by Spicer, 2001). This fact suggests that neural and neurohormonal regulation of the *Ligia* heart changes in association with the change of target tissue for regulation. We have previously shown that acceleratory and inhibitory nervous regulation of the *Ligia* heart changes in association with the cardiac pacemaker transfer during juvenile development (Sakurai *et al*., 1999; Yamagishi *et al*., 2001).

The neurogenic heart of decapods is regulated also by neurohormones including several amines and peptides released from the pericardial organ (Sullivan *et al*., 1977; reviewed by Cooke and Sullivan, 1982; McMahon *et al*., 1997). Among these neurohormones, the amines appear to affect the cardiac ganglion but not directly the myocardium (Wilkens, 1999). However, we showed previously that dopamine, one of the amines released from the decapod pericardial organ, modulates the neurogenic heart of adult *Ligia* affecting both the cardiac ganglion and myocardium (Yamagishi *et al*., 2004). The ability of dopamine to affect the two pacemaker tissues in the *Ligia* heart suggests that the modulation mechanism of dopamine changes according to the cardiac pacemaker transfer from the myocardium to the cardiac ganglion during development. We therefore examined the effects of dopamine on the *Ligia* heart of various developmental stages. The results show that the chronotropic effect of dopamine on the *Ligia* heart is reversed from negative to positive in association with the cardiac pacemaker transfer during juvenile development.

MATERIALS AND METHODS

Adult males and females of the littoral isopod crustacean *Ligia exotica* were collected on the Pacific coast at Shimoda and Kominato, Japan. The colony was maintained in the laboratory during the breeding period (April to September). Females that held eggs were

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kept individually in small plastic containers to obtain specimens of known developmental stages. More than 100 specimens of juveniles and immature adults (3 to 10 mm in body length) were used for the experiments.

The time course of juvenile development, the method of dissection and the anatomy of the heart were as described previously (Yamagishi and Hirose, 1997). Semi-isolated heart preparations were used for the experiments. The heart was isolated together with the dorsal carapace from the whole body as the heart was kept intact in the pericardial cavity. The preparation was fixed ventral side up in the Silgard seated experimental chamber by pinning through the dorsal carapace. The chamber was perfused continuously with aerated physiological saline having the following composition (in mM); NaCl 586, KCl 14, $CaCl_2$ 25, $MgCl_2$ 16.5, $MgSO_4$ 4.5, and Tris-HCl 5 (pH 7.4) (Yamagishi and Ebara, 1985). Dopamine (dopamine hydrochloride, Wako) was added at various concentrations to the saline just before use and was applied to the heart by changing the perfusing saline.

The membrane potential of the myocardial cells was recorded with a conventional glass microelectrode filled with 3 M KCl (electric resistance, 10 to 30 MΩ). The signals were amplified and displayed on a cathode ray oscilloscope. To determine instantaneous frequency of the myocardial action potential, a heart rate counter (Nihon Koden AT601G) was used. Data were recorded using a magnetic tape recorder and a chart recorder. All the experiments were performed at a temperature from 20 to 23°C.

RESULTS

After copulation, each female molts and holds approximately 80 to 120 fertilized eggs in the brood pouch situated on the ventral surface of the abdomen. The embryo develops in the egg for approximately 3 weeks before hatching as a juvenile. The newly hatched juvenile is approximately 3 mm in body length and has six pairs of legs. Several days after hatching, the juveniles are released from the mother's brood pouch. The juvenile stage lasts for approximately 3 weeks, during which time the juvenile moults twice before becoming an immature adult; it is then approximately 5 mm in body length and has seven pairs of legs. The immature adult takes 4 to 5 months to mature (approximately 20 mm in body length). Developmental stages of juveniles and immature adults were determined and expressed as the number of days after hatching (Yamagishi and Hirose, 1997). The beat frequency of the heart was high in early juveniles and appeared to decrease gradually during development. The beat frequency of the heart preparations used in the experiments was in the range of 168 to 452 / min.

We first examined the effects of dopamine on the heart of early juveniles (1 to 5 days after hatching): dopamine decreased the frequency of the myocardial action potential (Fig. 1A). In this case, application of 10^{-5} M dopamine for 30 s caused a 17% decrease in the frequency of the action potential (from 431 to 358 /min). The frequency recovered slowly to the control level during washout of dopamine. To determine the dose-response relationship, dopamine was applied at various concentrations for 30 s (n=13): the frequency of the myocardial action potential decreased in a concentration dependent manner with a threshold concentration of approximately 10^{-7} M (Fig. 1B).

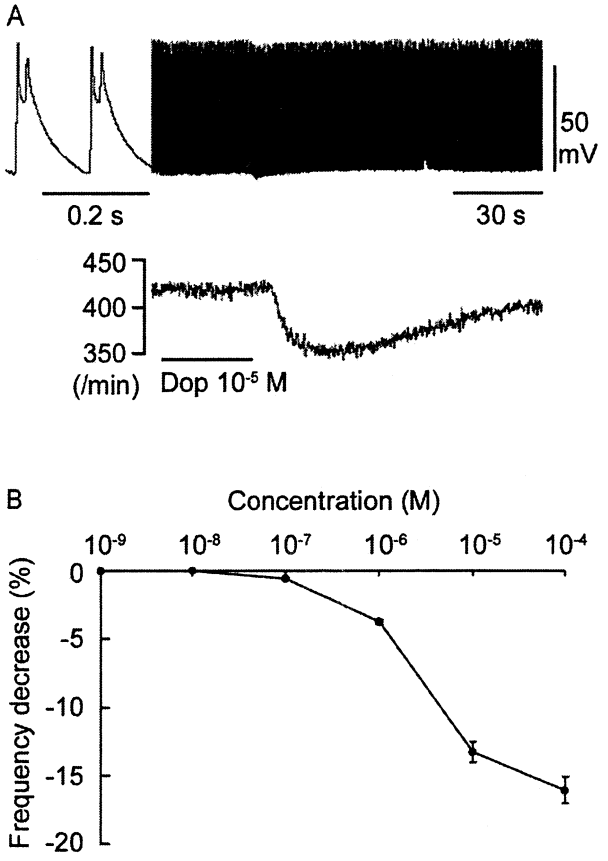

Fig. 1. Effects of dopamine on the heart rate of early juveniles. (A) Membrane potential of the myocardial cell (upper trace) and a plot of instantaneous frequency of the myocardial action potentials (lower trace). Dopamine (10^{-5} M) was applied during the period (30 s) indicated by the horizontal black bar. From a juvenile 4 days after hatching. (B) Relationship between rate of decrease (%) in the frequency of myocardial action potential and concentration of dopamine (M). Each data point shows the mean±SEM (N=13).

In addition to the frequency, dopamine changed also the contour of the myocardial action potential, increasing the duration and amplitude of the action potential plateau (Fig. 2A, upper and lower traces). In this case, application of 10^{-5} M dopamine for 30 s caused a 21% decrease in the frequency of the action potential (from 243 to 192 /min). At the same time, dopamine caused a 33% increase in the duration of the action potential plateau (from 120 to 160 ms at the point of half-amplitude of the plateau) with a slight increase of less than 5% in the amplitude. The changes in the duration and frequency of the action potential appeared with parallel time courses (Fig. 2B). With increasing dopamine concentration, the duration of the action potential lengthened, but the rate of duration increase varied among the preparations (3 to 43% in response to 10^{-5} M dopamine, n=22). With elongation of the action potential duration, the width of the heart during systolic contraction was observed to become shorter suggesting an increase in the force of the heartbeat.

We next examined the effects of dopamine on the heart of immature adults (more than 25 days after hatching).

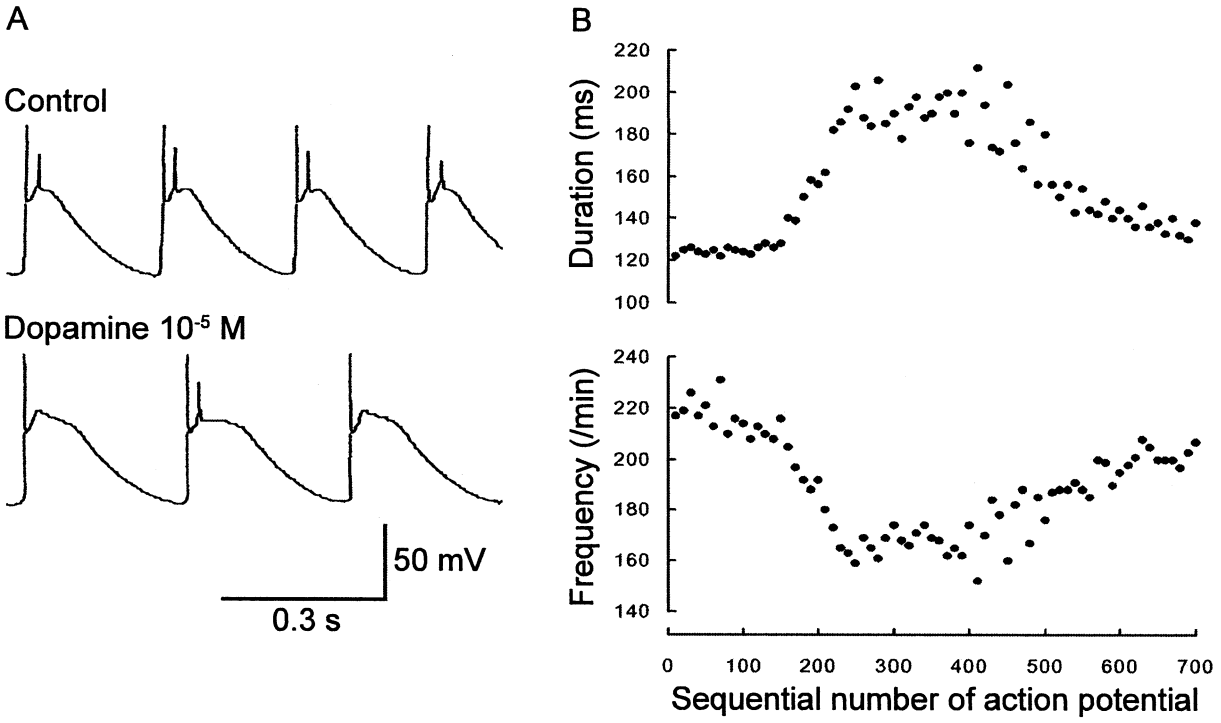

Fig. 2. Effects of dopamine on the myocardial action potential of early juvenile hearts. (A) Dopamine (10^{-5} M) was applied for 30 s while recording continuously membrane potential of the myocardial cell. Each trace shows action potentials recorded just before dopamine application (upper trace), at the peak of frequency decrease in response to dopamine (lower trace). From a juvenile 3 days after hatching. (B) Plots of changes in the duration and frequency of the successive myocardial action potentials in a response to dopamine (10^{-5} M, 30 s). The action potentials just after the application period of dopamine are numbered sequentially and every 10th action potential is plotted. Each data point shows duration of the action potential at the point of half amplitude of the plateau (upper graph) and its instantaneous frequency calculated as a reciprocal of the following inter action potential interval (lower graph). From a juvenile 3 days after hatching.

Dopamine increased the frequency of the myocardial action potential (Fig. 3A). In this case, application of 10^{-5} M dopamine for 30 s caused a 43% increase in the frequency of the action potential (from 242 to 347 /min). The frequency decreased rapidly to the control level during washout of dopamine. To determine the dose-response relationship, dopamine was applied at various concentrations for 30 s (n=9). Dopamine increased the frequency of the myocardial action potential in a concentration dependent manner with a threshold concentration of approximately 10^{-8} M (Fig. 3B). Dopamine increased also the duration and amplitude of the action potential plateau with a slower and longer time course than that of the frequency change (not shown). With elongation of the action potential duration, the width of the heart during systolic contraction was observed to become shorter suggesting an increase in the force of the heartbeat.

The above results suggest that the effect of dopamine on the action potential frequency of the myocardium is reversed from decrease to increase during juvenile development. We therefore examined the effect of 10^{-5} M dopamine on the frequency of the myocardial action potential in the heart of various developmental stages, from 1 to 40 days after hatching (n=80). The heart of early juveniles responded by a frequency decrease and that of immature adults (more than 25 days after hatching) by a frequency increase (Fig. 4A). The heart of middle and late juveniles responded by a frequency decrease or increase. Moreover, the heart of these juveniles often exhibited biphasic responses (Fig. 4B): the frequency first decreased and then increased (13 preparations, not included in Fig. 4A).

DISCUSSION

In the heart of early juveniles of *Ligia*, dopamine caused a decrease in the frequency of the myocardial action potential (Fig. 1). The *Ligia* heartbeat begins following an action potential of the myocardium (Yamagishi and Hirose, 1997). Moreover, the heart of early juveniles is myogenic and the heartbeat occurs according to endogenous pacemaker activity of the myocardium in the absence of the cardiac ganglion activity (Yamagishi, 1996; Yamagishi and Hirose, 1997). These facts indicate that dopamine causes a negative chronotropic effect (decrease in beat frequency) on the myogenic heart of early juveniles affecting the pacemaker activity of the myocardium. Moreover, dopamine changed also the contour of the myocardial action potential increasing duration and amplitude of the action potential plateau (Fig. 2). The myocardial action potential of *Ligia* is composed of a plateau potential and spike potentials superimposing on it (Yamagishi, 1996). This fact indicates that dopamine reinforces the plateau potential of the myocardium, as observed in the adult neurogenic heart (Yama-

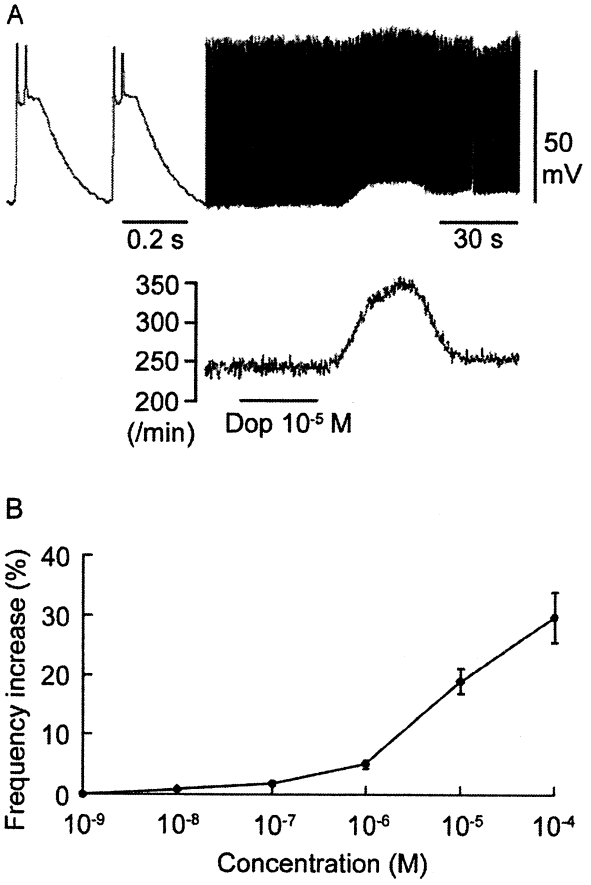

Fig. 3. Effects of dopamine on the heart rate of immature adults. (A) Membrane potential of the myocardial cell (upper trace) and a plot of instantaneous frequency of the myocardial action potentials (lower trace). Dopamine (10^{-5} M) was applied during the period (30 s) indicated by the horizontal black bar. From an immature adult 31 days after hatching. (B) Relationship between rate of increase (%) in the action potential frequency and concentration of dopamine (M). Each data point shows the mean±SEM (N=9).

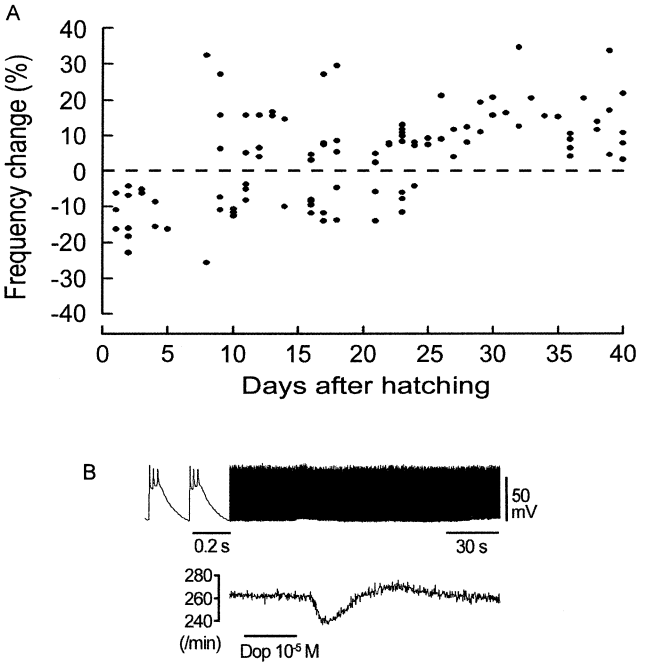

Fig. 4. Relationship between the effect of dopamine on heart rate and the developmental stage. (A) Maximum changes (%) in the frequency of the myocardial action potentials in response to dopamine were plotted against developmental stages (days after hatching). Dopamine (10^{-5} M) was applied for 30 s while recording the membrane potential of myocardial cell. The data were collected from 80 preparations including juveniles and immature adults (1 to 40 days after hatching). (B) Biphasic response to dopamine. Membrane potential of the myocardial cell (upper trace) and instantaneous frequency of the myocardial action potentials (lower trace) are shown. Dopamine (10^{-5} M) was applied during the period (30 s) indicated by the horizontal black bar. From a juvenile 23 days after hatching.

gishi *et al.*, 2004). Contractile force of the *Ligia* cardiac muscle depends on the absolute value of the membrane potential change (Sakurai and Yamagishi, 1998; Yamagishi *et al.*, 2004), as reported in crustacean skeletal (Orkand, 1962; Atwood and Dorai Raj, 1964) and cardiac muscles (Brown, 1964; Holley and Delaleu, 1972). Hence, reinforcement of the myocardial plateau potential results in a positive inotropic effect (increase in contractile force) on the heart. The results lead to the conclusion that, in the myogenic heart of early juveniles, dopamine causes a negative chronotropic effect affecting the myocardial pacemaker activity and a positive inotropic effect reinforcing the myocardial plateau potential.

In the heart of immature adults after juvenile development, dopamine increased the action potential frequency (Fig. 3) and reinforced the action potential plateau. In the neurogenic heart of mature adults, dopamine causes a positive chronotropic effect in the cardiac ganglion and a positive inotropic effect in the myocardium (Yamagishi *et al.*, 2004); this fact suggests that the heart of immature adults has been neurogenic already and the chronotropic effect of dopamine is reversed from negative to positive during juvenile development.

The heart of middle and late juveniles responded to dopamine by an increase or a decrease in the frequency of the myocardial action potential (Fig. 4A). Transfer of the cardiac pacemaker in the *Ligia* heart does not occur simultaneously among the juveniles released from the same mother but occurs individually during middle and late juvenile stages (Yamagishi and Hirose, 1997). Individual variation in the time course of pacemaker transfer would result in a variety of heart responses to dopamine among juveniles of the same stage. Moreover, biphasic responses of the action potential frequency to dopamine obtained from some preparations (Fig. 4B) might reflect an unstable condition of the heart at the beginning of generation of the cardiac ganglion activity; the neurogenic and myogenic activities appear asynchronously (Yamagishi and Hirose, 1997).

Fig. 5 is a schematic drawing of developmental changes in dopamine modulation of the *Ligia* heart concluded from the results of previous (Yamagishi *et al.*, 2004) and present studies. In the myogenic heart of early juveniles, dopamine affects the myocardium causing negative chrono-

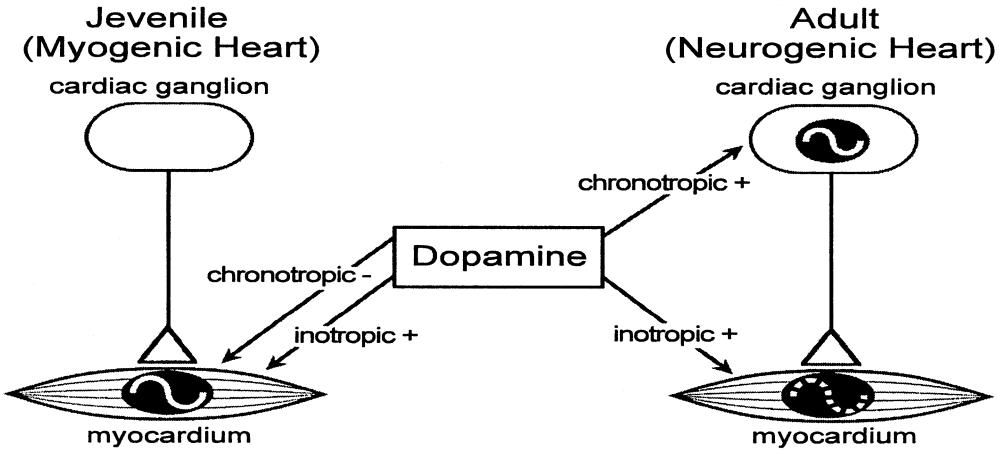

Fig. 5. A schematic drawing of developmental changes in dopamine modulation of the heart in *Ligia exotica*. See text for further details.

tropic and positive inotropic effects. Contrastingly, in the neurogenic heart of adults, dopamine affects both the cardiac ganglion causing a positive chronotropic effect and the myocardium causing a positive inotropic effect. Thus, the chronotropic effect of dopamine is reversed from negative to positive in association with the cardiac pacemaker transfer from the myocardium to the cardiac ganglion during juvenile development. Moreover, the chronotropic and inotropic effects of dopamine were similar in time course in the juvenile myogenic heart but different in the adult neurogenic heart. It has been shown in pacemaker bursting neurons and myocardial cells that prolongation of the plateau potential results in an increase in the following interburst (inter action potential) interval (crustacean cardiac ganglion neuron, Benson, 1980; molluscan pacemaker neuron, Pinsker, 1977; molluscan myocardial cell, Ebara and Ohshima, 1993). The chronotropic and inotropic effects of dopamine appeared to be parallel in time course in the juvenile myogenic heart (Fig. 2B); this fact suggests the presence of a correlation between duration and frequency of the action potential in the myocardial pacemaker activity. However, investigations on ionic channels involved in the pacemaker activity of the myocardium are required to understand the precise mechanism of dopamine modulation. While, in the adult neurogenic heart, the chronotropic effect of dopamine appears with a faster and shorter time course than that of the inotropic effect (Yamagishi *et al*., 2004); this fact may indicate that there is some difference in the signal transduction mechanism for dopamine between the cardiac ganglion neuron and myocardial cell.

In the *Ligia* heart, developmental changes associated with the cardiac pacemaker transfer have been found in acceleratory and inhibitory nervous regulation (Sakurai *et al*., 1999; Yamagishi *et al*., 2001). The *Ligia* cardioregulatory systems appear to change during development in association with the change of target tissue. Recently, it has been reported that, in the neurogenic heart of the decapod *Metapenaeus ensis*, the chronotropic effect of serotonin (5-hydroxytryptamine, 5-HT) on the heart is reversed from negative to positive during juvenile development (McMahon *et al*., 2002). Transfer of the cardiac pacemaker during development might occur in the neurogenic heart of decapods, but no direct morphological and electrophysiological evidence has been shown. Further investigations of comparative morphology and physiology of cardiac development in crustaceans are required.

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