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# Aminergic Modulation of the Myogenic Heart in the Branchiopod Crustacean *Triops longicaudatus*

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**ABSTRACTS**—Although crustaceans typically have a neurogenic heart, the primitive crustacean *Triops longicaudatus* has a myogenic heart with the heartbeat arising from the endogenous rhythmic activity of the myocardium. In the present investigation, the effects of six biogenic amines, epinephrine, norepinephrine, dopamine, octopamine, serotonin and histamine, on the myogenic heart of *T. longicaudatus* were examined. Epinephrine, norepinephrine, dopamine and octopamine accelerated the heartbeat, increasing both the frequency and amplitude of the action potential of the myocardium in a concentration dependent manner. The ability of epinephrine and norepinephrine to produce the acceleratory effects was more potent than that of dopamine and octopamine; the threshold concentrations of epinephrine and norepinephrine were approximately  $10^{-10}$  M and those of dopamine and octopamine approximately  $10^{-7}$  M. Serotonin weakly inhibited the heartbeat, decreasing both the frequency and amplitude of the myocardial action potential in a concentration dependent manner with a threshold concentration of approximately  $10^{-6}$  M. Histamine exhibited no effect on the heartbeat. The results provide the first evidence for direct effects of amines on the crustacean myocardium and suggest neurohormonal regulation of the myogenic heart in *T. longicaudatus*.

**Key words:** myogenic heart, modulation, biogenic amines, crustacea, *Triops longicaudatus*

## INTRODUCTION

The heart of many crustaceans is neurogenic with the cardiac ganglion acting as a pacemaker (reviewed by Maynard, 1960; McMahon *et al.*, 1997). The primitive crustacean (Branchiopoda) *Triops longicaudatus*, however, has no neurons in the heart and the heartbeat is myogenic with the myocardium serving a pacemaker function (Yamagishi *et al.*, 1997). Moreover, the crustacean neurogenic heart is regulated by inhibitory and acceleratory nerves from the central nervous system via synapses on the cardiac ganglion neurons or both the cardiac ganglion neurons and myocardial cells (reviewed by McMahon *et al.*, 1997). Gamma aminobutyric acid (GABA) is suggested to be a transmitter of the cardioinhibitory neurons and acetylcholine (ACh) or dopamine of the cardioacceleratory neurons (reviewed by Yazawa and Kuwasawa, 1992; McMahon *et al.*, 1997; Wilkens, 1999). However, morphological examination has revealed no neural elements in the heart of *T. longicaudatus* and neither GABA nor ACh affect the myogenic activity of the heart (Yamagishi *et al.*, 2000). It appears

unlikely that the heart of *T. longicaudatus* is regulated neurally from the central nervous system.

On the other hand, the crustacean neurogenic heart is also regulated neurohormonally by several amines and peptides released from the pericardial organ (reviewed by Cooke and Sullivan, 1982; McMahon *et al.*, 1997; Cooke, 2002). The amines, however, appear to affect the cardiac ganglion but not directly the myocardium (Wilkens, 1999). To explore the possibility of neurohormonal regulation of the heart of *T. longicaudatus*, the effects of six biogenic amines (epinephrine, norepinephrine, dopamine, octopamine, serotonin, histamine) on the myogenic heart were examined.

## MATERIALS AND METHODS

Adults of the freshwater tadpole shrimp *Triops longicaudatus* (LeConte), 17 to 26 mm in body length, were collected locally from rice fields and maintained in the laboratory (Yamagishi *et al.*, 1997). More than a hundred specimens were used for the experiments.

Anatomy of the heart and the method of dissection were described previously (Yamagishi *et al.*, 1997, 2000). Briefly, the heart was isolated together with the dorsal carapace and fixed in the Silgard seated experimental chamber (2.5 ml in volume), the ventral side up, by pinning through the dorsal carapace. In some cases, the heart was also used isolated completely. The chamber was continuously perfused (2–3 ml / min) with aerated physiological

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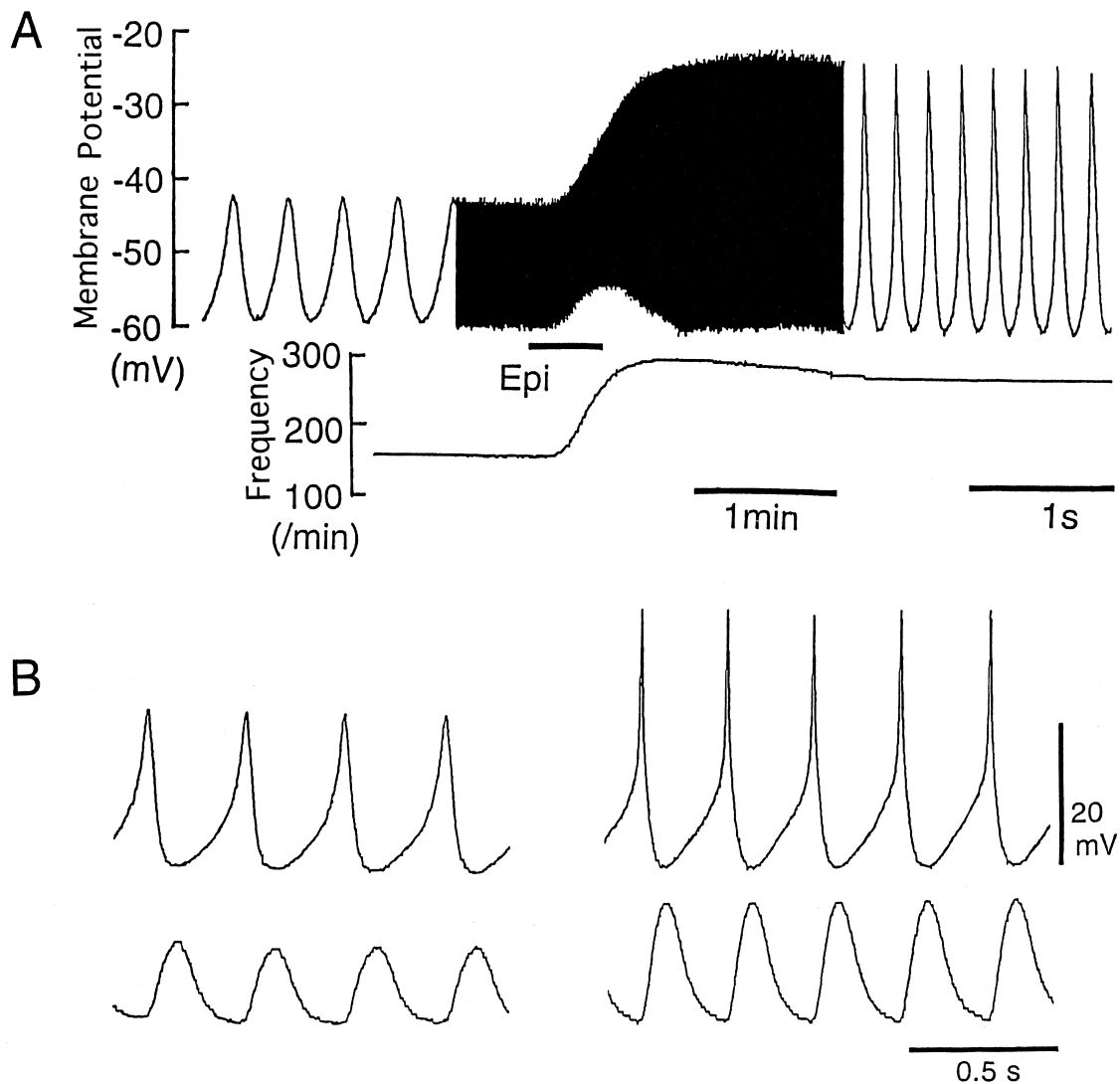
saline of the following composition (mM): NaCl 75, KCl 5, CaCl<sub>2</sub> 2, MgCl<sub>2</sub> 1, Tris-HCl (pH 7.4) 5 (Yamagishi *et al.*, 1997).

Intracellular potential of the myocardial cells was recorded using a conventional glass capillary microelectrode filled with 3 M KCl (resistance, 10–30 MΩ). Contraction of the heart was monitored by changes in the tip potential of a high-resistance microelectrode placed lightly on the ventral heart wall. To determine instantaneous frequency of the myocardial action potential, a heart rate counter (Nihon Kodan AT 601G) was used. The signals were displayed on a cathode ray oscilloscope, stored in an FM magnetic tape recorder and recorded on a chart recorder (thermal-dot alley type, GRAPHTECH, WR 7700).

The following chemicals were used for the experiments: epinephrine (Merck), norepinephrine (Aldrich), dopamine (Wako), octopamine (Wako), serotonin (5-HT) (Sigma), histamine (Wako). Each chemical was made up in saline just before use and was applied to the heart by changing the perfusing saline.

## RESULTS

The heartbeat of *T. longicaudatus* is myogenic and each heartbeat follows a spontaneous graded action potential of the myocardium (Yamagishi *et al.*, 1997). The frequency and amplitude of the myocardial action potential in the heart preparations used were in the range of 150 to 230 /min and of 14 to 22 mV, respectively. The maximum hyperpolarization between the action potentials was in the range of –56 to –64 mV. Among six biogenic amines examined, epinephrine, norepinephrine, dopamine and octopamine exhibited acceleratory effects and serotonin inhibitory effects on the heartbeat. Histamine had no effect at concentrations of up to 10<sup>–3</sup> M.



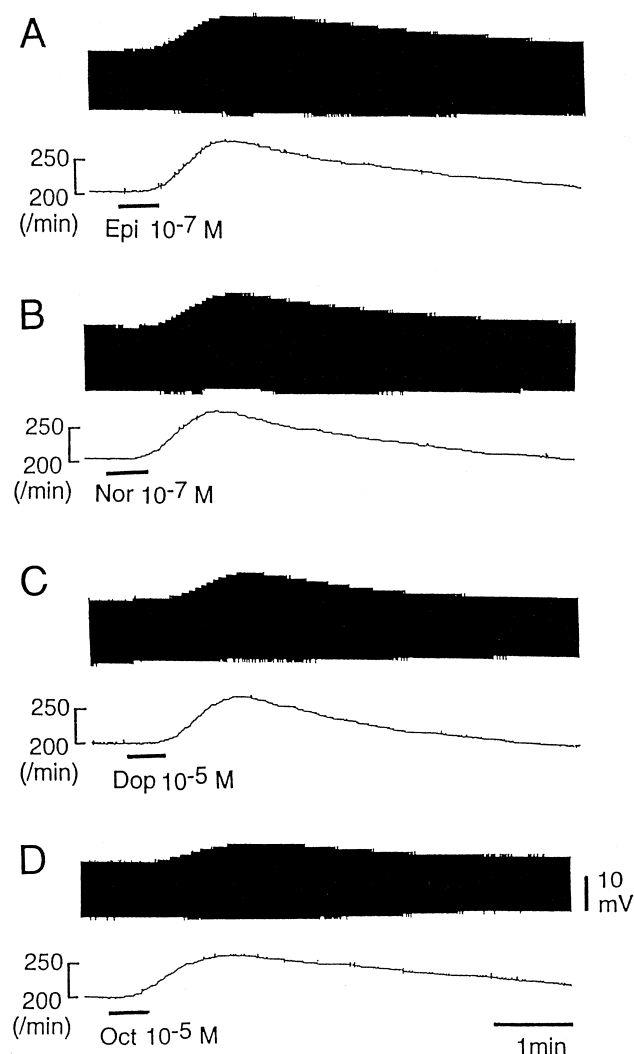
**Fig. 1.** Effects of epinephrine on myocardial electrical activity and contraction of the heart. (A) Membrane potential of the myocardial cell (upper trace) and frequency of the myocardial action potential (lower trace). Perfusing solution was normal saline except during the period (30 s) indicated by the horizontal bar under the record, when the saline contained 10<sup>–6</sup> M epinephrine (Epi). Note the different time scale for the central portion of the trace. (B) Membrane potential of the myocardial cell (upper trace) and contraction of the heart (lower trace) were recorded simultaneously. Before (left) and 1 min after application of epinephrine (10<sup>–6</sup> M, 30 s) (right) are shown. A and B were from different preparations.

### Acceleratory effects

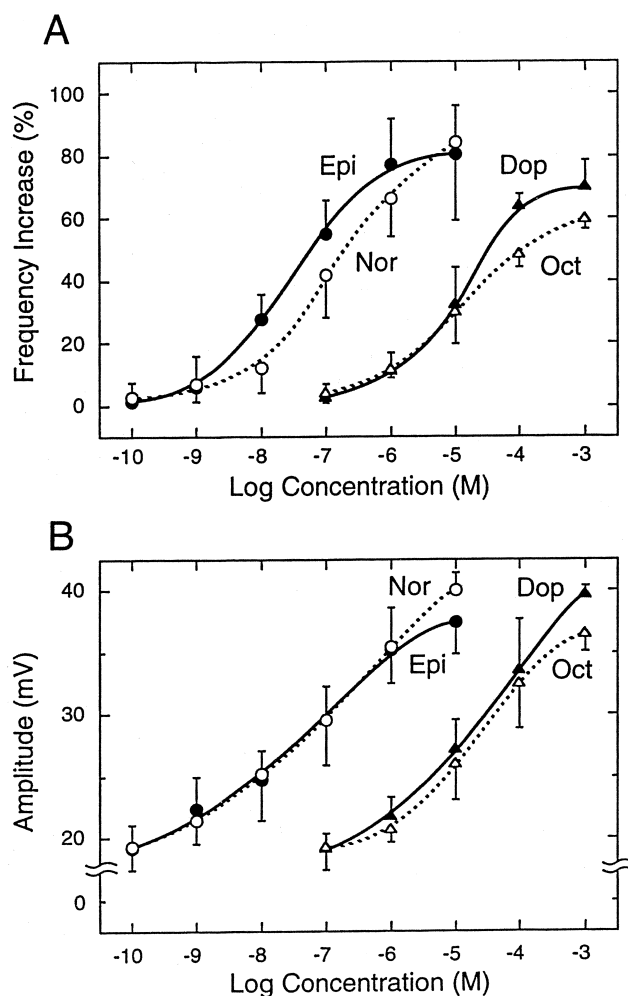
Fig. 1A shows a representative example of the acceleratory effects of epinephrine. Electrical activity recorded intracellularly from the myocardial cell (upper trace) and the frequency of the myocardial action potential (lower trace) are shown. Application of  $10^{-6}$  M epinephrine for 30 s caused a 77.1% increase in the frequency (from 162 to 287 /min) and an 111.1% increase in the amplitude (from 18 to 38 mV) of the myocardial action potential. After being returned to normal saline, the frequency and amplitude of the myocardial action potential decreased gradually to the control level observed before the application of epinephrine. In addition, epinephrine caused an initial depolarizing

response in the myocardial membrane; the maximum hyperpolarization of the myocardial membrane decreased slightly (approximately 6 mV) and returned soon to the control level. Simultaneous recordings of electrical and mechanical activities of the myocardium revealed that, by application of epinephrine, both the frequency and amplitude of the heartbeat increased in association with the increases in those of the myocardial action potential (Fig. 1B).

Fig. 2 shows the effects of the four acceleratory amines to the electrical activity of the myocardium obtained in the same heart preparation. Application of  $10^{-7}$  M epinephrine



**Fig. 2.** Effects of epinephrine, norepinephrine, dopamine and octopamine on myocardial electrical activity of the heart. Membrane potential of the myocardial cell (upper trace) and frequency of the myocardial action potential (lower trace) are shown in each record. Perfusing solution was normal saline except during the period (30 s) indicated by the horizontal bar under each record, when the saline contained the indicated amine. (A) Epinephrine (Epi)  $10^{-7}$  M, (B) Norepinephrine (Nor)  $10^{-7}$  M, (C) Dopamine (Dop)  $10^{-5}$  M, (D) Octopamine (Oct)  $10^{-5}$  M. All records were obtained from the same heart preparation.



**Fig. 3.** Dose-response relationships of epinephrine (Epi), norepinephrine (Nor), dopamine (Dop) and octopamine (Oct). (A) Relationships between rate of frequency increase of the myocardial action potential and amine concentration. Rate of increase (%) in the frequency of the myocardial action potential is plotted against log concentration (M) of the amine applied. Control frequency of the myocardial action potential before application of amines ranged from 146 to 237 /min. (B) Relationships between action potential amplitude and amine concentration. Amplitude (mV) of the myocardial action potential is plotted against log concentration (M) of the amine applied. Control amplitude of the myocardial action potential before application of amines ranged from 16 to 22 mV. In A and B, each plot shows the mean value (Mean  $\pm$  SEM) obtained from 5 to 16 preparations.

(A) and norepinephrine (B) caused a 27.7% (from 210 to 260 / min) and a 24.2% (from 211 to 262 / min) increases in the frequency and a 62.5% (from 16 to 26 mV) and a 42.1% (from 19 to 27 mV) increases in the amplitude of the myocardial action potential, respectively. Application of  $10^{-5}$  M dopamine (C) and octopamine (D) caused a 24.1% (from 203 to 252 / min) and a 23.4% (from 205 to 253 / min) increases in the frequency and a 36.8% (from 19 to 26 mV) and a 37.5% (from 16 to 22 mV) increases in the amplitude of the myocardial action potential, respectively.

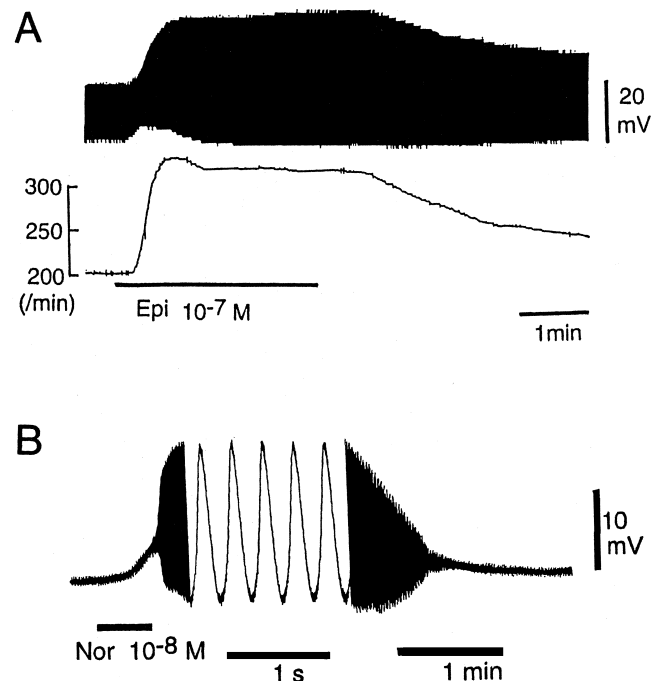
To obtain dose-response relationships of the acceleratory amines, each amine was applied at various concentrations. Both the frequency and amplitude of the myocardial action potential increase in a concentration-dependent manner (Fig. 3). The threshold concentrations of epinephrine and norepinephrine were approximately  $10^{-10}$  M; the effects of epinephrine saturated at approximately  $10^{-6}$  M and those of norepinephrine appeared to saturate at a higher concentration. On the other hand, the threshold concentrations of dopamine and octopamine were approximately  $10^{-7}$  M and the effects saturated at approximately  $10^{-3}$  M. The ability of epinephrine and norepinephrine to produce the acceleratory effects to the myocardial electrical activity was about  $10^3$  times as potent than that of dopamine and octopamine.

The time course of the effects of prolonged application of the four acceleratory amines was next examined. With epinephrine application for 3 min at a concentration of  $10^{-7}$  M, the frequency of the myocardial action potential increased rapidly from the pretreatment level of 202 / min to a maximum value of 329 / min, decreased slightly (310 / min) and then remained constant during the rest of the 3-min application period (Fig. 4A). The amplitude of the myocardial action potential increased rapidly from the pretreatment level of 14 mV to a maximum value of 36 mV and then remained constant during the application period. After return to normal saline, both the frequency and amplitude decreased gradually to the original values; the amplitude recovered more slowly than the frequency. Similar results were obtained for the other three acceleratory amines (data not shown).

The effects of the four acceleratory amines were further examined in the heart preparations that had become quiescent after perfusion for several hours. Norepinephrine was applied at a concentration of  $10^{-8}$  M for 30 sec to a quiescent heart (Fig. 4B). Norepinephrine induced a membrane depolarization followed by rhythmic firing of the action potential in the myocardial cell. After being returned to normal saline, the myocardial action potential became gradually smaller in amplitude and vanished. With a higher concentration of epinephrine, the frequency and amplitude of the myocardial action potential were larger and the period of the induced rhythmic activity was longer. Similar results were obtained for the other three acceleratory amines (not shown).

### Inhibitory effects

Fig. 5A shows the effects of serotonin on the myocar-

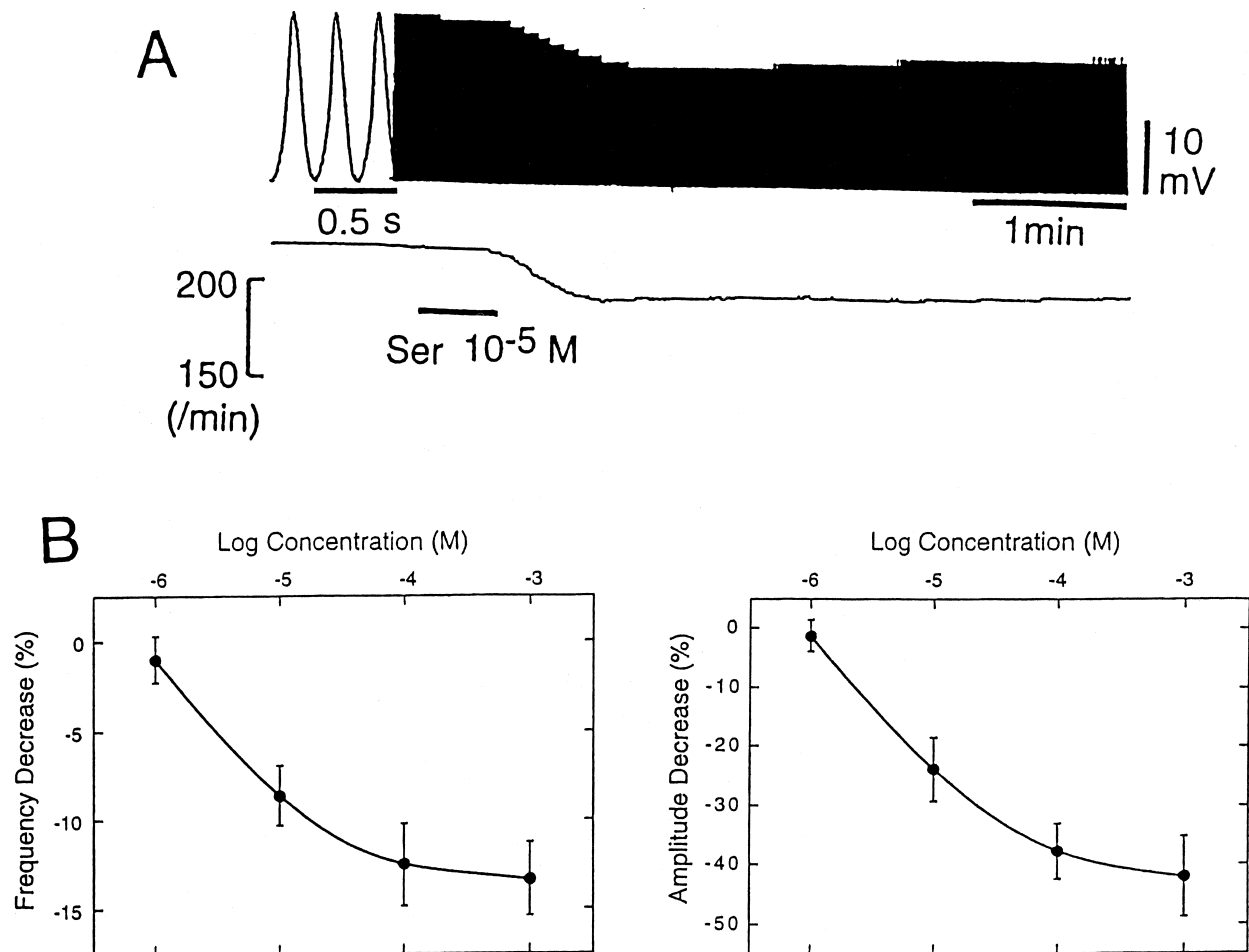


**Fig. 4.** (A) Effects of prolonged application of epinephrine on myocardial electrical activity of the heart. Membrane potential of the myocardial cell (upper trace) and frequency of the myocardial action potential (lower trace) are shown. Perfusing solution was normal saline except during the period (3 min) indicated by the horizontal bar under the record, when the saline contained  $10^{-7}$  M epinephrine (Epi). (B) Effects of norepinephrine on a quiescent heart. Membrane potential response of the myocardial cell to norepinephrine is shown. Resting potential of the myocardial cell was -52 mV. Perfusing solution was normal saline except during the period (30 s) indicated by the horizontal bar under the record, when the saline contained  $10^{-8}$  M norepinephrine (Nor). Note the different time scale for the central portion of the trace.

dial electrical activity of the heart. Application of  $10^{-5}$  M serotonin for 30 s caused a 12.0% decrease in the frequency (from 217 to 191 / min) and a 33.3% decrease in the amplitude (from 24 to 16 mV) of the myocardial action potential. The frequency and amplitude of the myocardial action potential recovered slowly to the original values after washout of serotonin. The frequency and amplitude of the heartbeat were decreased with decreasing those of the action potential of the myocardium (data not shown). The threshold concentration of serotonin inhibition was approximately  $10^{-6}$  M (Fig. 5B). With increasing serotonin concentration, the frequency and amplitude of the myocardial action potential decreased, whereas the maximum rate of the decrease was less than 20% in frequency and 50% in amplitude.

### DISCUSSION

The results of the present study showed that the heartbeat of the branchiopod crustacean *Triops longicaudatus* is modulated by several biogenic amines. There are no intrinsic neurons in the *Triops* heart and the heartbeat is myo-



**Fig. 5.** Effects of serotonin on myocardial electrical activity of the heart. (A) Membrane potential of the myocardial cell (upper trace) and frequency of the myocardial action potential (lower trace) are shown. Perfusing solution was normal saline except during the period (30 s) indicated by the horizontal bar under the record, when the saline contained  $10^{-5}$  M serotonin (Ser). Note the different time scale for the left portion of the trace. (B) (Left) Relationship between rate of frequency decrease of the myogenic activity and serotonin concentration. Rate of decrease (%) in the frequency of the myocardial action potential is plotted against concentration of serotonin applied. Control frequency of the myocardial action potential before application of serotonin ranged from 200 to 239 / min. (Right) Relationship between rate of amplitude decrease of the myocardial action potential and serotonin concentration. Rate of decrease in the amplitude of the myocardial action potential is plotted against concentration of serotonin applied. Control amplitude of the myocardial action potential before application of serotonin ranged from 15 to 24 mV. Each plot shows the mean value (Mean  $\pm$  SEM) obtained from 6 preparations.

genic with the myocardium serving a pacemaker function (Yamagishi *et al.*, 1997). This indicates that the amines modulate the myogenic heartbeat of *T. longicaudatus* by affecting directly the myocardium. Within the Arthropoda, the heart of many insects is myogenic and the heartbeat is modulated by some biogenic amines that affect directly the myocardium (reviewed by Miller, 1985). However, in the neurogenic heart of crustaceans, several biogenic amines modulate the heartbeat by affecting the cardiac ganglion (reviewed by McMahon *et al.*, 1997), but appear not to affect directly the myocardium (Wilkens, 1999). The observations presented in this study are the first evidence for direct effects of biogenic amines on the crustacean myocardium.

The heartbeat of *T. longicaudatus* is produced by spontaneous graded action potentials of the myocardium (Yamagishi *et al.*, 1997). Both the acceleratory and inhibitory amines modulated not only the frequency but also the ampli-

tude of the myocardial action potential. The degree of mechanical tension of the crustacean skeletal and cardiac muscle depends on the absolute value of the membrane potential (Orkand, 1962; Brown, 1964; Holley and Delaleu, 1972). In the neurogenic heart of decapods, the heartbeat is produced by periodic bursts of impulses generated in the cardiac ganglion via excitatory junctional potentials on the myocardium. Therefore, the amines produce chronotropic and inotropic effects to the neurogenic heartbeat by modulating the frequency and impulse pattern of bursting activity of the cardiac ganglion (reviewed by McMahon *et al.*, 1997; Wilkens, 1999; Cooke, 2002). In contrast, in the myogenic heart of *T. longicaudatus*, the amines produced chronotropic and inotropic effects on the heartbeat by modulating both the frequency and amplitude of the myocardial action potential in a concentration dependent manner.

Among some neurotransmitter candidates (glutamate,

Ach, GABA) in the crustacean cardiac and cardio regulatory nervous system, only glutamate induces a depolarizing response in the *Triops* heart; the myocardial action potential increases in frequency during depolarization with low concentrations and, during depolarization with higher concentrations, the action potential adapts with the heart resulting in a systolic arrest (Yamagishi *et al.*, 2000). On the other hand, the amines produced an initial depolarizing response and modulated both the frequency and amplitude of the myocardial action potential in a concentration dependent manner. Moreover, in the myocardium of a quiescent heart, the acceleratory amines induced a depolarizing response that was always followed by rhythmic action potentials. These observations suggest that the modulation mechanisms of the myocardial electrical activity are different between glutamate and biogenic amines.

Epinephrine, norepinephrine, dopamine and octopamine produced acceleratory effects on the myocardial electrical activity of the *Triops* heart; epinephrine and norepinephrine were more potent accelerators than dopamine and octopamine. In addition, serotonin produced inhibitory effects. In the neurogenic heart of decapods, these five amines produce acceleratory effects on the heartbeat by affecting the cardiac ganglion (reviewed by Krijgsman, 1952; Maynard, 1960; McMahon *et al.*, 1997); serotonin, dopamine and octopamine are more potent accelerators than epinephrine and norepinephrine (Yazawa and Kuwasawa, 1992). However, in the isopod *Bathynomus doederleini*, octopamine and serotonin accelerate the neurogenic heartbeat and dopamine, epinephrine and norepinephrine inhibit it (Tanaka *et al.*, 1992). Histamine inhibits the neurogenic heartbeat of the stomatopod *Squilla oratoria* (Ando *et al.*, 1995), but it has no effect on the myocardial electrical activity of the *Triops* heart.

Dopamine, octopamine and serotonin are present in the nervous system of decapods and are released from the pericardial organ as neurohormones (Sullivan *et al.*, 1977; reviewed by Cooke and Sullivan, 1982; McMahon *et al.*, 1997). Norepinephrine is found in the lobster cardiac ganglion (Ocorr and Berlind, 1983), but there have been no reports on the presence of epinephrine in the crustacean nervous system. Investigations on the presence and secretion of biogenic amines in the nervous system of *T. longicaudatus* are required.

The heart of many crustaceans is controlled neurally and neurohormonally from the central nervous system (McMahon *et al.*, 1997). However, the heart of *T. longicaudatus* appears not to be innervated by neural elements from the central nervous system (Yamagishi *et al.*, 2000). The results of the present study might indicate that the heart of *T. longicaudatus* is neurohormonally regulated by some biogenic amines.

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