Amine Modulation of the Neurogenic *Limulus* Heart

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SUMMARY

(1) The biogenic amines octopamine (OCT), dopamine (DA), epinephrine (E), and norepinephrine (NE) cause dose-dependent increases in both the rate and amplitude of contractions of the isolated *Limulus* heart–cardiac ganglion. Their relative ability to produce this excitation is OCT > DA ~ E > NE. (2) The excitatory effects of all these amines are antagonized by the α-adrenergic blocker phentolamine and the dopaminergic antagonist haloperidol. The β-adrenergic antagonist dichloroisoproterenol slightly reduces amine excitation, but is also a partial agonist. The β-adrenergic antagonist propanolol, the α-blocker phenoxybenzamine, and the serotonin antagonist metergoline are ineffective. (3) In addition to their excitatory effects, DA and, to a lesser extent, NE initially reduce contraction rate and amplitude. (4) The transient inhibition is eliminated selectively by metergoline and is unaffected by the other antagonists. (5) The amines all increase the frequency of cardiac ganglion electrical bursting activity, whether ganglia are isolated or attached to cardiac muscle. Dopamine and NE also transiently inhibit the cardiac ganglion. (6) The amines do not alter myocardial resting tension, contractility, or membrane potential. (7) These amines appear to exert their modulatory effects on *Limulus* heart by altering the properties of the neurons which comprise its cardiac ganglion.

INTRODUCTION

The heartbeat of the horseshoe crab *Limulus polyphemus* is neurogenic in origin. A cardiac ganglion on the dorsal surface of the heart generates the

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rhythmic electrical activity responsible for contraction of the heart muscle (Patten and Redenbaugh, 1899; Carlson, 1904). Study of cardiac ganglia of *Limulus* and other arthropods has helped to reveal how simple neural networks generate rhythmic electrical activity (Hagiwara, 1961; Maynard, 1966; Lang, 1971; Friesen and Stent, 1978; Hartline, 1979). However, little is known about how the central nervous system regulates these networks.

The activity of the *Limulus* cardiac ganglion is modulated by cardioregulatory nerves that originate in the central nervous system (Pax and Sanborn, 1964; Pax, 1969; Bursey and Pax, 1970; Watson, 1979). Cardioregulation presumably is mediated via chemical neurotransmitters or neurohormones. This is indicated by the sensitivity of the heart to pharmacological agents and the slow kinetics of the response to cardioregulatory nerve stimulation (Pax and Sanborn, 1967a, b; Pax, 1969). However, the chemical agents that regulate the *Limulus* heart are largely unknown.

Because bath application of γ-aminobutyric acid (GABA) or serotonin reduces heart rate in *Limulus*, both have been proposed as possible cardioinhibitors (Pax and Sanborn, 1967a, b; Abbott, Lang, and Parnas, 1969). Serotonin is the more likely candidate because it is found in the *Limulus* heart (Welsh and Moorhead, 1960) and its effects mimic those of cardioinhibitory nerve stimulation (Pax and Sanborn, 1967a, b).

An excitatory cardioregulatory agent has yet to be conclusively demonstrated in the *Limulus* heart. The heart is excited by acetylcholine (Garrey, 1942) and octopamine (Grega and Sherman, 1975), but physiological roles for these agents have been dismissed previously (Grega and Sherman, 1975).

Recent analysis with neurochemical techniques showed that the *Limulus* cardioregulatory nerves and cardiac ganglion contain epinephrine (EPI), norepinephrine (NE), dopamine (DA) (Watson et al., 1979; O'Connor, Watson, and Wyse, 1982), and octopamine (OCT) (Sullivan, personal communication; Edwards, Pierce, and Battelle, 1979). The presence of these amines suggest that they could be involved in cardioregulation in *Limulus*.

As part of a study of the neural control of the *Limulus* cardiac ganglion, the sensitivity of the *Limulus* heart to all four of these amines was examined. Each excites the heart, accelerating the rate and increasing the amplitude of heart contractions. This report describes the characteristics of these excitatory responses and shows that they originate in the cardiac ganglion. Preliminary accounts of some aspects of this work have appeared (Fetterer and Augustine, 1977; Augustine, Watson, and Fetterer, 1978; Watson et al., 1979).

METHODS

Tissue preparation

Male and female specimens of *Limulus polyphemus* with carapace widths of 5–23 cm were obtained from the supply department of the Marine Biological Laboratory, Woods Hole, MA, or from Martin Fish Co., Ocean City, MD. Animals were maintained in circulating natural or artificial (Instant Ocean) seawater at 10–15°C.

Hearts with their associated cardiac ganglia were removed from animals by the technique of Pax and Sanborn (1964). In most experiments, the cardiac ganglion was left attached to the myocardium to yield an "isolated heart" preparation. These preparations were placed in a Plexiglas chamber of ca. 100-mL volume, and perfused with the salines described below. In other experiments, where the properties of only cardiac ganglia or deganglionated myocardia were considered, the ganglion was dissected away from the muscle using the method of Abbott, Lang, and Parnas (1969). These preparations were placed in chambers of 5–10-mL volume.

Instrumentation

Contractions of isolated heart preparations were monitored with force transducers (Grass FT.03) attached to a lateral boundary of the heart between the fifth and sixth ostia. The resulting tension measurements were displayed on a polygraph recorder and rates were measured either manually or electronically with a rate meter (Grass 7P4 tachograph) or computer (see Watson and Wyse, 1978). Tension was recorded from deganglionated hearts held vertically against an array of Ag–AgCl electrodes (Sasner, 1973) connected to a Grass S9 stimulator. One end of the heart was clamped to the Lucite at the bottom of the electrode assembly and the other end suspended from a force transducer. Electrical pulses were applied to the heart in air and, between stimuli, the heart and electrode assembly were submerged in either normal saline or saline containing amines.

Extracellular recordings of the electrical activity of cardiac ganglia were made with a suction electrode placed on the central portion of the ganglion. Intracellular recordings were obtained from cardiac muscle cells using standard electrophysiological techniques (Abbott, Lang, and Parnas, 1969).

Solutions

Either natural or artificial seawater was used as standard physiological saline (Wyse, 1972). Salines containing drugs were prepared by diluting stock drug solutions at least 100-fold with seawater.

Many of the drugs were either slightly soluble in seawater or oxidized rapidly at neutral pH. Therefore, most drugs were first dissolved in a small quantity of carrier and then diluted with distilled water. Acetic acid (0.1*N*) was used as a carrier for most drugs. Spiramide and haloperidol were made up in 50% dimethyl sulfoxide, metergoline was dissolved in 0.1% ascorbic acid (pH 4.0), and phenoxybenzamine was dissolved in propylene glycol. Control experiments showed that none of these carriers affected heart rates in the concentrations used here. All drugs were obtained from Sigma Chemical Co., St. Louis, MO, with the exceptions of phentolamine (a gift from CIBA-Geigy, Summit, NJ), metergoline (a gift of Dr. Ellen Silbergeld, National Institutes of Health), and phenoxybenzamine (Smith, Kline and French Laboratories, Philadelphia, PA).

Experimental protocol

Preparations were treated with octopamine, dopamine, epinephrine, or norepinephrine by continuously perfusing the preparation chamber with saline containing one of these amines. Amine perfusion was maintained until heart rate reached a steady-state value, typically within 15 to 20 min. The chamber was then rinsed with normal saline for at least 1 h before application of another amine, to allow the preparations to recover their normal rate of beating.

Because of the wide range of contraction amplitudes in individual preparations, changes in heart contraction amplitude could not be quantitively compared. To compensate for the small differences in basal heart rate in different preparations, changes in contraction rates produced by amine treatment were expressed as percentages.

Cumulative dose–response curves for the amines were generated by treating hearts with a $10^{-9}M$ concentration of an amine and progressively increasing the bath concentration of this amine by one-half-log-unit increments until a maximal change in heart rate was observed. These dose–response curves were analyzed quantitatively using the computerized curve-fitting method of DeLean, Munson, and Rodbard (1978). For this cumulative dose–response procedure to accurately measure amine responses, responses must not be reduced by desensitization during cumulative treatments. Three observations suggest that little desensitization occurred. First, prolonged exposure (>30 min) to any of the amines resulted in maintained elevations in heart rate. Second, repeated applications of the same concentrations of amine produced equivalent changes in heart rate throughout the course of each experiment. Third, responses to a given concentration of amine were similar, whether applied alone or as part of a cumulative series of increasing concentrations.

To compare the effects of various pharmacological antagonists, preparations were first treated with an amine and then rinsed by perfusion with drug-free saline until the heart rate had returned to its pretreatment value. A low dose of antagonist was then applied for 15 min, followed by the control dose of amine plus antagonist. After again rinsing the preparation for 1 h, the responsiveness of the heart was reexamined by treating with the amine alone. This procedure was continued with increasing antagonist concentrations so that antagonist dose–response curves could be plotted. To avoid possible complications due to competitive agonist–antagonist interactions (Waud, 1976), we used amine concentrations that produced roughly equivalent increases in heart rate in the absence of antagonists ($10^{-7}M$ OCT, $10^{-6}M$ DA, $10^{-6}M$ E, and $5 \times 10^{-6}M$ NE).

RESULTS

Response of the Limulus *heart to biogenic amines*

Treatment of the *Limulus* heart–cardiac ganglion preparation with OCT, DA, EPI, or NE resulted in a net increase in the rate and amplitude of heart contractions (Fig. 1). At all concentrations examined, OCT produced the greatest increase in heart rate, followed by DA, EPI, and NE.

Differences in the response of the *Limulus* heart to the four amines are illustrated in the dose–response relationships of Figure 2. The dose–response data shown in this figure were analyzed quantitatively by the curve-fitting analysis of DeLean, Munson, and Rodbard (1978; see the Methods section). This analysis revealed that the efficacy (maximum excitatory response) of OCT, DA, and EPI were comparable, and were substantially greater than that of NE (Table 1). The ED_{50} for DA and NE were similar, ca. ten times higher than that of OCT and three times greater than that of EPI (Table 1). Thus OCT is approximately three to ten times more potent than the other three amines in increasing the rate of the *Limulus* heart.

The excitatory effects of all four amines were long-lasting (Fig. 3). Rinsing amine-treated preparations with saline resulted in a gradual, apparently exponential, recovery of heart rate (Fig. 4). The time constant for this exponential

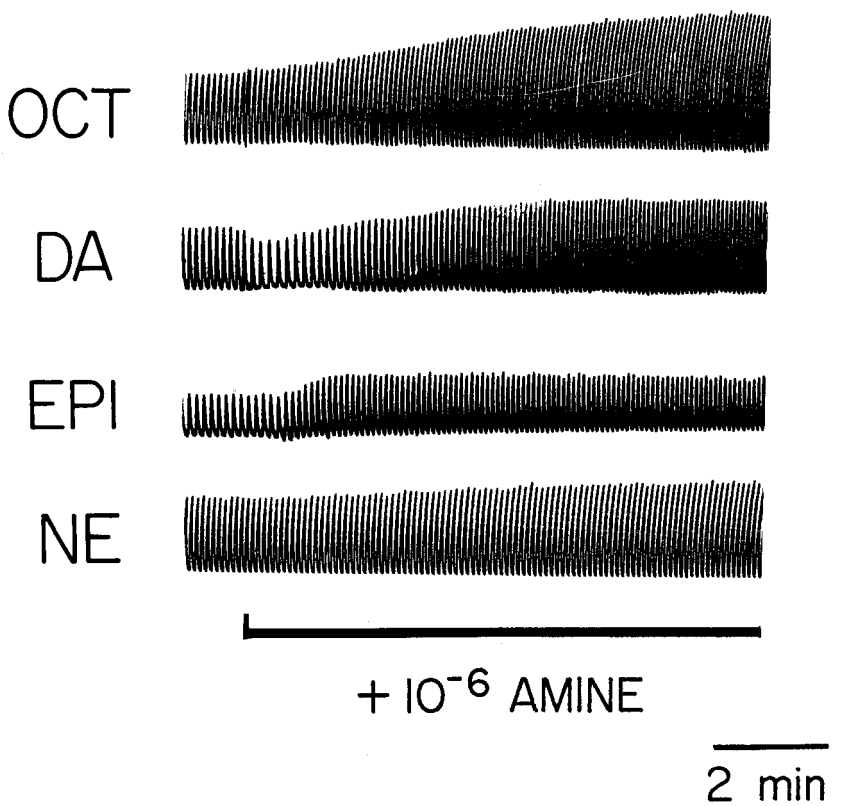

Fig. 1. The effect of amines on the heartbeat of isolated heart–cardiac ganglion preparations. Traces represent heart contractions, as measured by a force transducer attached to the lateral margin of the heart muscle. Bar indicates the time period that octopamine (OCT), dopamine (DA), epinephrine (EPI), and norepinephrine (NE) were added to the bathing medium at a concentration of $10^{-6}M$.

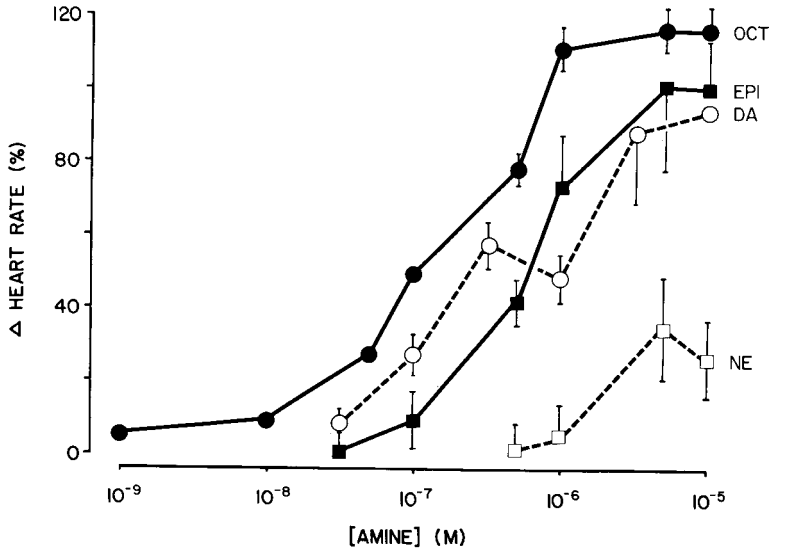

Fig. 2. Dose–response curves for the increases in heart rate produced by the four amines. Points represent mean steady-state changes in the rate of four to eight preparations, expressed as a percentage of control rate. Error bars indicate ±1 SEM when this value is larger than the symbols.

recovery was not identical for all amines; DA consistently elevated the rate for a longer period than did roughly equipotent doses of the other three amines (Figs. 3 and 4). The mean time constant for recovery was 20.5 min for $10^{-6}M$ DA (range 14.0–30.9 in 12 experiments), compared to 13.5 min (8.0–18.0; five experiments) for $10^{-7}M$ OCT, 15.8 min (12.0–23.2; seven experiments) for $10^{-6}M$ EPI, and 16.9 min (8.8–23.2; 12 experiments) for $5 \times 10^{-6}M$ NE.

In addition to the amines' different effects on heart rate, each amine had unique onset kinetics (Fig. 5). Octopamine produced a monotonic increase in heart rate that began immediately after drug perfusion and reached a maximum level within 10 min. The response to epinephrine was similar, although its onset was generally delayed by 1–2 min. Dopamine and, to a lesser extent, norepinephrine transiently decreased contraction rate and amplitude for up to 3 min before their excitatory effects began. In general, the inhibitory responses were more variable and less pronounced than the excitation produced by these two amines. Because the responses were multiphasic, one could not quantitatively compare their onset kinetics. However, the impression is that the differing kinetics represent distinct differences in the heart's responsiveness to the amines, rather than diffusion delays caused by bulk mixing within the preparation chamber.

TABLE 1
Relative Excitatory Activity of Amines on the *Limulus* Heart

Amine	Efficacy[a]	ED_{50}[b]
OCT	121 ± 9.5	0.2 ± 0.07
EPI	102 ± 3.6	0.6 ± 0.06
DA	140 ± 43.7	1.8 ± 2.4
NE	34 ± 6.8	2.3 ± 2.3

[a] Percent increase in heart rate ±1 SE.
[b] In μM ± 1 SE.

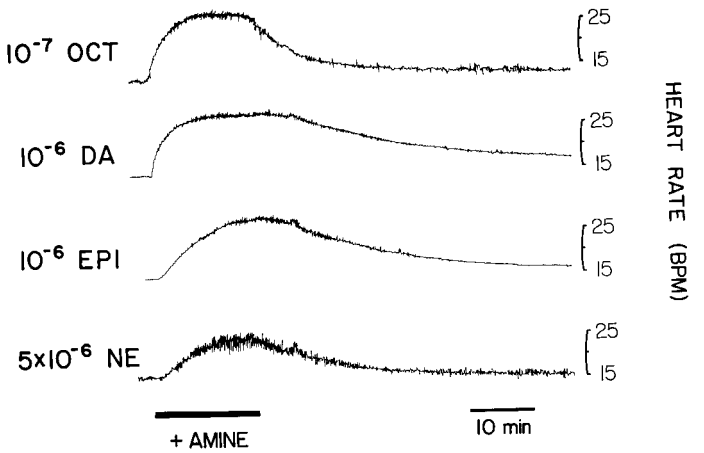

Fig. 3. Time course of amine excitatory effects. Amines were added during the time indicated by the bar and produced approximately equivalent increases in heartbeat rate in the four different preparations. Subsequent rinsing with amine-free seawater gradually reversed the effects.

Pharmacological properties of the amine responses

To characterize further the amine responses of the *Limulus* heart, the responses' sensitivity to pharmacological antagonists was examined. Representative antagonists of α- and β-adrenergic, dopaminergic, and serotonergic responses were tested at concentrations between 10^{-7} and $10^{-4}M$ to determine their ability to block responses of the *Limulus* heart to approximately equipotent doses of each of the amines.

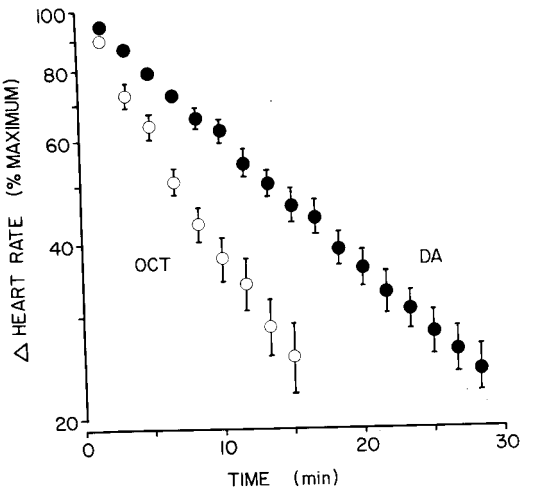

Fig. 4. Semilogarithmic plots of changes in heart rate following treatment with $10^{-7}M$ octopamine (○) and $10^{-6}M$ dopamine (●). Upon rinsing amine-treated hearts with seawater (beginning at time = 0 min), heart rate decreased exponentially. Points represent the mean change in heart rate measured every 100 s for five experiments with octopamine and 12 experiments for dopamine.

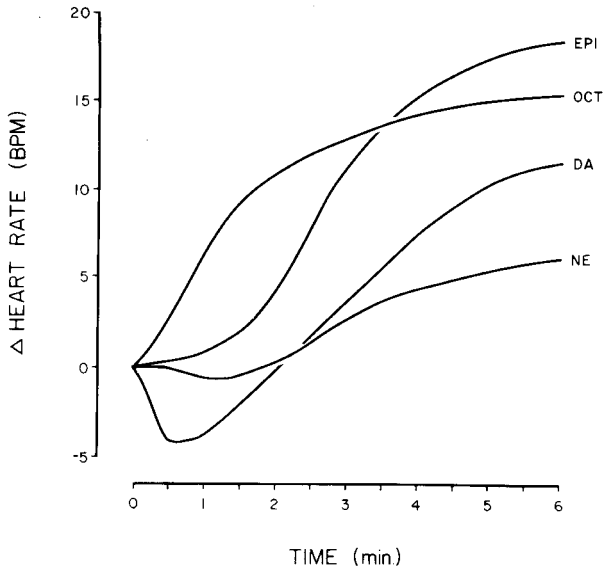

Fig. 5. Diagrammatic representation of amine response onset kinetics. At time zero four heart–cardiac ganglion preparations were treated with $5 \times 10^{-6}M$ concentrations of one of four amines. Tracings represent plots of the instantaneous change in heart rate produced by these amines, as calculated from extracellular recordings of cardiac ganglion electrical activity.

α-Adrenergic antagonists

Phentolamine and phenoxybenzamine are α-adrenergic antagonists of relatively high specificity in mammals (Goodman and Gilman, 1975; Walker and Kerkut, 1978). Phentolamine reversibly reduced the excitatory effects of all

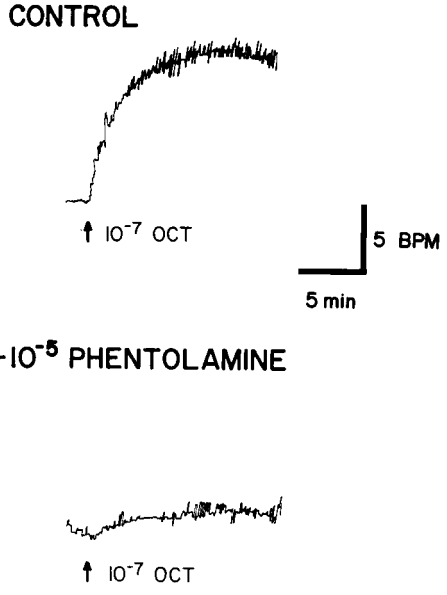

Fig. 6. Blockage of octopamine-induced excitation of the *Limulus* heart by the α-adrenergic antagonist phentolamine. The top tachygraph record (control) shows the heart rate change to bath application of $10^{-7}M$ octopamine (continuously, beginning at arrow), while the bottom record ($+10^{-5}M$ phentolamine) illustrates the response of the same preparation to $10^{-7}M$ octopamine after treatment with $10^{-5}M$ phentolamine.

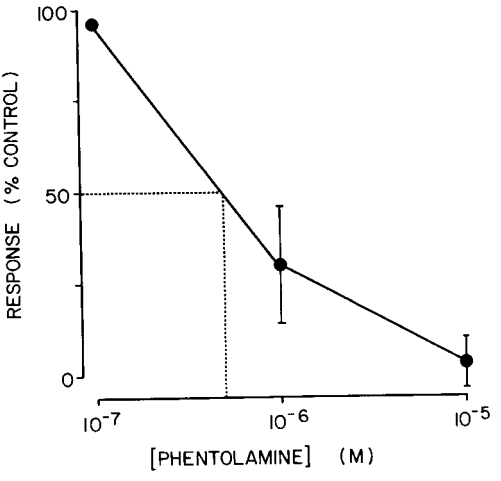

Fig. 7. Dose–response curve for the inhibition of the excitatory octopamine response by phentolamine. Ordinate expresses the relative change in heart rate produced by $10^{-7}M$ octopamine in the presence of phentolamine, compared to the response to octopamine alone. Each point represents the mean of two to four determinations at each phentolamine concentration. The ID_{50} for this inhibition, estimated by interpolation (···), is ca. $5 \times 10^{-7}M$.

four amines (Figs. 6 and 7). The concentration of phentolamine required to reduce the response by 50% (the ID_{50}) was estimated from plots comparable to Figure 7. The ID_{50} values for phentolamine suggest that the response to some amines, i.e., OCT and DA, are more sensitive to phentolamine than others, such as NE and EPI (Table 2). However, our method of estimating the ID_{50} is not sufficiently accurate to permit discrimination between ID_{50} values differing by less than approximately 50-fold.

Phenoxybenzamine was without effect at $10^{-5}M$, the highest concentration considered. Neither α-adrenergic blocker affected the transient inhibition produced by DA or NE. Neither phentolamine nor phenoxybenzamine had

TABLE 2
Summary of the Effects of Several Pharmacological Antagonists on the Excitatory Amine Responses of the *Limulus* Heart[a]

Antagonist	Amine			
	$10^{-6}M$ EPI	$10^{-7}M$ OCT	$10^{-6}M$ DA	$5 \times 10^{-6}M$ NE
α-Adrenergic				
Phentolamine	>10[b]	0.5	1	10
Phenoxybenzamine	*[c]	*	*	*
β-Adrenergic				
Propanolol	>100	>100	—[d]	—
Dichloroisoproterenol	50	>100	100	50
Dopaminergic				
Haloperidol	>10	>10	10	5
Serotonergic				
Metergoline	*	*	*	*

[a] Values represent the ID_{50}, namely, the concentration of antagonist (in μM) which reduced the response of the *Limulus* heart to the indicated amine by 50%. These values were determined from two to five replicates at each concentration of antagonist.

[b] >: Inhibited responses <50% at the highest concentration tested.

[c] *: Ineffective at $10\mu M$.

[d] —: Ineffective (i.e., produced <20% reduction) at $100\mu M$.

agonistic properties. However, at concentrations greater than $10^{-5}M$, phentolamine frequently produced irregularities in the heartbeat rhythm, possibly due to the known nonspecific effects of adrenergic antagonists on excitable membranes (Sada, 1978) and/or interference with synaptic mechanisms responsible for coordinating the heartbeat (Lang, 1971).

β-Adrenergic antagonists

Propanolol and dichloroisoproterenol were examined as representative β-adrenergic antagonists (Goodman and Gilman, 1975). Propanolol slightly reduced amine excitation at concentrations of $10^{-4}M$, but was ineffective at lower doses (Table 2). At high concentrations, dichloroisoproterenol also reduced the excitatory effects of amines (Table 2). This drug also had substantial agonstic activity, increasing heart rate by a mean value of 42.3% (SEM = 5.35) in eight experiments at $10^{-4}M$. Neither β-adrenergic antagonist altered the transient inhibition of DA or NE.

Dopaminergic antagonist

Haloperidol, an antagonist of dopamine-sensitive adenylate cyclase and DA receptors in the vertebrate brain (Woodruff, 1971; Steinsland and Hieble, 1978), blocked excitation weakly and was ineffective in blocking the transient inhibition (Table 2). Halperidol was also a slight agonist and was reversible.

Serotonin antagonist

Metergoline, an effective serotonin antagonist in the vertebrate CNS (Beretta, Ferrini, and Glasser, 1965; Sastry and Phillis, 1977), selectively and irreversibly blocked the transient inhibition produced by DA (Fig. 8) and NE without impairing the excitatory responses to any of the four amines (Table 2).

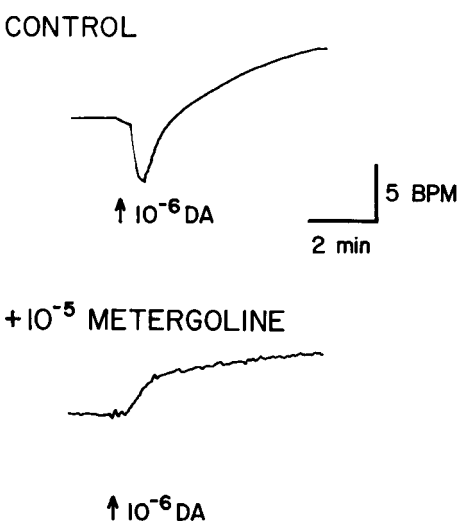

Fig. 8. Antagonistic effect of metergoline on the biphasic response to dopamine. The top tachygraph record (control) shows the changes in heart rate produced by application of $10^{-6}M$ dopamine (continuously, beginning at arrow). When dopamine was added in combination with $10^{-5}M$ metergoline (+ 10^{-5} metergoline) the transient decrease in rate was eliminated, but the secondary excitation was unaffected.

Site of amine action

The *Limulus* heart is neurogenic; thus agents which change its heart rate should act by altering the activity of its cardiac ganglion. Electrical activity recorded extracellularly from cardiac ganglia attached to isolated hearts was indeed altered by all the amines. An example of the action of DA is shown in Figure 9. As heart rate increased, cardiac ganglion burst frequency increased and burst duration and interburst interval decreased. To further test whether responses to the amines were localized in the cardiac ganglion, electrical activity was recorded from cardiac ganglia removed from the myocardium. The amines produced dose-dependent changes in the burst activity of such isolated ganglia which were similar to those observed in ganglia attached to heart muscle [Fig. 10(A)]. DA and NE also transiently inhibited isolated ganglia [Fig. 10(B)].

Although the amines appear to act upon the cardiac ganglion, they could also affect cardiac muscle directly. This possibility in *Limulus* was examined by determining the effect of amines on hearts with their cardiac ganglia removed. Treating deganglionated hearts with any of the four amines did not change resting tension or contractions elicited by electrical stimulation of the cardiac muscle. Further, intracellular recordings from individual muscle cells revealed no changes in their resting potentials after exposure to any of the four amines. These data indicate that the amines do not directly affect *Limulus* muscle fibers. When combined with responses of isolated cardiac ganglia to amines, these results suggest that modulation of the *Limulus* heart by biogenic amines probably results from direct action upon the neurons which comprise the cardiac ganglion.

DISCUSSION

OCT, DA, EPI, and NE all excite the isolated *Limulus* heart–cardiac ganglion preparation in a dose-dependent manner. This excitation is slow in onset and lasts for many minutes after the amines are removed from the perfusion chamber. Dopamine and NE produce an additional transient inhibition which precedes the excitatory response. These two effects are pharmacologically distinct, as indicated by their differential sensitivity to these amines and to aminergic antagonists. Both the excitatory and inhibitory responses appear to be localized to the cardiac ganglion, rather than the heart muscle itself.

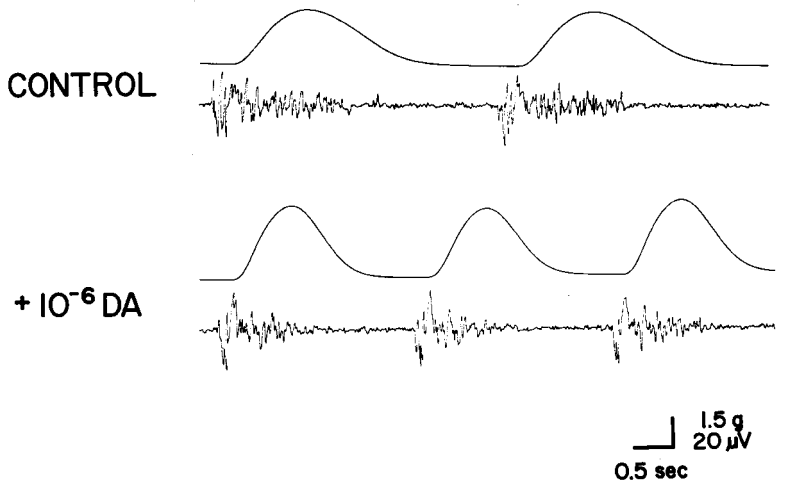

Fig. 9. Simultaneous recordings of myocardial tension (upper traces) and ganglionic electrical activity (lower traces) from an isolated heart in seawater (control) and five minutes after treatment with $10^{-6}M$ dopamine (+ 10^{-6} DA).

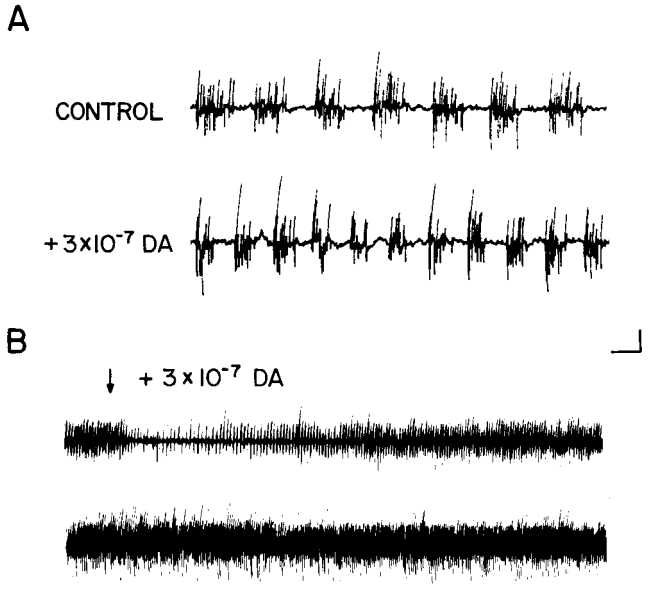

Fig. 10. Response of an isolated cardiac ganglion to dopamine. The upper portion (A) shows typical extracellular electrical recordings obtained from an isolated cardiac ganglion before (control) and after (+ 3×10^{-7} DA) addition of $3 \times 10^{-7} M$ dopamine. Calibration: 10 μV, 1 s. (B) is a continuous record showing the transient inhibition and secondary excitation of ganglionic activity produced by dopamine ($3 \times 10^{-7} M$). Calibration: 20 μV, 45 s.

The pharmacology of the excitatory amine responses of the *Limulus* heart is similar to that of a variety of invertebrate OCT responses. Locust muscle (Evans and O'Shea, 1978), the firefly lantern organ (Carlson, 1968; Oertel and Case, 1976), and several *Helix* central neurons (Batta, Walker, and Woodruff, 1979) are all more sensitive to OCT, but DA and NE also elicit substantial responses. In these systems, as well as in the *Limulus* heart, the α-adrenergic antagonist phentolamine is a more effective antagonist than the β-adrenergic antagonists. If the excitatory responses observed in *Limulus* are the result of amine–receptor interactions, one or several receptors may be involved. There appears to be some variability in the amines' sensitivity to pharmacological antagonists, and they have somewhat different onset kinetics. More detailed analyses, with further use of pharmacological antagonists, structure activity (Greenberg, 1970; Woodruff, 1971; Batta, Walker, and Woodruff, 1979), and binding studies (Snyder and Goodman, 1980) might help determine whether they are comparable to the OCT–receptor responses reported in other invertebrates.

The excitatory amine responses have slow onsets and prolonged time courses. The slow time course of these responses may be due to a variety of causes, such as limited access to amine-sensitive sites, slow binding to these sites, slow activation and inactivation of receptors, or activation of cellular metabolism (see Hill-Smith and Purves, 1978). Involvement of cyclic nucleotides in these slow responses is possible in light of evidence that biogenic amines stimulate cyclic nucleotide metabolism in tissues of a variety of arthropods (Nathanson and Greengard, 1974; Sullivan and Barker, 1975; Kravitz et al., 1976; Harmar and Horn, 1977; Grega, 1978; Nathanson, 1978) including *Limulus* (Atkinson, Herman, and Sheppard, 1977).

The biphasic effects of DA and NE on the *Limulus* heart qualitatively resemble the biphasic response to OCT observed in some decapod hearts (Grega and Sherman, 1975; Florey and Rathmayer, 1978). The inhibitory components could be due to a direct interaction with "receptors" mediating cardioinhibition or to release of inhibitory transmitter from presynaptic nerve terminals. This

does not appear to be the case in decapods, because picrotoxin does not affect the transient inhibition produced by OCT, but it does block the effect of inhibitory nerve stimulation (Florey and Rathmayer, 1978). The same is apparently true in *Limulus*. Metergoline blocked the transient inhibition observed with DA and NE, but it does not block the inhibitory effects of serotonin or GABA (W. Watson, unpublished observations), the two candidates for cardioinhibitory agents (Pax and Sanborn, 1967a, b; Abbott, Lang, and Parnas, 1969). Thus in *Limulus* and in some decapods this transient inhibition appears to be pharmacologically distinct from the inhibitory response to cardioregulatory nerves. Such transient inhibitory responses could therefore have a separate cardioinhibitory role.

The cardiac ganglion is a likely target of the amines, since they act on isolated and intact ganglia but apparently do not alter the contactility of heart muscle. Similarly, the decapod cardiac ganglion appears to be the main site of action of pericardial organ amines (Cooke, 1966), although in the neurogenic heart of the isopod *Porcellio* both the cardiac ganglion and musculature are affected by GABA and inhibitory nerve stimulation (Delaleu and Holley, 1976). Because the biogenic amines considered here act upon the isolated cardiac ganglion, the constituent pacemaker and/or follower neurons that make up the ganglion (Palese, Becker, and Pax, 1970; Lang, 1971) must be the amines' cellular site of action. Intracellular studies indicate that amines alter the properties of both pacemaker and follower neurons (Fetterer and Augustine, 1977; Augustine, Fetterer, and Watson, 1979; Augustine and Fetterer, in preparation).

These amine–cardiac ganglion interactions can account for the amine effects on heart rate, but the causes of amine-induced increases in contraction amplitude are uncertain. The pentapeptide proctolin excites the *Limulus* heart by direct effects on muscle contractility, rather than by modulation of the cardiac ganglion (Benson et al., 1981). The amines have no effect on cardiac muscle and thus probably enhance contraction amplitude by altering the properties of follower neurons or neuromuscular transmission. Preliminary experiments (Augustine and Watson, unpublished) indicate amines do potentiate cardiac neuromuscular transmission.

Although the experiments reported here show that these four amines affect the *Limulus* heart, their physiological roles *in vivo* are unclear. Acetylcholine excites the *Limulus* heart (Garrey, 1942) and acetylcholinesterase is present in the cardiac ganglion (Stephens and Greenberg, 1973). The concentration (1 in 10,000 or ca. $5 \times 10^{-4}M$; Garrey, 1942) and acetylcholine required to alter heart rate is substantially greater than that required for the amines, suggesting that acetylcholine may not play a physiological role in this system. However, ganglionic cholinesterase makes it difficult to compare the potency of acetylcholine and the amines. Grega and Sherman (1975) previously dismissed the possibility that OCT could be a cardioregulatory agent. However, in light of the pronounced effects of low concentrations of OCT, DA, EPI, and NE on the *Limulus* heart, and the presence of these amines in the cardiac ganglion and cardioregulatory nerves (O'Connor, Watson, and Wyse, 1981; R. Sullivan, personal communication; Edwards, Pierce, and Battelle, 1979), these compounds seem to be good candidates for excitatory cardioregulatory substances in *Limulus*.

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