Mechanism for Amine Modulation of the Neurogenic *Limulus* Heart: Evidence for Involvement of cAMP

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SUMMARY

The role of cyclic nucleotides as intracellular second messengers mediating the excitatory chronotropic and inotropic actions of octopamine (OCT) and dopamine (DA) on the neurogenic *Limulus* heart was investigated. Tissue levels of cAMP, but not cGMP, were significantly increased in isolated cardiac ganglia and cardiac muscle following 10 min exposure to 10^{-5} *M* OCT or 10^{-5} *M* DA. In both tissues, OCT elicited larger increases in cAMP than did DA. Amine-induced cAMP accumulation in the cardiac ganglion and in the cardiac muscle was prevented by the alpha-adrenergic blocker phentolamine. The adenylate cyclase activator forskolin and the phosphodiesterase inhibitor IBMX produced amine-like chronotropic and inotropic effects when applied to the isolated heart preparation. However, the kinetics of the responses differed for the two agents. Additional pharmacological agents (RO-20-1724, papaverine, SQ 20,009, and 8-parachloro-phenylthio cAMP) also had amine-like effects but to a lesser extent. The chronotropic, but not inotropic, effects of OCT and DA were potentiated in the presence of IBMX. These data suggest that a cAMP-dependent mechanism underlies the excitatory effects of the neuromodulators OCT and DA on the *Limulus* heart.

INTRODUCTION

In the horseshoe crab, *Limulus polyphemus*, the cardiac rhythm is modulated by several amines endogenous to the *Limulus* nervous system, including octopamine and dopamine (Edwards et al., 1979; Augustine et al., 1982; O'Connor et al., 1982). These amines act on several cellular sites within this neuronal/muscular system to produce long-lasting excitation of heart contraction rate and amplitude (Watson and Augustine, 1982). Heart rate is increased by aminergic actions on the cardiac ganglion (Augustine and Fetterer, 1985). Increases in the strength of heart contractions are due to effects of the amines on cardiac muscle contractility and neuromuscular transmission (Watson and Hoshi, 1981; Watson et al., 1985). Although the cellular targets of these amines have been identified, the intracellular mechanisms underlying their actions on these tissues have not been examined.

The role of cyclic nucleotides (cyclic adenosine monophosphate, cAMP, and cyclic guanosine monophosphate; cGMP) as second messengers mediating

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the intracellular effects of peptides and amines is well documented in a variety of vertebrate and invertebrate tissues (for reviews, see Berridge, 1975; Greengard, 1976). Cyclic AMP and cyclic GMP act as intracellular signals for hormones by initiating a series of protein phosphorylations that ultimately result in specific cellular responses (Williams and Rodnight, 1977; Levitan et al., 1983; Winegrad et al., 1983). As a result, hormones acting via cyclic nucleotides usually have potent and long-lasting effects even at relatively low concentrations.

Several previous studies have suggested that cyclic nucleotides are involved in the response of neurogenic hearts to amines. In some species of crabs and lobsters octopamine increases tissue levels of cAMP in the cardiac ganglion and in the cardiac muscle and it also has long-lasting effects on these hearts (Sullivan and Barker, 1975; Battelle and Kravitz, 1978). In *Limulus*, amine-induced excitation of heart contraction rate and amplitude is slow in onset and is long-lasting. For example, peak effect is 10–15 min after amine application, and effects typically last 30–60 min following removal of amines from the bath (Augustine et al., 1982). However, there are no studies to date that clearly define the role of cAMP in the response of neurogenic hearts to amines.

In this study we investigated the relative roles of cAMP and cGMP in amine modulation of the neurogenic *Limulus* heart. We present biochemical and pharmacological evidence which indicates that both the chronotropic and inotropic effects of amines on the *Limulus* heart involve cAMP-, but not cGMP-, dependent mechanisms. A preliminary account of portions of this work has appeared previously in abstract form (Groome and Watson, 1983).

METHODS

Animal Maintenance

Adult horseshoe crabs (*Limulus polyphemus*) were collected from Great Bay, NH, or obtained from the supply department, Marine Biological Laboratory, Woods Hole, MA. Horseshoe crabs were maintained on a diet of mussels (*Mytilus edulis*) in flow-through sea tables (ambient temperature) at the Jackson Estuarine Labs, University of New Hampshire or in recirculating sea tables (10–15°C) at the Zoology Dept, University of New Hampshire. Specimens of either sex (carapace width 15–20 cm) were used in this study.

Determination of cAMP and cGMP Levels in Limulus *Tissue*

Isolated cardiac ganglia or pieces of heart muscle (1 cm rings excised from the region between the 2nd to 6th ostia) were placed in vials containing 5 mL seawater at room temperature. The vials were continuously shaken for 1 h, at which time tests were initiated by the addition of amine solutions. The tissues were removed after a 10 min incubation and homogenized in ice-cold 6% trichloroacetic acid. The homogenates were centrifuged (cardiac ganglia at 10,000 *g*; cardiac muscle at 5,000 *g*) for 30 min at 4°C. The supernatants were extracted with four 5 mL washes of diethyl ether, and the aqueous phase was dried on a vacuum centrifuge and stored (−20°C) until assay. The tissue pellet was boiled in 1*N* NaOH for protein content determination (Lowry et al., 1951).

Levels of cAMP and cGMP in these tissues were determined by radioimmunoassay (RIA). Duplicate cAMP or cGMP standards (Sigma Chemical Co., St. Louis, MO) and samples were diluted in 50 μL RIA buffer B (0.05 *M* sodium acetate at pH 6.0, containing 0.1% gelatin and 0.1% sodium azide) and added to 10 × 75 mm incubation tubes. Tracer (10,000 cpm, ^{125}I-labeled cAMP or cGMP, Chemicon Inc., La Jolla, CA) in 100 μL buffer B containing 1% normal rabbit serum (NRS) was added, and the volume was brought to 600 μL with buffer B. Cyclic AMP or cyclic GMP antibodies (Chemicon) were diluted (cAMP, 1:400; cGMP, 1:200) in 100 μL buffer A

(0.02 *M* sodium phosphate at pH 7.4, containing 0.1% gelatin and 0.1% sodium azide) with 1% NRS and added to each tube, except for the blanks. All tubes were then vortexed and incubated for 1 h at room temperature.

Separation of bound and free ^{125}I was accomplished by adding 100 μL of secondary antibody (25% goat anti-rabbit gamma globulin in buffer A with 1% NRS) to each tube. After 30 min of incubation the tubes were centrifuged (1800 *g*, 4°C) for 30 min, and the supernatant was decanted. The pellets were solubulized (100 μL Protosol, 30 μL glacial acetic acid), mixed with 1 mL scintillation fluor, and counted (Beckman LS 7000).

Counts were converted to pmol cAMP or cGMP by linear regression of the working portion of the standard curve. A series of dilutions with extracts of *Limulus* cardiac ganglia and cardiac muscle tissue displayed parallel inhibition of tracer binding as compared with standards for both cAMP and cGMP antibodies (Fig. 1). Both cAMP and cGMP RIA's were sensitive to 0.1 pmol cyclic nucleotide. Neither cAMP or cGMP antibodies react with other cyclic nucleotides or cyclic nucleotide metabolic products (Chemicon).

Pharmacology

Limulus hearts were removed according to the method of Pax and Sanborn (1967). The isolated heart with associated cardiac ganglion was pinned to the Sylgard resin in the bottom of a small (10 mL) plexiglass chamber and its lateral boundary hooked via thread to a force transducer (Grass FT .03 C; Grass Instruments, Co., Quincy, MA). Rate and tension measurements were recorded on an oscillograph (Grass Model 79 D). Preparations were perfused continuously (flow rate 5 mL/min) with natural seawater (obtained from Portsmouth Harbor, Portsmouth, NH) at room temperature.

Pharmacological agents used in this study were dopamine, octopamine, 3-isobutyl 1-methyl-xanthine (IBMX), papaverine, theophylline, dibutyryl cAMP (Sigma), 8-*para*-chlorophenylthio cAMP (ICN Pharmaceuticals, Irvine, Cal.), forskolin (Calbiochem Biochemicals, San Diego, CA), SQ 20,009 (Squibb Inc., Princeton NJ), phentolamine (CIBA-Giegy Corp., Summit, NJ), and RO-20-1724 (a gift from Hoffman-LaRoche Inc., Nutley, NJ). Solutions were prepared by diluting an aliquot of a concentrated stock solution (forskolin in 95% EtOH, RO-20-1724 in dimethyl sulfoxide, amines in dH_20 with 1*N* acetic acid as carrier) in seawater or by adding the compound directly to seawater. In most experiments, drug solutions were added to the isolated heart preparation via the perfusion reservoir. For the potentiation experiments, a 1 mL aliquot of the test solution was applied directly to the heart while it was being continuously perfused. This permitted more accurate comparisons of each response, since effects were washed off relatively quickly. In all experiments, results were calculated as the maximal percent change in heart contraction rate and amplitude elicited by a particular test agent relative to values obtained immediately prior to addition of the compound.

RESULTS

Amine, Forskolin, and IBMX Effects upon Tissue Levels of cAMP and cGMP

Incubation of cardiac ganglia for 10 min in OCT (10^{-5} *M*) or DA (10^{-5} *M*) significantly increased levels of cAMP in this tissue (Table 1). OCT (4.2 × control levels) was more potent than DA (2.2 × control levels) in ganglion cAMP elevation, which is consistent with their relative capacities to increase heart rate (Augustine et al., 1982). Neither amine altered levels of cGMP in the cardiac ganglion (Table 1).

In cardiac muscle (Table 1), OCT elevated cAMP to a much greater extent (8.9 × control levels) than did DA (2.7 × control levels). No change in cardiac muscle cGMP content was observed after 10 min amine incubation. These data suggest that amine excitation of heart contraction rate and strength in *Limulus* may be mediated by simultaneous cAMP increases in the cardiac ganglion and cardiac muscle, respectively.

The alpha-adrenergic receptor antagonist phentolamine (10^{-4} *M*) inhibited the accumulation of cAMP induced by 10^{-5} *M* OCT or DA in the cardiac

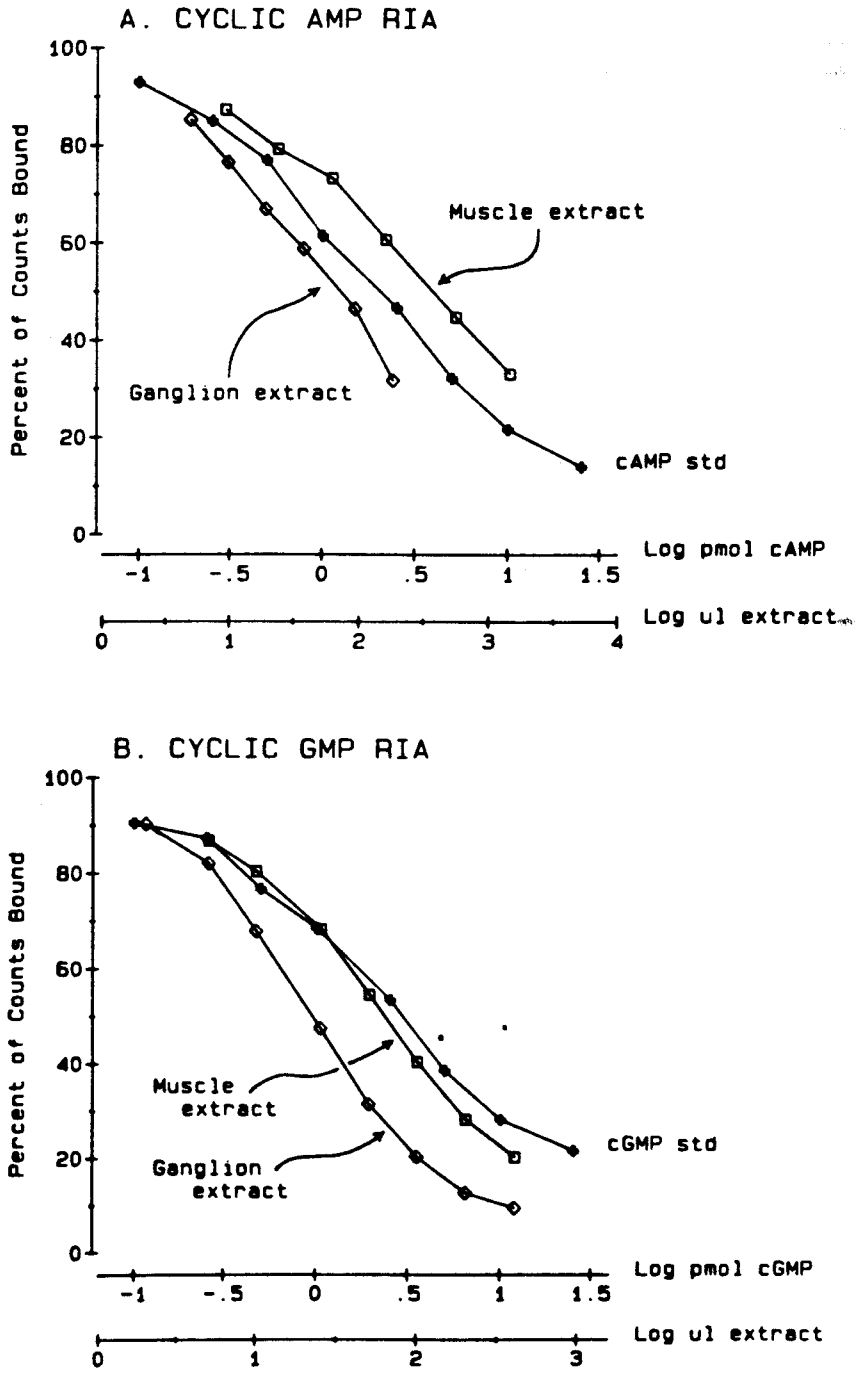

Fig. 1. Cyclic nucleotide radioimmunoassay (RIA) standard and extract dilution curves. (A) Displacement of ^{125}I-cAMP tracer from cAMP antibodies (diluted 1:400) occurs with standard cAMP; extracts of cardiac ganglia or cardiac muscle show parallel inhibition of tracer binding. (B) The RIA for cGMP (antibodies diluted 1:200) is similar in sensitivity and extract binding inhibition characteristics..

ganglion and in the cardiac muscle (Table 2). Suppression of amine-induced elevation of cAMP levels by phentolamine was more effective for OCT than for DA in either tissue.

The adenylate cyclase activator forskolin (5×10^{-6} *M*) and the phosphodiesterase inhibitor IBMX (10^{-3} *M*) also increased cardiac ganglion cAMP

TABLE 1
Changes in Levels of Cyclic Nucleotides in *Limulus* Cardiac Ganglia and Cardiac Muscle Induced by Amines, Forskolin, and IBMX

Tissue	Treatment	*n*	cAMP Levels	cGMP Levels
Cardiac Ganglion	Control	6	28.0 ± 10.6	5.8 ± 0.7
	Octopamine	6	117.0 ± 30.6[a]	4.1 ± 0.3
	Dopamine	6	61.8 ± 7.1[b]	5.2 ± 0.0
	Forskolin	6	76.9 ± 9.8[b]	4.2 ± 0.7
	IBMX	6	59.6 ± 8.4[b]	73.7 ± 19.0[a]
Cardiac Muscle	Control	6	6.1 ± 1.1	1.3 ± 0.2
	Octopamine	6	54.6 ± 9.6[a]	0.8 ± 0.1
	Dopamine	7	16.4 ± 2.6[b]	0.9 ± 0.1
	Forskolin	6	9.5 ± 1.5[c]	0.8 ± 0.1
	IBMX	6	11.0 ± 2.4[c]	5.2 ± 1.2[a]

Note: Values represent mean pmol cyclic nucleotide/mg protein ± SEM in tissues incubated for 10 min in control or test solutions (amines at 10^{-5} *M*, forskolin at 5×10^{-6} *M*, and IBMX at 10^{-3} *M*). Level of significance was determined using an unpaired Student's *t*-test.

[a] $p \leq 0.01$.
[b] $p \leq 0.03$.
[c] $p \leq 0.05$.

TABLE 2
Effect of Phentolamine on Amine-induced cAMP Accumulation in Cardiac Ganglia and Cardiac Muscle

Tissue	Treatment	*n*	cAMP Levels	Percent Inhibition by Phentolamine
Cardiac Ganglion	Control	4	24.5 ± 5.7	
	Phentolamine	4	35.5 ± 10.6	
	Octopamine	4	92.0 ± 21.8	
	Dopamine	4	71.2 ± 19.8	
	Octopamine and phentolamine	4	22.4 ± 2.4	100.0
	Dopamine and phentolamine	4	40.8 ± 19.6	62.5
Cardiac Muscle	Control	4	8.1 ± 1.4	
	Phentolamine	4	6.5 ± 0.5	
	Octopamine	4	51.2 ± 7.3	
	Dopamine	4	28.2 ± 2.7	
	Octopamine and phentolamine	4	8.5 ± 1.3	99.0
	Dopamine and phentolamine	4	14.2 ± 4.6	69.4

Note: Values represent pmol cAMP/mg protein ± SEM in tissues incubated for 3 min in various solutions (amines always 10^{-5} *M* and phentolamine always 10^{-4} *M*). Percent inhibition by phentolamine was determined as the percent reduction in cAMP accumulation (amine + phentolamine) in comparison to cAMP accumulation produced by amine only.

(Table 1) when applied to this tissue for 10 min. At these doses, forskolin (2.7 × control levels) was slightly more potent than IBMX (2.1 × control levels). Although forskolin did not elevate cGMP in the cardiac ganglion, IBMX markedly increased ganglion cGMP (12.7 × control levels).

Both forskolin and IBMX, at 5×10^{-6} *M* and 10^{-3} *M*, respectively, also increased cAMP levels in cardiac muscle (Table 1). In this tissue, IBMX increased cAMP levels to 1.8 × control levels, whereas forskolin increased cAMP levels to 1.5 × control. Forskolin was without effect on levels of cardiac muscle cGMP, whereas IBMX increased cardiac muscle cGMP significantly (4.0 × control levels). Thus, in both the cardiac ganglion and cardiac muscle, forskolin selectively increased cAMP content, whereas IBMX increased both cAMP and cGMP.

Comparison of Amine, Forskolin, and IBMX Effects on the Limulus *Heart*

The biogenic amines OCT and DA had powerful chronotropic and inotropic effects on the *Limulus* heartbeat [Fig. 2(A)]. Although DA also transiently inhibited the heart, both amines produced positive chronotropic and inotropic responses that were slow in onset and long lasting.

Forskolin, which increases intracellular levels of cAMP by stimulating adenylate cyclase (Seaman et al., 1981), and IBMX, which elevates cAMP by inhibiting cyclic nucleotide phosphodiesterases (Chasin and Harris, 1976; Levitan and Norman, 1980), elicited amine-like increases in the rate and strength of heart contractions [Fig. 2(B)]. The excitatory effects of both compounds were dose-dependent (Fig. 3), with forskolin approximately 2 log units more potent than IBMX. The greater potency of forskolin versus IBMX was especially prominent when comparing their effects on the strength of heart contractions.

The effects of both forskolin and IBMX were long lasting (Fig. 4). In order to compare the time course of the responses of the *Limulus* heart to these drugs, doses of each compound that produced comparable increases in heart rate were applied (5×10^{-6} *M* forskolin, 90.8 ± 10.0% SEM increase, $n = 17$ and 10^{-3} *M* IBMX, 89.3 ± 7.5% increase, $n = 16$). Under these conditions there were two major differences between the effects of forskolin and IBMX. First, increases in the rate of *Limulus* heart contractions elicited by IBMX were more rapid in onset, and in decline, than the comparable increase elicited by forskolin [Fig. 4(A)]. Secondly, although forskolin, in most preparations, elicited a gradual and prolonged increase in heart contraction amplitude, IBMX consistently produced enhancement of heart contractions for a short period only, followed by a prolonged decrease [Fig. 4(B)]. Such a biphasic response was occasionally observed upon forskolin application as well. OCT and DA also have been shown to have a biphasic effect on *Limulus* heart contraction amplitude in some preparations (Watson et al., 1985).

Effects of Cyclic Nucleotide Analogues and Phosphodiesterase Inhibitors

The cAMP analogue 8-*para*-chlorophenylthio cAMP (10^{-3} *M*) had no effect on heart contraction amplitude but did produce a 34.0 ± 9.9% increase in heart rate in 7 preparations. Dibutyryl cAMP (10^{-3} *M*) had no effect on the cardiac rhythm ($n = 7$).

Three phosphodiesterase inhibitors, in addition to IBMX, also elicited

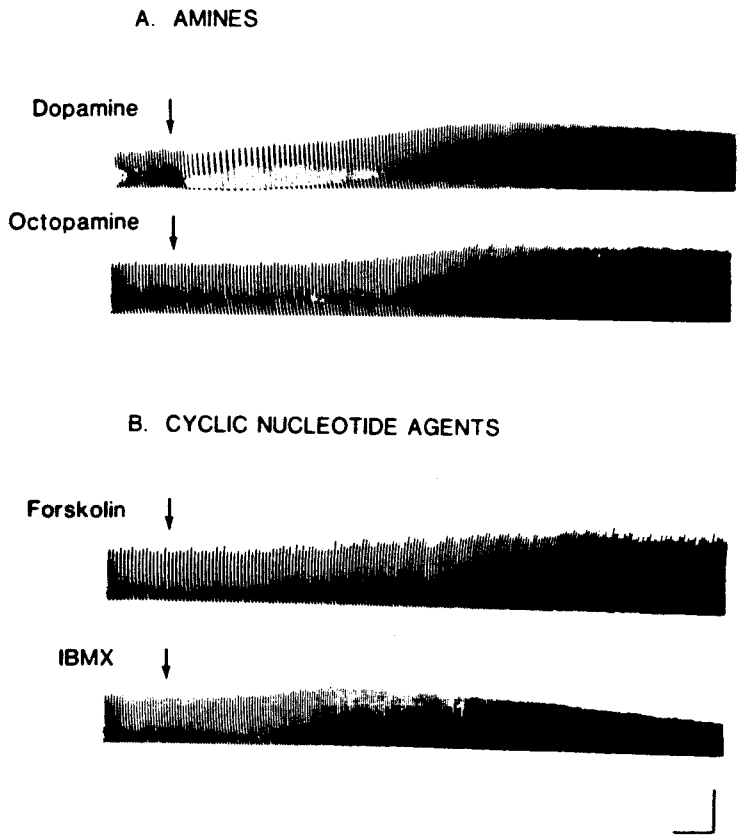

Fig. 2. Amine-induced increases in the rate and strength of contractions of the isolated *Limulus* heart are mimicked by pharmacological agents that influence cyclic nucleotide metabolism. (A) Dopamine and octopamine (10^{-6} *M*) both elicit long-lasting positive chronotropic and inotropic effects. In addition, dopamine transiently inhibits the heart. (B) Forskolin (5×10^{-6} *M*), which activates adenylate cyclase, produces amine-like increases in the rate and strength of heart contractions. The phosphodiesterase inhibitor 3-isobutyl 1-methylxanthine (IBMX) at 10^{-3} *M*, also has amine-like excitatory effects on the *Limulus* heart, but the increase in heart contraction amplitude is short-lived. Calibration: 1.5 g, 1 min.

some amine-like effects when applied to the isolated heart preparation. Papaverine (10^{-3} *M*) elicited a 10.5 ± 4.3% increase in heart rate while enhancing contraction amplitude 39.6 ± 7.8% (n = 13). RO-20-1724 (10^{-3} *M*), in 12 preparations, increased contraction rate only slightly (4.9 ± .4%) but did increase contraction amplitude 26.6 ± 5.4%. Finally, SQ 20,009 (10^{-4} *M*) had no effect on heart rate but did enhance contraction amplitude (36.1 ± 4.8% increase, n = 14). At higher doses SQ 20,009 consistently produced irregularities in the cardiac rhythm. Theophylline (10^{-3} *M*) had no effect (n = 6).

Potentiation of Amine Responses by IBMX

Octopamine, dopamine, or IBMX were topically applied to the isolated heart at doses that produced only slight increases in contraction rate and amplitude. Amines were then added with IBMX present. IBMX did not influence the effects of either amine on heart contraction amplitude [Fig. 5(A)]. However, IBMX did potentiate amine-induced effects on heart rate [Fig. 5(B)]. Dopamine produced a 21.4 ± 5.7% (n = 7) greater increase on

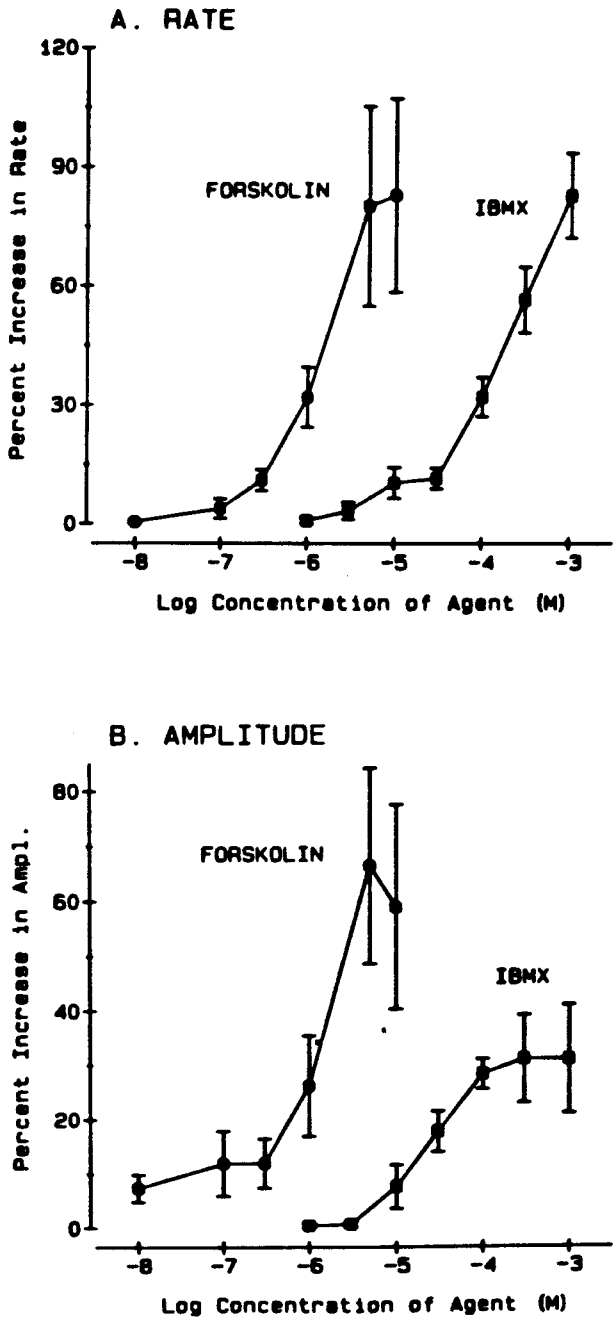

Fig. 3. The excitatory chronotropic and inotropic effects of forskolin and IBMX on the isolated *Limulus* heart are dose-dependent. Values represent the mean ± SEM of 12 trials at each dose. Forskolin is approximately 2 log units more potent than IBMX in its effects on heart contraction rate (A) and amplitude (B). The maximal effect on heart contraction rate is similar for both agents, whereas the maximal amplitude effect of IBMX is only half that elicited by forskolin.

heart rate when IBMX was present, and octopamine elicited a 13.5 ± 2.7% (n = 7) greater response with IBMX. At this low dose (1 mL, 10^{-5} M), IBMX itself had a minimal effect on heart rate (2.6 ± 1.7% increase). These results suggest that in the cardiac ganglion, but not in the cardiac muscle, IBMX suppressed phosphodiesterase activity sufficiently to permit additional cAMP accumulation with exposure to low doses of amine, leading to a greater effect on heart rate.

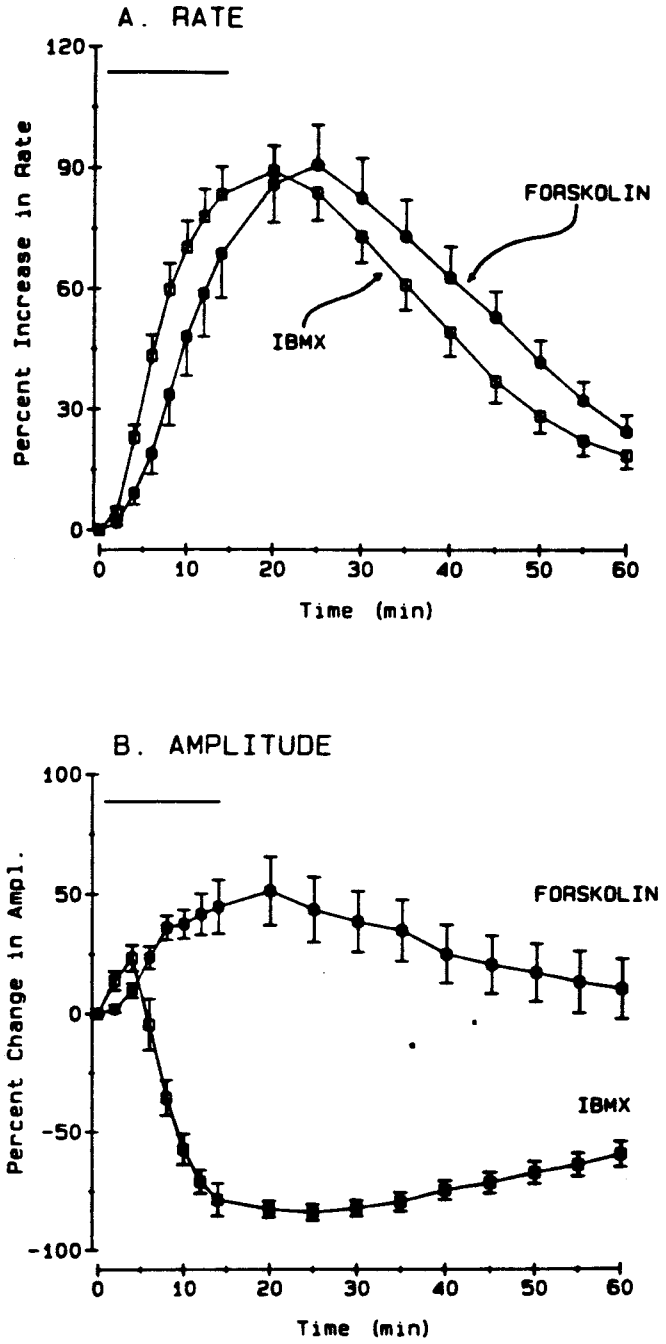

Fig. 4. Time course of the effects of 5×10^{-6} *M* forskolin and 10^{-3} *M* IBMX on heart contraction rate (A) and amplitude (B). Agents were perfused over isolated hearts for 15 min (indicated by the solid bar) and subsequently washed off. Mean rate and amplitude (± SEM) values were determined at 5 min intervals in 14 preparations. Forskolin produces long-lasting increases in both rate and strength of heart contractions. IBMX has a similar chronotropic effect, but its transient positive inotropic effect is followed by long-lasting inhibition.

DISCUSSION

Cyclic AMP, but not cyclic GMP, plays an important role as a second messenger mediating the excitatory effects of OCT and DA on the neurogenic *Limulus* heart. Both of these amines significantly increase intracellular levels of cAMP in the cardiac ganglion and in the myocardium. Furthermore,

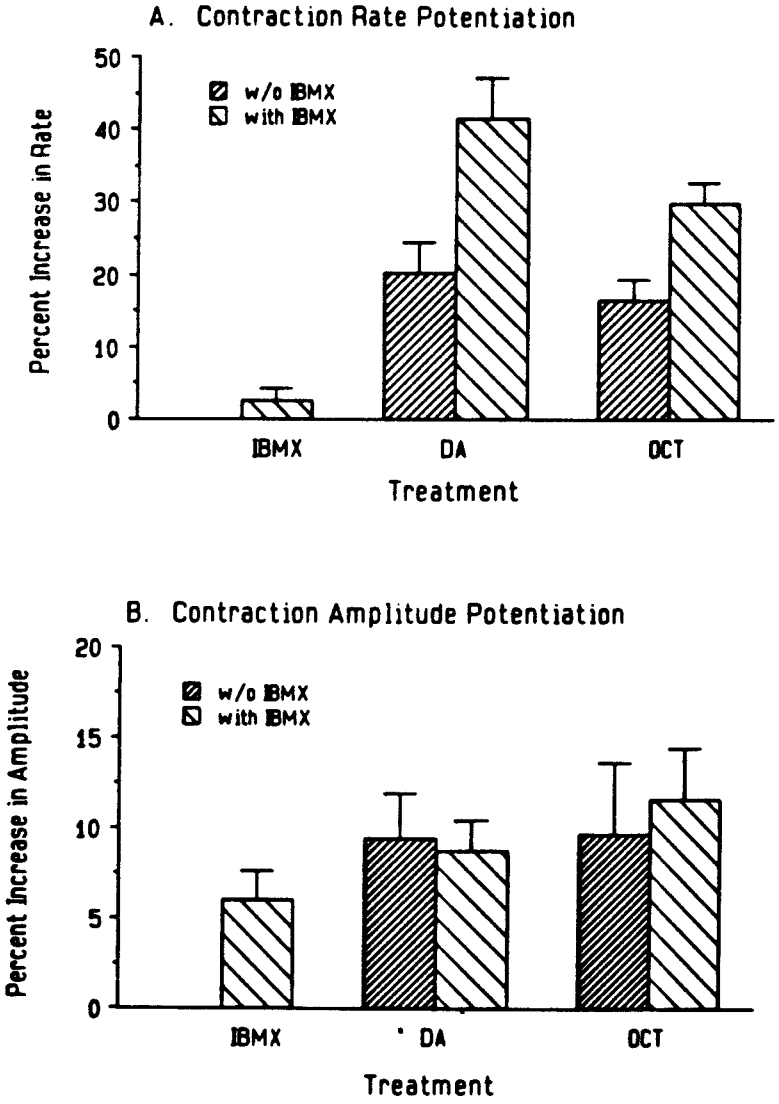

Fig. 5. The phosphodiesterase inhibitor IBMX potentiates the chronotropic (A) but not inotropic (B) effects of amines. In seven preparations DA or OCT was added to the isolated *Limulus* heart at doses that produced minimal increases in heart contraction rate and amplitude. In the presence of 10^{-5} *M* IBMX, enhancement of rate effects was observed for DA ($p \leq 0.01$) and OCT ($p \leq 0.03$). However, amine modulation of contraction amplitude was not affected by IBMX. Levels of significance were determined using a paired Student's *t*-test comparing the effects of amine + IBMX to the sum of the individual effects of an amine and IBMX.

amine-like excitation of the cardiac rhythm is observed with the application of a number of pharmacological agents that influence cAMP metabolism. Apparently, OCT and DA utilize a common second messenger, cAMP, to produce similar physiological effects on the cardiac rhythm in *Limulus*.

Amines also have pronounced effects on the neurogenic crustacean heart (Florey and Rathmayer, 1978; Cooke and Sullivan, 1982), and in both crabs and lobsters OCT elevates cardiac ganglion and cardiac muscle cAMP (Sullivan and Barker, 1975; Battelle and Kravitz, 1978). Therefore, in a variety of arthropods, as in vertebrates, cyclic AMP is an important second messenger mediating the modulation of cardiac output by amines.

Cyclic AMP Mechanism for Chronotropic Actions of the Amines

In *Limulus*, the site of inotropic amine action is the cardiac ganglion, which consists of pacemaker neurons and follower, or motor, neurons (Augustine et al., 1982). The increase in cardiac ganglion cAMP observed during exposure to DA and OCT may be due to cAMP elevation in the pacemaker neurons, follower neurons, or both. Several lines of evidence suggest that amines act to increase burst frequency in the *Limulus* cardiac ganglion by elevating cAMP levels in pacemaker neurons. First, pacemaker neurons appear to be responsible for controlling the rate of the cardiac rhythm in *Limulus* (Lang, 1971). Second, recent work indicates that amines act on pacemaker neurons to increase the rate of cardiac ganglion bursting (Augustine and Fetterer, 1985). Finally, forskolin and IBMX, agents that elevate levels of cAMP in the *Limulus* cardiac ganglion and cardiac muscle (Table 1), increase heart contraction rate in *Limulus*.

It may well be that follower cell cAMP is also increased by amines. The pacemaker neurons are very small (20–40μm) in comparison to the follower cells (60–150μm), so a selective increase in pacemaker cAMP might not be readily detected by RIA using the whole ganglion if there was no concomitant change in follower cell cAMP. However, increases in pacemaker, not follower, cell cAMP must underlie inotropic amine action.

The burst frequency of cardiac ganglia in some other arthropods is accelerated by octopamine and dopamine (Grega and Sherman, 1975; Miller et al., 1984). In addition, octopamine increases cAMP levels in the cardiac ganglion of several species of crabs and lobsters (Sullivan and Barker, 1975; Battelle and Kravitz, 1978). There is also some evidence for the role of cAMP in the excitatory action of a peptide in the lobster cardiac ganglion (Lemos and Berlind, 1981). However, although these data suggest a role for cAMP in these systems, this is the first study to demonstrate a cAMP-dependent mechanism for amine actions on an arthropod cardiac ganglion.

Cyclic AMP Mechanism for Inotropic Actions of the Amines

Regulation of the strength of heart contractions in *Limulus* involves cellular elements in both the cardiac ganglion and myocardium. Cyclic AMP may mediate amine effects at any or all of these locations. Octopamine and dopamine both act directly on cardiac muscle to increase the strength of contractions (Watson et al., 1985). In addition, amines influence heart contraction strength in *Limulus* indirectly by their actions on follower cell resting potentials (Augustine and Fetterer, 1985). Finally, amines modulate heart contraction amplitude in *Limulus* by increasing the size of excitatory junction potentials (EJPs) at the neuromuscular junction (Watson and Hoshi, 1981; Watson et al., 1985).

Whether or not cAMP is involved in amine actions on neuromuscular transmission or on follower neurons is uncertain. Cyclic AMP might mediate amine enhancement of EJPs in *Limulus* as it does in other invertebrate systems (Enyeart, 1981; Fujiwara and Kobayashi, 1983; Evans, 1984b). The data obtained in this study suggest that cAMP mediates the effects of amines on contractility. We demonstrated that amines raise intracellular cAMP levels in the cardiac muscle. Furthermore, forskolin and IBMX, agents that also increase cardiac muscle cAMP, have amine-like positive inotropic effects

on the *Limulus* heart. Cyclic AMP appears to be a common second messenger mediating the modulation of muscle contractility by amines. A cAMP-dependent mechanism underlying the action of amines on muscle contractility has been demonstrated for other invertebrate species (Weiss et al., 1979, Hess et al., 1981; Sawada et al., 1984; Weiss et al., 1985). In vertebrate cardiac muscle, the positive inotropic actions of catecholamines are also mediated by a cAMP-dependent mechanism (Tsien, 1977).

The role of cAMP as the second messenger underlying inotropic amine action in the *Limulus* system is apparent from the biochemical and pharmacological data presented. However, two findings are not consistent with this hypothesis. First, the cAMP analogues tested did not enhance contraction strength, as do the amines. Second, IBMX produced only a transient increase in heart contraction amplitude and was ineffective in enhancing the inotropic actions of amines. Work is presently underway to clarify these discrepancies and to determine the extent to which cAMP is involved in the action of amines at particular loci in the *Limulus* system to increase heart contraction strength. The accessibility of the constituents of the neurogenic *Limulus* heart for both physiological and biochemical studies should facilitate a comprehensive investigation of the role of cAMP in amine actions at multiple sites in this simple system.

Amine Receptors and Adenylate Cyclase

Although DA and OCT produce similar effects on the *Limulus* heart, and the chronotropic actions of both amines are blocked by phentolamine, it is not clear whether they are acting on the same receptors. According to our data, both OCT and DA are capable of activating receptors linked to adenylate cyclase. However, the only adenylate cyclase that has been partially characterized in *Limulus* is sensitive to OCT but not to DA (Atkinson et al., 1977). Phentolamine completely blocks the ability of OCT to increase cAMP in *Limulus* cardiac ganglia and heart muscle, but it only partially blocks cAMP accumulation induced by DA. These data suggest that in *Limulus*, as in another arthropod, the mosquito *Culex* (Pratt and Pryor, 1986), there are both DA- and OCT-sensitive adenylate cyclases. Thus, although OCT and DA probably share a common second messenger to produce similar physiological actions, they may activate this second messenger via different receptors.

Pharmacological Actions and Specificity

The best pharmacological evidence for cAMP involvement in excitatory chronotropic and inotropic amine action is the similarity of forskolin and amine effects on the cardiac rhythm and cyclic nucleotide levels. Forskolin, an activator of the catalytic subunit of adenylate cyclase (Seaman et al., 1981), precisely mimicked the excitatory chronotropic and inotropic effects of amines. In addition, forskolin, like the amines, increased levels of cAMP, but not cGMP, in the *Limulus* cardiac ganglion and in cardiac muscle. The phosphodiesterase inhibitors also may exert their effects by increasing levels of cAMP in the cardiac ganglion and/or muscle. IBMX produced significant elevations of cAMP in both these tissues, suggesting that cAMP might

mediate the positive chronotropic and inotropic response to IBMX. However, unlike forskolin, IBMX does not precisely mimic amine action on the *Limulus* heart, since this agent also evokes long-lasting inhibition of heart contraction amplitude.

The negative inotropic effects of IBMX may be a result of the marked effect of this agent on cGMP content in the cardiac ganglion and cardiac muscle. IBMX has similar biochemical and physiological actions on *Limulus* cardiac muscle to those reported for this agent on locust skeletal muscle (Evans, 1984a). In that system, IBMX increases muscle contractility via increased muscle cAMP, but at millimolar concentrations IBMX decreases contractility. Evans postulates that this effect is due to increased cGMP. It remains to be determined in either of these systems if increases in muscle cGMP are indeed responsible for inhibitory physiological actions elicited by IBMX

An alternative possibility is that the negative inotropic effect of IBMX is an indirect consequence of its pronounced rate effect. Amine-induced increases in heart rate are accompanied by a reduction in the number of action potentials per follower (motor) neuron burst (Watson and Augustine, 1982; Augustine and Fetterer, 1985). Occasional negative inotropic actions of amines appear to be a result of this action (Watson et al., 1985; Watson and Groome, unpublished). Experiments are presently underway to determine which of the aforementioned hypotheses is correct.

In summary, the positive chronotropic and inotropic effects of octopamine and dopamine on the neurogenic *Limulus* heart appear to be mediated by a cAMP-dependent process. Increases in pacemaker cell cAMP most likely mediate the positive chronotropic actions of amines on the cardiac ganglion. Cyclic AMP may also be involved in amine modulation of a number of cellular targets within this system to increase the strength of heart contractions.

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