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NEUROHORMONAL MODULATION OF THE *LIMULUS* HEART: AMINE ACTIONS ON CARDIAC GANGLION NEURONES

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SUMMARY

Octopamine (OCT), dopamine (DA), epinephrine (EPI) and norepinephrine (NE) are endogenous excitors of the *Limulus* heart. The cellular sites of action of these amines were investigated by recording responses of neurones in the cardiac ganglion. There was an increase in spike frequency in pacemaker neurones, accompanied by a depolarization and an increase in the rate of repolarization of the 'pacemaker potential'. DA also produced a transient decrease in pacemaker spike frequency which preceded its excitatory effect. All four amines produced slow, dose-dependent increases in the frequency of bursting activity in cardiac ganglion follower neurones. These increases were blocked by the antagonist phentolamine. DA and NE also produced transient decreases in burst rate which were blocked by the antagonist metergoline. Local application of DA on to pacemaker cells increased follower cell burst frequency, while similar applications on to follower cells did not affect burst frequency. This indicates that DA acts directly upon pacemaker neurones to increase cardiac ganglion burst activity. In addition to their pacemaker-mediated effects, the amines also had direct effects upon follower cells. These effects were examined after eliminating follower cell bursting activity with cobalt ions. OCT and, to a smaller extent, EPI, depolarized follower cells. DA and, occasionally, NE hyperpolarized follower cells. The hyperpolarizing DA response was due to a conductance increase and was blocked by metergoline and ergonovine.

INTRODUCTION

In addition to classical synaptic interactions, some neurones are capable of more global, neurohormonal communication (Barker & Smith, 1979; Kupferman, 1979; Kandel, Krasne, Strumwasser & Truman, 1979; Kravitz & Treherne,

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1980). This mode of communication imparts the ability to interact with many targets simultaneously and thus to modulate the activity of a collection of neurones. In this paper we study such a response.

The cardiac ganglion of *Limulus* generates the rhythmic beating of the tubular array of muscle cells which forms the heart. Biochemical, histological and pharmacological studies have identified a number of neurohormone candidates which modulate the activity of this system (see Watson & Augustine, 1982). Four of these endogenous amines, octopamine (OCT), dopamine (DA), epinephrine (EPI) and norepinephrine (NE), increase the rate and amplitude of the heartbeat at micromolar (or lower) concentrations (Augustine, Fetterer & Watson, 1982).

In this paper and the subsequent one (Watson, Hoshi, Colburne & Augustine, 1985) we have used electrophysiological techniques to identify the cellular sites of action of these amines. We demonstrate here that both types of neurone within the cardiac ganglion possess at least two pharmacologically distinct receptors sensitive to these amines. The combined responses of these neurones account for the ability of amines to increase heart rate. Actions of the amines upon cardiac neuromuscular transmission and cardiac muscle are responsible for increasing heartbeat amplitude (Watson *et al.* 1985). Thus amines simultaneously act upon multiple cellular targets to modulate the *Limulus* cardiac system.

Preliminary accounts of some of these results have appeared in abstracts (Fetterer & Augustine, 1977; Augustine, Watson & Fetterer, 1978; Augustine, Fetterer & Watson, 1979) and a review (Watson & Augustine, 1982).

METHODS

Experiments were performed on isolated cardiac ganglia of *Limulus polyphemus*. The ganglia were isolated as described by Augustine *et al.* (1982). Ganglia were pinned to silicone resin (Sylgard 184, Dow Chemical Co.) in a 5 ml capacity Plexiglas chamber containing physiological saline. This chamber was attached to a perfusion system which permitted exchange of bathing solutions without disrupting recording electrodes. Superfusion of a dye solution indicated that exchange within the chamber was complete within 45 s of initiating flow.

Somata of cardiac ganglion neurones were impaled with a glass microelectrode filled with 3 mol l^{-1} KCl or 2·5 mol l^{-1} potassium-acetate (5–20 MΩ resistance in sea water). Neurones could be identified in unstained ganglia by appropriate trans- or epi-illumination. Follower cells were much more readily located, and could be distinguished from pacemaker cells by their larger size (80–150 μm diameter; Bursey & Pax, 1970) and their characteristic 'bursting' electrical activity (Palese, Becker & Pax, 1970; Lang, 1971). Transmembrane potentials were recorded *via* a high impedence preamplifier with negative capacitance compensation and displayed on both a storage oscilloscope (Tektronix 5113) and a chart recorder (Brush 220, Gould Inst.). Burst frequency was measured manually or with a Grass 7P4 tachograph. In experiments where current was injected intracellularly, an amplifier (WPI Model no. 701) with a bridge circuit was used and a

Grass S-44 stimulator was used as a current source. To verify results obtained in this way, a second current-passing microelectrode was occasionally inserted into follower cells. This method, although more reliable than the use of a bridge circuit, was not routinely used because the thick connective tissue sheath surrounding each neurone (Bursey & Pax, 1970; Palese *et al.* 1970) made multiple electrode insertion difficult.

In some experiments dopamine was applied on to individual neurones by iontophoresis or pressure ejection. Iontophoretic application was accomplished by passing current from an isolated pulse generator (Grass S-44 with SIU-9 isolator in series with a 100 MΩ resistor) through a microelectrode containing 1 mol l^{-1} dopamine-HCl (Sigma Chemical Co.). Braking current was applied, when necessary, to reduce dopamine leakage from the electrode tip. Pressure ejection was accomplished with the device described by McCaman, McKenna & Ono (1977).

Filtered natural sea water ordinarily was used as physiological saline (Augustine *et al.* 1982). Artificial sea water (Instant Ocean) or *Limulus* saline (Chao, 1933) were used occasionally and yielded similar results. Amines and other drugs were generally applied to cardiac ganglia *via* bath superfusion. Drug sources and methods used to prepare drug solutions were as described by Augustine *et al.* (1982). All solutions were applied to preparations at room temperature (18–24 °C).

RESULTS

The *Limulus* cardiac ganglion consists of several hundred neurones (Bursey & Pax, 1970) which have been grouped into only two functional types (Watson & Augustine, 1982). *Pacemaker* cells are spontaneously active neurones responsible for generating the heartbeat rhythm, while *follower* cells are postsynaptic to pacemakers and are thought to be motor neurones (Palese *et al.* 1970; Lang, 1971). The synaptic organization of the *Limulus* ganglion thus appears to be simpler than that of crustacean ganglia, whose nine neurones interact in complex ways to produce the heartbeat rhythm (Maynard, 1966; Hartline, 1979; Benson & Cooke, 1984). We describe below experiments which indicate that the amines OCT, DA, EPI and NE affect the electrical properties of both types of neurones present within the *Limulus* cardiac ganglion. Like their effects on the *Limulus* heart and isolated cardiac ganglia (Augustine *et al.* 1982), the predominant effect of these amines was to increase the rate of neuronal activity.

Pacemaker neurone responses

It was difficult to insert microelectrodes into the small (30–60 μm diameter) pacemaker cells, so that our observations on pacemaker cells were limited to three experiments where we recorded simultaneously the activity of single pacemaker and follower cells. Pacemaker neurones generate overshooting action potentials which occur once per heartbeat cycle (Lang, 1971). Bath application of DA

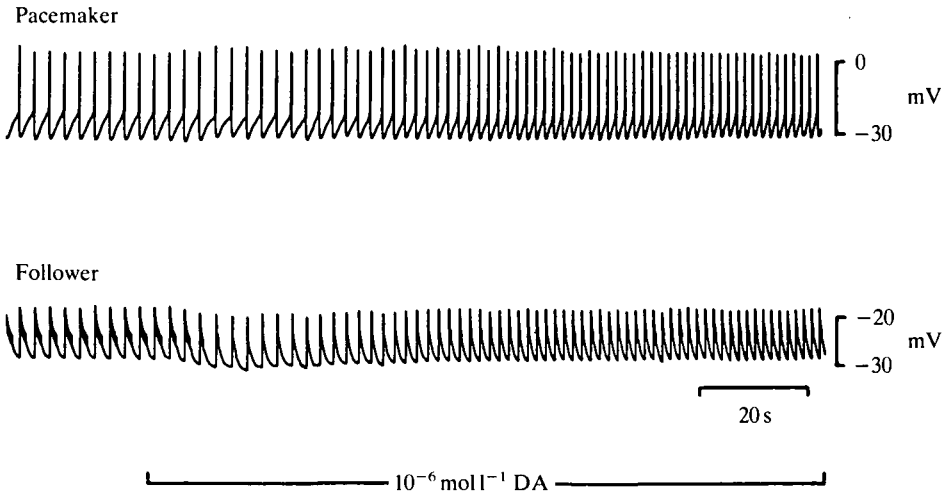

Fig. 1. Dopamine (DA) affects cardiac ganglion neurone activity. Simultaneous recording from pacemaker (top) and follower (bottom) neurones during bath perfusion of 10^{-6} mol l^{-1} DA revealed an increase in pacemaker spike frequency which coincided with increased follower cell burst frequency.

increased the frequency of pacemaker action potentials (Fig. 1). Similar effects were produced by OCT and EPI in other experiments. DA also produced a small, transient inhibition of cardiac ganglion activity which was reflected in a slight decrease in pacemaker action potential frequency (Fig. 1). No such inhibition was caused by OCT or EPI. Between pacemaker action potentials there is a gradually depolarizing 'pacemaker potential' (Lang, 1971). DA, OCT and EPI increased the rate of rise of this pacemaker potential and also produced a small overall depolarization, as illustrated for DA in Fig. 1. Such effects may underlie the amine-induced increases in pacemaker cell firing rate.

Simultaneous recordings from pacemaker and follower neurones showed that application of the amines caused simultaneous increases in the frequency of both pacemaker spikes and follower bursts, so that the interval between successive events in these two neurone types always declined in parallel. Increases in the frequency of follower bursts were accompanied by decreases in their duration, as described in greater detail below.

Follower neurone responses

Changes in follower cell bursts

The most prominent effect of the amines on *Limulus* follower neurones was an increase in the frequency of bursting activity (Fig. 2). At all concentrations OCT was most effective and NE was generally least effective (Fig. 3). Approximate ED_{50} values for the effects on burst frequency are: OCT, 6×10^{-8} mol l^{-1}; DA, 10^{-7} mol l^{-1}; EPI, 5×10^{-7} mol l^{-1}; and NE, 10^{-6} mol l^{-1}.

The amine-induced increases in burst frequency were rather slow in onset,

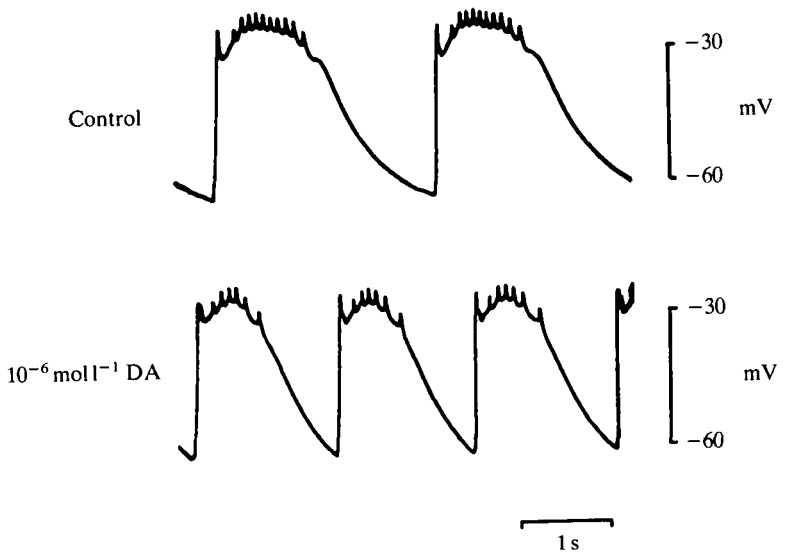

Fig. 2. Effect of dopamine (DA) on the bursting activity of a *Limulus* follower neurone. Intracellular recordings from a follower cell before (Control) and during bath application of 10^{-6} mol l^{-1} DA revealed a net increase in the rate of follower cell bursts.

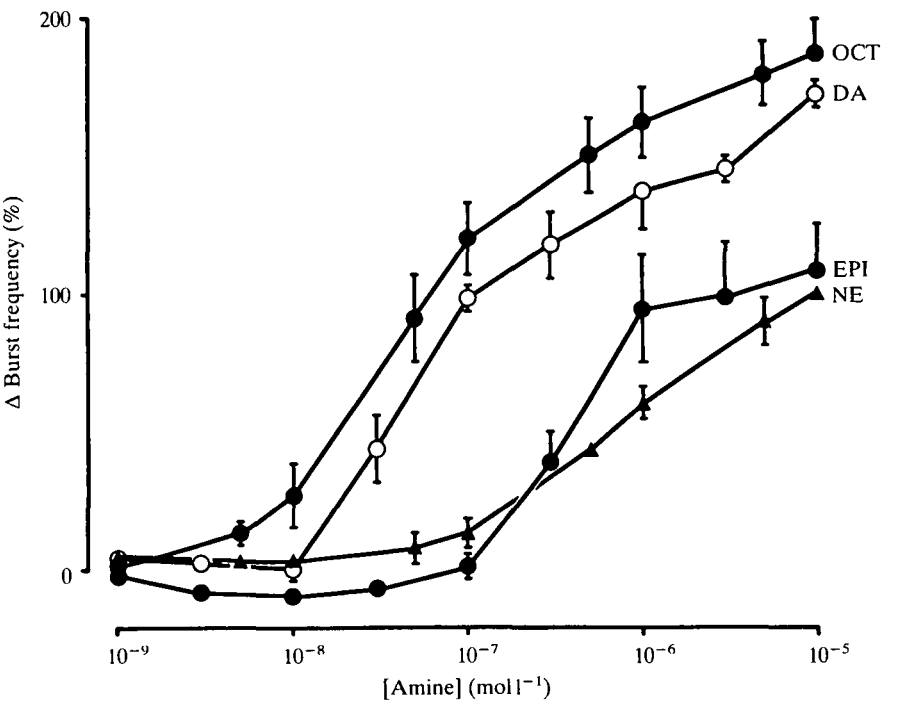

Fig. 3. Cumulative dose-response curves for amine-induced changes in follower cell burst rate. Points indicate mean values derived from two to four experiments, and error bars represent ±S.E.M. OCT, octopamine; DA, dopamine; EPI, epinephrine; NE, norepinephrine.

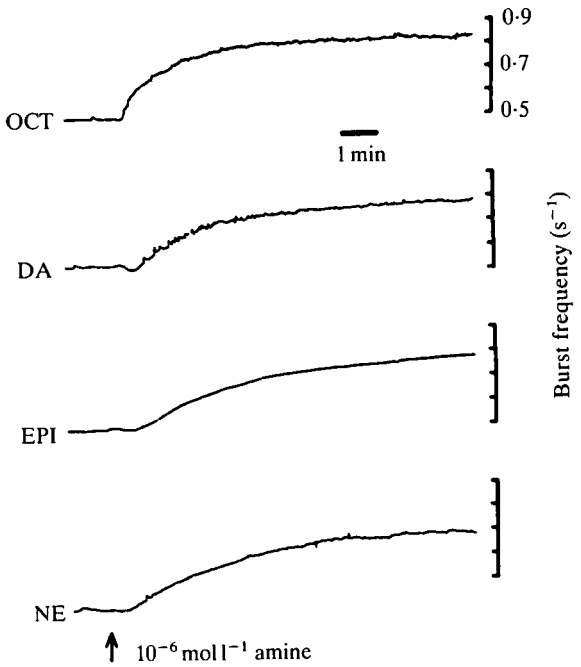

Fig. 4. Time course of amine-induced changes in follower burst frequency. Traces are rate meter recordings of burst frequency of a single neurone during bath application of 10^{-6} mol l^{-1} solutions of each amine. Octopamine (OCT) produced the most rapid, monotonic increase in burst rate, and dopamine (DA) produced a small, transient decrease in burst frequency which preceded its excitatory effect. EPI, epinephrine; NE, norepinephrine.

reaching a maximum over a period of 5–10 min (Fig. 4). These increases in burst frequency recovered even more slowly, requiring 20–60 min to return to control values after rinsing with amine-free saline. DA, and to a small extent NE, also produced small, transient decreases in burst rate which preceded their excitatory effects.

Coincident with the amine-induced increases in follower cell burst frequency, the duration of the slow plateau component of the burst decreased and the number of small antidromic spikes superimposed on this plateau also decreased (e.g. Fig. 2). Interburst interval, burst duration and number of spikes per burst generally decreased, in parallel, with increased amine concentrations (Fig. 5). Concentrations of OCT greater than 10^{-7} mol l^{-1} produced a progressive increase in the number of spikes per burst. This effect may be due to the depolarizing response of follower cells to OCT which is described below.

One prominent feature of the amine responses of the intact *Limulus* heart is the differential sensitivity of the excitatory and transient inhibitory components to pharmacological agents (Augustine *et al.* 1982). In intact hearts the increase in heart rate produced by any of the four amines studied here is preferentially blocked by phentolamine, an α-adrenergic antagonist which blocks octopamine responses in arthropods (Evans, 1981), while the transient inhibition of heart rate produced by DA and NE is eliminated by metergoline, an antagonist of serotonin

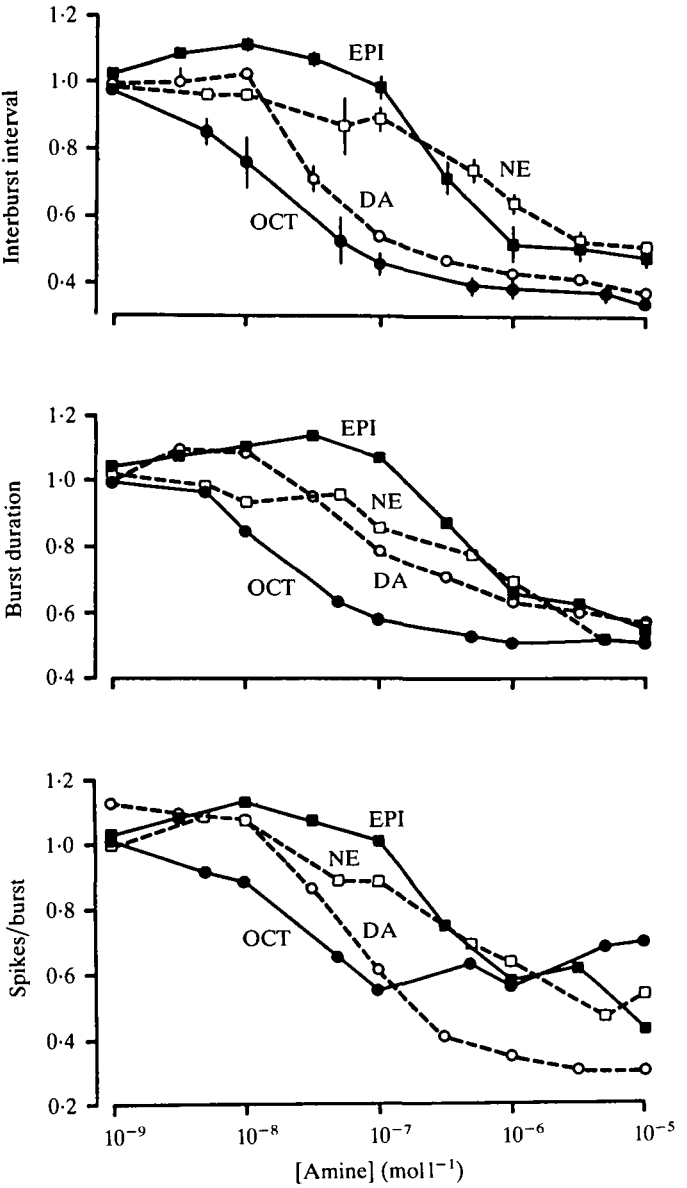

Fig. 5. Cumulative bath application of all four amines produced dose-dependent reductions in interburst interval (top), accompanied by decreases in the duration of the plateau component of the follower cell burst (centre) and the number of axonal spikes superimposed on each plateau (bottom). Points indicate mean values derived from two to four experiments and are normalized relative to values measured in amine-free saline. Error bars represent ±S.E.M. and, for clarity, are shown in only the top graph. Variability in the parameters shown in the lower two graphs was similar. EPI, epinephrine, NE, norepinephrine; DA, dopamine; OCT, octopamine.

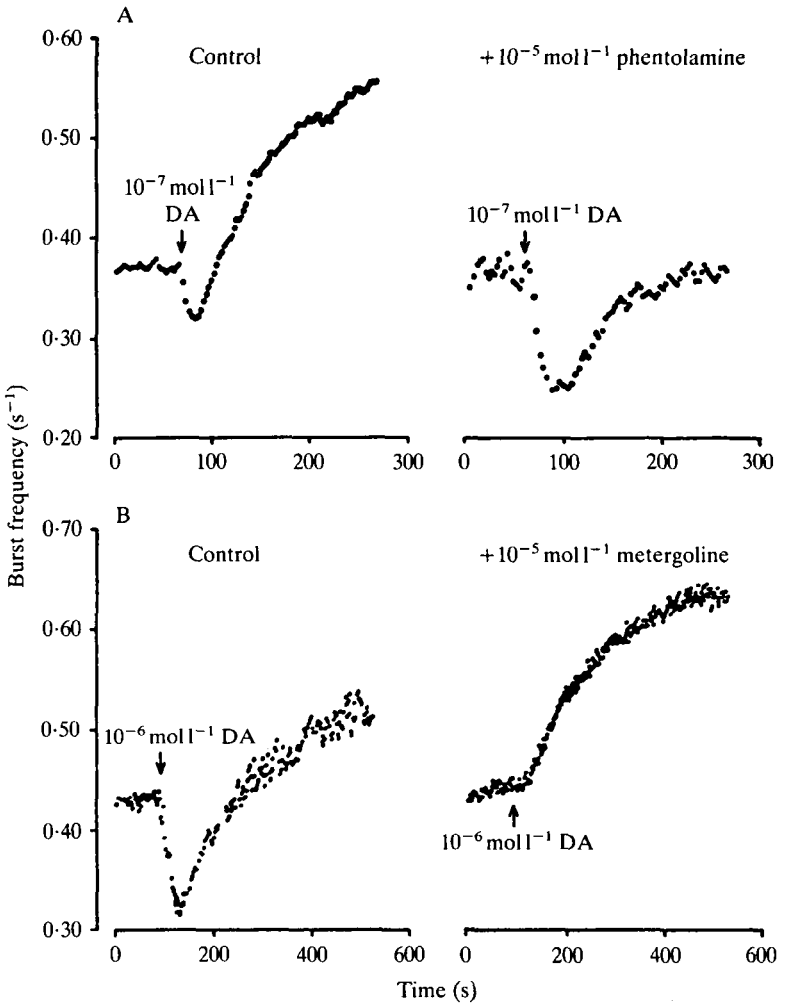

Fig. 6. Pharmacological separation of inhibitory and excitatory effects of DA on follower burst rate. Application of dopamine DA (continuously, beginning at arrow) produced a transient decrease in burst frequency which was followed by a net increase. The rate increase was reduced by 10^{-5} mol l^{-1} phentolamine (A), while the transient inhibition was eliminated by 10^{-5} mol l^{-1} metergoline (B). A and B are from different experiments.

responses of vertebrate central neurones (Beretta, Ferrini & Glasser, 1965). These antagonists also selectively blocked the excitatory and inhibitory effects of amines on follower cell bursts (e.g. Fig. 6). These results suggest the presence of at least two distinct amine responses, one excitatory and the other inhibitory. More detailed pharmacological studies will be needed to determine whether two or more distinct receptors are involved in these responses.

Potential changes

Bath perfusion of the amines gradually depolarized the membrane potential of follower neurones (Fig. 7). To quantify this effect, measurements were made in

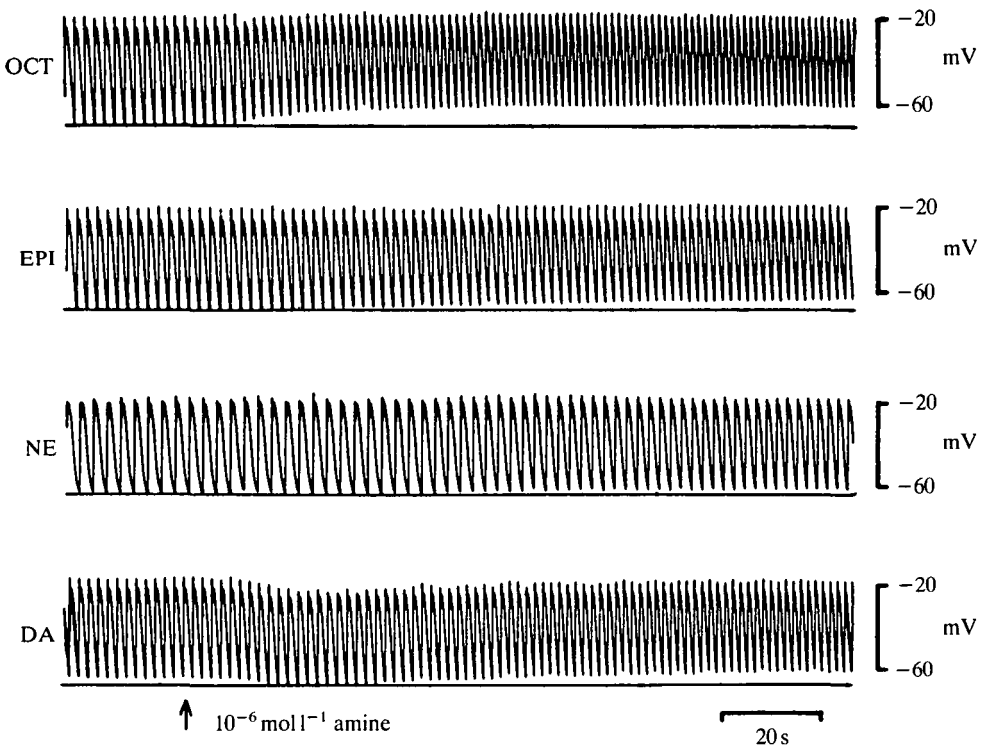

Fig. 7. Amines affected the membrane potential of follower neurones. Continuous bath perfusion (beginning at arrow) of 10^{-6} mol l^{-1} solutions of the amines indicated at the left changed the potential attained after each burst. All four amines depolarize, while dopamine (DA) (and occasionally norepinephrine, NE) transiently hyperpolarize. All four records were obtained from the same neurone; solid lines indicate the most negative potential of each trace. OCT, octopamine; EPI, epinephrine.

four experiments (of the 49 performed) where 10^{-6} mol l^{-1} concentrations of all four amines were sequentially applied to the same neurone. In these experiments the net depolarization was $3{\cdot}3 \pm 2{\cdot}1$ mV (S.E.M.) for OCT, $2{\cdot}9 \pm 1{\cdot}8$ mV for DA, $4{\cdot}4 \pm 2{\cdot}4$ mV for EPI and $2{\cdot}0 \pm 1{\cdot}6$ mV for NE. DA, and occasionally NE, also produced transient increases in the hyperpolarizing phase of the follower cell burst (Fig. 7). For the same four experiments described above this transient hyperpolarization was $-3{\cdot}8 \pm 1{\cdot}1$ mV for DA and $-0{\cdot}4 \pm 0{\cdot}4$ mV for NE. These hyperpolarizations transiently reduced the number of axonal spikes appearing on the burst plateau.

The large, oscillatory changes in follower cell conductance and potential which are associated with bursting activity made it quite difficult to characterize the effects of amines upon follower cells. To eliminate bursting activity the calcium channel blocker $CoCl_2$ (25–50 mmol l^{-1}) was added to the bathing medium. In this condition the follower cells became roughly 15 mV less negative than they were at the most hyperpolarized potential of a burst cycle and had a somewhat higher (typically less than 50 % greater) input resistance. These changes are

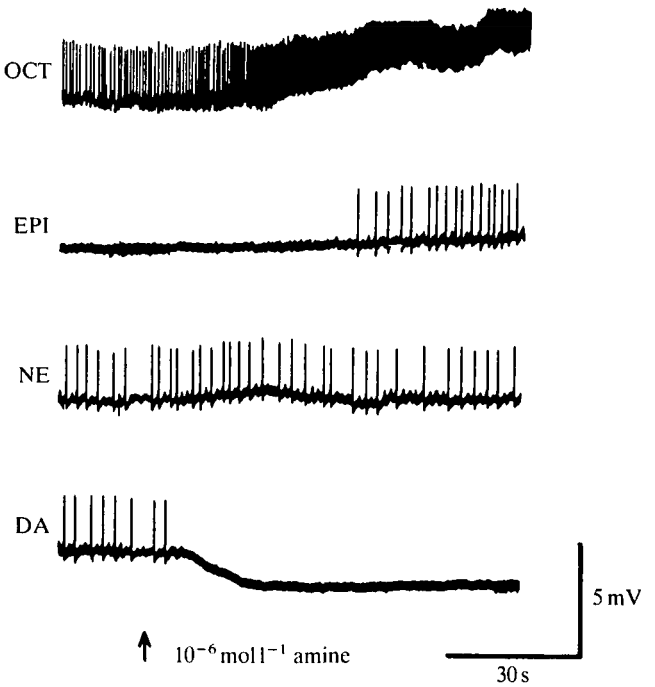

Fig. 8. Amines affected the membrane potential of follower neurones whose synaptic input and bursting activity have been blocked by 50 mmol l^{-1} cobalt. Octopamine (OCT) and epinephrine (EPI) depolarized, while dopamine, (DA) and occasionally norepinephrine (NE), hyperpolarized. All recordings are from the same cell; tops of axonal spikes are clipped in a portion of the OCT trace. Resting potential was −47 mV at the beginning of the traces.

presumably due to blockade of the ionic conductance changes underlying bursting (Augustine, 1979). In cobalt-containing saline, cells were either quiescent or spontaneously produced the tetrodotoxin-sensitive, antidromic spikes normally found on the burst plateau (Augustine, 1979). Perfusion of the four amines in the presence of cobalt revealed at least two types of potential changes (Fig. 8). OCT slowly depolarized follower cells and increased the frequency of axonal spikes. DA produced a rapid hyperpolarization which decreased spike frequency. This response was due to a conductance increase (Fig. 9). EPI produced a depolarization substantially smaller than that generated by an equal OCT concentration, while NE had little effect on membrane potential except for an occasional small, transient hyperpolarization. These effects were quantified by measuring the potential changes produced in four cells (of the 21 examined) bathed in cobalt-containing saline to which 10^{-6} mol l^{-1} of each amine had been added. In these experiments OCT depolarized $1{\cdot}9 \pm 0{\cdot}4$ mV and EPI depolarized $1{\cdot}0 \pm 0{\cdot}5$ mV. DA hyperpolarized these cells by $-2{\cdot}2 \pm 0{\cdot}3$ mV and NE produced a slight ($-0{\cdot}7 \pm 0{\cdot}6$ mV) hyperpolarization. The slow, gradual depolarization which normally occurred after applying DA or NE was eliminated by cobalt. Similar effects were also observed in neurones in which bursting was suppressed by exposure to saline containing high (200 mmol l^{-1}) Mg and low (2 mmol l^{-1}) Ca.

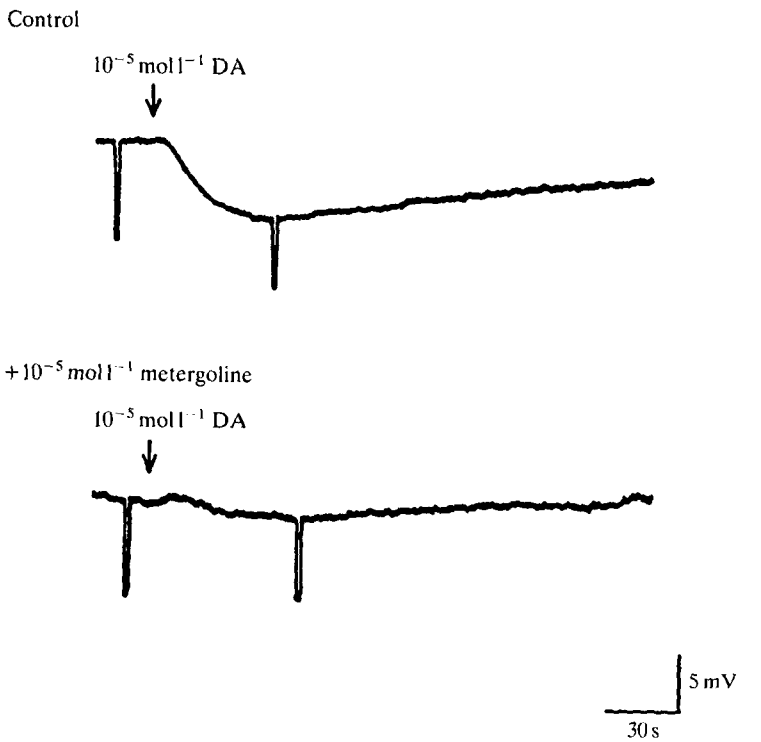

Fig. 9. Metergoline blocked the hyperpolarizing dopamine (DA) response of follower neurones. Bath perfusion of 10^{-5} mol l^{-1} DA hyperpolarized a cobalt-treated (50 mmol l^{-1} follower cell and produced a decrease in input resistance, reflected in the smaller potential changes in response to constant current pulses. Metergoline (10^{-5} mol l^{-1}) reduced the potential and conductance changes caused by DA.

We also examined some of the pharmacological properties of the hyperpolarizing DA response. This response was reduced by metergoline (Fig. 9), but was not affected by phentolamine (not shown). Ergonovine, an ergot which antagonizes DA responses in molluscan neurones (Ascher, 1972) and DA-induced increases in the *Limulus* heartbeat (Fetterer & Augustine, 1977), irreversibly blocked the follower cell DA response (Fig. 10). However, ergonovine also transiently hyperpolarized follower cells and is thus an agonist as well.

Sites of action

Because pacemaker neurones alone are thought to be responsible for generating the *Limulus* heart rhythm (Lang, 1971; Watson & Augustine, 1982), the amine-induced increases in heart rate and pacemaker firing frequency would be expected to be caused by direct action upon pacemaker neurones. However, because bath perfusion permits access of the amines to all ganglionic elements, it is possible that the effects are indirectly mediated. We examined this possibility (in eight experiments) by locally applying DA from micropipettes on to individual pacemaker and follower neurones, while monitoring ganglionic burst rate by recording follower cell activity.

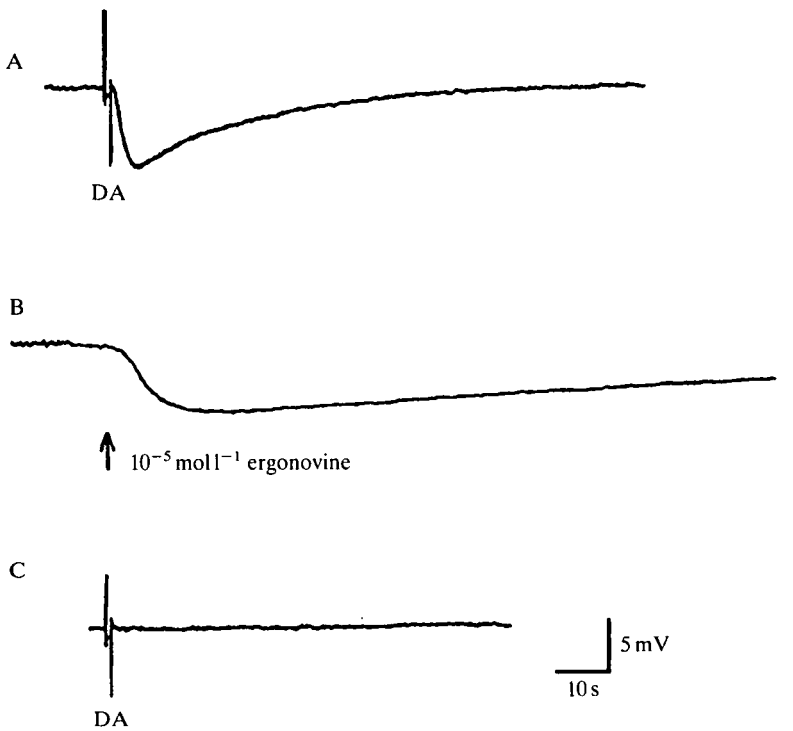

Fig. 10. Ergonovine irreversibly blocked the follower cell dopamine (DA) response. Iontophoretic application of DA on to a follower neurone bathed in 50 mmol l^{-1} cobalt produced a transient hyperpolarizing response (A). Bath perfusion of 10^{-5} mol l^{-1} mergonovine transiently hyperpolarized the follower cell (B) and eliminated the DA response (C). Resting potential at beginning of traces: A = −36 mV; B = −35 mV; C = −33 mV.

Ejection of DA on to small cells, near their cell bodies, increased follower burst frequency (Fig. 11), while similar ejections upon various regions of follower cells never altered burst frequency or duration (not shown). Transient decreases in burst rate were never observed with local DA application on to either neurone. These results indicate that (1) DA can act directly upon pacemaker cells to increase their firing rate, and (2) this effect of DA upon pacemaker neurones can subsequently increase activity in the follower neurones. Possible causes of the transient inhibition of pacemaker firing rate are considered in the Discussion.

Local application of DA on to pacemaker neurones also decreased follower cell plateau duration and decreased the number of spikes appearing on the plateau (Fig. 11). All these effects are likely to be mediated *via* pacemaker neurones, because DA should not have access to follower neurones in these conditions.

The amine responses of cobalt-treated follower neurones are presumably due to direct effects upon these cells, because cobalt treatment blocks pacemaker synaptic input on to follower cells (G. Augustine & D. McCulloh, unpublished observations). This was verified (in six experiments) by local iontophoretic application of DA on to follower cells (Fig. 10, top). Hyperpolarizing responses

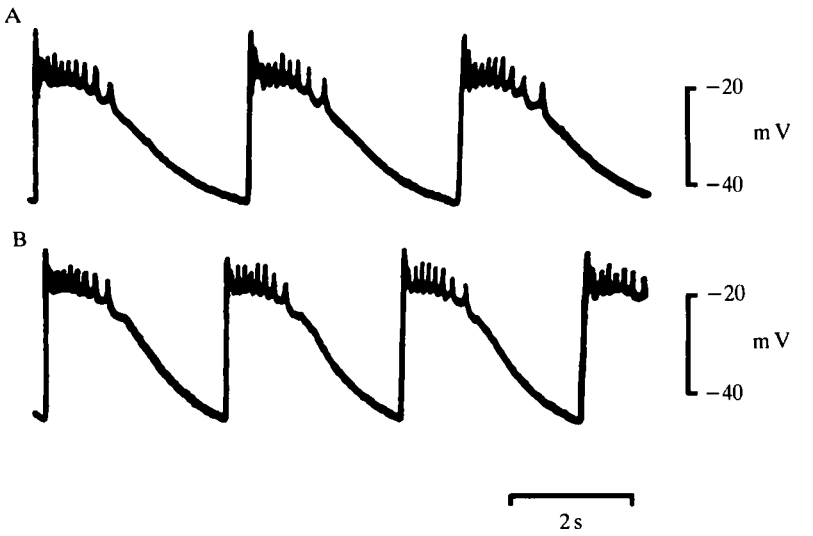

Fig. 11. Local dopamine (DA) application on to a pacemaker neurone increases follower burst frequency. DA was applied *via* pressure ejection on to a pacemaker neurone (near its cell body), while simultaneously recording the electrical activity of a follower neurone approximately 1 mm away. DA application produced a long-lasting elevation of follower burst frequency (B) over that measured before DA application (A).

could be consistently elicited by ejecting DA on to follower cell neurites, but applications on to follower cell somata were seldom effective. It therefore appears that amines also act directly upon follower cells and, at least for DA, sensitivity is greatly reduced at the cell body. Similar regional sensitivity to neurohormones has been reported for neurones in decapod cardiac ganglia (Cooke & Hartline, 1975; Miller, Benson & Berlind, 1984).

DISCUSSION

Sites of amine action

The amines OCT, DA, EPI and NE affect the physiological properties of *Limulus* cardiac ganglion pacemaker and follower neurones. Because local application of DA on to pacemaker cells (but not follower neurones) increases follower burst frequency (Fig. 11), it appears that the amine-induced increases in the frequency of pacemaker action potentials and follower cell bursts are due to amine excitation of pacemaker neurones. In addition to pacemaker-mediated effects, amines also directly elicit (at least) two distinct responses from follower neurones whose synaptic input and bursting activity have been blocked by cobalt. Thus these putative neurohormones affect the cardiac ganglion network at its two known cellular levels.

Although the *excitatory* effects of the amines on ganglionic activity are apparently mediated *via* pacemaker neurones, it is possible that the transient

inhibition of the ganglion produced by DA and NE is due to an effect upon follower neurones. Experiments where DA was locally applied on to pacemaker neurones did not provide any evidence for a direct inhibition preceding the excitatory response. Such a response could have been small and undetectable, or inhibitory 'receptors' could be located on an area of the pacemaker neurone which was not exposed to the local applications. Alternatively these responses could be mediated *via* follower neurones, because (1) the same amines which transiently inhibit ganglionic activity (i.e. DA and NE) also transiently hyperpolarize follower neurones and (2) metergoline blocks both the inhibition of ganglionic activity (Fig. 6) and hyperpolarization of follower neurones (Fig. 9) which are produced by DA. DA and NE are thus either evoking pharmacologically similar responses on both pacemaker and follower cells or are inhibiting pacemaker cells *via* followers. Unlike follower neurones of other cardiac ganglia (Otani & Bullock, 1959; Tazaki & Cooke, 1979; Benson, 1980), hyperpolarization of single *Limulus* follower neurones does not decrease the burst frequency of the entire ganglion (Palese *et al.* 1970; G. Augustine & D. McCulloh, unpublished observations). Thus the transient inhibitory response to DA and NE is the first suggestion of a follower-pacemaker feedback pathway in *Limulus*.

The amine-induced decrease in the plateau component of the follower cell burst appears to be, at least in part, a secondary consequence of increased pacemaker activity, since plateau duration decreased when DA was locally applied on to pacemaker neurones (Fig. 11). This may reflect the reciprocal relationship between duration and frequency described in other cardiac ganglia (Brown, 1964; Tazaki & Cooke, 1979; Benson, 1980).

Comparison to neurohormone actions in other cardiac ganglia

Cardiac ganglia of other arthropods have also served as model systems for the study of neurohormone action (Cooke & Sullivan, 1982). Recent studies have examined the sites of action of several putative neurohormones and have also found that neurohormones affect multiple neuronal targets within these cardiac ganglia (Miller *et al.* 1984; Benson, 1984; Sullivan & Miller, 1984). Our experiments complement these other studies by providing the first intracellular recordings of neurohormone actions upon pacemaker neurones (Fig. 1).

Relationship to amine responses of Limulus *heart*

The four amines examined here have several effects on the isolated *Limulus* heart (Augustine *et al.* 1982). They increase the rate and amplitude of heart contractions, while decreasing the duration of individual contractions. In addition, DA and NE transiently decrease the rate and amplitude of the *Limulus* heartbeat. The results presented in this paper are sufficient to explain some of these effects in terms of cellular actions of amines.

It is likely that the amine-induced changes in heart rate are due to actions on cardiac ganglion neurones, because of the numerous parallels between the amine

responses of the intact heart and the isolated cardiac ganglion. These parallels include: similarities in time course, presence of DA/NE inhibition, selective insensitivity to phentolamine (or metergoline) and relative sensitivity to amines (OCT > DA > EPI > NE). One apparent discrepancy is that EPI is less potent than DA on isolated ganglia (Fig. 3), although the two amines are approximately equipotent on the intact heart (Augustine *et al.* 1982). This could reflect a genuine difference between the two preparations, or could merely be a consequence of the wide variability of the *Limulus* EPI response (Augustine *et al.* 1982).

The effects of the amines on the amplitude and duration of heart contractions can be partially explained by the responses of the isolated cardiac ganglion. The decrease in contraction duration observed when amines increase heart rate is probably due to decreases in the duration of the follower cell plateau. This decrease in plateau duration is associated with a reduction in the number of spikes per burst (Figs 2, 5) which reduces the duration of cardiac neuromuscular transmission (Fig. 5 of Watson *et al.* 1985). The transient decreases in contraction amplitude produced by DA and NE may be due to their ability to transiently hyperpolarize follower cells; such a hyperpolarization reduces the frequency of axonal spikes (Fig. 8) and should decrease the number of junction potentials transmitted to the muscle. The increase in contraction amplitude is not simply explained by ganglionic effects: amines decrease the number of follower spikes per burst, which would probably decrease contraction amplitude. The following paper (Watson *et al.* 1985) demonstrates that increased contraction amplitude is due to an enhancement of cardiac neuromuscular transmission and heart muscle contractility.

In sum, we have characterized the actions of several putative neurohormones upon a simple neuronal network, and have ascribed the response of this network to actions upon its various cellular constituents. Our conclusions are compiled in Table 1.

Table 1. *Amine responses of the* Limulus *heart and cardiac ganglion network, summarized from this paper, Watson, Hoshi, Colburne & Augustine (1985) and Augustine, Fetterer & Watson (1982)*

Amines	Effect on whole heart contractions	Cellular actions	Pharmacological antagonists
OCT, EPI, DA, NE	↑rate	↑pacemaker spike frequency	phentolamine
OCT, EPI, DA, NE	↓duration	↓follower plateau duration (may be mediated *via* pacemakers)	phentolamine
OCT, EPI, DA, NE	↑amplitude	↑cardiac neuromuscular transmission and cardiac muscle contractility	none known
DA, NE	transient ↓ rate	↓pacemaker spike frequency (may be mediated *via* followers)	metergoline
DA, NE	transient ↓ amplitude	follower cells hyperpolarize and ↓spikes/burst	metergoline

OCT, octopamine; EPI, epinephrine; DA, dopamine; NE, norepinephrine.

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REFERENCES

ASCHER, P. (1972). Inhibitory and excitatory effects of dopamine on *Aplysia* neurones. *J. Physiol., Lond.* **225**, 173–209.

AUGUSTINE, G. J. (1979). Bursting electrical activity of *Limulus* cardiac ganglion neurons. *Am. Zool.* **19**, 859.

AUGUSTINE, G. J., FETTERER, F. & WATSON, W. (1979). Biogenic amine actions on *Limulus* cardiac ganglion neurons. *Soc. Neurosci. Abstr.* **5**, 493.

AUGUSTINE, G. J., FETTERER, R. & WATSON, W. H. (1982). Amine modulation of the neurogenic *Limulus* heart. *J. Neurobiol.* **13**, 61–74.

AUGUSTINE, G. J., WATSON, W. & FETTERER, R. H. (1978). Biogenic amines and cardioregulation in *Limulus*. *Am. Zool.* **18**, 640.

BARKER, J. L. & SMITH, T. G. (1979). Peptides as neurohormones. In *Approaches to the Cell Biology of Neurons*, (eds W. M. Cowan & J. A. Ferrendelli), Society for Neuroscience Symposia, Vol. 3, pp. 340–373. Bethesda, MD: Society for Neuroscience.

BENSON, J. A. (1980). Burst reset and frequency control of the neuronal oscillators in the cardiac ganglion of the crab, *Portunus sanguinolentus*. *J. exp. Biol.* **87**, 285–313.

BENSON, J. A. (1984). Octopamine alters rhythmic activity in the isolated cardiac ganglion of the crab, *Portunus sanguinolentus*. *Neurosci. Letts* **44**, 59–64.

BENSON, J. A. & COOKE, I. M. (1984). Driver potentials and the organization of rhythmic bursting in crustacean ganglia. *Trends Neurosci.* **7**, 85–91.

BERETTA, C., FERRINI, R. & GLASSER, A. H. (1965). 1-Methyl-8 β-carbobenzyloxy-aminomethyl-10-α-ergoline, a potent and long-lasting 5-hydroxytryptamine antagonist. *Nature, Lond.* **207**, 421–422.

BROWN, H. F. (1964). Electrophysiological investigations of the heart of *Squilla mantis*. I. The ganglionic nerve trunk. *J. exp. Biol.* **41**, 689–700.

BURSEY, C. R. & PAX, R. A. (1970). Microscopic anatomy of the cardiac ganglion of *Limulus polyphemus*. *J. Morph.* **130**, 385–396.

CHAO, I. (1933). Action of electrolytes on the dorsal median nerve cord of the *Limulus* heart. *Biol. Bull. mar. biol. Lab., Woods Hole* **64**, 358–382.

COOKE, I. M. & HARTLINE, D. K. (1975). Neurohormonal alteration of integrative properties of the cardiac ganglion of the lobster, *Homarus americanus*. *J. exp. Biol.* **63**, 33–52.

COOKE, I. M. & SULLIVAN, R. E. (1982). Hormones and secretion. In *The Biology of Crustacea*, Vol. 3, (eds H. Atwood & D. Sandeman), pp. 205–391. New York: Academic Press.

EVANS, P. D. (1981). Multiple receptor types for octopamine in the locust. *J. Physiol., Lond.* **318**, 99–122.

FETTERER, R. H. & AUGUSTINE, G. J. (1977). Dopamine cardioexcitation in *Limulus*. *Am. Zool.* **17**, 959.

HARTLINE, D. K. (1979). Integrative neurophysiology of the lobster cardiac ganglion. *Am. Zool.* **19**, 53–65.

KANDEL, E. R., KRASNE, F. B., STRUMWASSER, F. & TRUMAN, J. W. (1979). Cellular mechanisms in the selection and modulation of behaviour. *Neurosci. Res. Prog. Bull.* **17**, 521–710.

KRAVITZ, E. A. & TREHERNE, J. E. (1980). Neurotransmission, neurotransmitters, and neuromodulators. *J. exp. Biol.* **89**, 1–286.

KUPFERMAN, I. (1979). Modulatory actions of transmitters. *A. Rev. Neurosci.* **2**, 447–465.

LANG, F. (1971). Intracellular studies on pacemaker and follower neurones in the cardiac ganglion of *Limulus*. *J. exp. Biol.* **54**, 815–826.

MCCAMAN, R. E., MCKENNA, D. G. & ONO, J. K. (1977). A pressure system for intracellular and extracellular ejections of picoliter volumes. *Brain Res.* **136**, 141–147.

MAYNARD, D. M. (1966). Integration in crustacean ganglia. *Symp. Soc. exp. Biol.* **20**, 111–149.

MILLER, M. W., BENSON, J. A. & BERLIND, A. (1984). Excitatory effects of dopamine on the cardiac ganglia of the crabs *Portunus sanguinolentus* and *Podophthalmus vigil*. *J. exp. Biol.* **108**, 97–118.

OTANI, T. & BULLOCK, T. H. (1959). Effects of presetting the membrane potential of the soma of spontaneous and integrating ganglion cells. *Physiol. Zoöl.* **32**, 104–114.

PALESE, V. J., BECKER, J. L. & PAX, R. A. (1970). Cardiac ganglion of *Limulus*: intracellular activity in the unipolar cells. *J. exp. Biol.* **53**, 411–423.

SULLIVAN, R. E. & MILLER, M. W. (1984). Dual effects of proctolin on the rhythmic burst activity of the cardiac ganglion. *J. Neurobiol.* **15**, 173–196.

TAZAKI, K. & COOKE, I. M. (1979). Ionic bases of slow, depolarizing responses of cardiac ganglion neurones in the crab, *Portunus sanguinolentus*. *J. Neurophysiol.* **42**, 1022–1047.

WATSON, W. H. & AUGUSTINE, G. J. (1982). Peptide and amine modulation of the *Limulus* heart: a simple neural network and its target tissue. *Peptides* **3**, 485–492.

WATSON, W. H., HOSHI, T., COLBURNE, J. & AUGUSTINE, G. J. (1985). Neurohormonal modulation of the *Limulus* heart: amine actions on neuromuscular transmission and cardiac muscle. *J. exp. Biol.* **118**, 71–84.