Peptides, Vol. 3, pp. 485–492, 1982. Printed in the U.S.A.

Peptide and Amine Modulation of the *Limulus* Heart: A Simple Neural Network and Its Target Tissue

WINSOR H. WATSON, III[1]

Department of Zoology, University of New Hampshire, Durham, NH 03824

AND

GEORGE J. AUGUSTINE

Department of Biology, UCLA, Los Angeles, CA 90024

WATSON, W. H., III AND G. J. AUGUSTINE. *Peptide and amine modulation of the* Limulus *heart: A simple neural network and its target tissue.* PEPTIDES 3(3) 485–492, 1982.—The *Limulus* heart consists of a relatively simple neural network, the cardiac ganglion, and its target tissue, cardiac muscle. The large size and exceptional *in vitro* viability of this system has made it relatively easy to extract, purify, and identify endogenous compounds which alter cardiac function. These agents included peptides, such as protolin and *Limulus* chromatophorotropic factor, and amines such as dopamine, epinephrine, norepinephrine, octopamine, and serotonin. The accessibility and simple organization of the cardiac ganglion has also permitted clear identification of the sites of action of these amines and peptides. The *Limulus* heart is thus a very favorable system for studying peptide and amine neurohormones at the network, cellular and molecular levels.

Proctolin Catecholamines *Limulus* Cardiac muscle Cardiac ganglion Neurohormones Modulation

INVERTEBRATE nervous systems have proven useful for the identification of neuropeptides and their actions on neurons and other target tissues [21,23]. The primary advantage of working with invertebrates is the relative simplicity of their nervous systems. This simplicity permits localization of peptides and other neuroactive compounds to well-defined neural networks or even to single, identifiable neurons and also facilitates analysis of the actions of these compounds at network, cellular, and molecular levels.

During the past few years we and others have been using the neurogenic heart of the horseshoe crab, *Limulus polyphemus*, as a model system for investigating neurohormonal modulation of neural networks. The purpose of this paper is to summarize some of our results, and to introduce the system to others interested in similar questions.

We have focused most of our attention on the *Limulus* cardiac ganglion, a small neural network responsible for generating the rhythmic heartbeat of this animal. The *Limulus* cardiac ganglion is composed of a few hundred neurons, of only two functional types. This simple organization permits detailed physiological and pharmacological studies. The organization of the *Limulus* cardiac ganglion, the properties of its constituent neurons, and the characteristics of the cardiac muscle which it innervates are briefly described in the first section of this paper.

The large size, yet relatively simple organization, of the *Limulus* cardiac ganglion facilitates the separation and purification of neurohormones responsible for regulating cardiac activity. Exploiting these advantages, several peptides and amines have been discovered which modulate cardiac function and, due to the functional simplicity of the system, the cellular targets of these agents have also been identified. These studies are summarized in the second part of this paper.

THE *LIMULUS* HEART AND CARDIAC GANGLION

The *Limulus* heart is located on the dorsal surface of the animal, just beneath the carapace. It is a long tubular organ which is approximately 80% of the length of the animal. The

[1]Address reprint requests to Dr. Winsor H. Watson, Department of Zoology, University of New Hampshire, Durham, NH 03824.

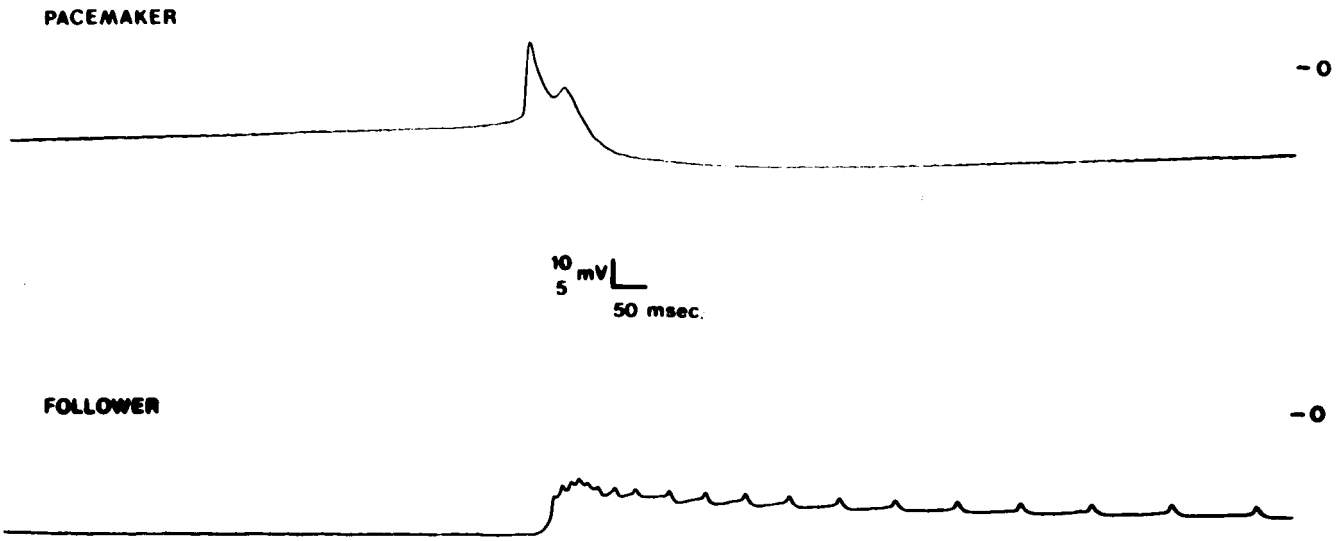

FIG. 1. Simultaneous intracellular recordings of pacemaker and follower neuron electrical activity. During each heartbeat cycle a slow pacemaker potential gives rise to a single overshooting spike in pacemaker cells. Pacemaker cells synaptically drive the follower cells, which produce a series of spikes superimposed on a plateau potential. These follower spikes are small because they are recorded from the follower cell soma, while the spikes are thought to be generated at an electrically distant site in the follower cell axon(s).

cardiac ganglion is on the dorsal surface of the myocardium and it extends the entire length of the heart. A thorough anatomical description may be found in Patten and Redenbaugh [41]. The heart and its associated cardiac ganglion can be easily removed from the animal, permitting its rhythmic heartbeats to be recorded *in vitro* with a force transducer. Such an "isolated heart" preparation is a useful and simple bioassay for cardioactive agents because it remains viable *in vitro* for up to 24 hours and its contractile activity provides a sensitive monitor of both cardiac ganglion and cardiac muscle activity.

The large size of the *Limulus* cardiac ganglion (approximately 1 mm wide and 15–20 cm long) is advantageous for both biochemical and physiological studies. Relatively large quantities of uncontaminated ganglionic tissue can be readily collected for extraction, separation and purification of endogenous neurohormones or neurotransmitters. The sites of action of these agents or synthetic compounds can be determined by applying them to the intact heart, the isolated cardiac ganglion, or the deganglionated myocardium. Thus neurohormonal changes in the output of the entire system can be explained in terms of the alterations produced in its constituent elements.

Cardiac Ganglion

Detailed anatomical studies of the *Limulus* cardiac ganglion have revealed the presence of a few hundred neurons, which are divisible into two distinct size classes [11,19]. The smaller neurons are either bipolar or multipolar and have cell bodies which range from 20–40 μm in diameter. The largest cells have somatic diameters of 60–150 μm, and have a variable number of neurites extending from the cell bodies. Many of the large cells send processes out of the ganglion and apparently innervate the cardiac muscle ([19], and Riordan and Augustine, in preparation).

These two types of cardiac ganglion neurons seem to have

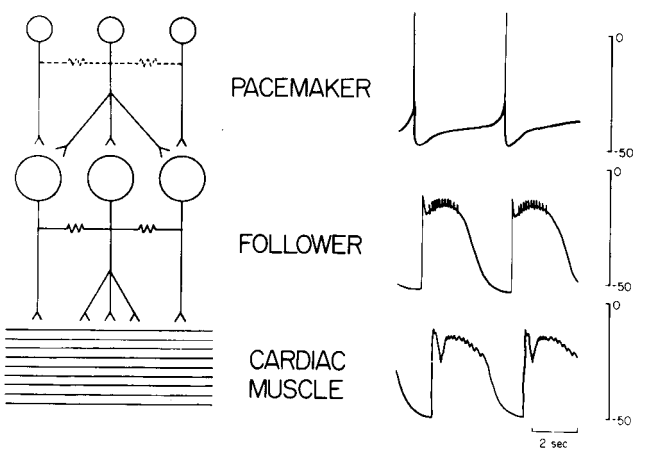

FIG. 2. Schematic organization of the *Limulus* cardiac ganglion network. The traces on the right represent the electrical activity of neurons and muscle fibers depicted in the diagram on the left. Pacemaker (small) cells produce a single overshooting spike during each heartbeat cycle. Pacemaker cells are probably electrically coupled to each other (dashed resistors connecting cells), and they innervate more than one follower (large) cell. Follower cell electrical activity is complex, consisting of synaptic input from pacemaker cells and endogenous voltage-sensitive conductance changes. The result of this activity is a train of action potentials which appear as small deflections upon the prolonged plateau. Follower cells are also electrically coupled to each other, as shown by the interconnecting resistors in the left diagram. Each follower cell innervates many cardiac muscle fibers. Input from several follower cells sums to produce a compound EJP in each muscle fiber.

distinctly different functional roles. Intracellular recordings from these neurons reveal that each has a characteristic type of electrical activity [31,39]. During a single heartbeat cycle,

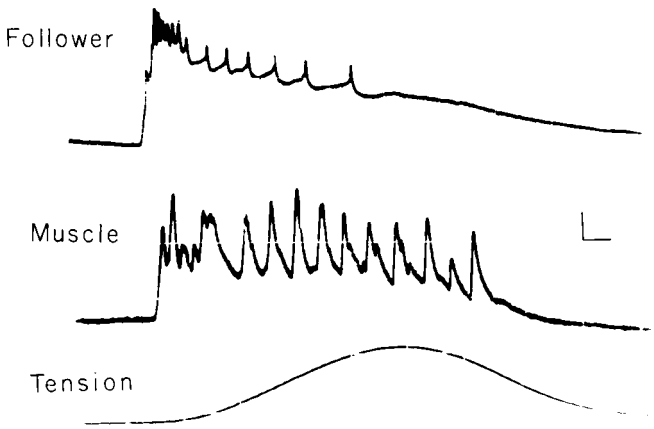

FIG. 3. Simultaneous recordings of follower cell and cardiac muscle cell electrical activity and cardiac muscle tension. Follower cell bursting activity (top) leads to a prolonged compound EJP (center) and a slow, prolonged contraction of the heart muscle (bottom). Calibration: horizontal: 0.1 sec; vertical: 10 mV (top), 5 mV (center), 1 g (bottom).

small neurons produce single overshooting action potentials, while large cells produce trains of action potentials superimposed upon a slowly decaying plateau potential (Fig. 1). These and other results indicate that the small cells are acting as pacemakers, by initiating the heartbeat rhythm, and that they synaptically drive the large cells which act as follower, or motor, neurons. The pacemaker-follower cell synapse is probably chemically mediated because the amplitude of its synaptic potential varies with the external calcium ion concentration (Augustine and McCulloh, unpublished results). The identity of the presumptive transmitter at this synapse is unknown. Follower cells, and probably pacemakers as well, are weakly coupled to each other by electrical synapses [31]. A summary of the organization of the *Limulus* cardiac ganglion network is shown in Fig. 2.

Neuromuscular Junction

The large follower neurons which send axons out of the ganglion are presumably the motorneurons innervating the cardiac muscle [31,39]. Each muscle fiber is innervated by several motor axons, so that electrical stimulation of motor nerve bundles produces one or more excitatory junction potentials (EJPs) in each muscle fiber [1,40]. Glutamate is thought to be the neuromuscular transmitter in *Limulus* ([40], and Watson and Hoshi, in preparation), as in other arthropods [52].

During the normal heartbeat summating compound EJPs occur in the electrically inexcitable heart muscle fibers (Fig. 3). The prolonged duration of the compound EJP results from the pattern of discharge of the motorneurons and the polyneuronal innervation of each muscle fiber. The prolonged depolarization of the *Limulus* heart muscle fibers, which is reminiscent of vertebrate cardiac action potentials, produces a slow wave of contraction in each cardiac muscle cell (Fig. 3).

Cardiac Muscle

Physical or pharmacological removal of the *Limulus* cardiac ganglion abolishes compound EJPs and terminates the cardiac rhythm [1,12]. This indicates that under normal circumstances the deganglionated heart musculature is not capable of producing rhythmic contractions. However, under certain conditions the heart muscle does contract rhythmically in the absence of input from the cardiac ganglion. Exposure of deganglionated cardiac muscle to calcium-free saline or to excessive stretch induces rhythmic action potentials and heart-beat contractions [32,47]. This demonstrates that *Limulus* cardiac muscle is capable of generating action potentials, but that this capacity is not ordinarily expressed. The neuropeptide proctolin is also capable of producing similar rhythmic contractions and action potentials in *Limulus* cardiac muscle ([55], and Watson and Hoshi, in preparation), which raises the possibility that expression of the excitability of these muscle fibers is under neurohormonal control *in vivo*. The electrical properties of *Limulus* heart muscle are analogous in many respects to vertebrate smooth muscle, which is also sensitive to proctolin [44,49]. The actions of proctolin and several other putative neurohormones on *Limulus* cardiac tissues will be discussed in the following section.

TABLE 1

CATECHOLAMINE CONTENTS OF *LIMULUS* NERVOUS TISSUE*

Tissue	Tissue Weight (mg)	DA	NE	E
Brain	73 ± 3	395 ± 120	160 ± 23	<0.1
Cardiac ganglion	10 ± 2	830 ± 63	228 ± 68	6 ± 2
Cardioregulatory nerve	9	125	96	25

*Values are expressed in ng/g wet weight [37].

NEUROHORMONAL MODULATION OF THE CARDIAC GANGLION AND MYOCARDIUM

The purpose of this section is to consider how the *Limulus* cardiac ganglion-myocardium system, described in the previous section, is modulated by neurohormonal agents. We focus on three aspects of this subject:

(1) identification of putative amine and peptide neurohormone candidates,

(2) actions of these agents on the entire cardiac system, as assayed by pharmacological effects on the isolated *Limulus* heart,

(3) the cellular sites of action of these agents.

We begin with a discussion of biogenic amines, because they were the first compounds identified within the *Limulus* cardiac ganglion, and then describe more recent work on peptides.

Amines

The *Limulus* central nervous system and cardiac ganglion contain dopamine (DA), norepinephrine (NE), epinephrine (E), octopamine (OCT) and serotonin [5, 15, 37, 57]. Their distribution in the CNS [37] corresponds to that reported in insects [28] and to a lesser extent crayfish [17]. The levels of DA and NE in *Limulus* nervous tissue (Table 1) are comparable to those reported for the CNS of other arthropods [20,29] and vertebrates [46]. However, the detection of E

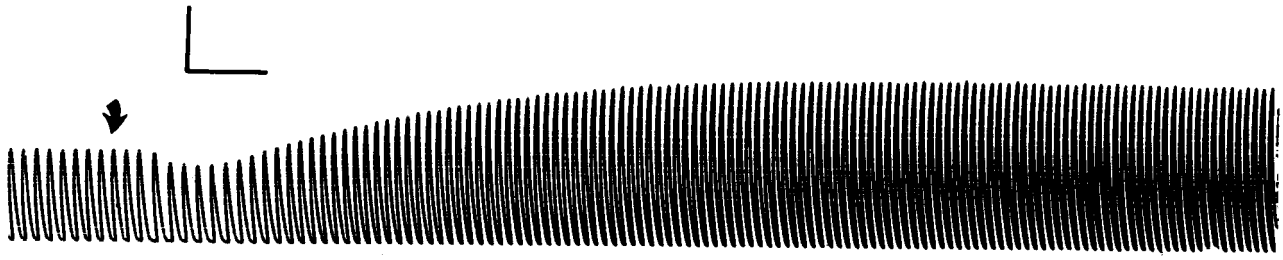

FIG. 4. Response of the isolated *Limulus* heart to dopamine. Bath application of 10^{-6} M dopamine (beginning at arrow) initially inhibits but then excites the *Limulus* heart. Calibration: horizontal: 30 sec; vertical: 0.5 g.

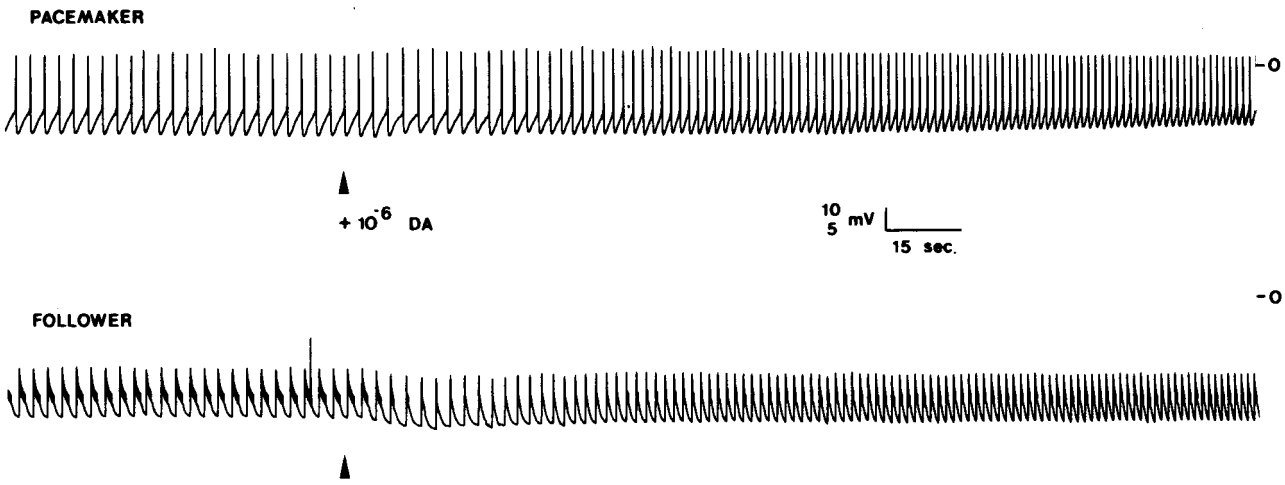

FIG. 5. Amines affect the electrical activity of both cardiac ganglion pacemaker and follower neurons. Dopamine causes a slight transient decrease, and a secondary increase in the rate of firing of pacemaker cells (top trace). This amine also transiently inhibits follower neurons (bottom trace).

is unusual because it has not been found in the CNS of other arthropods [16, 27, 28].

The presence of catecholamines in the cardioregulatory nerves and cardiac ganglion suggest a possible role in cardioregulation and this hypothesis is substantiated by our recent pharmacological studies. Application of DA, OCT, E, or NE increases the rate and amplitude of contractions of the isolated *Limulus* heart [2,22]. DA and NE also produce a transient inhibition which precedes a net increase in heart rate (Fig. 4). These excitatory and inhibitory effects are pharmacologically distinct; the α-adrenergic antagonist phentolamine selectively blocks the excitatory effect of all these amines, while the serotonin antagonist metergoline selectively eliminates the inhibitory response to DA and NE [2]. Thus there may be two or more distinct amine receptors in this tissue [4,18].

The cellular targets for the excitatory amine actions have been identified. NE, OCT, DA, and E all excite the isolated *Limulus* cardiac ganglion, but do not effect the contractility of the *Limulus* cardiac muscle [2]. Intracellular recordings (Fig. 5) reveal that both cardiac ganglion pacemaker and follower neurons are sensitive to amines [3]. Pacemaker neurons respond to amines with an increase in spike frequency; this is responsible for the amine-induced elevation of heart rate. OCT and E also directly excite follower neurons, while DA and NE transiently inhibit these cells. These direct effects on follower neurons probably account for the presence (or absence) of the transient decrease in heartbeat amplitude. All four amines also enhance cardiac neuromuscular transmission (Fig. 6, [53]), which is probably the cause of their ability to increase the strength of heart contractions. Amine modulation of heart rate and contraction amplitude is thus a result of their actions at several sites within the neural network.

Serotonin and the amino acid GABA inhibit the activity of the isolated *Limulus* heart [1,42]. The responses to these two agents are pharmacologically distinct, since only the action of serotonin is blocked by LSD [43]. They can also be distinguished from the transient inhibitory response to dopamine and norepinephrine, since the serotonin and GABA responses are not blocked by metergoline (Watson, unpublished results). Thus amines are capable of eliciting at least three distinct inhibitory responses when applied to the *Limulus* heart.

The cellular targets of these two inhibitory agents are not clear. Their effects are mediated, at least in part, via cardiac ganglion neurons since they inhibit the activity of isolated cardiac ganglia [42]. Preliminary intracellular recordings suggest that serotonin and GABA inhibit both pacemaker and follower neurons (Augustine, unpublished results). Possible effects on neuromuscular transmission or cardiac muscle have not yet been considered.

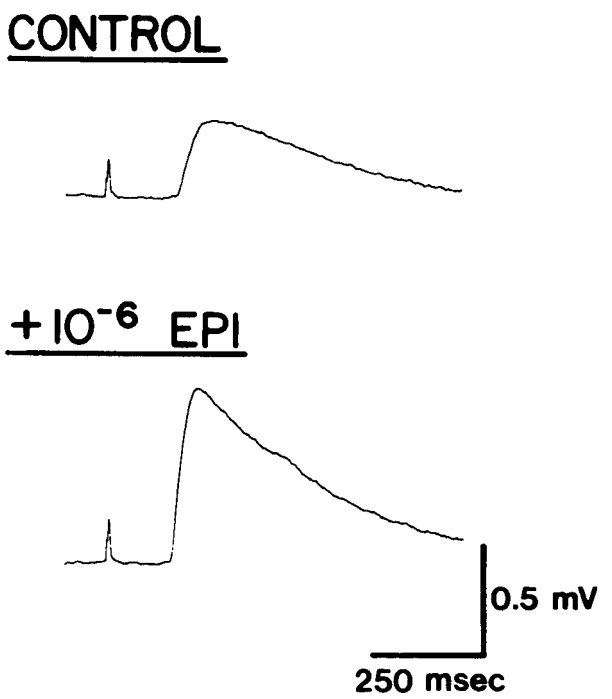

FIG. 6. Amines also enhance cardiac neuromuscular transmission. Unitary EJPs were elicited in *Limulus* cardiac muscle cells by stimulation of motor nerves. Bath application of amines (in this example, 10^{-6} M epinephrine) increases the amplitude of these unitary EJPs. Traces represent averages of 32 responses and the initial deflection in each record is the stimulus artifact.

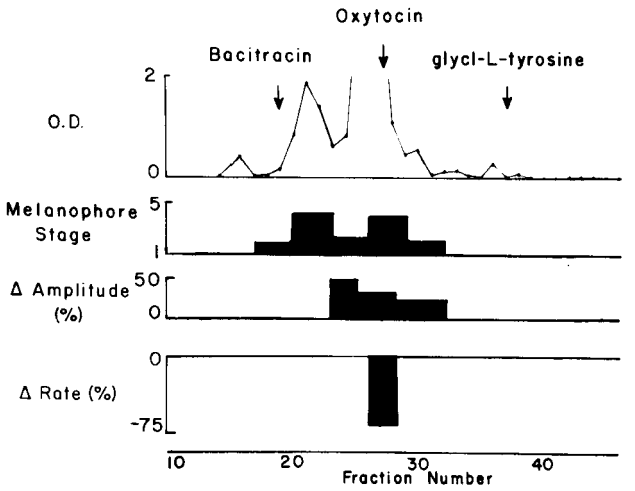

FIG. 7. Separation of LCF and proctolin-like activity from *Limulus* brain extracts. Extracts of ten *Limulus* brains were first passed through a G-25 gel filtration column, and then cardioactive fractions were pooled and separated further on a G-15 column. The column was calibrated with bacitracin (MW=1411), oxytocin (MW=1007) and glycl-L-tyrosine (MW=200). Graphs (from top to bottom) represent results of the following assays performed on aliquots of the G-15 fractions: optical density at 280 nm; changes in the darkness of chromatophores of fiddler crabs *Uca pugilator* (5=darkest); ability to increase the contraction amplitude of an isolated *Limulus* heart; ability to alter heart rate. While chromatophorotropic activity was widely distributed, cardioinhibitory activity was only found in a few fractions. Proctolin-like activity, defined as the ability to increase heartbeat amplitude but not rate, was also present in a fairly broad peak which overlapped, but did not coincide with, chromatophorotropic and cardioinhibitory activity.

Peptides

At least two cardioactive peptides have been identified in the *Limulus* nervous system. One of these resembles the pentapeptide proctolin. Proctolin (Arg-Tyr-Leu-Pro-Thr-OH) is found in many arthropod species, where it affects both nerve and muscle cells [10, 30, 35, 36, 38, 51]. A proctolin-like peptide has also been detected in the mammalian CNS and synthetic proctolin modulates the activity of mammalian smooth muscle [44,49]. The *Limulus* proctolin-like peptide is present in both the CNS and cardiac ganglion [7,54]. Each cardiac ganglion contains approximately 0.6 pmoles of the peptide, while the brain and circumesophageal ganglion contain considerably more. The endogenous peptide has an apparent molecular weight which is slightly greater than synthetic proctolin, but its susceptibility to enzymatic degradation (pronase and leucine aminopeptidase are effective, chymotrypsin and trypsin are not), and pharmacological actions on the *Limulus* heart and cockroach hindgut are nearly identical to synthetic proctolin. In recent studies with HPLC proctolin-like fractions were separated into two peaks of activity (Sullivan and Watson, unpublished). This may be due to the presence of two peptides and it many account for the broad peak of proctolin-like activity obtained with gel filtration of brain and cardiac ganglion extracts (Fig. 7).

The other peptidergic cardioactive factor that has been isolated from the *Limulus* CNS is called *Limulus* chromatophorotropic factor (LCF) because it causes the black melanophores of fiddler crabs to enlarge [9, 14, 45]. LCF is trypsin-sensitive and has a molecular weight of approximately 1000. However, its amino acid sequence is unknown and recent HPLC analysis suggests that it also may be more than one peptide [54]. LCF is present in the *Limulus* CNS in large quantities and may be localized within large, identifiable A_1 neurosecretory cells [33,48].

Both endogenous neuropeptides modulate the activity of the isolated *Limulus* heart. The proctolin-like peptide increases the strength of heart contractions, but does not affect heart rate [7,55]. Synthetic proctolin has an identical effect (Fig. 8), with a threshold concentration of 3×10^{-10} M. The inotropic effects of proctolin are long-lasting. The amplitude of heart contractions remain elevated for approximately 1 hr after treatment with 10^{-8} M proctolin.

Proctolin acts directly on *Limulus* cardiac muscle, because it enhances the contractility of deganglionated heart muscle and has no effect on the cardiac ganglion neurons or neuromuscular transmission [7,55]. At concentrations greater than 10^{-7} M, proctolin also induces rhythmic contractions and spike-like potentials in deganglionated heart muscles (Fig. 9, [24], and Watson and Hoshi, in preparation). As mentioned previously *Limulus* cardiac muscle fibers usually do not produce spikes *in vitro*, so it appears that proctolin is unmasking latent ionic conductances [6]. Proctolin has similar effects on other arthropod muscles [25,30].

Our initial experiments with LCF revealed that it had biphasic effects on the heart. Further purification with gel

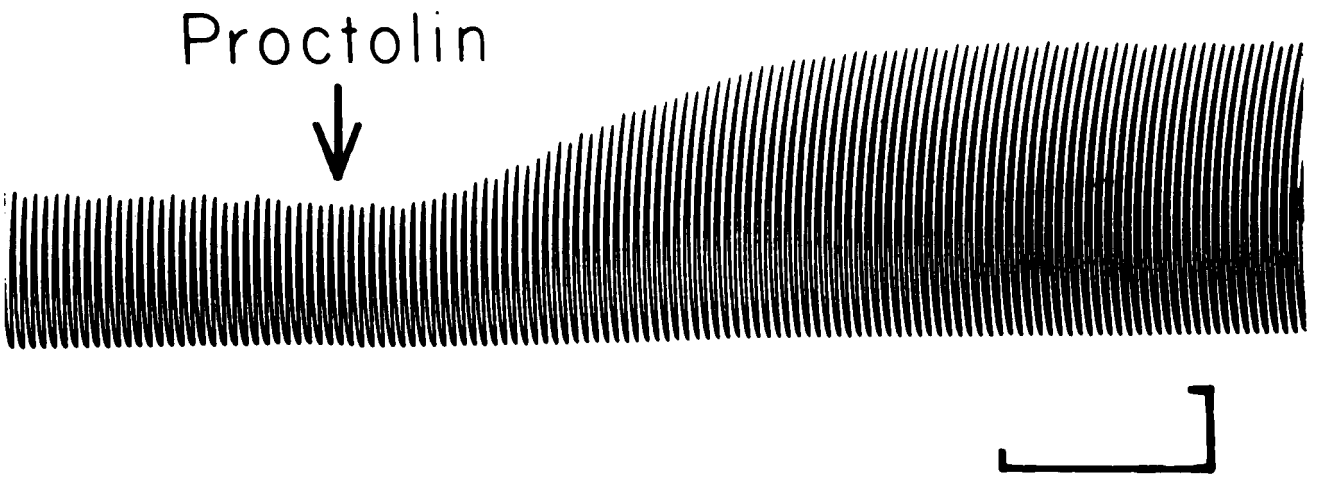

FIG. 8. Proctolin increases the strength of contractions of isolated *Limulus* hearts. Synthetic proctolin (10^{-8} M) was added at the arrow. Calibration: 1 g, 1 min.

filtration and HPLC separated LCF into excitatory, inhibitory, and melanophorotropic components. Both cardioactive fractions appear to act directly on the cardiac ganglion. However, neither cardioactive fraction appears to be responsible for the chromatophorotropic activity of LCF, since activity peaks for heart effects and chromatophorotropic activity do not completely overlap (Fig. 7). Thus LCF may be composed of 3 or more biologically active compounds.

CONCLUSIONS

In this paper we have reviewed some of our work on neurohormonal modulation of the *Limulus* heart and have used our data to illustrate some of the advantages invertebrate preparations have to offer to those interested in peptide and amine actions in the mammalian brain.

One of the primary ways that invertebrate nervous systems have been exploited is for the identification of novel neuropeptides. Invertebrate nervous tissue can be obtained in large quantities (and at little cost) from defined neural systems, such as the *Limulus* cardiac ganglion, or from single cells [34,38]. Small quantities of bioactive peptides can be detected using several convenient bioassays, such as the isolated *Limulus* heart. Because many neuropeptides appear to be conserved throughout the animal kingdom [8, 13, 44, 56, 58], peptides which are readily discovered in invertebrate species may eventually prove to be important agents in vertebrates as well.

Most of our knowledge of the mechanisms responsible for the modulatory effects of peptide and amine neurohormones has come from studies on single cells [26,50]. However, many peptides and amines are released globally to simultaneously influence entire neural networks. Thus a full understanding of the complex physiological and behavioral consequences of neurohormone action, which are likely to include emergent properties not evident from examination of single cells, must also include studies on neurohormone effects on larger neural ensembles. Several invertebrate preparations, including the *Limulus* cardiac ganglion, are well suited for this type of analysis.

We hope this short review will encourage others to take full advantage of the wide variety of invertebrate prepartions to study general questions related to the modulatory actions of neuropeptides and amines.

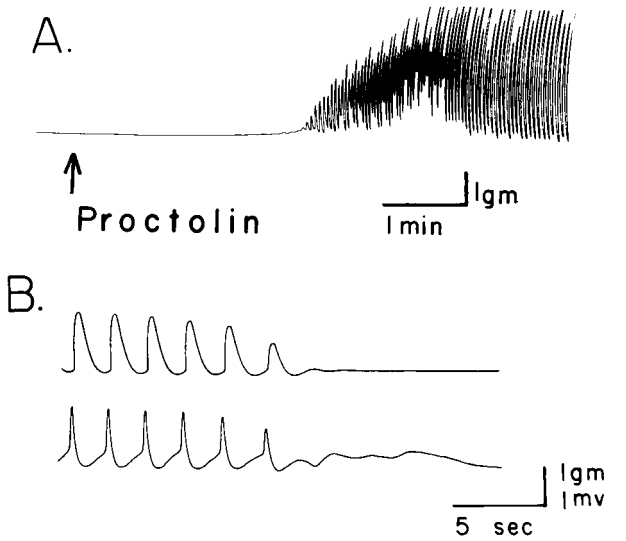

FIG. 9. Proctolin induces myogenic activity in *Limulus* heart muscle. A. Small contracture and rhythmic contractions are observed in deganglionated heart muscle following application of 10^{-6} M proctolin. B. Proctolin-induced contractions are accompanied by action potentials. Mechanical (top trace) and electrical (bottom trace) activity was recorded using a single sucrose-gap chamber. Recordings were obtained during the washoff of proctolin (10^{-6} M) and demonstrate the close correlation between mechanical and electrical activity during cessation of myogenic contractions.

ACKNOWLEDGEMENTS

We thank L. Beres and T. O'Donohue for critically reviewing this manuscript and J. Benson, R. Dores, R. Fetterer, T. Hoshi, D. McCulloh, K. Neill, E. O'Connor, T. O'Donohue, G. P. Riordan, R. Sullivan, E. Tillinghast, and G. Wyse for their collaboration on many of the studies summarized here. Work described in this paper was supported by a grant from the New Hampshire Heart Association, a Grass Foundation Fellowship, a Summer Faculty Fellowship, and MBL Steps Towards Independence Fellowship to W. Watson, and an MDA Postdoctoral Fellowship, Grass Foundation Fellowship, and NSF Graduate Fellowship to G. Augustine. This is contribution Number 178 of the Tallahassee, Sopchoppy and Gulf Coast Marine Biological Association, Inc.

REFERENCES

1. Abbott, B. C., F. Lang and I. Parnas. Physiological properties of the heart and cardiac ganglion of *Limulus polyphemus*. *Comp. Biochem. Physiol.* **28:** 149–158, 1969.
2. Augustine, G. J., R. Fetterer and W. H. Watson. Amine modulation of the neurogenic *Limulus* heart. *J. Neurobiol.* **13:** 61–74, 1982.
3. Augustine, G. J. and R. H. Fetterer. Neurohormonal modulation of the *Limulus* heart: amine actions on cardiac ganglion neurons. Submitted for publication.
4. Batta, S., R. J. Walker and G. N. Woodruff. Pharmacological studies on *Helix* neuron octopamine receptors. *Comp. Biochem. Physiol.* **64C:** 43–51, 1979.
5. Battelle, B. A. Neurotransmitter candidates in the visual system of *Limulus polyphemus:* synthesis and distribution of octopamine. *Vision Res.* **20:** 911–922, 1980.
6. Benson, J. A. Synaptic and regenerative responses of cardiac muscle fibres in the crab, *Portunus*. *J. comp. Physiol.* **143:** 349–356, 1981.
7. Benson, J. A., R. E. Sullivan, W. H. Watson and G. J. Augustine. The neuropeptide proctolin acts directly on *Limulus* cardiac muscle to increase the amplitude of contraction. *Brain Res.* **213:** 449–454, 1981.
8. Bodenmuller, H. and H. C. Schaller. Conserved amino acid sequence of a neuropeptide, the head activator, from coelenterates to humans. *Nature* **293:** 579–583, 1981.
9. Brown, F. A. and O. Cunningham. Upon the presence and distribution of a chromatophorotropic principle in the central nervous system of *Limulus polyphemus*. *Biol. Bull.* **29:** 409–418, 1941.
10. Brown, B. E. Occurrence of proctolin in six orders of insects. *J. Insect Physiol.* **23:** 861–864, 1977.
11. Bursey, C. R. and R. A. Pax. Microscopic anatomy of the cardiac ganglion of *Limulus polyphemus*. *J. Morph.* **103:** 385–396, 1970.
12. Carlson, A. J. The nervous origin of the heartbeat in *Limulus* and the nervous nature of co-ordination of conduction in the heart. *Am. J. Physiol.* **12:** 67–74, 1904.
13. Dockray, G. J., C. Vaillant and R. C. Williams. New vertebrate brain-gut peptide related to molluscan neuropeptide and an opioid peptide. *Nature* **293:** 656–657, 1981.
14. Dores, R. M. and W. S. Herman. The localization of two putative neurohormones in the central nervous system of *Limulus polyphemus*. *Comp. Biochem. Physiol.* **67:** 459–463, 1980.
15. Edwards, S. C., S. K. Pierce and B. A. Battelle. Hyposmotic stress-induced release of octopamine from isolated cardiac ganglia of *Limulus polyphemus*. *Am. Zool.* **19:** 859, 1979.
16. Elofsson, R. and N. Klemm. Monoamine-containing neurons in the optic ganglia of crustaceans and insects. *Z. Zellforsch.* **133:** 475–499, 1972.
17. Elofsson, R., D. Nasel and H. Myhreberg. A catecholaminergic neuron connecting the first two optic neuropiles (lamina ganglionaris and medulla externa) of the crayfish *Pacifastacus leniusculus*. *Cell Tissue Res.* **182:** 287–297, 1977.
18. Evans, P. D. Multiple receptor types for octopamine in the locust. *J. Physiol.* **318:** 99–122, 1981.
19. Fedele, M. Sulla innervazione intracardiaca del *Limulus polyphemus*. *Archo zool. ital.* **30:** 39–137, 1942.
20. Frontali, N. and J. Haggendal. Noradrenaline and dopamine content in the brain of the cockroach *Periplaneta americana*. *Brain Res.* **14:** 540–542, 1969.
21. Greenberg, M. J. and D. A. Price. Cardioregulatory peptides in molluscs. In: *Peptides: Integrators of Cell and Tissue Function,* edited by F. E. Bloom. New York: Raven Press, 1980, pp. 107–126.
22. Grega, D. S. and R. G. Sherman. Responsiveness of neurogenic hearts to octopamine. *Comp. Biochem. Physiol.* **52C:** 5–8, 1975.
23. Gaynes, L. W. Peptide neuroregulators in invertebrates. *Prog. Neurobiol.* **15:** 205–245, 1980.
24. Hoshi, T. and W. H. Watson. Proctolin induces myogenicity in the deganglionated *Limulus* heart. *Soc. Neurosci. Abstr.* **7:** 254, 1981.
25. Irving, S. N. and T. A. Miller. Octopamine and proctolin mimic spontaneous membrane depolarizations in *Lucilia* larvae. *Experientia* 36: 566–567, 1980.
26. Kehoe, J. S. and A. Marty. Certain slow synaptic responses: their properties and possible underlying mechanisms. *A. Rev. Biophys. Bioengng.* **9:** 437–465, 1980.
27. Kerkut, G. A., C. B. Sedden and R. J. Walker. The effect of Dopa, α-methyl dopa and reserpine on the dopamine content of the brain, of the snail, *Helix aspersa*. *Comp. Biochem. Physiol.* **18:** 921–930, 1966.
28. Klemm, N. Histochemistry of putative transmitter substances in the insect brain. *Prog. Neurobiol.* **7:** 99–169, 1976.
29. Klemm, N. and A. Bjorklund. Identification of dopamine and noradrenaline in the nervous structures of the insect brain. *Brain Res.* **26:** 459–464, 1971.
30. Kravitz, E. A., S. Glusman, R. M. Harris-Warrick, M. S. Livingstone, T. Schwarz and M. F. Goy. Amines and a peptide as neurohormones in lobsters: actions on neuromuscular preparations and preliminary behavioral studies. *J. exp. Biol.* **89:** 159–175, 1980.
31. Lang, F. Intracellular studies on pacemaker and follower neurones in the cardiac ganglion of *Limulus*. *J. exp. Biol.* **54:** 815–826, 1971.
32. Lang, F. Induced myogenic activity in the neurogenic heart of *Limulus polyphemus*. *Biol. Bull.* **141:** 269–277, 1971.
33. Leetma, B. A morphological and electrophysiological study of A_1 neurosecretory cells in *Limulus polyphemus*. Ph.D. Thesis, University of Massachusetts, Amherst, MA, 1976.
34. Lloyd, P. E., B. Masinovsky, R. E. McCaman and A. O. D. Willows. Coexistence of a neuropeptide and acetylcholine in an identified molluscan neuron. *Soc. Neurosci. Abstr.* **7:** 637, 1981.
35. Miller, T. Nervous versus neurohormonal control of insect heartbeat. *Am. Zool.* **19:** 77–86, 1979.
36. Miller, M. and R. E. Sullivan. Some effects of proctolin on the cardiac ganglion of the Maine lobster *Homarus americanus* (Milne Edwards). *J. Neurobiol.* **12:** 629–639, 1981.
37. O'Connor, E., W. H. Watson and G. Wyse. Identification and localization of catecholamines in the nervous system of *Limulus polyphemus*. *J. Neurobiol.* **13:** 49–62, 1982.
38. O'Shea, M. and M. E. Adams. Pentapeptide (proctolin) associated with an identified neurone. *Science* **213:** 567–569, 1981.
39. Palese, V. J., J. L. Becker and R. A. Pax. Cardiac ganglion of *Limulus*: intracellular activity in the unipolar cells. *J. exp. Biol.* **53:** 411–423, 1970.
40. Parnas, I., B. C. Abbott and F. Lang. Electrophysiological properties of *Limulus* heart and effect of drugs. *Am. J. Physiol.* **217:** 1814–1822, 1969.
41. Patten, W. and W. A. Redenbaugh. Studies on *Limulus*. II. The nervous system of *Limulus polyphemus* with observations upon the general anatomy. *J. Morph.* **16:** 99–200, 1899.
42. Pax, R. A. and R. C. Sanborn. Cardioregulation in Limulus. II. Gamma-aminobutyric acid, antagonists and inhibitory nerves. *Biol. Bull.* **132:** 381–391, 1967.
43. Pax, R. A. and R. C. Sanborn. Cardioregulation in *Limulus*. III. Inhibition by 5-hydroxytryptamine and antagonism by bromlysergic acid diethylamide and picrotoxin. *Biol. Bull.* **132:** 392–403, 1967.
44. Penzlin, H., H. Agricola, M. Eckert and T. Kusch. Distribution of proctolin in the sixth abdominal ganglion of *Periplaneta americana* L. and the effect of proctolin on the ileum of mammals. *Adv. Physiol. Sci.* **22:** 525–540, 1981.
45. Pezalla, P., R. M. Dores and W. S. Herman. Separation and partial purification of central nervous system peptides from *Limulus polyphemus* with hyperglycemic and chromatophorotrophic activity in crustaceans. *Biol. Bull.* **154:** 148–156, 1978.

46. Refshauge, C., P. T. Kissinger, R. Drieling, L. Blank, R. Freeman and R. N. Adams. New high performance liquid chromatographic analysis of brain catecholamines. *Life Sci.* **14:** 311–322, 1974.
47. Rulon, R., K. Hermsmeyer and N. Speralakis. Regenerative action potentials induced in the neurogenic heart of *Limulus polyphemus*. *Comp. Biochem. Physiol.* **39A:** 333–355, 1971.
48. Scharrer, B. Neurosecretion IV. Localization of neurosecretory cells in the central nervous system of *Limulus*. *Biol. Bull.* **81:** 96–104, 1941.
49. Schulz, H., H. Schwartzberg and H. Penzlin. The insect neuropeptide proctolin can affect the CNS and smooth muscle of mammals. *Acta biol. med. germ.* **40:** K1–K5, 1981.
50. Shain, W. and D. O. Carpenter. Mechanisms of synaptic modulation. *Int. Rev. Neurobiol.* **22:** 205–244, 1981.
51. Sullivan, R. E. A proctolin-like peptide in crab pericardial organs. *J. exp. Zool.* **210:** 543–552, 1979.
52. Takeuchi, A. and N. Takeuchi. The effect on crayfish muscle of iontophoretically applied glutamate. *J. Physiol.* **170:** 296–317, 1964.
53. Watson, W. H. and T. Hoshi. Amines increase the strength of *Limulus* heart contractions by enhancing neuromuscular transmission. *Biol. Bull.* **161:** 317, 1981.
54. Watson, W. H., III, K. Neill, E. Tillinghast, R. M. Dores, G. Augustine and T. O'Donohue. *Limulus* cardioactive peptides. *Soc. Neurosci. Abstr.* **7:** 254, 1981.
55. Watson, W. H., III, G. J. Augustine, J. A. Benson and R. E. Sullivan. Proctolin and an endogenous proctolin-like peptide enhance the contractility of the *Limulus* heart. Submitted.
56. Weber, E., C. J. Evans, S. J. Samuelsson and J. D. Barchas. Novel peptide neuronal system in rat brain and pituitary. *Science* **214:** 1248–1251, 1981.
57. Welsh, J. H. and M. Moorhead. The quantitative distribution of 5-hydroxytryptamine in the invertebrates, especially their nervous systems. *J. Neurochem.* **6:** 146–169, 1960.
58. Zipser, B. Identification of specific leech neurones immunoreactive to enkephalin. *Nature, Lond.* **283:** 857–858, 1980.