J. exp. Biol. **111**, 123–130 (1984)
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NEUROHORMONAL MODULATION OF THE CARDIAC OUTFLOW THROUGH THE CARDIOARTERIAL VALVE IN THE LOBSTER

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Accepted 29 February 1984

SUMMARY

1. Systolic pressure in the abdominal artery of the lobster *Panulirus japonicus* (Von Siebold) was decreased by contraction of the cardioarterial valve located at the posterior exit of the heart. Crustacean neurohormones, octopamine and proctolin, induced rhythmic contractions or contracture of the posterior valve but serotonin did not induce valve contracture.

2. Systolic pressure in the posterior artery was reduced when the anterior cardioarterial valves contracted at the same time as the posterior valve. Proctolin caused the anterior and posterior valves to contract. Octopamine caused only the posterior valve to contract and often caused the anterior valves to relax.

3. Proctolin induced depolarizing responses while serotonin often induced hyperpolarizing responses in muscle cells of the anterior and posterior valves. Octopamine hyperpolarized the resting membrane potential of muscle cells in the anterior valves and depolarized the membrane potential in the posterior valve.

4. In some intracellular recordings from the valve muscle cells, slow action potentials followed by a plateau were produced on slow sustained depolarization induced by proctolin or octopamine. The action potential was followed by a large contraction of the valve. Contracture of the valve was associated with a large sustained depolarization in single muscle cells of the valve.

INTRODUCTION

In decapod crustaceans, serotonin, octopamine and a proctolin-like peptide are released as hormones from the pericardial neurosecretory organs (Maynard & Welsh, 1959; Cooke & Goldstone, 1970; Sullivan, Friend & Barker, 1977; Sullivan, 1978, 1979; Kravitz *et al.* 1980; Schwarz, Lee & Kravitz, 1981). The effects of these hormones on heart beat have been studied extensively (Cooke, 1966; Cooke & Hartline, 1975; Battelle & Kravitz, 1978; Florey & Rathmayer, 1978; Lemos & Berlind, 1981; Miller & Sullivan, 1981; Kuramoto & Ebara, 1982*a*). In lobsters, we have found that octopamine causes a drop in systolic pressure in the posterior arteries even

Key words: Lobster, arterial valve, serotonin, octopamine, proctolin, circulation, neurohormone.

at concentrations (e.g. 5 nM) lower than that need to affect heart beat. The pressure drop is due to contraction of the cardioarterial valve located at the posterior exit of the heart (Kuramoto & Ebara, 1982*b*). In a lobster heart, there are five small anterior and a single posterior cardioarterial valves. Each of them consists of two membranous flaps, containing muscle fibres, which project into the lumen of the artery in the direction of blood flow. A contraction of valve muscle draws the aperture edges together to close the opening. Regulation of the valve contraction has not been studied in spite of its importance to our understanding of the circulation of lobsters (Maynard, 1960). The responses of the anterior and posterior valves to each of the three hormones are reported here. The results suggest that a change of tension in each of the valves induced by the hormones may alter the cardiac outflow through each of the arteries.

MATERIALS AND METHODS

All experiments were performed on the spiny lobster, *Panulirus japonicus*. The methods for isolating, perfusing and recording from the heart with intact valves were similar to those reported previously (Kuramoto & Ebara, 1984). The heart beat and the valve contraction *in situ* were recorded simultaneously with strain gauges and the pressure in the abdominal artery was monitored with a pressure sensor (Fig. 1). The anterior and posterior arteries with intact valves and adjoining connective tissue were isolated from the heart. Hormone responses to the anterior and posterior valves were recorded simultaneously in the perfusion chamber (10 ml). Intracellular recordings were made from valve muscle fibres using glass microelectrodes (20–30 MΩ) and conventional amplification circuitry.

RESULTS AND DISCUSSION

When octopamine (10 nM–1 μM) was introduced in the perfusion medium, the tension of the posterior valve increased and systolic pressure in the abdominal artery was reduced although the heart beat was enhanced (Fig. 2A). After the valve relaxed, the systolic pressure in the artery returned to its original value. Serotonin (10 nM–1 μM) did not increase valve tension, but sometimes relaxed the valve (Fig. 2B, middle trace). Therefore, the valve did not reduce the systolic pressure in the posterior artery (Fig. 2B, lower trace). Proctolin (1 nM–0·1 μM) induced contracture of the valve and systolic pressure in the artery decreased (Fig. 2C). Perfusion with proctolin caused a reduction in systolic pressure that was usually smaller than that induced by octopamine and often led to a transient increase in systolic pressure just after release of the valve contracture (Fig. 2C, lower trace).

In the isolated preparations, proctolin (0·1 nM–0·1 μM) induced rhythmic contractions and/or contracture of the anterior and posterior valves (Fig. 3A). Serotonin (10 nM–1 μM) did not induce contracture of the isolated anterior and posterior valves, although small single or repetitive contractions sometimes occurred (10%). Serotonin (1 μM) reduced the valve contracture induced by octopamine or proctolin (Fig. 3B). Therefore, serotonin might have an inhibitory effect on the valve contractions. Octopamine (10 nM–1 μM) induced rhythmic contractions and/or contracture

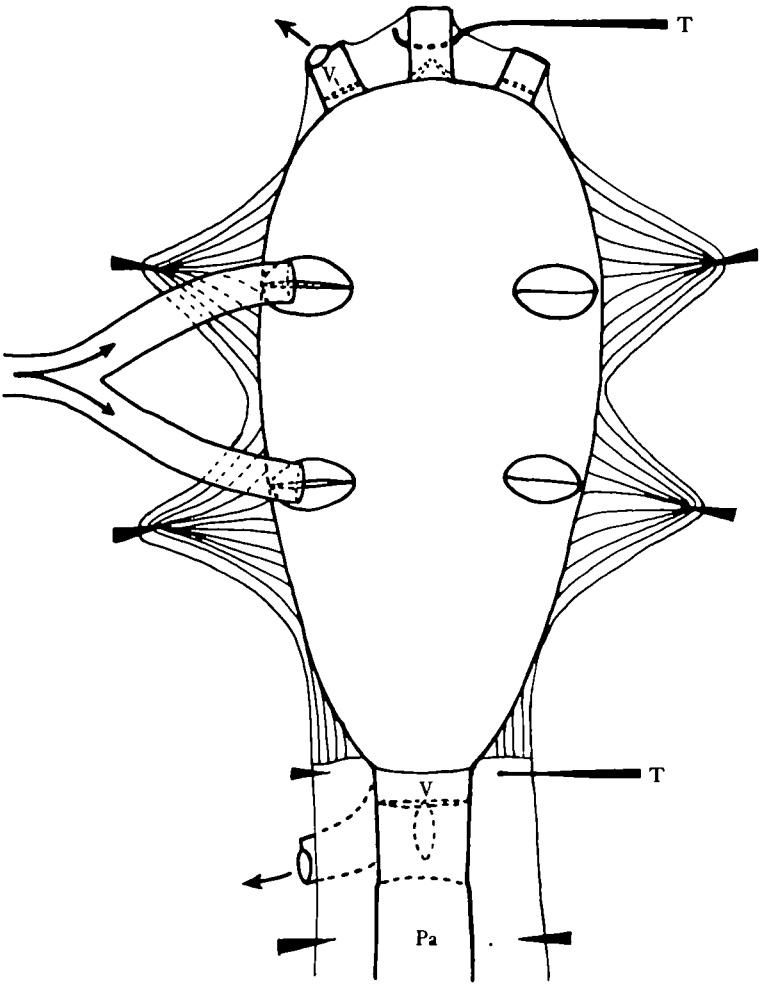

Fig. 1. Experimental arrangement. The isolated heart of the lobster is suspended in a chamber by the ligaments and connective tissue of the arteries. Flow of the perfusion media (arrows) is from the left ostia to the left antennary and the sternal arteries. The cardioarterial valves (V) are situated at the gates from ventricle to arteries. Changes of tension (T) produced by heart beat and valve contraction are recorded by strain gauges. Pressure in the dorsal abdominal artery (Pa) is monitored with a pressure sensor.

of the posterior valve (Fig. $3C_1$) and often relaxed the anterior valves (cf. Fig. $3C_2$). Octopamine (1 μM) suppressed the contracture of anterior valves induced by proctolin (Fig. $3A_2$) and also reduced spontaneous contractions of the anterior valves (Fig. $3C_2$). The octopamine effect was blocked by phentolamine (10 μM or less) but not by picrotoxin (10 μM).

In some intracellular recordings from the valve muscle cells, slow action potentials followed by a plateau were observed (Fig. 4A, upper trace). They were superimposed on spontaneous slow depolarizing potentials or slow, sustained depolarizations induced by octopamine. The action potential was followed by a large contraction of the valve (Fig. 4B). The contracture of the valve corresponded to a large sustained

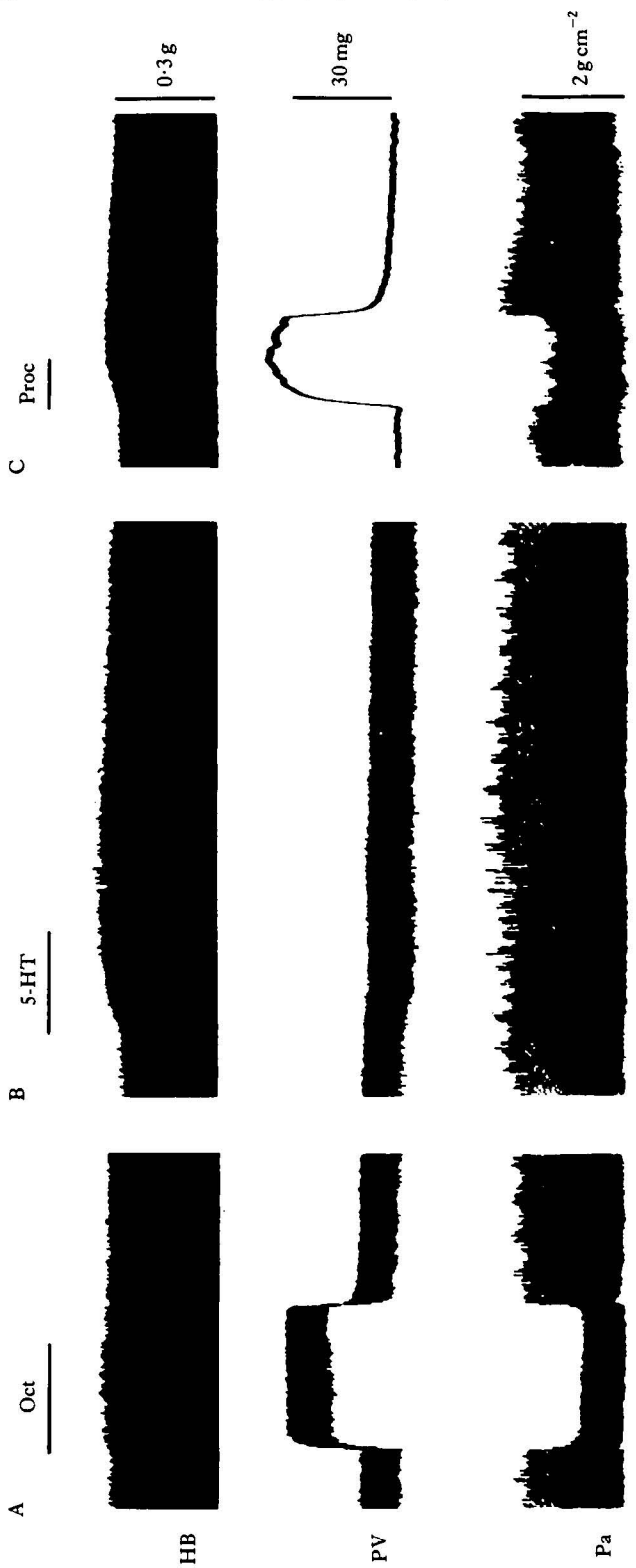

Fig. 2. Simultaneous recordings of heart beat (HB), contraction of the posterior valve (PV) and pressure in the abdominal artery (Pa). (A) Octopamine (Oct: 0·1 μM) enhances the heart beat and induces contracture of the valve, which reduces systolic pressure in the artery. (B) Serotonin (5-HT: 0·1 μM) also enhances the heart beat (same heart as in A) and appears to relax the valve slightly. Systolic pressure in the artery is not reduced by serotonin. (C) Proctolin (Proc: 10 nM) also enhances the heart beat (different heart from that in A and B) and induces contracture of the valve, but systolic pressure in the artery is not reduced as much as in A. Note a transient increase in systolic pressure just after relaxation of the valve. Although records of valve tension are often contaminated by the heart beat (A and B), the contamination is eliminated in this record.

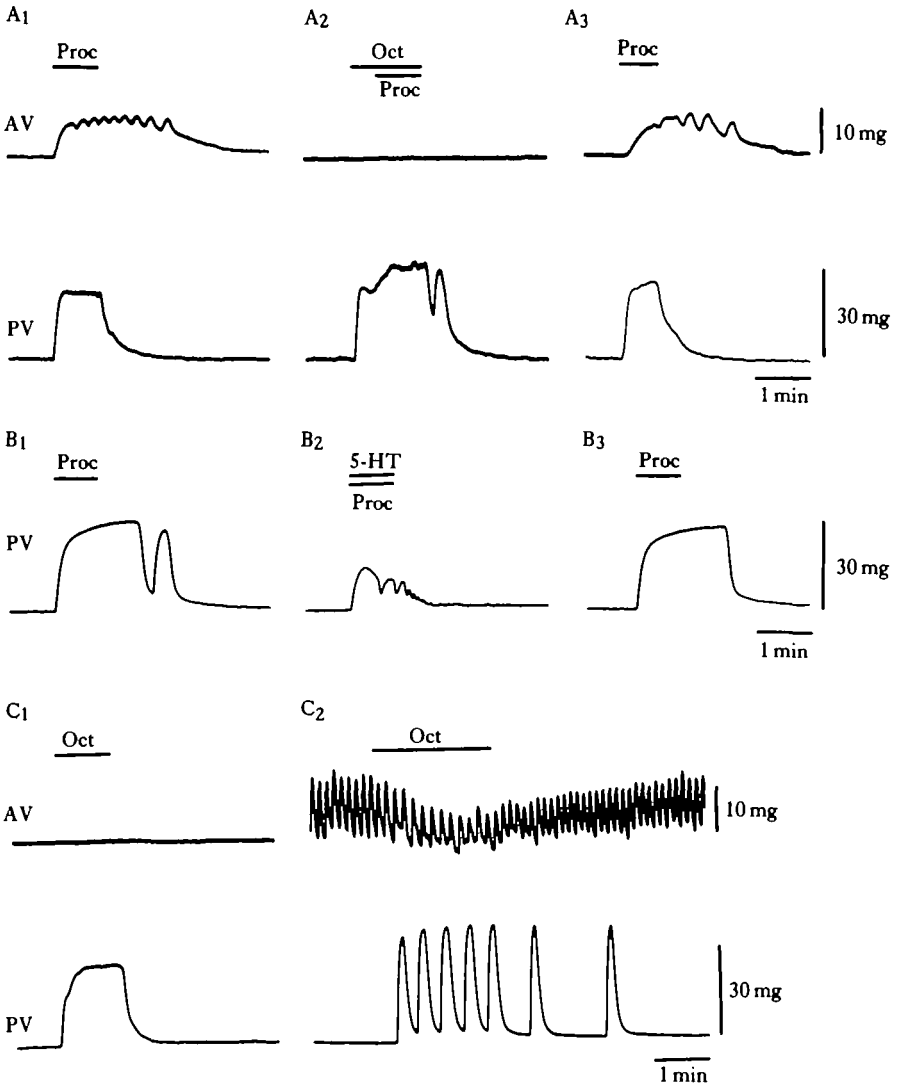

Fig. 3. Contraction and relaxation of the cardioarterial valves isolated from the hearts. (A_1) Contracture and rhythmic activity of the anterior valve (AV) and the posterior valve (PV) induced by proctolin (Proc: 10 nM). (A_2) The proctolin-induced contracture of the anterior valve is suppressed completely by octopamine (Oct: 1 μM), whereas the contracture of the posterior valve appears to be augmented. (A_3) Restoration of the response to proctolin after 10 min. (B_1) Contraction of the posterior valve (PV) induced by proctolin (Proc: 10 nM). (B_2) Serotonin (5-HT: 1 μM) reduces the contraction induced by proctolin (10 nM). (B_3) A normal response to proctolin recovered after 10 min. (C_1) Octopamine (Oct: 0·1 μM) does not induce any contraction of the anterior valve (AV) but induces contracture of the posterior valve (PV). (C_2) Octopamine (10 nM) reduces spontaneous contractions of the anterior valve (AV) by decreasing the tonus while inducing rhythmic contractions of the posterior valve.

depolarization (approx. 20 mV) in single muscle cells of the valve (Fig. 4B). A similar effect has been seen in the visceral muscle cells of the cockroach (Nagai & Brown, 1969). Proctolin induced depolarizing responses, while serotonin often induced hyperpolarizing responses in the muscle cells of the anterior and posterior valves (not

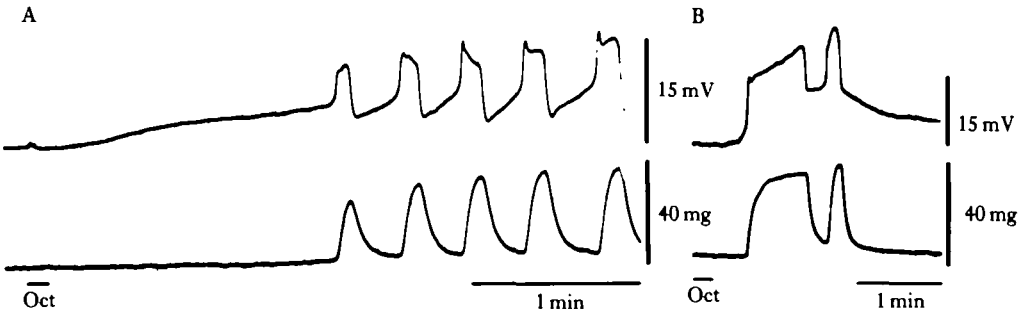

Fig. 4. Membrane potential changes in the valve muscle cell and corresponding contractions of the cardioarterial valve of the lobster. (A) Slow action potentials (upper trace) and corresponding contractions (lower trace) of the posterior valve induced by octopamine (Oct: 0·1 μM). The resting potential is −50 mV. (B) sustained depolarizations (upper trace) and corresponding contracture (lower trace) of the posterior valve induced by octopamine (Oct: 1 μM).

shown in Figures). Further, octopamine (0·1 μM) hyperpolarized the resting membrane potential of muscle cells in the anterior valves (e.g. from 53 to 62 mV) while it depolarized the membrane potential in the posterior valve. This suggests that the muscle cells of the anterior valves and of the posterior valve may have membranes whose specific ion permeabilities are affected by octopamine in different ways.

An increase in tension of the valves should produce a higher resistance to cardiac outflow through them. The biphasic nature of the proctolin effect on systolic pressure in the artery (Fig. 2C) can be explained by the fact that all of the valves are first caused to contract by proctolin and then relax. The small decrease in systolic pressure in the artery reflects an increase of perfusion pressure in the heart due to the high resistance to cardiac outflow. The transient increase of pressure in the artery after relaxation of the valves also reflects a high perfusion pressure in the heart which increases during the valve contracture. It is therefore suggested that the proctolin effect in intact hearts often involves an increase of perfusion pressure in the heart, which can enhance the heart beat, as reported previously (Maynard, 1960; Kuramoto & Ebara, 1984). In contrast, the octopamine effect which relaxes the anterior valves cannot increase perfusion pressure in the heart. Therefore, octopamine simply decreases the systolic pressure in the posterior artery (Fig. 2A).

The valve in the ophthalmic artery is innervated by a pair of the anterior cardiac nerves (Lemoine's nerve) and each of the other cardioarterial valves is innervated by a pair of the segmental nerves, as has also been described in *Homarus* (Alexandrowicz, 1932). The posterior valve is also innervated by a pair of the abdominal nerves. If all are motor neurones, contraction or relaxation of each valve may be mediated by one or two kinds of transmitters. Sullivan *et al.* (1977) have reported synthesis of octopamine in the nerves innervating the anterior cardioarterial valves. Therefore, octopamine may be a transmitter for the motor neurones regulating the anterior valves. Application of octopamine might therefore mimic the synaptic transmissions. However, synaptic potentials in the valve muscle have not been identified in any lobster. We have no evidence available at present that proctolin or serotonin is a transmitter for the motor neurones regulating the cardioarterial valves. Nevertheless, the slow membrane potential changes of the single muscle cells in response to each

The three hormones at low concentrations (cf. Fig. 4A) suggest that the hormones have a direct action and that there are three kinds of receptors on the muscle membrane. The presence of one or two kinds of receptors on the postsynaptic membrane of the valve muscle, can be inferred from the innervation; at least one of the three kinds of receptors can function as a hormone receptor. It is therefore conceivable that the tension of the cardioarterial valves is regulated by a form of remote hormonal modulation.

It has been demonstrated that the hormones octopamine and serotonin are released from the ligament nerve terminals at the perimeter of ostia in *Panulirus interruptus* (Sullivan *et al.* 1977; Sullivan, 1978). Therefore, the tension of the cardioarterial valves of *Panulirus* is probably changed if each of the hormones is released from the nerve terminals at concentrations of more than 10 nM. Serotonin enhanced the heart beat and often relaxed the valves (Fig. 2B). Therefore, serotonin release can increase the amount of cardiac outflow into the anterior and posterior arteries. On the other hand, octopamine increased tension of only the posterior valve and relaxed the anterior valves (Fig. 3C). This might give the cardiac outflow a bias from the posterior to the anterior direction. Moreover, the heart beat was also enhanced by octopamine (Fig. 2A). Therefore, octopamine release probably causes the powerful blood flow to the head of the lobster by preventing reverse blood flow from the posterior arteries. Proctolin (0·1 nM or more) contracted all of the valves and also enhanced the heart beat (Figs 2C, 3A). Therefore, proctolin release may enhance valve function and prevent backward blood flow during powerful movements of the lobster which may be caused by proctolin circulating through the body (Schwarz, Harris-Warrick, Glusman & Kravitz, 1980).

We are grateful to Dr Eisuke Munekata for providing the synthetic proctolin. Contribution from the Shimoda Marine Research Centre, No. 414.

REFERENCES

ALEXANDROWICZ, J. S. (1932). The innervation of the heart of the crustacea. I. Decapoda. *Q. Jl microsc. Sci.* **75**, 181–249.

BATTELLE, B. A. & KRAVITZ, E. A. (1978). Targets of octopamine action in the lobster; cyclic nucleotide changes and physiological effects in haemolymph, heart and exoskeletal muscle. *J. Pharmacol. exp. Ther.* **205**, 438–448.

COOKE, I. M. (1966). The sites of action of pericardial organ extract and 5-hydroxytryptamine in the decapod crustacean heart. *Am. Zool.* **6**, 107–121.

COOKE, I. M. & GOLDSTONE, M. W. (1970). Fluorescence localization of monoamines in crab neurosecretory structures. *J. exp. Biol.* **53**, 651–668.

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.

FLOREY, F. & RATHMAYER, M. (1978). The effects of octopamine and other amines on the heart and on neuromuscular transmission in decapod crustaceans; further evidence for a role as a neurohormone. *Comp. Biochem. Physiol.* **61C**, 229–237.

KRAVITZ, E. A., GLUSMAN, S., HARRIS-WARRICK, R. M., LIVINGSTONE, M. S., SCHWARZ, T. L. & GOY, M. F. (1980). Amines and a peptide as neurohormones in lobsters: action on neuromuscular preparations and preliminary behavioural studies. *J. exp. Biol.* **89**, 159–175.

KURAMOTO, T. & EBARA, A. (1982*a*). Effects of octopamine and serotonin on the ganglion cell discharges in the isolated heart of the lobster. *J. physiol. Soc. Japan Abstr.* **44**, 476.

KURAMOTO, T. & EBARA, A. (1982*b*). Contraction of the cardioarterial valve of the spiny lobster. *Proc. Jpn. Soc. gen. comp. Physiol. Abstr.* **4**, 40.

KURAMOTO, T. & EBARA, A. (1984). Effects of perfusion pressure on the isolated heart of the lobster, *Panulirus japonicus*. *J. exp. Biol.* **109**, 121–140.

LEMOS, J. R. & BERLIND, A. (1981). Cyclic adenosine monophosphate mediation of peptide neurohormone effects on the lobster cardiac ganglion. *J. exp. Biol.* **90**, 307–326.

MAYNARD, D. M. (1960). Circulation and heart function. In *The Physiology of Crustacea*, Vol. 1, (ed. T. H. Waterman), pp. 161–214. New York: Academic Press.

MAYNARD, D. M. & WELSH, J. H. (1959). Neurohormones of the pericardial organs of brachyuran crustacea. *J. Physiol., Lond.* **149**, 215–227.

MILLER, M. W. & SULLIVAN, R. E. (1981). Some effects of proctolin on the cardiac ganglion of the marine lobster, *Homarus americanus*. *J. Neurobiol.* **12**, 629–639.

NAGAI, T. & BROWN, B. E. (1969). Insect visceral muscle; electrical potentials and contraction in fibers of the cockroach proctodeum. *J. Insect Physiol.* **15**, 2151–2167.

SCHWARZ, T. L., HARRIS-WARRICK, R. M., GLUSMAN, S. & KRAVITZ, E. A. (1980). A peptide action in a lobster neuromuscular preparation. *J. Neurobiol.* **11**, 623–628.

SCHWARZ, T. L., LEE, G. & KRAVITZ, E. A. (1981). Proctolin-like immunoreactivity in the nervous system of the lobster *Homarus americanus*. *Soc. Neurosci. Abstr.* **7**, 253.

SULLIVAN, R. E. (1978). Stimulus-coupled ^{3}H-serotonin release from identified neurosecretory fibers in the spiny lobster, *Panulirus interruptus*. *Life Sci.* **22**, 1429–1438.

SULLIVAN, R. E. (1979). A proctolin-like peptide in crab pericardial organs. *J. exp. Zool.* **210**, 543–552.

SULLIVAN, R. E., FRIEND, B. J. & BARKER, D. L. (1977). Structure and function of spiny lobster ligament nerve plexuses: evidence for synthesis, storage and secretion of biogenic amines. *J. Neurobiol.* **8**, 581–605.