43 Mayeri, E., and Rothman, B. S., Neuropeptides and the control of egg-laying behavior in *Aplysia*, in: Model Neural Networks and Behavior, pp. 205–301. Ed. A. I. Selverston. Plenum, New York 1985.
44 Meglitsch, P. A., Invertebrate Zoology. Oxford Univ. Press, New York/London 1972.
45 Price, D. A., and Greenberg, M. J., The structure of a molluscan cardioexcitatory neuropeptide. Science *197* (1977) 670–671.
46 Price, C. H., and McAdoo, D. J., Anatomy and ultrastructure of the axons and terminals of neurons R3–R14 in *Aplysia*. J. comp. Neurol. *188* (1979) 647–678.
47 Redmond, J. R., and Bourne, G. B., Vascular resistance in the isolated gills of *Octopus macropus* and *Nautilus pompilius*. Pacific Sci. *36* (1983) 299–303.
48 Rittenhouse, A. R., and Price, C. H., Electrophysiological and anatomical identification of the peripheral axons and target tissues of *Aplysia* neurons R3–R14 and their status as multifunctional, multimessenger neurons. J. Neurosci. *6* (1986) 2071–2084.
49 Russel, C. W., and Evans, B. K., Cardiovascular anatomy and physiology of the black-lip abalone, *Haliotis ruber*. J. exp. Zool. *252* (1989) 105–117.
50 Sawada, M., Blankenship, J. E., and McAdoo, D. J., Neural control of a molluscan blood vessel, anterior aorta of *Aplysia*. J. Neurophysiol. *46* (1981) 967–986.
51 Schaefer, M., and Brownell, P. H., Modulation of a respiratory motor program by peptide secreting neurons in *Aplysia*. J. Neurobiol. *17* (1986) 121–126.
52 Scheller, R. H., Jackson, J. F., McAllister, L. B., Rothman, B. S., Mayeri, E., and Axel, R., A single gene encodes multiple neuropeptides mediating a stereotyped behavior. Cell *32* (1983) 7–22.
53 Schadwick, R. E., and Gosline, J. M., Elastic arteries in invertebrates: Mechanics of the octopus aorta. Science *213* (1981) 759–761.
54 Shadwick, R. E., Gosline, J. M., and Milsom, W. K., Arterial haemodynamics in the cephalopod mollusc, *Octopus dofleini*. J. exp. Biol. *130* (1987) 87–106.
55 Skelton, M., Alvezios, A., and Koester, J., Control of the cardiovascular system of *Aplysia* by identified neurons. Experientia *48* (1992) 809–817, this issue.
56 Teyke, T., Weiss, K. R., and Kupfermann, I., An identified neuron (CPR) evokes neuronal responses reflecting food arousal in *Aplysia*. Science *247* (1990) 85–87.
57 Umitsu, Y., Matsumoto, H., and Koike, H., Active contraction of nerve bundle and identification of a nerve contractor motoneuron in *Aplysia*. J. Neurophysiol. *58* (1987) 1016–1034.
58 Wiens, B. L., and Brownell, P. H., Anatomical and physiological characterization of cardiac innervation in the nudibranch, *Archidoris montereyensis*. J. comp. Physiol. *167* (1990) 51–60.

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Intrinsic properties and extrinsic neurohormonal control of crab cardiac hemodynamics

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Abstract. This report provides the first direct measurements of the stroke volume and total cardiac output of crustacean hearts, as recorded from a semi-isolated in vitro preparation. The responses to mechanical perturbations, changes in preload and afterload, show that these hearts do not possess automatic compensatory Frank-Starling-like mechanisms. Heart rate, reflecting the burst rate of the cardiac ganglion, is minimally affected by stretch. On the other hand, these hearts are exquisitely responsive to the neurohormones of the pericardial organs. Serotonin, CCAP and proctolin all produce positive chronotropic and inotropic effects, but the responses to each are unique. Two FMRFamide peptides were positively chronotropic, but negatively inotropic.
Key words. Cardiac output; cardio-regulatory nerves; hemodynamics; neurohormones; pericardial organ; Crustacea.

Introduction

The heart of decapod crustaceans consists of a muscular ventricle suspended by a three-dimensional array of alary ligaments and arteries within the pericardial cavity (see reviews by Maynard[26] and McMahon and Burnett[28]). The ventricle is composed of striated muscle fibers which are electrically coupled[4,9]. The heart is neurogenic with primary excitation arising from a small number of autorhythmic (pacemaker) neurons[2,10,26] located in the cardiac ganglion on the inner dorsal wall of the heart. Muscle excitation is via a group of large ganglion cells acting as motoneurones. The length of systolic contraction depends upon the duration of the ganglionic burst[3,7,18]. The relationship between ganglionic burst characteristics and strength of contraction has not been determined. During systole part of the energy is stored in the stretched elastic suspensory ligaments. During diastole this energy acts to restore heart volume and blood enters the heart via the ostial valves. The heart receives extrinsic input from the CNS via the paired cardio-regulatory nerves[14,15,24,25,27] and via neurohormones released from the pericardial organs (PO's) located on the lateral walls of the pericardial cavity (see reviews by Cooke[12]; Cooke and Sullivan[13]).

There is almost no knowledge concerning the mechanical aspects either of heart function or of hemodynamics within the open circulatory system[27,28]. Of the relatively few studies published to date, several show pressures developed by the heart in vivo[5,6,8,46]. There is only one direct measurement of cardiac output[11], but this confirms the many estimates made utilizing the Fick princi-

ple[29]. There are only two published values for blood flow velocity in any crustacean artery[5,6]. These areas, however, are the object of intense study in our laboratories.

The focus of the present paper is to review our recent work which examines the mechanical aspects of the decapod crustacean heart. Specifically, we will identify the intrinsic mechanical properties of the heart via a semi-isolated heart preparation and illustrate modulation by some PO neurohormones. The role of extrinsic control via the CNS[45] and control of cardiac distribution by peptidergic[30] and aminergic neurohormones[1] will be the focus of additional presentations at the associated satellite symposium at Shimoda.

Methods

The preparation we have developed consists of a crab heart, *Carcinus maenas*, suspended in the dorsal half of the thorax by its alary ligaments, dorso-anterior arteries and pericardial septum. Flow and arterial pressure are measured via the cannulated sternal artery. Intraventricular pressure is measured by a cannula inserted through a hepatic artery. All other arteries are tied off. The in situ heart maintained at 12–15 °C in an oxygenated saline bath remains viable for up to 12 h. Immediately after surgical isolation the heart continues to beat at rates (60–90 bpm) with cardiac outputs (90–130 ml/min/kg) similar to those observed in settled intact animals. During the following 30 min the rates and outputs may decline by 30–50%, but they remain stable at these new levels for many hours.

The variables we measure are heart rate (f_H), stroke volume (V_s), arterial pulse pressure (P_a) and ventricular pressure (P_v). In chart records the flow traces do not include a scale since V_s was calculated from the digitally integrated area under flow waveforms. From these data we calculate cardiac output ($\dot{V}_b$),

$$\dot{V}_b\ (\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}) = f_H \times V_s$$

Neurohormones were delivered to the heart either into the pericardial sinus by a perfusion cannula inserted through the pericardial septum or by a fine tipped cannula inserted directly into the ventricle through its ventral wall. The responses were essentially the same by each method, but the exact time of delivery was more precisely known with the latter technique. The data presented here are derived only from *Carcinus meanas*, but most of the tests have also been repeated, with similar results, on in situ hearts of the crayfish *Procambarus bandingi*.

Intrinsic attributes of cardiac regulation – f_H, V_s, P_v and P_a

The intrinsic variables which can be anticipated to be important for determining the pumping action in the heart include the output of the cardiac ganglion, the contractility of the myocardium, the amount of tension stored in the alary ligaments, and the properties of the ostial and cardioarterial valves.

External variables which might affect the heart would include factors influencing diastolic filling, loosely termed the *preload,* and factors which would affect systolic ejection, termed the *afterload* on the heart.

Preload. The preload, a concept taken from the vertebrate literature, but also applied to molluscan hearts[17,31,35,36] referring to venous return pressure and stretch of heart, must be redefined for the decapod heart. During diastole the heart fills passively as the relaxed ventricle is enlarged by the elastic recoil of the alary ligaments and anterior arteries. The alary ligaments are made up of thin sheets of connective tissue which in some species contain small bundles of striated muscle fibers[32,42]. The function of these muscles is presently unknown. One component of preload is the amount of stress stored in the alary ligaments either passively in response to systolic contraction or actively. End-diastolic volume must depend on the amount of ventricular expansion produced by the ligaments. During preliminary experiments in which the ventral alary ligaments were tonically stretched $\dot{V}_b$ was not changed. Conversely, the importance of the ligaments in cardiac filling is seen when the ligaments are progressively removed (fig. 1). As the ligaments are cut, both V_s and P_a fall linearly indicating that each ligament contributes about equally to end-diastolic volume. Ligament removal does not affect heart rate, indicating that within the physiological operating range during diastolic/systolic excursions the burst rate of the cardiac ganglion is unaffected by stretch.

Variation in pericardial sinus pressure is another possible component of preload. During diastole blood enters through the valved ostia driven by the pressure gradient between the pericardial sinus and the inside of the ventricle. In intact aquatic and land crabs, increases in pericardial sinus pressure occur during reversed ventilatory

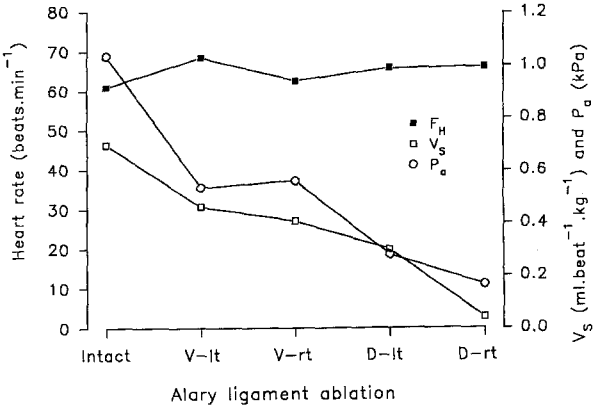

Figure 1. The effects of cutting the alary ligaments on cardiac performance. Codes: V-lt, both left ventrolateral ligaments cut; V-rt, both right ventro-lateral ligaments cut; D-lt, both left dorso-lateral ligaments cut; D-rt, both right dorso-lateral ligaments cut.

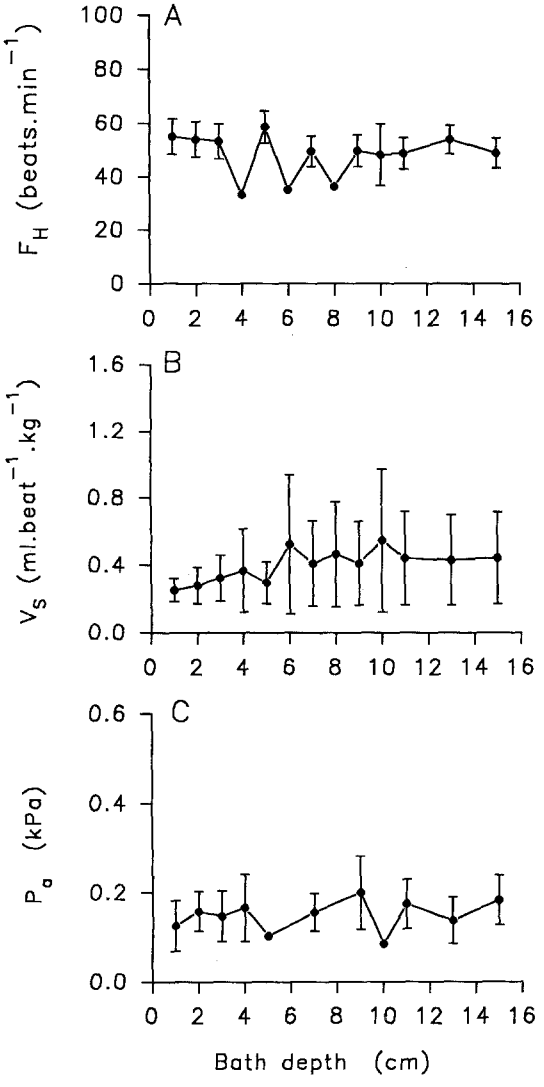

Figure 2. The effects of ambient pressure (bath depth) on cardiac performance. The return end of the arterial cannula always remained at the same level as the heart. Data presented as mean ± SEM, n = 5.

pumping when the transmural pressure gradient across the gills and branchiostegal lungs moves from negative to positive[6, 39, 46]. We attempted to simulate the resultant increases in pericardial sinus pressure by raising the height of the water column over the heart up to 15 cm, an ambient pressure similar to that measured from the pericardial sinus of intact crabs. None of the intrinsic variables, including $\dot{V}_b$, were affected by changes in ambient pressure (fig. 2).

Finally, the vertebrate equivalent of increasing preload by increasing venous return can be accomplished by directly perfusing the heart, although it should be remembered that this is a highly artificial perturbation since the ventricle normally fills passively via the ostia during alary ligament induced diastolic expansion. We found that the majority (6 of 10) of in situ 'healthy' hearts did not show changes in f_H when perfused at rates up to 4–5 times the extant $\dot{V}_b$ (fig. 3), nor do they produce double contractions[19, 21, 22]. Of the four hearts which showed positive

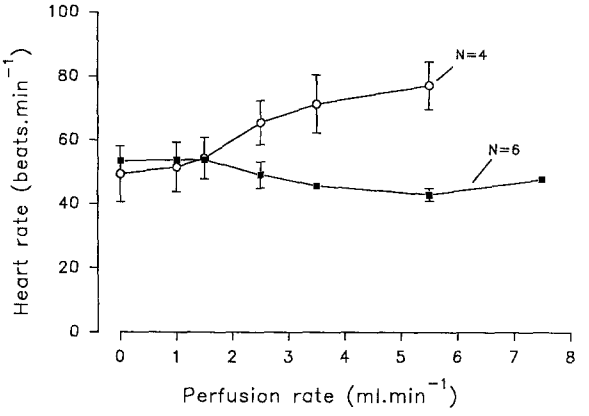

Figure 3. The effects of direct ventricular perfusion on heart rate. At high perfusion rates in situ hearts often begin to beat irregularly and slowly. Perfusate was delivered from a 26-ga syringe needle inserted through the ventral wall of the heart. Perfusate did not leak around the input cannula as ascertained by delivering pulses of saline colored with methylene blue. Data shown as mean ± SEM.

responses, two were in a weakened condition at the end of a several-hour-long experimental session.

These above data indicate that the decapod heart shows virtually no automatic adaptive responses to changes in the preload.

Afterload. Any increase in peripheral resistance to blood flow would increase the load on the contracting heart, i.e., the afterload. The question arises as to whether the decapod heart possesses the ability to autoregulate if faced with perturbations in afterload, either on an acute or a chronic basis. Changes in afterload could occur during locomotion, following expansion of crop and gut after feeding, during any condition which would alter blood volume and pressure, or during bouts of reversed ventilation when the resistance to blood flow through the gills may increase[28, 39, 40].

We increased afterload by connecting lengths of decreasing diameter polyethylene tubing to the sternal artery cannula. The resistance to flow through the stepped-down lengths of tubing is expressed as the quotient of perfusion pressure divided by flow. Heart rate was unaffected by these increases in afterload, while stroke volume decreased in an exponential fashion (fig. 4). Arterial pressure increased with increasing afterload. Cardiac output and stroke work also decrease with a similar magnitude to the fall in V_s.

Thus, this decapod heart possesses little or no autoregulatory capacity or ability to adjust its output to perturbations in peripheral resistance to arterial blood flow. We suggest that myocardial contractions directly follow the neural output of the cardiac ganglion and that this ganglion is insensitive to changes in afterload.

Extrinsic neurohormonal factors affecting cardiac performance

Since the heart shows no autoregulatory response to mechanically imposed perturbations, one must look to ex-

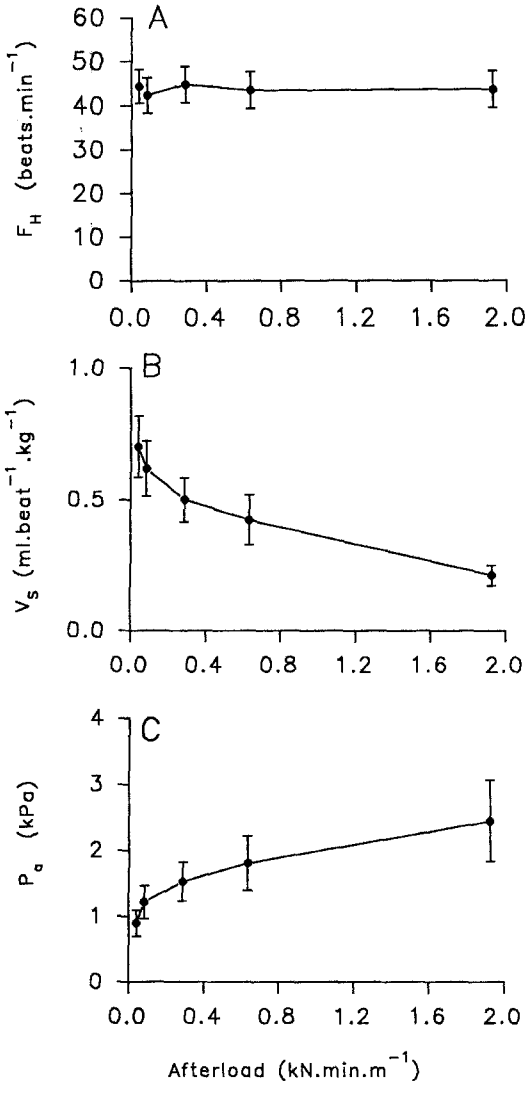

Figure 4. The effects of increasing arterial resistance (afterload) on pumping performance of in situ hearts. Arterial pressure and flow are reciprocally related. Data presented as mean ± SEM.

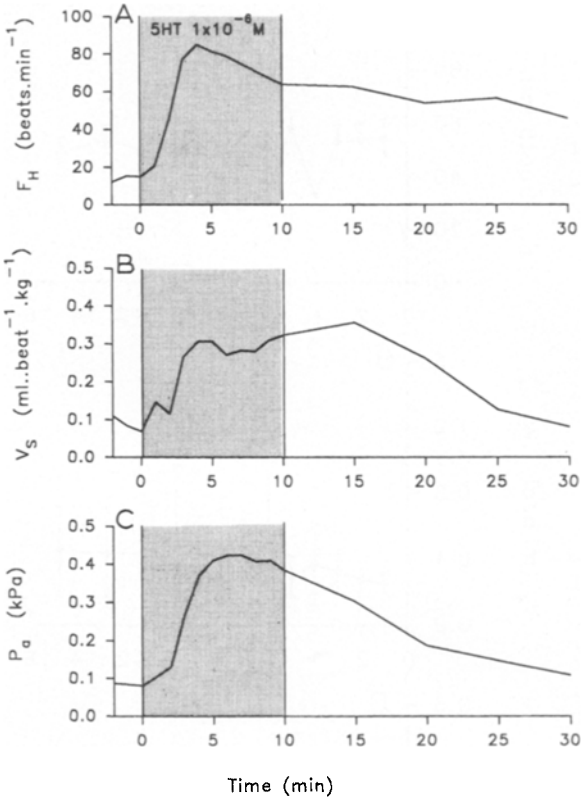

Figure 5. Serotonin (1×10^{-6} M) perfused into the pericardial sinus at the rate of 1.5 ml · min^{-1} causes increased f_H, V_s and P_a. The stroke volume prior to hormone, and before being scaled to a kg basis, was about 6 µl · beat^{-1}. f_H returned to the control after 60 min.

trinsic control systems to understand how cardiac performance is integrated with the whole organism. A variety of aminergic and peptidergic hormones are stored and released from the PO's, and they are known to increase heart rate and contractile force[16, 22, 23, 44] as well as regulate the cardioarterial valves[20]. We have examined the effects of the indoleamine 5-hydroxytryptamine (5HT) (Wilkens, MacLeod and Lee, unpubl.) and of four peptides[43] on hemodynamic variables. The peptides include crustacean cardioactive peptide (CCAP[37]), two FMRFamide related peptides F1 and F2 identified from crustaceans[41] and proctolin[37, 38]. Except for F1 and F2, these peptides are structurally unrelated, as illustrated below:

Proctolin (MW 648.82)

Arg-Tyr-Leu-Pro-Thr

F1 (MW 1065.57)

Thr-Asn-Arg-Asn-Phe-Leu-Arg-Phe-NH_2

F2 (MW 1211.5)

Ser-Asn-Arg-Asn-Phe-Leu-Arg-Phe-NH_2

CCAP (MW 956.07)

Pro-Phe-Cys-Asn-Ala-Phe-Thr-Gly-Cys-NH_2

Serotonin (5HT)

5HT has both chronotropic and inotropic effects on cardiac pumping performance. Figure 5 illustrates the responses of a single heart to a 10-min exposure to 1 mM 5HT. f_H, V_s, and P_a always increase in parallel and in this example the latter two parameters are maintained without adaptation for the duration of the stimulus. Heart rate sometimes adapted despite the continued administration of 5HT, but in the majority of cases remained elevated for the duration of exposures of up to 30 min. The ED_{50} for a 30-s exposure to 5HT is 2.6×10^{-7} M, the time from application to peak f_H is 54.2 ± 5.8 s (mean ± SD, n = 6) and the recovery time constant is 5.7 ± 2.0 min (n = 5).

Preliminary pharmacological tests indicate that 5HT may bind to more than one class of 5HT receptor. The effects of 5HT are reduced by up to 50% after the heart has been pretreated by ketanserin, propranolol, or MDL7222 (3-tropanyl-3,5-dichlorobenzoate [Research

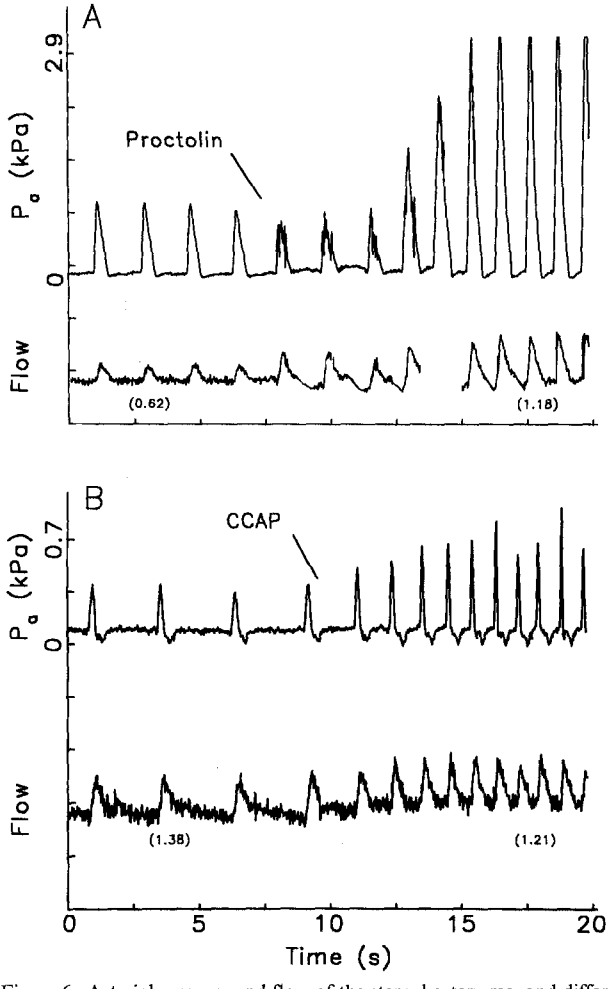

Figure 6. Arterial pressure and flow of the sternal artery respond differently to the infusion of *A* proctolin (1×10^{-6} M) and *B* CCAP (1×10^{-5} M) into the pericardial sinus. The volume in ml · kg^{-1} of inidividual flow pulses before and after hormone treatment is listed in parentheses. The time of hormone arrival is indicated by arrow. These records are from different hearts.

Biochemicals Inc.]), high affinity vertebrate $5HT_1$, $5HT_2$ and $5HT_3$ antagonists, respectively[33, 34].

Peptides

The responses to the peptide neurohormones are much quicker than those to 5HT. The pentapeptide proctolin produces greater inotropic than chronotropic effects (fig. 6A). The threshold dose is $<10^{-11}$ M. In this case V_s was increased by 90% within 6 beats after proctolin arrived. The time to peak response is short (< 10 s) and recovery following washout is complete in 3–4 min.

In contrast to proctolin, the nine amino acid CCAP exerts strong positive chronotropic effects on the heart and causes moderately increased amplitude and decreased duration of P_a waves (fig. 6B). CCAP has little effect on V_s and in this instance V_s was reduced by 12%. The time to peak response is < 10 s, while recovery following washout is complete in 4 min. The threshold for f_H responses is $< 10^{-9}$ M.

The FMRFamide related octapeptides F1 and F2 were tested at only two concentrations, 10^{-8} and 10^{-6} M. The responses to both were similar, those for F2 are presented here. F2 produced positive chronotropic effects at both doses (fig. 7). P_a was increased slightly at the lower dose, while at 10^{-6} M F2 dramatically reduced V_s, P_a and P_v. These reductions occurred in over half of the hearts tested. A similar reduction in myocardial contractility was also observed in 2 of 12 hearts exposed to $\leq 10^{-6}$ M proctolin. These reductions in contractility and flow at high f_H's suggest either that high doses of these peptides act at the CG to reduce the number and frequency of impulses per burst which would reduce excitation of the myocardium, or that they exert direct negative inotropic effects on the heart muscle.

In the absence of known antagonists for the peptide neurohormones, we attempted to determine whether each peptide binds to a unique receptor by testing the responses when different peptides where applied singly and in pairs. In the case of CCAP and F2, each applied at 10^{-8} M, the effects were similar in all cases (fig. 8). This observation is open to at least two interpretations. Either these two peptides bind to the same generalized peptide receptor or they each trigger the same post-receptor reaction(s). Either mechanism would explain the non-additive responses to paired presentation. It is hoped that further tests will clarify this point.

Preliminary HPLC analysis of saline collected from the pericardial sinus reveals that small quantities of 5HT and dopamine are continually released from the PO's (unpubl.). The concentrations recovered are in the picomolar range, but indicate that the heart may be exposed to small quantities of neurohormones at all times.

Conclusions

The neurogenic decapod crusteacean heart, when isolated from extrinsic neural or elevated concentrations of neurohumoral input, is capable of pumping performances similar to or slightly below those observed in intact animals. These basal levels of performance should be sufficient to supply the vascular needs of a settled animal, i.e., the cardiovascular needs of routine metabolism. The autonomous nature of the heart fulfills the requirements of a fail-safe system.

The semi-isolated heart is, however, not capable of autoregulatory responses to external mechanical perturbations of either preload or afterload and it is obviously not able to show adaptive responses to environmental challenges such as hypoxia or the need to fuel increased metabolism as during locomotor activity. To understand how the heart responds to these demands we must understand how it is controlled by and responds to the extrinsic inputs from the central nervous system and from neurohormones. We have outlined here some of the hemodynamic responses to neurohormones released from the pericardial organs. These observations allow us to identify and evaluate the intrinsic and extrinsic factors which can affect cardiac performance and assess their potential

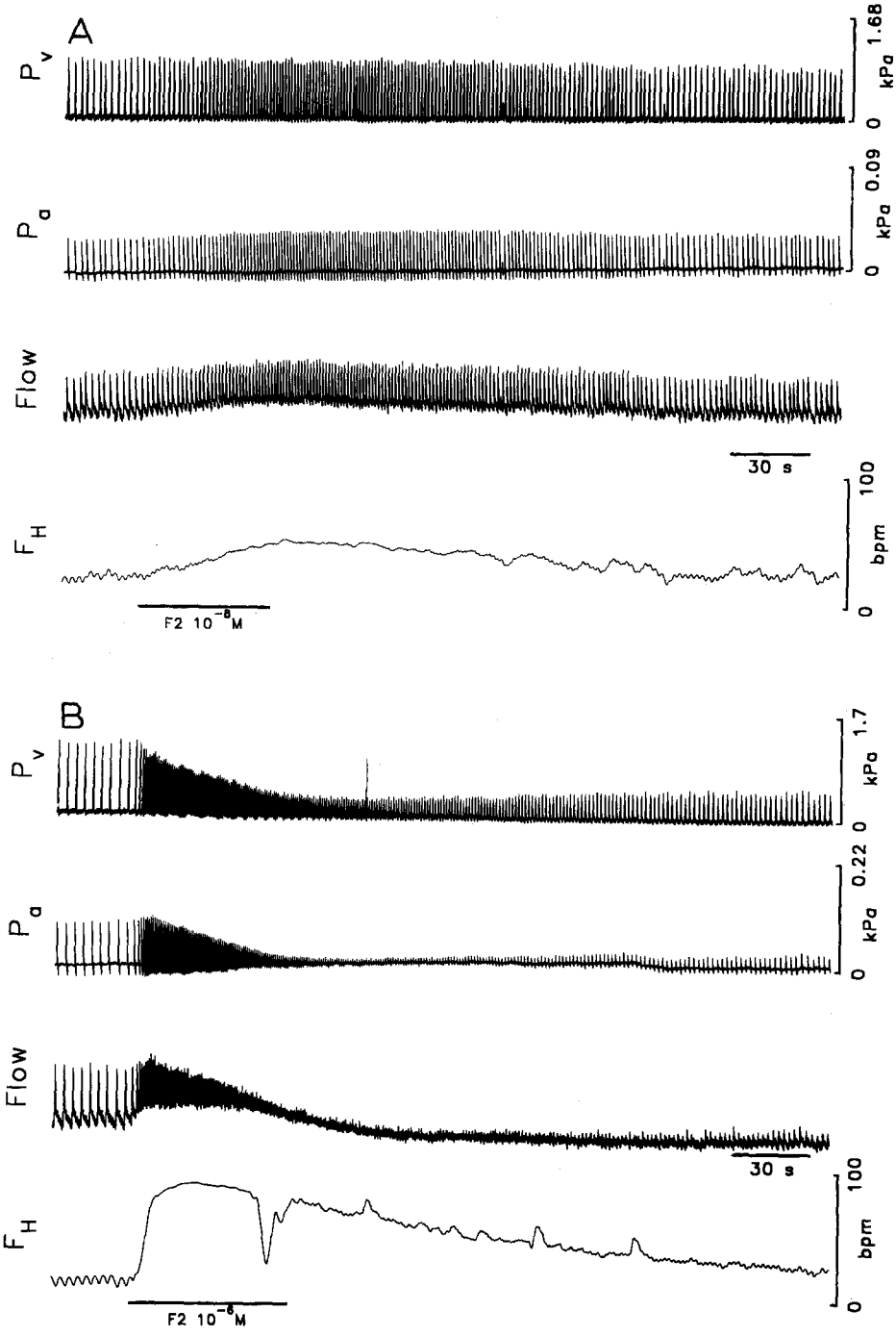

Figure 7. Examples of the responses of a heart to two concentrations of F2, *A* 1×10^{-8} and *B* 1×10^{-6} M. In *B*, f_H was dramatically elevated while V_s, P_a and P_v were equally dramatically reduced. The pulse-like fluctuations in the f_H trace are artifacts. Time calibration 30 s.

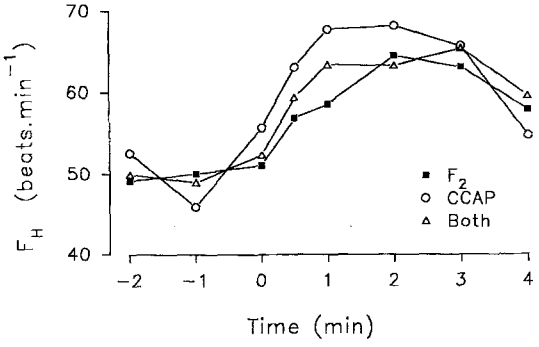

Figure 8. The effects on heart rate of applying CCAP and F2 (each at 10^{-8} M) either singly or in combination. For each trace the hormone arrived at time zero and was perfused for 3 min. Each point is the mean of 5 presentations to 3 hearts.

influence in setting cardiac performance to suit the animal's varying metabolic needs. A major challenge for the future is to devise experiments which will allow us to measure the extrinsic inputs to the heart in intact animals during natural acclimatization.

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1 Airriess, C., and McMahon, B. R., Aminergic modulation of circulatory performance in the crab *Cancer magister*, in: Comparative Physiology, vol. 11 Eds R. B. Hill, K. Kuwasawa and B. R. McMahon. Karger, Basel 1992.
2 Alexandrowicz, J. S., The innervation of the heart of the Crustacea. I. Decapoda. Q. Jl. microsc. Sci. *75* (1932) 181–249.
3 Anderson, M., and Cooke, I. M., Neural activation of the heart of the lobster *Homarus americanus*. J. exp. Biol. *55* (1971) 449–468.
4 Anderson, M. E., and Smith, D. S., Electrophysiological and structural studies on the heart muscle of the lobster *Homarus americanus*. Tiss. Cell *3* (1971) 191–205.
5 Belman, B. W., Some aspects of the circulatory physiology of the spiny lobster *Panulirus interruptus*. Mar. Biol. *29* (1975) 295–305.
6 Belman, B. W., and Childress, J. J., Circulatory adaptations to the oxygen minimum layer in the bathypelagic mysid *Gnathophausia ingens*. Biol. Bull. *150* (1976) 15–37.
7 Benson, J. A., Synaptic and regenerative responses of cardiac muscle fibres in the crab, *Portunus sanguinolentus*. J. comp. Physiol. *143* (1981) 349–356.
8 Blatchford, J. G., Hemodynamics of *Carcinus meanas* (L.). Comp. Biochem. Physiol. *39*A (1971) 193–202.
9 Brown, H. F., Electrophysiological investigations of the heart of *Squilla mantis*. II. The heart muscle. J. exp. Biol. *41* (1964) 701–722.
10 Bullock, T. H., and Horridge, G. A., Structure and Function in the Nervous Systems of Invertebrates, vol. 2, pp. 988–997. W. H. Freeman and Co., San Francisco 1965.
11 Burnett, L. E., deFur, P. L., and Jorgensen, D. D., Application of the thermodilution technique for measuring cardiac output and assessing cardiac stroke volume in crabs. J. exp. Zool. *218* (1981) 165–173.
12 Cooke, I. M., Studies on the crustacean cardiac ganglion. Comp. Biochem. Physiol. *91*C (1988) 205–218.
13 Cooke, I. M., and Sullivan, R. E., Hormones and neurosecretion, in: The Biology of Crustacea, vol. 2, pp. 205–290. Eds D. E. Bliss, H. L. Atwood and D. C. Sandeman. Academic Press, New York 1982.
14 Field, L. H., and Larimer, J. L., The cardioregulatory system of crayfish: Neuroanatomy and physiology. J. exp. Biol. *62* (1975) 519–530.
15 Florey, E., Studies on the nervous regulation of the heart beat in decapod crustacea. J. gen. Physiol. *43* (1960) 1061–1081.
16 Florey, E., and Rathmayer, M., The effects of octopamine and other amines on the heart and on neuromuscular transmission in decapod crustaceans: Further evidence for a role as neurohormone. Comp. Biochem. Physiol. *61*C (1978) 229–237.
17 Guyton, A. C., Textbook of Medical Physiology, 8th ed. W. B. Saunders Co., Philadelphia 1991.
18 Hawlett, M., Lobster heart: Electrophysiology of single cells including effects of the regulator nerves. Comp. Biochem. Physiol. *39*A (1971) 643–648.
19 Kuramoto, T., and Ebara, A., Effects of perfusion pressure on the isolated heart of the lobster, *Panulirus japonicus*. J. exp. Biol. *109* (1984a) 121–140.
20 Kuramoto, T., and Ebara, A., Neurohormonal modulation of the cardiac outflow through the cardioarterial valve in the lobster. J. exp. Biol. *111* (1984b) 123–130.
21 Kuramoto, T., and Ebara, A., Effects of perfusion pressure on the bursting neurones in the intact or segmented cardiac ganglion of the lobster, *Panulirus japonicus*. J. Neurosci. Res. *13* (1985) 569–580.
22 Kuramoto, T., and Ebara, A., Combined effects of 5-hydroxytryptamine and filling pressure on the isolated heart of the lobster, *Panulirus japonicus*. J. comp. Physiol. B *158* (1988) 403–412.
23 Kuramoto, T., and Yamagishi, H., Physiological anatomy, burst formation and burst frequency in the cardiac ganglion of crustaceans. Physiol. Zool. *63* (1990) 102–116.
24 Maynard, D. M., Activity in a crustacean ganglion. I. Cardioinhibition and acceleration in *Panulirus argus*. Biol. Bull. *104* (1953) 156–170.
25 Maynard, D. M., Activity in a crustacean ganglion. II. Pattern and interaction in burst formation. Biol. Bull. *109* (1955) 420–436.
26 Maynard, D. M., Circulation and heart function, in: Physiology of Crustacea, pp. 161–226. Ed. T. H. Waterman. Academic Press, New York 1960.
27 Maynard, D. M., Cardiac inhibition in decapod Crustacea, in: Nervous Inhibition, pp. 144–178. Ed. E. Florey. Pergamon Press, Oxford 1961.
28 McMahon, B. R., and Burnett, L. E., The crustacean open circulatory system: A reexamination. Physiol. Zool. *63* (1990) 35–71.
29 McMahon, B. R., and Wilkens, J. L., Ventilation, perfusion and oxygen uptake, in: Biology of Crustacea, vol. 5, pp. 289–372. Eds D. E. Bilss and L. Mantel. Academic Press, New York 1983.
30 McMahon, B. R., and Reiber, C., Peptidergic modulation of hemolymph flow in the lobster *Homarus americanus*, in: Comparative Physiology. vol. 11. Eds R. B. Hill, K. Kuwasawa and B. R. McMahon. Karger, Basel 1992.
31 Milnor, W. R., Hemodynamics, 2nd ed. Williams and Wilkins, Baltimore 1989.
32 Pearson, J., *Cancer*. Liverpool Marine Biological Committee Memoirs, No. 8. Williams and Nordgate, London 1908.
33 Peroulta, S. J., The molecular pharmacology of 5-hydroxytryptamine receptor subtypes, in: Serotonin Receptor Subtypes: Basic and Clinical Aspects, pp. 65–80. Ed. S. J. Peroutka. Wiley-Liss Inc., New York 1991.
34 Richardson, B. P., Engel, G., Donatsch, P., and Stakler, P. A., Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. Nature *316* (1985) 126–131.
35 Smith, P. J. S., Cardiac output in the Mollusca: Scope and regulation. Experientia *43* (1987) 956–965.
36 Smith, P. J. S., Integrated cardiovascular control in the Mollusca. Physiol. Zool. *63* (1990) 12–34.
37 Strangier, J., Hilbich, C., Beyreuther, K., and Keller, R., Unusual cardioactive peptide (CCAP) from pericardial organs of the shore crab *Carcinus maenas*. Proc. natl Acad. Sci. USA *84* (1987) 575–579.
38 Sullivan, R. E., A proctolin-like peptide in crab pericardial organs. J. exp. Biol. *210* (1979) 543–552.
39 Taylor, H. H., Pressure-flow characteristics of crab gills: Implications for regulation of hemolymph pressure. Physiol. Zool. *63* (1990) 72–89.
40 Taylor, H. H., and Greenaway, P., The role of the gills and branchiostegites in gas exchange in a bimodally breathing crab, *Holthuisana transversa:* Evidence for a facultative change in the distribution of the respiratory circulation. J. exp. Biol. *111* (1984) 103–121.
41 Trimmer, B. A., Kobierski, L. A., and Kravitz, E. A., Purification and characterization of FMRFamide-like immunoreactive substances from the lobster nervous system: Isolation and sequency analysis of two closely related peptides. J. comp. Neurol. *266* (1987) 16–26.
42 Volk, E. L., The role of suspensory ligaments in modifying cardiac output in crustaceans. MSc Thesis, University of Calgary, 1988.
43 Wilkens, J. L., and Mercier, A. J., Peptidergic modulation of cardiac performance in isolated hearts from the shore crab *Carcinus meanas*. Submitted.

44 Wilkens, J. L., Mercier, A. J., and Evans, J., Cardiac and ventilatory responses to stress and to neurohormonal modulators by the shore crab, *Carcinus maenas*. Comp. Biochem. Physiol. *82*C (1985) 337–343.
45 Wilkens, J. L., and Walker, R. L., Nervous control of crayfish cardiac hemodynamics, in: Comparative Physiology, vol. 11. Eds R. B. Hill, K. Kuwasawa and B. R. McMahon. Karger, Basel 1992.
46 Wilkens, J. L., and Young, R. E., Regulation of pulmonary blood flow and of blood pressure in a mangrove crab (*Goniopsis cruentata*). J. exp. Biol. *163* (1992) 297–316.

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Intrinsic and extrinsic neural and neurohumoral control of the decapod heart

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Abstract. The intra-cardiac nervous system of the decapod heart is composed of large and small ganglionic cells (LGCs and SGCs) and axons of extrinsic cardio-acceleratory and -inhibitory neurons (CAs and CIs). Candidate neurotransmitters for the neurons have been determined by pharmacological, cytochemical and immunocytochemical tests. SGCs may be cholinergic, LGCs and CAs are probably dopaminergic, and CIs are GABAergic. Serotonin and octopamine were cardio-excitatory neuromodulators of the heart. Proctolin, crustacean cardio-active peptide (CCAP), red pigment concentrating hormone (RPCH), and FMRFamide also had modulatory actions on the heart. Proctolin was the most potent peptide, which acted primary on the cardiac ganglion. Insect adipokinetic hormones had little effect on the heart.
Key words. Decapod heart; cardiac ganglion; cardio-acceleratory neuron; cardio-inhibitory neuron; neurotransmitters; neurohormones; neuropeptide.

Introduction

Since Carlson [5] reviewed studies on invertebrate hearts, a considerable number of papers on the neuroanatomy, physiology and pharmacology of crustacean hearts have been published. Those include studies of various cardio-active substances. Cooke [7], however, concluded in his review that the neurotransmitters are not established. The neuronal constituents of the intra-cardiac nervous system are small and large ganglionic cells (SGCs and LGCs, respectively) composing the cardiac ganglion itself, and axons of cardio-acceleratory and -inhibitory neurons (CAs and CIs, respectively) running from the central nervous system to make synaptic contact with the ganglion and myocardium. Activities of the neural constituents and the myocardium are modulated by neurohormones which are liberated by the neuro-secretory tissue, the pericardial organ, into the blood of pericardial sinus. Serotonin, octopamine and proctolin are major neurohormones found in the pericardial organ (see Cooke and Sullivan [9] for review).

In this report, we will describe effects of a variety of putative neurotransmitters and humoral substances on the heart of hermit crabs, and propose the most likely candidates for natural neurotransmitters of extrinsic inhibitory and acceleratory axons and of intrinsic LGCs and SGCs. Preliminary studies have appeared elsewhere [63, 64].

Materials and methods

Giant marine hermit crabs (*Aniculus aniculus* and *Dardanus crassimanus*) were treated as reported before [59, 64]. As far as the present report is concerned, there was no significant difference between the two species.

Electrophysiological and pharmacological methods were the same as those described previously [59]. Filtered natural seawater and artificial seawater (NaCl 526, KCl 11, $CaCl_2$ 18, $MgCl_2$ 24 [in mM], and Tris-buffer 5 mM at pH 7.4) were used as the perfusion medium for preparations.

The following chemicals were used: acetylcholine, atropine, scopolamine, pilocarpine, hexamethonium, nicotine, picrotoxin, serotonin, dopamine, l-noradrenaline, yohimbin (Wako); arecoline, carbamylcholine, muscarine, muscimol, octopamine, ergonovine, chlorpromazine, apomorphine (Sigma); methacholine (Nakarai); d-tubocurarine (Tokyo Kasei); phentolamine (Ciba Geigy); haloperidol (Dai-Nippon); fluphenazine (Yoshitomi, gift); methysergide (Sandoz); crustacean cardio-active peptide (CCAP) (KosmoBio-Bachem AG); red pigment concentrating hormone (RPCH) (Funakoshi-PLI); adipokinetic hormone II of *Schistocerca gregaria* (AKH-II), adipokinetic hormone I of the cockroach (AKH-I), FMRFamide, proctolin (Sigma).