lowed with suction electrode, sucrose gap, or microelectrode recording[10]. The resting potential of gastropod ventricular muscle fibers has been determined by microelectrode methods to be 65.6 ± 7.0 mV for *Dolabella auricularia*[21] and 61.2 ± 3.5 mV for *Lymnaea stagnalis*[5], which is very close to resting potential values determined by the sucrose gap method[10]. Recently, Brezden et al.[6] have also demonstrated that the membranes of gastropod myocardial cells provide an opportunity for gigohm-seal patch-clamp recording, after enzymatic isolation of ventricular muscle cells. Using this technique, a stretch-activated K^+ channel has been discovered in dispersed ventricular muscle cells of *Lymnaea stagnalis*[25]. Since the proteolytic enzymes used in dissociation may affect the electrophysiological activity of tissues[1] it was hoped that the lack of an external lamina might indicate that the *Busycon* preparation (figs 1–3) would be suitable for patch-clamp recording without dissociation. In fact, gigohm seals and channel recordings can be obtained from trabeculae in a ventricular preparation washed only with seawater (personal communication, Colin Leech) but the abundant fibers and blood cells, seen adhering to the muscle fibers (figs 1–3), seem to make it difficult to obtain good seals or large currents (fig. 4).

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Cardiac output in the Mollusca: Scope and regulation

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Summary. Different molluscan groups have evolved functionally specialised cardiovascular systems in response to varied behavioural and environmental demands, making the study of cardiovascular regulation in these animals a fascinating area for research. Currently, such research is frustrated by the lack of data on the in vivo performance of these systems, although, where examined, increased cardiac output appears to be accommodated by a change in stroke volume. This paper considers the in vivo regulation of cardiac output, primarily by extrapolating from in vivo experiments, and proposes the following three hypotheses for future study.
1. The increase in stroke volume is critically dependent on the phasic action of acetylcholine, expanding the end-diastolic volume of the ventricle for the same returning venous pressure.
2. Circulating cardioactive peptides will set the level of myocardial tone on a sliding scale, against which the action of both intrinsic and extrinsic factors are expressed.

3. In extreme cases, the inherent myogenicity of the heart may depend on the level of a circulating peptide. Here, the organ might be better described as humourogenic, rather than myogenic.
Key words. Mollusca; cardiac output; cardiac regulation; 5-HT; ACh; FMRFamide.

Introduction

The Molluscs span a remarkable range of morphologies, habitats and life styles, showing many specialisations in the cardiovascular systems but retaining indisputable common elements, as might be expected from a monophyletic group. A unique opportunity exists, therefore, to study the development of cardiac organs and vascular systems in relation to the wide range of functional demands which occur in this phylum. Control of the hearts, within widely different circulatory systems, is thus an interesting question, both from the comparative organisation and in terms of performance at the species level. Yet, despite the popularity of this group for cardiac studies, particularly pharmacological assays[24], we are still largely ignorant about the intrinsic and extrinsic control of cardiac output, and virtually nothing is known about how these mechanisms might be integrated to modulate cardiac performance.

A major problem is our ignorance of what parameters are regulated in vivo. This is the first question addressed in this paper. The next point to be considered is how cardiac output is modulated, by intrinsic and extrinsic factors, in the isolated heart, and then how these factors might be expressed in the whole animal. An unavoidable problem is that the conclusions not only have to be extrapolated from in vitro to in vivo experiments, and vice versa, but also from species to species. Gross generalisations are, therefore, inevitable in our present state of knowledge.

Scope of cardiac performance during exercise

The lack of data on how cardiac output (output = stroke volume × heart rate) is varied in vivo is perhaps not surprising as exercise, the most obvious behaviour where output might be expected to change, is hard to quantify in many molluscs. Progress is being made with some molluscs, such as *Aplysia* (see Koch, this issue) but the only case where there is anything approaching a comprehensive story, is for the cephalopod *Octopus vulgaris;* partly because this animal can be easily interpreted in vertebrate terms.

What questions should we ask when examining in vivo cardiac performance during exercise? First we must make the assumption that the blood, circulated by the heart, is required to deliver more oxygen to the tissues. This is not a straightforward assumption for many molluscs, and caution should be exercised in using what Greenberg calls an 'oxycentric' approach[7,36]. However, this assumption can be made, with reasonable confidence, for the highly active and vertebrate-like octopus[48].

Increased oxygen delivery to the tissues can be achieved by one of two methods: more oxygen can be extracted from the blood per circuit or more blood can be delivered to the active tissues in the same time. The mammalian strategy involves a combination of both methods: more oxygen is extracted from the blood, thus decreasing the venous reserve, as heart rate increases by as much as 130%. Limited increases in stroke volume (between 15 and 20%) result from a reduction in the end-systolic volume (data from Mountcastle[25]).

Limitations, imposed by mollusc design, force the octopus to solve the problem of increasing tissue oxygen delivery in a quite different manner. Even at rest, the venous O_2 reserve is restricted to 16% , with no significant change during exercise[12]. The arterial PO_2 may even *decrease* during short periods of exercise[40] (Erratum note: The minute scale in fig. 4 of reference 40 is an order of magnitude too large). Another remarkable difference is that the heart rate increases by only about 15%–20% over the resting level[47]. As Wells et al.[50] suggested, the three-fold increase in oxygen consumption during exercise can only be accommodated by increasing the stroke volume, in the order of 2–2.4 times. This has now been confirmed by both indirect Fick determination and with flow probes[12,49].

The difference between the mammalian strategy and that of the octopus can be illustrated by the ventricular work loops (fig. 1a and b), in particular, by the increased area for the active loop in the octopus (relating directly to the stroke volume change) and the large increase in the peripheral resistance (reflected by the pressure of ejection). A rise in the peripheral resistance is a consequence of the hydrostatic skeleton, which is common to all molluscs; in a mammal, peripheral resistance drops slightly during exercise.

In the octopus, therefore, the parameter of cardiac performance which changes most during exercise, is stroke volume. However, it is appropriate to question whether the octopus is typical of other molluscs. Again the lack of data frustrates comparison, although in one case (the valve snapping behaviour of the scallop) the work of Thompson, Livingston and de Zwaan[45] supports such a conclusion. They show, not only that ventricular volume can change by three-fold, but that this is achieved by an increase in the end-diastolic volume. This was assumed, for mechanical reasons, in drawing out the octopod work loop (fig. 1) by displacing the active loop to the right of the resting loop, rather than to the left as in the mammal.

Cardiac performance and its regulation in vitro

Stroke volume regulation is more easily examined in vitro, but the first problem is to find a suitable system for such a study. The octopus, although a fascinating and useful animal for in vivo work, has clear problems in vitro. The ventricle, when isolated and perfused, obeys Starling's Law and can remain active for up to two days[34,35] but unfortunately, it fails, by an order of magnitude, to produce ventricular power values equivalent to the in vivo levels[36]. This applies even when cannulated in a more complex fashion[4]. The reason for this failure may lie in the complexity of the cardiac structure. The ventricle has a thick, nontrabecular myocardium, well

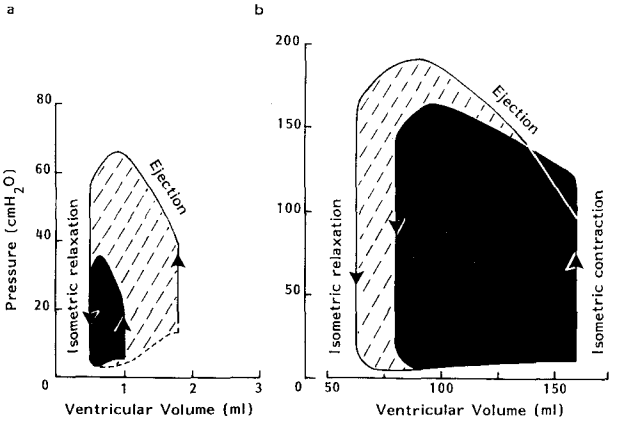

Figure 1. Work loops for the octopus (*a*) and human (*b*) ventricles. The loops during exercise (area of broken lines) behave differently for the two animals. The octopus shows a much greater change in the pressures and volumes during ejection (after Smith[36]). Human data come from Mountcastle[25] and octopus data from Wells[47,48].

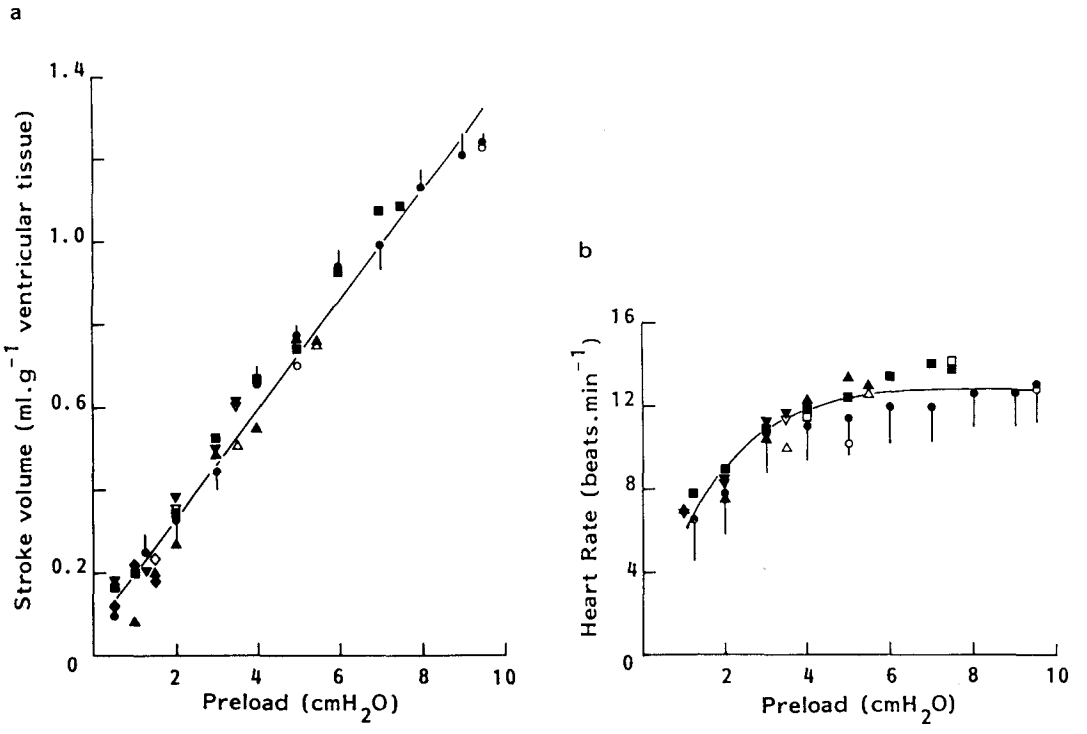

Figure 2. The effect of changing the preload level on the stroke volume (*a*) and heart rate (*b*) of the isolated *Busycon* heart at different preset afterloads. Afterload values are: 10 (●), 8 (■), 6 (▲), 4 (▼), and 2 (♦) cmH_2O. Curves and standard errors are fitted through the data from the highest afterload setting. Preload levels start at the upper end of the range and are reduced. Open symbols are for the performance when the preload is returned to its original setting (after Smith[36]).

vascularised by its own coronary supply, with specialised areas of innervation and, probably, discrete nodal areas for beat generation[39]. It is probable that localised sites for beat generation exist in many molluscs at the atrial-ventricular junction[21] (see also Kuwasawa this issue). Thus to get the octopod heart to preform adequately in vitro the same care should be taken as for a vertebrate heart with a compact myocardium. Therefore, to understand the intrinsic charac-

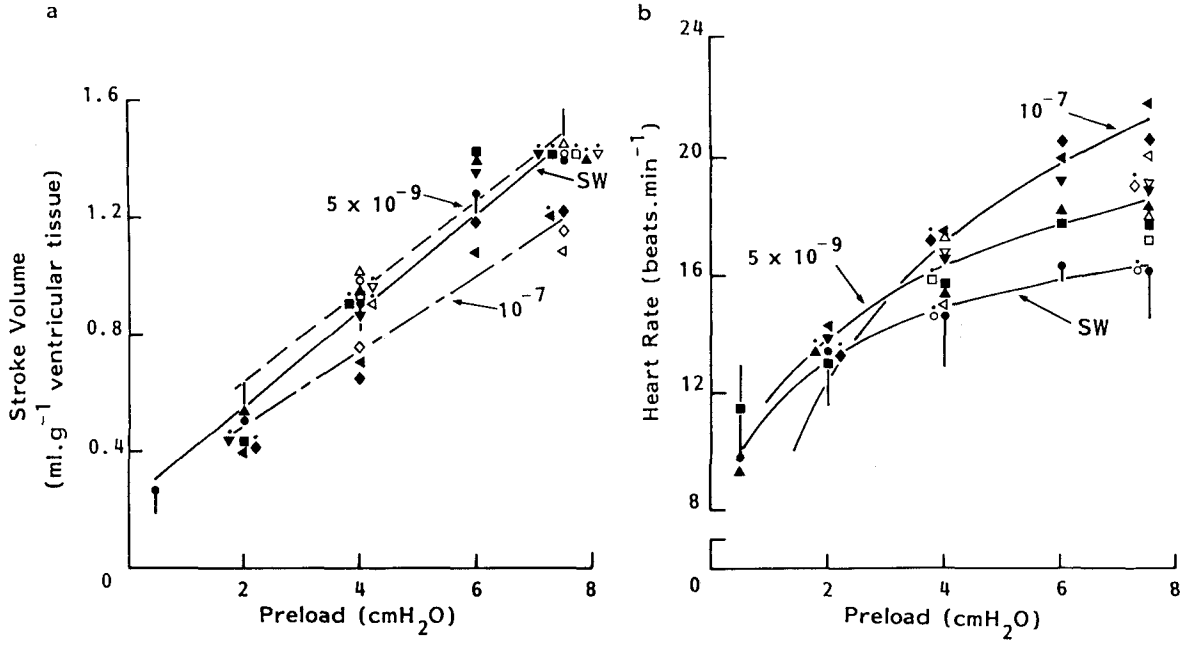

Figure 3. Relationships between preload, 5-hydroxytryptamine (5-HT) concentration and ventricular stroke volume (*a*) and heart rate (*b*) for the gastropod *Busycon canaliculatum*. 5-HT has a limited effect on both parameters, particularly at in vivo pressure levels (2–4 cm H_2O). 5-HT concentrations are: zero, ●; 10^{-10}, ■; 5×10^{-9}, ▲; 10^{-8}, ▼; 5×10^{-8}, ♦; 10^{-7} M, ◄. The afterload is held at 8 cm H_2O in all these experiments. Overlapping mean values (n = 5) are displaced to one side. Single standard errors are shown on one side of the control means and as in figure 2, open symbols are for returning preload levels (after Smith and Hill[41]).

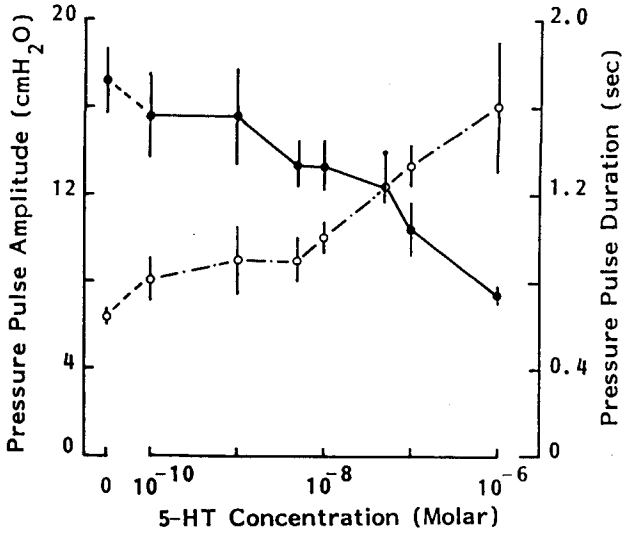

Figure 4. Changes in the aortic pressure pulse amplitude and duration, from the isolated *Busycon* heart, in response to different concentrations of 5-HT. The preload is set at 6 cmH_2O with an afterload of 8 cmH_2O. Standard deviations round the mean (n = 8) are included (after Smith and Hill[41]).

teristics of a molluscan myocardium it is better to start with the simpler hearts of lower molluscs. The gastropod heart is suitable for such a study; the genus *Busycon* offering advantages of size, availability, a reliable in vitro preparation[36] and a considerable literature on several aspects of cardiac biology[3, 22].

Intrinsic properties of the myocardium

When Patterson and Starling[30] examined the intrinsic properties of the dog heart, they concluded that returning venous flow and pressure could be instrumental in regulating the cardiac output, via stroke volume changes. This view, which is no longer applicable for mammalian hearts (due to the small in vivo changes of stroke volume), remains perfectly valid for the molluscan heart. Indeed, Skramlik[33] placed considerable emphasis on the role of pressure waves in controlling the contraction sequence of molluscan cardiovascular organs. Using the isolated systemic heart of the prosobranch gastropod, *Busycon canaliculatum*, the response of the heart to simple physical changes in the perfusion conditions can be examined and, by extrapolation, related to changes in the dynamic characteristics of venous blood, in vivo.

Both stroke volume and the heart rate in *Busycon* are affected by changes in the preload level (input perfusion pressure to the isolated heart), but are largely independent of the afterload values (output back pressure) over the pressure range expected in vivo (fig. 2 a and b). (A marine prosobranch gastropod might be expected to have an atrial pressure of between 1 and 4 cmH_2O, with aortic diastolic pressures in the region of 6 cm H_2O[1, 14]). The heart of *Busycon* conforms to Starling's Law, as do the ventricles of: the cephalopods, *Eledone*[35] and *Octopus*[4]; the gastropod *Aplysia*[43, 44]; and the bivalve *Mercenaria*[37]. Heart rate is insensitive to preload changes above 5 cm H_2O, but is maximally responsive over the in vivo range (fig. 2b).

What is clear from figure 2, is that significant changes in the stroke volume of a *Busycon* ventricle could only be achieved by fairly dramatic changes, in molluscan terms, of venous return pressure. Given that all molluscs, with the exception of the cephalopods, have what is loosely termed an open cardiovascular system (see Jones[15] for a criticism of this point), and even the cephalopods have very low venous and atrial pressures[36, 51], a mechanism of control relying on changes in the returning venous pressure, seems inherently unlikely. Therefore, to progress further in determining what might regulate cardiac output, the action of extrinsic factors on the heart should be considered.

Extrinsic control of cardiac output in vitro

Control of ventricular performance in the molluscs could, theoretically, be affected by a vast array of cardioactive substances, ranging from common neurotransmitters to circulating peptides (for a review see Jones[15]). Numerous studies have examined the action of cardioactive substances on the molluscan heart, but seldom in a way that can be related to the whole heart or the effect on output in vivo. In the next section, the results of testing the action of three substances, 5-hydroxytryptamine, acetylcholine and the tetrapeptide (PHE-MET-ARG-PHE-NH2) FMRFamide, on the perfused isolated heart of *Busycon canaliculatum* are discussed[41, 42]. The experiments were designed so that the results could be equated with in vivo performance.

1. 5-hydroxytryptamine (5-HT). 5-HT can act as an excitatory neurotransmitter in the molluscan myocardium, although, like numerous other cardioactive substances, its action can be species dependent[29]. In some cases 5-HT can be inhibitory. When this drug is perfused through the isolated *Busycon* heart it has no effect on stroke volume, throughout the concentration range used (10^{-10}–10^{-6} M: fig. 3a). Heart rate is dose dependent, showing a clear acceleration with 5-HT perfusion at preload levels of between 6 and 8 cm H_2O (fig. 3b). However, at the lower in vivo operating pressures (2–4 cm H_2O), heart rate is also insensitive to increased concentrations of this drug. On the basis of these results, it would appear that in vivo, 5-HT should have only a very limited effect on cardiac output.

Despite an apparent lack of effect on output per se, 5-HT does affect the manner in which the perfusate is ejected from the heart: the amplitude of the aortic pressure pulse rises dramatically, but the duration is greatly reduced (fig. 4). In other words, the blood is being ejected by the heart at a higher flow rate and pressure, in such a way that the volume ejected remains constant, while both the end-systolic and diastolic volumes are reduced. If we return to the original question, and are grossly simplistic in our outlook, then clearly 5-HT is not a compound which, on its own, could regulate cardiac output by an action on the stroke volume. What then could be the in vivo action of 5-HT? One of the inevitable consequences of movement in the molluscs is the increase in peripheral resistance (as shown in fig. 1 for the octopus). Under these conditions, an increase in blood pressure would seem essential if blood is to reach the exchange vessels. There may be little need for increased O_2 delivery in some of the less active species, or during behaviour such as withdrawal into the shell, where peripheral resistance would be expected to increase. Output might, therefore, remain constant. However, no matter what cardiac response is considered, it is unlikely that any transmitter will act alone in vivo.

2. Acetylcholine (ACh). ACh is frequently antagonistic to 5-HT in the molluscan heart. On the isolated *Busycon* heart, it acts to reduce both the stroke volume and the heart rate (fig. 5a and b), as well as increasing the duration and reducing the amplitude of the aortic pressure pulse (fig. 5c and d). Less perfusate is being ejected at a lower pressure. The method used in these experiments was different from that used in the 5-HT studies. For ACh, the hearts were perfused at a constant pressure (Preload = 4 cm H_2O; Afterload = 6 cm H_2O) and returned to seawater perfusion between each drug concentration. This not only controlled for any deterioration of the preparations during the assay, but also revealed an

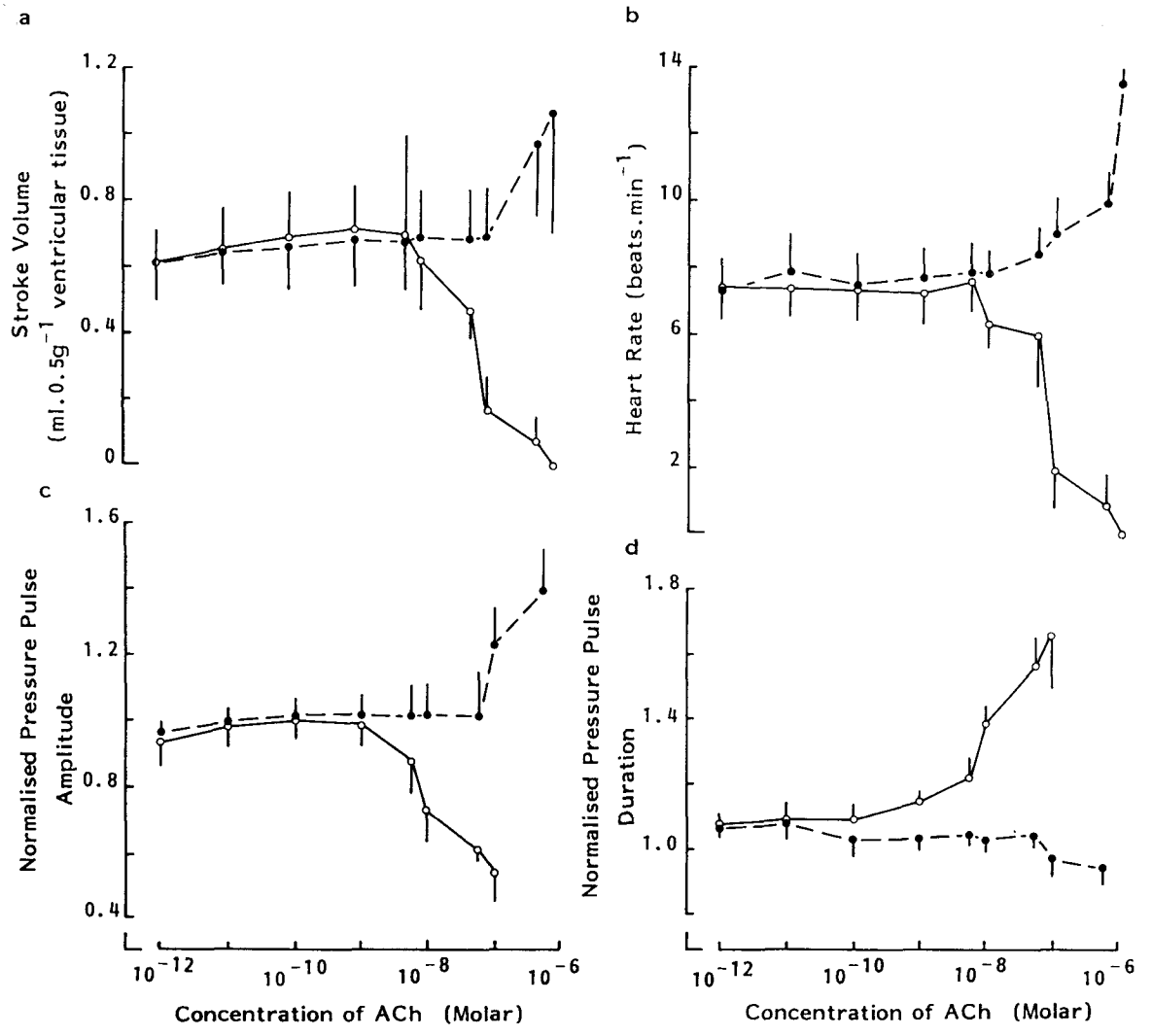

Figure 5a–d. The response of four parameters of *Busycon* cardiac performance to increasing concentrations of acetylcholine (ACh: ○) at preset perfusion pressures (preload and afterload are 4 and 6 cmH_2O respectively). The performance during a 10-min washout period, between ACh concentrations, is also shown (●). Standard errors are shown on one side of the mean values. (In *a* and *b*, n = 6; in *c* and *d*, n = 9: after Smith and Hill[42]).

after-effect for ACh on heart rate, pressure pulse amplitude and possibly stroke volume[42].

ACh is known to affect the configuration of the cardiac muscle action potential[9, 13, 54], shortening the duration of the sodium dependent plateau phase[17, 43] as well as reducing the force of contraction[9, 54]. Suction electrode recordings of the myogram from the beating, isolated heart, during perfusion with ACh, contradict these results (fig. 6a), although such recordings are usually considered to be an accurate representation of the cardiac action potential[13]. In the study reported here, the plateau increases in duration while the output, and pressure pulse amplitude, decrease. The action of 5-HT, on the myogram recorded from the isolated and perfused *Busycon* heart, also contradicts previously published results. Here, the amplitude of the pressure pulse increases while the duration of the plateau phase decreases [41]. It is normally supposed that the action potential duration relates directly to the force of contraction[10, 27]. The reason for these differences is not clear: it may simply lie in the variant definitions of force, but another possible explanation is the experimental design. Internal stretch on the myocardium can affect the configuration of the action potential in *Busycon*, and, in *Mercenaria*, can even convert the simple form recorded by the sucrose gap technique[2] into a complex spike and plateau[37]. Experiments on the isolated heart, with drug perfusion, represent an interaction of regulatory mechanisms, which in the case of 5-HT can clearly affect how we assess the action of a cardioactive substance.

A likely feature of any system that can regulate stroke volume by three times the resting level, at low vascular pressures, will be the alteration of myocardial tone, so as to increase the cardiac volume for the same returning venous pressure. ACh has this effect on the *Busycon* heart (fig. 5b). However, stroke volume does not increase, as the end-systolic volume also goes up with ACh concentration. Again, as with 5-HT, ACh alone cannot increase stroke volume, but it is now apparent that an interaction between these two transmitters might produce such a response from the heart.

3. FMRFamide. The tetrapeptide, FMRFamide, frequently mimics the action of 5-HT on the molluscan heart, but is not inhibited by the serotinergic antagonist methysergide[16, 28, 31] (see also Kobayashi in this issue). Certainly, at lower concentrations, its effect on the isolated heart of *Busycon* is similar to 5-HT, acting in the opposite manner to ACh. At concen-

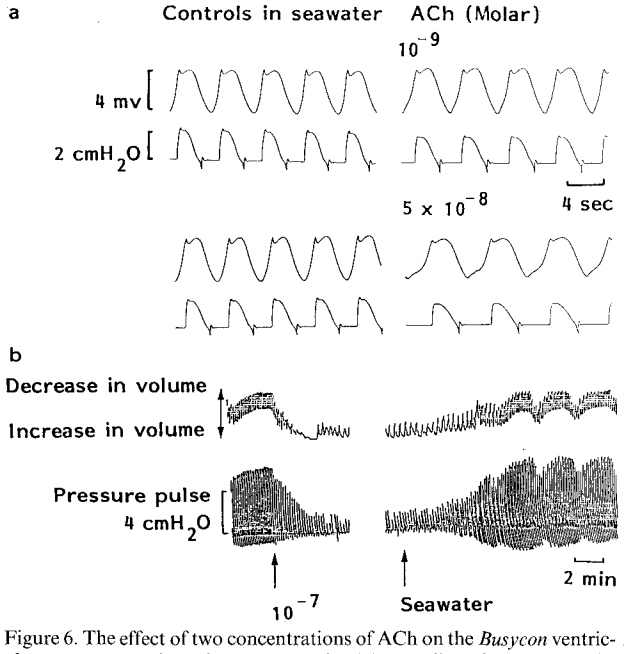

Figure 6. The effect of two concentrations of ACh on the *Busycon* ventricular myogram and aortic pressure pulse (*a*) as well as the effect on the ventricular volume at preset and unaltered perfusion pressure levels (*b*: after Smith and Hill[42]).

trations below 10^{-7}M, pressure pulse amplitude increases while the duration declines, heart rate is accelerated but stroke volume remains unchanged (fig. 7). The myogram form changes as with 5-HT perfusion, the amplitude increases, the duration shortens (fig. 8). However, above the concentration of 10^{-7} M, there is a change in the response to FMRFamide. Now, both rate and stroke volume decline. Biphasic responses of molluscan hearts (where increasing concentrations of cardioregulatory substance cause first inhibition or excitation, then reverse) have been noted before[29]. At first sight such an interpretation of the data in figure 7 might seem reasonable. However, the electrical activity of the heart during perfusion with increased concentrations of FMRFamide, indicates that we are not seeing inhibition as described for ACh. FMRFamide, at concentrations above 10^{-7} M, causes extreme excitation, extending the time course of the plateau phase of the action potential so that the heart does not relax (fig. 8a). The rate thus declines. This compound also reduces the end-diastolic volume (fig. 8b), so that stroke volume declines and the heart simply expels its entire content in the initial contraction.

An hypothesis for integrated control in vivo

Work on the isolated heart suggests that neither intrinsic factors nor the most obvious cardioactive substances, can on their own, regulate cardiac output by affecting stroke volume. However, in vivo, none of these factors will act at a constant level or in isolation from other parameters that

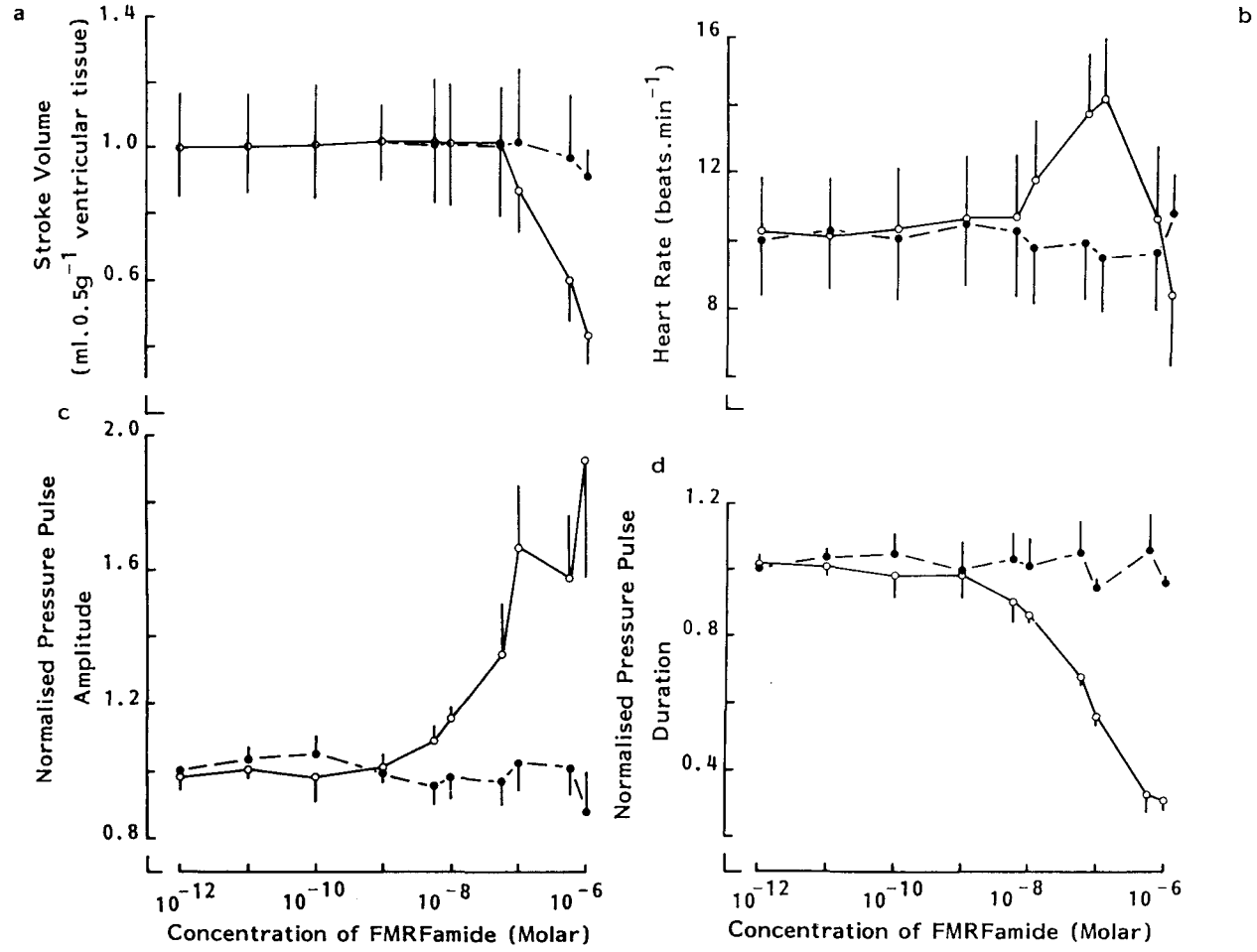

Figure 7a–d. The response of four parameters of *Busycon* cardiac performance to increasing concentrations of the tetrapeptide FMRFamide (○) at preset perfusion pressures (preload and afterload are 4 and 6 cm H_2O respectively). The performance during a 10-min washout period, between FMRFamide concentrations, in also shown (●). Standard errors are shown on one side of the mean values. (In *a* and *b*, n = 5; in *c* and *d*, n = 6: after Smith and Hill[42]).

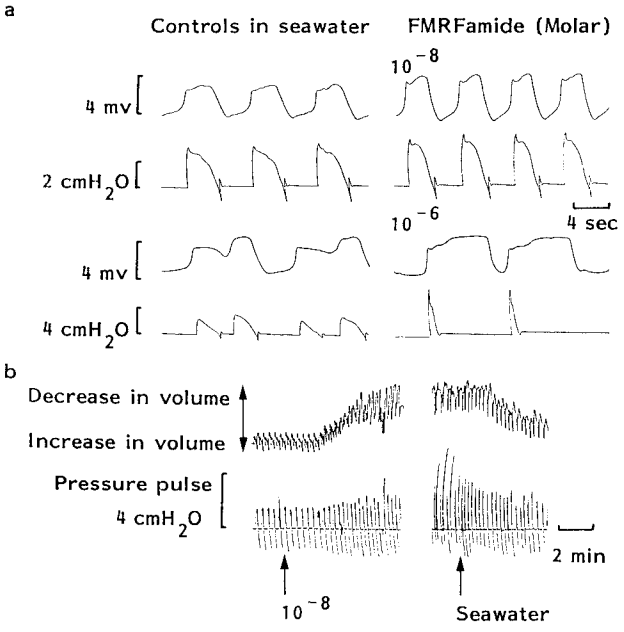

Figure 8. The effect of two concentrations of FMRFamide on the *Busycon* ventricular myogram and aortic pressure pulse (*a*) as well as its effect on the ventricular volume at preset and unaltered perfusion pressure levels (*b*: after Smith and Hill[42]).

influence cardiac performance. Regulation of stroke volume could be achieved, in vivo, by the phasic action of the cholinergic innervation at the end of systole, allowing a larger ventricular volume for the same returning venous pressure. A subsequent increase in the volume discharged, could be mediated either by rebound effect, as seen in the study reported here, or by aminergic activity at the onset of systole. In support of this hypothesis, cholinergic regulation of the ventricular relaxation state is already suggested by results from the opisthobranch *Aplysia*. Koester et al.[20] noted that a pattern of cholinergic neuronal discharge to the heart accompanies a stereotyped behaviour, during which fresh seawater flows over the gills. Simultaneously, the gills contract, pumping an increased volume of oxygenated blood to the heart, which is then in a relaxed state.

The action of FMRFamide, in vivo, is both confusing, as the target organ is not known, and fascinating, for its regulatory potential. Both FMRFamide and the FMRFamide-like peptides, such as pQDPFLRFamide, are present in molluscan blood at concentrations of at least 10^{-9} M[26, 32]. Further, Price et al.[32] show that levels are variable between individuals. The obvious possibility exists, therefore, that these compounds could have a continuous effect on the myocardium, setting a variable tone against which other cardioregulatory factors act.

Aplysia and a humourgenic heart?

Aplysia is perhaps the best studied of the molluscan genera with respect to the neural control of cardiac activity[6], and these studies are now being linked to behaviour and cardiac performance in vivo[18, 19] (see also Koch this issue). It is an obvious animal for a study of the in vitro ventricular energetics and modulation, as already reported for *Busycon*. However, there are problems with the isolated heart preparation. Both Hill[9] and Straub[43, 44] noted that the hearts of aplysiids can often be quiescent in isolation and Straub used the animals own blood to retain activity in the organ; a not uncommon solution to inactivity in molluscan heart preparations. The heart of *Aplysia* is difficult to maintain in vitro using techniques which work well for other gastropods, bivalves, and cephalopods. On isolation and perfusion with filtered seawater, some hearts are inactive although most preparations will beat for 60–90 min[38]. After arrest such preparations are not dead, for perfusion with either FMRFamide or 5-HT initiates sustained activity long enough to allow the effects of preload changes to be examined (fig. 9a). 5-HT stabilises the heart rate over a range of perfusion pressures, where-as FMRFamide allows it to vary. The latter type of response was seen in the two cases where spontaneous activity, in seawater alone, remained long enough for a similar experiment (fig. 9b).

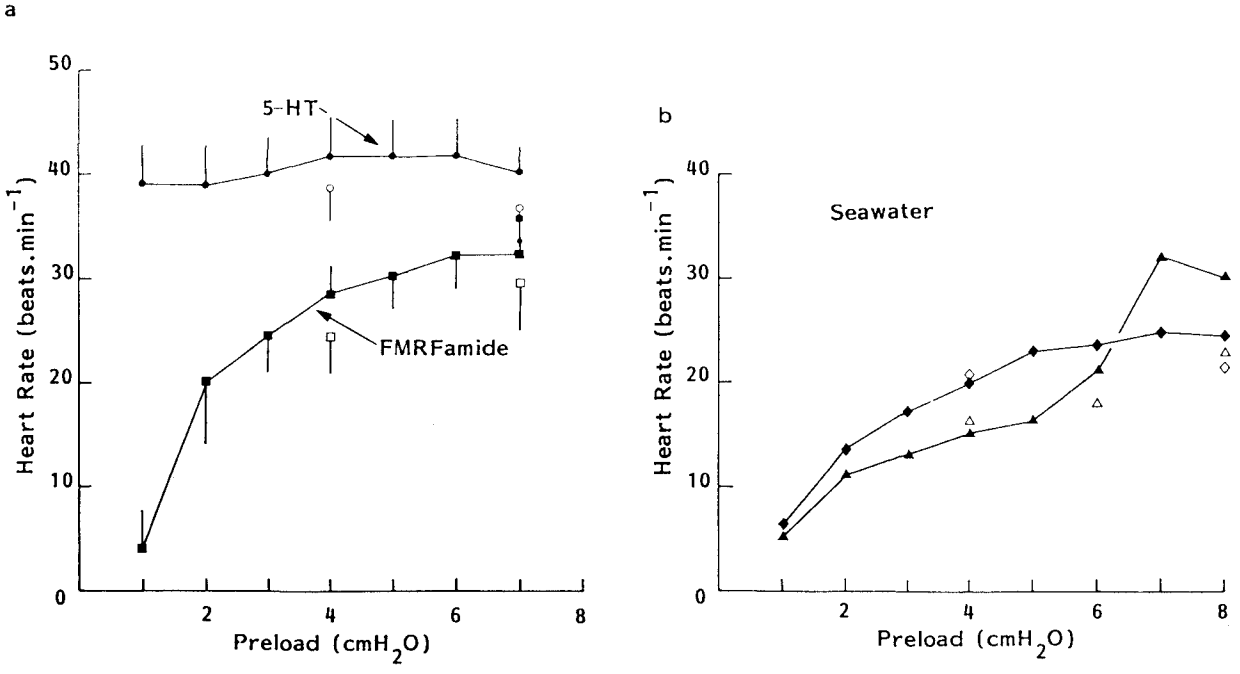

Figure 9. The heart rate response of the isolated systemic heart of *Aplysia dactylomela* at different preload levels. The afterload was zero (Smith, in prep.). The preparations are either perfused with a solution of 10^{-7} M FMRFamide or 5-HT (*a*: n = 5, standard errors plotted on one side of the mean values) or with filtered seawater (*b*).

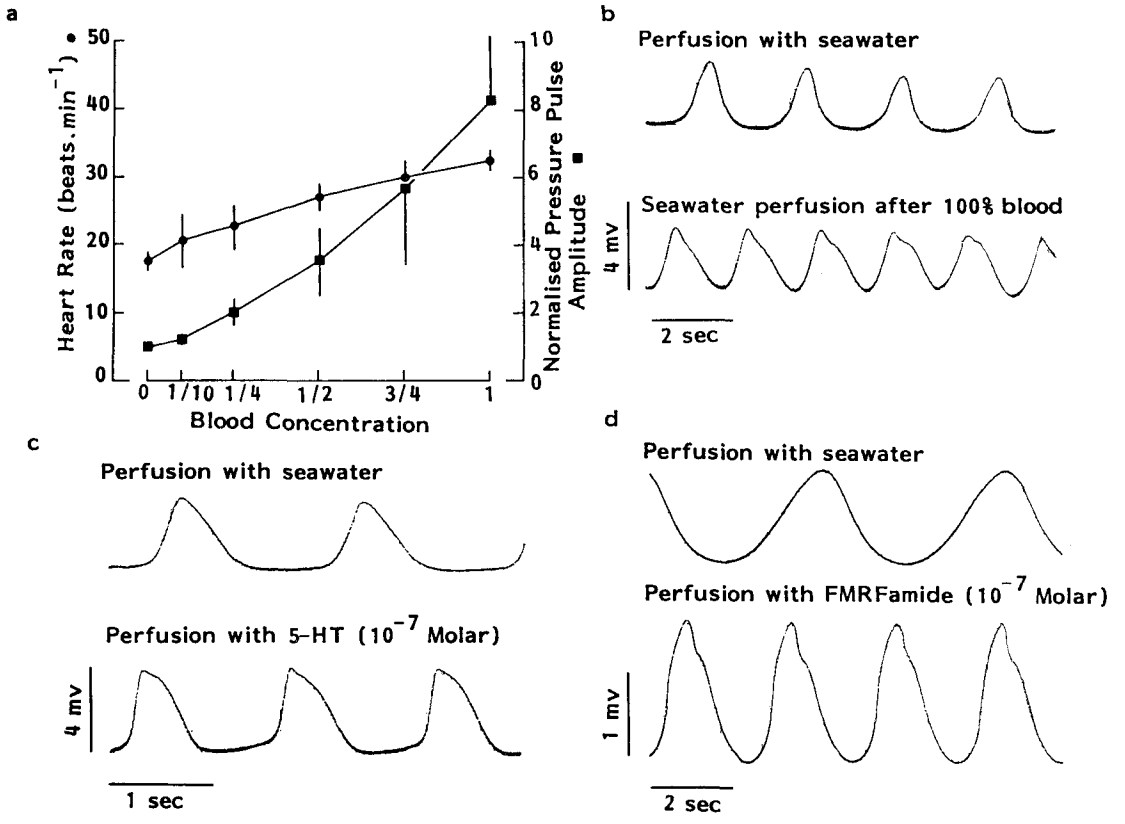

Figure 10. *a* The effect on the isolated systemic heart of *Aplysia dactylomela* of a 2-min perfusion with dilutions of its own blood. Measurements were made 10 min after the introduction of the blood mixture. Values are means from 4 preparations with standard errors plotted. *b* The effect of blood perfusion on the form of the ventricular myogram, recorded with a suction electrode from the beating heart. *c* As in *b* but for perfusion with 5-hydroxytryptamine. *d* As in *b* but for perfusion with FMRFamide.

The question is, why do the isolated hearts not keep contracting when perfused with seawater? If the animal's blood is introduced into the perfusion system, in a 2-min pulse, the performance is clearly enhanced (fig. 10a); an effect that can last for more than 20 min, even at a dilution of 25%. The dose dependence of the response to blood and the effect at low dilutions on the heart, *suggests* that this effect may not simply be ionic or osmotic, but result from the presence of an active component, such as an endogenous peptide. FMRFamide is, infact, present in the circulatory system of *Aplysia*[23]. Further evidence in support of a hypothesis that the heart of *Aplysia dactylomela* may be dependent upon a circulating factor for normal rhythmicity, comes from the myogram recorded from the beating heart. Here, there is an anomaly in the literature. Hill and Yantorno[11] reported that using an identical sucrose gap technique, the cardiac muscle of *Dolabella auricularia* has a normal spike and plateau action potential whereas that of the aplysiid, *A. californica*, has only a simple, fast form. The two genera are closely related, yet such a result implies fundamental changes in the cardiac muscle cell membrane. These differences could be artefactual, resulting from the abnormal stretch imposed on the myocardium by the technique (as discussed above for *Mercenaria*). However, when internally perfused and spontaneously beating in seawater, the *Aplysia* heart still shows only a simple myogram record (fig. 10b, c and d). Perfusion, with either the animals own blood or 5-HT, converts the myogram to the complex type (fig. 10b and c). Perfusion with FMRFamide also produces a complex waveform, but the case for the generation of the expected molluscan cardiac myogram is not so clear with this compound. What appears to be a spike phase is abnormally slow (fig. 10d).

The hypothesis that the heart of *A. dactylomela* is dependent on the presence of an exogenous factor, such as a circulating cardioactive substance, for normal rhythmicity, can only be tentative. However, the presence of peptides in the circulatory system at reasonable concentrations[32] would seem to make it inevitable that these compounds have a constant action on the heart. Welsh[52] proposed that neurohumours might act as long-range, long-duration mimics of some neurotransmitters. Although no longer considered mimetic, Greenberg and Price[8] reiterate that the peptides might generally regulate the level of visceral muscle excitability. Here might lie an additional reason for the poor energetic performance of the octopod heart. The action of a humour might differ in effect depending on the species, overlying, to a variable degree, the inherent myogenicity. In extreme cases, the term humourogenic might be deserved for some molluscan hearts.

The origin of cardiac rhythmicity is normally divided into two simple categories: myogenic (typical of vertebrates, tunicates and molluscs), where activity is based on the inherent rhythmicity of the muscle or neurogenic (as in the Crustacea), where the nervous system regulates the contraction directly. The above considerations suggest that the myogenicity in molluscs is modulated by the level of a circulating hormone or hormones. Other hearts can have their performance modulated by circulating cardioactive substances, such as adrenaline in mammals, catecholamines in the teleost[5] and peptides in the insect *Manduca*[46]. Perhaps, many myogenic hearts are subject to a tonic modulation of contractility by circulating cardioactive substances.

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Organization of the multifunctional neural network regulating visceral organs in *Helix pomatia* L. (Mollusca, Gastropoda)

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Summary. In *Helix pomatia* L. the overlapping neuronal network was found to regulate the visceral functions (e.g. cardio-renal, respiratory and genital functions). The neural network is organized around the multifunctional interneurons, which take part in forming both afferent and efferent pathways. The interneurons are sensitive to a wide range of neurotransmitters or transmitter candidates including low molecular weight substances (ACh, 5HT, DA, octopamine, glutamate) and several oligopeptides. In this system both selection of information and modification of membrane properties (for example habituation) are carried out by a combination of simultaneously liberated active agents.
Key words. Helix pomatia L.; neural network; habituating and non-habituating neurons; interaction of neurotransmitters and peptides; FMRFamide; opiate peptides; morphine; ACh; 5HT.

Introduction

In the subesophageal ganglion complex of *Helix pomatia* a network consisting of a number of identified neurons was found to regulate the visceral organs including the cardio-renal, respiratory and genital systems[24].
The gastropod central nervous system and semi-intact preparations are commonly used for studying questions connected with the transmission, interpretation and storage of information. This involves the problems of specificity or invariance of single units in the regulatory neural networks, or on the contrary, the variability and dynamic nature of the network elements[2,15,22,26].
The data support an emphasis on the concept that recognition, analysis and regulation occur at the level of neural networks building up from overlapping neural populations. Any idea that the same unit of the network can take part in the interpretation of various pieces of information, or in the regulation of different functions, contradicts the alternative idea of networks or units specialized for one single function. The aim of our investigations was to study the interrelation of the neurons regulating various visceral organs, e.g., cardio-renal, respiratory and genital. During the investigation special attention was paid to the interaction of neurotransmitters involved in the identified neural network.

Material and methods

The experiments were performed on active snails, *Helix pomatia* L., at room temperature (20–24 °C), throughout the year. For the investigations semi-intact preparations developed earlier [21,25] were used. The preparation employed contained: 1) cardio-renal system (e.g. heart, pericardium, blood vessels, kidney and liver), 2) respiratory system (e.g. pneumostoma surrounded with a piece of mantle and body wall), 3) genital organs (e.g. hermaphroditic gland, hermaphroditic duct, female duct, spermatheca, accessory genital mass and prostate gland).
During preparation, care was taken to preserve intact the connection of anal, right parietal and intestinal nerves, innervating the cardio-renal, respiratory and genital systems, respectively, with the central nervous system (CNS).
In the majority of experiments the intracellular activity of two identified central neurons, heart contractions and the extracellular activity of the corresponding nerve were recorded simultaneously. The intracellular activity of the neurons was registered with conventional glass microelectrodes, filled with 2.5 M KCl or 0.6 M K_2SO_4, which had resistances of 5–20 MOhm. A four-channel Tektronix oscilloscope, a Gould-Brush recorder and Dagan amplifiers were employed during experiments. The inputs from various visceral organs were activated by applying tactile stimuli to the peripheral receptors.
The application of low molecular weight neurotransmitters or peptides was carried out by two methods: 1) from a micropipette 50, 100 or 200 µl of substances in the range of 10^{-4}–10^{-8} M was applied to the surface of ganglia (drop application). 2) the microelectrode with 4–6 µm tip diameter filled with the drug was positioned on the soma membrane of the

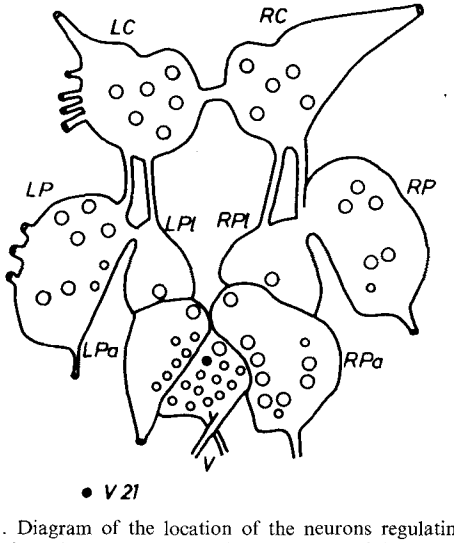

Figure 1. Diagram of the location of the neurons regulating visceral functions in *Helix pomatia* on the dorsal surfaces of the central ganglia. LC and RC, left and right parietal; LP and RP, left and right pedal; LPl and RPl, left and right pleural; LPa and RPa, left and right parietal; V, visceral ganglia.