J. exp. Biol. **148**, 461–475 (1990)
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NEURAL CONTROL OF HEART BEAT IN THE PTEROPOD MOLLUSC *CLIONE LIMACINA*: COORDINATION OF CIRCULATORY AND LOCOMOTOR SYSTEMS

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Accepted 13 September 1989

Summary

1. In the intact pteropod mollusc *Clione limacina* a correlation between heart beat and locomotor activity has been found. In freely swimming *Clione*, pauses in locomotor activity arose spontaneously with intervals of a few minutes. During these pauses, the heart stopped beating. A link between the heart and locomotor activity was also observed during defensive reactions: mechanical stimulation of the head resulted in the termination of both locomotion and heart beating, while stimulation of the tail accelerated both the locomotor and the heart rhythms. After transection of the medial abdominal nerve supplying the heart, the link between heart rate and locomotor activity disappeared. The heart rhythm accelerated during hunting behaviour of *Clione*.

2. Four efferent neurones controlling heart beat were found: one heart excitor (HE) in the left pedal ganglion, and three heart inhibitors (HI) in the left abdominal ganglion. The HE exerted an inhibitory action upon the HIs.

3. Spontaneous or reflex activation of the locomotor generator was accompanied by excitation of the HE and by inhibition of the HIs, while spontaneous or reflex inhibition of the locomotor generator was accompanied by inhibition of the HE and by excitation of the HIs. These effects were due, at least partly, to the direct action of the neurones of the locomotor generator upon the HE and HIs.

Introduction

In gastropods, the blood flows round the open circulatory system. This flow is mainly determined by beating of the heart, which consists of an auricle and a ventricle. As in vertebrates, the molluscan heart rhythm is myogenic (Hill and Welsh, 1966; Irisawa, 1978; Jones, 1983; Krijgsman and Divaris, 1955). The frequency and amplitude of the heart beat depend on a number of influences upon

Key words: pteropod mollusc, heart control, locomotion, identified neurones.

the myogenic pacemaker. These are: (1) nervous influences, (2) humoral influences, (3) metabolic influences, and (4) peripheral influences upon the blood flow, which affect the heart pacemaker because it is sensitive to stretch of the heart wall.

Among various heart-controlling mechanisms, the nervous ones have been studied in greatest detail. The most extensive studies of neuronal mechanisms controlling the heart were carried out on three species of gastropod mollusc: *Aplysia californica* (Kandel, 1976; Koester *et al.* 1979; Koester and Koch, 1987; Mayeri *et al.* 1974), *Helix pomatia* (S.-Rozsa, 1979, 1983, 1987; Zhuravlev and Safonova, 1984) and *Achatina fulica* (Furukawa and Kobayashi, 1987*a*,*b*). In the visceroparietal (abdominal) ganglia of these molluscs, neurones were found which accelerated or decelerated heart beating.

The present paper deals with the control of heart beat in the marine pteropod mollusc *Clione limacina*. *Clione* lives in the northern seas. It is a planktonic animal which stays at a constant depth because of rhythmic movements of its wings (Wagner, 1885; Arshavsky *et al.* 1985*a*; Satterlie *et al.* 1985). Sometimes the wing movements cease and the mollusc sinks by 20–50 cm; the wing movements are then resumed, and the mollusc returns to its initial depth (Litvinova and Orlovsky, 1985). These vertical migrations are repeated at intervals of a few minutes.

Clione is a predator. It subsists on the small pteropod mollusc *Limacina helicina* (Wagner, 1885; Lalli, 1970; Litvinova and Orlovsky, 1985). On contacting a *Limacina*, *Clione* everts three pairs of buccal cones with which it seizes the prey (see Fig. 3C). Protraction of the cones (which are normally retracted within the head) is brought about through an increase of blood pressure in the head cavity. Simultaneously with catching the prey, the frequency of wing oscillations increases 2–3 times, and *Clione* swims rapidly, keeping *Limacina* against its mouth: it seems likely that the opposing water flow presses *Limacina* to the mouth. The locomotor activity decreases after the prey has been swallowed. The locomotor activity of *Clione* is also affected by tactile stimuli evoking defensive reactions (Arshavsky *et al.* 1985*a*). Touching the head results in termination of the wing oscillations, and *Clione* sinks. In contrast, touching the tail results in acceleration of locomotion, and *Clione* swims away from the source of irritation.

In this work we have studied: (1) coordination of locomotor and heart activities in intact *Clione*; (2) efferent neurones controlling the heart rate; and (3) neuronal mechanisms responsible for coordination of the circulatory and locomotor systems. A preliminary account of these data has been published elsewhere (Arshavsky *et al.* 1988, 1989).

Materials and methods

Experiments were carried out at the White Sea Marine Biological Station *Kartesh* during the summer and autumn season. Adult *Clione limacina* (3–5 cm long) were used in the experiments. Both intact animals and reduced preparations were examined.

Experiments on intact animals (N=14)

Coordination of locomotor activity and heart rate was studied in intact molluscs which swam in a Petri dish (10 cm in diameter) in which they could swim normally. Two observers participated in these experiments. One pushed the button of an event recorder every time the wings moved up during the swim cycle; the second observer pushed the other button with each contraction of the heart (see Fig. 3). To evoke defensive reactions, the head or the tail of an animal was mechanically stimulated. When studying the hunting behaviour of *Clione*, several specimens of *Limacina* were put into the dish.

To study the morphology of the circulatory system, Indian ink was injected into the heart or body cavities in intact *Clione*, and ink spread was observed through the semi-transparent body walls.

Semi-intact preparations (N=40)

These consisted of the posterior part of the body (including the heart), the head and the central nervous system (Fig. 1). The nerves supplying the posterior part of the body and the head were left intact. The preparation was pinned to the bottom of a Sylgard-lined chamber filled with sea water. Heart contractions were recorded with a photocell. To facilitate the insertion of microelectrodes, the epineural sheath was locally softened with Pronase E (3.5 % solution for 3–5 min) which was gradually ejected from a small pipette (tip diameter about 20 μm).

Glass microelectrodes filled with 3 mol l^{-1} KCl (tip resistance, 20–60 MΩ) were

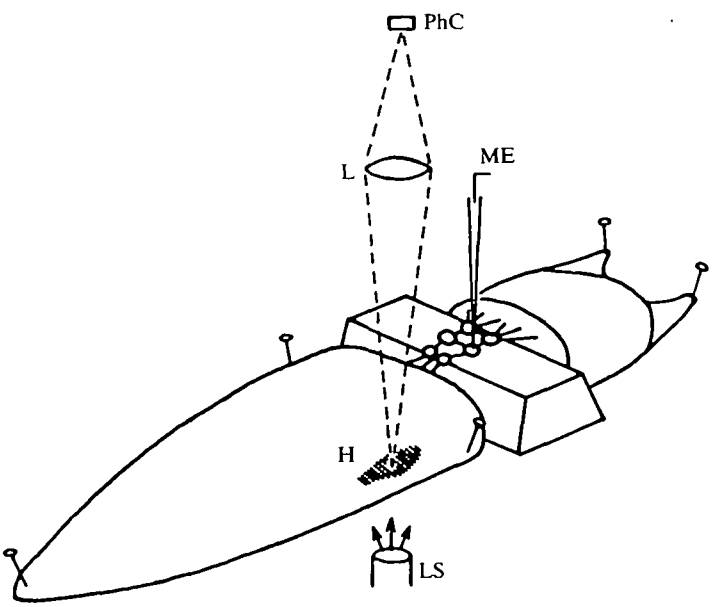

Fig. 1. Semi-intact preparation consisting of the head, the caudal part of the body and the central nervous system. PhC, photocell; L, lens; LS, light source; ME, microelectrode; H, heart.

used for intracellular recordings. The same electrode was used for both recording and current injection. The artefact caused by a polarizing current was partly compensated by means of a bridge circuit. For intracellular staining, microelectrodes filled with a solution of Lucifer Yellow were used; the dye was injected into the cell by passing a hyperpolarizing current (Stewart, 1978).

Isolated central nervous system (N=9)

This was used in preliminary experiments to find the neurones projecting to the heart. In these experiments, the dissected end of the heart branch of the medial abdominal nerve (9 in Fig. 2B) was put into either cobalt chloride or Lucifer Yellow solution, and neurones were retrogradely stained (for a description of the method see Benjamin *et al.* 1979; Sonetti *et al.* 1982; Stewart, 1981).

In some experiments ($N=5$) this preparation was used for studying a correlation between activity of the locomotor generator and that of neurones controlling the heart.

Neuronal activity was recorded with a pen recorder which was not rectilinear, and had a frequency range of 0–200 Hz which somewhat attenuated the amplitude of recorded spikes.

Results

Circulatory system of Clione

The circulatory system of *Clione* was first described by Wagner (1885); his findings were confirmed by our observations (Fig. 2A). The heart is located on the right side of the body. It consists of the auricle and the ventricle, with a valve in between. There is another valve between the ventricle and the aorta. The heart is enclosed in the pericardium. The aorta branches off into arteries leading to the visceral organs, the wings and the head. Like all gastropods, *Clione* has an open circulatory system. Blood from the arteries is collected in the haemocoel and then travels to the venous cavity. The latter is a narrow space between two muscle layers located under the skin throughout the body surface, except for the head. *Clione* has no special organ of respiration, and gas exchange occurs in the venous cavity (Wagner, 1885).

Adaptive changes in the heart rate

Observations on intact *Clione* demonstrated that, in many cases, the heart rate is linked with the level of locomotor activity. Simultaneous recordings of wing movements and heart contractions in *Clione* swimming in a Petri dish are shown in Fig. 3. Swimming was not continuous: it was occasionally interrupted by short pauses. The heart frequency during periods of swimming was about 0.5 Hz. During the pauses in locomotor activity, there were profound slowings of the heart rate. A similar relationship between the circulatory and locomotor systems was observed during defensive reactions (Fig. 3D). Tactile stimulation of the head resulted in termination both of the rhythmic wing movements and of heart

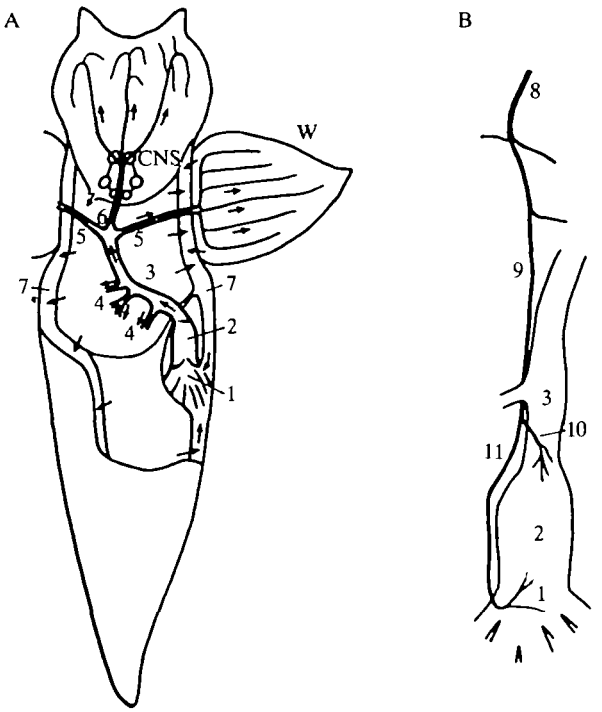

Fig. 2. (A) Schematic diagram of the circulatory system of *Clione* (modified from Wagner, 1885). Arrows show the direction of blood flow. (B) Heart innervation. CNS, central nervous system; W, wing; 1, auricle; 2, ventricle; 3, aorta; 4, abdominal arteries; 5, wing arteries; 6, head artery; 7, venous cavity; 8, medial abdominal nerve; 9, heart branch of this nerve; 10 and 11, ventricle and auricle branches of the nerve, respectively.

beating. Conversely, tactile stimulation of the tail resulted in activation of the locomotor system (escape reaction) and, in parallel, acceleration of heat beating.

However, heart rate was not always linked with the level of locomotor activity. During hunting behaviour of *Clione*, a short-term contact with *Limacina* could result in the dramatic activation of both locomotor and circulatory systems ('food arousal', see Weiss *et al.* 1982). A few minutes later the activity of the locomotor system decreased, while that of the circulatory system remained high (about 1 Hz; Fig. 3B,E). Throughout this period there was no correlation between the two systems. When *Clione* caught the prey, the locomotor system was strongly activated again, but the frequency of heart beating did not change (Fig. 3C).

When *Clione* was in the state of food arousal, or when it held the prey by the buccal cones (Fig. 3C), its defensive responses to tactile stimulation (highly pronounced in the absence of food arousal, Fig. 3D) were strongly suppressed. Under such conditions, only strong stimuli (pinching by forceps) could evoke weak defensive reactions (Fig. 3E); the reactions in this case were observed in the

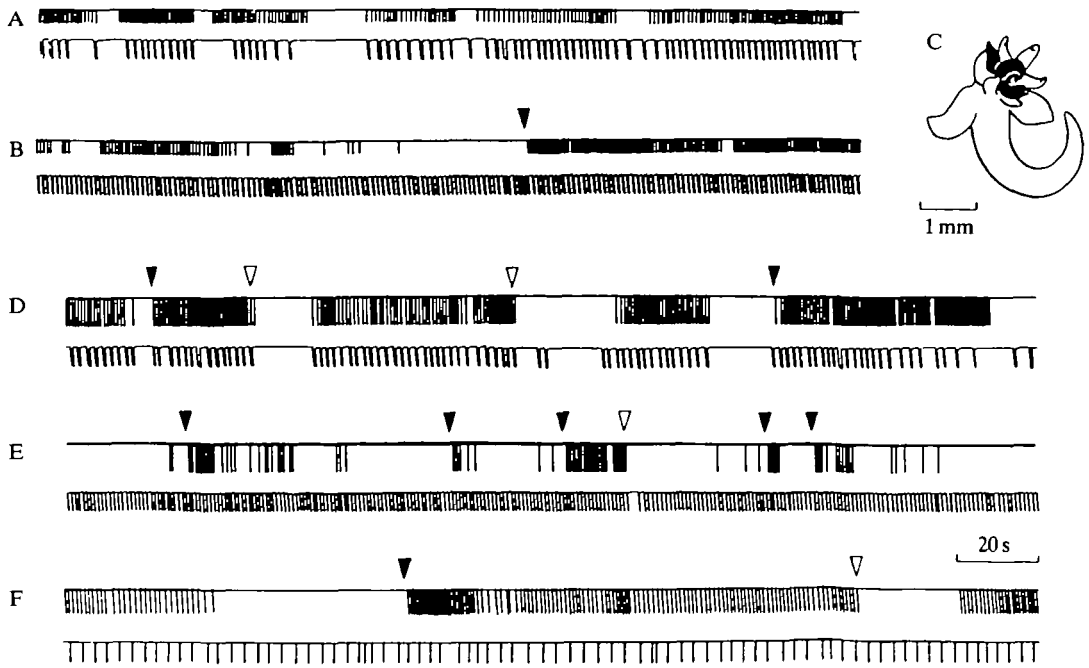

Fig. 3. Simultaneous recording of wing movements (upper trace) and heart contractions (lower trace) in *Clione* swimming in a Petri dish. (A) Undisturbed swimming before presentation of *Limacina*. (B) The same, 30 s after the first contact with *Limacina*. The black arrowhead shows the second contact with *Limacina* which is followed by ejection of the buccal cones and catching the prey. (C) *Clione* catching *Limacina* (drawing from a photograph). (D) Defensive reactions elicited by tactile stimulation of the tail (black arrowheads) or head (white arrowheads). (E) The same animal, 1 min after contact with *Limacina*. Tactile stimulation did not evoke reactions. Nociceptive stimulation (pinching by forceps) of the tail (black arrowhead) or the head (white arrowhead) evoked reactions in the locomotor system only. (F) After transection of the medial abdominal nerve (3 days before the experiment) tactile stimulation of the tail (black arrowhead) or head (white arrowhead) evoked reactions in the locomotor system but did not affect the heart rate. (A,B), (C,D) and (E) are three different experiments.

locomotor system only; the heart rate did not change. 20–30 min after swallowing *Limacina*, the heart rate returned to the initial level, and its relationship with locomotor activity was restored.

The influences upon the circulatory system described above were exerted *via* the medial abdominal nerve (n1 in Fig. 4). This was demonstrated in molluscs (N=8) in which this nerve was cut 1–5 days before the experiment. In these animals the heart either did not contract or contracted at a low frequency (maximally 0.3 Hz), and the dependence of the heart rhythm on the spontaneous or reflex changes of the intensity of locomotion was absent (Fig. 3F). Unfortunately, the hunting behaviour of animals with n1 transected was not studied.

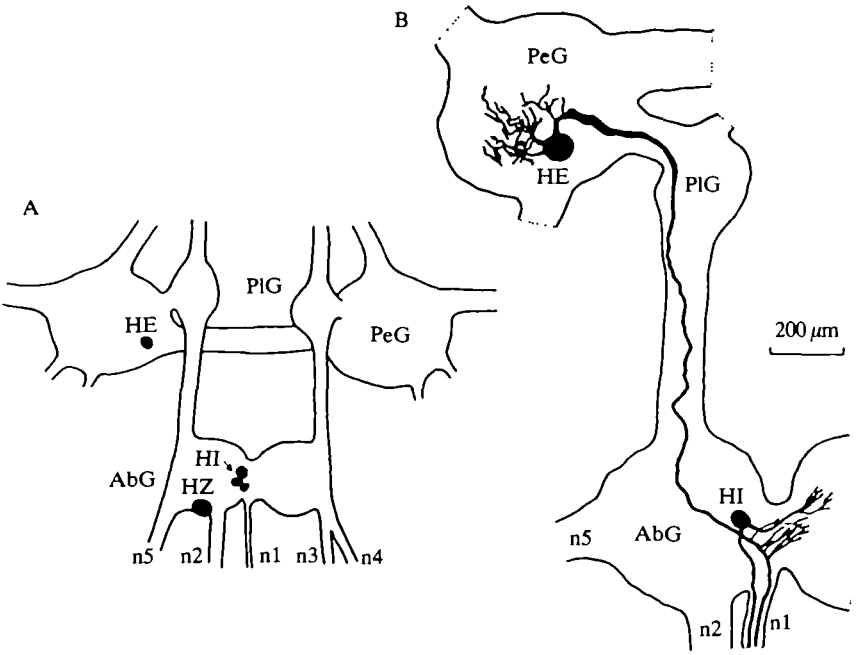

Fig. 4. (A) Location of neurones sending axons to the heart. (B) Morphology of the heart excitor (HE) and one of the heart inhibitors (HI) revealed by Lucifer Yellow. PlG, pleural ganglia; PeG, pedal ganglia; AbG, abdominal ganglia; n1, medial abdominal nerve; n2–n5, other nerves supplying visceral organs and muscles of the tail; HZ, large heart-innervating neurone.

Neurones controlling the heart

Innervation of the *Clione* heart is shown schematically in Fig. 2B. The medial abdominal nerve ramifies into three branches at the level of the posterior border of the wings. One of these branches (9 in Fig. 2B) goes to the heart and aorta. This branch approaches the heart from the ventral side of the aorta and then passes to its dorsal side. One part of this branch terminates at the ventricle–aorta border, while the other part circumvents the ventricle and enters the auricle. Electrical stimulation of the medial abdominal nerve evoked either excitation or inhibition of the heart, or mixed (excitatory–inhibitory) effects. Stimulation of the other abdominal nerves (n2–n5 in Fig. 4A) did not affect the heart rate.

Using the method of retrograde staining of neurones through the heart branch of the medial abdominal nerve, we localized the heart-innervating neurones. Differing numbers of neurones were stained in different experiments, probably because the dye had leaked to neighbouring nerve branches supplying other organs. In the present work we examined five neurones stained with Lucifer Yellow (neurones HE, HI and HZ in Fig. 4A) and found that their axons went into the medial nerve (Fig. 4B). The axons could be traced down to the ventricle–aorta border.

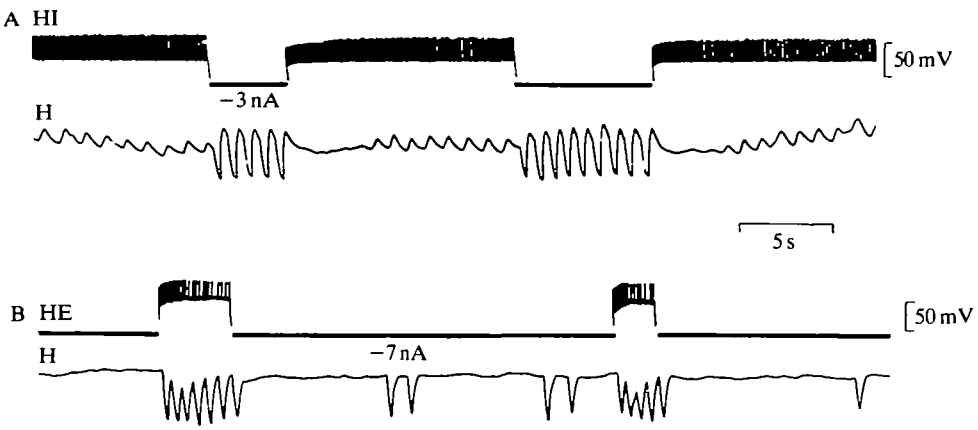

Fig. 5. Effects of the HI (A) and HE (B) on the heart rate. Upper traces, neurone activity; lower traces, heart contractions recorded with the photocell. In A, the neurone discharge was inhibited by pulses of hyperpolarizing current. In B, the constant-current hyperpolarization of HE was interrupted twice, both interruptions invoking neurone excitation. A and B are from different experiments. In this and subsequent figures, the period of current injection is marked by a solid line, the polarity and strength of the current being indicated.

The three HIs were located in a compact group on the dorsal side of the left abdominal ganglion and had a soma diameter of 20–30 μm. These neurones were found to be the heart inhibitors. Fig. 5A shows an instance in which the spontaneous activity of HI was high, and only the auricle contracted rhythmically. The discharge of the neurone was abolished by means of hyperpolarizing current, which resulted in the appearance of ventricle contractions. Strong excitation of the neurone after switching off the current (postinhibitory rebound) resulted in the complete termination of heart beating. Inhibition of the neurone by means of current injection was repeated once more with the same result. Paired recordings of HIs (N=7) demonstrated that these cells are not interconnected (Fig. 6A).

The heart-exciting neurone (HE) was a cell 30–40 μm in diameter located on the dorsal side of the left pedal ganglion (Fig. 4). In the example shown in Fig. 5B, the discharge of HE was abolished by constant hyperpolarizing current (7 nA). When HE was silent, heart activity was either absent or low. With excitation of HE (when the current was transiently switched off) the heart began to contract, the frequency of contractions reaching 1.5 Hz (i.e. the maximal rate observed in *Clione* under natural conditions). Recordings from HE–HI pairs (N=6) demonstrated that HE inhibits the HIs (Fig. 6B), whereas the HIs do not affect HE (Fig. 6C).

Besides HE and the HIs, one more heart-innervating neurone was identified (HZ in Fig. 4A). This was a large cell (80–90 μm) located near the caudal edge of the left abdominal ganglion. Though the axon of HZ could be traced down to the ventricle–aorta border, stimulation of this cell never affected heart beating.

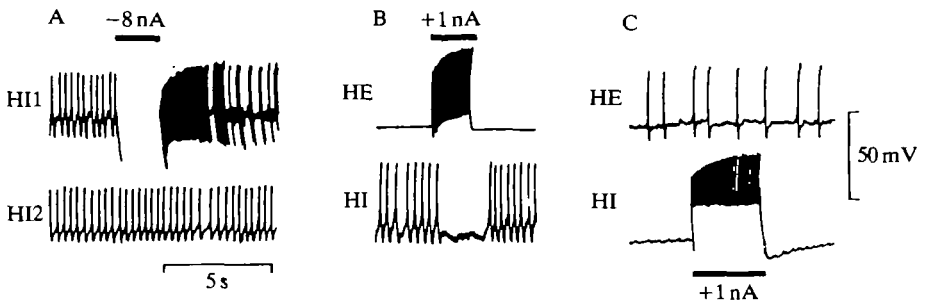

Fig. 6. (A) Absence of interaction between two HIs. (B) Inhibitory action of HE upon HI. (C) HI did not influence HE. All recordings are from different experiments carried out on the semi-intact preparation.

Neuronal mechanisms of heart–locomotion coordination

As demonstrated above, the heart rate and the frequency of wing oscillations changed in parallel both during undisturbed swimming and during defensive reactions. To understand the mechanism of this coordination, we recorded simultaneously the activity of HE and HI, as well as that of one of the big neurones of the pedal ganglia involved in the control of locomotor wing movements (i.e. the 1A motoneurone responsible for wing elevation and 2A motoneurone responsible for lowering: see Arshavsky *et al.* 1985*a*,*b*). Activity of the HE, HI and 1A neurones and heart contractions are shown in Fig. 7 (semi-intact preparation). From the record of activity of the 1A motoneurone it can be seen that in this experiment the locomotor generator worked irregularly, i.e. the bursts of activity alternated with 'silent' periods. Each 'locomotor burst' consisted of a series (up to 20) of locomotor cycles and lasted for a few seconds. In each locomotor burst the activity of the HE increased while that of the HI was inhibited. The heart contracted rhythmically during the bursts and was not active between the bursts. The same result was obtained when the locomotor burst was evoked by tactile

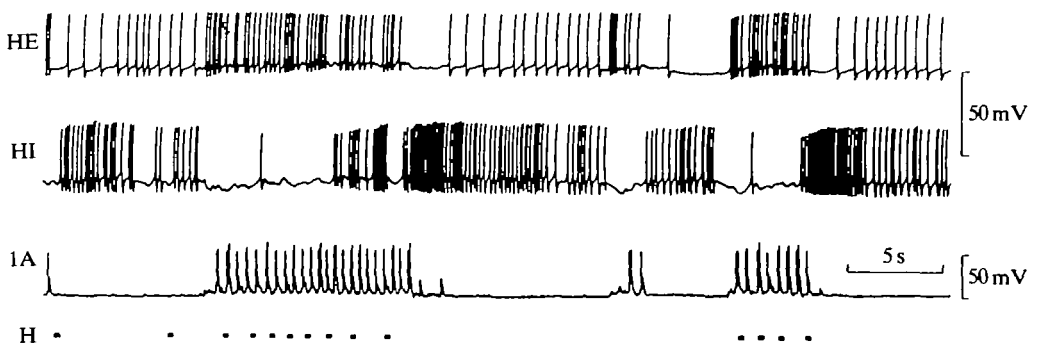

Fig. 7. Spontaneous changes in the locomotor generator activity are reflected in the activity of the heart-controlling neurones and in the heart rate (semi-intact preparation). Simultaneous recordings of the HE, HI and the wing motoneurone 1A, and the heart contractions (H).

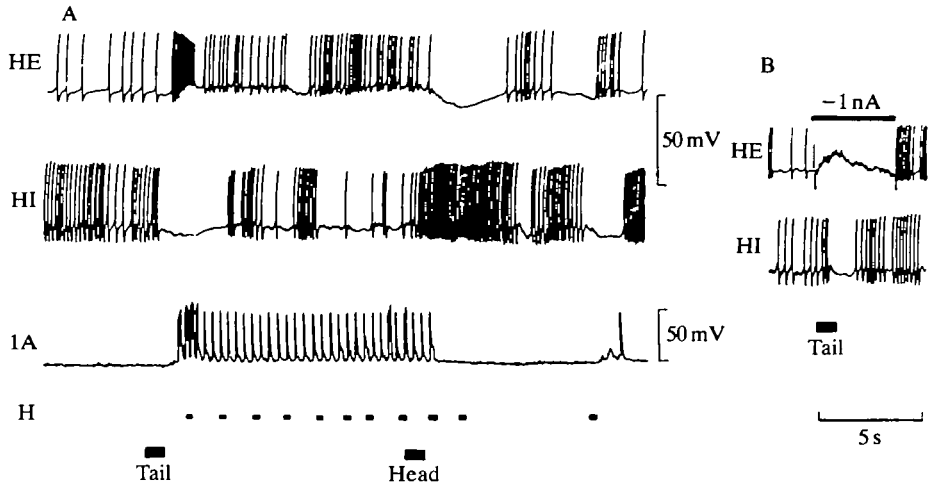

Fig. 8. (A) Peripherally evoked changes in the locomotor generator activity are reflected in the activity of the heart-controlling neurones and in the heart rate (semi-intact preparation). Simultaneous recording of HE, HI and the wing motoneurone 1A, and the heart contractions (H). Tail and Head, tactile stimulation of corresponding part of the body. (B) Tactile stimulation of the tail evoked inhibition of the HI in spite of inactivation of the HE by injection of the hyperpolarizing current.

stimulation of the tail (escape reaction) and terminated by tactile stimulation of the head (Fig. 8A; see also the effects of tail stimulation in Fig. 9A,B). Such a correlation between the activities of the HE, HI and locomotor neurones was also observed in the isolated nervous system during spontaneous locomotor bursts. The discharge frequency of the HEs during the locomotor bursts was usually 5–8 Hz (maximally 20 Hz), and between the bursts it was 0–1 Hz. The discharge frequency of HIs between bursts was usually 10–15 Hz, and it decreased during bursts to 0–3 Hz.

These data clearly demonstrate that activation of the locomotor generator in the pedal ganglia (of both central and reflex origin) is accompanied by activation of the heart excitor and by inhibition of the heart inhibitors. The latter effect is partly determined by the inhibitory action of the HE upon the HIs (see Fig. 6B). Besides inhibitory influences from the HE, the HIs also have another inhibitory input acting during the locomotor burst. Indeed, tail stimulation could produce inhibition of the HI even when the HE was inactivated by means of a hyperpolarizing current (Fig. 8B). In addition, inhibition of the HI which appeared before the HE was excited was sometimes seen (see effect of tail stimulation in Fig. 8A).

The linkage between the locomotor and heart-controlling systems described above could have two possible causes: (1) both systems could have common central and peripheral inputs (e.g. from the 'command neurones' and from the afferents responsible for defensive reactions); or (2) the locomotor generator itself could affect the HE and HIs. The influences of the locomotor generator upon the

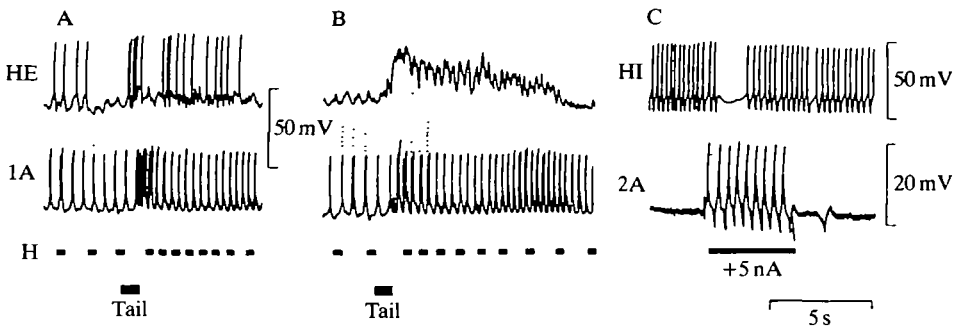

Fig. 9. Influences of the locomotor generator upon the heart-controlling neurones. (A,B) Simultaneous recordings of HE and the wing motoneurone 1A. Dotted lines connect periodic oscillations (presumed EPSPs) of the HE membrane potential with periodic discharges in the 1A motoneurone which reflect the locomotor rhythm. In B, HE was hyperpolarized by a current of 1.5 nA. (C) Inhibition of discharge in the HI during activation of the locomotor generator. The activation was produced by injection of a depolarizing current into the wing motoneurone 2A. (A,B) and (C) are from two different experiments; semi-intact preparation.

HE and HIs are shown in Fig. 9. In Fig. 9A, rhythmical oscillations of the membrane potential in the HE correspond to each locomotor cycle (monitored by rhythmical discharges of the 1A motoneurone). These oscillations became more pronounced when the HE was hyperpolarized and its discharges were suppressed (Fig. 9B). Thus, these oscillations seem to be locomotor-cycle-related excitatory postsynaptic potentials. Fig. 9C shows inhibition of the HI during the locomotor burst elicited by depolarization of the 2A locomotor motoneurone (this motoneurone is electrically connected with the type 8 interneurones responsible for rhythm generation; see Arshavsky *et al.* 1985*b*,*c*).

These results clearly demonstrate the existence of an excitatory input from the locomotor generator to HE and inhibitory input to the HIs. Whether there are common inputs to the locomotor and heart-controlling systems is yet to be resolved.

Discussion

Adaptive changes in the heart rate

In intact *Clione* we have found a number of adaptive changes in the heart rate related to changes in the animal's behaviour. Heart rate proved to be positively correlated with the level of locomotor activity both during spontaneous changes of this activity (locomotor bursts) and during defensive reactions. This correlation seems to be relevant for two reasons. (1) An increase in heart output is important for provision of oxygen and nutrients to active muscles. (2) In such a soft-bodied animal as *Clione*, the body and wing rigidity necessary for locomotion can only be maintained at a sufficiently high blood pressure. It seems likely that the high heart rate serves to increase the pressure (see Jones, 1983).

We found that the correlation between heart rate and locomotor activity disappears after transection of the medial abdominal nerve, and the frequency of heart beating becomes rather constant. This means that the adaptive changes of the heart rate during spontaneous 'locomotor bursts' and defensive reactions are determined by signals sent by the central nervous system, and that the efferent neurones with axons coming into the medial abdominal nerve are responsible for regulation of the heart rate.

Adaptive changes in heart beat during defensive responses have also been observed in other gastropods: *Aplysia californica* (Dieringer *et al.* 1978; Kandel, 1976), *Helix pomatia* (Safonova *et al.* 1984) and *Limax maximus* (MacKay and Gelperin, 1972). In these species, tactile stimulation of the head resulted in its withdrawal and in slowing of the heart rate. Stimulation of the tail elicited locomotion and accelerated the heart beat. However, in *Aplysia*, in contrast to *Clione*, the adaptive changes of the heart rate during the escape reaction persisted after denervation of the heart (Dieringer *et al.* 1978). These changes seem to be a secondary effect produced by increased peripheral resistance to blood flow.

In intact *Clione* we also found that contact with *Limacina* evokes a complicated behaviour pattern (food arousal), including a significant increase in the frequency of the heart beat. An increased blood pressure (which results from the increased activity of the heart) is necessary for the hydraulically controlled hunting apparatus (the buccal cones, Fig. 3C) of *Clione* to work. During hunting behaviour, the heart rate was shown not to be linked to the level of locomotor activity. This makes the functioning of the hunting apparatus independent of the locomotor activity.

Food arousal, including acceleration of the heart beat, has also been observed in *Aplysia* (Dieringer *et al.* 1978; Weiss *et al.* 1982). Activation of the circulatory system in *Aplysia* by food presentation is mediated mainly by the efferent neurones of the abdominal ganglia directly affecting the heart, since this activation considerably decreased after denervation of the heart. Acceleration of the heart beat after feeding has also been described for *Limax* (Grega and Prior, 1985), this effect being partly determined by the central nervous system. We did not study food arousal in *Clione* after cutting the medial abdominal nerve, and whether the acceleration of the heart rate in this case is determined by nervous influences upon the heart remains to be resolved.

Neurones controlling the heart

In the present study we have found four efferent neurones (one HE and three HIs) affecting the heart rate. It seems very likely that these cells, sending axons to the heart, are motoneurones, i.e. that they directly affect the heart muscle fibres. But one cannot exclude the existence of a peripheral nerve plexus mediating effects of HE and the HIs upon the heart. The influences of these efferent neurones are rather strong: by varying the activity of HE or HI within a physiological range we could strongly affect the heart beat (Fig. 5). We have also found a profound modulation of the discharge rate of the HE and HIs (determined

by central and afferent influences) in the semi-intact preparation, the changes of activity of HE and HI being accompanied by corresponding changes in the heart rate (Figs 7 and 8A). It seems very likely that the HE and HIs are indeed responsible for the control of the heart rate. But we cannot exclude the possibility that there might be other efferent neurones influencing the heart.

In other gastropods, nervous influences upon heart beat are also mediated by a small number of efferent neurones. In *Aplysia*, another opisthobranch mollusc, two heart excitors and two heart inhibitors have been found, all of them being located in abdominal ganglia (Kandel, 1976; Koester *et al.* 1979; Koester and Koch, 1987; Mayeri *et al.* 1974). In *Helix pomatia* (subclass Pulmonata), there are four heart excitors and two heart inhibitors (S.-Rozsa, 1979, 1983, 1987; Zhuravlev and Safonova, 1984). All of them are located in the visceral and parietal ganglia, which are homologues of the abdominal ganglia of *Clione* and *Aplysia*. In another pulmonate, *Achatina fulica*, heart inhibitors have not been found, but there are four heart excitors located in the right parietal ganglion (Furukawa and Kobayashi, 1987*a*,*b*). These cells, in contrast to heart-controlling cells in other gastropods, are electrically interconnected. In addition to the parietal neurones, two cells, stimulation of which accelerates the heart rhythm, have been found in the cerebral ganglia. Their effect is mediated mainly by the parietal heart excitors, but the cerebral neurones themselves also send axons to the heart. Thus, the number, location and functional characteristics of the efferent neurones controlling the heart vary over a wide range in different gastropod species.

Besides the heart excitor and heart inhibitors, one more neurone (HZ) was found in the left abdominal ganglion of *Clione*. Its axon projects to the heart, but stimulation of this neurone did not affect heart rate. Neurones sending processes to the heart but not affecting its beating have also been found in the right abdominal ganglion of *Aplysia* (cells R_7, R_8 and R_{15}; see Koester and Koch, 1987).

Neuronal mechanisms of heart–locomotion coordination

Study of the heart-controlling neurones (HE and HIs) in the semi-intact preparation has demonstrated that their activity is closely linked to that of the locomotor generator: when the generator is activated, HE receives an excitatory input and the HIs receive an inhibitory one (Figs 7 and 8A). It is due to these inputs that heart rate proves to be positively correlated with locomotor activity. What is the origin of the locomotion-related inputs to the heart-controlling system? In the HE we detected periodic EPSPs related to the locomotor rhythm (Fig. 9A,B). The inhibitory effect on the HIs, in contrast, could be obtained by 'direct' activation of the locomotor generator, without involvement of command neurones or afferents (Fig. 9C). The inhibitory influences upon the HIs are partly mediated by the HE (Fig. 6B). These data clearly show that the heart–locomotion synergy is due, at least partly, to the action of neurones of the locomotor generator upon the heart-controlling neurones. But we do not exclude the possibility that the locomotor and circulatory systems might also have common inputs from command

neurones and afferents, these inputs promoting organization of the heart–locomotion synergy.

Coordination between locomotor activity and heart rate is not present in all behavioural acts. During hunting behaviour, locomotor activity can vary independently of the heart rate (Fig. 3B,E). The neuronal basis of such an independence of the two systems will be the subject of future studies.

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