Control of the cardiovascular system of *Aplysia* by identified neurons

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Abstract. The neural network that controls the cardiovascular system of *Aplysia* adapts cardiovascular function to a variety of different physiological and behavioral situations. It (1) coordinates the cardiovascular system with the renal and respiratory systems; (2) modifies both systemic and regional blood flow during food-elicited arousal and feeding; and (3) changes the tension of longitudinal vascular muscle to adapt the arterial tree to changes in body shape. Indirect evidence suggests that the cardiovascular control circuit may also play a role in maintaining homeostasis during egg laying. Several putative neurotransmitters, including acetylcholine, serotonin, R15α1 and R15α2 peptides, have been localized to identified neurons in this circuit.

Key words. Aplysia; gastropod; acetylcholine; serotonin; R15α peptides; heart; vascular system; respiratory system; feeding; arousal; kidney; co-localization.

Molluscan hearts have traditionally proven valuable as bioassays for the isolation and identification of neurotransmitters[38,55,81]. With the introduction of the marine opisthobranch gastropod *Aplysia californica* as a preparation for neurobiological studies, it has become possible to examine in cellular detail how various transmitters are used to control cardiovascular function. The large size of *Aplysia* neurons has allowed a number of investigators to work out in detail the neural circuits that modulate the cardiovascular system, and to use neurochemical, pharmacological, and molecular biological techniques to study individual cardiovascular control neurons. The primary focus of this review will be on studies of how the cardiovascular control circuit of the *Aplysia* nervous system is used to maintain homeostasis in response to various changes in physiological or behavioral states. We will emphasize the roles played by various neurotransmitters, including acetylcholine, serotonin, R15α1 peptide, or R15α2 peptide. The anatomy of the *Aplysia* circulatory system is described in the accompanying paper by Brownell[12] and in an earlier review[44]. The latter paper also reviews in depth the details of the pharmacology and innervation of the cardiovascular system.

Several types of motoneurons innervate the cardiovascular system

Ten types of motoneurons that innervate the cardiovascular system have been identified in *Aplysia*[1,58,66,67,69,71,75,76]. In addition, neurons L2 and R7 and R8 terminate in the heart and may also modulate cardiac function[69,75]. All of these neuronal types are found in the abdominal ganglion, with the exception of a pair of vasomotoneurons in the pedal ganglia. The locations of the known cardiovascular output neurons, as well as some of the interneurons that control their activity, are illustrated in figure 1.

Heart beat is inhibited during respiratory pumping

An important function of the neural input to the heart is to coordinate the cardiovascular and respiratory systems during respiratory pumping. Respiratory pumping consists of a brief, synchronous contraction of the gill, siphon and parapodia. This stereotyped set of actions has been postulated to enhance the circulation of blood through the gill and the flow of seawater through the mantle cavity[17]. The frequency of occurrence of pumping increases in response to hypoxia[52], hypercarbia[21], feeding[27,45], tactile stimulation of the mantle organs[63], and release of defensive secretions into the mantle cavity[83]. Therefore, respiratory pumping may have either respiratory or defensive functions, depending on the context in which it occurs.

During each respiratory pumping episode, contraction of the gill ejects a large bolus of blood from the gill. The passage of this blood through the heart, into the arterial system, is facilitated by the transient inhibition of the heart by a pair of cholinergic heart inhibitor (HI) mo-

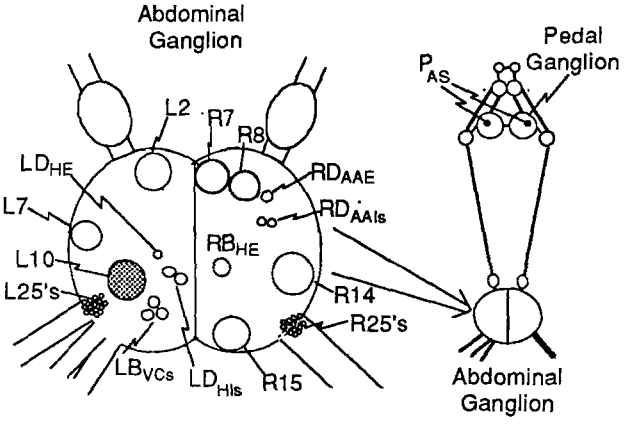


Figure 1. Identified motoneurons and interneurons that terminate in the cardiovascular system of *Aplysia californica* are located primarily in the abdominal ganglion[1,16,41,46,58,67–69,71,75,76]. The ganglia of the central nervous system are shown, dorsal side up. Most of the identified cardiovascular control neurons are in the abdominal ganglion, which innervates primarily the heart and proximal aortae, as well as other mantle organs. The P_{AS} neurons, found in the more rostral pedal ganglia, innervate the more distal regions of the arterial tree[76]. All neurons shown here are found on the dorsal surface of the ganglia, except L10, which is on the ventral surface[46].

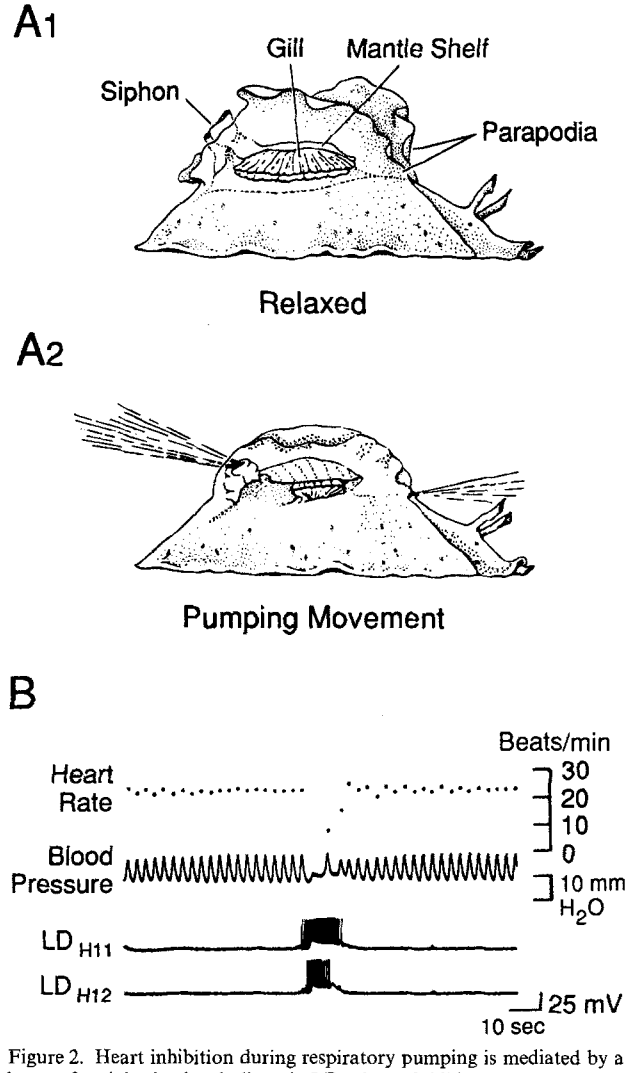


Figure 2. Heart inhibition during respiratory pumping is mediated by a burst of activity in the cholinergic LD_{HI} heart inhibitory motoneurons. *A* Side view of an *Aplysia* in which the parapodia are rendered transparent to reveal the gill and siphon in the *A1* relaxed state and *A2* the maximally contracted state during an episode of respiratory pumping[35]. *B* At the peak of the gill and siphon contractions, the heart is inhibited by the two LD_{HI} neurons[46]. The bursts in the LD_{HI} cells are driven by the R25/L25 network[16,41].

toneurones in the LD cluster[46] (fig. 2). These LD_{HI} cells are driven into a brief burst by activity in the R25/L25 network, which triggers the pumping behavior[16,41]. The R25/L25 neurons also modulate the activity of several other motoneurons that innervate the vascular system, parapodia, gill, siphon, pericardium and kidney[34,42,43,50,62,71,76]. The R25/L25 network thus both activates and coordinates a variety of systems that are involved in respiratory pumping behavior.

Regional blood flow is modulated in phase with the biting cycle during feeding

One function of the vascular control system is to optimize perfusion of the muscles involved in feeding and digestion. This function may be quite important, because *Aplysia*, which is a herbivore, spends up to several hours a day eating[49]. When presented with an abundant supply of seaweed, a hungry *Aplysia* eats in a steady, rhythmic fashion. Based on the movement of the radula, each cycle of the biting rhythm can be divided into two phases, protraction and retraction[48]. Each of these two half cycles lasts 3–6 s. During the retraction phase, vascular resistance increases in the head and most of the cardiac output is diverted to the gut. As a result, the muscular buccal mass, which moves the radula, is starved of the blood flow required to support feeding activity[39]. This momentary circulatory imbalance is compensated for during the protraction phase of each biting cycle by excitation of cholinergic LB vasoconstrictor (VC) motoneurons. When the three LB_{VC} neurons are activated during the protraction phase of the biting cycle, they constrict the base of the abdominal aorta, shifting blood flow to the feeding musculature in the head region[40]. When protraction gives way to retraction, the synaptic inputs to the LB_{VC} cells change from excitation to inhibition. This alternating excitation and inhibition of the vasoconstrictor motoneuron pool contributes to the rapid switching of cardiac output between the two vascular beds during the two phases of the biting cycle (fig. 3).

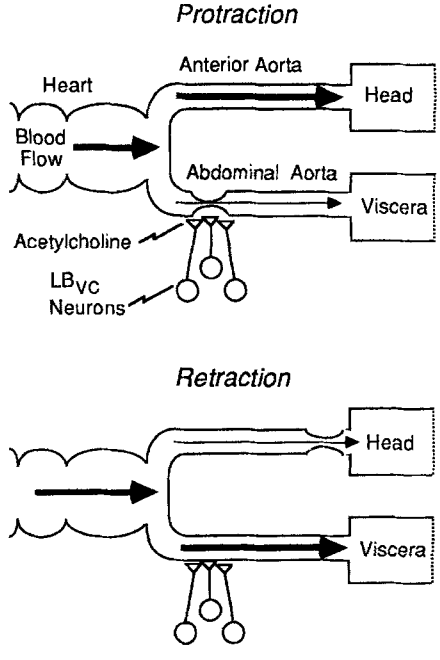


Figure 3. The three cholinergic LB_{VC} vasoconstrictor neurons provide rapid regional adjustments of blood flow that compensate for changes in vascular resistance that accompany biting. During the retraction phase of biting (bottom), the vascular resistance of the anterior aorta increases, directing blood flow to the viscera at the expense of the biting musculature in the head. This transient reduction in flow to the head is compensated for during the protraction phase of biting (top) by a burst of activity in the LB_{VC} neurons. Activity in the LB_{VC} cells causes constriction of the base of the abdominal aorta, redirecting blood flow from the viscera to the head. These alternating shifts of blood flow, which occur every few seconds, cause the cardiac output to be 'time-shared' between the two vascular beds[39,40]. (The third major output of the heart, the gastroesophageal artery, is also innervated by LB_{VC} neuron and is presumed to behave similarly to the abdominal aorta.)

Adjustments of the arterial system to postural changes

One function of the cardiovascular control system in *Aplysia* is to adapt the shape of the arterial tree to match changes in posture. *Aplysia* lacks a rigid skeleton, so its body wall can assume a wide range of shapes. The neck in particular is quite plastic. It undergoes a significant decrease in length when the animal changes from an aroused state, in which it actively searches for food or for a mate, to a vegetative, relaxed state (fig. 4), or to a defensive posture[83]. Moreover, in the aroused state the extended neck can move from side to side in arcs of as much as 60–70° from the centered position[80].

The arterial tree is adapted to changes in posture in part by the activity of two bilaterally symmetrical pedal arterial shortener P_{AS} motoneurons. These two cholinergic motoneurons, which are located in the pedal ganglia (fig. 1), innervate the longitudinal muscle of the rostral arterial system. Both neurons innervate the rostral anterior aorta and the ipsilateral left or right pedal-parapodial artery[76]. (See paper by Brownell[12], in this issue, for diagram of arterial system). Both neurons fire whenever the neck shortens (fig. 5), but when the animal turns to one side, the ipsilateral motoneuron is activated while the contralateral neuron is inhibited. It is presumed that these activity patterns prevent any slack that might occur in the arteries as the animal withdraws its head or turns. Such a motor effect may reduce the possibility of arterial kinking, which could occlude blood flow or make it more turbulent[76].

Cardiovascular changes generated by neuron R15 may contribute to egg laying behavior

The endogenously bursting neuron R15 has two types of synaptic action that affect the cardiovascular system:

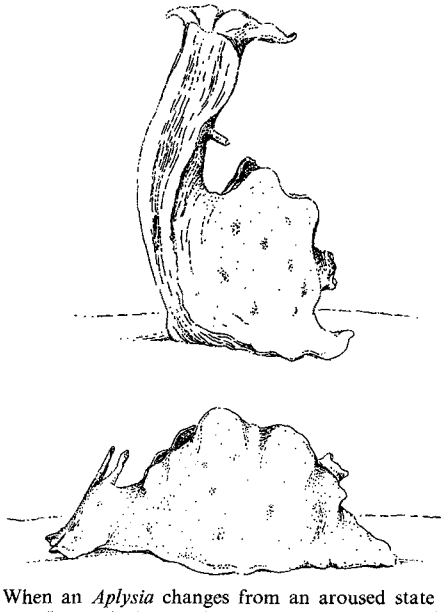


Figure 4. When an *Aplysia* changes from an aroused state (top) to a quiescent state (bottom), the anterior aorta undergoes a significant shortening. The longitudinal muscle of the anterior aorta contracts when the head is withdrawn, to compensate for the shortening of the neck[76].

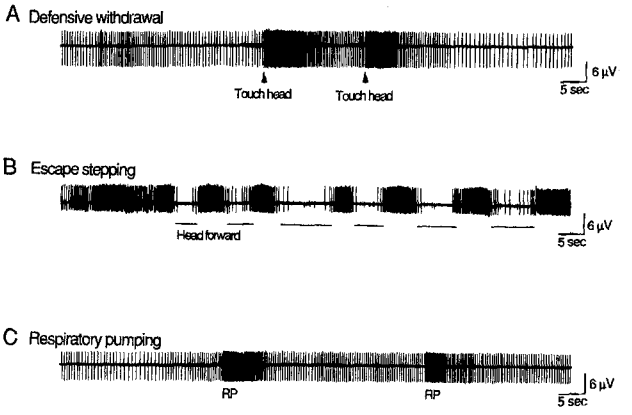


Figure 5. The cholinergic P_{AS} arterial shortener motoneurons fire when the neck of the animal shortens. This presumably takes up the slack in the anterior aorta, reducing the chance that it will kink or twist. This recording was made in an intact animal from a fine nerve branch that innervates the anterior aorta. The only axon in this branch that is large enough to be recorded is that of the right P_{AS} neuron. *A* Head withdrawal can be evoked by tactile stimulation of the head. *B* During escape stepping, the head attaches to the substrate and pulls the posterior part of the animal forward, causing the neck to shorten. *C* The head withdraws slightly during respiratory pumping[76].

1) Activity in R15 causes constriction of the base of the left pedal-parapodial artery. This constriction shunts blood toward the right pedal-parapodial artery, which perfuses the right side of the anterior body wall[12], including the genital groove[76] (fig. 6B). 2) Activity in R15 also increases the frequency of occurrence of respiratory pumping episodes by exciting the R25/L25 network. The activation of the R25/L25 network by R15 decreases profoundly over the course of several minutes (fig. 6C). Some, and perhaps all, of this decrement results from desensitization of postsynaptic responses of the R25/L25 neurons to the transmitter of R15[4].

The modulatory inputs and outputs of R15 have led to the hypothesis that it plays a role in maintaining homeostasis during egg laying. R15 is excited by the neuroendocrine bag cells when they are triggered into a population burst in vitro[10] (fig. 6A). Since such a bag cell burst initiates egg laying when it occurs in vivo[24, 64], it has been hypothesized that R15 is excited during egg laying[10]. If so, it could generate an increase in respiratory pumping[4] and increase blood flow to the genital groove[76]. The increased pumping may enhance respiration, and the rerouting of blood may support the activity of the cilia in the genital groove, which propel the egg cordon forward during oviposition. It remains to be determined whether R15 activity actually increases during egg laying and whether its actions on respiratory pumping and blood flow are indeed expressed during egg laying in vivo.

An interesting feature of R15 is that it appears to use as a transmitter R15α1 peptide, which is one of two peptides generated by the alternative splicing of an mRNA precursor. The precursor of the mRNA that encodes the R15α1 peptide is spliced differently in other neurons in *Aplysia* to form R15α2 peptide. The 38-amino acid

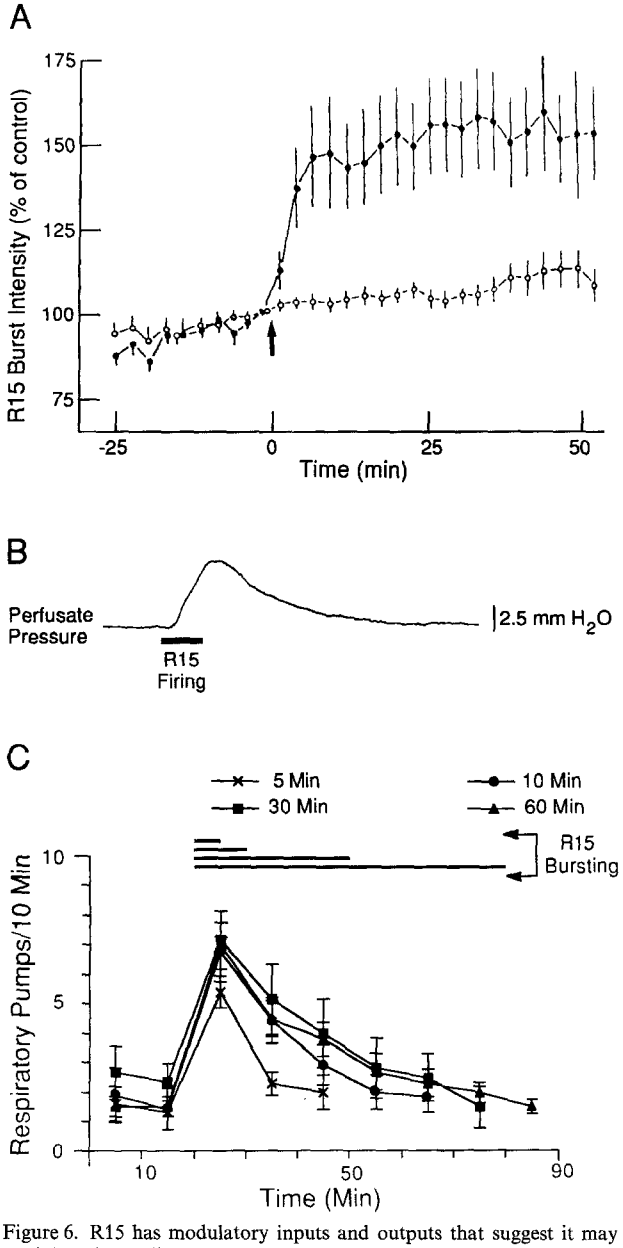


Figure 6. R15 has modulatory inputs and outputs that suggest it may modulate the cardiovascular system during egg laying behavior. *A* When the neuroendocrine bag cells fire in a burst in vivo they initiate egg laying behavior[24]. When they are triggered to fire in a burst in vitro, as shown here, they also excite R15. In the experimental group (•) a population burst was triggered in the bag cells at time zero (arrow); no burst was triggered in the control group (o)[10]. *B* When R15 fires in vitro, it causes constriction of the base of the left pedal-parapodial artery. The result is to increase the pressure (and flow) in the right pedal-parapodial artery, which perfuses the right body wall including the genital groove. R15 was released from hyperpolarization during the time marked by the bar, resulting in 9 spontaneously generated bursts of action potentials[76]. *C* When R15 fires in vitro, it increases the frequency of occurrence of spontaneous episodes of respiratory pumping. This effect increases when the duration of R15 activity is allowed to increase from 5 to 10 min. However, burst periods of 30 or 60 min are no more effective than one of 10 min. This profound synaptic decrement results in part from desensitization of the postsynaptic response to R15's transmitter in the R25/L25 neurons, which trigger respiratory pumping[4].

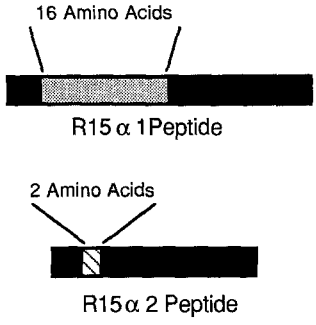


Figure 7. The R15α1 and R15α2 neuropeptides are encoded by the same mRNA precursor, which is alternatively spliced in different neurons. In R15, the α1 version of the peptide is synthesized[13, 84]. In other neurons, such as RB_{HE}, the precursor is spliced differently, resulting in a 2-for-16 amino acid substitution that shortens the overall length of the peptide from 38 amino acids to the 24 amino acid, α2 form[13, 77].

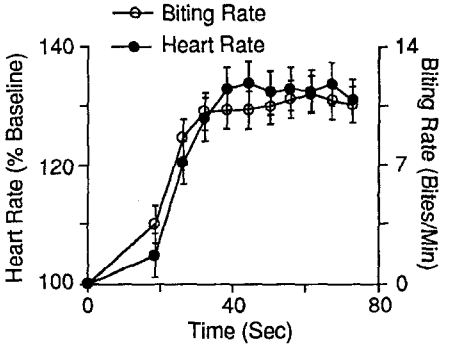
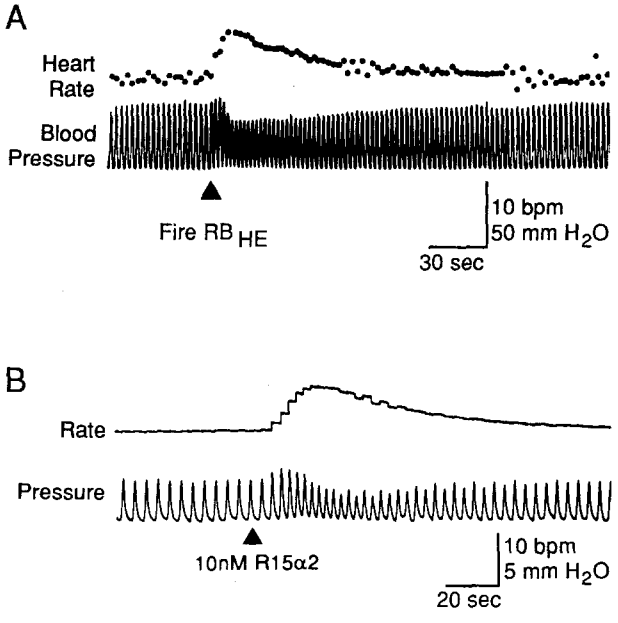


Figure 8. Heart rate and biting rate increase in parallel during the initial stages of food-elicited arousal in intact *Aplysia*. A small piece of seaweed was placed on the animal's lip, starting at time zero, and kept there for the time required to elicit 10 bites[22]. In vitro experiments showed that at least part of this heart rate increase is caused by an increase in firing of RB_{HE}[40].

R15α1 peptide differs from the R15α2 peptide by the substitution of a 16-amino acid segment for a 2-amino acid segment (fig. 7). This structural difference appears to be functionally important, as the two peptides differ in some respects from one another in their pharmacological actions and their distributions within the nervous system[2].

Heart rate is increased during food-elicited arousal and feeding

The RB heart exciter (HE) neuron, RB_{HE}, has been implicated in physiological adjustments that take place when the animal is aroused by food and when it eats. When the animal bites, in addition to the vascular switching that is described above, there is also a tonic increase in blood flow to the head[39]. This increase in perfusion is mediated in part by the increase in heart rate that occurs in response to food stimuli (fig. 8). Inputs from the head ganglia excite RB_{HE} when the animal is food-aroused, contributing to this increase in heart rate[40]. RB_{HE} synthesizes both serotonin and the R15α2 peptide[53, 77]. Both putative transmitters appear to have similar effects



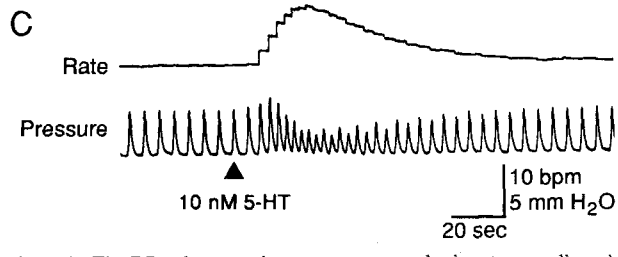


Figure 9. The RB_{HE} heart excitatory neuron synthesizes two cardioexcitatory substances, both of which mimick its synaptic actions. *A* Firing 18 spikes in RB_{HE} in a period of 2.6 s causes a long-lasting increase in heart rate, accompanied by a slight decrease in beat strength. Myocardial contractions are increased in intensity as the muscle is lengthened [78], so the negative inotropic effect of RB_{HE} activity may result from the decrease in filling time. *B, C* Injecting a 50-µl bolus of 10 nM R15α2 peptide or serotonin into the perfusion line generates a change in heart beat that mimicks the effect produced by firing RB_{HE}. The data in *A* were obtained from a preparation different from that used in *B* and *C* [53, 58, 77].

on the heart – primarily an increase in rate associated with a slight decrease in strength of beat (fig. 9).

Neurons L10 and RB_{HE} integrate cardio-renal function

The synaptic connections made by and received by RB_{HE} suggest it also plays a role in controlling renal function. In *Aplysia*, the ultrafiltrate of blood that forms the prourine is generated by filtration across the wall of the heart and/or the crista aortae [7, 26]. Neuron L10, which excites RB_{HE} (fig. 10), plays an important role in controlling renal function in *Aplysia*. Biochemical and pharmacological evidence indicate that L10 is cholinergic [9, 31, 37], but it also synthesizes the neuropeptide myomodulin [3, 61]. When L10 bursts spontaneously, it causes localized contractions within the kidney, opens the renal pore (allowing urine to leave the kidney) [42], and increases heart rate by exciting RB_{HE} [46] (fig. 11). The effect on heart rate is

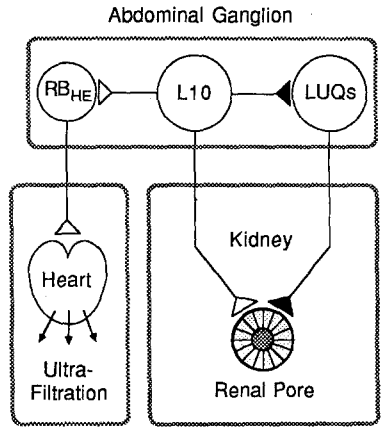


Figure 10. Cardio-renal function is integrated by neurons located in the abdominal ganglion. Neuron L10 opens the renal pore by its direct excitatory actions in the periphery, and by inhibiting the LUQ neurons that cause the pore to close. L10 also excites the RB_{HE} heart exciter neuron, causing an increase in heart rate that is postulated to enhance the rate of renal filtration. Open triangles represent excitation, closed triangles inhibition. Part of L10's excitatory action on renal pore opener muscle is mediated by an identified peripheral motoneuron (not shown) that L10 excites directly [42].

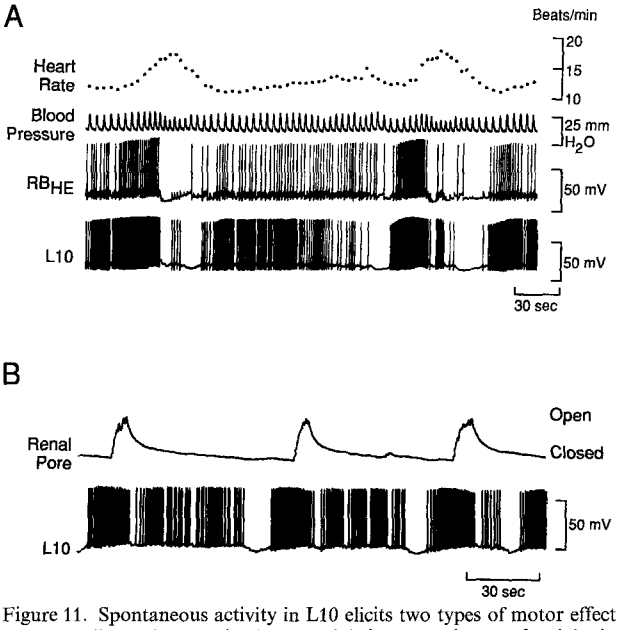


Figure 11. Spontaneous activity in L10 elicits two types of motor effect on the cardiorenal system in vitro. *A* High frequency bursts of activity in L10 that are sufficiently intense to strongly excite RB_{HE} cause slow, long-lasting increases in heart rate [46]. These increases in heart rate may increase renal filtration. *B* High frequency spontaneous bursts in L10 also causes transient openings of the renal pore [42].

presumed to enhance renal filtration. This constellation of synaptic actions has led to the hypothesis that activity in L10 enhances renal excretion [42].

The sensory input that controls L10 activity has not been studied in detail. However, it is known that L10 is excited when perfusion of the arterial supply to the abdominal ganglion is interrupted in vitro, suggesting that L10 may be sensitive to blood pressure or to oxygen or CO_2 levels [29].

Other neurons, transmitters and neurohumoral agents contribute to cardiovascular control

The examples of identified neurons and their transmitters given above have focussed on neurons with functional roles in cardiovascular control that are at least partly understood. However, they make up but a small fraction of the neurons that innervate the cardiovascular system. There are several additional known inputs whose features are much less clearly defined: 1) L7 is a multimodal motoneuron that directly excites the heart and the abdominal aorta[1]. 2) LD_{HE} causes a transient increase in heart rate[58]. 3) L2, a peptidergic endogenously bursting neuron[28,86], has axon terminals in the heart[75]. 4) Two neurosecretory neurons, R7 and R8, also terminate in the heart[69]. They express a gene that encodes a cardioexcitatory neuropeptide[18], and may utilize glycine as a transmitter as well[65]. 5) R15 has axon terminals in the heart[67]. 6) Immunocytochemical evidence indicates that small cardioactive peptide[55] (SCP)-like immunoreactive axon branches from an unidentified neuron terminate in the heart (unpublished observations). 7) Antisera to the neuropeptide FMRFamide binds to the somata of peripheral neurons in the heart, in the paired valves between the auricle and ventricle[33]. FMRFamide-like immunoreactivity was also found in nerve terminals in the abdominal aorta[1], the tentacular arteries, and a restricted portion of the distal anterior aorta[76]. 8) Longitudinal muscle of the proximal anterior aorta receives a variety of synaptic inputs, including excitatory junction potentials from RD_{AAE} and inhibitory junction potentials from the RD_{AAI} neurons[71] (fig. 1). 9) R14, a peptidergic neuron that is thought to also use glycine as a transmitter, excites longitudinal muscle at the base of the anterior aorta[66,69,73]. 10) Immunocytochemical and histochemical evidence suggest there are serotonin-containing terminals from unknown sources at several sites in the cardiovascular system besides the heart. These terminals are found in the crista aortae (unpublished), the abdominal aorta and gastroesophageal artery[1], the rostral anterior aorta, tentacular arteries, and circum-oral arteries[76]. 11) Dopamine-like histofluorescent nerve terminals are found at the base of the proximal anterior aorta, throughout the genital artery[1], and in the tentacular and circum-oral arteries and a restricted region of the distal anterior aorta[76]. 12) Antiserum for the neuropeptide buccalin binds to nerve terminals in the genital artery[60]. 13) Antiserum for the neuropeptide myomodulin binds to nerve terminals in the genital and gastroesophageal arteries and the abdominal aorta[61]. 14) R15α peptide-like immunoreactive nerve terminals were found in the abdominal and proximal anterior aortae and the genital and gastroesophageal arteries[2], the mantle artery, and the artery that perfuses the abdominal ganglion[76].

Immunohistochemical and immunocytochemical results must be interpreted with caution, because of the possibility of nonspecific reactions. Moreover, some of the nerve terminals found in the cardiovascular system may release substances that have systemic, rather than local, effects. Nevertheless, the highly selective distribution of staining for specific substances, combined with the electrophysiological data, suggests that there is a great deal of regional differentiation of cardiovascular innervation patterns in *Aplysia*.

Conclusions

Valve-like control of arterial blood flow

Although vascular muscle is found throughout the arterial system of *Aplysia*, it is not uniformly distributed. For example, there is a concentrated band of muscle at the base of the abdominal aorta that acts like a sphincter. When the LB_{VC} cells activate this muscle during the protraction phase of biting, they completely shut off blood flow to the entire vascular bed perfused by this artery[40,58]. Likewise, when R15 is active, it constricts a localized region, only a few mm in length, at the base of the left pedal-parapodial artery[76]. In this regard, the circulatory system of *Aplysia* resembles that of crustacea, which possess neurally controlled valves at the proximal ends of their arteries. Neural activation of these valves, similar to the local activation of vascular sphincters at the proximal ends of arteries in *Aplysia*, selectively directs blood flow to different regions of the vascular system[59]. Although the valves in crustacea and the vasoconstrictor cuffs in *Aplysia* are quite different in structure, they are similar in being located at the root of an arterial tree. It will be interesting to determine how general this localization of vasoconstrictor muscle to the proximal ends of arteries is in *Aplysia* and in other gastropods.

Functional significance of variations in duration of transmitter action

The neurons that innervate the cardiovascular system in *Aplysia* are adapted to their functional roles in part by the transmitter-receptor systems that they use. Acetylcholine, which has a short-lasting postsynaptic action at most cholinergic synapses in *Aplysia*, is used to mediate the transient heart inhibition during respiratory pumping, the brief constrictions of the abdominal aorta during the protraction phase of biting, and the rapid changes in arterial length associated with postural changes. These actions are generated by the LD_{HI}, LB_{VC}, and P_{AS} neurons, respectively. The rapidity of action of these three cell types suggests that the acetylcholine they release directly activates postsynaptic channels.

RB_{HE}, which is activated tonically during feeding and which mediates slow, long-lasting effects, contains two slow-acting transmitters, serotonin and R15α2 peptide. Both have actions on heart beat that take up to a few minutes to decay. The slow kinetics of serotonin action are consistent with pharmacological and biochemical evidence suggesting that its effects on heart rate medi-

ated by activation of cyclic adenosine-monophosphate[23, 56, 57, 72]. It remains to be determined how much and under what conditions serotonin and the R15α2 peptide contribute to the synaptic actions of RB_{HE}. The effects of the instantaneous firing pattern of RB_{HE} is smoothed out appreciably by the slow kinetics of its postsynaptic actions[46]. Therefore, RB_{HE} is not utilized to generate rapidly varying changes in heart rate such as might accompany abrupt movements generated by somatic muscle. Rather, it generates the tonic changes in heart rate that adapt circulatory function to relatively slowly changing conditions.

R15 has even slower postsynaptic effects than does RB_{HE}. The excitation of the R25/L25 network by a brief period of R15 activity decays over the course of tens of minutes[4]. The most striking feature of R15's synaptic action is its refractoriness to repeated activation, which seems to result primarily from postsynaptic desensitization[4, 76]. It seems likely that when R15 fires in vivo, desensitization of its postsynaptic actions will have an important effect on the duration of its physiological effects.

Taken together, these data illustrate the broad range over which the kinetics of transmitter action determine the functioning of different parts of the cardiovascular control network. Along with repetitive firing properties and patterns of synaptic connections, the kinetics of synaptic action are important determinants of the degrees of freedom available to this neural network.

Co-localization of transmitters

It has recently become clear that, as in other nervous systems, co-localization of conventional transmitters and neuropeptides is a ubiquitous phenomenon in the *Aplysia* nervous system[20, 31, 74, 79], as it is in most animals that have been examined in detail. The recent results from the circulatory control system of *Aplysia* reinforce that conclusion. RB_{HE} synthesizes serotonin and the R15α2 peptide, both of which mimic its motor effects on the heart. Analysis of mRNAs expressed in the abdominal ganglion suggests that RB_{HE} also expresses the R15β and R15γ peptides[13], although they have not yet been shown to be bioactive.

L10 synthesizes both acetylcholine and myomodulin[3, 31], but only acetylcholine has been shown to mimic synaptic actions of L10[9, 37]. If myomodulin also acts as a transmitter or modulator at L10's output synapses in the abdominal ganglion, its actions must be rather subtle. Perhaps it is released from L10's terminals in the periphery[42], where it might have effects on the muscle or epithelial cells of the kidney sack.

The functions of two of the three neuropeptides synthesized by R15 also remain unknown. Most of R15's known synaptic actions can be mimicked by the R15α1 peptide, which it contains[4, 76]. However, R15 has been shown to also synthesize the R15β and R15γ peptides[84]. They are not known to mimic or to modulate any of its synaptic actions. Little work has been done in his regard, however, and the R15β and R15γ peptides remain candidates for mediating the non-decrementing synaptic actions produced by R15[11]. A complete analysis of the functions of the identified cells in the cardiovascular control circuit will require determination of the stoichiometry of release of different co-localized transmitters.

Multiaction neurons

Many neurons that control motor output in *Aplysia* can be classified as pure motoneurons or interneurons. In contrast, many of the output neurons in the cardiovascular control circuit appear to be hybrid motoneurons/interneurons. For example, RB_{HE}, the LD_{HI} neurons, and the LP_{AS} neurons are not known to make any connections within the nervous system, and are thought to function as pure motoneurons. However, three of the cardiovascular control neurons that synapse on muscle in the periphery also make synaptic connections in the central or peripheral nervous system. For example, L7, which makes direct synaptic connections to a variety of muscle fiber types[1, 19, 82], also acts as a pre-ganglionic neuron, synapsing onto gill motoneurons in the branchial ganglion[51]. L10 synapses onto motoneurons within the CNS[36], as well as onto muscle fibers and an identified peripheral motoneuron in the kidney[42]. R15 also makes both central and peripheral synapses[4–6, 11].

Variety of cardiovascular control mechanisms in gastropods

The variety of identified and unidentified neurons innervating the heart and arteries of *Aplysia* indicate that control of its cardiovascular system is a highly complex process. The heart receives several types of excitatory input, as well as inhibitory input. Evidence described above suggests that these inputs are activated in various combinations to support different behavioral or physiological functions. They are supplemented by excitatory neurohumoral factors that can also modulate heart beat[22, 47, 78]. Several types of cardiac control neurons, many with similar properties to those found in *Aplysia*, have been identified in other gastropods[8, 14, 15, 30, 70, 85, 87]. These species have been studied less extensively than has *Aplysia*, but the available evidence suggests that their cardiac control systems will prove to be as important as that of *Aplysia* for adapting the animal to changing physiological conditions[8, 25, 32]. There is a high degree of regional differentiation in the neural inputs to the vascular system in *Aplysia*. This local variation in type of neural input allows the animal to exert precise control over the regional distribution of blood flow in response to both tonic and phasic challenges to homeostasis. Neurohormonal factors also modulate vasomotor tone in *Aplysia*[54]. These results suggest that studies of the neural control of regional blood flow in other gastropods will be essential for gaining a full understanding of their cardiovascular function.

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