Mechanisms of circulatory homeostasis and response in *Aplysia*

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Abstract. This review concerns the organization and function of arterial vasculature in *Aplysia californica*, especially the vasomotor reflexes that support circulatory homeostasis, and fixed patterns of response that may reroute blood flow during changes in behavioral state. The observations presented here raise three hypotheses for further study: 1) Arterial vasculature is functionally organized with precisely structured, independently regulated subdivisions; these are most evident for arterial systems serving digestive and reproductive processes; 2) arterial musculature is inherently responsive to local pressure changes, having both static and dynamic reflexes that promote efficient, evenly-distributed flow of blood; and 3) complex, long-lasting behaviors like egg laying have, as part of their makeup, equally prolonged and stereotypical changes in the pattern of circulation. Taken together, these observations support the view that maintenance and adjustment of blood flow in gastropod molluscs is an unexpectedly complex and highly integrated component of behavior.

Key words. Aplysia; gastropod; mollusc; cardiovascular; circulation; anatomy; homeostasis; bag cells; egg-laying behavior.

Introduction

Animal behavior generally involves regional and tissue-specific changes in metabolic rate[21], implying a need for precise regulation of the volume and pattern of blood flow as activities change. Behavioral physiologists have largely ignored this potential component of a well-integrated motor response, even in studies of invertebrates where the neuronal mechanisms of behavior are most thoroughly investigated. This may be due to a perception that vascular systems in invertebrates are diffuse and passive structures, too small or fragile for study by standard physiological procedures. This is certainly not the case in cephalopods[22, 54] and, as this and the previous paper by Skelton et al. show, it is not true of larger gastropods. In the latter, the neural and endocrine processes regulating circulatory pumps and vessels are particularly accessible to physiological analysis, and the quantifiable behavior of gastropod cardiac systems is already well recognized[24, 33, 58]. To progress into a neuroethological analysis of circulatory regulation we must now select experimental models where both the heart and the vasculature can be studied in detail. Indeed, based on what we know from extensive investigations of vertebrates[21] and consideration of theory[37], it is the vascular motor system that is most likely to show intricate patterns of response during changes in behavioral state.

In this regard, only in gastropod molluscs have mechanistic studies of cardiovascular regulation progressed meaningfully beyond the heart[23, 30]. In particular, the gastropod *Aplysia* has proven to be an advantageous system for study due to its large size, compartmentalized visceral anatomy and limited behavioral repertoire. Circulatory organs of *Aplysia* can be dissected and manipulated in a functional state and its major arteries are large (> 3 mm diameter, > 10 cm length in adults) and sturdy enough to permit freehand dissection and use of pressure and tension recordings[42]. The most unique advantage of this invertebrate, however, is the substantial accumulation of information concerning the organization and functioning of its neural and endocrine systems[26], several of which trigger or control discrete behaviors[4, 11, 27, 51, 56]. This relatively detailed understanding of the neural basis of *Aplysia*'s behavior is a unique platform for investigating the cellular mechanisms of circulatory homeostasis and response in this animal.

In this review of *Aplysia*'s vascular anatomy and physiology we highlight evidence that circulation in gastropod molluscs is a functionally organized and dynamic process. We anticipate that descriptions of neural and hormonal factors controlling circulation, excretion and respiration in *Aplysia* will continually improve and that a comprehensive understanding of circulatory regulation, perhaps to the level of a quantitatively accurate simulation of homeostatic reflexes and behavioral response, may soon emerge.

Vascular anatomy

A detailed understanding of circulatory behavior depends importantly on an accurate anatomical description of the channels through which blood flows. High-resolution anatomical studies (generally those utilizing gelatinous inks or synthetic resins to generate etchable casts) of gastropod vasculature are notably scarce in the literature, but each of the three extant subclasses – the Prosobranchia[4, 9, 49], Opisthobranchia[18] and Pulmonata[6, 7, 17] – have representative studies for comparison[44]. As a rule, branching patterns vary greatly within and between the subclasses, but within one species group the individual anatomies are strikingly regular[17]. Reproductive organs (gonads, gametic ducts, penis) are generally supplied by discrete arteries or arterial branches whereas digestive organs (esophagus, crop, stomach, intestine, hepatopancreas) are commonly vascularized by small branches of several larger arteries. These anatomical ar-

rangements may be important for differential distribution of blood to organs with specialized and interdependent functions, like those of the reproductive tract. Circulation to these systems could be simply controlled by vasoconstrictor muscles on their common artery. The locations of vasoconstrictor muscles has not been systematically studied in any gastropod, however, so our understanding of circulation dynamics is constrained to functional interpretations of vascular anatomy. A notable exception to this is the study by Koch and Koester of feeding-induced rerouting of blood flow in *Aplysia*[28,30]. The importance of adequate circulation in molluscs is nowhere more evident than in the intricate ramifications of terminal arteries; all organs and active tissues are vascularized to some extent, generally to within a few tens of microns of their constitutive cells[49]. In gastropods the arteries terminate in tissue sinuses or lacunar spaces approaching the dimensions (10–20 μm) of mammalian capillaries. The density of terminations varies between tissues[6], with central ganglia showing some of the most elaborate vascularization[7]. Ganglionic arteries commonly branch and enter peripheral nerves, or terminate in lacunar spaces of the sheath overlying neuronal somata. The ganglionic neuropil is unvascularized[20]. Muscle fibers in the sheath and connective nerves are contractile[1,57] and may regulate tissue blood flow by constricting the terminal vascular spaces.

For *Aplysia* the most complete description of arterial anatomy is still that of Eales[18]. Three major arteries – the anterior aorta, gastroesophageal and abdominal arteries – are described and diagrammed in that work and elsewhere[27,47] but details of their branching patterns and organ associations are lacking. In figure 1 we present a two-dimensional representation of the arterial system of an adult (500 g) *Aplysia californica*, with individual vessel dimensions (diameters and lengths at maximum expansion) and branch points accurately rendered to scale. It is clear from this representation that large arteries or their primary branches are associated with discrete groups of functionally-related organs. For example, the gastroesophageal artery supplies blood to tissues involved in early digestion (salivary gland, crop and gizzard), while the abdominal artery supplies organs involved in later phases of the nutrient assimilation process (stomach, cecum, intestine and digestive gland). The end of this artery also supplies the gamete producing tissues (ovotestis) but not other reproductive tissues involved in later phases of reproduction (egg-string formation). These tissues (hermaphroditic ducts and accessory glands, seminal receptacle) all receive blood from a separate vessel, the genital artery, which can be considered a fourth major artery originating at the bulbar region of the anterior aorta. The aorta also gives rise to discrete arteries supplying organs of defense (opaline gland and ink gland in mantle cavity), copulation (penile artery), feeding (buccal mass and oral veil) and locomotion (left and right parapodia and pedal body wall). Each of the cephalic ganglia receives a small arterial branch at some distance from the aortic truck, but the abdominal ganglion, which contains most of the neurons regulating

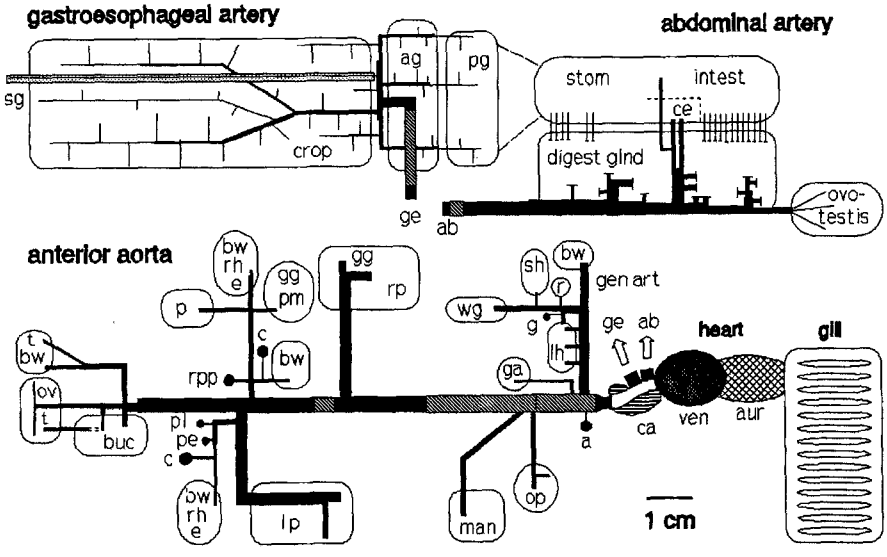

Figure 1. Two-dimensional representation of arterial branching in *Aplysia californica*. Lengths and diameters of major arteries were measured at maximal extension following injection of an agar-ink mixture (see Furgal and Brownell[20] for methods) into a 500 g animal and accurately reconstructed here as a dendrogram. For clarity, the gastroesophageal (ge) and abdominal (ab) arteries are shown displaced from their normal attachment sites on the post-ventricular aortic bulb. Cross-hatching indicates arterial vasoconstrictors examined in this study. 'T' endings of vessels in digestive gland indicate portal vessels passing through digestive diverticula to intestine. Auricle, gill and other organs are not drawn to scale. Abbreviations: a, abdominal ganglion; ag, anterior gizzard; aur, auricle; c, cerebral ganglion; ca, crista aortae; ce, cecum; buc, buccal mass; bw, body wall; e, eye; lh, large hermaphroditic duct; lp, left pedal/parapodial body wall; g, genital ganglion; ga, gametolytic gland; gen art, genital artery; gg, genital groove; man, mantle organs; op, opaline gland; ov, oral veil; pg, posterior gizzard; p, penis; pl, left pleural ganglion; pm, penis retractor muscle; ps, penis sheath; r, seminal receptacle; rh, rhinophore; rp, right pedal/parapodial body wall; sg, salivary gland; t, tentacle; ven, ventricle.

circulatory functions[34], receives its blood directly from the aorta near its union with the heart. In this position the abdominal ganglion and its complement of visceromotor neurons are well situated to monitor pressure and chemical indicators of cardiorespiratory functions[20]. Thus, by its anatomical arrangement, the vascular system of *Aplysia* appears to be functionally organized. As such, it is conceivable that a small set of vasoconstrictor muscles placed strategically at the branch points of primary arteries could strongly influence the pattern of blood flow within the animal. A growing body of evidence now supports this view[29,41,55].

Vascular hemodynamics

The structure of vasculature is related to its function through the study of hemodynamics[16]. The viscoelastic properties of molluscan vasculature were first studied only a decade ago and have been limited essentially to large cephalopods[9,22,53,54]. In gastropods and bivalves, hemodynamic studies have focused on the heart and pericardial organs[10,15,24,35]. Shadwick and Goslin[53] showed that the dorsal aorta of *Octopus* behaves as a highly distensible tube, with rubber-like elastic qualities comparable to the arteries of vertebrates[16]. The aorta readily receives blood ejected from the contracting heart, storing part of the stroke volume (through elastic expansion of vessel walls) for subsequent release to peripheral circulation during diastole. In this way the pulsatile output of the molluscan heart is smoothed and peripheral blood flow continues throughout the cardiac cycle[25]. The dynamics of arterial flow are difficult to measure[36] but it is assumed that individual arteries of molluscs behave as a single elastic chamber of high compliance – a 'Windkessel'[37,54]. Since blood acts as an incompressible fluid driven by infrequent pressure pulses of low frequency content (< 1 Hz) and long wavelength relative to artery length, pressure pulses conduct virtually instantaneously throughout the arterial tree and their shape is minimally affected by vascular impedance[54].

Our investigations of the mechanical properties of major arteries in *Aplysia* suggest they too function as high-compliance elastic tubes coupled to flow resistive elements in the peripheral tissues. When volumes of blood or saline are injected into larger arteries they expand uniformly to limiting capacity, then empty slowly over several seconds. Figure 2 shows the relaxation of pressure in two of the major arteries after injection of an 'impulse' of blood. It is evident that relaxation is not a simple exponential function of time, as expected for purely passive recoil of

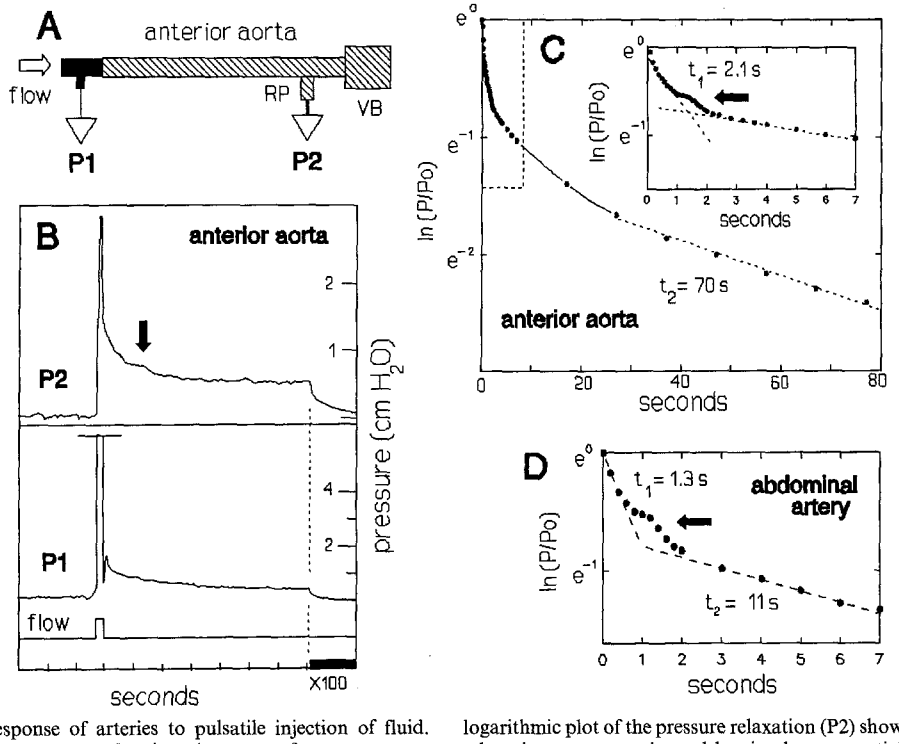

Figure 2. Dynamic response of arteries to pulsatile injection of fluid. *A* Diagram of experimental setup showing placement of pressure transducers P1 near the cannulated origin of the anterior aorta (where fluid pulses were injected) and P2 inserted into the right pedal/parapodial artery (RP) approximately 5 cm from the injection site. The vascular bed (VB) distal to RP remained intact. *B* Pressure in the artery at P2 (upper panel) increased abruptly with pulse injection of 0.2 ml of saline (lower trace) and showed fast and slow components of relaxation thereafter. 7 s into relaxation (dashed line) chart speed for this record was decreased 100-fold to reveal a slow exponential decline in pressure. *C* Normalized logarithmic plot of the pressure relaxation (P2) shown in *B*. Fast and slow relaxation are approximated by simple exponential functions with time constants (t) of 2.1 and 70 s, respectively. Inset graph shows an expanded plot of this data (area within dashed lines). Note brief period of non-linearity (arrow) during the transition between fast and slow phases of relaxation (1–2 s after pulse injection). This discontinuity appeared reproducibly as a small 'hump' in the arterial pressure recordings (arrow in *B*). *D* The abdominal artery showed similar dynamic responses to pulse injections of saline, with fast (t_1 = 1.3 s) and slow (t_2 = 11 s) phases of relaxation. P_0 = maximal pressure recorded at t = 0.

an expanded elastic tube, but a multiphasic process with at least three phases. The initial and major relaxation occurs within the first few seconds and shows an exponential time course with time constants of 1–2 s (T_{ant} = 2.1 s for the anterior aorta and T_{ab} = 1.3 s for the smaller abdominal artery). After several seconds relaxation slows by an order of magnitude (T_{ant} = 70 s; T_{ab} = 11 s) as the injected volume gradually drains through a high peripheral resistance.

Interestingly, between these fast and slow phases of relaxation there appears in both arteries a brief period of arterial 'stiffening', beginning about one second after pulse application and lasting about one second. This is apparent in pressure recordings as a small but reproducible 'hump' in the relaxation curve (fig. 2B), and as a discontinuity in semi-logarithmic plots of these data (fig. 2C,D). The properties of this anomaly suggest that it may function to improve fluid conducting efficiency of *Aplysia*'s arteries, particularly for second-long pulses of blood injected at frequencies between 0.5 and 1 Hz (heart beat duration and frequency for *Aplysia*). In theory, well-designed arteries should present minimal resistance to flow and fill uniformly throughout their length without 'ballooning'[22]. To achieve this, the arterial walls in vertebrates are constructed of highly compliant materials with low modulus of elasticity (elastin), and fibers of high elastic modulus (collagen) that abruptly stiffen the arterial wall as it expands radially[16]. This dynamic increase in stiffness is evident in the J-shaped stress-extension behavior of molluscan (cephalopod) vessel walls[53], suggesting that perhaps all animals with pulsatile pumps have evolved arterial pressure reservoirs with these properties. The behavior of *Aplysia*'s arteries (fig. 2) indicates that in addition to these passive properties, there is a pressure-sensitive active process in operation, probably mediated by momentary contraction of arterial musculature. In the intact animal these pressure-stimulated contractions would develop and subside with each heart beat, thereby mechanically coupling arterial stiffening to the pressure peak of each cardiac cycle. The net effect should promote even distribution of the ejected blood throughout the vascular tree.

An important and unresolved issue in molluscan circulatory physiology concerns the degree to which the pattern of tissue blood flow is regulated. In theory[16], flow resistance of the larger arteries should be insignificant in comparison to that of the smaller terminal branches and lacunar endings. This brings into question the relative importance of large artery vasoconstriction as a means of regulating regional volume and distribution of blood. Nevertheless, all major arteries in *Aplysia* have powerful vasoconstricting musculature that appears to be active under physiological conditions[28]. On the other hand, the low-pressure, 'open' vascular systems of gastropods could lack the high peripheral resistance necessary for flow regulation to occur at this level as it does in vertebrates[21]. Peripheral vascular resistance has been measured in several molluscs[8,18,47] but only indirectly in *Aplysia*[30]. It is noteworthy that some gastropods may have peripheral resistances exceeding those observed in mammals[8], reflecting the fine dimensions of the terminal lacunar endings of these animals[49]. In the high-pressure, closed circulatory system of *Octopus*, peripheral resistance (gill) is reduced by micromolar serotonin[47], indicating that tissue blood flow can be regulated in terminal vasculature of molluscs by release of neurotransmitters or circulating hormones.

Homeostatic regulation of circulation

What we know of the mechanisms underlying circulatory homeostasis comes almost exclusively from studies of vertebrates[5,21]. We expect these reflexive processes to be similar in all larger animals, however, since they use the same basic machinery to solve the fundamental problems of fluid transport[37]. The control mechanisms for regulation of blood flow generally operate at two levels: *intrinsic reflexes* originating from receptors within the arterial system, and *extrinsic reflexes* originating from a diversity of non-vascular sources[5]. The latter reflexes are especially important for differential distribution of blood in accordance with regional metabolic needs, while intrinsic reflexes commonly promote even distribution of blood within a vascular bed. In our incipient investigations of homeostatic circulatory regulation in *Aplysia* we find evidence that both mechanisms are functioning.

Intrinsic reflexes. Since the anterior aorta functions as a manifold for distribution of blood to several smaller arteries (fig. 1), vasoconstrictive musculature along this vessel should act to promote even distribution of blood throughout its various arborizations. The pressure-sensitive behavior of one such vasoconstrictive band is shown in figure 3. By its position just distal to the origin of the right pedal/parapodial artery, contraction of this vasoconstrictor should shunt blood away from cephalic organs (including most of the central nervous system) and toward organs vascularized by posterior branches on the aorta. A plot of vessel diameter against arterial pressure shows this vasoconstrictor (d2 in fig. 2A,B) has a relatively low (1.8 cm H_2O) and distinct threshold of activation and a high degree of hysteresis consistent with its all-or-none behavior. This appears to be a stretch-activated reflex inherent to the arterial musculature, although denervation of the artery (from cephalic ganglia) had the effect of increasing threshold of activation. It is interesting to note that mean arterial pressure in intact animals (4–7 cm H_2O, Koch and Koester[28], Koester and Koch[30]) chronically exceeds threshold for the reflex, indicating that the distal aorta would be tonically vasoconstricted until arterial pressure in the intact animal fell below the threshold for relaxation ($\sim$ 1.5 cm H_2O). These characteristics would insure adequate, but not excessive, blood flow to cephalic organs and the central nervous system. Regions of the aorta adjacent to this

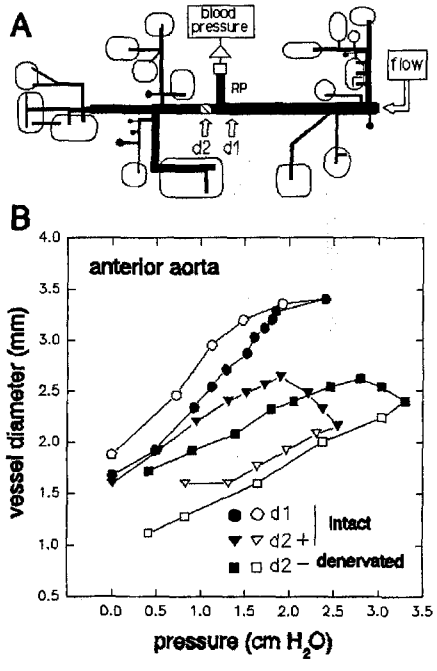

Figure 3. Pressure-induced vasoconstriction reflex in the distal anterior aorta. *A* Schematic of experimental setup for *B* showing anterior aorta and the location of a vasoconstrictor band (at d2) just distal to origin of the right pedal/parapodial artery (RP). A transducer inserted into RP measured local arterial pressure (adjusted by flow into artery). *B* As pressure increased from 0 to 2.5 cm H_2O, the diameter of the aorta just proximal to RP (d1 in A) was a smooth, positive function of pressure (●) to the limit of distension (3.4 mm); it showed minimal hysteresis (○) as pressure returned to 0. Just distal to RP (d2 in A), the same increase in arterial pressure (▼) induced strong vasoconstriction of a circumaterial muscle cuff. Contraction began as pressure exceeded 1.8 cm H_2O and was sustained as pressure returned below this threshold (▽). Denervation of the anterior aorta did not eliminate this pressure-induced vasoconstriction but its threshold for activation increased to 2.8 cm H_2O.

band but proximal to the parapodial artery branch (d1 in fig. 2A,B) expand and relax in the manner of passive elastic tubes[22], indicating that pressure-sensitive reflexes of this kind are likely to be localized specializations of the artery wall.

Extrinsic reflexes. Like the brainstem in vertebrates, the abdominal ganglion of *Aplysia* contains much of the neural circuitry controlling cardiovascular and related visceromotor functions of the animal[26]. The ganglion is vascularized by a distinct artery branching from the base of the anterior aorta (fig. 1) from which it receives blood freshly discharged from the heart. For both anatomical and physiological reasons, therefore, the abdominal ganglion is a likely site for extrinsic reflexes that alter circulatory and related homeostatic processes as a function of blood pressure and chemical composition.

For experimental applications it is possible to control ganglionic circulation by cannulating the ganglion's artery and manipulating the flow rate and content of fluids passing through it using a peristaltic pump and sample injection valve (fig. 4A). This preparation is mechanically stable and permits simultaneous recordings of intracellular neuronal activity, aortic blood pressure and contractile activities of the respiratory organs[20]. As shown in figure 4B and D, cessation of ganglionic circulation has an excitatory effect on three major fluid pumps in the animal – the gill, heart, and pericardial membrane. The most immediate response is vigorous pumping of the gill which forcefully ejects blood through the heart into the aorta (blocked in these pressure recordings by a gill catheter). This respiratory pumping behavior is triggered by ganglionic interneurons[14,31] that inhibit the heart and trigger contraction of the pericardium. The latter effect forces additional blood from the heart and may help clear the pericardial cavity of ultrafiltrate, thus further reducing cardiac resistance to refilling. The heart's excitatory response develops more slowly, but, unlike the gill and pericardial effects, it lasts for the duration of ganglionic ischemia (fig. 4D).

These compensatory responses of *Aplysia*'s circulatory pumps to reduced ganglionic circulation appear to be mediated by direct action of blood pressure on key regulatory neurons[20]. Other neurons in the ganglion, some of which control circulation-related processes like excretion and respiration, are also affected. One example is shown in figure 4C, where aortic pressure (and ganglionic circulation) was manipulated by increasing the flow rate of blood into the aorta (using a peristaltic pump). One of the affected neurons was the identified cell L5, a putative renal pore closer motorneuron[32]. L5 was strongly inhibited by this hypertension stimulus, apparently by a nonsynaptic mechanism capable of hyperpolarizing the cell beyond its reversal potential for chloride (note reversal of the fast component of L10's synaptic potential in this cell and see fig. 8.19 in Kandel[26]). The opposite (excitatory) response was observed in another renal pore closer neuron, cell L3, when ganglionic blood flow alone was decreased[20]. In the intact animal these neuronal reflexes should positively couple renal clearance to fluctuations in blood pressure and thus facilitate regulation of blood volume.

Vascular response

A central aim of our research on *Aplysia*'s cardiovascular system is to understand how it responds as a motor system during changes in behavioral state. *Aplysia* is certainly one of the best research models available for physiological analysis of behavior[26] and these advantages apply to neuronal studies of circulatory motor pattern generation. Moreover, *Aplysia* displays a remarkably stereotyped and long-term transformation in behavioral state as it undergoes the hours-long process of laying eggs[4,19]. During egg laying, 10^5–10^6 fertilized ova are released and packaged by the reproductive organs[18,26] while other behaviors, most notably feeding behavior, are suppressed. This abrupt switching of activities presents the circulatory system with a clear physiological challenge: to adjust blood flow from a pattern appropriate to feeding and digestion to one that matches the

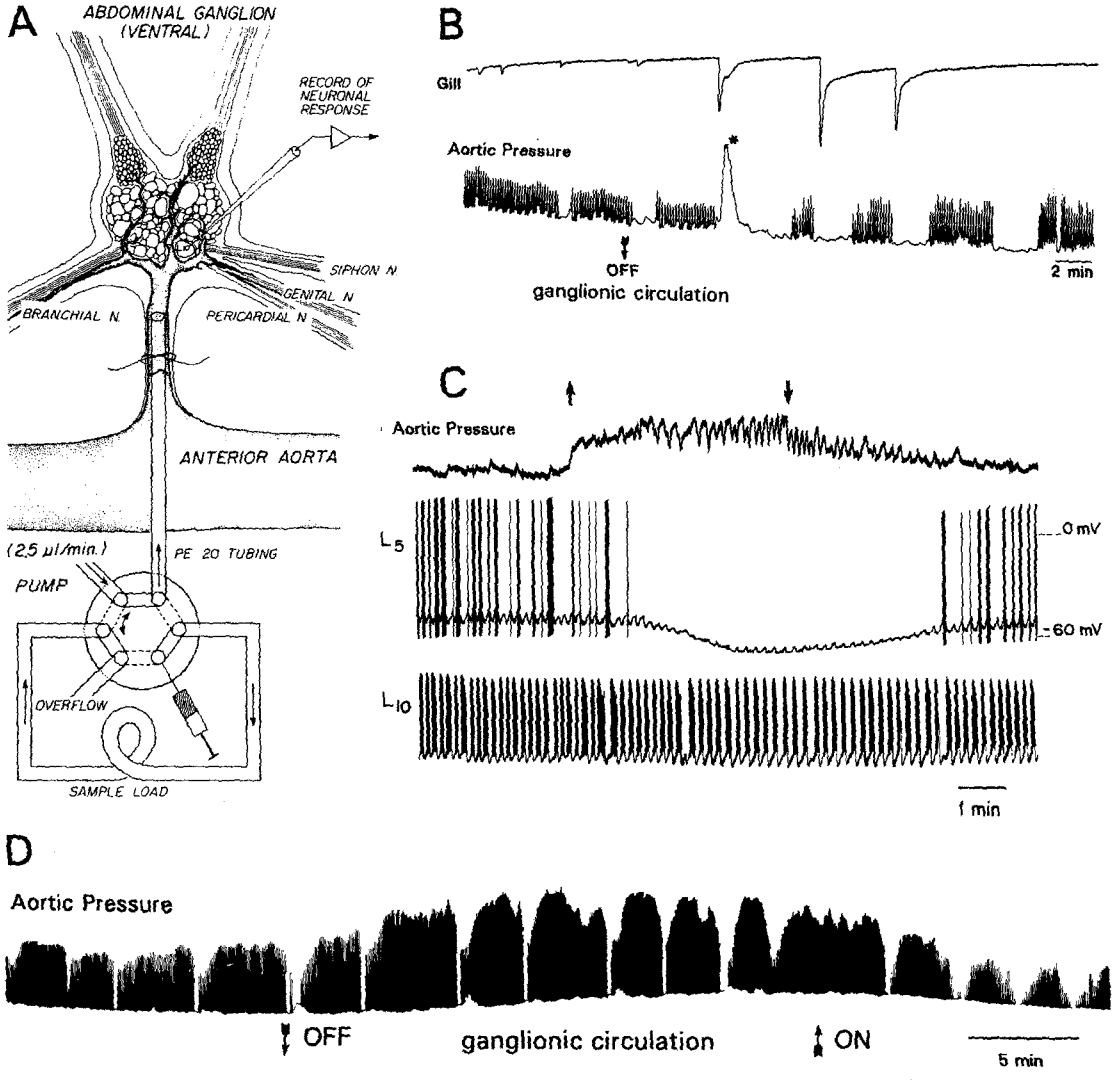

Figure 4. Circulation through abdominal ganglion and neuronal reflexes mediating circulatory homeostasis. *A* Diagrammatic representation of vascular terminations in the abdominal ganglion and the cannulation procedure for recording neuronal responses to changes in ganglionic circulation. In semi-intact preparations, activities of pericardial and branchial organs innervated by the ganglion are continuously monitored by pressure and displacement transducers. *B* When microinfusion of blood into the ganglion was interrupted (beginning at arrow) respiratory pumping of the gill increased (abrupt deflections in displacement recording). Cardiac activity (pulsatile deflections in pressure recording from anterior aorta) also gradually increased (see *D* below) following the initial period of respiratory pumping which inhibited the heart. The pericardial membrane often contracts during the initial respiratory pump, forcing additional blood into the anterior aorta (pressure pulse indicated by *) and ultrafiltrate from the pericardial cavity into the kidney. *C* Mild distension of the anterior aorta by infusion of blood at its origin strongly inhibited cell L5, a putative renal pore closer motorneuron[32], without markedly affecting cell L10, a heart excitor interneuron that also causes opening of the renal pore. *D* Termination of ganglionic blood flow (down arrow) has sustained positive inotropic and chronotropic effects on the heart as seen in this 50-min recording from the base of the anterior aorta. Return of ganglionic circulation (up arrow) reverses these effects.

emergent metabolic demands of an egg-laying organism. As part of this transformation, the vascular system is likely to show fixed patterns of action comparable to motor programs of better-studied somatic behaviors.

Hormonal mechanisms. In *Aplysia*, egg-laying behavior is triggered by identified clusters of electrically-coupled neurons, the bag cells of the abdominal ganglion[26], which synthesize and release egg-laying hormone (ELH) and several other peptides encoded by a single gene[52]. In reduced preparations of *Aplysia* it is possible to electrically trigger a physiological discharge of the bag cell peptides[43], in a manner similar to the natural event preceding egg-laying behavior[19]. Our studies[11–13,40,41,51] of this preparation show that central neurons and peripheral tissues controlling circulatory functions are primary targets of these peptidergic cells.

Figure 5 shows the vascular contractile activity evoked by stimulation of the bag cell neurons in a semi-intact preparation. Of the three major arteries, only the anterior aorta and gastroesophageal artery responded to bag cell activation (BCA). Both arteries showed strong increases in phasic contractile activity (recorded here as axial tension) which began at the distal end of each artery and migrated toward the heart as a synchronous contraction of axial and circumferential muscle. The anterior aorta also showed a tonic increase in tension that distinguished

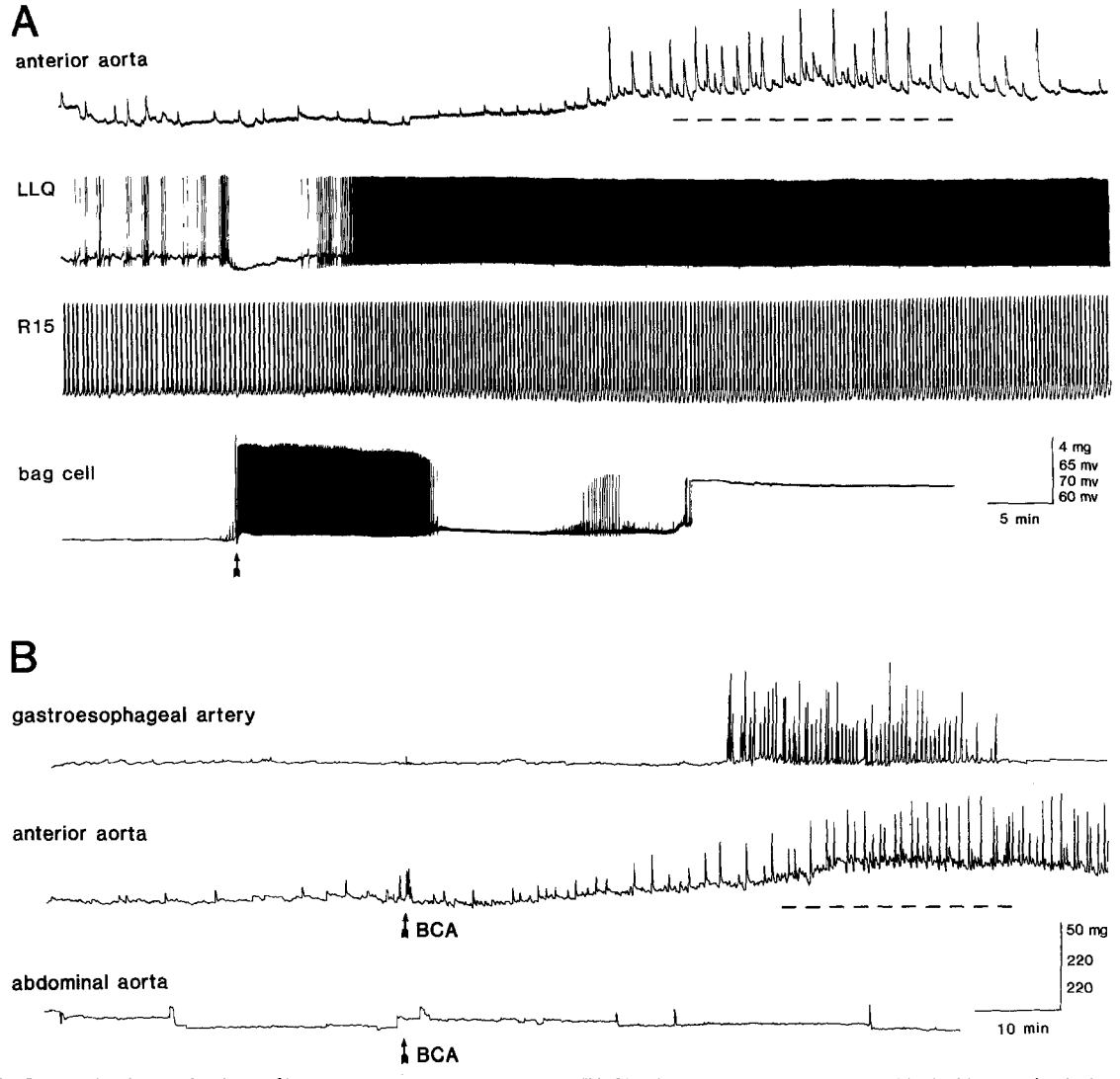

Figure 5. Neuroendocrine mechanisms of long-term vasomotor response. *A* Electrical stimulation (at arrow) of bag cell activity (spike burst lasting about 15 min in lower trace) activates phasic and tonic contractile activity (recorded as axial tension) of arteries in an innervated semi-intact preparation. Bag cell activation (BCA) also stimulated increases in spontaneous activity of neurosecretory neuron R15 and an unidentified cell (LLQ). The artery's response was not blocked by severing its innervation from the ganglion. *B* Two of three major arteries in *Aplysia* show phasic contractions following BCA. Only the anterior artery showed sustained tonic contraction. The abdominal artery was unresponsive. Arrow indicates BCA onset for each recording. The two upper traces were recorded simultaneously from the same preparation.

its response (fig. 5B). Arterial contractions began 20–30 min after BCA onset and continued for 30 min to several hours thereafter. The time-course and amplitude of response was unaffected when nerves to the arteries were severed indicating that this is a hormone-mediated action of the bag cell neurons[41]. Given the anatomical relationships of these arteries in the intact animal (fig. 1), this pattern of vasoconstriction should divert blood away from anterior (cephalic) organs, and from digestive organs served by the gastroesophageal artery; flow to the genital artery (at the base of the aorta) and to the abdominal artery should consequently increase. This long-term rerouting of circulation may be important compensation for the expected increase in metabolic activities of reproductive and nutrient storage organs (hepatopancreas) during egg laying (served by genital and abdominal arteries).

Rerouting of blood flow has been observed in conjunction with feeding behavior of *Aplysia*[28, 30] which appears to have its own neuronal trigger for activation[56]. For this behavior blood pressure and flow in the anterior aorta is increased in synchrony with the bite cycle of feeding structures in the head, ostensibly through neurally-controlled vasoconstriction of the appropriate arteries[29]. Thus, partitioning of blood flow between the major arteries of *Aplysia* appears to be a dynamic process acutely regulated during feeding (through neural pathways) or for hours-long intervals during egg laying (through hormonal actions on arterial musculature).

Neuronal mechanisms. Bag cell activation (BCA) has several long-lasting effects on neurons in the abdominal ganglion, including neurosecretory cell R15 and a left

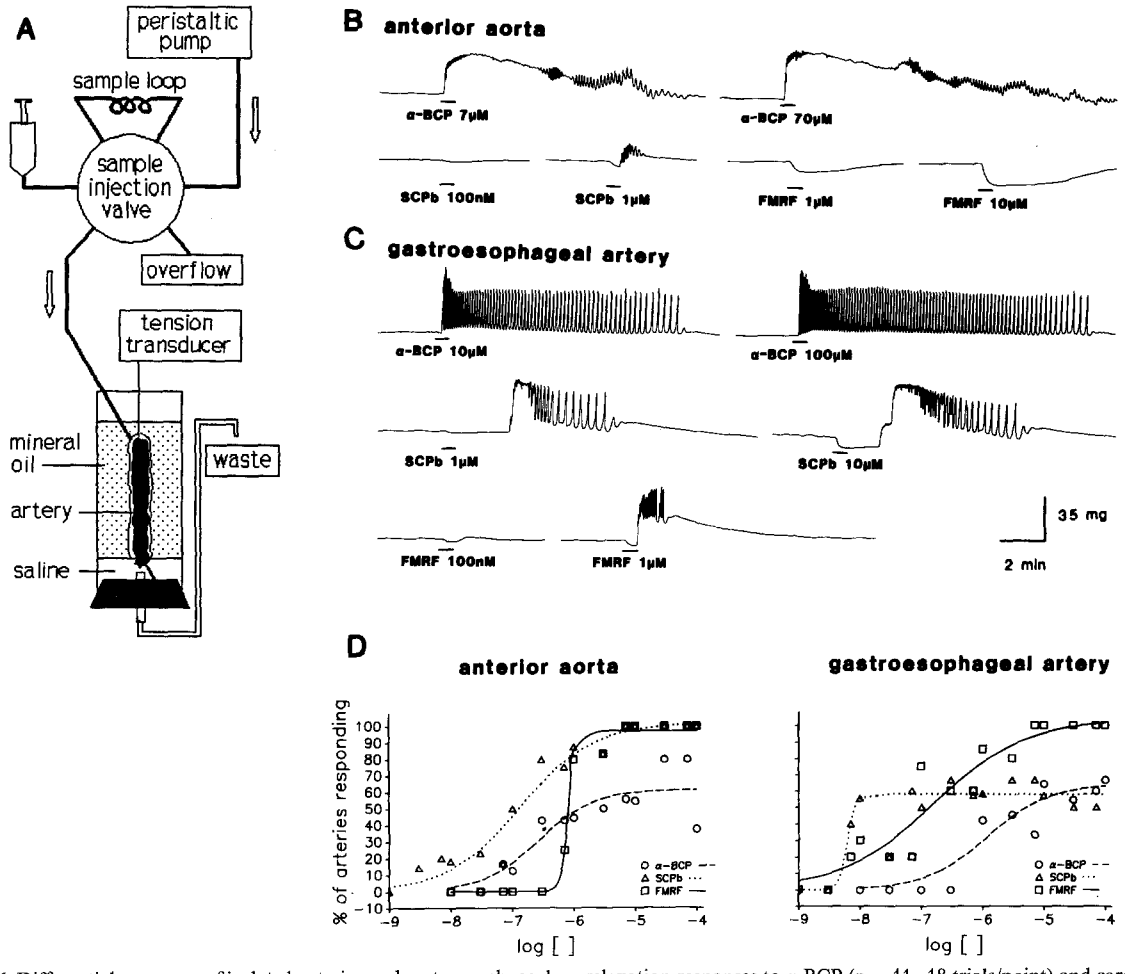

Figure 6. Differential responses of isolated anterior and gastroesophageal arteries to vasoactive neuropeptides. *A* Apparatus for bioassay of neurotransmitters on arteries. Isolated segments of arteries attached to a tension transducer are immersed in mineral oil and continuously superfused with a thin layer of saline. A 30–40 s pulse of saline containing neurotransmitter of known concentration is introduced into the flow stream over the artery (bars beneath tension records in *B,C*) without interrupting flow. *B* Segments of anterior aorta generally showed tonic contraction or relaxation responses to α-BCP (n = 11–18 trials/point) and cardioactive peptides SCP_B (n = 3–6/pt) or FMRFa (n = 4–5/pt) applied at ED_{50} (left trace of each pair) and 10 × ED_{50} (right trace) concentrations. *C* Similar concentrations of these peptides generally stimulated phasic contraction from segments of gastroesophageal artery. *D* Dose-response curves for anterior and gastroesophageal artery segments superfused with varying concentrations of the three neuropeptides.

lower quadrant cell (LLQ) shown in the example of figure 5A. R15 innervates many different tissues including the heart, pericardium, reproductive organs and all major arteries[3, 55]. Activation of this neuron stimulates respiratory pumping of the gill[2], which has non-respiratory[38] functions as a circulatory pump[51]. R15 also stimulates vasoconstriction of the left pedal/parapodial artery which may aid in hydrostatic swelling of the genital groove prior to egg laying[55]. Among the LLQ cells excited by the bag cells are vasomotor neurons ($LB_{VC1,2,3}$) and the single motorneuron (LC_P) controlling pericardial tension[40]. Thus, in addition to its direct (hormonal) actions on vasculature, the bag cell system appears to act through indirect (neurohormonal) channels to initiate changes in circulatory behavior.

The importance of neural factors for the regulation of peripheral circulation in *Aplysia* is also indirectly indicated by a growing body of neuroanatomical and pharmacological evidence. The arteries appear to be richly innervated throughout their length, particularly at branch points where regulation of musculature can most affect the pattern of flow[1, 46, 48, 50, 55]. Many of these innervating fibers are peptidergic by immunocytochemical criteria. In pharmacological studies, we have tested for transmitter receptors on isolated segments of major arteries using a low-volume perfusion bath for pulse applications of known transmitters. Examples of our results for three neuropeptide transmitters are shown in figure 6. One of the bag cell transmitters, α-BCP[43], induced artery-specific patterns of vasocontraction similar to those observed following excitation of the bag cells, but thresholds for these actions were higher than expected for hormonal transmitters. Perfusion of cardioactive transmitters SCP_B[39] and FMRFa[45] gave lower threshold, dose-dependent actions on the arteries, generally relaxing arterial tension at sub-micromolar doses and inducing contractions at higher dose. More extensive assays[40] show that arterial musculature and vasomotor

neurons[13] are responsive to several common transmitters. This is consistent with our view that vascular functions are influenced by a complex array of neuronal inputs.

Toward a comprehensive analysis of circulatory behavior

Given the substantial advantages of *Aplysia* for mechanistic studies of animal behavior, further research on its cardiovascular systems will likely reveal physiological processes that link behavioral change to circulatory response. Our objective in this review was to highlight basic aspects of vascular anatomy and physiology that must be understood as a foundation for comprehensive understanding of this process. Transport and distribution of blood is a quantifiable component of animal behavior and one that has predictable 'goals' in the context of a well-orchestrated physiological response. In pursuing the neuronal and hormonal mechanisms of long-term changes in cardiovascular behavior we discover that fundamental aspects of vascular anatomy and physiology have not been adequately researched; to proceed with the former we must build a better foundation in the latter.

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Intrinsic properties and extrinsic neurohormonal control of crab cardiac hemodynamics

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

Introduction

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

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