Multi-author Reviews

Control of circulation in invertebrates

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This Multi-author Review is based on a series of lectures concerning control of circulation in invertebrates, which were presented at a symposium of the 3rd International Congress of Comparative Biochemistry and Physiology, Tokyo, August 25–30, 1991. These lectures formed the background for a satellite symposium in workshop format: Phylogenetic models in functional coupling of the CNS and the cardiovascular system, held in Shimoda, August 31-September 2, 1991. The review reflects the pioneer leadership in comparative physiology of Dr. Kizo Matsui, Professor Emeritus of Tokyo Kyoiku University. We wish to dedicate this publication to Dr. Matsui on the occasion of his 80th birthday.

Introduction: Recent advances in comparative physiology of cardiovascular control

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The wide range of cardiovascular demands encountered across the phylum Mollusca provide an opportunity to survey the adaptation of cardiovascular systems to functional needs[24]. In our first contribution, M. J. Wells contrasts the performance of the hearts of *Nautilus, Octopus* and squids. Although power output is low, Wells finds that cardiac outputs of *Nautilus* and *Octopus* exceed those of fish, while squid cardiac output almost reaches mammalian levels. In the course of cephalopod evolution, cardiac power has been increased by an increase in frequency rather than stroke work. Wells[28] points out that the first cephalopods almost certainly evolved in hypoxic habitats. Taking *Nautilus* as a model for an early cephalopod, Wells looks at an animal which must survive serious hypoxia or periods when it is unable to ventilate. Under those circumstances, *Nautilus* may be well served by its enormous blood volume and intermittent behavior. Wells calculated that the oxygen in the blood may be just enough for a few minutes of aerobic activity, followed by oxygen uptake during a quiet period. Armstrong and Smith[1] show that the molluscs provide ideal models for the study of stroke volume regulation. This must involve the interaction of several intrinsic and extrinsic control mechanisms. Of these, the cellular neuronal and hormonal control mechanisms can be studied with advantage in *Aplysia californica*[18]. In our second paper, M. Skelton, A. Alevizos and J. Koester provide an account of the way in which cardiovascular function is adapted to physiological demands by a network of identified neurons. Neural control of vasoconstrictor cuffs determines blood flow to entire arterial trees. As we will see later, in principle this resembles the economical crustacean system more than that of the cephalopods, where the muscular arteries are under the control of more than a million neurons[29]. In our third paper, P. H. Brownell and S. H. Ligman review reflex control of the arterial vasculature of *Aplysia californica*, a favorable system because of the compartmentalization of the visceral circulation and the large size of the major arteries, combined with the depth in which neural and endocrine control systems have been studied. They note that gastropod arteries may terminate in tissue spaces approximating the dimensions of mammalian capillaries. Ichinose et al.[14] have found that neurotransmitters of central motor neurons can induce strong contraction or relaxation of the smooth muscle of the anterior aorta of *Aplysia kurodai.*

The Crustacea present an interesting contrast to the Mollusca, in that the control systems which respond to cardiovascular demands are, in a sense, servosystems. That is, to meet circulatory requirements, the CNS must control the autorhythmic intrinsic cardiac ganglion[30]. An additional interesting feature of the Crustacea is the potential for circulatory switching[30]; that is, redirection of central blood flow by valve action. In our fourth paper, J. L. Wilkens and B. R. McMahon describe extrinsic neurohumoral control of crab hemodynamics. McMahon[22] points out that decapod crustaceans labor under something of a disadvantage in central distribution of the circulation among the seven pathways to discrete peripheral regions. Lacking smooth muscle, regional blood flow cannot be controlled by peripheral vasoconstriction. Thus the innervated muscular cardioarterial valves are left to determine the distribution of flow of the arterial hemolymph. During behavioral shifts in activity patterns, flow patterns change very rapidly and this may be under neural control at the valves, since valve nerves evoke excitatory or inhibitory junctional potentials in the muscle fibers of crustacean valve flaps[19]. This mode of neural control can activate circulatory flow to a specific target organ[8]. In addition to direct neural control, cardioactive peptides and amines affect valves differentially and may strongly affect flow[22]. However controlled, differential arterial flow is an important component of response to hypoxia in decapod crustaceans[23]. This cardiovascular compensatory response to hypoxic stress resembles cardiovascular responses to exercise and to diving in higher animals[6].

Control of the decapod heart involves control of the autorhythmic cardiac ganglion, a topic discussed in our fifth paper, by T. Yazawa and K. Kuwasawa. The authors distinguish between probable sets of neurotransmitters by which the ganglionic cells interact and probable sets of neurotransmitters by which extrinsic axons modulate the activity of the cardiac ganglion. In the giant isopod, *Bathynomus doederleini*, acetylcholine (ACh) may be the excitatory extrinsic transmitter substance while τ-amino-n-butyric acid (GABA) may be the inhibitory extrinsic transmitter[25]. Applied peptides also modulate ganglionic activity. Apparently neurohumors and neurendocrines overlap, since the pericardial organs may liberate both into the pericardial sinus. Of the peptides found in the pericardial organs, proctolin has the lowest threshold for cardioexcitation and may act on cardiac ganglion neurons rather than on cardiac muscle cells. J. L. Wilkens and R. L. Walker[31] have addressed the consequences of cardio-regulatory neural input for crayfish hemodynamics. Extrinsic neural excitation and inhibition predominantly affect rate of beating. However, the release of serotonin (5HT) from the pericardial organs affects both rate and force. This is an independent mode of extrinsic control, which does not change the cardiac response to neural control. C. N. Airriess and B. R. McMahon[2] have examined in vivo circulatory effects of the pericardial monoamines, in *Cancer magister*. Infusion of 5HT, which may be considered to mimic the release of 5HT from pericardial organs, causes tachycardia of rapid onset and long duration, plus a differential effect on distribution of hemolymph to arterial trees.

In this multi-author review, we have concentrated on the central aspect of regulation of cardiac output and arterial pathways, but of course the central control mechanisms respond not only to demands from perfused regions but also to the total peripheral resistance or pressure in the hemocoel. The general internal pressure fluctuates continually in a mollusc enclosed in a muscular body wall or compressed into a shell[12]. Similarly, the dorsoventral muscles of crabs affect hemolymph volume and pressure[27]. In aquatic crabs, the dorsoventral muscles may control internal hydrostatic pressure and gill volume[26]. The small number of central neurons in the crustaceans has provided a favorable opportunity for studies of redistribution of central output and control of cardiac hemodynamics[22, 23, 31]. In cephalopods[28], muscular activity entails both a rise in hydrostatic pressure on blood vessels and a demand for increased blood flow in the active musculature. However, extensive neural control of the smooth muscle of arteries and veins allows more than a million central neurons to participate in regulation of peripheral resistance during activity. In octopods[28] there is also possible central neural control of force of contraction of the systemic and branchial heart and provision for extensive hormonal control[21]. Although there is an extensive bioassay literature, these possibilities need to be investigated by methods which can detect changes in stroke volume.

Peptides have long been suspected of playing a role in cardiac regulation[9]. Molluscan cardioactive peptides, in particular, have attracted a great deal of attention and work in this field has been reviewed repeatedly[11, 17]. The role of peptides in endocrine control of cephalopod hearts is of particular interest, since the rich innervation of the neurosecretory system of the vena cava must be centrally coordinated with the extensive innervation of the cardiovascular system. Bioassay responses of cephalopod hearts to peptides have been summarized by Kling and Jakobs[15]. However, the physiological mechanisms by which peptides regulate cardiovascular functions are much better understood in gastropods with giant identifiable neurons. Brownell, Morgan and Ligman[5] have reviewed *presynaptic* cardiovascular effects of peptides; that is, effects of peptides on circulatory and respiratory motor neurons. Identified cells surveyed were responsive to modulation by endogenous neuropeptides applied through the vascular supply to the abdominal ganglion of *Aplysia californica*. Both presynaptic and postsynaptic sites of action of neuropeptides have been identified, involving CNS neurons and myocardial cells of *Achatina fulica*[16]. If one goes beyond the gastropods with identifiable neurons, a wide range of physiological diversity has been observed in a phylogenetic survey of the cardiac effects of neuropeptides in the Polyplacophora, Gastropoda, Bivalvia and Cephalopoda[20].

Among gastropods with identifiable CNS neurons the pond snail, *Lymnaea stagnalis*, has recently provided the favorite preparation for studying regulation by neurons releasing the cardioactive tetrapeptide FMRFamide. At the level of the myocardial cell membrane, FMRFamide stimulates two non-voltage-gated channels which carry Na in the absence of Ca and a 'calcium' channel which passes unitary barium currents[4]. The action of FMRFamide is presumably mediated through a second messenger, since the currents are activated after a latency by FMRFamide applied on the membrane at some distance from the recording site. Similarly, responses of molluscan smooth muscle to FMRFamide are accompanied by very little depolarization and thus may be mediated by second messengers. Questions arise regarding the interaction of extrinsic control pathways mediated at receptors for postjunctional excitable membrane responses, ionic channels, and cellular biochemistry. This field of inquiry is reviewed in our sixth paper, in which B. L. Brezden and D. R. Gardner summarize the present state of knowledge of single channel properties of ventricular muscle cells of *Lymnaea stagnalis*. The authors modestly disclaim any comprehensive evaluation of the field, but actually survey our overall knowledge of the morphology, electrophysiology and pharmacology of their chosen cell type. Their single channel studies clarify the dependence of resting potential on conductance in potassium-selective channels and demonstrate that the longest-lived closed kinetic state is affected by membrane stretch. This stretch-sensitivity is probably not an artefact of the patch-clamp technique, and yet, in our final paper, C. E. Morris questions a mechanotransducer role for stretch-sensitive potassium channels. The *L. stagnalis* cardiac muscle preparation has also been useful for distinguishing between potential-dependent calcium-channels and receptor-operated calcium channels, whereas the evidence for the existence of the latter in vertebrate cells[22] has been rather indirect. Overall, it appears that peptidergic as well as non-peptidergic control of the *L. stagnalis* heart involves action of an array of modulators on a number of ionic channels.

In this review we see that concepts developed at the cellular level can be applied to the analysis of control of whole working hearts and are essential to understanding control of the cardiovascular system in the intact animal. The long-term objectives of comparative cardiovascular physiology have to do with the evolution of the control of cardiovascular work to fit the very different conditions faced in diverse ecological niches. This is a drive for knowledge which can serve as a paradigm of physiological evolution. For instance, we must study cardiovascular function in a variety of molluscs, since physiological activity places such widely differing demands on, for instance, the heart of a clam, an actively swimming scallop, a predator gastropod, a squid, or an octopus. Control of the isolated heart of *Aplysia* was an object of the classic investigations of Straub at the turn of the century. Now many investigators, whose work is reviewed here, have made the *Aplysia* myocardium perhaps the best understood of any cardiac tissue, with regard to control of function by central motor neurons and interneurons. Other investigations, reviewed here, have led to a comparable understanding of intraganglionic control of function in the neurogenic crustacean myocardium. In both cases, rapid advances can now be expected in understanding the hierarchy of cardiovascular control, from the cellular level to the whole organism.

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The cephalopod heart: The evolution of a high-performance invertebrate pump

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Abstract. Cephalopods typically have high metabolic rates. They have blood in which the oxygen carrier is haemocyanin, a pigment that is found only in solution and which never seems to be present in concentrations that will transport more than 4–5 vols % of oxygen. Their hearts must in consequence have very high cardiac outputs. In this account the performance of the heart of *Nautilus*, the only surviving ectocochleate, is contrasted with the performance of the hearts of coleoids, *Octopus* which has a relatively low metabolic rate (for a coleoid) and squids which have very high oxygen uptakes by any standards. In all these animals, heartbeat frequency is temperature-dependent and the additional oxygen demand in exercise is met very largely by a 2–3-fold increase in stroke volume. With the exception of *Nautilus*, cephalopods tend to utilise nearly all of the oxygen transported in the blood even at rest; they show very limited factorial scopes. Specific power output has, however, increased dramatically from 2.7 mW g^{-1} in an active *Nautilus* to 5.5 mW g^{-1} in *Octopus* and up to 20 or 30 mW g^{-1} in species of *Loligo*. The increase is almost entirely due to a 10-fold increase in heartbeat frequency. It is argued that frequency cannot be used as a means of responding to extra demand in an animal that must also carry automatic compensation for changes in metabolic rate dependent upon the ambient temperature, and that the use of frequency in some squid may be associated with a reduced temperature tolerance. Cephalopod systemic hearts do not scale directly with body mass, like the hearts of fish and the higher vertebrates. Smaller cephalopods have relatively larger hearts (as $\text{Mass}^{0.9}$). A typical 100-g coleoid would have a heart mass of 0.15 g. Oegopsid squids appear to be exceptional with hearts twice as large.

Key words. Cephalopod evolution; cardiac performance; scaling of hearts.

Introduction

Cephalopods include the largest invertebrate animals. They are active predators, preying on fish and invertebrates. With the single known exception of *Nautilus*, which lives for 10 to 20 years, they grow very rapidly, breed in a single season at an age of one to three years and then die (for summaries of the life histories of the better-known species, see Boyle[4]).

Growth rates that can raise an animal like *Octopus cyanea* from a few milligrams to several kilograms within a matter of ten months (Van Heukelem[30], but see Boyle[4] for many other examples) are associated with high metabolic rates. These in turn imply high capacity ventilatory and circulatory systems. A great deal of oxygen must be extracted and distributed through bodies that are often of very considerable size.

Distribution is made difficult by the low oxygen carrying capacity of cephalopod bloods. The pigment is always haemocyanin and this is always in solution, never in cells. Concentration is presumably limited by viscosity and the blood oxygen capacity, even in the most active squid, seems never to exceed 4 or 5 volumes per cent. Cardiac outputs have to be enormous by the standards of invertebrate animals or fish, reaching levels more typical of mammals than molluscs. This paper considers the changes in performance of the systemic heart that must have come about in the course of cephalopod evolution, and contrasts the situation in *Nautilus*, as a possible model for the situation in primitive ectocochleate cephalopods, with the state of affairs that we find in modern coleoids.

The animals concerned and the information available

Information about the performance of the systemic heart of *Nautilus* comes almost entirely from two sources: data collected in the course of the 1976 *Alpha Helix* expedition to the Philippines by the late Kjell Johansen and his colleagues (Bourne, Redmond and Johansen[3]; Johansen, Redmond and Bourne[12]) and data, most of it as yet unpublished, collected by the present author and colleagues in 1989 and 1991 at the Motupore Island Research Department of the University of Papua New Guinea. The heart of *Nautilus* is readily accessible through a hole in the shell (see operational details given