Central Control of the Cardiovascular and Respiratory Systems and Their Interactions in Vertebrates

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vertebrates and traces their evolution through the vertebrate groups, from primarily water-breathing fish and larval amphibians to facultative air-breathers such as lungfish and some adult amphibians and finally obligate air-breathers among the reptiles, birds, and mammals. A comparative account of respiratory rhythm generation leads to consideration of the changing roles in cardiorespiratory integration for central and peripheral chemoreceptors and mechanoreceptors and their central projections. We review evidence of a developing role in the control of cardiorespiratory interactions for the partial relocation from the dorsal motor nucleus of the vagus into the nucleus ambiguus of vagal preganglionic neurons, and in particular those innervating the heart, and for the existence of a functional topography of specific groups of sympathetic preganglionic neurons in the spinal cord. Finally, we consider the mechanisms generating temporal modulation of heart rate, vasomotor tone, and control of the airways in mammals; cardiorespiratory synchrony in fish; and integration of the cardiorespiratory system during intermittent breathing in amphibians, reptiles, and diving birds. Concluding comments suggest areas for further productive research.

I. INTRODUCTION

This review explores the mechanisms of central control and coordination of the respiratory and cardiovascular systems in vertebrates. Animals have evolved sophisticated control mechanisms enabling them to match their rates of oxygen uptake to their rates of aerobic metabolism. As the relative effectiveness of respiratory gas exchange over lungs or gills is determined not only by their physical dimensions but also by the rates and patterns of their ventilation and perfusion, it is essential that these latter components are controlled, both individually and in relation to one another. It has long been recognized that the overall rates of flow of air or water and of blood over the respiratory surfaces are matched according to their respective capacities for oxygen so that the ventilation-to-perfusion ratio varies from ~1 in air-breathers to 10 or more in water-breathers, with the bimodal, air/water-breathers among the lungfishes and amphibians showing variable ratios (286, 498). However, these overall ratios ignore the pulsatile and sometimes intermittent nature of both ventilation and perfusion. Careful study of respiratory and cardiac rhythms often shows them to be temporally related in ways that may optimize respiratory gas exchange. Control of these cardiorespiratory interactions resides in the central nervous system that integrates different inputs from a range of central and peripheral receptors and coordinates central interactions between pools of neurons generating the respiratory rhythm and determining heart rate variability. Thus, although the absence of a heart beat signifies death, a high and unvarying heart rate can indicate incipient brain death.

Many aspects of the brain circuitry of this remarkably sensitive system seem to have been highly conserved throughout evolution. Thus the regulatory mechanisms that operate in the central nervous systems of lower chordates such as the elasmobranch fishes show a remarkable degree of homology with those that operate in mammals, including humans (606). Homology literally means “of the same essential nature” having affinity of structure and origin. In this review, we examine apparent homologies in the cardiorespiratory control system of vertebrates, in terms of the location and phenotype of the neuronal substrate, the pattern of central nervous system (CNS) connections, the development and conservation of fundamental rhythms of nerve discharge, and neuroeffector mechanisms. Of course, we are aware that a strictly phylogenetic approach to comparative physiology is inappropriate, since parallel evolution can result in clear homologies of structure and function in distantly related species. Accordingly, we have emphasized the topographical similarities and their apparent evolution and treated the functional role of central nervous connections separately, while drawing parallels with their structural bases. We have not attempted to draw a phylogenetic tree for control of cardiorespiratory function; rather, we have explored its evolutionary roots. We show that there are considerable similarities in the topography and functional characteristics between groups of neurons in the hindbrain and spinal cord of the different vertebrate groups. However, there are also significant differences, the problem then being to know when it is reasonable to generalize and when not.

There are important differences in the construction of the respiratory and cardiovascular systems in vertebrates, related to their modes of respiration. Fish typically propel water unidirectionally over the gills, using ventilatory muscles that operate around the jaws and skeletal elements in the gill arches lining the pharynx. Adult amphibians, which lack a diaphragm, retain the buccal force pump for tidal lung ventilation; their larvae are aquatic gill-breathers. Thus, in fish and amphibians, the major respiratory muscles are cranial muscles, innervated by motoneurons with their cell bodies in the brain stem. Reptiles retain an elaborate buccal, hyoidean force pump, but ventilate the lungs primarily with a thoracic aspiratory pump, although they typically lack a diaphragm. Mammals have respiratory lungs, and ventilation is accomplished by coordinated contractions of diaphragmatic, intercostal and/or abdominal muscles innervated from the spinal cord, with only some accessory respiratory muscles (e.g., for control of the glottis) innervated by cranial nerves. Consequently, medullary respiratory neurons send axons down the spinal cord to innervate spinal...
motoneurons. The respiratory system in birds resembles that of mammals, except that they lack a diaphragm and the lungs are ventilated by volume changes in the air sacs.

The cardiovascular system is undivided in a typical fish, with the heart delivering blood into the branchial vasculature and an arterioarterial respiratory route conducting blood directly from the gills to the systemic circuit. A parallel arteriovenous route through the branchial circulation is probably nutritive, rather than constituting a functional shunt past the respiratory route. In contrast, mammals and birds have a completely divided circulatory system, with separate pulmonary and systemic circuits. Air-breathing fish, amphibians, and most reptiles have more or less incompletely divided circulatory systems, allowing differential perfusion of the pulmonary circuit. This ability may be an essential component of their intermittent patterns of ventilation, often associated with periods of submersion. Amphibians may, in addition, utilize bimodal respiration. Larval amphibians possess gills, often in combination with developing lungs, while adult amphibians can switch between cutaneous and lung breathing (e.g., during graded hypoxia or submersion) so that the distributing effect of vascular mechanisms are of paramount importance.

Despite these major differences in the construction and mode of operation of their respiratory and cardiovascular systems, evidence is accumulating that the vertebrates share some important similarities in the mechanisms of central generation of the respiratory rhythm, control of the cardiovascular system and, more specifically in the present context, in the central nervous and reflex generation of cardiorespiratory interactions. The central theme of this review is the evolution of the mechanisms of integration and coordination that match blood flow to ventilatory movements, a relationship probably fundamental to the success of vertebrates. Accordingly, we address such questions as the origin and nature of tonic nervous activity to the heart, to blood vessels, and to the airways. It may be that our review of the evolutionary relationships between cardiorespiratory control systems in vertebrates will illuminate our current inadequate understanding of the fundamental mechanisms underlying the observed interrelationships between respiratory control and cardiac control.

Knowledge of this complex area is of course dominated by the results of medically oriented research on mammals. To thoroughly review the mammalian literature is beyond the scope and length constraints of the current account. Instead, reference will be made in the relevant sections to recent extensive reviews. Readers requiring a more detailed account of the mammalian literature thus have points of access to that debate, without unduly lengthening the current review, or unbalancing it in relation to the available information from “lower” vertebrates. Each aspect of the review, therefore, begins with a summary of our current understanding of the extensive mammalian literature. This then underpins the subsequent comparative survey of the other vertebrate groups, considered in turn from fish, through amphibians and reptiles to birds, in relation to our more thorough understanding of the mammalian pattern. The treatment of each group is necessarily uneven because of the limitations on our knowledge so that the sections on “fish” are sometimes divided between elasmobranchs and teleosts and sometimes not. It must be emphasized here that, unlike the mammals and birds, the so-called “lower vertebrate” groups have a complex phylogeny; that is to say that fish, amphibian, or reptile is an umbrella term describing very diverse groups of animals, some relatively little studied. Because the respiratory and cardiovascular systems and their innervation in the lower vertebrates are less well known than those of mammals, some brief descriptions of selected examples are included to illuminate the account of the mechanisms of their control.

Some consideration of the mechanisms of ventilation and of the generation of the respiratory rhythm in the CNS is a necessary prelude to a review of the control of cardiorespiratory interactions. Consequently, a very brief overview of this area in mammals leads to a comparative account of our more limited understanding of the mechanisms in lower vertebrates, which includes descriptions of their patterns of ventilation and their origins in the brain stem, plus a consideration of the factors determining the onset and frequency of bouts of intermittent breathing in air-breathing fish, amphibians, and reptiles.

We then describe the innervation of the reflexogenic areas supplying the cardiovascular and respiratory systems and implicated in the generation of cardiorespiratory interactions, including central and peripheral chemoreceptors, arterial baroreceptors, and mechanoreceptors supplying the respiratory system. There follows a review of the evidence for functional chemoreceptors and mechanoreceptors in fish, including air-breathing fish, and in amphibians, which considers the developing roles for central chemoreceptors, lung stretch receptors, and arterial baroreceptors as the vertebrates evolved from primarily water-breathing to facultative and then to obligate air-breathing forms.

A review of the efferent innervation of the cardiovascular and respiratory systems is initiated by consideration of the cranial autonomic outflow. Beginning with a detailed description of the central locations of vagal preganglionic neurons (VPN) in mammals, which emphasizes the importance of the nucleus ambiguus (nA), a comparative account of the central origins of vagal efferents innervating the cardiovascular and respiratory systems in lower vertebrates follows. This considers evidence of a developing role in the control of cardiorespiratory interactions for neurons relocated from the dorsal motor nucleus of the vagus (DVN) into the nA. Description of the sympa-
thetic innervation of the cardiorespiratory system explores evidence for the existence of functionally organized specific groups of cells, including the possible functional importance of the arrangement of their dendritic fields, in the control of heart rate, vasomotor control, and the control of airway resistance.

The review culminates in a consideration of the central control of cardiorespiratory interactions in mammals, with a necessarily less detailed comparison of the mechanisms in lower vertebrates. This account begins with a consideration of control of the heart then progresses to a review of the role of central interactions and reflex inputs in the generation of cardiorespiratory modulation of heart rate, vasomotor tone, and control of the airways in mammals. Discussion of the role of neurons and their connections within the nucleus of the solitary tract (NTS) and the ventrolateral medulla in the generation of cardiorespiratory interactions is followed by a consideration of the generation of respiratory oscillations in sympathetic cardiovascular neurons. A comparative account of the central and peripheral interactions resulting in cardiorespiratory synchrony in fish is followed by consideration of the interactions responsible for control of the cardiorespiratory responses of intermittent breathers among the amphibians, reptiles, and diving birds.

We conclude the review with a summary of the apparent evolutionary changes in the control systems described in lower vertebrates, toward the more fully investigated systems in mammals, which attempts to identify areas that merit the urgent attention of comparative physiologists. The identification of these areas is made in the knowledge that comparative studies are becoming ever harder to fund from the agencies that support academic research. It is our task to emphasize that such studies are not only of great intrinsic interest but can further illuminate our understanding of mammalian, and therefore human, systems.

II. PATTERNS OF VENTILATION AND CENTRAL RESPIRATORY PATTERN GENERATION

Mammals characteristically display continuous, rhythmic, inspiratory breathing to maintain their relatively high rates of oxygen uptake and carbon dioxide excretion. Exceptions are the fetus and neonate which show intermittent cycles of breathing related to sleep states (260) and diving or hibernating mammals which suspend or markedly reduce breathing and heart rates for varying periods but otherwise show typical cardiorespiratory control mechanisms. Patterns of ventilatory mechanics are defined solely in terms of the time spent in inspiration and expiration and the rate of air flow. Combinations of these variables produce the more familiar components of breathing, namely, frequency, tidal volume, and minute ventilation. From neurophysiological data, the mammalian ventilatory cycle has been divided into three distinct neural phases in which each phase reflects a “state” of the oscillating network rather than a particular configuration of the motor output. In other words, a cycle phase in this context means a recurring episode when one or more groups of neurons in the network discharge a characteristic pattern of action potentials (528, 529). These phases have been defined as inspiration, post-inspiration (passive expiration), and expiration (active expiration). The postinspiratory phase is a period of inspiratory braking, which is also referred to as the first stage of expiration (EI) (364, 528).

Pattern is more complex in arrhythmic or episodic breathers, such as the amphibians and reptiles, where the components of breathing frequency also include number of breaths per episode and an apneic or nonventilatory period of variable duration. Kogo and Remmers (364) have recently discussed the similarity of the respiratory phases between amphibians and mammals. Their intracellular extracellular recordings of respiratory neurons in bullfrogs provide solid evidence to argue that lower vertebrates also have a three-phase respiratory cycle. According to their analysis, the first phase is expiration, and it occurs when the glottis is first opened. This is then followed by inspiration, which is produced by the brisk activation of the buccal levators to push air back into the lungs. The last phase is a period of breath holding, during which neurons other than those involved in the production of the two other phases were shown to be active. This phase corresponds to the postinspiratory phase described previously for mammals. They conclude their discussion by stating that this analysis is consistent with that of Pack et al. (487), who suggest that lungfish, which also have a buccal force pump, have a postinspiratory phase.

The mechanisms underlying respiratory rhythmogenesis in mammals are now being resolved (67, 529, 585), and even less is known about respiratory rhythmogenesis in nonmammalian species. Recordings from isolated brain stem-spinal cord preparations in lamprey (539), bullfrog (427), and turtle (178) have shown rhythmic respiratory-related discharges in spinal and cranial motoneurons. Because these preparations can produce a respiratory rhythm in the absence of afferent feedback (with the possible exception of input from central oxygen chemoreceptors, when present) it would appear that a central respiratory pattern generator is present in all vertebrates. At the same time, because it is possible to eliminate breathing by artificially meeting the convective requirements of an animal (e.g., external membrane lung, unidirectional gill or lung ventilation; for review, see Ref. 440), it would appear that the CPG requires some external stimulus to trigger respiratory events.
A. Mammals

The neural substrate responsible for respiratory rhythm generation and mediation of respiratory reflexes lies within the brain stem of mammals. Groups of respiratory premotoneurons and neurons innervating upper airway muscles are found in the caudal medulla near the nA and the Bo¨ tzinger complexes. In addition, at least one site of respiratory rhythmogenesis has been identified, in neonatal mammals, in the “pre-Bo¨ tzinger” complex which is situated in the reticular formation of the rostral medulla, at the level of the hypoglossal nuclei (585). These outflows probably derive, in an evolutionary sense, from the branchial motoneurons of more primitive, gill-breathing vertebrates that retain their primary roles in respiratory rhythm generation in present-day fish and larval amphibians. Accordingly, the reticular formation is thought to be the site both of the primary respiratory rhythm generator in fish and amphibians and of the respiratory and suckling rhythms in neonatal mammals.

Because the detailed organization of central respiratory control in mammals has been exceedingly well reviewed recently (67, 200, 529), a brief synopsis, for comparison with nonmammalian vertebrates, will be sufficient here. Two models have been proposed to try to explain respiratory rhythmogenesis in mammals. One proposes that the central respiratory rhythm generator consists of burster or pacemaker neurons, which show spontaneous rhythmic oscillations in membrane potential in the absence of synaptic inputs or alternatively require a tonic excitatory input before they exhibit rhythmic oscillatory activity. The second postulates that respiratory rhythm is produced by neural networks that exhibit oscillatory behavior due to synaptic interactions alone. Indeed, although pacemaker-like neurons have been identified in the pre-Bo¨ tzinger region in neonatal mammals in vitro, when sensory input was removed (585), most recently Richter (529) has argued for a hybrid of these two in vivo, whereby under normal conditions of sensory input, the synaptic interactions between respiratory neurons override the effects of pacemaker inputs. In their recent review of the literature on the central control of breathing in mammals, Bianchi et al. (67) have proposed that respiratory rhythm is not generated by a single conditional pacemaker process. Their argument was based on the assumption that brain stem respiratory activity results from the sequential activation of many populations of neurons to produce a three-phase motor act (breathing) in which each process is conditioned by the previous one and initiates the next. An alternate view would be that the coordination of the groups of respiratory neurons would be performed by a different entity. This entity would be responsible for processing the relevant sensory signals and would ensure precise spatial and temporal pattern of muscle activation during each breath so that the respiratory system meets the demand of the organism. It is to help understand the relationship between respiratory rhythm and pattern that the concept of a central respiratory pattern generator has emerged (203, 439). Because the mechanisms underlying the generation of central respiratory rhythms are not the prime subject of this review, central pattern generation will be referred to nonspecifically and the generator designated as the CPG.

B. Cyclostomes

This group of vertebrates is composed of the myxinoids (e.g., Myxine, the hagfish) and the petromyzontes (e.g., Lampetra, the lamprey). They are jawless fishes, possibly related to the primitive, extinct agnathans, but with highly specialized life-styles. In the hagfishes, water is drawn in through the nostrils by the action of a muscular membrane known as the velum and exits from a series of gill pouches via a single external opening. The ammocoete larva of the lamprey has a series of finely divided gill slits which it ventilates unidirectionally by means of the velum. Water flow is utilized both for gas exchange and filter-feeding. Adult lampreys are ectoparasites and have powerful suckers around the mouth with which they attach themselves to their fish hosts. The gills are enclosed in a series of pouches that are ventilated with tidal flow of water in and out of the small external openings of each pouch. Inspiration is the active phase with muscles in the walls of the pouches contracting against the elastic recoil of the branchial basket.

Spontaneous bursts of respiration-related activity have been recorded from the isolated brain stem of the lamprey. Recording sites included respiratory motor nuclei in the caudal half of the medulla, innervating the VIIth, IXth and Xth cranial nerves and sites near the trigeminal (Vth) motor nuclei, in the rostral half of the medulla (538, 541, 622). Periodic bursts of small spikes recorded from the rostral medulla, at the levels of the V motor nuclei, continued after isolation of the isthmic-trigeminal region by transections and occurred before bursts recorded from the IX and X cranial nerve roots. Electrical stimulation of this area excited respiratory motoneurons monosynaptically and could entrain or reset the respiratory rhythm. These observations suggest that the motor pattern for respiration is at least partly generated and coordinated in the rostral half of the medulla in the lamprey and is transmitted to respiratory motoneurons through descending pathways (539).

C. Fish

Water contains less oxygen per unit volume than air and yet is considerably more dense and viscous. Consequently, fish have to work relatively hard to extract suf-
efficient oxygen from water and normally exhibit continuous rhythmical breathing movements of the buccal and septal or opercular pumps. Fish use cranial muscles for gill ventilation. These are innervated by a dorsal group of cranial nerves exiting from the brain stem and termed the branchial nerves (536). This series of nerves contains sensory fibers and in most cases visceral motor components (Fig. 1). The nerves innervating respiratory muscles include the trigeminal Vth which provides the major innervation to the mouth region of all vertebrates, including the maxillary branch to the upper jaw and mandibular branch to the lower jaw, responsible for motor control of the jaw-closing muscles. Jaw opening is passive in routine aquatic ventilation (31, 285). The facial VIIth nerve provides the hyomandibular branch to the branchial muscles in the hyoid arch, including the levator hyoidei and, in teleosts, the opercular muscles. The glossopharyngeal IXth and the vagal Xth cranial nerves innervate the gill arches and in particular provide afferent innervation of the mechanoreceptors and chemoreceptors important in ventilatory control and efferent innervation to intrinsic respiratory muscles in the gill arches. These branchial nerves have their efferent cell bodies and afferent sensory projections located dorsomedially in the brain stem, close to the fourth ventricle, in a rostrocaudal sequential series (607; Fig. 2).

Rhythmic ventilatory movements continue in fish after brain transection to isolate the medulla oblongata, although changes in pattern indicate that there are influences from higher centers (563). Central recording and marking techniques have identified a longitudinal strip of neurons with spontaneous respiration-related bursting activity, extending dorsolaterally throughout the whole extent of the medulla (36, 564, 565, 636). These neurons make up elements of the trigeminal Vth, facial VIIth, glossopharyngeal IXth, and vagal Xth motor nuclei, which drive the respiratory muscles, together with the descending trigeminal nucleus and the reticular formation (Fig. 1). All the motor nuclei are interconnected, and each receives an afferent projection from the descending trigeminal nucleus and has efferent and afferent projections to and from the reticular formation (29). The intermediate facial nucleus, which receives vagal afferents from the gill arches that innervate a range of tonically and physically active mechanoreceptors (164) as well as chemoreceptors (607), projects to the motor nuclei (34). Finally, areas in the midbrain such as the mesencephalic tegmentum have efferent and afferent connections with the reticular formation (33, 335). The respiratory rhythm apparently originates in a diffuse respiratory pattern generator in the reticular formation, and this remains functional under anesthesia (29).

Some fish will exhibit episodic breathing patterns when exposed to particular environmental conditions such as hyperoxia. Carp were shown to possess a group of neurons with phase-switching properties, situated in the dorsal tegmentum at the level of the caudal midbrain. This group of respiratory rhythmic neurons (termed type A neurons) do not sustain continuous respiration but appear to play a key role in the control of episodic breathing. Indeed, type A neurons fire just before the onset of a breathing bout during intermittent respiration. Furthermore, stimulation of this area of the brain stem, during a ventilatory pause, brings forward the onset of the next breathing bout (334).

Central recordings from the medulla oblongata of the carp suggested that adjacent neurons have different firing patterns (30). These authors identified the target muscle for individual motoneurons by simultaneous recordings of neuronal activity and electromyograms (EMG) from the respiratory muscles. In contrast, retrograde intra-axonal transport of horseradish peroxidase (HRP) along nerves that innervate the respiratory muscles revealed that in the brain stem of elasmobranchs the neurons in the various motor nuclei are distributed in a sequential series (607). Recordings of efferent activity from the central cut ends of the nerves innervating the respiratory muscles of the dogfish Scyliorhinus canicula (52) and the ray Raia clavata (E. W. Taylor and J. J. Levings, unpublished data) have revealed that the branches of the Vth, VIIth, IXth,
and Xth cranial nerves fire sequentially in the order of the sequential rostrocaudal distribution of their motoneurones in the brain stem and rostral spinal cord. The resultant coordinated contractions of the appropriate respiratory muscles may relate to their original segmental arrangement before cephalization, an arrangement which is retained in the hindbrain of the fish in the sequential topographical arrangement of the motor neurones, including the subdivisions of the vagal motoneuron (Fig. 2). This traditional view of the origin of the jaws and visceral arches and their innervation (161) has recently been questioned on the basis of developmental studies of the role of neural crest cells (215). These suggest a separate origin for the jaws as feeding structures, independent of the visceral arches, which combined ventilation with filter-feeding, a view supported by study of marker genes (586). A possible evolutionary antecedent of the jaws may be the velum of filter feeding protochordates or larval cyclostomes (M. A. Smith, personal communication).

Both elasmobranchs and teleosts can recruit an additional group of muscles into the respiratory cycle to provide active jaw occlusion. These are derived phylogenetically from the forward migration of four anterior myotomes (the hypaxial muscles) to form a complex ventral sheet of muscle, inserted between the pectoral girdle, the lower jaw, and the ventral processes of the hyoid and branchial skeleton. They are associated primarily with suction feeding and ingestion in water-breathing fishes.
(285) but can be recruited into the respiratory cycle during periods of vigorous, forced ventilation such as may occur following exercise or deep hypoxia (32, 285). These muscles are innervated by the hypobranchial nerve, which contains elements of the occipital nerves and the anterior spinal nerves (Figs. 1 and 2). The hypobranchial nerve in fish is the morphological equivalent of the hypoglossal nerve that innervates the muscles of the tongue in reptiles, birds, and mammals. These muscles are utilized in suckling by infant mammals, an activity likely to require its own central oscillator, which is thought to reside in the reticular formation.

In the dogfish and ray, rhythmic opening and closing of the mouth occurs during ingestion of food, implying the central generation of a feeding rhythm (391). The neural mechanisms operative in the control of masticatory rhythms in fish remain unexplored, although it has been argued that the respiratory and feeding rhythms in fish are generated by separate groups of interneurons (32). It is now well established that in mammals the masticatory rhythm is generated in the hindbrain, in the reticular formation (481), and the same has been suggested for birds (181). It is interesting, in this regard, that the CPG in fish is thought to reside in the reticular formation (29).

Preliminary studies on dogfish, in which simultaneous recordings were made of efferent activity in the central cut end of a branchial branch of the vagus and of a branch of the hypobranchial nerve in decerebrate, paralyzed fish, confirmed that the hypobranchial nerve is inactive during normal fictive ventilation (Taylor and Levings, unpublished data). Short sequences of bursting activity were elicited in the silent hypobranchial nerve by activation of tongue mechanoreceptors and skin stretch receptors on the jaw (stimuli associated with feeding). Periods of spontaneous, respiration-related bursting activity could be elicited by stimulation of gill proprioreceptors and chemoreceptors (this latter response to experimental oxygen deprivation) and by intravenous injection of norepinephrine, which increases ventilation in dogfish, possibly due to central stimulation of respiratory neurons (517, 615). The mechanisms involved in recruitment of hypobranchial motoneurons into the respiratory rhythm have not been studied.

D. Air-Breathing Fish

Air-breathing fish retain gills, ventilated by cranial muscles, for the uptake of a variable proportion of their oxygen requirements, dependent on species and conditions, and excretion of most of their carbon dioxide. Gulping of air is achieved through the action of the same muscles in all air-breathing fish. These are elements of the jaw musculature, innervated by cranial nerve V, together with hypobranchial musculature, identified by Liem (396, 397) such as the geniohyoideus and sternohyoideus muscles. The combined action of jaw and hypobranchial muscles in the generation of feeding or air-gulping, independently of the visceral arches, may derive from their separate embryological and evolutionary origins (586). Liem (397) described the sequence of events associated with air-breathing in the primitive actinopterygian, the bowfin (Amia calva), a fish that utilizes a well-vascularized swimbladder as an air-breathing organ (ABO), and suggested that the action of air-breathing would require little change in the pattern of neural control required for suction feeding and/or coughing, with the exception of control over glottal opening. Brainerd (82) has suggested separate origins for air-pumping mechanisms in actinopterygian fishes (derived from the suction feeding/coughing pumps) and sarcopterygian lung fish and amphibians (the branchial irrigation pump). However, both pumps utilize the same sets of muscles and possibly the same central oscillators. In the bowfin, there appear to be two types of air breath, one that involves exhalation followed by inhalation (designated “type I” air breaths by the authors) and one that simply involves inhalation (“type II” air breaths), and it is suggested that type I breaths are respiratory in nature, whereas type II breaths have a buoyancy-regulating function (265).

Reorganization of the CNS associated with the evolution of air-breathing has been poorly studied in fish. It has been suggested that the African lungfish (Protopterus aethiopicus) possesses two separate central rhythm generators, one for gill ventilation and the other for air-breathing (205). With regard to actinopterygian, air-breathing fishes, there is probably a CPG for gill ventilation located in the reticular formation of the hindbrain, similar to that of water-breathing fish (29). In the bowfin, catecholamine infusion stimulates gill ventilation, apparently via a central mechanism, but has no effect on air-breathing in normoxia or hypoxia (422, 423), indicating that central sites controlling gill ventilation and air-breathing are pharmacologically and possibly spatially different. The central sites responsible for control over air-breathing reflexes in fish are still unknown. Some authors have suggested that air-breathing is critically dependent on afferent feedback (568, 575, 577) and, as stated above, is simply a reorganization of coughing and suction-feeding movements requiring relatively little neural reorganization (397, 575, 577). In the bowfin, spectral analysis indicates that there is an inherent rhythmicity to type I (i.e., respiratory-related) air-breathing, both in normoxia and hypoxia (267). These authors suggest, however, that this periodicity may be driven by changes in blood oxygen status that occur during the interbreath interval, rather than by a CPG for air-breathing.

E. Amphibians

Amphibian tadpole larvae have gills ventilated by activity in cranial muscles, with branchial performance...
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comparable to teleost fish, but carry out a large proportion (60%) of respiratory gas exchange over their permeable skin. As development proceeds, the lungs assume increasing importance in oxygen uptake, although the skin remains the major exchange surface until metamorphosis is nearly complete (91). In adult amphibians, most oxygen is taken up from the lungs, ventilated by the buccal cavity, but the skin retains a predominant role in the excretion of carbon dioxide (80%, $r = 7.5$).

The sequence of air flow in the breathing cycle of lungfish and amphibians such as bullfrogs is similar. Unlike air-breathing fish, which must open their mouth to aspirate ambient air into their buccal cavity at the onset of the breathing cycle, frogs aspirate air via nostrils. Even though this modification imposes a slight resistance to gas flow, it eliminates the energy expenditure associated with gulping air at the water surface (213, 214). Lung ventilation usually occurs episodically in bullfrogs. A breathing cycle begins by activation of the buccal depressor muscles that brings buccal pressure below ambient, and air is aspirated into the buccal cavity via the nostrils. The laryngeal dilator muscles then contract to open the glottis, and this allows outflow of pulmonary gas that exits by the nostrils. Subsequent closure of the nostrils coincides with a brisk contraction of the buccal levators, which pushes the bolus of gas through the glottis and into the lungs. The glottis then closes, and the inflated lung is held at a positive pressure. Lung inflation cycles, in which a series of inhalations occur without an intervening expiratory phase, are associated with experimental hypoxia or hypercapnia (640).

Typically, after a bout of lung breathing, there follows a series of elevations and depressions of the floor of the buccal cavity, called buccal oscillations. These small-amplitude, low-pressure buccal movements may help flush the buccal cavity from the previous expiration, before the next air breath (166, 168, 213, 214, 648), although their primary role may be olfaction (650). It has been suggested that they may be remnants of the mechanisms of gill ventilation used by the premetamorphic tadpole stages, and homologous to gill ventilations in fish, and that their rhythm may reflect vestiges of the central rhythm generator for gill ventilation (209, 395, 487, 576).

Buccal oscillations and lung ventilations are produced by the same muscles. The primary difference between these two events is the force of the contraction and the positions of the glottis and nares. Lung ventilations are associated with more forceful contractions with the glottis open and nares closed; buccal oscillations are associated with less forceful contractions with the nares open and the glottis closed. In resting animals, buccal oscillations occur more or less continuously and are interrupted by periodic lung ventilations, which normally occur at a time when another buccal oscillation would have been initiated. Regardless of the level of respiratory drive, there appears to be an intrinsic rhythm to lung inflation events, increasing respiratory drive simply appears to result in this rhythm being expressed a greater percentage of the time.

These observations suggest at least two possible scenarios. The first is that there is a single central rhythm generator whose output is integrated with inputs from higher centers and peripheral feedback (mechano- and chemoreceptors) at two distinct pattern generators. At low levels of respiratory drive, only output from the pattern generator driving buccal oscillations is produced, but as respiratory drive increases, output is generated from the pattern generator driving lung inflation, which leads to the increase in the force of buccal contraction and the switch in the state of the nares and glottis. The other possibility is that there are two distinct rhythm generators, with expression of the lung rhythm being conditional upon a higher level of central and/or peripheral receptor input. However, the fact that lung ventilation always occurs at a time when a buccal oscillation would otherwise have occurred suggests that if there are separate rhythm generators, they are entrained to a large degree. Kinkead (354) has described some circumstantial evidence for the existence of two central respiratory rhythm generators in the bullfrog. Hypercapnia had no effect on the frequency of lung inflations but reduced both the occurrence of buccal oscillations and their instantaneous frequency when they did occur. This might suggest that there are separate rhythms for lung inflation and buccal oscillation, which can be uncoupled.

Recently, a number of investigators have used in vitro preparations of the larval or adult anuran brain stem to examine the mechanisms of respiratory rhythmogenesis (209, 427–429, 491). Recordings of fictive breathing in isolated brain stem preparations revealed spontaneous neural output from the roots of cranial nerves V, VII, X, and XII. However, these bursts were synchronous, implying that the spatiotemporal relationships between bursts of activity in these nerves in the intact animal rely on feedback from peripheral receptors. Microinjections of glutamate into rostral areas of the bullfrog brain stem, near the VII motor nucleus, caused a brief excitation of fictive breathing (427). Interestingly, this area corresponds to the pre-Bötzinger area of the reticular formation in the mammalian brain stem, considered to be the primary site for respiratory rhythmogenesis in the neonate (e.g., Ref. 523). The CPG in fish is thought to reside in the reticular formation (29). Other pharmacological investigations support the suggestion that the neural networks associated with respiratory rhythmogenesis may be well conserved during vertebrate evolution (640).

Extracellular recording from in vitro brain stem as well as spinal cord preparations of Rana catesbeiana tadpoles and adults revealed that it is possible to manipulate the two types of neural activity associated with
buccal or lung breathing independently, using pharmacological agents (209, 428, 487, 585, 637). Superfusion of an in vitro brain stem-spinal cord preparation from the bullfrog tadpole with chloride-free saline eliminated the rhythmic bursts associated with gill ventilation while augmenting lung bursts, indicating that the former arise from a GABAergic, network-type rhythm generator, whereas lung ventilatory rhythms arise from pacemaker cells (209). This apparent discrimination is of interest in comparison to the situation described in fish, where gill ventilation may depend on pacemaker cells located in the reticular formation, and in adult mammals, where lung ventilation may be dependent on activity in neural networks. The evolution/development of air-breathing rhythms may have required a new motor pattern in the CNS rather than one that evolved from progressive modification of the branchial rhythm generator (354, 577). This may have evolved from the generator for the feeding rhythm that can be recruited by the respiratory CPG during forced ventilation in fish or when air-breathing fish gulp air at the water surface, as described above.

A recent report by Brainerd and Monroy (83) described activity in hypaxial muscles during exhalation in salamanders, possibly representing a primitive condition, intermediate between the buccal force pump of fish and the thoracic/abdominal aspiration pump of reptiles, birds, and mammals. Although similar data are not available for anuran amphibians, which may have lost this function, these data raise important considerations regarding the evolution of the control of ventilation in amphibians, which imply that descending fibers from the brain stem, innervating spinal motoneurons can have important roles in some species, anticipating their roles in the supposedly more advanced tetrapods.

Amphibians often breathe intermittently, with bouts of ventilation interrupted by quiescent periods or, in aquatic species or stages, submersion. Intermittent breathing patterns are common in lower vertebrates, such as reptiles and amphibians, and contrast with the continuous breathing patterns of nondiving birds and mammals in their apparent lack of constancy and intrinsic rhythm. Many researchers have ascribed the genesis of breathing episodes in amphibians and reptiles to the inherent oscillations of blood oxygen and/or CO₂/pH levels associated with intermittent breathing, rather than to the action of a “mammalian-type” central control mechanism. In this model, lung ventilations are induced when a certain arterial P O₂ (PaO₂) or arterial P CO₂ (PaCO₂) threshold is reached and breathing ceases when the blood gas values have been brought back within a certain range (79, 568, 649). The observation that breathing is completely suppressed when convective requirements are met by unidirectional ventilation (354, 357, 580, 649) indicates that lung ventilation is conditional upon a minimal stimulatory input (439, 576, 580, 649). However, these experiments were conducted with some degree of lung inflation, which may have overridden peripheral chemoreceptor drive. Preliminary evidence from experiments on decerebrate, paralyzed, and unilaterally ventilated toads suggests that pulmonary stretch receptor inputs may be important in the initiation of breathing. When fictive ventilation had been suppressed by unilateral ventilation, it was induced by lung deflation (640). Clearly, chemoreceptor and lung mechanoreceptor inputs influence the respiratory CPG, but their central interactions are unknown.

Several studies suggest that episodic breathing does not necessarily reflect the phasic nature ofafferent chemoreceptor or mechanoreceptor inputs. Unidirectionally ventilated toads (580, 640, 649) can still display episodic breathing or fictive ventilation, although this experimental procedure has been assumed to maintain blood gases constant, and in paralyzed animals, lung distension constant, and thus produces only tonic chemoreceptor and mechanoreceptor input. These data imply that the mechanisms underlying episodic breathing may be an intrinsic property of the central respiratory control system, a view which seems confirmed by the observation that the motor output from a brain stem-spinal cord preparation of the bullfrog was episodic, in the absence of any possible feedback from the periphery (354). The central generation of these episodic breathing patterns has been localized to the nucleus isthmi in the brain stem of the bullfrog (354, 356). This mesencephalic structure is the neuratomographical equivalent of the pons in mammals, which contributes to the control of breathing pattern (202).

Whether episodic air-breathing is generated by central or peripheral mechanisms, it is vulnerable to inputs from centers higher in the CNS. In their recent review, Burggren and Infantino (90) described how adult male newts reduced air-breathing frequency to maximize time for courtship behavior toward females during the breeding season. Foraging or searching for prey can impact on surfacing behavior in amphibians. Larval salamanders supplied with benthic food showed reductions in buoyancy (which reflects degree of lung inflation) and frequency of air breaths compared with plankton feeders. These larvae also reduced air-breathing frequency during daylight hours, presumably to reduce the risk of aerial predation. A similar interpretation was placed on the very different periods of surface breathing between day and night in Xenopus laevis (288).

F. Reptiles

Reptiles are typically committed air-breathers, having dry scaly skin and well-developed lungs. They are an ancient and highly polyphyletic class of vertebrates. Extant members show diverse respiratory and cardiovascular mechanisms, including some they share with the am-
phibians, such as an incompletely divided circulatory system and periodic ventilation, often combined with periods of submersion. Accordingly, generalizations regarding the topography and control of their cardiorespiratory systems must be avoided. The anapsids or turtles and tortoises are the most primitive extant group of reptiles. However, their ventilatory mechanisms are highly specialized to account for their shell. This incorporates their vertebrae and ribs so that the lungs cannot be ventilated by movements of the thoracic cage as in other tetrapod vertebrates, and lung ventilation is greatly restricted when the animal retreats into its shell. In the tortoise, *Testudo*, the forelimbs move in and out as the animal breathes; the turtles have sheets of muscle wrapped around the viscera or under the skin at the anterior and posterior openings of the shell, which contract alternately to ventilate the lungs. Thus the respiratory muscles are elements of the limb or body wall musculature, innervated by spinal nerves. Many of the freshwater turtles are extremely tolerant of anoxia, experienced when denied access to air by submersion under ice in frozen ponds. In the crocodilians, which have a divided circulatory system and may be more closely related to the birds rather than other reptiles, breathing movements are driven by muscles of the body wall moving the liver, which is attached to a transverse connective tissue sheet resembling a mammalian diaphragm.

Lizards, in common with all other reptiles (except some crocodilians), lack a diaphragm. However, unlike modern amphibians, they do have ribs, and lung ventilation has long been considered to be generated by intercostal muscles acting on the rib cage, with a primitive buccopharyngeal or gular pump, like that described in amphibians, utilized primarily for olfaction. As lizards run in a serpentine manner, employing segmental muscles from the body wall, it was asserted by some investigators that they are unable to breathe while running. Recently these views have been questioned. Whole animal plethysmography, together with recordings of EMG from respiratory muscles, in the agamid lizard, *Uromastyx microlepis*, revealed that the prevailing mode of ventilation in the lightly anesthetized animal involved the intercostal muscles in triphasic lung inflation and deflation, with both passive and active expiratory stages, interrupted by periods of breath-hold (7). However, an alternative mode of ventilation involved a gular pump that alternated with the costal pump. After a short passive expiration, a bout of buccal pumping caused a progressive increase in lung volume, followed by breath-hold (Fig. 3). Gular pumping commenced as lightly anesthetized lizards were warmed from 30 to 35°C, as part of their normal daily cycle of temperature variation, and could be induced by tactile stimulation of conscious lizards. A parallel study, using X-ray imaging of varanid lizards, *Varanus exanthemi-
has revealed that when at rest they rarely used an accessory gular pump. However, during recovery from exercise, all animals used gular pumping in addition to a costal pump, with between one and five gular pumping movements following costal inspiration. These clearly caused lung inflation, with caudal translation of the visceral mass (84). More recently, these lizards have been shown to employ gular pumping when walking, thus overcoming the supposed mechanical constraint on active lung ventilation during exercise (486).

The existence of anatomically and functionally separate thoracic and gular respiratory pumps in lizards would seem to require separate sites of central respiratory rhythm generation. However, this interesting possibility remains unexplored. Putative sites of respiratory pattern generation, having similarities in neural organization and activation to those extensively documented for mammals, have been described for turtles (603). However, the direct contribution of these populations of neurons and their potential integration of sensory information in determining the generation of respiratory movements remain unclear (439). In turtles, the basic output of the CPG is episodic, even under experimental conditions when all sensory feedback appears tonic (178). Experiments performed on reptiles demonstrated that mild anesthesia and brain stem section at the level of the rostral rhombencephalon (metencephalon) abolish these breathing episodes, i.e., the animals now breathe in an uninterrupted fashion (461–463). Vagotomy also affects the breathing pattern by reducing the number of breaths per episode in crocodilians (461–463). It is interesting to note, however, that vagotomy had no effect on the breathing pattern when it was performed after episodic breathing had been abolished by a caudal midbrain transection (463).

As in amphibians, it has been suggested that the initiation of bouts of discontinuous breathing may owe more to thresholds for stimulation of central and peripheral chemoreceptors than to patterns dictated by a central rhythm generator (439). This may enable the flexibility of response essential for an ectothermic vertebrate, since the thresholds for stimulation will vary with temperature, in accordance with the animal’s oxygen demand. However, unidirectionally ventilated alligators display episodic breathing (179) so that centrally generated rhythmicity may have a role in its initiation.

### G. Birds

Birds, like their endothermic relatives the mammals, typically breath continuously and rhythmically, to supply their high demand for oxygen, thus sustaining their high metabolic rate. In both groups, ventilation is cyclic, with air sucked into the lungs during inspiration and expelled at expiration. However, birds do not have a diaphragm, and lung volume appears to vary little over the respiratory cycle. Instead, tidal volume is taken up by thin-walled, highly extensible air sacs. Respiratory gas exchange takes place over the walls of the well-vascularized parabronchi in the lung, which, because of the unique structure of the respiratory apparatus, are ventilated unidirectionally. The walls of the parabronchi bear air capillaries, the functional equivalent of mammalian alveoli, which are in intimate contact with blood capillaries, providing highly effective exchange conditions between blood and air, described as cross-current flow (553).

The respiratory rhythm in birds is assumed to arise from a CPG, evidenced by the virtually constant periods of inspiration and expiration recorded from birds at various levels of ventilatory output (440). Other respiratory variables, such as tidal volume and interbreath interval, do vary, presumably under the influence of inputs from central and peripheral receptors. Breathing hyperoxic gas mixtures reduces ventilation in birds, implying a chemoreceptive drive to ventilation in normoxia (553). Diving birds can show prolonged apneas, associated particularly with forced submersion or extended “escape” dives, during which stimulation of water receptors in the airways overrides respiratory drive. Control of the complex suite of reflex cardiorespiratory responses shown by diving birds to forcible submersion in the laboratory (apnea, profound bradycardia, and marked increase in peripheral resistance) and their very different responses during telemetered natural dives have been comprehensively reviewed (98, 589).

The pools of neurons in the medulla that generate the patterned activity driving the respiratory muscles in birds appear to resemble those described in mammals (153). A pneumotaxic center, similar in location and functional characteristics to that previously described in mammals, has been postulated to exist in dorsal mesencephalic regions of the brain (553). Sections caudal to this region abolish rhythmic respiratory activity that can, however, be reinstated by rhythmic electrical stimulation of the vagus nerves. The normal respiratory period in birds may be set by cyclical changes in lung CO₂ levels. In a unidirectionally ventilated bird preparation, when insufflated CO₂ levels were raised to stimulate spontaneous breathing cycles, then periodically varied around this level, the respiratory movements of the bird were found to lock onto the imposed fluctuations in CO₂ (553).

It has long been recognized that lung ventilation may be coordinated with wing beat in birds. Compressive effects of wing upstroke and expansive effects of downstroke may assist airflow through the lung, in coordination with activity in respiratory muscles. The correspondence between the two rhythms varies from a ratio of 1:1 in crows and pigeons up to 5:1 (wing beats per breath) in ducks and pheasants (95). Bats, as flying mammals, show similar patterns of coordination and also share a relatively
large heart and increased blood oxygen capacity with their flying cousins, the birds. Respiratory frequency increases immediately upon take off in pigeons, indicating that a combination of central and peripheral nervous mechanisms, as well as mechanical considerations, is likely to be influencing the relationship. Stimulation of ventilation with CO₂ during flight did not alter the phasic coordination patterns between respiratory and wingbeat cycles in either pigeons or magpies (77), suggesting that neural interactions between control centers in the CNS are important. A potent influence of locomotor centers in the brain stem upon respiratory center motor output (or vice versa) in geese and ducks has been demonstrated by Funk et al. (207, 208). Their studies on decerebrate geese indicated that, in the absence of feedback from flapping wings, there was a predominantly 1:1 ratio between the two motor outputs, implying direct recruitment of one by the other. The various patterns of coordination seen in free-flying birds clearly require feedback from peripheral receptors.

III. AFFERENT INNERVATION OF THE CIRCULATORY AND RESPIRATORY SYSTEMS

A. Mammals

The activity of the different types of sensory receptors in the cardiovascular system and the airways of mammals has been described in several reviews (123, 489, 490, 544). In addition, the cardiovascular and respiratory responses evoked in mammals by stimulation of arterial chemoreceptors have been reviewed very recently (138, 415). Accordingly, the well-known characteristics of these mammalian sensors and the responses they engender are not described here but are referred to, for comparison, in the descriptions of their equivalents in nonmammalian vertebrates, in which their roles are still not yet fully understood.

The central projections from the various reflexogenic sites in the mammalian cardiorespiratory system are, however, of direct relevance to the current account. A wide variety of afferent fibers transmitting sensory information arises from the heart, vascular, and ventilatory systems of mammals. Arterial baroreceptors are located in the walls of the carotid sinus and aortic arch while arterial chemoreceptor afferents are located in the carotid and aortic bodies, and probably elsewhere in the circulation. Both the atria and ventricles of the heart contain mechano- and chemoreceptive afferents in their walls. Within the respiratory system there is a wide variety of sensory afferents (both mechano- and chemosensitive) throughout the respiratory tract, from the nasal cavity to the alveolar walls. These circulatory and respiratory afferents include both myelinated and unmyelinated nerve fibers and are located mainly in the trigeminal, glossopharyngeal, and vagus nerves. In addition, activity in several types of somatic afferent can have actions on either or both the respiratory and cardiovascular systems. In general, in both systems, the different afferents can be split into those involved in homeostasis, which monitor ongoing activity, whereas others, involved in defensive type reflexes, are only activated by more aversive types of stimuli (120, 121).

Afferents from receptors in the cardiorespiratory system, travelling in the cranial nerves, terminate in the brain stem, in the NTS, and, to some extent, in the trigeminal nucleus. These make multiple synapses in distinct regions of the NTS and show a large amount of overlap in their terminal fields. This allows convergence of input onto postsynaptic neurons in the NTS and may form part of the neural substrate by which various afferent inputs are integrated into physiological response patterns, since it is well known from reflex studies that simultaneous activation of several afferent inputs may interact in either a positive or negative manner (see Ref. 121). At least some of these interactions occur as the afferent information arrives at the CNS. There is some evidence for polysynaptic convergence and interactions of afferent inputs on postsynaptic NTS neurons, but the extent of these interactions is still a matter of debate and has been discussed in detail previously (121, 328).

The topography of these central terminations has been studied by a variety of techniques. The earliest studies have been summarized and discussed previously (328). More detailed information has now become available and will form the basis of this description. Histological studies employing degeneration (129), or more recently anterograde axonal transport of neuronal markers (337), have demonstrated that vagal afferents terminate predominantly in the rostral two-thirds of the NTS, whereas glossopharyngeal afferents terminate in the caudal two-thirds, overlapping in the region around obex. In addition, there was a certain degree of topography of termination within the different subnuclei of the NTS, based on organ of innervation (118, 204, 337). Although there are different degrees of input to the different subnuclei of the NTS, there is no clear anatomical separation between the terminations of afferent fibers from the respiratory or circulatory systems.

These histological studies give little information about the function of the visualized afferents, a major restraint since both the vagus and glossopharyngeal nerve contain a large number of functionally different afferent fibers. Electrophysiological techniques have been used to map terminations of afferents, whose function had been identified (Fig. 4). This antidromic mapping technique has been used to delineate the terminal fields of slowly adapting (176) and rapidly adapting (156) pulmonary stretch
receptor afferents, arterial baroreceptor and chemoreceptor afferents (174), and bronchial and pulmonary C-fiber afferents (370). Slowly adapting pulmonary stretch receptor afferents terminate rostral to obex, mainly in the ipsilateral medial subnucleus with some innervation of the lateral and ventrolateral subnuclei. This latter region is the location of the dorsal respiratory group (67, 188, 189). In contrast, rapidly adapting pulmonary stretch receptor afferents terminate more caudally, rostral and caudal to obex, mainly in the ipsilateral commissural nucleus, with less dense innervation of the medial and ventrolateral subnuclei and the contralateral commissural nucleus. Bronchial and pulmonary C-fiber afferents only project to medial regions of the NTS spanning the obex region. Unlike myelinated pulmonary afferents, there are no terminations in the lateral, ventrolateral, or ventral subnuclei of the NTS. Caudal to obex, the terminal fields are localized to the dorsal part of the commissural nucleus. This projection of C-fiber afferents is not dissimilar to that of arterial chemoreceptor afferent fibers that terminate in the medial and dorsomedial NTS and in the commissural nucleus (320).

Although antidromic activation can delineate the terminal regions of functionally identified afferent fibers, there are limitations when the question of the fine branches is addressed. The organization of preterminal processes and distribution of synaptic boutons for single pulmonary stretch receptor afferents (both slowly and rapidly adapting) has been described (338, 339) by microinjecting an HRP conjugate into axons impaled in the solitary tract, allowing direct visualization of the terminal fields of the labeled afferents. The intermediate, ventral, ventrolateral, and interstitial nuclei were the only regions of the NTS receiving terminals of slowly adapting receptor afferents, whereas rapidly adapting receptor afferents terminated in the intermediate, dorsal, and dorsolateral subnuclei more caudally. Similar studies (55, 557) have shown that laryngeal afferents terminate mainly in the ventral and ventrolateral NTS, with some projections to the interstitial, dorsolateral, medial, and dorsomedial nuclei.

Little is known about the postsynaptic neurons activated by stimulation of bronchial or pulmonary C-fiber afferents, although neurons in the commissural and caudal part of the medial NTS can be activated by stimulation of C fibers in pulmonary branches of the vagus (58). Although some of these neurons also received input from nonmyelinated afferents arising in the heart, they never received input from myelinated afferents, from either the heart or lungs (58). In recent studies we have confirmed that some neurons with these same properties are indeed activated when phenylbiguanide is injected into the right atrium (315, 317), whereas inspiratory neurons in the ventrolateral NTS are inhibited by this stimulus (314). Finally, arterial baroreceptor terminals are restricted to the ipsilateral NTS, rostral to the obex. The dorsolateral and dorsomedial subnuclei are the most often innervated, and the commissural nucleus also received some innervation (318). The central terminations of afferents arising in the heart have not been studied in such detail, but type A atrial receptor afferents have been shown to terminate in the dorsolateral and ventrolateral subnuclei (S. Donoghue and D. Jordan, unpublished observations).

In many species, activation of receptors in different parts of the upper respiratory tract evokes similar cardiorespiratory responses (see Ref. 135). In dogs, cats, and monkeys, stimulation of afferents in the superior laryngeal nerve (SLN) or nasal mucosa results in apnea, bradycardia, and vasoconstriction. The trigeminal nucleus is one site where convergence of such afferent information may take place, since it receives afferent input from some vagal and glossopharyngeal fibers (351, 626) and SLN (258, 599). Indeed, some SLN fibers bifurcate, one branch terminating in the NTS and the other in the rostral trigeminal nucleus (114). Neurons in both the rostral and caudal sensory trigeminal nuclei have been reported to receive a convergent visceral and somatic inputs from stimulation of the SLN, glossopharyngeal nerves, tooth pulp, and cutaneous facial mechanoreceptors (282, 561). In addition, Jordan and Wood (332) reported a group of neurons

<table>
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<th>RECEPTOR TYPE</th>
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<td>medial</td>
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<td>myelinated aortic baroreceptor</td>
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<td>myelinated carotid baroreceptor</td>
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<td>unmyelinated carotid baroreceptor</td>
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<td>unmyelinated bronchial receptor</td>
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<td>unmyelinated pulmonary receptor</td>
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FIG. 4. Summary of major regions of termination within nucleus tractus solitarius (NTS) of cat cardiovascular and pulmonary afferents as determined by antidromic mapping studies. Relative density of ipsilateral (●) and contralateral (●) regions of termination is denoted by number of dots, and most extensive regions of termination are shaded. [Modified from Jordan (321).]

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in the rostral trigeminal nucleus that were activated by SLN stimulation and mechanical stimulation of the nasal mucosa but were unaffected by tactile stimulation of other parts of the face. Finally, stimulation of trigeminal afferents has been shown to evoke a short latency response in vagal nerves (238).

Clearly, the neurons of the NTS and trigeminal nucleus are not simple relay stations. Integration between different afferent inputs can occur here, and there is some degree of functional organization within these nuclei. Unfortunately, such detailed information is not available from the other vertebrate groups, where similar studies have yet to be performed.

B. Fish

1. Chemoreceptors

Oxygen-sensitive chemoreceptors exert dominant control over cardiorespiratory reflexes in fish. The typical response to ambient hypoxia is a reflex bradycardia and increased ventilatory effort (607). Many studies support the existence of peripheral oxygen receptors on or near the gills of fish, and these were recently reviewed (93). However, the precise anatomical sites and functional properties of these peripheral chemoreceptors in fish remain uncertain. Saunders and Sutterlin (551) observed an increase in “breathing amplitude” in the sea raven when the dorsal aorta was perfused with hypoxic blood, and also when perfusing the dorsal aorta with normoxic blood during ambient hypoxia, which they regarded as evidence for both central and peripheral sites of oxygen receptor activity. In the sturgeon, cyanide stimulated ventilation, both when added to inspired water and when injected intra-arterially, indicating the presence of oxygen receptors sensitive to both internal and external milieu (425). In contrast, Eclancher and Dejours (182) observed a ventilatory and cardiac response only to an intravascular injection of cyanide; no response was evident to cyanide in the ventilatory water stream of teleosts, indicating that the Po2 receptors are located internally. Daxbock and Holeton (159) found that irrigation of the anterior region of the respiratory tract of the trout with hypoxic water caused a reflex bradycardia but no change in ventilation, whereas McKenzie et al. (425) found that cyanide added to the water stimulated a transient bradycardia in the sturgeon, whereas intra-arterial infusion was without effect on heart rate, implying that different receptors are involved in the induction of the two overt responses to hypoxic exposure. Ventilation rate in trout varied inversely with blood oxygen content, independently of partial pressure, indicating that arterial receptors respond to rate of delivery of oxygen to the receptor site (511). There is some evidence for receptor sites outside the branchial apparatus, including the proposed existence of venous oxygen receptors in fish (50, 617). Alternatively, Bamford (36) concluded that the most important site of oxygen detection in the trout is the brain.

The gill arches in fishes are innervated by cranial nerves IX and X, and it is these nerves that innervate the carotid and aortic bodies of mammals. Bilateral section of IX and X abolished the hypoxic bradycardia in the trout (584) but did not in elasmobranchs (547). Butler et al. (103) found it necessary to bilaterally section cranial nerves V, VII, IX, and X to abolish the hypoxic bradycardia in the dogfish and concluded that the oxygen receptors are distributed diffusely in the orobranchial and parabranchial cavities. Laurent et al. (384) recorded oxygen chemoreceptor activity from branches of cranial nerve IX innervating the pseudobranch in the tench. This organ is derived from the spiracle, which is open in elasmobranchs, and because it receives arterialized blood flowing from the gills, it is ideally suited to monitor blood oxygen levels. Although Smith and Davie (583) concluded that oxygen receptors were innervated by the IXth cranial nerve in the salmon, bilateral denervation of the pseudobranch in the trout had no effect on the changes in ventilation volume after exposure to hypoxia and hyperoxia (514). Afferent activity has been recorded from the branchial branch of the vagus innervating the first gill arch of tuna and trout (93, 443). Receptors that increased their rate of discharge in response to a decrease in the rate of perfusion or oxygen level of the perfusate also responded to ambient hypoxia. Fibers responding to hypoxic water showed an exponential increase in rate of discharge to decreasing external oxygen partial pressure, with a sensitivity similar to that exhibited by mammalian carotid body chemoreceptors (93).

Although fish have been shown to respond to hypercapnia, there is no clear evidence of a role for central chemoreceptors in the control of ventilation in fish (93, 232, 512). Although hypercapnic acidosis stimulated ventilation in channel catfish, *Ictalurus punctatus* (92), the response was abolished by cranial denervation, indicating that it resulted from stimulation of peripheral chemoreceptors, innervated by cranial nerves IX and X. These may be the same receptors that respond to oxygen. Mammalian carotid and aortic receptors respond both to oxygen and CO₂/pH (571).

Similarly, there is no evidence that chemoreceptor stimulation produces behavioral arousal in fish similar to the visceral alerting response that accompanies the stimulation of carotid chemoreceptors in mammals (415, 416). In fact, the unrestrained dogfish responds to environmental hypoxia with a reduction in activity, which remains suppressed throughout the hypoxic period, despite an increase in circulating catecholamines (431). This is analogous to the “playing dead” response shown by many animals, including some mammals (see Ref. 319), and would seem to be the opposite of a defense or alerting
response. The absence of clear visceral alerting or baroreceptor responses in dogfish (see sect. viB2) precludes their interference in chemoreceptor-induced changes in ventilation or heart rate.

2. Mechanoreceptors

The respiratory muscles in fish contain length and tension receptors, in common with other vertebrate muscles, and the gill arches bear a number of mechanoreceptors with various functional characteristics. Satchell and Way (550) characterized mechanoreceptors on the branchial processes of the dogfish, and Sutterlin and Saunders (598) described receptors on the gill filaments and gill rakers of the sea raven. De Graaf and Ballintijn (163, 164) described slowly adapting position receptors on the gill arches and phasic receptors on the gill filaments and rakers of the carp. They interpreted their function as maintenance of the gill sieve and detection of and protection from clogging or damaging material. Mechanical stimulation of the gill arches is known to elicit the "cough" reflex in fish (e.g., Ref. 547) and a reflex bradycardia (430, 604). These mechanoreceptors will be stimulated by the ventilatory movements of the gill arches and filaments, but there is no direct evidence that they contribute to respiratory control on a breath-by-breath basis (93). Stimulation of branchial mechanoreceptors by increasing rates of water flow may be the trigger for the cessation of active ventilatory movements during "ram ventilation" in fish (306, 511).

Despite the early recordings of apparent pressoreceptor responses in elasmobranch fish (e.g., Ref. 293), evidence for the involvement of baroreceptors in vasomotor control in fish remains contentious. The evolution of a role for baroreceptor afferents and for vasomotor control, exercised via the sympathetic nervous system, in control of the cardiovascular system, may be associated with the evolution of air-breathing. The gills of fish are supported by their neutral buoyancy in water. Ventilation of the gills generates hydrostatic pressures that fluctuate around, but predominantly above, ambient. Arterial blood pressures in the branchial circulation of fish and the pressure difference across the gill epithelia are relatively low, despite the fact that the highest systolic pressures are generated in the ventral aorta, which leaves the heart to supply the afferent branchial arteries. Consequently, the need for functional baroreceptors in fish is not clear.

Increased arterial pressure has been shown to induce a bradycardia in both elasmobranchs (409, 410) and teleosts (457). However, in both cases, the increase in pressure required to cause a significant reduction in heart rate was relatively high (10–30 mmHg), and dogfish seem not to control arterial pressure after withdrawal of blood (50, 585). In teleosts, injection of epinephrine, which raised arterial pressure, caused a bradycardia, abolished by atropine (516), whereas low-frequency oscillations in blood pressure, similar to the Mayer waves in mammals, were abolished by injection of the a-adrenoreceptor antagonist yohimbine (659). These data imply active regulation of vasomotor tone, and the balance of evidence indicates that functional arterial baroreceptors may exist in the branchial circulation of teleost fishes (24, 93).

The branchial branches of cranial nerves IX and X provide the afferent arm for the reflex changes in ventilation and heart rate after stimulation of the gill arches or increases in arterial pressure. Central stimulation of branchial nerves in both elasmobranchs (409, 668) and teleosts (456) caused a bradycardia. However, this could have stimulated mechanoreceptor and/or chemoreceptor afferents (see above). Afferent information reaching the brain in the IXth and Xth cranial nerves is also known to influence the respiratory rhythm, with fictive breathing rate slowing in teleosts and increasing in elasmobranchs after transection of the branchial nerves or paralysis of the ventilatory muscles (29, 52, 306). Central stimulation of branchial branches of the vagus in the dogfish with bursts of electrical pulses entrained the efferent activity in neighboring branchial and cardiac branches (607). The entrained activity in the cardiac vagus drove the heart at rates either slower or faster than its intrinsic rate (see sect. viB).

The branchial branches of cranial nerves IXth and Xth supplying the gill arches of fish project to the sensory nuclei lying dorsally and laterally above the sulcus limitans of His immediately above the equivalent motor nuclei in the medulla. These have an overlapping, sequential rostrocaudal distribution in the brain stem. Other than this generalization, the central projections of the sensory afferents contributing to cardioventilatory control in fish have not been identified (93).

C. Air-Breathing Fish

It has generally been considered that hypoxia, consequent upon stagnation of tropical freshwater habitats, was the environmental spur for the evolution, in the Devonian era, of the ABO of air-breathing fishes. A contrary view proposes that lungs evolved in vertebrates primarily to supply oxygen to the heart, before the evolution of the coronary vessels (193). Stimuli for air-breathing in fish include hypoxia and hypercapnia, both modulated by increased temperature and exercise, which increase oxygen demand and CO2 production (306, 513, 575, 577). It is not yet established whether the increases in air-breathing observed under these circumstances are stimulated by changes in oxygen availability, delivery, or demand or whether there are also responses to changes in blood pH or Pco2. However, in the bowfin Amia calva, there is evidence that air-breathing is only stimulated by changes
in water or blood oxygen status, not by changes in plasma acid-base status (422), and further evidence suggests that bowfin do not possess any central chemosensitivity controlling gill ventilation or air-breathing (265).

Application of the oxygen chemoreceptor stimulant sodium cyanide (NaCN) into the ventilatory water stream of longnose gar (Lepisosteus osseus) inhibits gill ventilation and elicits air breathing, whereas NaCN given into the bloodstream (dorsal aorta) stimulates both gill ventilation and air-breathing (579). In the bowfin, only externally applied NaCN elicits air-breathing, whereas both external and internal NaCN stimulate gill ventilation (423). Both gar and bowfin utilize a well-vascularized swimbladder as an ABO, and there are, to date, very few studies on the distribution of receptors stimulating air-breathing in fish that use other types of ABO. In the obligate air-breather the African lungfish, there are air-breathing responses to both internally and externally applied NaCN (376), whereas in the facultative air-breather Ancistrus, which possesses suprabranchial chambers, hypoxia stimulates air-breathing but increased temperature and exercise do not (231).

The sites and afferent innervation of oxygen-sensitive chemoreceptors that stimulate gill ventilation and air-breathing have been studied in various species of gar and in the bowfin. They are found diffusely distributed in the gills and pseudobranch, innervated by cranial nerves VII, IX, and X (423, 574, 579). In gar and bowfin, gill denervation (with pseudobranch ablation in the latter case) almost completely abolished air-breathing in normoxia and abolished responses to hypoxia and NaCN (423, 574), indicating that such responses are indeed dependent on afferent (oxygen chemoreceptor) feedback (568, 575, 577). Smatresk et al. (579) suggested that in gar there is central integration of input from internally and externally oriented receptors whereby internal receptors set the level of hypoxic drive and external receptors set the balance between air-breathing and gill ventilation.

Air-breathing can also be stimulated by other factors, such as water-borne irritants (576) and stretch receptors in the swimbladder of another primitive actinopterygian, the spotted gar (Lepisosteus oculatus), exhibit sensitivity to CO₂ (578).

There is no experimental evidence for baroreceptor responses in air-breathing fish. Most air-breathing fish supply their various ABO from the systemic circulation. Lungfish and all of the tetrapods have distinct pulmonary arteries and veins in association with true lungs, having highly permeable surfaces; the lungfish Proopterus has a diffusion distance of 0.5 μm over the ABO, which is similar to the mammalian lung (458). However, possibly because they retain gills, lungfish have similar, relatively low blood pressures in the respiratory and systemic circuits and may as a consequence not have a functional requirement for baroreceptor responses to protect their lungs against edema, resulting from hypertension. It could of course be argued that control of blood pressure in a relatively low pressure system requires sensitive pressoreception. This remains to be demonstrated.

D. Amphibians

1. Chemoreceptors

In their detailed review (650), West and Van Vliet considered the roles of peripheral chemoreceptors and baroreceptors in cardiorespiratory control in amphibians, while the factors influencing the progressive transition from water to air-breathing during amphibian metamorphosis were reviewed by Burggren and Infantino (90). Although chemoreceptive responses have been described previously, the specific role of peripheral oxygen receptors in the regulation of breathing in amphibians has only recently been identified (79). Jia and Burggren (304) measured the time course of reflex changes in ventilation in unanesthetized larval bullfrogs at various developmental stages. Inspiration of hypoxic water or NaCN caused rapid increases in the rate of gill ventilation, whereas hyperoxic water reduced ventilation. These rapid responses to hypoxia were eliminated by ablation of the first gill arches, suggesting that they are the site of the oxygen-sensitive chemoreceptors (305). A residual slow response was interpreted as stimulation of a second population of receptors, possibly monitoring the cerebrospinal fluid (CSF). The rapid responses to hypoxia are blunted in later stage bullfrog larvae, in which the lungs are developing and the gills degenerating (304). In an earlier study of the bimodally breathing bullfrog tadpole, mild aquatic hypoxia was found to increase gill ventilation, but more severe hypoxia promoted high frequencies of lung ventilation and a suppression of gill ventilation (645), which was in response both to lung inflation per se and the resulting increase in Po₂ (646).

In the neotenous, gill-bearing axolotl, Ambystoma mexicanum, both gill ventilation and air-breathing were stimulated by cyanide, infused either into the ventilatory water stream or into the bloodstream (424). Cardiac responses were complex with an initial bradycardia, presumably in response to stimulation of peripheral chemoreceptors, followed by a tachycardia at the first air breath, possibly in response to stimulation of lung stretch receptors, a situation comparable to the mammalian response to hypoxia (144, 145). Heart rate in the bullfrog tadpole did not change during aquatic hypoxia, with access to air (645).

Although their larvae may retain functional oxygen receptors on the gill arches, the carotid labyrinth is putative sites for oxygen receptors in adult amphibians. They are situated at the bifurcation of the internal and
external carotid arteries and innervated by branches of the glossoaryngeal nerve, which projects its afferent fibers to the NTS in the brain stem (594). These receptors are functionally similar to the mammalian carotid bodies, as they also respond to hypercapnia, and their discharge can be modulated by sympathetic stimulation (294, 295). More recent studies have also shown that the receptors are sensitive to oxygen partial pressure, rather than content (650), a finding consistent with the results of whole animal study of the stimulus modulation of the hypoxic ventilatory response in toads (639). Elevated arterial levels of CO₂/H⁺ increase discharge rate from the carotid labyrinth of toads (650).

Although carotid labyrinth denervation caused a significant reduction of resting ventilatory activity, in comparison with sham-denervated animals, it had no significant effect on the ventilatory response to hypoxia in Xenopus laevis or Bufo marinus (190, 649). These findings indicate that the carotid labyrinth influences respiratory drive but it is not essential for the control of ventilation during hypoxia in anurans. Given that hypoxia is a poor ventilatory stimulant in frogs, it is not surprising that the current consensus, from studies performed on whole conscious animals, tends to minimize the importance initially accorded to the carotid labyrinth in the regulation of ventilation. However, blood gas levels in adult amphibians are not determined solely by rates of lung ventilation; instead, the degree of shunting of blood through the pulmonary circuit and/or the cutaneous vessels may have a major role in determining oxygen and CO₂ levels. The degree of shunting is likely to be referred to input from peripheral chemoreceptors. In the adult bullfrog, more blood is directed toward the lungs during aquatic hypoxia, while aerial hypoxia elicits an increase in cutaneous perfusion (80). The return of blood to the right side of the heart from the cutaneous circulation may specifically serve to improve oxygen supply to the myocardium, which in amphibians is devoid of a coronary circulation (193).

Other putative oxygen-chemosensitive areas associated with the aortic trunk have been identified in toads (294, 631). Furthermore, it appears that the pulmocutaneous arteries of anurans may also be the site of an intrarterial chemoreceptive zone. Injection of NaCN into the pulmocutaneous arches of anesthetized bullfrogs and conscious toads stimulated ventilation (398, 631). In the absence of recordings from pulmocutaneous chemoreceptors, however, the role of the pulmocutaneous artery as a chemoreceptive site in amphibians is conjecture (650). The relative contribution of the latter two chemoreceptive sites to the hypoxic ventilatory or cardiovascular responses in amphibians is yet to be investigated.

2. CO₂/H⁺ receptors

Perfusion of the brain in anesthetized toads with artificial CSF having low pH/high CO₂ significantly increased ventilation in normoxia (580). A similar response was recorded from the in vitro preparation of the bullfrog brain stem (355, 451). It appears that, as in most vertebrates (other than some fish) investigated thus far, toads have central chemoreceptors, probably located on the ventral surface of the medulla, which respond to acidic/hypercapnic challenge. Repeating these experiments in unanesthetized animals (85) indicated that the contribution of peripheral receptors to respiratory drive was secondary to the role of central chemoreceptors that contributed ~80% of the total hypercapnic respiratory drive in the toad, a similar proportion to that observed in mammals.

This dominant role for central chemoreceptors in the generation of respiratory drive in amphibians appears at metamorphosis. An in vitro preparation of the isolated brain stem from the bullfrog tadpole displayed coordinated, rhythmic bursting activity in cranial nerves V, VII, and X, which could be characterized as representing fictive gill or lung ventilation. In early stage larvae, variations in pH of the superfusate were without effect on gill or lung burst frequency. Later stage larvae showed an increasing predominance of neural lung burst activity, which markedly increased in acid pH (625). The onset of episodic breathing patterns during metamorphosis was coincident with developmental changes in the nucleus isthmi in the bullfrog, and it seems possible that this region of the brain stem is involved in integration of central chemoreceptor information (354).

3. Pulmonary stretch receptors

Pulmonary stretch receptors (PSR) constitute another important source of feedback, contributing to the control of breathing in amphibians. There are three different types of PSR in amphibians responding to 1) the degree of lung inflation, 2) the rate at which lung volume changes, or 3) both stimuli (312, 444). These receptors are innervated by afferent fibers in the pulmonary vagi (312, 444) that project to the solitary tract in the brain stem (594). The receptors are mostly slowly adapting, and their firing rates decrease when the intrapulmonary CO₂ concentration is increased (371, 444). Although pure chemoreceptors sensitive to CO₂ have not been identified in the lungs of frogs, the discharge of stretch receptors is sufficiently modulated by CO₂ to have noticeable effects on the breathing pattern. Vagotomy abolished the increase in breathing frequency that anuran amphibians usually exhibit during hypercarbia (85, 580, 649), suggesting that pulmonary vagal input is necessary for the production of normal respiratory chemoreflexes.

Pulmonary afferent fibers play a key role in the termination of lung inflation in the adult and inhibition of buccal oscillation in the premetamorphic tadpoles. The evidence is that pulmonary deafferentation by vagotomy
in *Xenopus* results in an increase in the number of inspirations in a ventilatory period and overinflation of the lungs (190). Sectioning the pulmonary branch of the vagus nerve leads to an increase in the amplitude and frequency of resting ventilation in the bullfrog (358, 359), indicating that PSR feedback modulates breathing pattern; however, these data also suggest that PSR feedback is not responsible for the onset/termination of the breathing episodes. This matter remains unresolved as recent studies of decerebrate, paralyzed anurans showed that lung inflation inhibited fictive breathing (364, 640), as would be predicted from work on mammals, while another study, of a similar preparation, indicated that lung inflation stimulated fictive breathing (359).

Amphibians that breath discontinuously, often in association with periods of submersion, typically display large increases in heart rate and pulmonary blood flow at the onset of bouts of lung ventilation. However, the contribution of lung stretch receptors to this response is not resolved (650). Whereas artificial lung inflation increased heart rate in anesthetized toads, in conscious *Xenopus laevis*, denervation of PSR did not abolish the increase in heart rate associated with lung inflation (190), and in lightly anesthetized animals, artificial lung inflation did not affect heart rate, though pulmocutaneous blood flow increased, presumably due to shunting (186). A similar response was demonstrated in *Bufo marinus* (647).

E. Reptiles

1. Peripheral oxygen receptors

Scattered groups of glomus cells have been identified in the connective tissue surrounding the main and collateral branches of the carotid arteries in lizards. This area is profusely innervated by the superior laryngeal branch of the vagus nerve (535) and possibly the glossopharyngeal nerve (4). Although activity in these putative receptors has not been recorded, denervation of this area abolished the increase in ventilation shown by lizards when hypoxic or hypercapnic blood was injected into the carotid arch (130). In turtles, chemoreceptive tissue has been located on the aortic arch innervated by the superior and inferior branches of the vagus nerve (295). The former nerve is thought to correspond to the aortic nerve of mammals, whereas the latter arises from the ganglion trunci of the vagus. The inferior branch also innervates chemoreceptors located on the pulmocutaneous artery of the turtle (3, 298). These receptor groups have been shown to respond to changes in oxygen level, but their roles in establishing resting ventilatory drive or in reflex responses to hypoxia are unknown (440). All primary afferent fibers of the glossopharyngeal and the majority of vagal afferent fibers enter the NTS in the monitor lizard (38).

Oxygen uptake in reptiles is dependent on $P_{O_2}$ down to a critical level, below which aerobic metabolism is depressed (439). This critical $P_{O_2}$ varies with temperature and can be correlated with changes in the relative affinity for oxygen (measured as $P_{50}$) of the animal’s hemoglobin (568). Species having hemoglobin with a relatively high affinity for oxygen, such as the turtle *Chrysemys picta* (229), have greater hypoxic tolerance, and therefore a lower threshold, but often show a more marked ventilatory response at threshold than species having lower affinity hemoglobin, such as the lizard *Lacerta viridis* (466). Current theory suggests a role for heme protein in the functioning of peripheral chemoreceptors (442). These data suggest that the peripheral oxygen receptors respond to a reduction in oxygen content (i.e., hypoxemia) or to rate of delivery of oxygen to the receptor, which includes blood flow, rather than to systemic hypoxia (i.e., a reduction in $P_{O_2}$). Similar characteristics have been attributed to arterial chemoreceptors in fish (511, 512) and in birds and mammals (440).

Reptiles, in common with some air-breathing fish and amphibians, have pulmonary and systemic circulations that are incompletely separated so that some systemic venous blood can bypass the lungs to reenter the systemic circulation, while some arterialized blood can reenter the pulmonary circulation. Consequently, arterial blood gas composition is affected by the degree of admixture of oxygenated arterialized blood and oxygen-depleted venous blood, rather than lung gas composition alone, as it is in mammals. This presents the intriguing possibility that regulation of these central vascular shunts, with reference to peripheral chemoreceptors, may play an important role in control of arterial blood gas composition in reptiles, independent of ventilatory control (88, 640).

2. $CO_2/H^+$ receptors

The hypercapnic ventilatory response is well developed in reptiles, and changes in $P_{CO_2}$ rather than $P_{O_2}$ provide the dominant drive to breathe (439, 441). Central chemical control of ventilation in an ecotothermal, air-breathing vertebrate was first demonstrated in the unanesthetized turtle, *Pseudemys scripta elegans* (271). Perfusion of the lateral and fourth cerebral ventricles with artificial CSF caused an increase in ventilation to four times control, following a calculated $\Delta pH$ change of only 0.02 units. Inhalation of gas mixtures enriched with $CO_2$ stimulates ventilation and affects pulmonary vagal activity in crocodilians (441, 503). It causes ventilation volume to rise, decreases periods of breath hold, and increases the number of breaths in each breathing episode. Snakes and lizards may respond to environmental hypercarbia, which stimulates lung receptors, with decreased ventilation but show a marked increase in response to venous $CO_2$ loading (441).
F. Birds

1. Peripheral oxygen receptors

In birds, chemoreceptive tissue is concentrated around the common carotid arteries, close to the thyroid glands, and is innervated by vagal branches from the nodose ganglion. These receptors are thought to be homologous to the carotid bodies of mammals (459). Glomus tissue, which may be homologous to the aortic bodies of mammals, has been described within the aortic walls of birds (618), and a recording from a putative aortic chemoreceptor in a duck has been reported (483). All receptors respond to changes in \( P_{O_2} \) but in some birds, the threshold for a hypoxic ventilatory response is more strongly correlated with blood oxygen content (78). The carotid chemoreceptors contribute to resting ventilatory drive and may be solely responsible for reflex responses to hypoxia or hyperoxia (81). Hypoxia stimulates breathing in conscious, anesthetized, or decerebrate birds (553). There are species differences in oxygen chemosensitivity. For example, Pekin ducks showed a much higher cardiac chronotropic sensitivity to hypoxia during forcible submergence than Canada geese (590).

2. \( CO_2/pH \)

In birds, as in reptiles and mammals, the reflex effects of central chemoreceptor stimulation, following changes in \( P_{aCO_2} \) or arterial pH, appear to predominate over all other receptor inputs. Cerebral perfusion and/or denervation of peripheral receptors in ducks indicated that central chemoreceptors are primarily responsible for resting respiratory drive as well as reflex responses to hypercapnia and acidosis (445). As in mammals, the glomus tissue associated with oxygen chemoreception is also sensitive to changes in \( CO_2 \) or pH. In addition, birds possess intrapulmonary chemoreceptors that show discharge rates inversely proportional to the level of \( CO_2 \) in inhaled gas mixtures (553). They are located within the lung, innervated by the vagus nerve and to some extent by the cardiac sympathetic nerve (187). These receptors are silenced by high levels of \( CO_2 \) in the airway but are insensitive to changing \( P_{aCO_2} \) and their responses to changes in lung volume or pressure are not significant within physiological limits (198, 199, 553). Thus, unlike mammals, lung stretch receptor inputs are not important in the regulation of breathing or cardiorespiratory interactions in birds. This relates to the fundamental morphological and functional characteristics of the compact bird lung, which is ventilated unidirectionally by volume changes in the air sacs and is not itself stretched (553).

Because some birds, like the bar-headed goose, \( Anser indicus \), fly to extremely high altitudes, they regularly undertake exercise in hypobaric hypoxia (97). Their adaptations to this environment include increased heart size and blood oxygen affinity, compared with other birds or with mammals. They have been found to maintain arterialized blood \( P_{O_2} \) very close to inspired levels in hypoxia, by effective hyperventilation, with an associated reduction in blood \( P_{CO_2} \) and a respiratory alkalosis. This is achieved without the reduction in cerebral blood flow typically associated with hypocapnia in mammals, and indeed, hypoxia causes a greater increase in cerebral blood flow than is observed in mammals so that blood flow to the brain may be maintained at high altitude. A critical difference between these high-flying birds and other birds or mammals appears to be their greater tolerance of hypocapnia, which is likely to reside at the level of their central chemoreceptors in the brain.

IV. CRANIAL AUTONOMIC INNERVATION OF THE CARDIORESPIRATORY SYSTEM

A. Mammals

1. Topography of the vagal motor column

The central origin of the motor fibers innervating the heart and airways in mammals has been well documented by localizing the retrograde labeling following application of HRP or a conjugate to either the whole vagus nerve, its individual branches, or directly into the relevant targets. Neurons with vagally projecting axons are found predominantly ipsilaterally in both vagal motor nuclei, the DVN and the nA, and in the region joining these two, the intermediate zone.

The nA, as its name implies, is a diffuse region extending throughout the ventrolateral medulla, from the level of the facial nucleus to the first cervical segment of the spinal column. It has been described by several authors, and the terminology used was somewhat confusing until a study in the rat (68) resulted in a description which is beginning to be used by other authors and will be used here. The nuclear regions of the nA are defined on the basis of the location of neurons projecting in the glossopharyngeal and vagal nerves and their branches. There are two major longitudinal divisions of the nucleus. The dorsal division has three subdivisions, the compact, semi-compact, and loose divisions (rostral to caudal, respectively), and comprises the somatomotor innervation of the pharyngeal and thoracic viscera. The ventral external formation comprises parasympathetic preganglionic neurons. The different subdivisions can be discerned by the size of the neurons, their dendritic organization, and the projection of their axons. There is less distinction in the subregions of the DVN (206), but some differences do exist, and there are suggestions of a topographical localization of DVN neurons, based on target organ. Even if such an organization exists, the dendritic fields of many...
DVN neurons are very extensive, often projecting into adjacent DVN subnuclei or other medullary nuclei such as the NTS (206, 562).

Vagal-projecting neurons are found throughout the dorsal nA and in the external division ventrolateral to the principal column. They are densest in the DVN and extend lateral to its boundaries into the region bordering the NTS and the reticular formation between it and the nA. A similar distribution of vagal-projecting neurons has been described in rats (126, 340), cats (336), dogs (116), ferrets and mink (522), neonatal pigs (276), and Old and New World monkeys (257). The central distribution of vagal motoneurons includes those innervating both intrathoracic and abdominal organs, but there is no certainty that individual organs are represented at all sites. More specific studies have examined the possibility that there is a topographic organization based on the organ of innervation. Although the overall pattern of labeling is similar, the detailed organization of the innervations do differ between species.

2. Innervation of the heart

In a number of studies, tracers have been applied to the cardiac vagal branches or to the heart itself. Although some neurons in the DVN and intermediate zone were labeled, it is clear that the region of the nA provided the major cardiac innervation in most mammals. In rats (298, 446, 479, 480, 593), cats (59, 117, 222, 223, 330, 337, 449, 596), dogs (59, 274, 275, 500), and neonatal pigs (276), the majority of labeled neurons were located in the external subnucleus of the nA. Neurons within the compact region of the nA, if labeled, were always in the minority. When the DVN was labeled, it was usually the lateral and dorsal regions that contained the majority of labeled cells. The relative contribution of DVN and nA to the cardiac vagi seems to vary between species. The DVN contains relatively more cardiac-projecting neurons in rats, and least in pigs and dogs, with cats lying somewhere in between. In the cat (59, 330), cardiac VPN (CVPN) are found predominantly in the nA, with up to 78% of cardiac neurons found in this location. This compares with 45% of CVPN located ventrolaterally in the dogfish and ~30% in Xenopus, indicating that in mammals, compared with lower vertebrates, a greater proportion of cardiac vagal motoneurons originate in this division. However, the ventrolateral group of vagal peranglionic neurons in the dogfish are all CVPN (see sect. nC). Only one set of studies in cats (623, 624) denied the role of the nA in cardiac control, but this has not been confirmed.

3. Innervation of the airways

Similar anatomical studies have delineated the motor innervation of the airways in air-breathing mammals. Because these have been described in detail recently (322), only a summary is provided here. In rats, Bieger and Hopkins (68) demonstrated that the compact region of the nA contained esophageal motoneurons, whereas pharyngeal motoneurons were located in both the more caudal semicompact formation and in a group of neurons rostral to the compact group and overlying the facial nucleus. Glossopharyngeal motoneurons were also found at this latter site and in the external formation where laryngeal motoneurons were also localized in addition to rostral part of the semicompact formation and the dorsal part of the caudal formation. The external and dorsal divisions differ in that the latter innervates striated muscles of the esophagus, larynx, and pharynx, whereas the former is the origin of parasympathetic preganglionic neurons. A similar localization of motoneurons innervating the rat esophagus, pharynx, and cricothyroid muscle has been demonstrated (10). Preganglionic neurons innervating the trachea are located in the compact formation, in the area ventral to it, and in the rostral part of the medial NTS, but not in the DVN (263). This description of the nuclear arrangement of the rat nA is similar to that described in rabbits (296, 385, 386) and cats (242, 336, 337, 478, 479). The ambiguous origin of the laryngeal motor innervation was confirmed, with some cells also being labeled in the rostral DVN. Neurons innervating the trachea overlapped those innervating the larynx in the rostral nA. In addition, cells in the nucleus retroambigualis caudal to the obex also provided an innervation of the extrathoracic trachea, whereas the DVN at around obex level provided some innervation of the intrathoracic trachea. Bronchial motoneurons were found mainly in the DVN, with limited numbers in the rostral nA, whereas those labeled following injections into lung tissue were located in both DVN and nA. This was partly confirmed when tracer was applied to the individual pulmonary branches of the vagus in cats (59, 330). Labeled neurons in the nA were found over a 10-mm distance spanning the obex in the external formation, but there was a trough in the distribution of the cells projecting to the pulmonary branches between 1 and 3 mm rostral to obex, where neurons projecting to the cardiac branches were located (Fig. 5). These pulmonary neurons outnumbered those labeled in the lateral DVN. Thus, in the cat, both pulmonary (68%) and cardiac (78%) VPN are found predominantly in the nA (330). The labeling seen in dogs, following tracers applied to the superior or recurrent laryngeal nerves (638) or pulmonary branches of the vagus (57, 261), is not unlike that described in cats.

B. Cyclostomes

The heart of myxinoids is aneural, that is, it is not innervated by the vagus or the sympathetic nervous system (115, 237), whereas the heart of the lamprey (al-
though similarly devoid of a sympathetic supply) is innervated by the vagus (19, 521). The cardiac fibers leave the thin non-myelinated epibranchial trunk of the vagus and run to the median jugular vein. In the wall of this vein, the nerve fibers form a loose network with one or two main bundles (192).

The main effect of vagal stimulation in lampetroids is an acceleration of the heart with an accompanying decrease in the force of contraction (191). Acetylcholine induces an acceleration of the heart, a response unique among vertebrates. Nicotinic cholinergic agonists, such as nicotine, have the same effect (19, 191). The excitatory effect of vagal stimulation or nicotinic agonists can be blocked by nicotinic cholinergic antagonists such as tubocurarine and hexamethonium (19, 191, 405).

According to Ariens Kappers (16, 17), the CNS of the cyclostomes represents the prototype of the vertebrate brain. The hindbrain is identical in superficial appearance to that of the rest of the vertebrates, with vagal rootlets leaving on either side to innervate the viscera. No study has been made of the topographical representation of vagally innervated structures within the vagal motor column of cyclostomes. In Lampetra, two separate divisions of the vagal motor column have been identified using normal staining techniques: a rostral and a caudal motor nucleus of X (467). The caudal motor nucleus of X, which cannot be delineated from the spinal visceromotor cells, is thought to represent a splanchnic center, and the rostral nucleus is considered to be branchiomotor in nature (i.e., to innervate the branchial pouches) (5). The location of the caudal motor nucleus in cyclostomes, which centers around the obex, is similar to the region of the DVN in the cat (59) and to the nucleus motorius nervi vagi medialis (Xmm) in the dogfish (53) in which the cell bodies contributing axons to the cardiac vagi are found.

C. Elasmobranch Fish

Innervation and control of the cardiorespiratory system in the cartilaginous elasmobranch fishes differs in important respects from that in the teleosts and in the air-breathing fishes. Accordingly, they are each described separately in the following account. The elasmobranchs are phylogenetically the earliest group of vertebrates in which a well-developed autonomic nervous system with clearly differentiated parasympathetic and sympathetic components has been described (465). They are also the earliest group known to have an inhibitory vagal innervation of the heart.

In the elasmobranch fish Scyliorhinus canicula, the vagus nerve divides to form, at its proximal end, branchial branches 1, 2, 3, and 4 that contain skeletonmotor fibers innervating the intrinsic respiratory muscles of gill arches 2, 3, 4, and 5, respectively (Fig. 1). The first gill arch is innervated by the glossophrangeal (IXth cranial) nerve. The vagus also sends, on each side of the fish, two branches to the heart. One arises close to the origin of the visceral branch of the vagus, the other from the post-trematic projection of the fourth branchial branch of the vagus (614). The two cardiac vagi pass down the ductus Cuveri and then break up into an interwoven plexus on the sinus venosus, terminating at the junction with the atrium (665). The sinoatrial node is thought to be the site
of the pacemaker in elasmobranch fishes (542, 551). The remainder of the vagus is termed the visceral branch, and this innervates the anterior part of the gut down to the pylorus and the anterior part of the spiral intestine (665).

Stimulation of the vagus nerve, as well as application of acetylcholine, has an inhibitory effect on heart rate. The effects are antagonized by atropine, implying that the effect is mediated by muscarinic cholinoceptors as in the higher vertebrates (99, 113, 308, 406, 408, 614). Variations in the degree of cholinergic vagal tonus on the heart, in virtue of their locations, be the homologs of the mammalian DVN and nA, respectively (49, 581), and will be referred to as such in the following descriptions of their topography and functional roles.

Retrograde intra-axonal transport of HRP along branches of the vagus nerve showed that the vagal motor column of the dogfish, Scyliorhinus canicula, extends over 5 mm in the hindbrain from 2 mm caudal to 3 mm rostral of obex (657). This agrees with the extent described by Smeets and Niewenhuys (581) for fish of similar size. Caudal to obex there appeared to be two distinct groups of vagal motoneurons, the majority found dorsomedially, and a smaller ventromedial group, both close to the lateral edge of the fourth ventricle. However, the ventromedial group was continuous with cells in the spino-occipital motor nucleus and may constitute a forward extension of this nucleus, contributing axons to the hypobranchial nerve which innervates the ventral muscles of the orobranchial cavity (392). The majority of vagal motoneurons caudal to obex contributed axons to the visceral branch of the vagus including the visceral cardiac branch. Visceral cardiac motoneurons were found solely in the dorsomedial division of the vagal motor column (i.e., the DVN).

Rostral to obex the medial motoneurons were no longer distinguishable into dorsal and ventral divisions; instead, a single column of medial cells was found clustered close to the ventrolateral edge of the fourth ventricle (constituting the DVN). Most of the vagal motoneurons were found in the DVN, with the caudal one-third contributing axons to the branchial cardiac branch and to the visceral branch while the rostral two-thirds contributed axons to the four branchial branches of the vagus (Fig. 6). There is a clear sequential topography in the rostrocaudal distribution of the cell bodies supplying ax-
ons to each of the gill arches, with a small degree of overlap between the pools of neurons supplying adjacent branches of the vagus (657). This sequential topography extends rostrally so that visceromotoneurons supplying axons to the glossohypogeanal, IXth, facial, VIIth and mandibular, Vth cranial nerves, innervating respiratory muscles, are distributed in discrete nuclei in a rostrocaudal array (Fig. 2).

A clearly distinguishable group of cells was identified that had a scattered ventrolateral distribution, outside the DVN, over a rostrocaudal extent of ~1 mm, rostrally from obex. They contributed axons solely to the branchial cardiac branch of the vagus, innervating the heart (53,49). Although the cells in this lateral division comprised only 8% of the total population of VPN, they supplied 60% of the efferent axons running in the branchial cardiac nerve, with the other 40% supplied by cells in the rostromedial division. When the medial cells contributing efferent axons to the heart, via the visceral cardiac branches, were taken into account, then the lateral cells were found to supply 45% of vagal efferent output to the heart. Thus CVPN providing axons to the branchial cardiac nerve are found rostromedially in the elasmobranch equivalent of the DVN and solely comprise the lateral division or nA of the vagal motor column (Fig. 6). It is thought that this dual location of CVPN has important functional implications (see sect. vB).

D. Teleost Fish

In teleost fish, the vagus innervates the gills, the heart, and the viscera (pharynx, esophagus, stomach, and swimbladder). The cardiac branches of the vagi follow the ductus Cuvieri to the sinus venosus and atrium, but vagal fibers do not reach the ventricle. A ganglion in the vagal pathway lies close to the sinoatrial border and appears to consist solely of nonadrenergic cell bodies (212, 272, 273, 382, 545, 546, 664).

The vagus in teleosts is cardioinhibitory as in all vertebrates, with the exception of the cyclostomes. This inhibitory effect is due to the release of acetylcholine affecting muscarinic cholinceptors associated with the pacemaker and atrial musculature (110, 212, 272, 273, 509, 516, 666). Although the negative inotropic influence of the vagi does not reach the ventricle, cardiac output is greatly affected by the inotropic control of the atrium, which directly regulates the filling of the ventricle (307, 313), although this has recently been questioned (J. B. Graham, personal communication).

In contrast to elasmobranchs, where the branchial branches of the vagus are solely skeletomotor (432), the branchial branches in teleosts have both a vasomotor and skeletomotor function (493). The vagus supplies vasomotor fibers to the branchial circulation that have been shown to innervate sphincters at the base of the efferent filament arteries (470).

Early topological studies of the brain of teleosts identified a single vagal motor nucleus (see Ref. 607). However, application of HRP and immunocytochemistry revealed a lateral subnucleus of the vagal complex that provided axons to respiratory muscles in goldfish (452). Application of HRP to the whole vagus nerve and to selected branches of the vagus in cod (Gadus morhua) and rainbow trout revealed that vagal motoneurons were located over a distance of 2.8 mm in the ipsilateral hindbrain from 1.2 mm caudal to 1.6 mm rostral to obex and that ~11% of these neurons were located ventrolaterally, whereas the others were found in a dorsomedial location clustered close to the edge of the fourth ventricle, identified as the DVN (658). The lateral group of vagal motoneurons was divided into two groups: a caudal group extending for ~1 mm (from 0.75 mm caudal to 0.25 mm rostral of obex) and a more rostral group that extended for ~0.75 mm (0.75–1.5 mm rostral of obex). When HRP was applied to the cardiac branch of the vagus, labeled neurons were found in the caudal lateral division as well as in the DVN. The application of HRP to one of the branchial branches of the vagus also labeled both lateral and dorsomedial cells, this time with the lateral cells located in the more rostral group of cells.

The identification of lateral CVPN in teleosts is similar to our findings in elasmobranchs. In contrast, however, some branchial motoneurons are located in the lateral division in teleosts, whereas they are confined to a medial location in elasmobranchs. This may reflect the observation that the branchial branches of the vagus serve both a vasomotor and skeletomotor function in teleosts but only a skeletomotor function in elasmobranchs (see above) so that the medial neurons may give rise to skeletomotor fibers, whereas the lateral neurons may give rise to vasomotor fibers. This remains to be demonstrated experimentally.

In teleosts, there is also a sequential topographic representation of the vagus within the vagal motor column. The most rostral neurons give rise to fibers supplying the most proximal organs (the gill arches), and the caudal neurons give rise to fibers innervating the viscera. The cardiac neurons are located in the middle of the vagal motor column (607). In both classes of fish in which the topography of the vagal motor column has been studied, there is a sequential representation of the vagal branches.

E. Air-Breathing Fish

After application of HRP to the second and third branchial branches of the vagus nerve in the bowfin, Amia calva (Fig. 7), retrogradely labeled cell bodies were found in the DVN over a rostrocaudal distance of 4 mm...
either side of obex (613). There was a sequential topography in the distribution of the cell bodies innervating branchial nerves 2 and 3, as described in dogfish and cod (607). In addition, motor cell bodies were located in lateral locations outside the DVN as described in teleosts (e.g., cod) and all tetrapod vertebrates (608). In the bowfin, some of these lateral cells were of an unusual appearance with large cell bodies and thick, branching dendrites. In one fish, anterograde labeling of the sensory projections of branchial branch 3 of the vagus was observed. This consisted of a diffuse array of fine dendrites and small cell bodies in the sensory vagal nucleus on the laterodorsal edge of the fourth ventricle above the DVN, which extended for \( \pm 2 \) mm in the brainstem immediately rostral of obex.

Application of HRP to the nerve supplying the glottis and ABO revealed cell bodies in a ventrolateral location in the brainstem and the ventral horn of the anterior spinal cord over a rostrocaudal distance of 5.3 mm, predominantly caudal of obex (Fig. 7). From their location it is possible to identify them as cell bodies that typically supply axons to the hypobranchial nerve (i.e., occipital and anterior spinal nerves). Consequently, it is apparent that the glottis and ABO are innervated by nerves of the hypobranchial complex, which provides nerves to elements of musculature normally associated with feeding movements in water-breathing fish. Given that feeding-type movements are implicated in air-breathing in bowfin (397; and see above), the observed hypobranchial innervation of the glottis and ABO may imply nervous coordination of air-gulping and glottal opening, which would ensure effective ventilation of the swimbladder. Indeed, even in mammals, there are functional similarities and a close connection between the central nervous mechanisms controlling breathing and those controlling swallowing (302).

Careful observation of the sections of bowfin brain after application of HRP to the nerve supplying the glottis and ABO revealed an additional group of stained cell bodies that could be identified topographically as preganglionic vagal motoneurons in the DVN (613). This implies that there is a vagal element to the efferent innervation of the ABO, as described by Allis (8). These cell bodies probably provide efferent axons to smooth muscle in the swimbladder wall comparable to the vagal efferents controlling reflex bronchoconstriction in the mammalian lung (559). An afferent vagal supply to the ABO would seem axiomatic but was not revealed by the study of Taylor et al. (613).

**F. Amphibians**

In adult air-breathing amphibians, the vagus innervates the hyoid apparatus and larynx, both structures derived from the larval branchial arches. It then passes on to innervate the viscera, including the heart and lungs. The ventricle in the amphibian heart is completely undi-

![Diagram](http://physrev.physiology.org/)
vided and receives oxygen-depleted systemic blood from the right atrium plus oxygen-rich pulmonary blood from the left atrium. The proportion of the blood ejected from the heart at systole that enters either the pulmonary or the systemic circuit is determined by the relative resistance to flow of each circuit, which is largely determined by the contraction of a smooth muscle sphincter on the pulmo-
cutaneous artery, innervated by the vagus nerve. Vagal stimulation both slows the heart and causes constriction of the pulmocutaneous sphincter. Both responses are primarily cholinergic, although other transmitters such as somatostatin and galanin are coreleased from vagal nerve terminals at both sites (640).

The central topography of the vagal motor column in amphibians is of current interest. In the African clawed toad *Xenopus laevis*, there are two cell groups in the medulla oblongata constituting the motor nuclei of the vagus and the glossopharyngeal nerves, one group in the most superficial zone of the central gray and a second group more laterally in the white matter overlying this central gray (468). A more recent study has confirmed that VPN are located ipsilaterally in the hindbrain over a distance of 2.5 mm with ~32% of all cell bodies identified in a ventrolateral location (640). Each target organ, including the heart and lungs, is innervated by VPN in both the DVN and the nA (Fig. 8), in roughly similar proportions (i.e., ~2:1). The increase in the proportion of lateral VPN, over the condition described in fish, may be partially attributable to a change from gill to lung breathing (609).

As the amphibians metamorphose from an aquatic larval stage to air-breathing adults, they provide an ideal model for testing the hypothesis that progressive ventrolateral location of VPN relates in part to the evolution/development of lung breathing (608). Of particular interest is the axolotl, *Ambystoma mexicanum*, which is neotenous. It retains larval features into the adult (i.e., sexually mature) stage including external gills and gill clefts in the pharynx. The axolotl can be induced to metamorphose into a salamander-like animal by treatment with analogs of the hormone thyroxine, when they lose their gills and leave water to become committed lung breathers. Before metamorphosis, all VPN are in a medial nucleus within the central gray representing the DVN, and there is a clear sequential rostrocaudal distribution of VPN supplying the first, second, and third branchial branches of the vagus rostral of obex, reminiscent of the

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**FIG. 8.** Rostrocaudal distribution of preganglionic vagal motoneurons either side of obex in hindbrain of the ray, *Raja baetis* (a); axolotl, *Ambystoma mexicanum* (b); clawed toad, *Xenopus laevis* (c); and cat, *Felis cattus* (d). Continuous lines indicate cell bodies in DVN; divided lines indicate cell bodies in ventrolateral nuclei associated with nucleus ambiguus (nA). There is a sequential topographic representation of vagally innervated structures in dogfish and in oxolotl, with all vagal presynaptic neurons (VPN) to branchial arches sequentially distributed rostral of obex. However, in axolotl, pulmonary VPN are widely distributed through DVN. This sequential topography remains discernible in *Xenopus* because structures derived from branchial arches (hyoid and larynx) have their VPN rostral of obex, but is virtually lost in cat where all structures are represented over a wide rostrocaudal extent in vagal motor column, both in DVN and nA. [From Taylor (608).]
arrangement described for the dogfish and ray brain stems, with the cardiac and gastric branches of the vagus located more caudally (Fig. 8). The pulmonary branch, supplying the reduced lungs, is widely distributed on either side of the obex. After metamorphosis, there is an increase in the number of VPN, and a proportion of them (~15%) is found in a more lateral location in the white matter of the medulla (287, 640). Presumably, this relocalization of VPN has a functional relevance that may in part relate to the switch from gill to lung breathing (609).

G. Reptiles

The vagus nerve in reptiles runs to the heart, trachea, lungs, pulmonary and coronary vasculature, thymus, thyroid, and gut, supplying preganglionic fibers. The vagus has an inhibitory effect on heart rate (Testudines, Ref. 438; Crocodilia, Refs. 217, 281; Sauropterygia, Refs. 352, 379; Serpentes, Ref. 216), an effect blocked by atropine (e.g., Ref. 216) and therefore cholinergic, as in all other gnathostomes. In addition, a tachycardial response has been reported following vagal stimulation in some species (64, 216, 352, 438). This may be attributable to the existence of a connection between the vagus and sympathetic fibers (64, 216, 352, 438). However, in reptiles, there is little, if any, sympathetic contribution to the cervical vagus nerve, and the sympathetic fibers join the vagus nerve near the heart in both the Crocodilia (217, 218) and the Lacertilia (64, 352, 438). In contrast, a mixed vagosympathetic trunk has been reported in widely different species, including amphibians (112, 217) and mammals (134).

Control of pulmonary blood flow in reptiles is achieved by vagal cholinergic constriction of the pulmonary artery (86, 87, 567, 651, 652). Peripheral electrical stimulation of the vagus or intravenous injection of acetylcholine results both in bradycardia and an increase in pulmonary vascular resistance, which reduces pulmonary blood flow (269). These cardiovascular changes are abolished by administration of atropine. Blood flow is also under adrenergic control. Intravenous injection of epinephrine causes a tachycardia and a reduction in pulmonary vascular resistance, resulting in an increase in pulmonary blood flow (269). Electrical stimulation of vagal afferents in the turtle results in similar cardiovascular changes that are blocked by administration of bretylium (124), suggesting that the cardiovascular changes often associated with brief periods of ventilation may be adrenergically mediated (642). There is also evidence for involvement of nonadrenergic noncholinergic (NANC) factors in the regulation of systemic and pulmonary vascular resistances in reptiles, which may influence patterns of cardiac shunting (642).

Few experimental studies have investigated the central projection of the vagus nerve in the brain stem of reptiles. It is still unresolved which fibers, pathways, and nuclei in the brain stem are specifically related to the efferent and afferent fibers and whether the information obtained from mammals is comparable to that in reptiles. Nevertheless, some information has been obtained regarding the central representation of the cranial nerves (IXth, Xth, XIIth) including sensory nuclei (18, 43, 131, 167), motor nuclei (70, 350, 629), and both sensory and motor nuclei (37, 38, 390).

Applying HRP techniques to some species of reptiles has revealed the central projections of a number of cranial nerves such as the trigeminal nerve (38), facial nerve (37), laryngeal nerve (38, 350), vagus nerve (38, 390), accessory nerve (38), and hypoglossal nerve (38, 350). Barbas-Henry and Lohman (38) described the motor nuclei and the primary projections of different cranial nerves in the monitor lizard and found that the motor nuclei of nerve IX are located ventrally in the brain stem, both medially in the glossopharyngeal part of the nA and laterally in the nucleus salivatorius inferior, whereas the motor nuclei of the Xth nerve are represented in the motor nucleus of the vagus and in a lateral group of cell bodies. The motor nuclear complex of nerve XII consists of a large dorsal nucleus and small ventral nucleus that extends from the medulla oblongata into the first segment of the cervical spinal cord.

In reptiles, early studies described two divisions (medial and ventrolateral) of the vagal motor column in a variety of species, which were provisionally designated as the DVN and nA (5, 15, 18). The pattern of labeling is on the whole similar to that observed after applying HRP to the vagus nerve in mammals, birds, amphibians, and fishes, except for minor differences, such as degree of representation of VPN in the lateral nA or the existence or absence of VPN in other nuclei, such as the nuclei of the spinal accessory nerve. The lateral division, although present in turtles, was more prominent in a lizard and an alligator but absent from a snake (70). A nA was identified adjacent to the DVN in the tortoise (131), and between 36 and 50% of VPN are located in the nA of the terrapin (390). Vocal control neurons are located in the nA of the gekko (350), and comparable motoneurons are present in the nA of mammals.

An initial HRP study of the vagal motor column in the agamid lizard Uromastyx microlepis (M. Al-Ghamdi and E. W. Taylor, unpublished data) revealed that the majority of VPN are in the DVN with a small proportion (6%) ventrolaterally located in the nA. However, there was a clear separation of VPN in the DVN into two distinct groups with a lateral group making up ~13% of the whole. Together with the cells in the nA and reticular formation, laterally displaced cells make up ~20% of the total VPN. All of these cells were cytoarchitecturally distinct from the cells in the medial DVN. An exploratory injection of
wheat germ agglutinin-HRP into the heart marked a few CVPN in the DVN rostral of obex, but none in the nA.

Electrical stimulation of the brain stem caused a pronounced bradycardia, as well as vasomotor responses, in spontaneously breathing, anesthetized pond turtles, *Cyclemys flavomarginata*. The cardioinhibitory response depended on the integrity of the vagus nerves and was particularly marked upon stimulation of areas in the caudal medulla corresponding to the nA, NTS, and DVN (281).

Thus the situation in reptiles seems to vary between species, and the somatotopic representation of the vagus is not yet known. The basis of this variation is likely to be that they are not a homogeneous group, because the present-day reptiles are separated by wide evolutionary divisions (536). The chelonians (turtles and tortoises) are anapsids, a group regarded as primitive, having arisen from close to the ancestral reptilian stock (evolved from primitive amphibians). The snakes and lizards are diapsids, from the same reptilian stock that produced the archosaurs. These in turn evolved into the ruling reptiles (“dinosaurs”) represented today by the crocodiles and alligators and, on another evolutionary line, the birds. Mammals are recognized as having evolved from a separate, primitive reptilian stock, the synapsids. These were remote in evolutionary terms from the lines leading to the present-day reptiles and the birds but may have been closer to their amphibian ancestors and to the primitive chelonians. Thus the disposition of VPN may have phylogenetic as well as functional correlates.

**H. Birds**

Cranial nerves IX and X are closely related, both topographically and functionally, in birds. Their motor nuclei lie in a continuous zone in the medulla oblongata, and they separately supply preganglionic fibers to the heart, lungs, blood vessels, and alimentary canal. An adjacent area in the hindbrain contains motoneurons supplying special visceral efferent fibers to the pharynx, larynx, and palate. Their fibers leave the hindbrain by a series of rootlets that coalesce at the proximal ganglion. This ganglion contains the cells of visceral and somatic sensory neurons which, together with cell bodies of vagal sensory neurons in the more distal nodose ganglion, send afferent projections into the NTS (348).

Vagal preganglionic neurons in the pigeon were localized in the DVN, in an area identified in the ventrolateral medulla as the avian homolog of the nA in mammals (18, 71) and the region of the reticular formation extending between the DVN and the nA (119, 347, 349). The DVN in pigeon is composed of 11 cytoarchitecturally distinct subnuclei in which individual target organs have discrete and topographic representation (347, 349). Some neurons supplying the viscera are located rostral to obex, whereas others are located in a typically sequential position caudal to obex. A lateral subgroup projects to the heart. Similar dorsoventral and rostrocaudal gradients of target representation are seen within vagal afferent projections to the NTS (14, 348). Such distinct topographic separation of groups of neurons innervating specific target organs has clear implications for the central coordination of their functioning.

Until recently, there were no figures available for the proportions of vagal neurons located in the medial (DVN) or lateral (nA) divisions of the vagal motor column in birds. However, current work on the tufted duck, *Aythya fuligula*, has revealed that VPN are located over a rostrocaudal extent of ~5 mm around obex in the medulla, with 3% of cell bodies in the nA and a diffuse area identified as the reticular formation. The majority (97%) of cells are in the DVN where they are separable into subnuclei, according to their cytoarchitecture and topography (72). As yet, the viscerotopic distribution of these cells is uncertain or in dispute.

Although of particular interest to this review, currently there is not a consensus on the topographic location of CVPN in birds. After electrophysiological and retrograde degeneration studies in the pigeon, CVPN were reportedly found exclusively in the DVN, located rostral to obex (119, 555, 556). Here they may be located with, and possibly influenced by, activity in respiratory motoneurons, although their central interactions remain unknown. The reported concentration of the majority of VPN in the DVN as well as the location of the CVPN rostrally in the DVN is similar to the agamid lizard (see sect. vG) but in sharp contrast to mammals, where over 30% of VPN and the majority of CVPN are located in the nA. This may reflect the diverse evolutionary background of these two homeothermic groups, referred to above. However, this conclusion was questioned by Cabot et al. (107) who demonstrated, using fragment C to tetanus toxin as a neural tract tracer, that the majority of CVPN were in a caudal subdivision of the nA in the pigeon, which may be homologous to the ventrolateral nucleus of the nA in mammals. A smaller fraction (10–30%) was located within the ventrolateral subnucleus of the DVN. Studies on the tufted duck recorded an apparent compromise between the two previous studies on pigeon, since ~77% of CVPN were located in the ventral subnucleus of the DVN, with 21% in the nA and 2% in the reticular formation (72). This distribution represents a relative concentration of CVPN into areas outside the DVN as the mean number of CVPN in the DVN represents only ~3% of the total population of VPN, whereas in the nA, CVPN represent ~30% of total VPN. It is important to our further understanding of the central control of the heart and of cardiorespiratory interactions in birds that the exact topographical location of CVPN, in relation to other neu-
rons such as the respiratory CPG, is resolved for a number of species.

V. SYMPATHETIC INNERVATION OF THE CARDIORESPIRATORY SYSTEM

A. Mammals

There is very little information on the central location of sympathetic neurons in lower vertebrates. However, this location has been described in a number of tetrapods and most extensively in various species of mammals. For a comprehensive account the reader is referred to the review by Coote (127). The advent of retrograde labeling of cell bodies with HRP and its conjugates, or fluorescent dyes applied to preganglionic areas, has provided a more detailed picture of the location of preganglionic neurons and their anatomical organization. These techniques have been utilized on the rat (13, 228, 278, 505, 506), guinea pig (426), rabbit (499, 633), and cat (507).

The sympathetic preganglionic neurons (SPN) lie in clusters in four topographically defined nuclei in the intermediate gray on either side of the spinal cord. These four nuclei, named in turn from the edge of the gray matter to the central canal, are the intermediolateralis thoraco lumbalis pars funiculus (ILF), intermediolateralis thoraco lumbalis pars principalis (ILP) or lateral horn, intercalatus spinalis (IC), and intercalatus spinalis pars para ependymatis (ICPe) or central autonomic area. Quantitatively, the majority of SPN are found in the ILP. The arrangement is likely to be the same in all mammals (127).

The rostrocaudal location of SPN in mammals is limited rostrally by the cervical segments, the last cervical segment being the most rostral in which these cells have been identified, for example, cat and rabbit T₁ (507, 633), rat C₈ (279, 505, 506), and guinea pig C₈ (540). The rostral location of SPN seems to be fixed at the last cervical or first thoracic segment, regardless of species or class. The sympathetic neurons come to lie at these locations by a process of migration. Studies in the rat using acetylcholine transferase as a marker (494) indicate that the SPN arise from the ventral ventricular zone of the developing neural tube, migrate radially into the ventral horn, then are displaced dorsally, and finally, a smaller number migrate medially to occupy sites between the ILP and central canal (413, 414). On reaching the ILP, these neurons become increasingly multipolar and may undergo a change in alignment from a dorsoventral and mediolateral orientation to a rostrocaudal one (414, 506). Dendritic orientation also changes from first being dorsolongitudinally organized to more mediolaterally organized as the rat matures (413, 414). There is also the development of new rostrocaudal dendrites (413, 506).

1. Topographical distribution of sympathetic preganglionic neurons

It has been suggested that a clustering of SPN into small groups is related to similarity of function of the neurons (13, 492). This remains to be substantiated. However, there is now good evidence that SPN projecting to different ganglia or to the adrenal medulla are organized into discrete rostrocaudally orientated columns (Fig. 9) (13, 301, 505). The idea of a viscerotopic organization of the cell groups has been extended to the four subnuclei (46–48, 426, 492). Thus SPN projecting to the inferior mesenteric ganglion of the guinea pig are found mainly in the IC and ICPe (133), whereas hindlimb vasomotor neurons appear to mainly occupy the ILP and ILF (426). Also, those neurons supplying the hypogastric nerve in the cat are located in the lower lumbar spinal cord, just medial to
the main portion of the ILP (46) or very medially just dorsal to the central canal in the rat (259). However, it cannot be quite as simple as this, since more recent studies in the rat show that neurons supplying the adrenal medulla, the stellate ganglion, superior cervical ganglion, celiac ganglion, aortic-renal ganglion, or superior or inferior mesenteric ganglion are represented in each of the four subnuclei (279, 506, 591).

A further extension of the idea of functionally organized specific groups of cells concerns their morphology. Two types of neurons, one with round-bodied and one with fusiform somata are most commonly described. A third, rather rare, larger cell type has also been observed (23, 506). These three types have been shown in the projections to the stellate ganglion, superior cervical ganglion, and the adrenal medulla (506). Of interest were the observations in the rat that round-bodied somata are the sole type in the IC, and fusiform somata are the sole type in the ICPe, whereas both types are present in the ILP and ILF (506). Although at present there is no strong reason to connect this morphology with electrophysiology, it is of interest that cat upper thoracic SPN can be classified into three types on the basis of their electrical properties (169). However, the biophysical codes are too few to account for the range of sympathetic functions.

Target specificity has also been related to chemical coding of these neurons. An elegant study in the guinea pig revealed that substance P-immunoreactive SPN projected selectively to postganglionic vasodilator neurons containing vasoactive intestinal polypeptide. In contrast, SPN that were immunoreactive to antibodies to calcitonin gene-related peptide were found to project to postganglionic vasoconstrictor neurons containing neuropeptide Y (NPY) as well as norepinephrine (224, 225). Rat secretomotor preganglionic terminals in the superior cervical ganglion are selectively immunoreactive to calretinin antibody (243). Using a conceptually similar approach in the cat, Krukoff et al. (367, 368) measured four peptides (neurotensin, substance P, enkephalin, and somatostatin) in SPN throughout the thoracolumbar spinal cord. This study failed to show any obvious peptide-specific preganglionic neurons associated with specific viscera. It also appears that nitric oxide synthase is specifically associated with a subpopulation of SPN that projects to adrenal medulla and viscera (244). As with the biophysical properties, at present too few chemical codes have been identified to account for the range of sympathetic functions. Nonetheless, this promising direction of research might be combined with another elegant and powerful technique of transneuronal labeling with neurotropic virus (592). So far, studies on the projections to the kidney and the adrenal medulla in rat, rabbit, and hamster have generally confirmed the viscerotopic organization of the SPN (165, 333, 554).

One of the more interesting features of recent studies of SPN has been the descriptions of their dendritic arbors, which appear to be more extensive than previously assumed. The dendritic arbor and its orientation have functional importance that is relevant to understanding the organization of the columns of spinal neurons that are target specified (506). In mammals (rat, rabbit, and guinea pig), primary dendrites number from six to eight and branch extensively after passing medially, laterally, and rostrocaudally (23, 279, 506, 634). It is now clear that a similar arrangement occurs in the cat (507). The lateral dendrites pass through the bundles of descending axons in the lateral funiculus; the medial dendrites converge from different clusters of neurons to cross the intermediate gray matter in bundles, which on reaching the central canal turn and pass up and down, while some extend further to the lateral horn of the opposite side. The longitudinally oriented dendrites are extensive, running for considerable distances between groups of SPN. These orientations may allow for reception of similar inputs by functionally similar SPN.

2. Sympathetic innervation of the heart

Almost all vertebrates have an excitatory sympathetic nerve supply to the heart. Sympathetic nerve fibers from the stellate ganglia innervate the atria and ventricles as well as the sinoatrial node in reptiles, birds, and mammals. Sympathetic fibers travel by two pathways: a direct one which mainly supplies the nonconducting tissue of the heart and an indirect one where a branch of the stellate ganglion joins the vagus nerve and from where fibers travel to the pacemaker regions and conducting tissue of the heart (342). In amphibians and teleost fish, the cardiac sympathetic innervation is often in the same trunk as the vagus (383). Cyclostomes, Elasmobranchs, Dipnoans, and some teleosts (particularly pleuronectids) lack adrenergic innervation of the heart (383). In those vertebrates without a sympathetic innervation of the heart, the effect of circulating or locally released catecholamines is also excitatory (472). Cardiac cells containing epinephrine or norepinephrine are found in all vertebrates, including lampreys (73). This phylogenetic variability is described in some detail below.

In amphibians, reptiles, birds, and mammals, the sympathetic excitatory influence on the heart is twofold in that it increases both rate and force. These actions in mammals, where the most detailed studies have been conducted, are to some extent dependent on whether right or left sympathetic supply to the heart is activated. In the dog, monkey, and probably human, the cardiac sympathetic projection has highly localized terminal arbors and is capable of causing sharply discrete alterations in the performance of segments of the myocardium (518). Broadly, stimulation of the right sympathetic nerves to the heart in the dog, for example, causes increased heart...
rate via accelerations of cardiac pacemaker activity. It also augments atrial inotropism. Stimulation of the left sympathetic cardiac nerves has a major facilitatory effect on ventricular contractions and also increases conductance and rate via the atrioventricular node (518, 519).

In mammals, postganglionic sympathetic axons innervating the respiratory and cardiac structures in the thoracic cavity are located in independent sympathetic nerves or travel to the effector with the thoracic vagus nerves, via the sympathetic branch from the stellate ganglion (197, 341, 342, 495, 600–602). It is estimated that the thoracic vagus in cats contains ~1,500 sympathetic fibers (197, 450). In comparison, the left inferior cardiac nerve, an independent sympathetic branch of the stellate ganglion, contains on average 2,600 sympathetic axons (374). It has been pointed out by Balkowiec and Szulczyk (28) that this large proportion of sympathetic fibers travelling with the vagus nerve to the heart may contribute to the axo-axonal synapses observed to occur between these nerves (183), providing a morphological basis for the observed cardiac vagal-cardiac sympathetic interaction (252, 366, 502, 524, 644). An electrophysiological study of single sympathetic fibers in the thoracic vagus by Balkowiec and Szulczyk (28) showed they were a homogeneous population, displaying cardiac pulse and respiratory-related rhythmicity in their discharge pattern and decreasing their activity in response to peripheral arterial chemoreceptor stimulation. Consequently, it was argued that they supply a single cardiac effector. It was assumed that the most likely effector was the conducting tissue, because stimulation of the sympathetic fibers in the thoracic vagus is most effective at producing increases in heart rate in dogs (520), cats (56, 341), and sheep (635).

3. Sympathetic nerve supply to airways

There is a large amount of literature detailing the physiology and pharmacology of the smooth muscle and secretory responses evoked by stimulation of airway autonomic nerves in mammals. However, these have been comprehensively reviewed (44, 45, 300, 388), and this review only provides a summary of this work.

The presence and extent of innervation of the smooth muscles by sympathetic nerves varies from species to species. It has been demonstrated in the trachea of guinea pigs (35) and rabbits (172) as well as in bronchi of dogs (360), cats (573), and humans (152, 378, 488). It has been found only rarely or not at all in the bronchi of rats (27, 35, 172), guinea pigs (484), rabbits (412), and humans (172). Even where an innervation has been demonstrated, it is rarely very extensive.

Although sympathetic nerves modulate transmission in enteric ganglia (659), the presence or absence of a sympathetic innervation of airway ganglia has been widely debated. An adrenergic innervation of airway ganglia has been suggested in kittens (360), humans (527), and calves (299) but has been discounted in dogs (299), cats (132), guinea pigs, rats, and mice (35). Even in calves, presumed adrenergic endings were found adjacent to only 10% of principal ganglion cells (299). However, in all species studied, adrenergic innervation of the airway vasculature has been demonstrated, and in many species, SIF cells are present in the ganglia. It is possible that when an adrenergic innervation of the ganglion cells has been demonstrated, the fibers arise from these SIF cells rather than an extrinsic sympathetic innervation.

B. Cyclostomes

The heart of cyclostomes, which is without a sympathetic nerve supply, contains large quantities of epinephrine and norepinephrine stored in chromaffin cells (2, 552). Depletion of this catecholamine store with reserpine causes a marked slowing of the heart rate in these animals (73). Epinephrine, norepinephrine, isoprenaline, and tyramine all stimulate the lampetroid heart, although the effects are less pronounced than the acceleration produced by acetylcholine. The effect of the adrenergic agonists is blocked by propranolol, suggesting an effect via β-adrenoceptors in lampreanoids as in the higher vertebrates (19, 191, 464). The isolated heart of myxinoids is insensitive to exogenously applied acetylcholine and the catecholamines. However, injected catecholamines have marked cardiostimulatory effects in the intact animal, whereas the β-adrenoceptor antagonist sotalol causes a markedly negative chronotropic effect. These responses have been attributed to stimulation of the effects of catecholamines released from chromaffin stores within the heart of the normal animal (474).

Little is known about the origin and nature of vasomotor nerves in cyclostomes, and there is no evidence for vasomotor innervation of their branchial vasculature. However, spinal autonomic nerve fibers, containing adrenergic elements, innervate some blood vessels in lampreys (230), and both catecholamines and acetylcholine increase vascular resistance in Myxine (21).

C. Elasmobranch Fish

The sympathetic system of elasmobranchs consists of an irregular series of ganglia, approximately segmental, lying dorsal to the posterior cardinal sinus and extending back above the kidneys (667). These paravertebral ganglia are arranged approximately segmentally, except in the most anterior part, and there are one or two ganglia connected to each spinal nerve by white rami communicantes (469). The existence of recurrent gray rami communicantes has been denied (665). The spinal autonomic
outflow in elasmobranchs appears to occur chiefly in the ventral roots of the spinal nerves (665), but a dorsal outflow to blood vessels is not excluded (497). The segmentally arranged ganglia are irregularly connected longitudinally and with the contralateral paravertebral ganglia, but there are no distinct sympathetic chains of the type found in higher vertebrate groups. There are no prevertebral (collateral) ganglia in elasmobranchs (469, 473).

The most anterior pair of paravertebral ganglia of elasmobranchs are the axillary bodies that are situated within the posterior cardinal sinuses. These are made up of ganglion cells and also large masses of catecholamine-storing chromaffin cells. The axillary body ganglia receive white rami communicantes from several of the anterior spinal nerves and give off the anterior splanchnic nerves. These are composed mainly, if not entirely, of postganglionic fibers. The left anterior splanchnic nerve crosses to join the right, forming a plexus along the celiac artery to the gut and the liver (465, 665). An anastomosis between the vagus and the anterior splanchnic nerves occurs in some elasmobranchs (666). The paravertebral ganglia behind the axillary bodies are smaller and lie in the dorsal wall of the cardinal sinuses. The suprarenal chromaffin tissue is often associated with the ganglia (as in the axillary bodies) but may exist separately (469).

A peculiarity of the sympathetic system of elasmobranchs is that it does not extend into the head. This condition is unique among vertebrates, but it is not clear whether it is primary or the result of a secondary loss (667). Contributions to the vagi or direct cardiac nerves from paravertebral ganglia are, with rare exceptions (e.g., Mustelus, Ref. 497), absent (297, 406–408, 497, 665). There are only single observations of fibers from the axillary bodies to the heart (Mustelus, Ref. 497). As a result, there is no direct sympathetic innervation of the heart or the branchial circulation in elasmobranchs (465), and there is no evidence for branchial vasomotor control, other than by circulating catecholamines (102, 155).

Circulating catecholamines exert a tonic influence on the cardiovascular system in elasmobranchs under resting, normoxic conditions (572). In elasmobranchs like the dogfish, which again have no cardiac sympathetic innervation, circulating catecholamines are important for maintaining and increasing heart rate (517). It has recently been demonstrated that circulating catecholamines modulate vagal control of heart rate in dogfish. The degree of inhibition of an in situ preparation of the dogfish heart during peripheral stimulation of a cardiac branch of the vagus was found to be modulated by circulating levels of norepinephrine (C. Agnisola and E. W. Taylor, unpublished data). In addition, an adrenergic influence on the heart may be exerted by specialized catecholamine-storing endothelial cells in the sinus venosus and atrium. These cells are innervated by cholinergic vagal fibers (493, 543). The effects of epinephrine and norepinephrine on the elasmobranch heart are somewhat variable, so the possibility of a selective cardiac control via the two naturally occurring amines exists, although the mechanisms of this action remain unknown (469).

D. Teleost Fish

Historically, a sympathetic cardioacceleratory innervation had been generally assumed to be lacking in teleosts (510). However, the sympathetic chains extend into the head where they contact cranial nerves, forming a vagosympathetic trunk (225), and adrenergic fibers have been found to innervate the heart of some teleosts (e.g., trout, Refs. 212, 664). An adrenergic tonus on the hearts of cod (Gadus) and goldfish (Carassius) has been demonstrated, but the relative importance of the neuronal and humoral adrenergic control of the heart remains uncertain (469). The positive chronotropic and ionotropic effects on the teleost heart produced by adrenergic agonists and adrenergic nerves are mediated via β-adrenoceptor mechanisms associated with the pacemaker and the myocardi al cells (111, 212, 272, 516). β-Adrenoceptors generally mediate positive chronotropy in fish and amphibians, whereas α-adrenoceptors mediate negative chronotropy in fish (194, 195, 469). As a whole, the teleosts may be considered as the earliest group of vertebrates in which there is both sympathetic and parasympathetic control of the heart.

There is adrenergic innervation of the branchial and systemic vascular beds in teleost and other actinopterygian fishes. They contain adrenergic fibers that innervate vessels in both the arterioarterial, respiratory circuit and the arteriovenous, nutritive circulation in the gill filaments, possibly controlling and directing blood flow through these alternate pathways (454). Thus the patterns of blood flow in the gills are regulated by vagal cholinergic (see sect. νD) and sympathetic adrenergic fibers that increase vascular resistance by stimulation of muscarinic and α-adrenoceptors, respectively, or decrease it by β-adrenoceptor stimulation (196, 469, 471, 511).

E. Amphibians

The most primitive arrangement of the sympathetic ganglia in amphibians is found in the urodeles (the newts and salamanders). Ganglionic sympathetic chains extend from the level of the first spinal nerve down into the tail. Except in Necturus, the sympathetic trunk does not project into the cranial region, although there are connections with the vagus nerve in all species examined. In Triturus, the subclavian plexus gives rise to cardiac sympathetic nerves. In anurans, all sympathetic innervation to the heart travels in the vagal trunk, whereas urodeles
possess both a vagosympathetic and direct cardiac sympathetic nerves (94, 469). As in fish, chromaffin tissue occurs in the walls of the posterior cardinal veins.

In anurans such as the frog (*Rana pipiens*) and the bullfrog, sympathetic outflow extends from the 2nd to the 10th spinal nerve along the shortened spinal column. The vagus nerve is a major pathway for postganglionic sympathetic fibers reaching the stomach, lungs, and heart so that it constitutes a vagosympathetic trunk (225). Sympathetic preganglionic neurons in spinal segments 2 and 3 innervate the first sympathetic ganglion that sends postganglionic fibers to heart and lungs (663), as well as upper digestive tract and structures in the head. The preganglionic neurons are located between segments 3 to 8 and mainly lie in the ILP and IL in about equal numbers (277, 534).

All the postganglionic neurons in the sympathetic chain of amphibians synthesize epinephrine. Epinephrine is the most important neurotransmitter of the positive chronotropic and ionotropic effects of sympathetic innervation on the heart, but in some species, there is corelease of NPY and ATP (455). In the toad and the bullfrog, the SPN are segregated into larger B and smaller C neurons (277, 454). The small C neurons, with slow conduction velocities, contain the neuropeptides NPY and galanin and innervate blood vessels (277, 454).

**F. Reptiles**

The spinal sympathetic pathways of reptiles and birds have clearly defined paravertebral chains, and some share features not seen in other vertebrates. The sympathetic trunk extends cranially and probably makes extensive connections with cranial nerves (3). The arrangement of the sympathetic ganglia is similar in lizards and chelonians (270). Both have a large ganglion at the level of the branchial plexus, which gives rise to cardiac and pulmonary nerves. A large ganglion in the same position in crocodilians, which corresponds to the stellate ganglion of mammals, gives rise to a prominent cardiac nerve. Catecholamine-synthesizing autonomic neurons project from the paravertebral sympathetic ganglia to all chambers of the reptilian heart. Their nerve fibers either run with the vagus in a vagosympathetic trunk or project directly from the sympathetic chain to the heart. These spinal autonomic neurons exert positive inotropic and chronotropic effects on the reptilian heart, mediated by stimulation of β-adrenoreceptors (455).

In reptiles (e.g., terrapin, *Tryonix sinensis*), SPN are located in the intermediolateral gray matter of the spinal cord, with a majority of neurons in the ILP and IC cell columns and a smaller population dorsal to the central canal (389). In the lower spinal cord segments 13–18 of the turtle, the sympathetic neurons form a small cluster in an area lateral to the central canal, perhaps equivalent to IC (375).

**G. Birds**

In birds, the sympathetic chain extends as a series of segmental ganglia from upper cervical to sacral levels, with the most rostral ganglion lying in the skull between the origins of the glossopharyngeal and vagus nerves. However, the preganglionic neurons have a more restricted rostrocaudal distribution (e.g., chicken and pigeon C14, Refs. 105, 280), and there is no cervical preganglionic contribution to the cervical sympathetic ganglia (225). This is despite birds having more cervical segments than mammals (e.g., 14 in pigeon, 15 in chickens). Most postganglionic neurons in the sympathetic chains contain catecholamines and innervate structures such as blood vessels. In contrast to reptiles, there are well-developed prevertebral ganglia in birds. Splanchnic nerves from the paravertebral chain supply a ganglionated plexus that surrounds the aorta and the origins of the celiac and mesenteric arteries (225).

In some avian species there is no lateral horn in the spinal cord, and the main location of SPN is in the central autonomic area. However, a few neurons in birds are present in more lateral nuclei such as the IC in the pigeon; in the chick, they also appear in an area close to the lateral border of the gray matter equivalent to the ILP (106, 107, 280). Therefore, although SPN are mainly located medially in birds and laterally in mammals, reptiles, and amphibians, their distribution areas are strikingly similar.

In pigeons and chickens, the dendritic arbors of sympathetic preganglionic neurons pass medially, laterally, and rostrocaudally in the spinal cord. The lateral dendrites of the principal nucleus above the central canal converge into bundles that traverse the entire width of the intermediate gray matter and often project into the lateral funiculus. These bundles are like rungs of a ladder in the longitudinal horizontal plane, similar in appearance to those in mammals, although projecting outward (laterally) rather than inward (medially). The medial dendrites pass to the contralateral part of the central nucleus where they form a network (280).

The spinal sympathetic neurons of the upper thoracic segments in birds innervate all chambers of the heart via the stellate ganglia, also contributing to the intracardiac plexus formed by vagal neurons (62). As in mammals, the cardiac sympathetic neurons are tonically active and elicit both chronotropic and inotropic responses, via the release of norepinephrine acting on β-adrenoreceptors (62, 309, 455, 628).

Little is known of the sympathetic innervation of the lungs and airways and pulmonary vasculature in birds.
There is a sparse sympathetic innervation that is without effect on bronchial muscle (66) but supplies the scattered bundles of smooth muscle fibers in the air sacs (245) and the smooth muscle of the oblique septum separating the air sacs from the abdominal cavity (62, 245). A sympathetic vasoconstrictor supply to pulmonary blood vessels has been described (60, 61, 62, 353).

VI. CENTRAL CONTROL OF CARDIORESPIRATORY INTERACTIONS

The importance of a linkage between the mechanisms controlling the respiratory and the cardiovascular systems has long been recognized, since in many physiological responses there are appropriate changes in both systems. For example, during exercise there is a close matching of the respiratory and cardiac outputs, which generate the increase in oxygen uptake and transport. In addition, many reflex responses evoked by stimulation of peripheral afferents evoke change simultaneously in the two systems. This linkage is engendered in the mechanisms that ensure optimal ventilation-perfusion matching within the lungs of air-breathing mammals. It is now becoming clear that there is a similar matching of the counterperfusions with water and blood of the gills of aquatic animals, so (for example, in dogfish) gill ventilatory movements and heart rate are often coordinated (548, 604). Centrally, there are also mechanisms by which these ventilatory and vascular control systems are coupled. They have been discussed in several reviews (121, 135, 137, 329, 532, 605, 607). Although the modification of cardiovascular control by the respiratory system seems to dominate, it is also clear that stimulation of cardiovascular afferents can modify the respiratory system. For example, when arterial baroreceptors are stimulated by rises in arterial pressure, respiratory output is depressed. Even at rest, the coupling between the respiratory and cardiovascular systems may manifest itself. Changes in heart period in phase with breathing (respiratory sinus arrhythmia) are due to respiratory modulation of the vagal parasympathetic innervation of the heart. Similar modulation can also be seen in the activity of many sympathetic nerves (see below), and this may, in part, explain the rhythmical changes in arterial pressure that are sometimes observed, although mechanical changes within the thoracic cavity affecting venous return to the heart are also involved. In both vagal and sympathetic outflows, the respiratory modulation of activity is due in part to activity in the “central respiratory network” and partly to sensory input related to lung inflation or gill ventilation. For a detailed discussion of the autonomic control of the heart and circulation in various vertebrates, the reader is referred to several very comprehensive reviews (135, 194, 307, 313, 383, 457, 469, 470, 472, 570, 607, 609).

A. Mammals

1. Control of heart rate

In mammals, the level of background resting activity in cardiac vagal nerves, and consequent cardiac vagal tone, appears to vary from species to species and also within species. In dogs and humans, heart rate increases markedly after injection of atropine, whereas in anesthetized cats, there is little change in heart rate when atropine is applied (289). In addition, in any particular organ, the relative tone in vagal and sympathetic innervations also varies. Even in those animals in which cardiac vagal tone is low, there is a predominant vagal tone in airway smooth muscle innervation, with sympathetic activity having little, if any, direct action here (322).

Vagal tone appears to derive from ongoing activity in peripheral sensory receptors and from other groups of central neurons (Fig. 10). One important ongoing excitatory drive to cardiac vagal outflow arises from the arterial baroreceptors, the level of vagal drive to the heart being related to the level of arterial blood pressure. Baroreceptor denervation reduces (421) but does not abolish cardiac vagal discharge (290), implying that other inputs must also be important. The arterial chemoreceptors may provide one alternative drive. They have ongoing activity at rest, and hypocapnia produced by hyperventilation produces a tachycardia and reduction in cardiac vagal efferent activity (139, 264). The fact that anesthetics reduce vagal tone (289) may suggest that tonic influences also arise from other parts of the nervous system. In this respect, decerebration produces a vagally mediated fall in heart rate (41), suggesting that there is a tonically active descending inhibitory pathway. This may originate in the hypothalamic defense area, since lesions here also produce cardiac slowing (402). Conversely, stimulation here inhibits cardiac vagal activity (326). This may act via the inhibitory neurotransmitter GABA, since GABA antagonists attenuate the tachycardia evoked by defense area stimulation (41). Vagal tone is greatly increased by stimulation of the superior laryngeal nerve because it induces apnea by inhibiting both inspiratory activity and the consequent lung inflation. This results in an increase in activity in cardiac vagal efferent fibers (329). Evidence suggests that superior laryngeal nerve stimulation may also excite CVPN directly and via a subpopulation of postinspiratory respiratory neurons that show firing patterns similar to CVPN, a situation reminiscent of the synchronous firing of respiratory motoneurons and CVPN in the DVN of the dogfish (see sect. VB).

In mammals, respiratory-related changes in heart period are due mainly to alterations in vagal drive to the
heart, so it is not surprising that respiratory-related variations in activity were seen in recordings from cardiac vagal efferent fibers (154, 291, 292, 303, 346, 373). The fibers fired preferentially during expiration and were also powerfully excited by stimulating the arterial chemoreceptors and baroreceptors, which would account for the cardiac-related component of their activity. The respiratory-related activity survives section of the vagus peripheral to the recording site and is thus of central origin. However, there is also a component due to activity in pulmonary afferents, since vagotomy does abolish the respiratory component if the animals are first hyperventilated to neural apnea. Koepchen et al. (362, 363) proposed that the respiratory modulation of vagal outflow could be explained either by a direct respiratory modulation of the preganglionic neurons or if the excitatory reflex inputs were somehow “gated” before arriving at the preganglionic neuron, or by a combination of the two. Experiments performed in vivo using intracellular recordings from identified CVPN in cats have demonstrated that these neurons do indeed receive a respiratory-related input. During each inspiration, their membrane potential is hyperpolarized due to the arrival of acetylcholine-mediated inhibitory postsynaptic potentials (226) which makes the neurons less amenable to excitatory inputs during inspiration.

A clear role for central respiratory drive modulating ongoing cardiac vagal drive has been elucidated. In addition, however, activity in afferents arising in the lungs also contributes to respiratory sinus arrhythmia (11, 12). Lung inflation inhibits cardiac vagal efferent activity (290, 303, 501) and evokes a tachycardia (142, 143, 211, 255). The effects of lung inflation may be so powerful that they reverse the bradycardia evoked by arterial chemoreceptor stimulation into a tachycardia (137, 143). Unlike the actions of central respiratory drive, the modulatory effects of lung inflation on cardiac vagal outflow do not appear to be imposed at the level of the preganglionic neurons (501).

There are several sites at which the respiratory modulation of reflexes may be imposed before the preganglionic neurons. The NTS where many cardiorespiratory afferents terminate is also the site of the dorsal respiratory group, so this is one possible site. Detailed descriptions of the neural organization and pharmacological modulation of transmission within the NTS have appeared (322, 323, 328, 387). Within the NTS, modulation may occur by presynaptic actions on the sensory terminals themselves, or alternatively, at postsynaptic sites on NTS neurons (see Fig. 11). Afferents from the lungs and airways travelling in the superior laryngeal and vagus nerves are amenable to presynaptic influences, both from central respiratory drive and from activity in other vagal afferents (39, 532). Recent studies have demonstrated that many NTS neurons receiving SLN input also receive convergent input from afferents travelling in the carotid sinus, aortic, and vagus nerves (158, 435). Arterial baroreceptor and chemoreceptor terminals appear to be unaffected by central respiratory activity (324, 327, 530) so that respiratory modulation of these reflexes must occur at a later site in the reflex pathway. This is unlikely to be within the NTS itself since NTS neurons receiving baroreceptor (436, 437) or chemoreceptor (433) inputs failed to show any such modulation of the membrane potential, in phase with either central respiratory drive or lung inflation. Although laryngeal afferent inputs converge onto NTS cells, which also receive input from either lung stretch afferents (P cells, Ref. 63) or central respiratory drive from dorsal inspiratory neurons (173), there is also a population of NTS neurons that receives laryngeal input which do not receive any identified respiratory-related activity (158, 434, 435). Clearly, at least some afferent input can pass the NTS without being modulated.
by respiratory activity. It should not be forgotten that respiratory modulation of baroreceptor firing, induced by the mechanical events of inspiration and expiration altering venous return and hence cardiac filling, cardiac output and blood pressure, is likely to be an important contributor to respiratory sinus arrhythmia in humans (65, 162, 627).

The effectiveness of certain cardiac reflexes is markedly modified by respiration. Brief stimuli applied to the arterial baroreceptors or chemoreceptors only evoke falls in heart rate if they are applied during expiration, with stimuli given during inspiration being less effective or totally ineffective (154, 264). Because the preganglionic neurons themselves are under respiratory control, it might be predicted that any cardiac reflex that is mediated by these CVPN would be modulated by respiration. Indeed, stimulation of trigeminal receptors in the facial skin, receptors in the nasopharynx and larynx, and cardiac C-fiber receptor stimulation all evoke reflex excitation of cardiac vagal outflow that is modified by respiratory drive. The degree of respiratory modulation imposed by lung inflation and central respiratory drive on the bradycardia evoked by stimulation of the arterial baroreceptors and chemoreceptors, cardiac receptors, and pulmonary C-fiber afferents has been compared quantitatively (136, 141). The arterial chemoreceptor-evoked bradycardia was almost abolished by lung inflation and during inspiration, whereas those evoked by stimulating the baroreceptors and cardiac C fibers were reduced by 50–60%. Surprisingly, that evoked by stimulation of pulmonary C-fiber afferents was unaffected by respiration. Several possibilities exist. Stimulation of the pulmonary C fibers may uncouple the linkage between the respiratory and cardiac control mechanisms; stimulation of the different reflexes may modify the central respiratory control system differently, or the bradycardia evoked by one group of afferents may be mediated by a different group of preganglionic neurons to those activated by the other afferents. Comparing the respiratory responses evoked by stimulation of baroreceptors and pulmonary C fibers, Daly et al. (140) could find no evidence for differential effects on the respiratory pattern that would explain the different respiratory modulations of the evoked bradycardias. With respect to the suggestion that there may be two separate populations of cardiac vagal motoneurons, it has been demonstrated recently in both cats and rats that C-fiber cardiac preganglionic neurons in the DVN are activated by stimulation of pulmonary C fibers but are unaffected by the arterial baroreceptors or the respiratory cycle (315–317). The ongoing activity of these neurons is rather regular, unlike those located in the nA that fire with respiratory- and cardiac-related rhythms.

Thus, as demonstrated in the dogfish (see sect. viB), mammals appear to have two separate groups of CVPN that have either tonic or phasic firing patterns and may be topographically separated into groups in the DVN or the nA. This separation arises during embryological development as neurons that form the nA migrate ventrolaterally from a more dorsomedial position, possibly the equivalent of the DVN, in the fetal brain stem (656). Power spectral analysis of recordings of heart rate and breathing movements in human neonates revealed that respiratory sinus arrhythmia (RSA) is a major contributor to heart rate variability (HRV) in healthy term (38–40 wk gestation) newborn infants (621). Although RSA was detected in the near-term fetus (>35 wk), it was not discernible in the fetus before this gestational age or in early premature neonates (<30 wk), appearing later in postnatal development (at ~33 wk). Thus the contribution of RSA to HRV varies both with pre- and postnatal age, which may reflect

![Diagram](http://physrev.physiology.org/)

**FIG. 11.** Diagrammatic representation of organization of second-order neurons within NTS (shaded) that receive monosynaptic inputs from slowly (SAR) and rapidly adapting (RAR) lung stretch receptor afferents and superior laryngeal nerve afferents (SLN). At least 2 discrete groups of P cell (P) are found. One receives SAR input alone, whereas the other also receives SLN inputs. Pβ neurons (a subgroup of dorsal respiratory neurons) receive monosynaptic SAR input and, in addition, an input related to central respiratory activity (CIA). Putative inputs to a group of output neurons (C), thought to be responsible for triggering a cough, are also illustrated. [Modified from Jordan (322, 323).]
a maturational development of the underlying mechanisms (609). This is more likely to reflect myelination of nerve fibers than ventrolateral migration of CVPN at this late stage of development. However, the onset of air-breathing at metamorphosis in the axolotl was accompanied by ventrolateral relocation of a subpopulation of VPN (287, 608).

2. Control of the airways

The respiratory-related modulation of cardiac vagal outflow is not unique. Tracheal muscle tension fluctuates in phase with central respiratory drive (448), and reflexes that increase respiratory drive also increase tracheal tension. Vagal efferent fibers innervating airway smooth muscle have an inspiratory pattern of firing that is augmented when central respiratory drive increases and that is inhibited by moderate degrees of lung inflation (303, 331, 420, 653, 654). The origin of the respiratory-related inputs to the motoneuronal pools is, as yet, unknown. However, there is a close association between the ventral respiratory group and the regions of the α8 containing the VPN (67, 184). Also, because the respiratory rhythm is thought to be generated in the region of the rostroventral medulla (529, 585), it is possible that this is the more likely site. In fact, Mitchell and Richardson (448, 525) have argued that there are probably more than one “respiratory oscillator” within the brain stem. In addition to the eupneic CPG, there is also a slower, and phylogenetically older, CPG for breathing at metamorphosis in the axolotl was accompanied by the ventrolateral migration of a subpopulation of VPN (287, 608).

3. Ongoing activity in cardiac sympathetic and vasomotor nerves

There is a large body of evidence that sympathetic nerves supplying the heart and blood vessels in most vertebrates studied show a continuous activity on the range of 0.1–7 Hz referred to as cardioaccelatory or vasomotor tone (see Refs. 127, 455 for review). For example, a 2- to 6-Hz periodicity is entrained to the cardiac cycle via inhibitory feedback from the arterial baroreceptors (219). This cardiac-related inhibition is dominant in muscle and splanchnic sympathetic vasoconstrictor nerves and probably in cardiac sympathetic nerves (475).

Spontaneous activity of most sympathetic cardiovascular neurons exhibits a respiratory modulation. This has been shown in a number of mammals such as the dog, cat, rabbit, and rat (6, 74–76, 227, 254, 482, 669–671). The mechanisms involved in the respiratory modulation of sympathetic cardiac and vasomotor discharge are complex, probably depending on several components interacting predominantly at the medullary level (253, 531). In general terms, there are two inputs to consider: one a central feed-forward excitatory input related to central respiratory drive and another peripheral feedback mechanism related to lung inflation. This is currently a topic of much interest and debate, and as yet, there is no consensus because of the lack of hard data. It is possible that respiratory neurons of the medulla make synaptic contact with vasomotor neurons situated nearby or both groups of neurons receive synaptic input from a common rhythm generator either directly or via bulbospinal pathways (22, 40, 125, 504, 532, 587). These respiratory inputs may have substantial effects on the excitability of SPN in the spinal cord (170, 399).

At least two afferent influences are likely to be important in a peripheral feedback mechanism. One is stretch receptor afferent input excited by lung inflation. During lung inflations, which were dissociated from central respiratory drive in artificially ventilated cats, the majority of SPN in the third thoracic segment were hyperpolarized (399). However, a minority were depolarized. This might relate to the finding in other studies that...
lung inflation decreases sympathetic activity to the heart yet increases vasoconstrictor activity to peripheral vascular beds (135). A second important peripheral mechanism is that dependent on alterations in baroreceptor afferent activity, caused by changes in arterial pulse pressure consequent on the fluctuations in left ventricular filling induced by ventilation (177, 251, 253, 627).

The evidence relating to central and peripheral influences on sympathetic activity to the heart and blood vessels has been comprehensively reviewed (135, 250, 253, 415); therefore, only a brief summary will be provided here. A respiratory-related discharge has been recorded in whole cervical and abdominal sympathetic nerves in cat and rabbit (6); in postganglionic cardiac and renal sympathetic nerves in cat (25, 345); in mixed pre- and postganglionic splanchic, adrenal, cervical, and lumbar nerves; and in postganglionic cardiac and renal nerves in the rat (482, 669, 670). Evidence obtained in cats and dogs supports the idea that the pattern of respiratory modulation is linked to the function of the sympathetic neurons (74–76, 253). Recordings from cardiac sympathetic nerves and sympathetic vasoconstrictor nerves to skeletal muscle and to skin in the cat indicate that the inspiratory-related pattern of activity is strong in neurons that are involved in cardiovascular regulation (25, 28, 241). Furthermore, it appears that the inspiratory influence is important in determining heart rate and vascular tone. When the central respiratory drive is abolished experimentally (by stimulating the laryngeal nerves), phasic activity in sympathetic nerve fibers is abolished, and there is a small decrease in heart rate and a substantial vasodilatation in hindlimb muscles, even though the tonic activity of sympathetic fibers often increases (22).

Studies in cat and dog show that sympathetic axons supplying the heart and pulmonary vessels have similar activity patterns to those supplying other vascular beds (180, 293, 240, 560, 653, 655). In the case of cardiac sympathetic innervation, studies show that even those nerves that probably have different influences on the heart, like those travelling from the right stellate ganglion but joining the right vagus nerve to supply pacemaker and conducting tissue of the heart, have a similar cardiac rhythm and respiratory periodicity to those nerves in the left sympathetic outflow, which mainly supply the myocardium and increase the force of contraction (28). This ongoing activity appears to be largely dependent on a group of spinally projecting neurons in the ventral medulla oblongata, although spinal sympathetic neurons are able to generate a small degree of “spontaneous” activity and forebrain regions may also contribute (127, 148).

The evidence showing the significance of the ventral medullary region in mammals has been extensively reviewed (109, 148, 403, 404, 588). In summary, the key region lies close to the ventral surface of the medulla, extending from the ventral portion of the inferior olive to the caudal border of the facial nucleus. It is close to the ventral group of respiratory neurons, which are anatomically associated with the Na wherein also lie cardiac vagal motoneurons (188, 532). Electrolytic or chemical lesion of this area abolishes sympathetic vasomotor tone (151, 419), as does application of inhibitory amino acids restricted to this region (160, 247, 403, 418, 537). Increases in sympathetic vasomotor activity are obtained after application of excitatory amino acids to this region (236, 403, 418, 537).

The weight of evidence favors the idea that the ventral medulla oblongata has separate populations of neurons controlling the adrenal medulla, different vascular beds, cardiac acceleration, cardiac slowing, and ventilation, which in turn are connected to their specific target sympathetic neurons in the spinal cord (508). This principle of organization, which may be present throughout the vertebrates, is depicted schematically in Figure 9. Each of these populations of neurons receives a variety of common afferent inputs including arterial baroreceptors, arterial chemoreceptors, trigeminal receptors, and somatosensory receptors (135, 149, 171, 201, 233, 248, 249, 253, 372, 415, 417, 597, 620). It is probable that most, if not all, these afferents converge at the NTS from where they diverge to influence the various functionally distinct populations of cardiorespiratory neurons.

Such an organization is observed in the baroreceptor reflex control of cardioacceleratory sympathetic neurons and cardiac vagal motoneurons. Thus bilateral lesions in a circumscribed region of the ventral medulla oblongata block the vasomotor component of the baroreceptor reflex, leaving the cardiac vagal component intact, whereas a kynurenic acid lesion of Na blocks the cardiac vagal component but leaves the vasomotor component (147, 235, 236).

The question of how tonic activity is generated by the medullary cardiovascular neurons is a topic of current interest. In vitro studies in which intracellular recordings are made from ventrally situated neurons in a cranial slice of the medulla oblongata of rats show that some of these neurons have pacemaker-like activity (343, 394). However, an in vivo intracellular study of identified ventral medulla vasomotor neurons was unable to find evidence of pacemaker-like potentials in these cells (400), thus indicating that tonic activity was dependent on synaptic input. Some of these may be respiratory related (253) and depend on a common cardiorespiratory neural network (532), or alternatively on an independent network oscillator that can become entrained to respiration (42, 219, 220, 411, 475, 476, 672).

The respiratory modulation of ventral medullary vasomotor neurons, observed in cat, rat, and rabbit, is preserved to a variable degree throughout the multisynaptic pathway to the peripheral effectors (253). Some sympathetic outflows, like those to the heart, pulmonary vessels,
skeletal muscle, vascular bed, and kidney, show stronger respiratory-related oscillations in activity than those to other regions (253). A reason for this could be that the respiratory influence at the brain stem level is reinforced at the spinal level by direct respiratory-related input to selected sympathetic neural networks (170, 227, 399, 532, 673).

4. Integrative control

Stimulation of a variety of cardiorespiratory afferents evokes changes in both respiratory and cardiovascular outflows and, at least at the peripheral level, these have been carefully reviewed (256, 415, 440, 529). For example, in addition to altering heart rate and vascular resistance, arterial chemoreceptor stimulation augments respiratory drive. Because the cardiovascular response is modified by respiration, the overall response evoked by chemoreceptor stimulation is complex. The precise effect on the cardiovascular system depends on the level of respiratory drive at the time and on the magnitude of the evoked increase in pulmonary ventilation. In some animals, tachycardia is evoked, whereas in others, a biphasic response or a bradycardia is produced. In fact, the primary response to stimulating the chemoreceptors is a slowing of the heart, and this is always seen if respiration is controlled. However, if respiration is allowed to increase, then this may mask the bradycardia and lead to tachycardia. This has been extremely well documented in recent reviews (137, 138, 146, 415). Similar interactions can be seen in the airway effectors. Stimulation of pulmonary C fibers in apneic animals evokes a constrictor response, but in animals with central respiratory activity, the same stimuli evoke an apnea and its concomitant relaxation of the airways, which fully masks the primary constrictor response (262). These interactions are not simply experimental curiosities; they do occur under normal physiological circumstances. During breath-hold diving, for instance, apnea is evoked by stimulation of facial receptors innervated by trigeminal afferents. The breath-hold leads to a progressive stimulation of the arterial chemoreceptors that would be expected to stimulate breathing. However, the simultaneous stimulation of the facial receptors blocks this respiratory component of the chemoreceptor reflex while at the same time augmenting the cardiac component, to induce a bradycardia (96, 98, 121, 185).

We have described how in mammals there is a tonic cardioacceleratory and vasomotor activity, which is coupled to the respiratory cycle by central and peripheral mechanisms. There are so far no studies in vertebrates other than mammals in which the patterns of sympathetic cardiac and vasomotor activity have been correlated with ventilatory control, of either gills or lungs. Although studies on other vertebrates are sparse, it seems likely that tonic activity in cardiac sympathetic and vasomotor nerves is a common feature. Adrenergic blockade leads to vasodilatation of most vascular beds in fish (470, 472) and in amphibians (453). Interestingly, the branchial vessels of fish constrict after adrenergic blockade because the sympathetic supply dilates the gill vessels via a β-adrenoceptor (470). Removal of the influence of sympathetic nerves either with reserpine or by pithing in frogs leads to a vasodilatation (455), and α-adrenoreceptor blockade causes a decrease in heart rate in several amphibians (455). Furthermore, electrophysiological recordings from sympathetic nerves supplying blood vessels in frogs reveal ongoing activity that may or may not be grouped synchronously with the heart beat (630). The main effect of sympathetic activation on the pulmonary vessels of reptiles is a vasodilatation, brought about by noradrenergic stimulation of β-adrenoreceptors (455). There are no studies in other vertebrates linking sympathetic cardiac and vasomotor activity with neurons controlling ventilation (gill movements or lung inflation). Neither are there any studies showing the location of presympathetic neurons, apart from one report on the toad (species not indicated) showing that synchronous bursts of activity and somatic or visceral afferent evoked responses are abolished only after removing the caudal part of the medulla (474). A review of the organization of pathways between the brain and spinal cord in amphibians and reptiles (619) did not identify respiratory or vasomotor neurons.

B. Fish
1. Cardiac vagal tone

As described previously, the heart in all fish except cyclostomes and in all tetrapods is supplied with inhibitory parasympathetic innervation via the vagus nerve. The inhibitory effect is mediated via muscarinic cholinoreceptors associated with the pacemaker and atrial myocardi um (272). The heart in vertebrates typically operates under a degree of inhibitory vagal tone that varies with physiological state and environmental conditions. Heart rate in the dogfish varied directly with PO2; hypoxia induced a reflex bradycardia, a normoxic vagal tone was released by exposure to moderate hyperoxia, and extreme hyperoxia induced a secondary reflex bradycardia, possibly resulting from stimulation of venous receptors. All of these effects were abolished by injection of the muscarinic cholinergic blocker atropine (607). In addition, cholinergic vagal tone, assessed as the proportional change in heart rate following atropinization or cardiac vagotomy, increased with increasing temperature of acclimation (100, 607, 614). These data indicate that variations in the degree of cholinergic vagal tone on the heart serve as the predominant mode of nervous cardioregulation in elasmobranchs and that the level of vagal tone on
the heart varies with temperature and oxygen partial pressure. A similar reliance of cardiac vagal tone on inputs from peripheral receptors has been identified in mammals (see sect. VI).

In the teleost fish, the heart receives both a cholinergic vagal supply and an adrenergic sympathetic supply. Available data on the extent of vagal tone on the teleost heart give a wide range of values revealing species differences and the effects of different environmental or experimental conditions. Variation in vagal tone affects heart rate, and a vagal, inhibitory, resting tonus has been demonstrated in some species (e.g., Carassius, Ref. 110). In the trout, vagal tone on the heart, although higher than in the dogfish at all temperatures, decreased at higher temperatures. However, the cardioacceleration induced by epinephrine injection into atropinized fish increased with temperature (660). In contrast, an inhibitory vagal tonus was significantly greater in warm-acclimated than in cold-acclimated eels, and blocking vagal function with benzetimide reduced a nearly complete temperature compensation (558). These data indicate that adaptation of heart rate to temperature in the eel was largely mediated by the parasympathetic nervous system. Further evidence for temperature-related changes in heart rate being determined centrally was provided by work on Antarctic fishes, which indicated that the very low resting heart rates in normoxia at around 0°C are attributable to very high levels of vagal tone (20, 607). An exception to this general rule is the sturgeon, which exhibited no change in normoxic heart rate after atropinization (425).

2. Cardiorespiratory synchrony

As mentioned at the outset of this review, the matching of the flow rates of water and blood over the countercurrent at the gills of fish, according to their relative capacities for oxygen, is essential for effective respiratory gas exchange (498). The pumping action of the heart generates a pulsatile flow of blood, which in fish is delivered directly down the ventral aorta to the afferent branchial vessels. To optimize respiratory gas exchange, this pulsatile blood flow should probably be synchronized with the respiratory cycle, which typically consists of a double pumping action, with a buccal pressure pump alternating with an opercular or septal suction pump to maintain a constant but highly pulsatile water flow throughout the respiratory cycle. The flow is maximal early in the respiratory cycle and declines during the last two-thirds of a cycle (283, 286). Thus the supposed functional significance of cardiorespiratory synchrony relates to the importance of continuously matching relative flow rates of water and blood over the countercurrent at the gill lamellae to optimize respiratory gas exchange.

A link between heart beat and ventilation in fish was first noted in 1895 by Schoenlein (cited in Ref. 548), who described 1:1 synchrony in Torpedo marmorata. This observation has been repeated (604). The original observation triggered numerous investigations of the occurrence and mechanisms underlying cardiorespiratory synchrony in fish. Recordings of differential blood pressure and gill opacity in the dogfish revealed a brief period of rapid blood flow through the lamellae early in each cardiac cycle (548), and because the electrocardiogram tended to occur at or near the mouth-opening phase of the ventilatory cycle, this could result in coincidence of the periods of maximum flow rate of blood and water during each cardiac cycle (565, 569). A clear coupling appears to exist since the heart tends to beat at a particular phase of the breathing cycle, for example, immediately after the opening of the mouth. The improvement in gill perfusion and consequent oxygen transfer resulting from pulsatile changes in transmural pressure and intralamellar blood flow (196) may be further improved by synchronization of the pressure pulses associated with ventilation and perfusion. Cardiorespiratory synchrony may, by a combination of these effects, increase the relative efficiency of respiratory gas exchange (i.e., maximum exchange for minimum work).

However, ventilation rate is usually two to three times faster than heart rate in experimental dogfish so that if one ventilatory cycle coincides appropriately with heart beat, then the second or third in a sequence will occur at a totally inappropriate phase of the cardiac cycle (565). Hughes (284) explored evidence for phase coupling between ventilation and heart beat in dogfish released into a fish box that included a movement restrictor. Sophisticated analysis using event correlograms revealed that in some cases the heart tended to beat in a particular phase of the ventilatory cycle for short periods. Use of polar coordinates revealed some significant coupling at varied phase angles between the two rhythms, with individual fish varying in both the degree of coupling and the phase angle, during a period of observation. In the restrained dogfish, ventilation rate was approximately twice heart rate, and these showed a drifting relationship (604, 610). Experimentally restrained dogfish show no hypoxic ventilatory response (99) and no evidence of maintained cardiorespiratory synchrony (284, 610). However, unrestrained fish show reduced normoxic ventilation rates, synchronous with heart beat, as described previously, and also exhibit a ventilatory response to hypoxia (431).

The absence of synchrony, or even consistent close coupling, as opposed to a drifting phase relationship, was most often attributable to changes in heart rate, which was more variable than ventilation rate in prepared dogfish (284, 604, 610). Because they lack sympathetic innervation to the heart, this may be reliably interpreted as variations in cardiac vagal tone. A decrease in vagal tone on the heart, such as that recorded during exposure to moderately hyperoxic water, caused heart rate to rise.
toward ventilation rate (50, 604), suggesting that when vagal tone was relatively low, a 1:1 synchrony could occur. When cannulated dogfish were allowed to settle in large tanks of running, aerated seawater at 23°C, they showed 1:1 synchrony between heartbeat and ventilation for long periods (604). This relationship was abolished by atropine, confirming the role of the vagus in the maintenance of synchrony. Whenever the fish was spontaneously active or disturbed, the relationship broke down due to a reflex bradycardia and acceleration of ventilation so that the 2:1 relationship between ventilation and heart rate characteristic of the experimentally restrained animal was reestablished. Thus it is possible that the elusiveness of data supporting the proposed existence of cardiorespiratory synchrony in dogfish was due to experimental procedures that increase vagal tone on the heart.

The heart in the dogfish operates under a variable degree of vagal tone (see sect. viB1). This implies that the cardiac vagi will show continuous efferent activity. Recordings from the central cut end of a branchial cardiac branch of the vagus in decerebrate, paralyzed dogfish revealed high levels of spontaneous efferent activity, which could be attributed to two types of unit (52, 53, 611). Some units fired sporadically and increased their firing rate during hypoxia. Injection of capsaicin into the ventilatory stream of the dogfish, which was accompanied by a marked bradycardia, powerfully stimulated activity in these nonbursting units recorded from the central cut end of the cardiac vagus (318). Consequently, we suggested they may initiate reflex changes in heart rate, as well as playing a role in the determination of the overall level of vagal tone on the heart, which as stated previously seems to vary according to oxygen supply. Other, typically larger units, fired in rhythmic bursts that were synchronous with ventilatory movements (607). The firing rates of the nonbursting units recorded from the central cut end of the ipsilateral branchial cardiac branch, presumably due to stimulation of mechanoreceptor afferents (607). The firing of the spontaneous, respiration-related units was abolished by atropine, confirming the role of the vagus in the maintenance of synchrony. Whenever the fish was spontaneously active or disturbed, the relationship broke down due to a reflex bradycardia and acceleration of ventilation so that the 2:1 relationship between ventilation and heart rate characteristic of the experimentally restrained animal was reestablished. Thus it is possible that the elusiveness of data supporting the proposed existence of cardiorespiratory synchrony in dogfish was due to experimental procedures that increase vagal tone on the heart.

The separation of efferent cardiac vagal activity into respiration-related and nonrespiration-related units was discovered to have a basis in the distribution of their neuron cell bodies in the brain stem. Extracellular recordings from CVNP identified in the hindbrain of decerebrate, paralyzed dogfish by antidromic stimulation of a branchial cardiac branch revealed that neurons located in the DVN were spontaneously active, firing in rhythmic bursts that contributed to the respiration-related bursts recorded from the intact nerve (51, 54). Neurons located ventrolaterally outside the DVN were either spontaneously active, firing regularly or sporadically but never rhythmically, or were silent. Thus the two types of efferent activity recorded from the cardiac nerve arise from the separate groups of CVPN, as identified by neuroanatomical studies (607).

Activity recorded from the central cut end of the cardiac vagus, or centrally from CVPN, in the decerebrate, paralyzed dogfish is likely to be centrally generated. In the intact fish, stimulation of peripheral receptors will affect patterns of activity. All of the spontaneously active CVPN from both divisions and some of the silent CVPN fired in response to mechanical stimulation of a gill arch, which implies that they could be entrained to ventilatory movements in the spontaneously breathing fish (607). Support for this idea was provided by phasic electrical stimulation of the central cut end of a branchial branch of the vagus in the decerebrate dogfish (M. J. Young, E. W. Taylor, and P. J. Butler, unpublished data). This entrained the efferent bursting units recorded from the central cut end of the branchial cardiac were also increased, suggesting that chemoreceptor afferents were being stimulated as well. Satchell (548) described a cyclical pattern of cardiac inhibition in the dogfish that related to dilatation of the pharynx at each inspiration. This led to phasic increases in vagal tone, superimposed on a tonic background of vagal activity, which he suggested may relate to blood pressure, although there is no evidence of baroreceptor inputs in elasmobranch fish (see above).

Consequently, normal breathing movements in the intact fish may indirectly influence cardiac vagal outflow, and subsequently heart rate, by stimulating branchial mechanoreceptors. Thus the typical reflex bradycardia in response to hypoxia may arise both directly, following stimulation of peripheral chemoreceptors, and indirectly, via increased stimulation of ventilatory effort, which by stimulating branchial mechanoreceptors may increase vagal outflow to the heart. This is reminiscent of, but opposite in kind to, the hypoxic response in the mammal, where stimulation of lung stretch receptors causes an increase in heart rate (144).

These data support a previous conclusion that synchrony in the dogfish was reflexly controlled, with mechanoreceptors on the gill arches constituting the afferent limb and the cardiac vagus the efferent limb of a reflex arc (548). However, the spontaneous, respiration-related bursts recorded from the branchial cardiac nerve continued in decerebrate dogfish, after treatment with curare, which stopped ventilatory movements, suggesting that they originated in the brain stem. Direct connections between bursting CVPN and RVM are possible in the dogfish hindbrain, as both are located in the DVN with an overlapping rostrocaudal distribution (see sect. ivC). Because the bursts are synchronous, the innervation of CVPN is likely to be excitatory rather than inhibitory as described for the mammal, and it is equally possible that a direct drive from a central pattern generator operates both on the RVM and the CVPN (607). The interactions
that may determine the patterns of activity in dogfish CVPN are summarized in Figure 12.

These data from elasmobranchs suggest that cardiorespiratory synchrony, when present, is due primarily to central interactions generating respiration-related activity in CVPN located in the DVN, which are then effective in determining synchronous heart beating when overall cardiac vagal tone, attributable primarily to activity in CVPN located outside the DVN, is relatively low in normoxic or hyperoxic fish. Synchrony will be reinforced in the spontaneously breathing fish by rhythmic stimulation of branchial mechanoreceptors (as described above).

Confirmation that the heart may beat at a rate determined by bursts of efferent activity in the cardiac vagi was obtained by peripheral electrical stimulation of these nerves in the prepared dogfish. Although continuous vagal stimulation normally slows the heart, it proved possible to drive the denervated heart at a rate either lower or somewhat higher than its intrinsic rate with brief bursts of stimuli, delivered down one branchial cardiac vagal branch. At a rate several beats higher than its intrinsic one, the heart responded to alternate bursts of electrical pulses so that it began beating at half the rate of the bursts (Young et al., unpublished data). Interestingly, similar results were obtained from a mammal. In the anesthetized dog, electrical stimulation of the vagus nerve toward the heart with brief bursts of stimuli, similar to those recorded from efferent cardiac vagal fibers, caused heart rate to synchronize with the stimulus, beating once for each vagal stimulus burst over a wide frequency range (393).

Work on teleosts has stressed the importance of inputs from peripheral receptors in the genesis of cardiorespiratory synchrony. Efferent nervous activity recorded from the cardiac branch of the vagus in the tench was synchronized with the mouth-opening phase of the breathing cycle (509). It was suggested that this activity maintains synchrony between heart beat and breathing movements and that both a hypoxic bradycardia and synchrony were mediated by reflex pathways. Randall and Smith (515) described the development of an exact synchrony between breathing and heart beat in the trout during progressive hypoxia. In normoxia, heart rate was faster than ventilation; hypoxia caused an increase in ventilation rate and a reflex bradycardia that converged to produce a 1:1 synchronization of the two rhythms. Both the bradycardia and synchrony were abolished by atropine. In addition, they were able to demonstrate 1:1 synchronization of hypoxic heart rate with pulsatile forced ventilation, which was clearly generated by reflex pathways, presumably arising from mechanoreceptors on the gills, because the spontaneous breathing efforts of the intubated fish were out of phase with imposed changes in water velocity and were without effect on heart beat (515). It is interesting in this regard that heart rate was observed to rise immediately upon the onset of ram ventilation in the trout, implying a reduction in vagal tone (607). Because this can be attributed to the effect of cessation of activity both in the CPG and in the respiratory apparatus, it implies that respiratory activity to some extent generates cardiac vagal tone. This is the obverse of the situation in mammals, where cessation of ventilation, for example during SLN stimulation (see above), increases vagal tone on the heart (329).

Thus we are left with an apparent conflict of evidence on the mode of generation of cardiorespiratory synchrony. In elasmobranchs it may be centrally generated in inactive, normoxic, or hyperoxic fish when cardiac vagal tone is low, whereas in teleosts it appears during hypoxia and is generated reflexly by increased vagal tone. The
differences between these two groups of fish may be real, and it is of interest that branchial denervation increases fictive ventilation rate in elasmobranchs but decreases it in teleosts. However, it is as likely that further experimentation will establish that both central and peripheral mechanisms are important in each group. When cod were cannulated and released into large holding tanks of normoxic seawater, they showed periods of 1:1 synchrony (311, 607). The importance of these observations is that they measured dorsal aortic blood flow, which was markedly pulsatile in phase with variation in buccal pressure, confirming a role for cardiorespiratory synchrony in the generation of concurrent flow patterns of ventilation and perfusion over the gills. Thus both unrestrained dogfish and cod can show synchrony, and as our understanding of the underlying mechanisms increases, it seems likely that elasmobranchs and teleosts will share common characteristics with respect to the generation and potential physiological advantages of cardiorespiratory synchrony.

What emerges from our present understanding is that a potent mechanism for the generation of cardiorespiratory synchrony in fish exists in the form of entrainment of the heart by the bursting units present in recordings of efferent activity in the cardiac vagi, whether these are generated by central interactions, reflexly by stimulation of branchial mechanoreceptors, or most likely by a combination of central and peripheral mechanisms. Entrainment of the heart with the bursts of efferent, respiration-related activity in the cardiac vagi could explain the 1:1 synchrony observed in “settled” normoxic dogfish and cod and in hypoxic trout. As discussed above, cardiorespiratory synchrony may serve to optimize the effectiveness and/or efficiency of respiratory gas exchange and transport in fish.

C. Air-Breathing Fish

Air-breathing fish use their gills for breathing water and intermittently ventilate an accessory ABO. Usually the circulation to the ABO is derived postbranchially. Thus the entire cardiac output is directed toward the gills, but only a portion perfuses the accessory ABO. Selective perfusion may depend on sympathetic α-adrenergic control (195), but because there is a reflex increase in perfusion of the ABO associated with each air breath, often without changes in cardiac output, some local efferent neural mechanism is likely to be important (307, 513). Lungfish intermittently breathe air, during which there can be up to a fourfold increase in lung perfusion and increased cardiac output associated with each breath. Control of pulmonary blood flow involves branchial shunts that are neurally regulated and cholinergic vasoconstriction of the pulmonary artery (205, 310).

D. Amphibians

A recent authoritative review considered the influences of phylogeny, ontogeny, and season on central cardiovascular function in amphibians (89). The author presented a detailed synopsis of our current understanding of the progressive development of vagal, cholinergic and sympathetic, adrenergic control of the heart in amphibians, based largely on the bullfrog tadpole. Early-stage, fully aquatic larvae show no evidence of reflex adjustments of heart rate. Cholinergic sensitivity of the cardiac pacemaker increases during larval development, and a vagal tone on the heart is first apparent at the onset of air-breathing. At metamorphosis, there is a sharp decrease in cholinergic sensitivity, and adult bullfrogs show no resting vagal or adrenergic tone on the heart. However, heart rate varies during intermittent lung ventilation in adult anurans (see below), and an exercise tachycardia results in part from β-adrenergic stimulation and a diving bradycardia from increased vagal tone. These changes during ontogeny are summarized in Figure 13, which is taken from Burggren’s review (89).

Cardiac vagal tone varies widely with temperature in some amphibians. Injection of atropine into Xenopus caused a doubling of mean heart rate from 6 to 12 beats/min at 5°C. At 15°C, the increase was from 9 to 35 beats/min and at 25°C from 12 to 70 beats/min (609, 612). Vagal control has a major modulating effect on the temperature dependency of heart rate with Q10 values between 5 and 10.220.33.5 on October 18, 2017 http://physrev.physiology.org/ Downloaded from

Amphibians have evolved control mechanisms that relate to whether they are more or less committed to lung breathing. Those that are less committed to air-breathing or have no lungs, like the lungless salamanders (Plethodontidae), rely solely on sympathetic adrenergic regulation of cutaneous blood flow to control blood gases (195). Amphibians have evolved control mechanisms that relate to whether they are more or less committed to lung breathing. Those that are less committed to air-breathing or have no lungs, like the lungless salamanders (Plethodontidae), rely solely on sympathetic adrenergic regulation of cutaneous blood flow to control blood gases (195). In intermittent lung breathers like the frog and toad, control of pulmonary blood flow is achieved by a strong vagal cholinergic vasoconstriction of the pulmocutaneous artery that is extrinsic to the lung (112, 647). Vasoconstriction in the pulmonary circuit reduces pulmonary blood flow and increases systemic recirculation of oxygen-poor blood from the right atrium, whereas decreased vagal tone on the pulmonary artery is associated with the increased pulmonary blood flow observed during lung ventilation (647). Adrenergic sympathetic vasoconstriction of the cutaneous circulation contributes secondarily...
to increases in pulmonary blood flow (647). The extent to which withdrawal of vagal tone versus increased sympathetic tone contributes to the increased heart rate and pulmonary blood flows associated with lung ventilation is not resolved, but it may be primarily due to release of vagal tone, because vagotomy or injection of atropine reduces or abolishes cardiorespiratory coupling (640).

Although most anuran larvae show unchanging heart rates during episodic lung ventilation (89), there is a clear cardiorespiratory coupling in adult anurans, in that intermittent lung ventilation is matched by intermittent increases in pulmonary blood flow, without compromising systemic blood flow (383, 566). The mechanisms underlying these relationships are unknown. Recent experiments demonstrated increases in heart rate and pulmonary blood flow during bouts of fictive breathing in decerebrate, paralyzed and through ventilated toads, indicating central control of cardiorespiratory interactions (640). These may in part arise from the overlapping central topography of CVPN and pulmonary VPN (see sect. IV F). Alternatively, stimulation of lung stretch receptors during bouts of breathing may result in release of vagal tone on the heart and pulmonary artery. Artificial inflation of the lungs in anesthetized frogs and toads elicited cardiovascular responses similar to those observed in normally breathing animals, which were abolished by deep anesthesia or injection of atropine (640). However, in conscious Xenopus, denervation of pulmonary stretch receptors did not abolish the increase in heart rate associated with lung inflation (190).

Some of the cardiac responses to intermittent lung ventilation may be generated directly by mechanical or chemical factors (640). In anesthetized toads, artificial lung inflation caused increased pressure in the left atrium and an elevated heart rate that was not abolished by atropine injection, implying that direct mechanical effects on venous return to the heart may contribute to cardiorespiratory coupling. An alternative mechanism has been proposed for anesthetized and unidirectionally ventilated toads, in which hypoxia and hypercapnia reduced pulmonary blood flow. Some of this response may be locally mediated, by a direct effect on vascular tone.

The existence of phase coupling of heart beat with ventilation in amphibians is contentious, although recent observations (T. Wang, E. W. Taylor, S. Reid, and W. K. Milson, unpublished data) indicated that coupling was present for periods of time in decerebrate, paralyzed, and unilaterally ventilated toads, implying generation by central interactions.

E. Reptiles

Reptiles are typically periodic breathers, and during bouts of breathing, the degree of shunting of blood flow to

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**DEVELOPMENTAL LANDMARKS**

No. 13. Ontogeny of cardiac regulation in bullfrog, *Rana catesbeiana*. Horizontal bar represents an individual's lifespan. Major developmental landmarks as well as appearance, modification, and/or disappearance of cardiac regulatory mechanisms are shown. Vertical arrows represent a single observation of indicated event. A vertical arrow combined with a horizontal arrow indicates onset of a continuing process. [From Burggren (89). Copyright 1995 Springer-Verlag.]
the lung increases. Vasomotor control is important in diverting blood between the pulmonary and systemic systems (662). In turtles and lizards, the net direction and magnitude of shunt flow is affected by resistance in the pulmonary circuit, relative to the systemic circuit, by active vagal, cholinergic regulation of pulmonary arterial resistance (268).

Reptiles show clear examples of cardiorespiratory coupling. In the free diving turtle, *Trachemys scripta*, pulmonary blood flow increased more than threefold at the onset of breathing, during recovery from breath-holds lasting longer than 5 min (641). Systemic blood flow also increased during ventilation. These increases were accomplished entirely through changes in heart rate during ventilation, with stroke volume unchanged. Systemic blood flow always exceeded pulmonary flow so that a net right to left cardiac shunt prevailed, regardless of ventilatory state. Nevertheless, because pulmonary flow increased markedly during ventilation, the ratio of pulmonary to systemic flow increased from 0.3 to 0.8. These cardiovascular changes associated with intermittent lung ventilation in discontinuous breathers have been referred to as cardiorespiratory synchrony (e.g., Ref. 641), which is a different use of the term compared with one-to-one synchrony in fish (see sect. VI). In both the turtle, *Pseudemys scripta*, and the tortoise, *Testudo graeca*, the onset of lung ventilation was closely accompanied by a tachycardia (86). As stimulation of pulmonary stretch receptors, arterial chemoreceptors and baroreceptors, or water receptors was without effect on heart rate, it was concluded that this ventilation tachycardia resulted from central interactions between respiratory and cardiac neurons in the medulla. Because the breathing tachycardia was unaffected by β-adrenergic blockade, it seems that all changes in heart rate were mediated by alterations in vagal tone. This was borne out by the observation that efferent vagal activity decreased progressively as heart rate increased at the onset of ventilation. Injection of atropine increased heart rate during apnea to the rate observed during breathing, when vagal tone is low. Heart rate fell slightly before and markedly after hatching in the snapping turtle, *Chelydra serpentina*, indicating the establishment of a vagal tone on the heart, coincident with the onset of lung breathing (69).

**F. Birds**

Neural control of the avian heart was reviewed by Cabot and Cohen (108). The heart in birds is innervated by branches of the vagus nerve that exert a cholinergic, tonic inhibitory influence on heart rate so that bilateral vagotomy causes a marked tachycardia (628). Electrical stimulation of either vagus elicits a profound bradycardia or cardiac arrest in birds. However, there is evidence that functional vagal input to the heart may be asymmetric, with the nerve on one side (often the right) exerting most of the inhibitory influence over heart rate. Cardiac vagal tone is reportedly high in many birds, with bilateral vagotomy causing a tripling of heart rate in the pigeon and duck. Direct evidence of cardiac vagal tone was obtained from the pigeon, in which the majority of CVPN were reckoned to be active in the unanesthetized bird (108). This observation may now be open to question due to the current debate regarding the central location of CVPN in birds (see sect. vG).

The nature of cardiorespiratory interactions in birds has been elucidated to some extent by study of the responses to submersion of diving species (96, 98, 589). Both central and peripheral respiratory drives are overridden by submersion of diving birds. Simultaneous stimulation of water receptors in the facial skin, innervated by the trigeminal (Vth) cranial nerve and in the respiratory tract, innervated by the glossopharyngeal (IXth) and vagus (Xth) cranial nerves, invokes a reflex apnea. There is some evidence that the cardiovascular responses to submersion (bradycardia and vasoconstriction in most vascular beds) may arise, in part, directly from stimulation of water receptors. However, the reflex apnea, because it is associated with cessation of central respiratory drive and phasic stimulation of lung receptors, is most likely a necessary prelude to the full development of these responses. The full development of a diving bradycardia in the mallard duck, *Anas platyrhynchos*, was dependent on the cessation of central respiratory activity and of respiratory movements, and artificial lung inflation during submersion markedly diminished the cardiovascular response (101). Ducks, in common with diving mammals such as seals, usually enter a dive in the expiratory phase. Consequently, their CVPN are likely (on the basis of the mammalian model) to be accessible to afferent inputs, rather than refractory, as they would be if dives were executed in the inspiratory phase (96). The progressive systemic hypoxia, developed during prolonged submersion, then stimulates peripheral chemoreceptors, causing a profound, vagally mediated bradycardia. Penguins and whales, however, dive on inspiration and show a relatively slowly developing bradycardia (96).

Clear indications of respiration-related oscillations in heart rate, similar to the respiratory sinus arrhythmia described in mammals, were recorded in spontaneously breathing ducks. The peaks of the accelerations in heart rate were clipped off when water was poured down an orally facing tracheal cannula, suggesting that they were generated by the intact respiratory rhythm and lost during stimulation of receptors normally responding to submersion, which induce apnea (101). This implies that inhibitory activity in CVPN is modulated by respiratory activity, and as this modulation reduces vagal tone, it is likely to resemble the situation described in mammals where ac-
tivity in inspiratory neurons inhibits CVPN (see sect. viA). There was a slight increase in heart rate on surfacing from a period of forced submersion in ducks with denervated lungs, which was interpreted as evidence for central interactions between inspiratory neurons and vagal cardio-motor neurons (101).

VII. CONCLUDING COMMENTS

As animals our lives are marked by rhythms, and the rhythmical activities of ventilation and heart beat are tangible evidence of the life force in each of us. What we cannot judge by merely feeling or listening are the subtle processes of generation, regulation, and integration of these internal rhythms. Present evidence suggests that in all vertebrates, from jawless fishes to mammals and birds, innately rhythmic neuronal systems in the brain stem generate the respiratory rhythm. The central oscillator driving gill ventilation in fish, initiating lung-breathing episodes in amphibians, and reptiles and promoting ventilation and suckling in neonatal mammals may reside in the reticular formation, suggesting that this center of rhythmicity has a long evolutionary history. Fish use respiratory muscles, innervated by cranial nerves, for gill ventilation, but can recruit hypaxial feeding muscles, into forced ventilation. These same muscles are used to gulp air at the water surface by air-breathing fish and for buccal and lung ventilation in amphibians. They are innervated by the hypobranchial nerve, comprising occipital and anterior spinal nerves, which is the forerunner of the hypoglossal nerve, innervating the tongue of advanced tetrapods. The respiratory, thoracic pump, characteristic of mammals and birds, which requires that the CPG in the brain stem supplies descending fibers to innervate spinal motoneurons, appears in reptiles but may be forshadowed in some amphibians, whereas some reptiles retain the use of a buccal pump. Evidence favors the existence of separate central respiratory rhythm generators for gill/ buccal cavity and ABO/lung ventilation, in air-breathing fish and amphibians, which may be the evolutionary antecedents of the separate areas generating inspiratory rhythms in mammals. The relative importance of afferent input from peripheral mechano- and chemoreceptors in initiating and sustaining ventilation remains unresolved. This whole area presents exciting and important opportunities for continued research, because the mechanisms of control of respiratory rhythms in all vertebrate groups remain incompletely understood. It is, of course, a prime example of an area in which an evolutionary approach to comparative studies is likely to increase our understanding of fundamental mechanisms, as is illustrated by the recent advances of Feldman and his group working on neonatal mammals and Remmers and his group on the frog brain stem (203, 429).

In mammals, the responses to stimulation of arterial chemoreceptors, baroreceptors, and lung stretch receptors are well characterized. Tracing their afferent projections into the NTS has revealed elements of a topographic separation of fibers innervating different organs and from different vagal branches. Slowly adapting pulmonary stretch receptor afferents (PSRA) project rostral of obex; rapidly adapting PSRA more caudally, and bronchial and pulmonary C-fiber afferents project to medial regions of the NTS around obex, together with arterial chemoreceptor afferents. Afferent projections from the upper respiratory tract converge in the trigeminal nucleus. The detailed topography of these projections is likely to be fundamental to their functional roles in controlling the cardiorespiratory system. Similar detail of central projections from reflexogenic sites in the cardiorespiratory system is lacking for other vertebrates, and this is a fertile area for further study.

Peripheral receptors in fish are less well characterized. Only mechanoreceptors and peripheral chemoreceptors sensitive to oxygen partial pressure, both diffusely distributed on the gill arches, have been positively identified by nerve transection and recording. There is strong circumstantial evidence for oxygen content receptors in the arterial and possibly in the venous system of fish, but they have not as yet been localized or characterized. Both peripheral chemoreceptors and pulmonary stretch receptors, which were described as slowly adapting and modulated by CO₂, have been identified and characterized in frogs and toads. Both of these receptor types project to an area of the hindbrain identified as the NTS. The situation so well described for mammals seems to have a long ancestry, extending back to or possibly beyond the evolution of air-breathing.

Central chemoreceptor control of ventilation, although seemingly unimportant in fish, predominates in air breathers from amphibians, through reptiles, to birds and mammals. A clue to the origins of a functional role for central chemoreceptors is provided by ontogenetic studies on amphibians. Fictive ventilation, measured from the bullfrog brain stem, is insensitive to hypercapnic acidosis in early larval stages but becomes progressively sensitized as lung ventilatory bursts begin to predominate over gill bursts, during metamorphosis. This developing sensitivity is coincident with recognizable topographical changes in the nucleus isthmi, an area of the brain stem recognized as being associated with the integration of chemoreceptor responses and the generation of episodic breathing in amphibians and the functional equivalent of the pons in the mammalian brain stem. It also may relate to the ventrolateral relocation of VPN at metamorphosis, noted in the axolotl. Because the ventrolateral medulla is the site of central chemoreception in mammals, it is an entertaining notion that there may be a direct correlation between the relocation of VPN and the onset of central...
chemoreceptor drive in amphibians. Once again, this area presents itself as ripe for further comparative studies of the ontogeny and phylogeny of central chemoreceptor control, to further our understanding of its fundamental nature and functional roles.

Evidence of the existence of baroreceptor responses in fish remains controversial, and the evolution of a role for baroreceptor afferents and for vasomotor control, exercised via the sympathetic nervous system, in control of the cardiovascular system, may be associated with the evolution of air-breathing. The gills of fish are neutrally buoyant, and ventilation of the gills generates hydrostatic pressures in their dense respiratory medium that match blood pressures in the branchial circulation so that the pressure differential is relatively low. Lungs, in contrast, are held in air that provides no support and allows the delicate respiratory surfaces to leak tissue fluid at a rate determined by their permeability, which of course is high, and the pressure gradient from blood to air, which consequently must be closely controlled between defined limits. Possibly because they retain gills, lungfish have similar, relatively low blood pressures in the respiratory and systemic circuits. Differences in pressure between the circuits appear in the amphibians and reptiles but are complicated by the presence of the variable cardiac shunt that can create similar pressures with unequal flows in each circuit. In the mammals, their requirement for a fast circulation time to satisfy their high metabolic rate results in very high arterial blood pressures in the systemic circuit, compared with lower vertebrates. However, the pressures developed in the pulmonary circuit are a little lower than those measured in fish so that there is a 10-fold difference between the pressures developed in the systemic and pulmonary circuits. This difference is of course made possible by their completely separated circulatory system in which flows must be equal on each side while pressures are very different. Despite this separation, it is important that blood pressure is maintained below a maximum to protect against leakage from the lung or lung damage. Consequently, there are clear roles for baroreceptors monitoring blood pressure and vasomotor control of peripheral resistance in lung breathers, which are not present in gill breathers. Control of minimum pressures are of course equally important, particularly for kidney and brain function, and no doubt these requirements also vary between fish and mammals.

Regulation of heart frequency is essentially similar in mammals, birds, reptiles, amphibians, teleosts, and elasmobranchs. With the exception of the jawless fishes, the cyclostomes, all vertebrates have an inhibitory muscarinic, cholinergic supply to the heart via the vagus nerve. A cardioexcitatory β-adrenergic innervation is provided via sympathetic nerve trunks in all vertebrates other than the cyclostomes and elasmobranchs. Vagal inhibition of heart rate seems to predominate in most vertebrates, but cardiac sympathetic excitation becomes more prominent in endotherms. The heart in most vertebrates operates under fluctuating levels of inhibitory vagal tone. This varies with temperature, hypoxia, disturbance, and anesthesia and fluctuates with the breathing cycle, the source of cardiorespiratory synchrony in fish and respiratory sinus arrhythmia in mammals. Sympathetic outflow to the heart and peripheral circulation, responsible for vasomotor tone, as well as outflow to the upper respiratory tract, also shows fluctuating levels of activity, with respiration-related components. It is interesting to hypothesize that respiration-related rhythmicity in the nervous supply to the cardiovascular system may serve to optimize its functional integrity, as well as respiratory gas exchange and transport. These possibilities would seem to invite further functional studies.

The central origins of fluctuations in vagal tone on the heart, and the consequent variability of heart rate, together with the origins of fluctuations in vasomotor tone, in the different vertebrate groups have been a central theme of this review. Central interactions appear to originate partially as a result of the convergence of sensory projections noted above, and the specific, and sometimes overlapping, distribution of preganglionic and visceral motoneurons in the brain stem and spinal cord. For example, comparative neuroanatomical studies have revealed that the distribution of VPN between the DVN and the ventrolateral nA varies between groups. In elasmobranch fish, only 8% of VPN are in the nA, but they are all CVPN. This ventrolateral group of cells seems to determine the reflex responses of the heart to external stimuli, such as hypoxia, whereas the CVPN in the DVN show respiration-related activity, which may generate cardiorespiratory synchrony. This topographical and functional separation of CVPN has since been found to be reflected in the brain stem of mammals, in which two functionally separate populations of CVPN have been identified, only one of which responds to pulmonary stretch receptor inputs. The most recent evidence suggests that, as in dogfish, these are topographically separated between the nA and DVN, indicating that functional separation of VPN may be fundamental to their control functions. This clear link between the primitive elasmobranch fishes and the mammals is bridged by an intriguing phylogenetic progression. In bony fishes, ~12% of VPN are located ventrolaterally outside the DVN; these are predominantly CVPN, but some cells supply axons to branchial (respiratory) branches of the vagus. This proportion rises to 15, 20, and even 30% in the different classes of amphibians with the ventrolateral relocation of VPN outside the DVN occurring at metamorphosis, concurrent with the onset of episodic lung breathing and central chemoreceptor responses. This proportion stabilizes at 30–40% in mammals, with up to 80% of CVPN located in the nA, together with respiratory motoneurons, from which they
receive inhibitory inputs, generating respiratory sinus arrhythmia, an arrangement established during embryological development. However, this neat progression is confused in the markedly polyphyletic reptiles, with the turtles and some lizards having high proportions of VPN in the nA, and other lizards and alligators having <5% of VPN in this location. This alternate pattern characterizes those near ancestors of the dinosaurs, the birds, with <3% of VPN found in the nA of the duck. Interestingly though, a large proportion of the VPN in the nA of the duck are CVPN. Separate functions have yet to be assigned to the CVPN in the dual locations in the brain stems of amphibians, reptiles, and birds. Further study of this area seems likely to be of very great interest and of importance to our understanding of the development and evolution of the control systems associated with air-breathing. The amphibians should be a primary target for these studies because they metamorphose from committed gill breathers to facultative lung breathers, and we already know that this process is accompanied by topographical changes in appropriate regions of the CNS. A combination of neuranatomical, neurophysiological, and functional studies of the kind previously applied to mammalian species should uncover new and fascinating insights into this exciting area of study as amphibian ontogeny, at least in part, recapitulates vertebrate phylogeny.

REFERENCES


