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Review

Control and interaction of the cardiovascular and respiratory systems in anuran amphibians

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Abstract

In anuran amphibians, respiratory rhythm is generated within the central nervous system (CNS) and is modulated by chemo- and mechanoreceptors located in the vascular system and within the CNS. The site for central respiratory rhythmogenesis and the role of various neurotransmitters and neuromodulators is described. Ventilatory air flow is generated by a positive pressure, buccal force pump driven by efferent motor output from cranial nerves. The vagus (cranial nerve X) also controls heart rate and pulmocutaneous arterial resistance that, in turn, affect cardiac shunts within the undivided anuran ventricle; however, little is known about the control of central vagal motor outflow to the heart and pulmocutaneous artery. Anatomical evidence indicates a close proximity of the centers responsible for respiratory rhythmogenesis and the vagal motoneurons involved in cardiovascular regulation. Furthermore, anurans in which phasic feedback from chemo- and mechanoreceptors is prevented by artificial ventilation exhibit cardiorespiratory interactions that appear similar to those of conscious animals. These observations indicate interactions between respiratory and cardiovascular centers within the CNS. Thus, like mammals and other air-breathing vertebrates, the cardio-respiratory interactions in anurans result from both feedback and feed-forward mechanisms.

Keywords: Amphibian; Anuran; Ventilation; Cardiovascular; Cardiac shunts; Blood flows; Central rhythm generation; Afferent regulation; Central interaction; Cardiac vagal motoneurons

1. Introduction

In vertebrates, the respiratory and cardiovascular systems supply oxygen to the tissues and are responsible for removal of metabolically produced CO_2. Coordinated responses of these systems are important to accommodate the delivery and removal of respiratory gases whenever metabolic demands are increased or during altered gas composition in the environment. Accordingly, it is not surprising that the cardiovascular and respiratory systems of all vertebrates are functionally linked and that they often exhibit tight interactions. For example, changes in heart rate with ventilation occur in all vertebrate groups and, in spite of the large anatomical differences, it is believed that the essential components of the cardiorespiratory control systems are similar among the different vertebrate taxa (e.g. Ref. [97]).

Anurans (i.e. frogs and toads) have aquatic, gill-breathing larvae and as adults are amphibious, inhabiting both terrestrial and aquatic habitats. Gas exchange is bimodal as they use both the structurally simple lungs, ventilated by muscles in the buccal cavity, and the skin for oxygen uptake and CO_2 excretion. Their cardiorespiratory systems are complex in comparison to other vertebrates being characterized by an incom-

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pletely divided ventricle with blood flow to the lungs, skin and body controlled independently. Thus, cardiorespiratory responses to increased metabolism or hypoxia can consist of increased ventilation, increased heart rate and/or redistributions of blood flows (see Ref. [106] for theoretical considerations). Nevertheless, because amphibians are positioned centrally in the phylogeny of air-breathing vertebrates, an understanding of the mechanisms controlling their cardiorespiratory systems may reveal the fundamental properties that were associated with the evolutionary emergence of air-breathing.

In the following review we consider some aspects of the cardiorespiratory responses and their underlying control in anuran amphibians. Recently, West and Van Vliet [109] provided an excellent and in-depth review of the role of peripheral chemoreceptors and baroreceptors in amphibians. In the present account we focus on the control of cardiorespiratory interactions, the role of centrally generated rhythms and the central integration of afferent input.

2. General considerations

As a common feature, most cardiorespiratory responses are initiated by chemo- and/or mechanoreceptors that sense, for example, changes in blood gas composition or lung stretch (afferent pathways in Fig. 1). Central integration of the afferent feedback from these receptors ultimately leads to modified motor output to the respiratory muscles and efferent control of the cardiovascular system (efferent pathways in Fig. 1). In addition to reflexes that are mediated by receptors, cardiorespiratory responses can be generated within the central nervous system (CNS) and, thus, serve as a feed-forward mechanism. These two mechanisms are not mutually exclusive and it is entirely possible that both occur simultaneously in many instances.

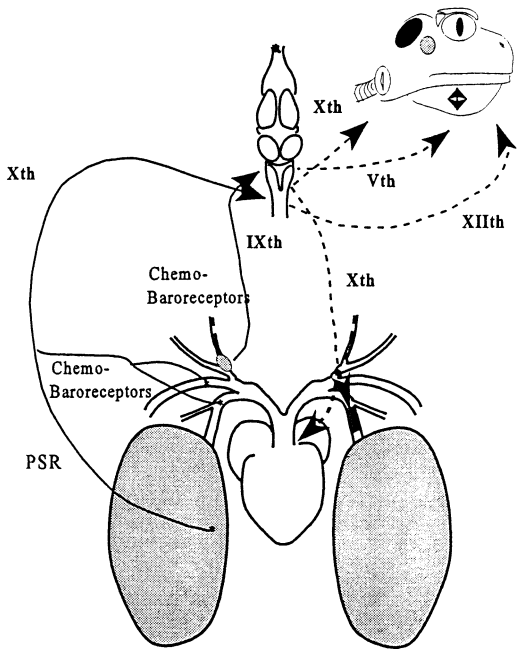

Fig. 1. The afferent and efferent nervous pathways involved in control of the cardiovascular and respiratory systems of anuran amphibians. Afferent pathways are shown on the left side of the diagram as solid lines, whereas the efferent pathways are depicted as the dotted lines on the right side of the figure. Roman numerals refer to cranial nerves.

3. Control of ventilation in anurans

3.1. Generation of central respiratory rhythm

The neural substrate for the generation of the respiratory rhythm in vertebrates is contained exclusively within the brainstem [9]. Thus, although structures higher in the CNS (e.g. forebrain, midbrain) certainly influence breathing, they are not essential for respiratory rhythmogenesis. In reduced preparations from a wide range of vertebrate species, in which the brainstem is isolated (see below), respiratory-related neural outputs appear remarkably similar. This has led to the hypothesis that the neural circuits generating breathing and other motor functions have been highly conserved during vertebrate evolution [100]. This intriguing suggestion is difficult to test experimentally in the absence of adequate information about the location and functional properties of the neurons that generate central respiratory rhythm in vertebrates. Although there is a growing body of information concerning the mechanisms that generate the respiratory rhythm in the brainstem of mammals (e.g. Refs. [2,15,75]), very little is known about similar mechanisms in non-mammalian vertebrates.

There are essentially two hypotheses that explain respiratory rhythmogenesis in the brainstem: neural network interactions that require synaptic inhibition and pacemaker neurons that generate an intrinsic rhythm independent of synaptic interactions [75,76]. In mammals, both mechanisms may play a role in generating central respiratory rhythm. Regardless of the underlying mechanism, the output of the respiratory rhythm generator is considered responsible for driving a respiratory pre-motor network characterized as a central pattern generator (CPG). Thus, the neural elements that generate central respiratory rhythm are distinct from those that regulate the timing and pattern of the respiratory CPG. A variety of intrinsic modulatory and feedback mechanisms shape the timing and burst characteristics of the respiratory CPG, so that transmission of respiratory drive to the motoneurons that innervate respiratory muscles allows for appropriate adjustments of ventilation.

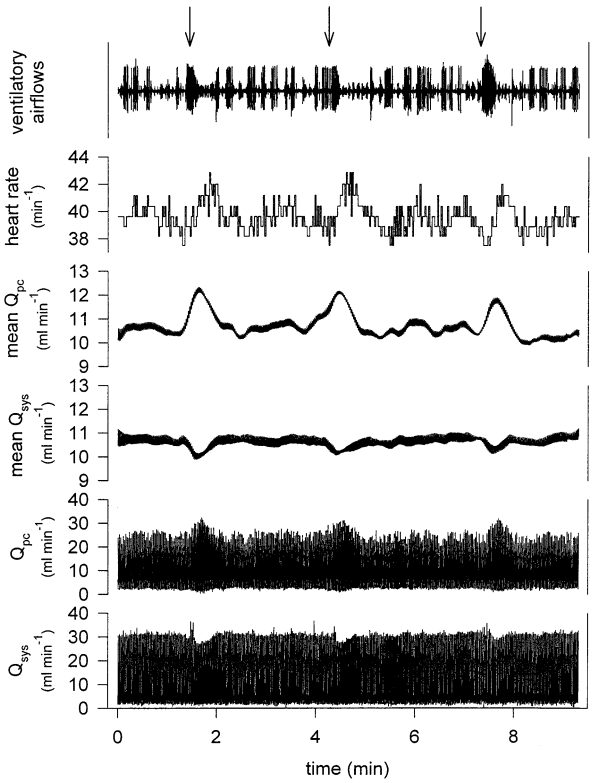

Fig. 2. Cardiorespiratory coupling in a resting, undisturbed and conscious toad (*Bufo marinus*) maintained at 25°C and while breathing room air. This animal was equipped with Transonic flowprobes on the pulmocutaneous artery and the systemic arch. Following surgery the toad was allowed to recover for 48 h before measurement. Ventilatory air-flows were measured with the pneumotachographic technique (e.g. Ref. [105]). Lung inflation cycles are indicated by the arrows (Hedrick, Andersen and Wang, in prep.).

3.2. Ventilatory patterns in anuran amphibians

Anuran amphibians typically display three distinct types of ventilatory behaviors: buccal oscillations, lung ventilations and lung inflation cycles [19,46,104]. Buccal oscillations are rhythmic elevations and depressions of the buccal musculature that result in tidal airflow between the atmosphere and buccal cavity through the open nares. The function of buccal oscillations is unknown, but the may play a role in olfaction [109] or serve to flush the buccal cavity during non-ventilatory periods [46]. Lung ventilations are intermittent or episodic ventilatory events also generated by the buccal pump. A lung ventilatory cycle begins with a more forceful buccal depression drawing air into the buccal cavity. Immediately following this inhalation, the glottis opens, allowing exhaled lung gas to escape through the open nares [46]. Lung inflation is then accomplished by simultaneous narial closure and buccal elevation which forces buccal air into the lungs, through the open glottis. The glottis then closes and the inflated lung is held at a positive pressure. Lung inflation cycles are essentially a series of lung ventilation cycles with no associated expiratory phase, so that the lungs are progressively inflated. Lung inflation cycles may be a behavioral defense response, but also occur in resting animals and become the dominant breathing pattern in some animals under experimental conditions such as hypoxia or hypercapnia ([60,105,107]; see also Fig. 2). The typical breathing pattern in anurans consists of buccal oscillations interrupted by single lung breaths or episodic breathing bouts consisting of two, three or more breaths in succession [68].

Expiration in anurans is entirely passive, with airflow generated by elastic recoil of the lungs and body wall when the glottis opens [19,46]. However, during sustained high rates of vocalization in *Hyla versicolor* and *H. chrysoscelis*, hypaxial musculature (external oblique) is recruited and provides muscular effort for active expiratory airflow during the calling cycle [31]. This suggests that, at least during vocalization, hypaxial muscles participate in expiratory control, although it remains to be determined whether they are involved in breathing under other conditions. If active expiration in anurans were to be demonstrated, it would imply that descending fibers from the brainstem, innervating spinal motoneurons have an important role in ventilation. Interestingly, in metamorphosed tadpoles, lung ventilation is associated with nervous activity in spinal nerve II [29,103].

3.3. Central control of ventilatory patterns

The central nervous control of breathing in anurans has been little studied and much more is known about central control of breathing in mammals (recently reviewed by Refs. [2,75]). However, in recent years several laboratories have started to use reduced preparations from anurans as models for examining central respiratory rhythm generation and pattern formation in vertebrates. These preparations include decerebrate, paralyzed and artificially ventilated animals as well as brainstem preparations from larval (tadpoles) and adult anurans that exhibit spontaneous, respiratory-related neural outputs in vitro.

The buccal and glottal musculature which generates ventilatory events in adult anurans are innervated by cranial nerves V, X and XII ([19,79]; Fig. 1). In tadpoles, which use a combination of gill and lung ventilation (e.g. Refs. [29,103]), the gill musculature is also innervated by cranial nerve VII, as described in fish [84]. In decerebrate, paralyzed and artificially-ventilated adult anurans, neural activity from cranial motoneurons that control respiratory muscles exhibits a precise spatial coordination and timing that is identical to the pattern of muscle activation observed in conscious animals [19,57,79]. Thus, recording neural efferent activity from these cranial nerves provides a useful neural index for breathing, characterized as central respiratory drive.

This reduced preparation allows for greater experimental control and manipulation of variables such as blood gases and lung volume than is possible in the conscious animal.

The in vitro brainstem preparation from anuran amphibians has proven very useful for examining the basic mechanisms for respiratory rhythm generation [27,58,64–66,74]. Whereas conscious [19] and decerebrate, paralyzed anurans display identical neuromuscular coordination of respiratory motor output [57], the in vitro brain stem preparation exhibits some important differences. Although it produces a spontaneous, respiration-related neural output from cranial nerves V, VII, X and XII, referred to as 'fictive breathing', burst activity with each fictive breath is often initiated simultaneously in all cranial nerves [27,54,65]. Thus, it appears that removal of peripheral sensory feedback disrupts the timing of the pre-motor neural network that drives respiratory motoneurons leading to a loss of the precise spatial and temporal motoneuronal output in vitro compared to that normally observed in vivo. This suggests that afferent feedback from peripheral receptors may be necessary for the precise timing of buccal and glottal musculature during normal ventilation. However, pulmonary and cardiac vagotomy does not disrupt the normal timing of respiratory output in decerebrate, paralyzed bullfrogs [57]. Recently, Kimura et al. [51] used a modified in vitro/in situ preparation of *Rana pipiens* that exhibits normal spatio-temporal activation of efferent cranial nerve output. In their study, perfusion of the brainstem with strychnine, a glycine receptor antagonist, changed this normal activation cycle to a synchronous activation of cranial nerve outputs. Although it is not clear to what extent this preparation has intact peripheral afferent pathways, their data suggest that glycinergic, Cl^--sensitive inhibitory mechanisms in the central nervous system (CNS) may be responsible for the precise timing of respiratory motor output in anurans.

Anuran amphibians are particularly good models for examining the ontogenetic changes that occur in the CNS during the transition from aquatic to aerial breathing. Torgerson et al. [101] have recently shown that fictive breathing in *Rana catesbeiana* tadpoles exhibits dramatic changes in central chemosensitivity to CO_2 during developmental metamorphosis. These findings are in agreement with previous experiments on intact animals. Moreover, with the appearance of central chemosensitivity, there appears to be a concomitant regression of the gill CPG and increased functional role of the lung CPG [101]. Perfusion of the tadpole brainstem preparation with Cl^--free solutions or with antagonists of GABA-ergic or glycinergic-dependent Cl^- conductance channels abolished fictive gill ventilation, while fictive lung bursts remained [27]. These data suggest there are two distinct CPGs that regulate gill and lung ventilation in the tadpole brainstem. Because Cl^--dependent synaptic inhibition did not abolish fictive lung ventilation, the data further suggest that the lung CPG may be driven by 'pacemaker-like' rhythm-generating neurons. A similar mechanism has been proposed for respiratory rhythmogenesis in mammals [75,89]. By contrast, the gill CPG in tadpoles may be controlled by conventional synaptic inhibition [27]. It has been hypothesized that buccal oscillations and lung ventilation in adult anurans represent output from two distinct CPGs [52]; however, there are no conclusive data to support this hypothesis.

Recent experiments have examined the role of various neurotransmitter systems in the control of fictive breathing in the anuran brainstem. Fictive breathing frequency is increased by a number of neurotransmitters/neuromodulators including glutamate [64], nitric oxide (NO) [35] and β-adrenoceptor stimulation [71] and inhibited by GABA [64]. Microinjections of glutamate into rostral areas of the bullfrog brainstem, near the VIIth motor nucleus, caused a brief excitation of fictive breathing [64]. Interestingly, these areas of glutamatergic stimulation correspond approximately to the site of the pre-Bötzinger complex in the mammalian brainstem that is considered to be the primary source of respiratory rhythmogenesis [75,89]. In amphibians, rostral areas of the brainstem appear to be important for the generation of respiratory rhythmogenesis. However, these areas appear to be more diffusely organized, compared with the mammalian brainstem.

Respiratory rhythm generation and neurotransmission of respiratory drive in neonatal mammals requires the excitatory amino acid, glutamate, acting primarily at non-*N*-methyl-D-aspartate (non-NMDA) receptors [75]. Topical application of antagonists to non-NMDA receptors or microinjections of antagonists into the pre-Bötzinger complex abolish respiratory rhythm in the in vitro neonatal rat brainstem [26,33,75]. In adult mammals, however, NMDA receptor blockade is also required to completely abolish respiratory rhythm in vivo. In anurans, it is not clear whether the excitatory effects of glutamate microinjection [64] are mediated through stimulation of NMDA and/or non-NMDA receptors. Inhibition of neuronal nitric oxide synthase (nNOS) reversibly abolishes fictive breathing in the bullfrog brainstem in vitro [35], suggesting that NMDA receptors are important for respiratory rhythm generation. Because NO is produced through glutamatergic stimulation of the NMDA receptor [8], abolition of respiratory rhythm by nNOS blockade suggests that central respiratory rhythmogenesis in anurans may be functionally coupled to NMDA receptors. However, the link between glutamate, NMDA receptors and NO production has not been established in the amphibian brain. The observations that NO is an excitatory neurotransmitter for breathing in the amphibian brainstem

[35] is consistent with experiments in mammals where NO has been shown to be excitatory and important for the transduction of hypoxic stimuli in the CNS [32]. The observations that glutamate and NO stimulates, and GABA inhibits, fictive breathing in the amphibian brain is consistent with the effects of these neurotransmitters in the mammalian brain [2]. Thus, there may be some common control mechanisms with regard to generation of central respiratory rhythm in mammals and some ectothermic vertebrates. The comparable roles of excitatory and inhibitory amino acids in the brainstem of vertebrates is consistent with the hypothesis that neural circuits and their control are conserved features of vertebrate brain evolution [100]. Clearly more work is necessary to establish the nature and evolution of the mechanisms for generating central respiratory rhythmogenesis in vertebrates.

4. Receptors involved in the regulation of ventilation

A number of receptors modify the ongoing respiratory rhythm generated in the brainstem to maintain adequate airflow to meet metabolic demands (reviewed by Refs. [45,68,69,84]). The major pathways influencing ventilation are peripheral chemoreceptors in the central vasculature, central chemoreceptors in the brain and lung mechanoreceptors [109]. However, other receptors, such as those in the olfactory region, have also been shown to influence ventilation in anurans [16,55,78].

It has been hypothesized that oscillations in blood gases (PaO_2 and $PaCO_2$) and/or pH are partly or entirely responsible for the intermittent breathing patterns characteristic of anurans (see Ref. [84]). However, whenever these oscillations in blood gases are prevented by unidirectional ventilation (UDV) of the lungs in conscious or decerebrate animals, intermittent breathing patterns are not fundamentally altered, unless made hypocapnic and/or hyperoxic by UDV, thus reducing chemoreceptor drive, breathing generally ceases (e.g. Refs. [53,110]). This has been interpreted to suggest that a minimal level of peripheral and/or central chemoreceptor input to the CNS is required for respiratory rhythm generation.

4.1. Central chemoreceptors and control of ventilation

Anurans exhibit strong responses to hypercapnia, and the central chemoreceptors responding to changes in PCO_2/pH are an important source of respiratory drive. In lightly anesthetized or conscious toads, perfusion of the brainstem with artificial cerebrospinal fluid (aCSF) equilibrated with high PCO_2/low pH significantly increased ventilation [6,7,87]. In these studies, central chemoreceptors were shown to contribute approximately 70–80% to overall ventilatory drive, and this contribution was largely independent of body temperature between 15 and 35°C [7].

The in vitro brainstem preparation of *R. catesbeiana* has been used to examine central chemosensitivity and whether temperature plays a role in modifying central chemoreceptor drive. Fictive breathing is increased by low pH/high PCO_2 aCSF [54,71] indicating that central chemoreceptors remain functional in this preparation. However, the chemoreceptor response is blunted compared with conscious animals. Central chemoreceptor responsiveness in this preparation is enhanced by vagal afferent stimulation indicating that peripheral feedback is important for full expression of central chemoreceptor drive for breathing [54]. Increased temperature at a given pH of the aCSF surrounding the brainstem also increases fictive breathing in this preparation [71]; although these changes occurred over a limited range of temperatures in vitro, compared with conscious animals. For example, fictive breathing in the bullfrog brainstem shows little or no activity below 10°C and above 30°C, suggesting that temperature alters ion conductances and/or has oxygen diffusion limitations at high temperatures as a result of increased metabolism, in the absence of a functional circulatory system [71]. At room temperature (23°C), the in vitro tadpole brainstem preparation is well oxygenated ($PO_2 > 150$ mm Hg) at all tissue layers when superfused with aCSF equilibrated with 90% oxygen [102]. Therefore, it is not clear whether diffusion limitations occur at higher temperatures in anuran brainstem preparations, or whether temperature and/or metabolic CO_2 production have direct effects on the ion conductances that regulate respiratory rhythm.

4.2. Pulmonary stretch receptors

Lung stretch mechanoreceptors also play a role in the control of breathing, but there are conflicting data on the effects of lung inflation/deflation in anurans. The amphibian lung has rapidly-adapting and slowly-adapting stretch receptors that respond to lung inflation and deflation [61,63,70,92]. Based upon this background information, and knowledge of the Breuer-Hering reflex in mammals, it is expected that lung inflation would inhibit breathing and deflation would stimulate breathing. Indeed, in decerebrate, paralyzed anurans, lung inflation inhibits, and deflation stimulates, fictive breathing in most, but not all, preparations in the expected manner ([57], Wang, Taylor, Reid and Milsom, in prep.). Further, artificial lung inflation in decerebrated and paralyzed frogs evokes burst activity in the laryngeal branch of the Xth cranial nerve that innervates the glottis [59]. In addition, artificial lung inflation and electrical stimulation of the afferent branch of the vagus elicited glottal closure in decerebrated frogs [59]. Collectively, these observations are consistent with the

existence of a glottic constrictor reflex to lung inflation being involved in terminating lung inflation. By contrast, Kinkead and Milsom [56] have shown that lung inflation stimulates breathing in the same type of preparation. It is not clear what accounts for these disparate results using similar preparations, but more studies are clearly necessary to define the roles of lung mechanoreceptors in the control of breathing.

In decerebrate, paralyzed and artificially ventilated (UDV) toads (*B. marinus*) pulmonary stretch receptor feedback exerts a powerful control over fictive breathing; that is, when breathing is 'turned off' by a reduction of chemoreceptor drive, lung deflation is capable of stimulating breathing (Wang, Taylor, Reid and Milsom, in prep.). Chemoreceptor and lung mechanoreceptor inputs are, therefore, likely to interact centrally and influence the respiratory CPG; however, the interactions between these afferent pathways in the CNS are unknown. Furthermore, these interactions implies that studies using unidirectionally ventilated animals should take into account the role of PSRs in modulating the inputs from other receptors.

4.3. Olfactory receptors

Olfactory receptors sensitive to CO_2 powerfully inhibit breathing in conscious bullfrogs [16,55]. Thus, exposure to a hypercapnia strongly inhibits breathing despite the large accumulation of CO_2 and $[H^+]$ in the blood; this inhibition was removed by denervation of the olfactory tract [55]. More studies are needed to define the role, mechanisms and significance of these receptors.

5. Cardiovascular anatomy and cardiac shunts in anurans

5.1. Anatomy of the heart and central blood vessels

The adult anuran heart and major arteries are depicted in Fig. 1, but because the cardiovascular anatomy has been reviewed previously (e.g. Refs. [44,77,82,83]) it will only be summarized briefly below. The ventricle is completely undivided and receives oxygen poor systemic venous blood from the right atrium and oxygen rich pulmonary venous blood that returns from the lungs via the left atrium. During systole, blood is ejected through the contractile conus arteriosus into the bilateral truncus arteriosi that each divide into a carotid arch, a systemic arch and a pulmocutaneous artery. Within the conus arteriosus an endothelial ridge (the spiral valve) attaches along one side and separates systemic and pulmocutaneous blood flows. The pulmocutaneous artery divides into separate pulmonary and cutaneous arteries before reaching the lungs, allowing selective perfusion of the lungs or skin. The oxygen rich blood returning from cutaneous perfusion empties into the systemic venous circulation, elevating oxygen levels of the right atrial blood above mixed venous levels.

5.2. Definitions and quantification of cardiac shunts in anuran amphibians

The cardiac anatomy of anurans allows for mixing of systemic and pulmonary blood within the ventricle and the conus arteriosus. Thus, systemic venous blood can re-enter the systemic circulation (right-to-left shunt), while pulmonary venous blood can re-enter the pulmonary circulation (left-to-right shunt). Shunt flows affect blood gas composition in the systemic and pulmonary arteries; a R-L shunt reduces arterial oxygen levels (and increases CO_2 levels) relative to the left atrium, whereas a L-R shunt increases oxygen levels relative to the right atrium. Due to these effects, it is possible to quantify the shunts from measurements of blood oxygen contents at various locations in the circulatory system. For example, the fractional R-L shunt ($Q_{\text{R-L}}/Q_{\text{sys}}$) can be quantified from measurements of O_2 content in arterial blood and in the two atria; L-R shunt ($Q_{\text{L-R}}/Q_{\text{pul}}$) can be calculated from measurements of O_2 content of pulmonary arterial blood and in the two atria. In a theoretical study, Tazawa and Johansen [99] provided the equations necessary to evaluate cardiac shunts in amphibians. Calculations of cardiac shunts based on oxygen contents provide a proportional measure of the shunt flow relative to the arterial blood flow, but cannot be used to quantify the actual shunt flow rates. These can only be obtained from simultaneous, direct measurements of systemic and pulmonary blood flows, using blood flow probes, or estimated from measurements of oxygen uptake and arterial-venous oxygen content differences (i.e. using the 'Fick principle'). Alternatively, shunts can be estimated from microsphere injections (see Ref. [67] for a study on frogs).

Measurements of systemic and pulmonary blood flows can quantify the net shunt flow, which is defined as the difference between Q_{pc} and Q_{sys} ($Q_{\text{net shunt}} = Q_{\text{pc}} - Q_{\text{sys}}$). It is important to emphasize that this measure alone does not reveal the extent of actual mixing because R-L and L-R shunts can occur simultaneously (bidirectional shunting). Under conditions of bidirectional shunting, a negative net shunt merely entails that the L-R shunt is larger than the R-L shunt. A physiological interpretation of net shunts is more complicated in anurans, compared to reptiles, because the cutaneous blood flow joins the systemic venous blood and enters the heart from the right atrium. Thus, if Q_{pc} equals Q_{sys} (i.e. no net shunt), the inflow of blood to the ventricle from the right atrium will exceed inflow from the left

atrium. In this situation, the R-L shunt is larger than the L-R right shunt, even though there is no net shunt. Obviously, if cutaneous blood flow is small, the source of error is negligible. However, as a general feature, measurements of net shunt flows in amphibians will tend to underestimate the R-L shunt relative to the L-R shunt, if the cutaneous blood flow is not taken into account.

A thorough description of blood flows and the extent of mixing between the two circulatory systems in anurans is complicated by the requirement for a combination of blood flow measurements together with some marker, such as oxygen content or injection of microspheres. In turtles, the R-L and L-R shunts are inversely related, so that there is no R-L shunt whenever the net L-R shunt flow is very high and vice versa [36,41]. Given such relationships, it may be plausible to predict the actual shunt flows on the basis of blood flow measurements. Nevertheless, the existence of similar relationships in amphibians remains to be verified.

5.3. Measurements of cardiac shunts in anuran amphibians

Although the anuran heart possess a large potential for mixing of systemic and pulmonary blood, dye injections and measurements of blood oxygen levels show that there exists a selective distribution of blood flows in both anesthetized and conscious animals (e.g. Refs. [4,18,34,42,85,86,98]; cf. Ref. [25]). Nevertheless, no studies to date provide a thorough description of cardiac shunts in conscious animals. This is perhaps not surprising given the considerable technical problems associated with simultaneous measurements of at least two blood flows (Q_{pc} and Q_{sys}) and O_2 contents at four sites (systemic and pulmonary arteries as well as both atria). However, in a study on freshly pithed frogs (*Rana catesbeiana*), Tazawa et al. [98] determined blood oxygen contents from the left atrium, the sinus venosus, the cutaneous vein as well as the aortic and pulmocutaneous arteries and calculated blood flows on basis of pulmonary and cutaneous oxygen uptake. In these animals, 16% of the systemic venous return re-entered the systemic circulation (R-L shunt), whereas 9% of the pulmonary venous blood was recirculated in the lungs (L-R shunt). As pointed out by Tazawa et al. [98], their study on pithed animals did not discern the changes in cardiac shunt patterns that may be associated with the changes in blood flows during ventilation in conscious animals. This important aspect has only been investigated in one study [67], where the shunt patterns were studied by microsphere injections into the left and right atria through indwelling catheters in conscious and voluntarily diving frogs (*R. catesbeiana*). As in the study by Tazawa et al. [98], the conscious frogs exhibited bidirectional shunting, but the degree of mixing was much larger and there were large differences among individual animals. During breath hold (diving) almost 70% of the systemic venous blood was shunted back into the systemic circulation, while the L-R shunt only amounted to 23%. During ventilation the L-R shunt increased whereas the R-L shunt was reduced. Thus, as expected from measurements of blood flows, it appears that R-L shunt dominates during breath hold when Q_{pc} is reduced, while L-R increases during ventilation. This pattern is consistent with other ectothermic vertebrates (e.g. Ref. [44]).

The underlying mechanism that accounts for the separation of blood flows in the anuran heart is not clear. However, it appears to involve laminar flow patterns maintained by the trabeculate nature of the myocardium (e.g. Refs. [44,82,83]), and it is possible that most of the mixing occurs within the conus arteriosus ([62,86], cf. Ref. [25]). As pointed out by Shelton [83], the conditions for blood flow separation would appear to be optimal whenever blood flows from the right and left atria are approximately equal.

5.4. Regulation of pulmonary blood flow and net shunts in amphibians

Because blood flows to the systemic and pulmocutaneous arteries are supplied by a single pressure source, the overall distribution of blood flows between these circuits is determined by the relative magnitudes of the vascular resistances in the systemic and pulmocutaneous vascular beds [62]. Thus, whenever the resistance in the pulmocutaneous circulation (R_{pc}) is low compared to that in the systemic circulation (R_{sys}), Q_{pc} will be high compared to Q_{sys} and a net L-R shunt would prevail. In anurans, it is well established that R_{pc} can be altered through a contraction of smooth muscle in the pulmocutaneous artery [20,88] and that this muscular sphincter, innervated by the vagus nerve, can control pulmonary blood and the net shunt flow [20,21,62,88]. Thus, increased vagal tone on the pulmonary artery reduces pulmonary blood flow and increases systemic recirculation of oxygen-poor blood from the right atrium (right-to-left cardiac shunt). In contrast, there is evidence that a decrease in vagal tone on the pulmonary artery is associated with the increased pulmonary blood flow observed during ventilation [81,108].

In addition to the shunt between the systemic and the pulmonary circulations, anurans can shunt blood between the cutaneous and pulmonary circulations. In the anesthetized toad, *Bufo marinus*, approximately 20% of Q_{pc} is directed towards the skin, while the remaining 80% of Q_{pc} enters the pulmonary circulation [108]. Similar distributions have been estimated by injecting radioactive microspheres directly into the pulmocutaneous artery of conscious *Rana catesbeiana* [3]. Further-

more, more blood was directed towards the lungs during aquatic hypoxia and aerial hypoxia elicited a proportional increase in cutaneous perfusion [3]. This ability is probably as a result of a reciprocal innervation and control of cutaneous versus pulmonary vascular resistances.

5.5. Innervation of the heart and major arteries

As in mammals, spinal sympathetic stimulatory and cranial (vagal) parasympathetic inhibitory nerves innervate the heart of anuran amphibians. Before reaching the heart, the sympathetic nerves join the vagus nerve and form a vagosympathetic trunk that innervates the atria, the ventricle and the sinus venosus. The vagal innervation has negative chronotropic and inotropic effects that are predominantly mediated by acetylcholine acting on muscarinic receptors ([28]; reviewed by Ref. [72]). In *Bufo marinus*, other transmitters such as somatostatin and galanin are co-released from the vagal nerve terminals and somatostatin has been shown to inhibit the heart in this species [13]. In other species, there does not appear to be co-release of somatostatin [14]. Adrenaline is the most important neurotransmitter for the positive chronotropic and inotropic effects exerted by the sympathetic innervation [1], but in some species neuropeptide Y and ATP are co-released with adrenaline [5,38,72].

Apart from the cardiac innervation, the vagosympathetic trunk innervates the pulmonary artery at the point of the sphincter as described above [10,20,30,73] and vagal stimulation elicits pulmonary arterial contraction at this point. This response is predominantly cholinergic, and mediated by muscarinic receptors, because it is mimicked by application of ACh and blocked by atropine. However, immunohistochemical studies on toads indicate co-localization of somatostatin and galanin [30,73] and it has been suggested that somatostatin contributes to the contraction of the pulmonary artery [72]. In addition, the smaller pulmonary arterial vessels receive adrenergic innervation and electrical stimulation of these nerves cause a vasodilation, while adrenaline appears to elicit a contraction of the main pulmonary arterial vessel [11,12,37].

In resting animals, both the sympathetic and the parasympathetic innervations influence heart rate and blood flows. Thus, injection of the muscarinic blocker, atropine, increases heart rate and pulmonary blood flow, while adrenergic blockade causes an immediate decrease in heart rate [21,88,96,108]. The extent to which withdrawal of vagal tone versus increased sympathetic tone accounts for the increased heart rate and pulmonary blood flows associated with ventilation (described in the following section) has not been determined.

6. Central location of cardiac vagal motoneurons

Cardiorespiratory coupling in anurans suggests that efferent control of the heart and respiratory system may occur via interactions within the CNS. Control of heart rate is accomplished to a large extent by the action of the vagus nerve (Xn) which also innervates the glottis, lungs and gut. In mammals, vagal preganglionic neurons (VPM) arise both from the dorsal vagal motonucleus (DVN) and the nucleus ambiguus (NA). The heart is innervated by axons having their cell bodies in either location, while VPM located in the NA play a crucial role in regulation of the glottis and laryngeal musculature, modulating airflow in the upper respiratory tract. The NA is anatomically very near the areas of the ventral respiratory group (VRG; e.g. Pre-Botzinger complex) that are considered the primary areas for respiratory rhythmogenesis in mammals. The NA is also the site of location for VPM supplying axons to the heart and lungs. For example, in the cat up to 78% of cardiac and up to 68% of pulmonary neurons are located together in the NA [48]. Central recordings of spontaneous and induced neuronal activity has provided strong evidence of a role in the generation of respiratory sinus arrhythmia and the respiratory 'gating' of the baroreceptor-cardiac reflex, for a direct, cholinergic, inhibitory input to cardiac vagal motoneurons (CVPM) from neighbouring inspiratory neurons [49]. These interactions take place in the NA [48]. Similar dual locations for VPM and CVPM have been reported in anuran amphibians [94,111].

In the aquatic amphibian, *Xenopus laevis*, labelling of neurons supplying the vagus nerve, using retrograde transport of horseradish peroxidase (HRP) along efferent axons, revealed that the vagal motonucleus is located in the medulla oblongata over a rostro-caudal distance of about 4 mm, either side of obex, with over 90% of neuron cell bodies rostral of obex. A sensory projection of the vagus was revealed, by anterograde transport of HRP along afferent axons, with diffuse labelling of the dorsal visceral sensory nucleus over a rostro-caudal distance of about 2 mm, rostral of obex in an area identified as the nucleus of the solitary tract (NTS), (Figs. 3 and 4). Labelling of discrete branches of the vagus revealed a partially sequential distribution of neuron cell bodies, with those innervating the laryngeal and hyoid branches located most rostrally, followed by cells innervating the heart and the abdominal viscera (Fig. 5). The larynx and hyoid are derived from elements of the larval gill arches so that the rostral location of their motoneurons may reflect the phylogenetically primitive arrangement described in fishes, in which the four branchial branches of the vagus are supplied with axons having their cell bodies in a sequential series, rostral of obex [93]. This arrangement is

retained in the neotenous, gill-bearing axolotl, *Ambystoma mexicanum* [94,97]. Cells innervating the lungs had a wide rostrocaudal distribution throughout the vagal motor column, which overlapped with that of the cardiac cells ([39]; see Fig. 5). These pulmonary fibres may include those innervating the sphincter on the major pulmonary artery, as well as the smooth muscle of the lung. Stimulation of the intracranial roots of the vagus in *Bufo* caused pulmonary vasoconstriction plus a marked relaxation of the lung [11]. The central origins of this dual innervation merit specific study.

All branches of the vagus in *Xenopus* are supplied by neurons with their cell bodies in either a medial nucleus, within the central grey, equivalent to the mammalian dorsal vagal motonucleus (DVN) or in a ventro-lateral nucleus, outside the central grey, which may be the amphibian equivalent of the NA (see Figs. 3 and 4). In this respect *Xenopus* resembles mammals such as the cat, in which each branch of the vagus is represented in all identified motonuclei [50]. In *Xenopus*, the proportion of cell bodies in the ventro-lateral location was 31% overall but varied from 17% in the pulmonary branch to 43% in the gastric branch. The cardiac vagus had 26% of its CVPM in the NA. A similar division of VPM between the DVN, and a putative NA, with 20% in this ventro-lateral nucleus, was described in *Bufo marinus* [40,95]. In contrast to these pipid and bufonid anurans, a single motor column was described for the ranid species *Rana pipiens*. Within this column cardiac and pulmonary vagal motoneurons showed an overlapping distribution [91], although CVPM were located rostral to pulmonary cells and laryngeal cells were found caudally in the motor column, in contrast to their rostral location in *Xenopus* (Fig. 5). The possibility that these neuranatomical differences between ranid and other anurans are associated with functional differences in the control of cardiorespiratory interactions remains to be investigated.

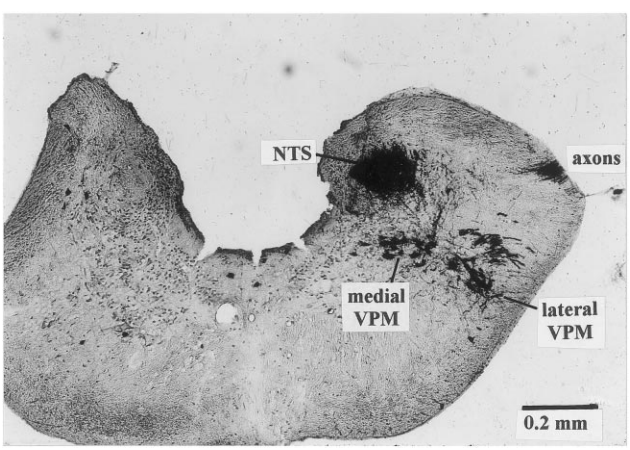

Fig. 3. Transverse section (60 μm) through the medulla oblongata of *Xenopus laevis*, taken approximately 1 mm rostral of obex showing the location of vagal preganglionic motoneurons (VPM). VPM were labelled using retrograde axonal transport of horseradish peroxidase (HRP) that had been applied to the individual branches of the vagus nerve in anesthetized animals. Following 10 days recovery, the animals were killed and the brainstem cut in serial transverse sections (60 μm) and stained for HRP using TMB (see Ref. [95], for a description of methods). Motor and sensory projections of the vagus are marked with a dark reaction product after histochemical treatment. Note the two discrete groups of HRP labeled VPM, with the lateral located in the white matter and the medial group in the central grey, and the sensory projection into the NTS (see text).

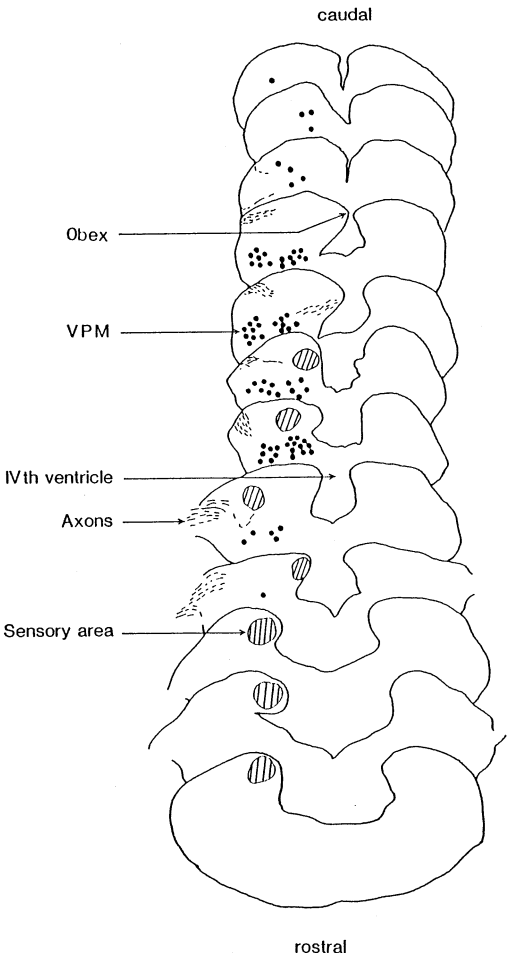

Fig. 4. Traces of representative transverse sections through the brainstem of *Xenopus laevis*, either side of obex, to show the location of the cell bodies of vagal motoneurons (VPM) in the DVN and NA and sensory areas in the NTS, lateral to the IVth ventricle

The neuranatomical evidence that cardiac and respiratory VPM are located in close proximity in the anuran brainstem implies that some cardiorespiratory interactions in anurans may be generated by synaptic interactions between central neurons generating efferent outflow to the heart and respiratory apparatus. This may be of particular importance in amphibians as their major respiratory muscles, as well as the airways, are innervated by cranial nerves, including the vagus, which have their cell bodies in the brainstem, in close proximity to CVPM. However, at present, there is no physio-

logical evidence for such a synaptic interaction between the populations of vagal preganglionic motoneurons controlling cardiovascular and respiratory function in amphibians.

7. Coupling between heart rate, blood flows and ventilation

The intermittent ventilatory patterns of anurans are often associated with concomitant cardiovascular changes. An example from a conscious and undisturbed toad (*Bufo marinus*) is shown in Fig. 2. In this record,

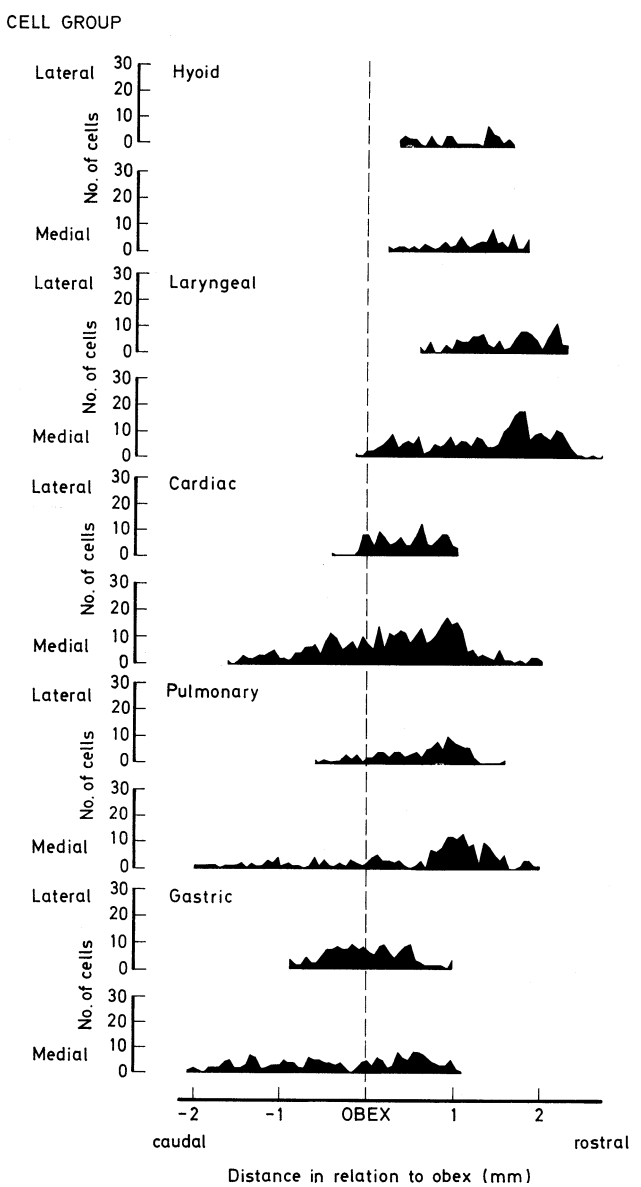

Fig. 5. The rostrocaudal extent, either side of obex, of cell bodies of vagal preganglionic motoneurons, following retrograde transport of HRP along selected branches of the vagus nerve in *Xenopus laevis* ($N = 22$). Distributions of cells in the medial (DVN) and lateral (NA) nuclei are plotted separately.

Q_{pc} increased approximately 20% during and following three lung inflation cycles, while there were no cardiovascular changes during the other shorter and less intense ventilatory periods. The increased blood flow to the lungs arose from an increased f_H and a reduction in Q_{sys}. Similar cardiorespiratory coupling has previously been shown in other anurans [81,108] and numerous other ectothermic vertebrates [43,44]. The increased f_H and Q_{pc} during ventilation is comparable to respiratory sinus arrhythmias in mammals. However, in mammals the increased f_H is manifested during inspiration, whereas the elevated f_H and Q_{pc} are maintained throughout the ventilatory periods in amphibians and reptiles. This difference apart, the underlying mechanisms for cardiorespiratory interactions appear similar in all vertebrates, suggesting structural and functional homologies [97].

The increased f_H and Q_{pc} during ventilation is primarily a result of a release of vagal tone on the heart and pulmonary artery. Thus, vagotomy or atropine injection reduces or abolishes cardiorespiratory coupling; however, the underlying mechanisms are not known. In mammals, several factors contribute to respiratory sinus arrhythmias: (1) central communication between the respiratory centres and the cardiac vagal motoneuron pools in the medulla; (2) a secondary result of pulmonary stretch receptor stimulation acting as feed back signal; (3) mechanical changes following ventilatory movements of the thoracic cavity; and (4) local effects of CO_2 and O_2 on the pulmonary vasculature. As outlined below, it appears that all these factors exert a role in the generation of cardiorespiratory interaction in anuran amphibians, but their individual contribution is at present difficult to discern. Thus, given the limited information firm conclusions are tenuous.

7.1. Centrally-generated cardiorespiratory coupling

Cardiorespiratory coupling in amphibians may originate from direct influences of activity in the centers responsible for respiratory rhythm generation on the cardiac and pulmonary arterial vagal motoneurons. These interactions occur within the CNS and are independent of afferent feedback. The possibility of a centrally generated coordination of respiratory motor activity and release of vagal tone on the heart and pulmonary artery has received very little attention in amphibians. However, recent experiments on decerebrated, paralyzed and UDV toads (*B. marinus*) have shown that such central interactions may be present (Fig. 6). In this preparation, fictive breathing was measured as neural activity from the mandibular branch of the trigeminal nerve, but because the animals were paralyzed, the input from lung stretch receptors and chemoreceptors were tonic and did not change during ventilation. Even in the absence of such phasic input,

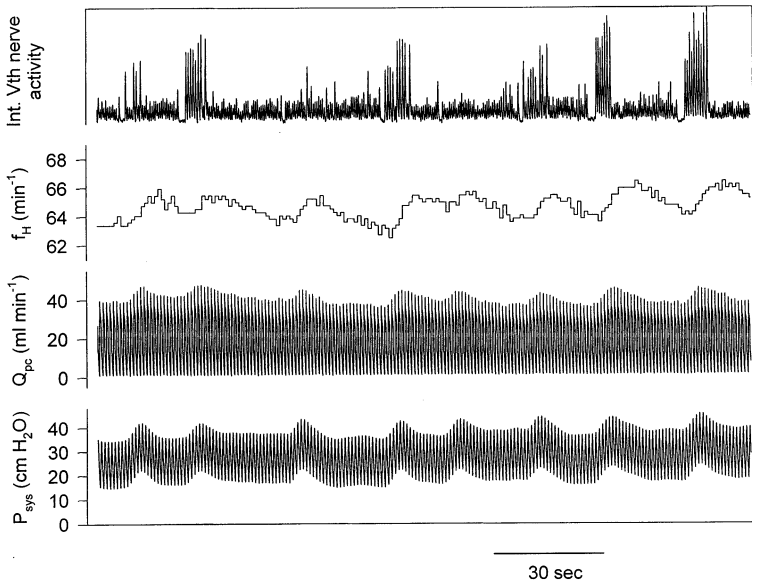

Fig. 6. Cardiorespiratory coupling in a decerebrate, paralyzed and unidirectionally ventilated (2.5% CO_2, balance air) toad (*Bufo marinus*) maintained at room temperature (22–23°C). A Transonic flowprobe was placed around the pulmocutaneous artery and the systemic blood pressure was measured in the femoral artery. Fictive breathing was measured as efferent neural output from the mandibular branch of the Vth cranial nerve (Wang, Taylor, Reid and Milsom, in prep.).

Q_{pc} and f_H increased during the breathing bouts, suggesting that some of the cardiovascular changes during ventilation originate centrally.

The mechanisms responsible for central cardiorespiratory coupling in *B. marinus* remain to be investigated. As shown in Fig. 3 for *Xenopus*, the cardiac vagal motoneurons are located close to the anatomical areas that generate breathing. Thus, it is possible that initiation of ventilatory motor activity is associated with a nervous interaction with the vagal motoneurons that lead to a release of vagal tone on the heart (i.e. by an inhibition of CVPM). In addition, the central interaction may include more complicated pathways where respiratory activity differentially modulates the afferent inputs to vagal motoneurons. These mechanisms have been described in mammals and there exist several reviews on this topic ([47,49,90]; see Ref. [97] for comparison among vertebrates).

7.2. Effects of pulmonary stretch receptors stimulation by lung inflation

During breathing, changes in lung volume stimulate pulmonary stretch receptors and it is possible that their afferent activity serves as a feedback mechanism to the cardiovascular centers that, in turn, release cardiac vagal tone on the heart and pulmonary artery. In some experiments on anaesthetized *Bufo*, *Rana* and *Xenopus*, artificial lung inflation elicits cardiovascular responses that are similar to those observed during spontaneous breathing and these responses were abolished following atropine injection and during deep anesthesia (e.g. Refs. [20,21,108]). These findings demonstrate a central integration of pulmonary stretch receptor stimulation and an involvement of the vagal innervation in the efferent control. However, in conscious *Xenopus*, denervation of pulmonary stretch receptors does not abolish the increase in heart rate associated with lung ventilation [23].

7.3. Mechanical effects

Changes in thoracic pressure and volume during pulmonary ventilation may alter the venous return to the heart and change cardiac output through the classical Frank-Starling mechanism. In anesthetized toads (*Bufo arenarum*), artificial lung inflation increased left atrial pressure and elevated heart rate [80]. Since these responses persisted following atropine injection and removal of the bulbomesencephalic centers, it was concluded that direct mechanical effects on venous return to the heart contribute to the respiratory sinus arrhythmia [80]. The possible effects of ventilation on thoracic pressure and volume should be particularly large during inflation cycles, when intrapulmonary pressure are maximal [105,107]. A contribution of these mechanical effects to respiratory sinus arrhythmia in conscious animals may be particularly important under these circumstances.

7.4. Local effects within the pulmonary circulation

Apart from nervous control, resistance to pulmonary blood flow can be altered within the pulmonary circula-

tion *per se*, where lung gas composition may exert a direct influence on vascular tone. In reptiles, birds and mammals, the pulmonary vasculature constricts during hypoxia causing an elevated resistance to pulmonary blood flow [17,22,24]. This response is locally mediated. It is not known whether the amphibian lung responds similarly. In anesthetized and unidirectionally ventilated toads, hypercapnia and hypoxia, alone or in combination, reduced pulmonary blood flow [108] and it is possible that some of this response is locally mediated.

8. Conclusion and perspectives

As in other vertebrates, respiratory rhythm in anuran amphibians is generated within the CNS that receives afferent input from peripheral and central chemo- and mechanoreceptors. While the role of the CNS in respiratory control is becoming increasingly well described, almost nothing is known about the role of cardiovascular centers in the regulation of heart rate and blood flow. The anatomical evidence reviewed in the present account shows a close proximity of the centers responsible for respiratory rhythmogenesis and the vagal motoneurons involved in cardiovascular regulation. This observation, in concert with the demonstration that paralyzed and UDV toads exhibit cardiorespiratory interactions in the absence of phasic receptor input, strongly indicates central communication between respiratory and cardiovascular centers within the brainstem. The reflex roles of peripheral receptors in respiratory control are well described; whereas, the attendant changes in blood flow, cardiac shunts and heart rate remain to be understood. Thus, at present, it is not known to what extent cardiovascular responses are directly influenced by peripheral chemoreceptors or whether they are a secondary consequence of increased respiratory drive.

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