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Review

How important is the CO_2 chemoreflex for the control of breathing? Environmental and evolutionary considerations☆

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A B S T R A C T

Haldane and Priestley (1905) discovered that the ventilatory control system is highly sensitive to CO_2. This "CO_2 chemoreflex" has been interpreted to dominate control of resting arterial PCO_2/pH (P_aCO_2/pH_a) by monitoring P_aCO_2/pH_a and altering ventilation through negative feedback. However, P_aCO_2/pH_a varies little in mammals as ventilation tightly couples to metabolic demands, which may minimize chemoreflex control of P_aCO_2. The purpose of this synthesis is to (1) interpret data from experimental models with meager CO_2 chemoreflexes to infer their role in ventilatory control of steady-state P_aCO_2, and (2) identify physiological causes of respiratory acidosis occurring normally across vertebrate classes. Interestingly, multiple rodent and amphibian models with minimal/absent CO_2 chemoreflexes exhibit normal ventilation, gas exchange, and P_aCO_2/pH_a. The chemoreflex, therefore, plays at most a minor role in ventilatory control at rest; however, the chemoreflex may be critical for recovering P_aCO_2 following acute respiratory acidosis induced by breath-holding and activity in many ectothermic vertebrates. An apparently small role for CO_2 feedback in the genesis of normal breathing contradicts the prevailing view that central CO_2/pH chemoreceptors increased in importance throughout vertebrate evolution. Since the CO_2 chemoreflex contributes minimally to resting ventilation, these CO_2 chemoreceptors may have instead decreased importance throughout tetrapod evolution, particularly with the onset and refinement of neural innovations that improved the matching of ventilation to tissue metabolic demands. This distinct and elusive "metabolic ventilatory drive" likely underlies steady-state P_aCO_2 in air-breathers. Uncovering the mechanisms and evolution of the metabolic ventilatory drive presents a challenge to clinically-oriented and comparative respiratory physiologists alike.

1. Introduction

The primary role of the respiratory system is to acquire O_2 to support aerobic metabolism and to eliminate CO_2 for acid-base balance. In recent years, respiratory physiologists and neurobiologists have made major progress in understanding how the respiratory control system generates the rhythm of lung breathing, senses respiratory gases, and manifests plasticity. Although we have learned a great deal about mechanisms that build important aspects of the respiratory control system in several vertebrate species, how these processes integrate to match ventilation to metabolic demands and regulate blood gases still remains elusive (Haouzi, 2015). The purpose of this paper is to critically evaluate the role of arterial CO_2/pH feedback *via* central and peripheral chemoreceptors in performing the primary function of the respiratory system; that is, matching ventilation to metabolism and maintaining arterial pH/PCO_2 (pH_a/P_aCO_2) at normal values.

Haldane and Priestley (1905) discovered that pulmonary ventilation in humans is highly sensitive to inspired CO_2. This seminal finding spurred over 100 years of work into understanding brainstem and peripheral mechanisms that sense CO_2 tensions of arterial blood to alter ventilation through negative feedback. Subsequently, many brain structures and molecules have been demonstrated to underlie this CO_2 chemoreflex. The mechanisms involved in altering ventilation through the CO_2 chemoreflex have traditionally been interpreted as playing a primary role in maintaining and defining the P_aCO_2/pH_a set point in healthy air-breathing vertebrates at rest. Additionally, mechanisms underlying the CO_2 chemoreflex are often interpreted as providing a "tonic drive" that is essential for normal air breathing. These views abound in the recent CO_2 chemoreception literature and they are often explicitly stated or strongly implied (Gestreau et al., 2010; Guyenet and Bayliss, 2015; Hartzler and Putnam, 2009; Hennessy et al., 2017; Hoffman et al., 2016; Mulkey et al., 2004; Nattie and Li, 2012; Puissant et al., 2015; Ruffault et al., 2015; Santin and Hartzler, 2013; Spyer and Gourine, 2009; Su et al., 2007; Sundin et al., 2007; Teran et al., 2014).

Several recent reviews have focused on cellular sensory mechanisms, brain structures, and phylogenetic trends of CO_2 chemoreception

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(Guyenet and Bayliss, 2015; Huckstepp and Dale, 2011; Milsom, 2010; Nattie and Li, 2012). Instead of focusing on these well-described topics, in this paper I present an argument intended to challenge the interpretation that the CO_2 chemoreflex plays a primary role in maintaining steady-state ventilation and P_aCO_2 under normal conditions. As such, I review the respiratory phenotype of several recently described rodent and amphibian models with severely blunted ventilatory responses to CO_2 (~65–100% reduction) to gauge the necessity of this system for production of both normal ventilation and gas exchange in adult and developing air-breathing vertebrates. Next, I review generalizable environmental scenarios and physiological characteristics across vertebrate classes that may have favored selection for an arterial-side CO_2 detection system to recover acid-base status following acute respiratory acidosis. I then conclude with a revised hypothesis regarding the role the CO_2 chemoreflex in regulating lung ventilation during and after the evolutionary transition from water to land in vertebrates.

2. Problems with interpreting the CO_2 chemoreflex as critical for normal ventilation and acid-base homeostasis

The notion that the CO_2 chemoreflex plays a feedback role in matching ventilation to metabolism for the preservation of arterial CO_2/pH likely stems from the original work performed by Haldane and Priestley (1905), who speculated: "Normal hyperpnea, such as that due to muscular work, may be explained as follows. The venous blood, returning to the lungs in larger quantity, and probably also more highly charged with CO_2, causes a rapid rise in the alveolar CO_2 pressure, and consequent rise in the arterial CO_2 pressure. The respiratory centre is thus stimulated to increased activity, with consequent lowering of the alveolar CO_2 pressure, until a point is struck at which an equilibrium is maintained between the effect of the increased supply of venous blood in raising the arterial CO_2 pressure and that of the increased respiratory activity in lowering it." We now know and acknowledge that arterial blood gas regulation does not require the CO_2 chemoreflex during "muscular work" (Forster et al., 2012) and that CO_2 homeostasis is indeed multifaceted (Guyenet, 2014). Yet, the transduction of subtle fluctuations in P_aCO_2 by mechanisms underlying the CO_2 chemoreflex is still viewed as paramount for ventilatory control of CO_2 homeostasis at rest (Guyenet and Bayliss, 2015; Teran et al., 2014). This interpretation implies that negative feedback mechanisms correct miniscule respiratory-driven (*i.e.*, CO_2 rather than bicarbonate) deviations from an arterial CO_2/pH set-point through ventilation to determine and maintain steady-state P_aCO_2 at rest. This relationship exists because hydrated CO_2 is in equilibrium with carbonic acid which dissociates into bicarbonate (HCO_3^-) and a proton (H^+). Therefore, changes in CO_2/pH of arterial blood, cerebrospinal fluid, and brain interstitial fluid that bathes peripheral and central chemoreceptors can signal homeostatic alterations in breathing through the CO_2 chemoreflex (Fig. 1). Respiratory-driven pH disturbances (*e.g.*, alveolar hypoventilation and hyperventilation) undoubtedly utilize these chemoreceptors. However, the extent to which subtle changes in P_aCO_2 engage the CO_2 chemoreflex to make a major contribution to steady-state ventilatory control of CO_2 homeostasis at rest is unclear (Haouzi, 2015) and should be questioned.

One important argument against resting P_aCO_2 homeostasis *via* the CO_2 chemoreflex is that ventilation couples tightly to metabolic demands; therefore, P_aCO_2 does not substantially change in continuously breathing mammals. This has been historically acknowledged during exercise in humans when CO_2 production increases well above its resting value (Dempsey et al., 2014; Forster et al., 2012). During low to moderate intensity steady-state exercise and postural changes, ventilation matches metabolic demands to eliminate CO_2 at the lung and, consequently, there is minimal-to-no change in P_aCO_2. If variations in P_aCO_2 do happen during exercise, they (1) likely occur at transitions between exercise workloads, (2) exhibit inter-individual differences in their magnitude and direction, and (3) change by ~1.5 mm Hg or less in the opposite direction consistent with a role in matching ventilation to metabolic demands (Forster et al., 1986). Increased exercise intensity in humans results in hyperventilation (*i.e.* ventilation overshoots the requirements for CO_2 elimination), whereas several non-human mammals hyperventilate at low to moderate exercise intensities. Taken together, the CO_2 chemoreflex may represent a counter measure to fine-tune the exercise hyperpnea, but it is not primarily responsible for coupling ventilation to CO_2 production for P_aCO_2 homeostasis across a range of metabolic rates (Forster et al., 2012). Therefore, it does not follow that feedback *via* arterial blood gases is essential when the system is unchallenged at rest, but not during large increases in CO_2 production. Distinct mechanisms that match ventilation to CO_2 production would make better candidates for maintaining steady-state P_aCO_2. As suggested by Dempsey et al. (2014), small deviations in CO_2 production may be capable of driving ventilatory changes to maintain P_aCO_2 by, somehow, sensing CO_2 delivery to the lungs (Phillipson et al., 1981a; Phillipson et al., 1981b). This idea was previously tested in awake sheep by modifying venous CO_2 using an extracorporeal loop prior to delivery into the lungs. In these experiments, scrubbing CO_2 from the venous circulation depressed breathing, while mimicking slightly less than a doubling of $\dot{V}co_2$ increased ventilation. In response to these manipulations, ventilation matches pulmonary CO_2 exchange and P_aCO_2 is generally maintained (for details, caveats, and other examples see section below titled "How is ventilation coupled to CO_2 production?"). These results imply that central and peripheral chemoreceptors detecting changes in arterial blood gases are on the wrong side of the circulatory system to actively participate in moment-to-moment ventilatory adjustments for the maintenance of steady-state P_aCO_2 at rest.

As with any other experimental approach to establish physiological necessity, a reasonable test of the hypothesis that "the CO_2 chemoreflex plays a large role in controlling ventilation to maintain steady-state P_aCO_2" would involve measuring ventilation, gas exchange, and arterial blood gases in animals lacking CO_2 chemoreflexes. Several mammalian and amphibian models have recently been shown to exhibit severely blunted CO_2 chemoreflexes (~65–100% reduction) (Kumar et al., 2015; Noronha-de-Souza et al., 2006; Puissant et al., 2015; Ramanantsoa et al., 2011; Santin and Hartzler, 2016a; Sun and Ray, 2017). These models provide an opportunity to infer the relative contribution of the CO_2 chemoreflex to the control of ventilation for maintenance of normal, steady-state gas exchange and P_aCO_2/pH_a. Given that these animal models have little to no ventilatory response to large CO_2 challenges (4–8% CO_2 in air), it is difficult to imagine a 1–2 mm Hg change in arterial P_aCO_2 that would occur normally at rest, at least in mammals (Forster et al., 1982; Klein et al., 1982), could meaningfully engage the chemoreflex. If continuous feedback from the CO_2 chemoreflex is the primary driver of normal ventilation, one would predict that an animal lacking ~65–100% of its CO_2 chemoreflex would have disrupted ventilation, gas exchange, and P_aCO_2 homeostasis. In the following section I will evaluate these studies to infer the relative contribution of negative feedback through CO_2 chemoreceptors to normal ventilation and P_aCO_2/pH_a homeostasis in juvenile and adult animals.

3. Does the CO_2 chemoreflex play a role in maintaining steady-state P_aCO_2 and matching ventilation to CO_2 production in adults?

Five relatively recent studies describe animal models with a severe reduction in ventilatory CO_2 sensitivity *via* different mechanisms and time scales (Table 1). First, GPR4 knockout (KO) and GPR4/TASK-2 double KO mice have a ~65% and ~85% reduction in the ventilatory response to CO_2, respectively (Kumar et al., 2015). Mice lacking pH-sensitive G-protein coupled receptors (GPR4) and K^+ channels (TASK-2) have blunted ventilatory responses to inspired CO_2 through a reduction in the CO_2/pH sensitivity of retrotrapezoid nucleus (RTN) neurons. Next, the Brown Norway (BN) rat strain is insensitive to

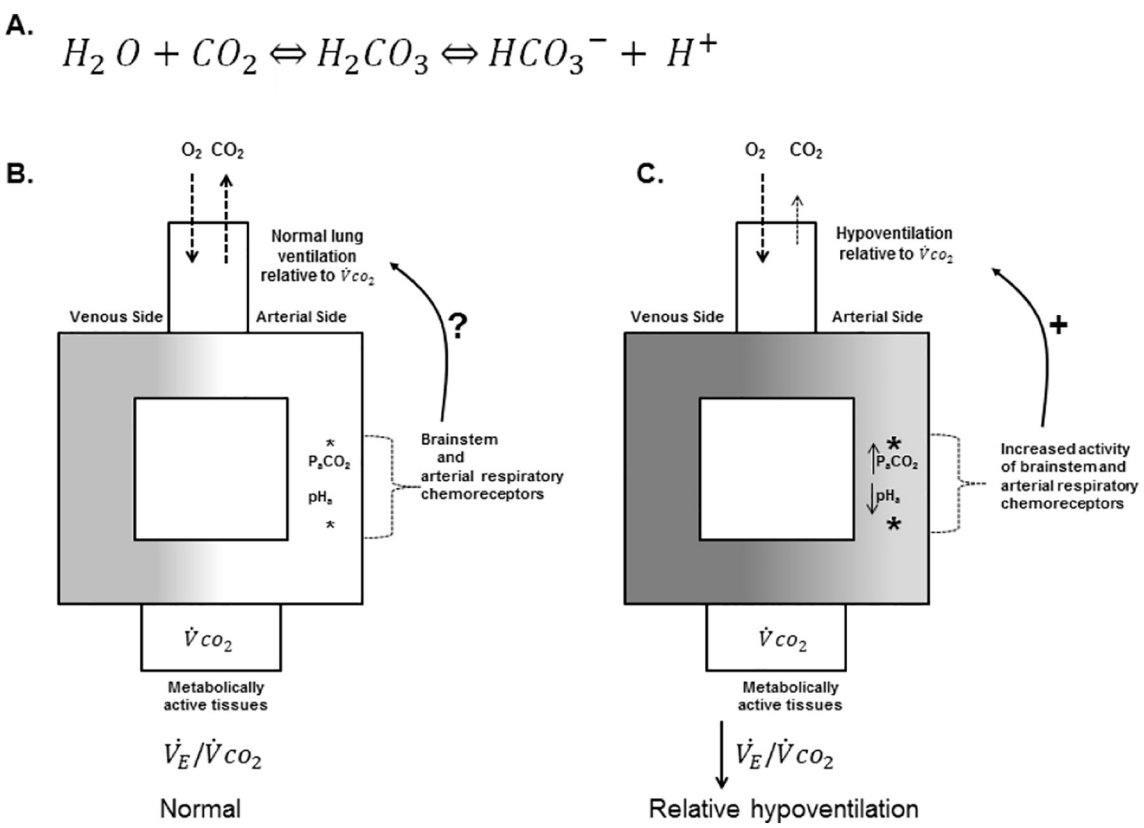

Fig. 1. Generalized relationship for ventilation, metabolism, and arterial PCO_2/pH (P_aCO_2/pH_a) in air-breathing vertebrates. A. demonstrates the equilibrium reaction for CO_2 and H^+/HCO_3^- in aqueous solution. B. and C. are simplified models illustrating the relationship between ventilation, metabolism, P_aCO_2/pH_a under normal, steady-state conditions and during a relative hypoventilation, respectively. In B. alveolar ventilation matches the rate of tissue CO_2 production. Consequently, steady-state P_aCO_2 and pH_a remain at normal values. The extent to which small imperfections in the coupling of ventilation-metabolism sufficiently alters P_aCO_2/pH_a to activate the CO_2 chemoreflex (represented by asterisk on the arterial side of the circulation) for a major role in steady-state ventilatory control and P_aCO_2 maintenance at rest is unclear (as indicated by a question mark). C. shows a hypoventilation relative to CO_2 excretion demands. When $\dot{V}_E/\dot{V}co_2$ decreases, P_aCO_2/pH_a will rise and fall (indicated by gray shading), respectively, leading to a respiratory acidosis that will acutely activate the CO_2 chemoreflex (larger asterisk).

elevated inspired CO_2 due to serotoninergic dysfunction (Puissant et al., 2015). Third, the use of pharmacogenetic technologies have allowed predominately catecholaminergic neurons, as well as other non- catecholaminergic neurons, to be acutely silenced within minutes upon intraperitoneal injection of an otherwise biologically inert ligand (Sun and Ray, 2017). Mice with acutely silenced catecholaminergic neurons have a ~70% reduction in the ventilatory response to CO_2. Fourth, toads with lesioned noradrenergic neurons of the locus coeruleus have a ~75% reduction in the ventilatory response to inspired CO_2 when assessed five days after the lesion. Lastly, bullfrogs recently emerged from an overwintering-like habitat have a ~80% reduction in stimulatory response to CO_2 challenge when assessed at a warmer body temperature (~24 °C). This deficit corresponded with an absence of CO_2/pH-sensitivity of the respiratory network *in vitro* and lower CO_2/pH sensitivity of neuronal firing in locus coeruleus neurons (Santin and Hartzler, 2016a; Santin and Hartzler, 2016b). Each of these models undoubtedly exhibit species-specific idiosyncrasies; however, similarities emerge among each model, which allows for comparison regarding ventilatory control. First, the loss of ventilatory CO_2 sensitivity in these models appears to emanate from central, as opposed to peripheral, origin. Second, when assessed, hypoxic ventilatory responses in all but one study (Sun and Ray, 2017) are normal. Therefore assessing ventilation, gas exchange, and/or blood gases in these models provides insight into the role of the CO_2 chemoreflex in producing normal ventilation.

3.1. Methodological considerations and interpretation

Three caveats must be mentioned. First, lesioning a chemosensory brain region not only disrupts chemosensory function, but may also disrupt respiratory rhythmogenesis independent of chemosensory function. For example, Nattie and Li (2006) lesioned several putative CO_2 chemosensory structures in the ventral medulla, resulting in a ~60% reduction in the hypercapnic ventilatory response and mild hypoventilation (− 8% decrease in ventilation relative to sham). Large-scale destruction of brainstem nuclei makes it difficult to determine whether hypoventilation originates from altered CO_2/pH sensitivity *per*

Table 1
Rodent and amphibian models with severely blunted ventilatory responses to inspired CO_2.

Animal	Reason for reduced CO_2 chemoreflex	Time scale	% reduction in ventilatory response to CO_2	Reference
Mouse	GPR4 gene KO	Chronic	~65%	Kumar et al. (2015)
Mouse	GPR4/TASK2 gene KO	Chronic	~85%	Kumar et al. (2015)
Mouse	Pharmacogenetic silencing of catecholaminergic neurons	Acute	~70%	Sun and Ray (2017)
Rat	Strain differences (Brown Norway *vs.* Sprague Dawley)	Chronic	~90–100%	Puissant et al. (2015)
Bullfrog	Overwintering environment	Chronic	~80%	Santin and Hartzler (2016a)
Toad	Pharmacologic lesion of noradrenergic neurons	Acute	~75%	Noronha-de-Souza et al. (2006)

se, aberrant network function independent of CO_2/pH, or both. In contrast, experiments using GPR4 and TASK-2 knockout mice elegantly delete chemosensory molecules without interrupting neuron and network integrity (Kumar et al., 2015). Furthermore, studies that compare strain differences and environmentally-induced CO_2 insensitivity phenotypes (Puissant et al., 2015; Santin and Hartzler, 2016a) did not require damage of neural tissue to elicit reductions in ventilatory CO_2 sensitivity. Caution must therefore be exercised when interpreting the resting ventilatory phenotype of animals with lesioned or silenced neurons involved in generating the hypercapnic ventilatory response (Noronha-de-Souza et al., 2006; Sun and Ray, 2017). Second, cells involved in generating the CO_2 chemoreflex undoubtedly alter their activity in response to stimuli other than CO_2/pH of arterial blood (*e.g.*, neuromodulators, synaptic inputs, temperature, *etc.*). As such, neurons identified as "respiratory chemoreceptors" may function as polymodal receptors and/or integrators. These cells could be involved in matching ventilation to metabolism for maintenance of steady-state P_aCO_2 through mechanisms outside of mediating the CO_2 chemoreflex (Kanbar et al., 2016). Here I focus only on their role as CO_2/pH sensors which underlie the ventilatory response to changes in P_aCO_2 because their detection of subtle P_aCO_2 changes is hypothesized to maintain resting CO_2 homeostasis through negative feedback. Third, I only discuss measurements of arterial blood gases obtained from chronic indwelling catheters in unrestrained animals because this approach is less stressful and provides the most accurate measurement of P_aCO_2/pH_a in rodents (Iversen et al., 2012). If arterial blood gases were not provided, I interpret the connection between ventilation and metabolic rate ($\dot{V}_E/\dot{V}co_2$) since this determines P_aCO_2/pH_a at steady-state (Fig. 1). However, both $\dot{V}_E/\dot{V}co_2$ and arterial blood gases are sometimes not reported.

3.2. Minute ventilation

The first important commonality among these models is that animals with abnormally small CO_2 chemoreflexes have relatively normal minute ventilation ($\dot{V}_E$; volume of air consumed per minute) when breathing room air (Kumar et al., 2015; Noronha-de-Souza et al., 2006; Puissant et al., 2015; Santin and Hartzler, 2016a; Sun and Ray, 2017). Overwintered bullfrogs, male BN rats, toads with pharmacologically lesioned LC neurons, and mice with pharmacogenetic silencing of mechanisms underlying the CO_2 chemoreflex all have similar respiratory frequencies and tidal volumes that maintain $\dot{V}_E$ (Hodges et al., 2002; Noronha-de-Souza et al., 2006; Puissant et al., 2015; Santin and Hartzler, 2016a). Interestingly, female BN rats had greater levels of ventilation through an increased tidal volume. Altogether these findings provide evidence that animals with severely blunted CO_2 chemoreflexes have normal (or elevated in the case of female BN rats) minute ventilation.

3.3. Matching ventilation to metabolism

It is commonly stated or strongly implied that mechanisms underlying the CO_2 chemoreflex determine resting ventilation and maintenance of P_aCO_2/pH_a. If this is the case, then the CO_2 chemoreflex should play a role in matching ventilation to metabolic demands because P_aCO_2 is determined by ventilation relative to metabolic CO_2 production. Of the models analyzed here, BN rats with practically undetectable ventilatory responses to CO_2 have metabolic rates (measured as oxygen consumption; $\dot{V}o_2$) similar to Sprague-Dawley rats with intact CO_2 chemoreflexes (Hodges et al., 2002; Puissant et al., 2015). Consequently, P_aCO_2/pH_a does not differ between BN and Sprague-Dawley rats (Hodges et al., 2002). Supporting these observations in BN rats with serotonergic dysfunction, adult mice lacking serotonin-producing neurons have blunted hypercapnic ventilatory responses and exhibit normal $\dot{V}_E/\dot{V}o_2$ at rest compared to wild-type mice (Hodges et al., 2008). Additionally, Sun and Ray (2017) show that mice with an acutely silenced chemoreflex have a normal $\dot{V}_E/\dot{V}o_2$. This suggests that normal coupling of ventilation and metabolic rate in models with chronically depressed CO_2 chemoreflexes are not a result of a long-term compensation. These examples demonstrate that mice and rats match ventilation to metabolism for regulation of P_aCO_2/pH_a despite missing ~70–~100% of the CO_2 chemoreflex.

Like BN rats and mice, adult bullfrogs with severely blunted CO_2 chemosensitivity also have a $\dot{V}_E/\dot{V}co_2$, $\dot{V}_E/\dot{V}o_2$, and respiratory exchange ratio (RER; $\dot{V}co_2$: $\dot{V}o_2$) comparable to control bullfrogs with normal ventilatory responses to CO_2. This suggests that ventilation matches requirements for CO_2 elimination at rest in bullfrogs with severely blunted CO_2 chemoreflexes (Santin and Hartzler, 2016a). One issue with this interpretation is that the lungs may account for a relatively small fraction of the total CO_2 elimination (~25–75% at 20 °C) (Gottlieb and Jackson, 1976). Therefore, $\dot{V}_E/\dot{V}co_2$ in resting bullfrogs does not truly represent ventilatory control with respect to total CO_2 production as much of the excreted CO_2 occurs *via* the skin. Although the lungs do not account for total CO_2 excretion, this does negate the lungs as an important eliminator of CO_2. Gottlieb and Jackson (1976) showed that inhibiting lung ventilation in adult bullfrogs results in a profound respiratory acidosis. Moreover, increases in calculated alveolar ventilation maintains a nearly constant P_aCO_2/pH_a during venous hypercapnia elicited by cutaneous CO_2 loading (Jackson and Braun, 1979). Although a large portion of CO_2 loss in bullfrogs occurs *via* cutaneous gas exchange, these studies demonstrate that lung ventilation contributes to normal CO_2 excretion in bullfrogs. Therefore, similar $\dot{V}_E/\dot{V}co_2$ ratios in bullfrogs lacking a large percentage of the CO_2 chemoreflex compared to normal frogs suggests that the lung ventilatory component of CO_2 excretion does not depend on CO_2 feedback under resting conditions.

3.4. Breathing pattern and breath characteristics

Although CO_2 feedback through the chemoreflex is not critical for producing normal minute ventilation and matching ventilation to metabolic rate, the CO_2 chemoreflex may be able to fine-tune the organization and timing of breaths at rest. For example, male BN rats a have greater inspiratory time for normal breaths, as well as longer sighs. However, sighs occurred at a similar frequency between SD and BN rats (Hodges et al., 2002). In mice with acute pharmacogenetic reduction of the chemoreflex, breath timing surprisingly became more regular in room air, but these mice did not experience an increase in frequency or duration of apneas compared to controls in room air. In contrast, while breathing 100% O_2, GPR4 KO mice exhibited an enhanced number of ventilatory pauses (~10 apneas h^{-1} in WT *vs.* ~60 apneas h^{-1} in GPR4 KO; defined as > 1.2 s respiratory pause) (Kumar et al., 2015). Anuran amphibians with severely blunted CO_2 chemoreflexes have normal ventilatory patterns (Noronha-de-Souza et al., 2006; Santin and Hartzler, 2016a). The ventilatory pattern in bullfrogs, however, is highly variable among animals (personal observation, JMS), suggesting that aspects of the breathing patterns altered in frogs with reduced CO_2 chemoreflexes could be overlooked. Collectively, rodents with severely reduced CO_2 chemoreflexes have differences in organization or timing of ventilatory behaviors when breathing room air (Hodges et al., 2002; Sun and Ray, 2017) and hyperoxia (Kumar et al., 2015). These changes appear to be distinct to each model and, therefore, may reflect species differences and/or chronic *vs.* acute reductions in CO_2 chemosensitivity. Since ventilation, and the coupling of ventilation to metabolism, is normal in these models, the physiological significance of these changes in breathing pattern is unclear.

3.5. CO_2 chemosensitivity and sleep

The bulk of the available evidence indicates that ventilation adequately matches metabolic CO_2 production in awake animals with severely diminished CO_2 chemoreflexes. However, an important

Table 2
PHOX2B mutant mice do not have CO_2 chemosensitivity at birth and only undergo partial recovery at older ages despite normal ventilation in room air.

PHOX2B mutant mice		
Age	$\dot{V}_E$ (~% reduction relative to wild type)	CO_2 chemoreflex (~% reduction relative to wild type)
P2	40%	100% (no ventilatory response)
P9	20%	100% (no ventilatory response)
P22	0% (normal)	80%
Adult	0% (normal)	60%

consideration, especially for mammals, is that these studies did not determine aspects of ventilatory control during non-REM (NREM) sleep where the CO_2 chemoreflex can potentially play a much larger role in breathing stability (Dempsey, 2005). Control of ventilation during NREM sleep has classically been attributed to CO_2/pH stimuli because: (1) there is a loss of "wakefulness drive" to breathe (Skatrud and Dempsey, 1983), (2) a sensitive hypocapnic apneic threshold contributes to sleep apnea in humans (Xie et al., 1997), and (3) humans hypoventilate upon the transition from wakefulness into NREM sleep causing P_aCO_2 to rise (Robin et al., 1958). Additionally, ventilatory control *via* the CO_2 chemoreflex is thought to be critical in humans as those who suffer from congenital central hypoventilation syndrome (CCHS) have severely blunted CO_2 chemoreflexes and often stop breathing during sleep when they are not supported by a mechanical ventilator. Interestingly, chronic reductions in the CO_2 chemoreflex of the previously discussed rodents have a similar (or greater) magnitude of reduction in the ventilatory response to CO_2 challenge as in awake, genotyped CCHS patients (~78%, reduction, on average compared to control subjects) (Carroll et al., 2014). Unlike CCHS patients, these rodents control ventilation adequately enough during sleep to survive and become adults that can participate in experiments that assess ventilation. If CO_2/pH stimuli are viewed as the primary driver of rhythmic breathing during NREM sleep, it may seem peculiar that these animals with a chronic, large reduction in the CO_2 chemoreflex lived to adulthood and did not die from respiratory insufficiency during sleep.

A couple of possibilities may explain this observation. First, if mechanisms underlying the CO_2 chemoreflex are the primary driver of rhythmic breathing during sleep, rodents with extremely low ventilatory responses to CO_2 while awake may retain them during NREM sleep. This possibility is not unreasonable since the sleep-wake dependence of multiple chemosensitive regions is well-established. For example, in adult rats, focal acidosis of the RTN increases ventilation during wakefulness but not during sleep (Li et al., 1999), while acidification of the medullary raphé increases ventilation during sleep but not wakefulness (Nattie and Li, 2001). Furthermore, intense stimulation of the RTN in rats increases ventilation across all sleep-wake states (Burke et al., 2015). Thus, adequate ventilation during sleep could simply reflect the presence of the CO_2 chemoreflex during NREM sleep. Second, if adult animals do in fact have diminished CO_2 chemoreflexes across all sleep-wake states, it is plausible that relative insensitivity to CO_2 has reduced the hypocapnic apneic threshold and protected these animals against apneas and breathing instability. Breathing stability during NREM sleep depends on two important interacting factors: ventilatory CO_2 sensitivity, termed, "controller gain" and the influence of background respiratory drive on P_aCO_2, termed, "plant gain". Decreasing ventilatory responsiveness to CO_2 and increasing the background level of respiratory drive protect animals against ventilatory instability by broadening the gap between resting P_aCO_2 and P_aCO_2 at apnea (Dempsey, 2005). Therefore a reduced CO_2 chemoreflex in the presence of a maintained background respiratory drive could stabilize breathing by reducing the apneic P_aCO_2 threshold. If this possibility is correct, additional factors besides CO_2/pH stimuli must provide sufficient background respiratory drive during NREM sleep in animals with minimal ventilatory responses to CO_2. It is clear that study of these rodent models with minimal ventilatory responses to CO_2 could provide new insights into the role of CO_2 chemosensors during sleep.

4. Does CO_2 chemoreception play a role in matching ventilation to CO_2 production in neonates?

The previous section described that ventilation-metabolism matching for the preservation of arterial CO_2/pH under normal conditions does not critically rely on CO_2 feedback in awake, adult vertebrates. However, the CO_2 chemosensory system undergoes changes during postnatal life (Putnam et al., 2005) due to developmental and/or environmental alternations associated with maturation. Therefore, it is worth assessing the available evidence regarding the role of the CO_2 chemosensory system in stabilizing ventilation during early postnatal life.

Development of mechanisms underlying the ventilatory response to CO_2 in air-breathing vertebrates has been mainly studied in rodents (Putnam et al., 2005) and to a lesser extent, anuran amphibians (Infantino, 1992; Rousseau et al., 2016; Taylor et al., 2003a; Taylor et al., 2003b; Torgerson et al., 1997). In rats, mice, and bullfrogs the ventilatory response to hypercapnia *in vivo* tends to increase during postnatal or metamorphic development until reaching an adult sensitivity (Cerpa et al., 2017; Davis et al., 2006; Infantino, 1992; Ramanantsoa et al., 2011). Most studies that have manipulated the CO_2 chemoreflex in intact, developing animals were performed using transgenic mice. First, Ramanantsoa et al. (2011) tracked changes in the ventilatory response to CO_2 in mice containing a mutated transcription factor, *PHOX2B*, that causes CCHS in human patients (Table 2). This mutation leads to a reduced ventilatory response to CO_2 by eliminating retrotrapezoid nucleus neurons. Second, Cerpa et al. (2017) followed development of CO_2 chemoreception in mice lacking serotonergic neurons (*Lmx1b*$^{(f/f/p)}$ mutant mice; Table 3). Is the CO_2 chemoreflex critical in these juvenile rodents?

Mice harboring the *PHOX2B* mutation had a severe CO_2-insensitivity phenotype. In this model, mice did not exhibit ventilatory responses to hypercapnia (8% CO_2 in air) until P22 (postnatal day 22). At this time point, ventilatory responses to CO_2 remained reduced by 80% compared to wild-type (WT) mice at this age and stayed 60% below WT during adulthood. In contrast to *PHOX2B* mutants, WT mice had robust CO_2 chemoreflexes that increased in magnitude throughout postnatal life (Ramanantsoa et al., 2011). Unlike the adult models discussed in this review, ventilation in room air was reduced in *PHOX2B* mutant mice at P2, but returned to ~80% of WT values by P9 (Table 2). It is unclear whether reduced ventilation in room air for P2 and P9 mutant mice occurred because of a lack of CO_2 chemoreception, absence of an essential component for normal rhythmogenic output caused by the *PHOX2B* mutation, or a combination of both. Because ventilation nearly recovered by P9 on a background of CO_2-

Table 3
Lmx1b$^{(f/f/p)}$ mutant mice cannot match ventilation to metabolic demand ($\dot{V}_E/\dot{V}o_2$) in the first few days of postnatal life when CO_2 chemoreflexes are normal. At P21 ventilation matches metabolic demands, but the CO_2 chemoreflex remains approximately half of its normal value.

Lmx1b$^{(f/f/p)}$ mutant mice		
Age	$\dot{V}_E/\dot{V}o_2$ (~% reduction relative to wild type)	CO_2 chemoreflex (~% reduction relative to wild type)
P4	50%	0% (normal)
P7	50%	0% (normal)
P12	0% (normal)	0% (normal)
P21	0% (normal)	50%

insensitivity, it seems likely that loss of RTN neurons causes dysfunction in the respiratory network independently of absent CO_2 chemosensitivity. Regardless of the cause underlying low baseline ventilation, most *PHOX2B* mutant mice in this study lived to adulthood without ventilatory responses to CO_2 in the first few postnatal weeks. The authors concluded that central CO_2 chemoreception is dispensable for survival in postnatal life and into to adulthood (Ramanantsoa et al., 2011).

Unlike the *PHOX2B* mutant mice lacking ventilatory responses to CO_2 in the early postnatal stage, mice without serotonergic neurons have normal ventilatory responses to CO_2 when compared to WT mice (Table 3). However, serotonin-deficient mice fail to develop full ventilatory chemosensitivity at a later neonatal age, P21, compared to WT (Cerpa et al., 2017). Despite a normal ventilatory response to CO_2, mice lacking serotonin neurons do not match ventilation to metabolism until P12 (*i.e.*, $\dot{V}_E/\dot{V}o_2$ was ~50% of the WT value). Inadequate ventilation-metabolism matching manifests as a greater number of prolonged apneas (~5–10 s duration; ~1 prolonged apnea per minute), which likely generates a respiratory acidosis in early neonates (Cerpa et al., 2017; Hodges et al., 2009). Interestingly, at P21 ventilation now matched metabolic demands, yet the CO_2 chemoreflex was reduced. Thus, mice lacking serotonergic neurons seem to regulate acid-base status normally through ventilation despite a dysfunctional CO_2 sensory system at older ages; however, P4–P7 mice severely hypoventilate at an age when CO_2 chemosensitivity is otherwise normal.

What can we learn from these studies using transgenic neonatal mice? First, it is unlikely that CO_2 chemosensitivity plays a large role in coupling ventilation to metabolism at rest. *PHOX2B* mutant neonatal mice lacking ventilatory responses to CO_2 have levels of ventilation approaching normal due to a recovery of chemoreflex-independent ventilatory dysfunction after P2 before CO_2 chemosensitivity partially recovers at later ages (Ramanantsoa et al., 2011). Second, breathing stability and ventilation-metabolism matching requires serotonergic neurons, but not a full CO_2 chemoreflex. This conclusion was drawn because mice without serotonergic neurons severely hypoventilate (*i.e.*, a 50% reduction in the air convection requirement for O_2) despite having intact CO_2 chemoreflexes (Cerpa et al., 2017; Hodges et al., 2009). When ventilation matches metabolism at older ages, ventilatory responses to CO_2 are abnormally low in these mice (Cerpa et al., 2017; Hodges et al., 2008). Taken together, these findings imply that the CO_2 chemoreflex is not essential to couple ventilation to metabolism in neonatal mice.

To summarize, the available evidence indicates that ventilation, gas exchange, and acid-base status (when assessed) are normal in animals with abnormally low ventilatory responses to CO_2 in diverse animal models across taxa and development. Of note, the most severe respiratory phenotype described here (~1 prolonged ~10s apnea per minute; $\dot{V}_E/\dot{V}o_2$ ~50% of control) originates from lack of serotonergic neurons early in development, but not decreased CO_2 chemosensitivity (Cerpa et al., 2017) (Table 3). CO_2/pH feedback does not likely play a primary role in coupling ventilation to metabolism for the maintenance of steady-state P_aCO_2 under resting conditions.

5. How is ventilation coupled to CO_2 production?

If the CO_2 chemoreflex does not make a large contribution to maintaining resting P_aCO_2, what mechanisms do? Three feedback or feedforward mechanisms seem to be important in the neural control of ventilation during changes in metabolism: pulmonary CO_2 exchange, central command (*i.e.*, supramedullary inputs to the respiratory control system), and group III and IV thin fiber afferents (*i.e.*, sensory neurons connecting the muscle microenvironment to the respiratory control system *via* relay neurons in the spinal cord) (Dempsey et al., 2014). Since these mechanisms presumably interact to match ventilation to metabolism during exercise, it is reasonable that they may be engaged to couple ventilation to CO_2 production at or near rest. Group III/IV muscle afferents have received a fair amount of attention, especially in mammals. In humans, reducing afferent feedback resulted in a hypoventilation relative to CO_2 production (as determined by an increase in end tidal CO_2) and blunted cardiovascular responses to exercise throughout a range of intensities (Amann et al., 2010). These afferents have also been suggested to match ventilation to CO_2 production induced by muscle contraction in anesthetized sheep. Importantly, this mechanism operated in response to small changes in CO_2 production near rest (Haouzi and Chenuel, 2005). Group III/IV thin fiber afferents are intriguing because they: (1) are sensory neurons embedded in the interstitial space of skeletal muscle and intimately interact with arterioles and venules of the capillary bed (Stacey, 1969), (2) may detect chemical and mechanical stimuli generated in proportion to metabolism at their site of production (*e.g.*, H^+, ATP, blood flow, temperature) (Haouzi et al., 1999; Li and Sinoway, 2002; Sinoway et al., 1993), (3) synapse onto interneurons that project to brain regions involved in respiratory control, including CO_2/pH sensitive RTN neurons that regulate respiratory motor output (Kanbar et al., 2016), and (4) operate independently of the CO_2 chemoreflex because their stimulation increases ventilation to match increases in $\dot{V}co_2$ with arterial blood gases perfusing the central and peripheral chemoreceptors clamped at normal values (Haouzi and Chenuel, 2005). Group III/IV afferents may therefore represent an elegant mechanism allowing ventilation to precisely match metabolic demands caused by light activities near rest through mechanical and/or chemical sensing of factors proportional to metabolism in muscle.

The mechanisms enabling group III/IV afferents to match ventilation to CO_2 production have been suggested to involve sensing venous distention caused by changes in muscle blood flow in response to muscle contraction (Haouzi and Chenuel, 2005; Haouzi et al., 1999). The molecular mechanisms underlying mechanical sensitivity of group III/IV afferents for ventilatory control are unknown. However, in adult rats, Copp et al. (2016) demonstrated that an inhibitor of mechanosensitive cation channels, Piezo I and II, blunted the cardiovascular pressor reflex, a response also mediated in part by group III/IV thin fiber afferents. Since Amann et al. (2010) showed that both cardiovascular and ventilatory increases during moderate intensity exercise depend on afferent feedback, mechanosensitive ion channels expressed on the terminals of group III/IV afferents could contribute to coupling ventilation to metabolism if the same receptors are involved in ventilatory control. Maintenance of steady-state P_aCO_2 through ventilation may therefore be driven by mechanical feedback at the level of the capillary bed (Haouzi and Chenuel, 2005), in part, *via* Piezo mechanoreceptors. This idea is intriguing because, if supported, maintenance of P_aCO_2 would be a byproduct of molecular mechanisms underlying neuronal mechanosensitivity to blood flow rather than what is truly homeostatic feedback through CO_2/pH stimuli.

Matching of ventilation to $\dot{V}co_2$ through mechanoreceptors expressed in group III/IV thin fiber afferents is an attractive hypothesis. However, results are inconsistent as to whether an intact spinal cord is required to elicit increases in ventilation during electrically-induced muscle contractions of the lower limbs (Adams et al., 1984; Brice et al., 1988; Comroe and Schmidt, 1943; Cross et al., 1982). Since ventilation has, in some cases, been shown to increase during muscle contraction without spinal transmission from the muscle to brainstem, additional mechanisms must also be involved in matching ventilation to small changes in metabolism. It has been known for years that manipulating CO_2 in the venous blood and changing pulmonary blood flow alters ventilation (Green and Sheldon, 1983; Phillipson et al., 1981a; Phillipson et al., 1981b). In the studies on awake sheep by Phillipson et al., an extracorporeal loop was used to progressively remove CO_2 from the venous circulation, which caused ventilation to cease while P_aCO_2 remained normal. When CO_2 was added to venous circulation ventilation increased, while again maintaining P_aCO_2. However, an approximate doubling of resting $\dot{V}co_2$ led to a respiratory acidosis in this preparation. Interestingly, a ventilatory control mechanism with sensitivity to pulmonary CO_2 exchange may also exist in bullfrogs. When

hypercapnia of the venous circulation was induced by loading across the skin, alveolar ventilation increased to eliminate metabolic CO_2 and all of the added CO_2, while P_aCO_2 remained largely unchanged (Jackson and Braun, 1979). One hypothesis that could support these findings is that sensory neurons near the alveoli of mammals (*i.e.*, pulmonary C-fibers, also known as J receptors) could somehow play a role regulating ventilation to meet CO_2 excretion demands around resting values (Dempsey et al., 2014). Recently, genetically identified pulmonary C-fibers induced to express photo-sensitive cation channels allowed these neurons to be activated by light. During stimulation by light, ventilation increased demonstrating that activation of genetically identified pulmonary C-fibers is sufficient to alter breathing (Chang et al., 2015). Therefore it is plausible that presently unknown mechanisms expressed on the sensory terminals C-fibers may provide the respiratory control system with a readout of CO_2 exchange at the lung. Of note, activation of pulmonary C-fibers probably does not involve direct sensing of CO_2/pH because rather large changes in CO_2/pH do not significantly alter their firing rates in anesthetized rats (Lin et al., 2005).

6. Ectothermic vertebrates routinely uncouple ventilation from metabolism and experience fluctuations in P_aCO_2

For most endotherms (*i.e.*, mammals and birds) that continuously breathe fresh air, CO_2 feedback seems to play a small role in steady-state CO_2/pH homeostasis and must rely heavily on mechanisms that match ventilation to metabolic rate (or related stimuli proportional to metabolism). Although ventilation performs this role adequately at rest in frogs and toads with severely blunted CO_2 chemoreflexes (Noronha-de-Souza et al., 2006; Santin and Hartzler, 2016a; Santin and Hartzler, 2016b), most ectotherms (*e.g.*, amphibians, reptiles, and fish) exhibit behavioral and physiological characteristics that regularly limit gas exchange and/or access to fresh air. As a result of uncoupling ventilation from metabolism (Fig. 1C), P_aCO_2/pH_a in these animals changes substantially during daily life. Furthermore, animals across vertebrate classes have CO_2/pH chemoreceptors capable of increasing ventilation of lungs or gills (Milsom, 2010; Milsom, 2012), suggesting that the acute respiratory acid-base disturbances that occur naturally could be compensated through classic negative feedback mechanisms after removal of the limitation to gas exchange. Similar scenarios also apply to select mammals and birds such as diving and burrowing species; however, limitations to gas exchange do not reflect typical avian and mammalian physiology. The following section will discuss potentially generalizable environmental and physiological constraints on gas exchange for ectotherms, resulting in normal changes in P_aCO_2/pH_a that may require a CO_2 chemoreflex for blood gas regulation. However, it is important to keep in mind that ectotherms inhabit diverse environments that result in strikingly different demands on their respiratory systems. Furthermore, many of scenarios described below have been studied in a relatively low number species, suggesting that the full breadth of ectothermic physiological responses cannot be properly considered.

6.1. Breath-hold diving

Many air-breathing ectotherms hold their breath as a normal part of the episodic respiratory pattern, in response environmental hypercapnia, and during behaviors that preclude lung ventilation. Many species of amphibians and reptiles dive for tens of minutes to hours, which undoubtedly causes a respiratory acidosis. Amphibians and reptiles dive for several reasons including, but not limited to aquatic lifestyle, predator avoidance, foraging, and drowning prey (Cott, 1961; Gregory, 1979; Houghton et al., 2000). Although acid-base homeostasis is critical, there appears to be a trade-off between diving behaviors and temporary disturbances in acid-base homeostasis.

The extent of the respiratory acidosis during a dive will depend on a combination of factors including the dive duration, metabolic rate, and extrapulmonary gas exchange. Although bullfrogs use cutaneous gas exchange, P_aCO_2/pH_a increases from ~11 mm Hg/7.9 at 25 °C to ~18 mm Hg/7.63 after a relatively short, 11 min, dive at 25 °C (Lillo, 1978). *Xenopus laevis* also exhibits a large respiratory acidosis during a 30 min dive with P_aCO_2 increasing from ~16 mm Hg to ~28 mm Hg and pH_a decreasing from ~7.8 to ~7.6. After surfacing, minute ventilation increases 10-fold compared to pre-dive values and P_aCO_2 returns to baseline within ~2 h (Boutilier and Shelton, 1986). Similar trends for respiratory acidosis have been observed during long submergences in reptiles including certain turtles (Bagatto and Henry, 1999; Burggren and Shelton, 1979; Robin et al., 1981), alligators (Andersen, 1961), and snakes (Seymour and Webster, 1975). Intriguingly, the respiratory acidosis during dives occur within a range where chemosensory neurons generating the hypercapnic ventilatory response in anuran amphibians are highly sensitive to CO_2/pH (Santin and Hartzler, 2013; Santin et al., 2013). It is, therefore, reasonable to speculate the hyperventilation following a dive recovers P_aCO_2 by, what is truly, homeostatic feedback from CO_2 chemoreceptors as predicted by Haldane and Priestley (1905).

Submergence does not always lead to a respiratory acidosis. Amphibians, such as aquatic salamanders (Toews et al., 1971), and turtles (Gordos et al., 2004) with highly efficient extrapulmonary gas exchange do not increase P_aCO_2 during submergence. Additionally, adequate cutaneous gas exchange paired with low metabolic rates, typically associated with cold temperatures (< ~5 °C), prevents respiratory acidosis in submerged frogs (Tattersall and Ultsch, 2008), indicating that cutaneous gas exchange matches or exceeds CO_2 excretion demands (Tattersall and Boutilier, 1999a). It is worth mentioning that even if a respiratory acidosis did occur at low body temperatures, chemoreceptors in amphibians would not respond to CO_2/pH (Morales and Hedrick, 2002; Santin et al., 2013) resulting in no ventilatory stimulation (Bicego-Nahas and Branco, 1999). With these exceptions in mind, respiratory acidosis induced by diving is relatively common among ectotherms. Therefore, compared to terrestrial mammals, breath-hold diving should activate the chemoreflex to make a large contribution to the overall acid-base regulation of these animals. However, the link between activation of the CO_2 chemoreflex and regulation of P_aCO_2 following a dive has not been definitively established.

6.2. Episodic breathing

Independent of diving, ectotherms tend to have episodic breathing patterns with variable amounts of time between breathing episodes (Milsom, 1991). Although this breathing strategy is common to many ectotherms, episodic breathing can occur in otherwise continuous breathing mammals and birds during early development and at low body temperature (Fong et al., 2009). Although select scenarios, such as reduced body temperature, may induce episodic breathing in endotherms, it is reasonable to suggest that episodic/intermittent breathing is a trait largely characteristic of ectothermic air-breathing vertebrates.

In episodic breathers, blood and tissue CO_2 stores fluctuate because an episodic pattern of lung ventilation is less efficient for CO_2 excretion compared to evenly spaced breaths, even at identical ventilation rates in the same individual (Malte et al., 2013). Modeling studies predict that the hypercapnic acidosis that occurs with episodic breathing can be accounted for by a ventilation-perfusion mismatch as well as the high solubility of CO_2 for body fluids (Malte et al., 2016). Thus, the same level of ventilation in an episodic pattern causes a transient respiratory acidosis because blood cannot carry CO_2 stored in the tissues to the lungs quickly enough to maintain steady-state. Continuous breathing, in contrast, prevents tissue CO_2 loading by maintaining an adequate relationship between ventilation and perfusion. Hyperventilation relative to metabolism in subsequent breathing episodes would be

required to clear the resulting hypercapnic acidosis generated by episodic breathing. In addition to a compensatory response of hyperventilation to transient hypercapnia, Malte et al. (2013) demonstrated that turtles, *Trachemys scripta*, reduced the respiratory exchange ratio (RER) by decreasing metabolic CO_2 production when ventilated episodically. Lowering CO_2 production during episodic breathing patterns may, therefore, act as a mechanism to combat the ensuing respiratory acidosis generated by episodic breathing. Although the underlying mechanism that lowers $\dot{V}co_2$ remains unknown, these results suggest that episodic breathing can somehow shift the use of fatty acids as a metabolic fuel to reduce the concomitant respiratory acidosis. Acid-base regulation through breathing pattern *per se* is interesting and warrants further study.

Because CO_2 accumulates during a non-ventilatory period, the logical cause for a relative hyperventilation immediately following a breath-hold (*i.e.*, the next episode) is negative feedback from CO_2 (and O_2) chemoreceptors. This seems unlikely because ectotherms still exhibit episodic breathing when blood gas oscillations are dampened by unidirectional ventilation (Kinkead and Milsom, 1994). Furthermore, bullfrogs with severely blunted ventilatory responses to inspired CO_2 still exhibit episodic breathing (Santin and Hartzler, 2016a). Instead of chemosensory feedback, episodic breathing seems to be an intrinsic property of the respiratory rhythm generator and is likely under the control of other peripheral influences (Gargaglioni et al., 2007; Kinkead and Milsom, 1996; Winmill et al., 2005). Although hypercapnia resulting from intermittent breathing does not explain the generation of breathing episodes, CO_2 oscillations may provide a stimulus to central and peripheral chemoreceptors, which could increase its role in ventilatory control of acid-base balance compared to most endotherms lacking large fluctuations in arterial blood gases. It seems reasonable to speculate that the CO_2 chemoreflex may influence ventilation at some point after the breath-hold, with strength correlating with breath-hold duration. For example, an 8 h non-ventilatory period (Coelho and Smatresk, 2003) may require greater amounts of CO_2 chemoreflex input to correct the respiratory acidosis when breathing resumes compared episodes occurring at a rate of one to two per minute (Kinkead and Milsom, 1996; Santin and Hartzler, 2016c). Breath-holding and intermittent ventilation, common to air-breathing ectotherms, provides an opportunity to assess the contribution of the CO_2 chemoreflex under conditions that might play a physiological role in ventilatory control of acid-base status.

6.3. Exercise

The respiratory system limits CO_2 excretion during activity in vertebrates (Hedrick et al., 2015; Hillman et al., 2013). An inability to sufficiently match ventilation to CO_2 production during activity will produce a respiratory acidosis and CO_2 chemoreflex activation. It is well-established that low to moderate intensity exercise in mammals and birds results in an isocapnic or hypocapnic hyperpnea, thus preventing respiratory acidosis (Brackenbury and Gleeson, 1983; Fedde et al., 1989; Forster et al., 2012). Therefore, the CO_2 chemoreflex does not play a major role in generating the hyperpnea of exercise in mammals (Forster et al., 2012). Does exercise result in similar matching of ventilation to CO_2 production in ectotherms?

6.3.1. Non-avian reptiles

Different reptile species avoid a respiratory acidosis during exercise through an isocapnic or hypocapnic hyperpnea despite drastically different environmental and mechanical constraints on ventilation. Lung ventilation during exercise in some reptiles may be limited by their anatomical structures because the muscles involved in ventilation are also used for locomotion (Carrier, 1987; Jackson and Prange, 1979). Regardless, of the squamates studied (*i.e.*, lizards and snakes), each increased ventilation during exercise, hyperventilated relative to CO_2 production and, when measured, experienced hypocapnia (Mitchell et al., 1981; Secor et al., 2000; Wang et al., 1997). The strategy used to avoid respiratory acidosis appears to differ among species. Savannah monitor lizards, *Varanus exanthematicus*, increase ventilation with metabolism across a range of exercise intensities (Wang et al., 1997). Similarly, iguanas, *Iguana iguana*, match ventilation to CO_2 excretion requirements across a span of exercise intensities. However, with more strenuous activity, ventilation and metabolism both decrease proportionally, thus avoiding respiratory acidosis (Mitchell et al., 1981; Wang et al., 1997). Like squamates, crocodilians also hyperventilate during exercise (Farmer and Carrier, 2000). Aquatic turtles differ in that they experience a respiratory acidosis while swimming, presumably because they have to surface to breathe (Butler et al., 1984). On land however, sea turtles cannot ventilate their lungs while locomoting because limb muscles are involved in both terrestrial locomotion and ventilation, as previously mentioned. Despite this anatomical constraint, sea turtles exercising on land adopted an intermittent pattern of locomotion to allow breathing in the absence of limb movement. Surprisingly, this strategy results in a hypocapnic hyperventilation, demonstrating an adequate ability to meet CO_2 excretion demands during exercise (Jackson and Prange, 1979). In contrast, terrestrial turtles can locomote and breathe simultaneous during activity (Landberg et al., 2003) suggesting the ability to increase ventilation in order to maintain acid-base balance. Like endotherms, most of the evidence indicates that squamate, crocodilian, and testudine reptiles can match ventilation to CO_2 excretion requirements of exercise without generating a respiratory acidosis. This is interesting because the coupling of ventilation to metabolism likely occurs through disparate ventilatory control and behavioral mechanisms, despite different constraints on ventilation and locomotion.

6.3.2. Amphibians

Unlike reptiles, amphibians do not match ventilation to CO_2 excretion across a range of CO_2 loads. Although anurans amphibians (*i.e.*, frogs and toads) increase breathing frequency during activity, pH_a decreases during forced activity mainly due to metabolic acidosis, but also a respiratory acidosis (McDonald et al., 1980; Withers et al., 1988). Like anurans, P_aCO_2 rises despite an increase in lung ventilation frequency during activity in salamanders that primarily use their skin to breathe (Boutilier et al., 1980). Thus, arterial side CO_2 chemoreceptors could respond to elevated P_aCO_2 and contribute to hyperpnea of exercise and recovery from respiratory acidosis following exercise. However, increased ventilation in response to hypercapnia during exercise is more likely to have physiological significance in anurans because skin-breathing salamanders have poorly vascularized lungs that play only a small role in CO_2 regulation (Guimond and Hutchison, 1973). Thus gas exchange abilities of the lung paired with the relative amount of cutaneous gas exchange determines the extent to which ventilation can match CO_2 production in various amphibian species. Since P_aCO_2 increases in response to exercise, the respiratory system of amphibians seems to have a lower capacity for coupling ventilation to CO_2 production, especially compared to endotherms and non-avian reptiles.

In contrast to large CO_2 loads during exercise, bullfrogs may have at least some capacity to alter ventilation in response to small changes in metabolism. Increasing CO_2 flux across the skin leads to venous hypercapnia enabling CO_2 to return to the lungs *via* the venous return (Jackson and Braun, 1979) as occurs during normal increases in metabolism. CO_2 loading through the skin of bullfrogs results in an approximately isocapnic doubling of alveolar ventilation and an increase in the pulmonary respiratory exchange ratio from ~0.55 to 1.3. Therefore, bullfrogs were able to eliminate all of the exogenous CO_2 as well the CO_2 produced through metabolism by increasing lung ventilation. The ability to elevate pulmonary CO_2 excretion and maintain P_aCO_2 indicates that ventilation doubled without contribution from arterial-side CO_2/pH chemoreceptors. These findings are consistent with ventilation matching $\dot{V}co_2$ at rest in bullfrogs with severely blunted ventilatory responses to CO_2 (Santin and Hartzler, 2016a; Santin and

Hartzler, 2016b). However, this response is not universal in amphibians. In a basal anuran, *Pipa carvalhoi*, as well as aquatic salamanders, hypercapnia that presumably elicited venous and arterial hypercapnia does not stimulate ventilation (Fonseca et al., 2012), which leads to a respiratory acidosis (Heisler et al., 1982; Toews, 1971). Therefore ventilation may be able to match small changes in CO_2 production in at least some, but not all amphibians, and its capacity appears to be substantially less than that of non-avian reptiles and endothermic mammals.

6.3.3. Jawless, cartilaginous, and bony fishes

In fish, O_2 chemoreceptors primarily drive ventilation to regulate arterial PO_2. However, it is now recognized that many water and bimodal (air and water) breathing fish have CO_2/pH chemoreceptors capable of altering ventilation of gills and air-breathing structures during environmental hypercarbia (Perry and Abdallah, 2012). All classes of fish studied experience changes in P_aCO_2 during exercise (Brauner et al., 2000; Tufts, 1991; Wood and Munger, 1994) and also increase ventilation during aquatic hypercarbia. Can the mechanisms that stimulate ventilation during externally applied CO_2 increase gill ventilation or air breathing to correct a respiratory acidosis following intense exercise?

The answer to this question is not straightforward for two main reasons. First, some fish have a strong Root effect that acts to decrease O_2 saturation of hemoglobin during hypercapnia. Thus a respiratory acidosis could stimulate ventilation through concurrent hypoxia due to the Root effect, the respiratory acidosis *per se*, or both. Although the Root effect may account for part of the ventilatory response to hypercapnia (Smith and Jones, 1982), there are many cases where gill ventilation frequency and/or amplitude increase during hypercapnia without changes in PO_2 or O_2 content (see Table 2 from Gilmour, 2001). Additionally, of the species from this table, elasmobranchs lack Root effect hemoglobins, supporting the argument for CO_2/pH sensors generating ventilatory responses to hypercapnia. Second, CO_2/pH sensors in the gills may be oriented externally or internally to sense either water or blood CO_2, respectively. If CO_2 sensors in the gill arches are positioned externally to sense CO_2 tensions of the water, an internally derived respiratory acidosis may not reach the CO_2 sensors to increase gill ventilation. Of the experiments that separated internal *vs.* external CO_2 sensing of the gills in fish, the vast majority of studies indicated that gill CO_2 sensors face externally, which may preclude them from responding to internally generated arterial acid-base disturbances (see Tables 6–8 from Milsom, 2012). However, there is contradictory evidence that supports the presence of internal CO_2/pH sensors. In rainbow trout, an obligate water breather, and gar, a facultative air breather, exhaustive exercise led to a respiratory acidosis. In response to this arterial acid-base disturbance, ventilation of water, and air in the case of gar, remained elevated in the post-exercise period until P_aCO_2 recovered (Burleson et al., 1998; Wood and Munger, 1994). When the respiratory acidosis was prevented by carbonic anhydrase, which facilitates CO_2 excretion, the post-exercise hyperpnea was blunted in trout (Wood and Munger, 1994). This provides strong evidence that the respiratory acidosis of heavy exercise in fish may be compensated through hyperventilation generated by internal CO_2/pH chemoreceptors.

In summary, fish generally experience respiratory acidosis in response to exercise, but the extent to which an internally-derived hypercapnic ventilatory response could act homeostatically to correct this disturbance is unclear. Although there is evidence presented that argue against this possibility, Wood and Munger (1994) provide the most convincing evidence that ventilatory correction of a respiratory acidosis could be mediated by internal CO_2/pH sensors in fish. This implies that hypercapnia caused by exercise provides the CO_2 chemoreflex with a physiological CO_2 error signal in the earliest water-breathing vertebrates.

6.3.4. Usefulness of flexibility and plasticity of the CO_2 chemoreflex

Ectotherms have remarkably flexible and plastic CO_2 chemosensory systems in response to changing environments. Flexibility refers to a built-in ability to modulate CO_2 chemosensitivity during acute environmental challenges and plasticity refers to a change in response to a prior experience. Given that these animals seem to routinely use this system to correct respiratory disturbances to blood gases, what kinds of advantages might the ability to modulate chemosensitivity provide? First, decreased CO_2 chemosensitivity might allow for longer breath holds by reducing the drive to breathe during respiratory acidosis. Because diving is an important adaptive response for predator avoidance in ectothermic animals (Gregory, 1979; McIntyre and McCollum, 2000), an ability to overcome a powerful drive to breathe might serve as a predator avoidance strategy. In turtles, *Trachemys scripta elegans*, ventilatory chemosensitivity is reduced at night, facilitating longer submergence times when turtles are at high risk for predation (Reyes and Milsom, 2009). Plasticity that reduces CO_2 chemosensitivity may also play a similar role in bullfrogs after overwintering submergence when faced with terrestrial predators in the spring (Santin and Hartzler, 2016a). Second, chemosensitivity may be altered in proportion with a need to use lungs for the elimination of CO_2. For example, chemosensory neurons that control ventilation in bullfrogs have sensitivities proportional to their body temperature, such that decreases in temperature no longer allow CO_2 to stimulate ventilation (Bicego-Nahas and Branco, 1999; Morales and Hedrick, 2002; Santin et al., 2013). Since submerged frogs at cold temperatures can compensate a respiratory acidosis in response to activity using their skin alone (Tattersall and Boutilier, 1999b), having the flexibility to depress CO_2 sensitivity might enable frogs to avoid the energetic costs of intermittently swimming to the surface to excrete CO_2 *via* the lungs after vigorous activity. Conversely, increasing CO_2 sensitivity would be inherently valuable at warm temperatures, as excess CO_2 must be excreted by the lungs in order to correct an acute respiratory acidosis. In clinical scenarios, deviations from "normal" CO_2 chemosensitivity tend to occur in pathological contexts that lead to reduced arousal or increased chemoreflex gain, especially during sleep (Dempsey et al., 2004; Guyenet, 2014). Although these scenarios are pathological in humans and rodent models, they seem to manifest during situations associated with inadequate gas exchange and intermittent ventilation (*e.g.*, congestive heart failure, sudden infant death syndrome, sleep apnea). Given the variable gas exchange abilities and requirements of ectothermic air-breathers, it is interesting to speculate that plasticity and flexibility of the chemosensory system could be advantageous in animals exploiting diverse ecological niches each with unique and variable convective environments. Environmental pressures associated with present-day cardiorespiratory disease might "unlock" mechanisms that modulate chemosensitivity in a way that is typically associated with a variable need for CO_2/pH to drive ventilation in ectotherms; however, in humans and other mammals with radically different environmental demands, chemoreceptor plasticity coexists with pathologies analogous to normal physiological scenarios of ectotherms. Perhaps a better understanding of how and/or why CO_2 chemoreceptors respond to environmental challenges in ectotherms could paradoxically provide clues into the underlying mechanisms of altered CO_2 chemoreflex gain observed in respiratory pathologies of mammals.

7. Evolutionary considerations

Nattie and Li (2012) stated that "central chemoreception grew out of the demands posed by air *vs.* water breathing, homeothermy, sleep, optimization of the work of breathing with the 'ideal' arterial PCO_2, and the maintenance of the appropriate pH at 37°C". However, data presented in this review indicate that the CO_2 chemoreflex, and by extension CO_2/pH sensing by central chemoreceptors, is not required for steady-state ventilation, gas exchange, and blood gas homeostasis across air-breathing vertebrate taxa, at least while awake (Hodges et al.,

2002; Kumar et al., 2015; Noronha-de-Souza et al., 2006; Puissant et al., 2015; Ramanantsoa et al., 2011; Santin and Hartzler, 2016a; Santin and Hartzler, 2016b; Sun and Ray, 2017). This contrasts with the notion that CO_2 chemoreception evolved with the regulatory demands of air breathing to maintain an 'ideal' P_aCO_2 value. To place CO_2 chemoreception *via* the chemoreflex in the appropriate evolutionary context, an improved framework is needed.

7.1. Did the CO_2 chemoreflex link ventilation to CO_2 excretion demands posed by the water-to-land transition? A revised hypothesis

A brief discussion regarding the well-described CO_2 dilemma encountered during the water-to-land transition is warranted (Witzmann, 2016). Because CO_2 is ~30 times more soluble in water compared to O_2, water-breathers passively eliminate CO_2 while satisfying their O_2 demands through gill ventilation. Consequently, water-breathers typically have low P_aCO_2 values. In air, O_2 is more abundant and a lower lung ventilation rate adequately supplies the tissues with O_2 to support aerobic metabolism. However, the transition to air breathing would have resulted in the need to compensate a chronic respiratory acidosis due to hypoventilation of the lungs relative to CO_2 production. The mechanisms used to compensate the chronic respiratory acidosis of tetrapods transitioning to land may have involved both bicarbonate retention through endogenous or exogenous sources and calcium carbonate from the breakdown of mineralized tissues (Janis et al., 2012; Ultsch, 1996; Witzmann, 2016).

The CO_2 retention that occurred during the water-to-land transition required a switch from O_2 to CO_2 as the primary regulated gas. Because central CO_2 chemoreceptors exist in all air-breathing tetrapods, lungfishes, and some facultative air-breathing ray-finned fishes, the ability for central CO_2 sensors to modulate ventilation rate clearly predates the origin of tetrapods (Milsom, 2010). This supports the attractive idea that central CO_2 chemoreceptors provided the major stimulus to breathe air in response to CO_2 excretion demands associated with terrestrial life (Hoffman et al., 2016; Remmers et al., 2001; Smatresk, 1990, 1994; Wilson et al., 2000). I argue that this may not have been so. The animal models described in this review that have apparently normal ventilatory control and gas exchange despite negligible CO_2 chemoreflexes contradicts the view that central respiratory chemoreception was, and still is, required to adequately eliminate CO_2 when breathing air. With this point in mind, the hypothesis that central CO_2 chemoreceptors underlying the chemoreflex solved the CO_2 dilemma associated with terrestriality needs to be revised.

Under what conditions might respiratory chemoreceptors and subsequent CO_2 chemoreflex activation have been used during the evolutionary transition from water to land? A respiratory acidosis, specifically an acidification of arterial pH caused by hypoventilation relative to CO_2 production, is the primary physiological stimulus for CO_2 chemoreflex activation in vertebrates. Consequently, the role of central respiratory chemoreceptors may have varied in different groups of early tetrapods depending on the extent and timing of base retention that compensated the chronic respiratory acidosis. Recently, Witzmann (2016) outlined possible scenarios for CO_2 elimination based on fossil evidence for internal gills, skin composition, ventilatory mechanics, and degree of terrestriality in three groups of early tetrapods: stem tetrapods (extinct, aquatic tetrapods), temnospondyls (extinct, primitive amphibians), and stem amniotes (extinct, primitive amniotes). This scheme described by Witzmann (2016) can be used to infer the extent of the chronic respiratory acidosis and subsequent CO_2 chemoreflex activation in different groups of early tetrapods. First, the stem tetrapods may have been mostly aquatic, resembling lungfish rather than extant amphibians. It is therefore plausible that these animals encountered mainly acute, but not chronic respiratory acidosis typically ascribed to early tetrapods. Assuming similarity to lungfish, stem tetrapods may have ventilated their lungs ~1–14 times per hour to eliminate ~20–70% of their metabolic CO_2, depending on the temperature (Amin-Naves et al., 2004). It is possible that fluctuations in acid-base status of arterial blood during these rather long inter-breath intervals might have provided an acute stimulus to respiratory chemoreceptors to initiate the next breath. This could be analogous to blood gas variations experienced by extant ectotherms with long non-ventilatory periods. Because these animals were primarily aquatic, if they did venture onto land, hypoventilation relative to CO_2 production may have acutely stimulated the chemoreflex, but this respiratory acidosis would have probably been dissipated quickly upon return to the water. In contrast to aquatic stem tetrapods, temnospondyls and stem amniotes may have chronically hypoventilated relative to CO_2 production while breathing air, at least at some point during their evolutionary transition to terrestrial life. Temnospondyls retained gills through much of their evolutionary history and may have slowly transitioned to a land-dwelling adult stage (Witzmann, 2016). Thus compensation of the chronic respiratory acidosis may have occurred gradually in the amphibian lineage and respiratory CO_2 chemoreceptors may have provided a "drive to breathe" throughout much of the evolution of amphibians. On the other hand, stem amniotes lost their gills early and terrestrialized rapidly in their evolutionary history (Witzmann, 2016). In the early amniotes, only a transient period may have existed where a respiratory acidosis would have stimulated ventilation through CO_2 chemoreception. Therefore, the "transition phase" of the water-to-land transition would have created a respiratory acidosis to stimulate ventilation. This is compatible with the hypothesis that respiratory chemoreception linked ventilation to CO_2 challenges during a shift to air-breathing (Hoffman et al., 2016; Remmers et al., 2001; Smatresk, 1990, 1994). However, this hypothesis is specific only to the evolutionary transition from water-breathing to air-breathing when a respiratory acidosis would have been prevalent.

What happened upon "completion" of the transition? Regardless of the time frame or mechanism, once early tetrapods retained enough base to buffer the chronic respiratory acidosis and establish a new steady-state, CO_2 chemoreceptors would have no longer provided a significant stimulus for maintaining ventilation to meet gas exchange demands. This is clear because extant semi-terrestrial amphibians and fully-terrestrial rodents have a normal ventilatory "drive" and effectively eliminate CO_2 through lung ventilation independent of the CO_2 chemoreflex. Although these extant animals absolutely differ from the earliest terrestrialized tetrapods, they paint a vivid picture of CO_2 excretion demands in air-breathing animals that require partial to exclusive use of lung ventilation for CO_2 elimination. Upon completion of the water-to-land transition, the relationship between ventilation and metabolic rate and extrapulmonary CO_2 loss, but not the CO_2 chemoreflex, would be the main determinant of steady-state P_aCO_2. This conclusion that central CO_2 chemoreception does not tie ventilation to CO_2 excretion requirements of air breathing starkly contrasts with the dominant hypothesis. Unlike the present model, I argue that for extant air-breathers, steady-state ventilation must be driven by mechanisms independent of the CO_2 chemoreflex; however, situations that lead to an acute respiratory acidosis would still engage respiratory chemoreceptors (for examples see section titled "Ectothermic vertebrates routinely uncouple ventilation from metabolism and experience fluctuations in P_aCO_2"). From fish to mammals at any stage in vertebrate evolution, it appears that the CO_2 chemoreflex has always influenced ventilation in an exclusively compensatory manner, regardless of whether the respiratory acidosis occurred over acute or long evolutionary time scales. Once the disturbance is removed and steady-state is reestablished, mechanisms that match ventilation to metabolic demands and extrapulmonary CO_2 loss dominate CO_2 excretion requirements on land.

7.2. Ventilatory sensors of organismal metabolism: the missing link?

If mechanisms underlying the CO_2 chemoreflex do not couple ventilation to CO_2 excretion demands when breathing air on land, what

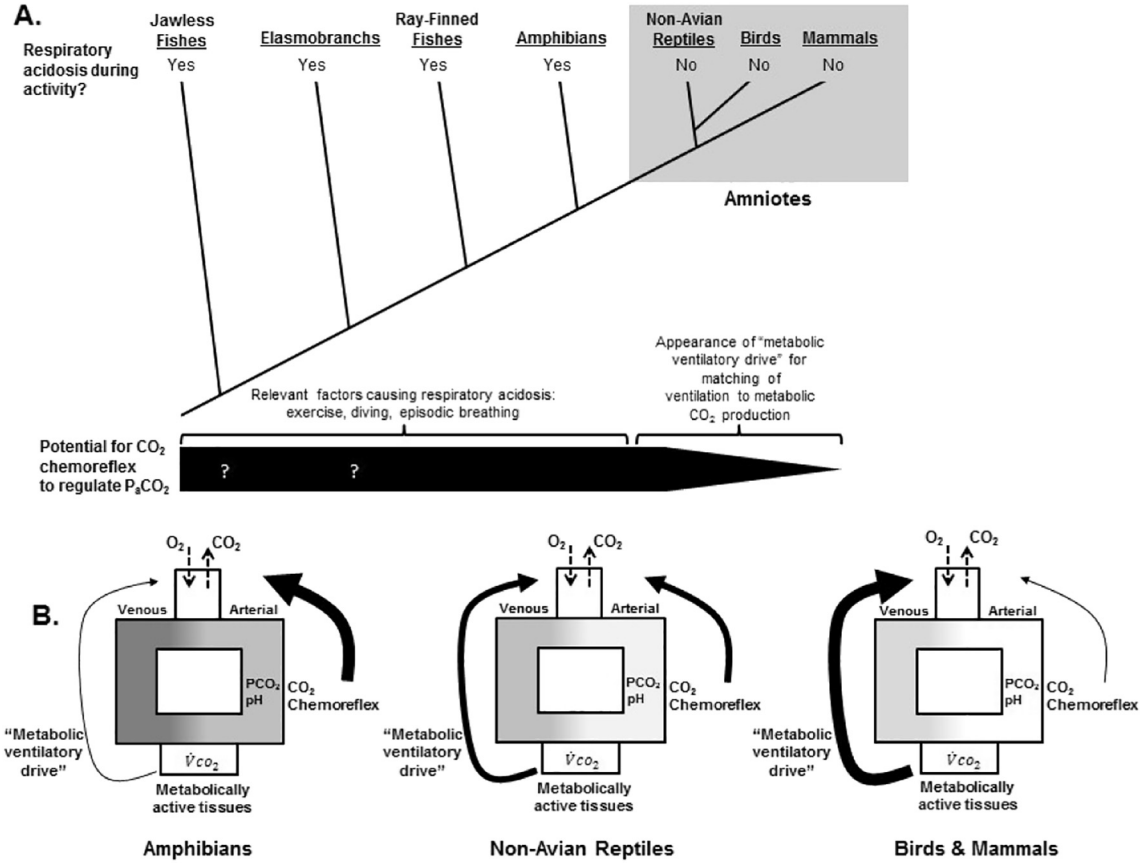

Fig. 2. Phylogenetic trends in matching ventilation to metabolism during activity coupled with the likelihood of encountering a respiratory acidosis during daily life. A. depicts a simplified phylogeny of vertebrates. Whether or not these animals experience a respiratory acidosis in response to activity is noted across the top by "yes" or "no". All amniotes assessed thus far can match ventilation to metabolic demands across a range of exercise intensities, thus preventing a respiratory acidosis, while fish and amphibians cannot. The thickness of the black bar across the bottom of the cladogram estimates the propensity for CO_2 chemoreflex activation during daily life across vertebrates. Because ectotherms have physiology and/or lifestyles that preclude tight regulation of arterial blood gases, the CO_2 chemoreflex likely plays a large role in the regulation of blood gases (thick portion of the black bar). Question marks signify uncertainty due to lack of data. In contrast, mammals and birds tightly couple ventilation to metabolic demands, and therefore are unlikely to rely on CO_2/pH feedback from chemoreceptors detecting arterial blood gases under normal conditions (narrowing of black bar). B. displays simplified models illustrating a hypothetical shift from a major reliance on chemoreceptors detecting arterial blood CO_2/pH to the "metabolic ventilatory drive" across air-breathing tetrapods. Thickness of the arrows represents the hypothetical relative contribution of each mechanism to overall ventilatory control of CO_2 homeostasis. Gray scale of the animated circulation represents regular swings in arterial blood gases with darker shades signifying increasing severity of the respiratory acidosis. Because amphibians have only a small capability to match ventilation to metabolic demands, arterial CO_2/pH will vary, forcing amphibians to rely heavily on CO_2/pH chemoreceptors for correcting acid-base disturbances. In non-avian reptiles, ventilation can better match metabolic demands during activity, but lifestyles and breathing patterns of these animals still produce substantial arterial Pco_2 changes. Thus both the metabolic drive and chemoreflex drive likely contribute to the maintenance of arterial CO_2/pH in these animals. In most endothermic mammals and birds, metabolic ventilatory drive seems to dominate ventilatory control, preventing significant changes in arterial blood gases, and consequently the CO_2 chemoreflex plays on a minor role in maintaining steady-state P_aCO_2 in these animals.

does? I hypothesize that neural mechanisms coupling ventilation to tissue metabolic requirements (*i.e.*, a "metabolic ventilatory drive") may, in part, define steady-state CO_2 homeostasis in air-breathing vertebrates. Specifically, these neural mechanisms must provide the respiratory control system with a signal proportional to organismal metabolic rate to appropriately regulate steady-state ventilation and gas exchange. Such a metabolic ventilatory drive must be distinct from the chemical drive provided by mechanisms detecting arterial P_aCO_2/pH through the CO_2 chemoreflex because animals lacking the CO_2 chemoreflex have an apparently normal metabolic ventilatory drive. Mechanisms underlying this distinct metabolic ventilatory drive may include, but are not limited to, sensory afferents relaying information about metabolic activity of skeletal muscle as well as CO_2 exchange at the lung to the respiratory control system (see section titled "How is ventilation coupled to CO_2 production?"). Because all extant amniotes, but not amphibians, can match ventilation to metabolism across a wide-range of activity, neural mechanisms underlying this metabolic ventilatory drive may have evolved in the ancestors of the amniotes. Accordingly, phylogenetic bracketing for the presence of a respiratory acidosis during activity shows an organized clustering (Fig. 2A). Fish and amphibians experience respiratory limitations to CO_2 transport with activity, while amniotes meet CO_2 excretion demands over a wider range of exercise intensities. As aerobic capacity increased throughout tetrapod evolution (Bennett, 1978), greater demands were placed on the respiratory system to eliminate larger quantities of CO_2 (Hedrick et al., 2015; Hillman et al., 2013). Anatomical changes in the production of air flow (*i.e.*, buccal *vs.* aspiration pump), locomotor posture, accessory breathing muscles, and respiratory-locomotor coupling (Brainerd and Owerkowicz, 2006) would have improved CO_2 release. I propose that neural mechanisms underlying the "metabolic ventilatory drive" would have been critical to connect these mechanical modifications to increased ventilation rates for CO_2 elimination. In addition to underlying ventilatory control of steady-state P_aCO_2, the distinct metabolic drive to breathe may also have been a key neural substrate necessary for expanding the aerobic capacity of mammals and birds.

The phylogenetic trend for the presence of a metabolic ventilatory drive may apply to ventilation-metabolism matching over larger increases in CO_2 production. Whether this is the case for small changes in metabolic rate near rest is less clear as bullfrogs can couple ventilation to increases in venous CO_2 around baseline to prevent changes in P_aCO_2 (Jackson and Braun, 1979). This suggests that a small metabolic ventilatory drive may be an ancestral trait of tetrapods. However, this

interpretation is tempered because an aquatic basal anuran, *Pipa carvalhoi*, and aquatic salamanders do not seem to have this ability (Fonseca et al., 2012; Heisler et al., 1982; Toews, 1971). Therefore three untested, potentially interacting, possibilities exist for amphibians: (1) a small metabolic ventilatory drive may be variable across amphibians, (2) the response observed in bullfrogs may represent a derived trait of modern anurans, and (3) cutaneous gas exchange in primarily aquatic species reduces the need for ventilation to match CO_2 elimination, resulting in a lack of a metabolic ventilatory drive.

Although mechanisms underlying the "metabolic ventilatory drive" may have improved the coupling of ventilation to CO_2 production, there would still be a clear advantage to retain P_aCO_2/pH_a chemoreceptors if life histories and other behavioral constraints regularly led to fluctuations in P_aCO_2/pH_a. One major question warrants speculation: why do terrestrial mammals have an elaborate CO_2 detection system if these mechanisms are not required for both resting ventilatory control of P_aCO_2 (Hodges et al., 2002; Kumar et al., 2015; Puissant et al., 2015; Ramanantsoa et al., 2011; Sun and Ray, 2017) and ventilatory responses to exercise, the largest physiological increase in CO_2 production (Forster et al., 2012)? Two speculative hypotheses should be considered. The CO_2 chemoreflex may have been important in the ancestors of present day mammals because of environmental and/or physiological limitations on CO_2 excretion. Because this system had probable adaptive value for correcting acute arterial blood gas disturbances throughout much of tetrapod evolution, mechanisms underlying the CO_2 chemoreflex may have become fixed in well conserved developmental programs within the respiratory network. Therefore, the ventilatory sensitivity to CO_2 in terrestrial mammals may be an evolutionary holdover based on needs of ancestors. Second, internal and/or external factors associated with terrestrial life at warm temperatures may drive expression of a CO_2-sensitive breathing control system. This hypothesis is supported by adult bullfrogs that lack central CO_2 sensitivity immediately after emerging from a cold, submerged overwintering environment when assessed at a warm temperature (Santin and Hartzler, 2016b). This is unusual because air-breathing tetrapods typically have respiratory control systems modulated by CO_2 (Milsom, 2010), especially at high body temperatures. Although a bit speculative, this raises the possibility that factors associated with terrestrial life and/or warm temperatures *per se* could, in part, drive expression of central CO_2 sensitivity through phenotypic plasticity. These two hypotheses are consistent with data presented in this review. Certainly these scenarios are not mutually exclusive and there remains room for additional hypotheses.

8. Conclusion

Haldane and Priestley (1905) were correct when they stated ventilation responds to small changes in P_aCO_2. However, the chemoreflex does not appear to be the primary determinate of normal steady-state P_aCO_2 through ventilatory control. This interpretation would not have been apparent without models that exhibit severely blunted CO_2 chemoreflexes. It can be enticing to assign specific functions to present-day traits in the animals we study. Yet, based on the various evolutionary histories of these animals, as well as their relationship with the environment, there may be innumerable reasons as to why behaviors appear the way they do and why they exist, even if it is not immediately obvious (Gould and Lewontin, 1979). Using humans as a model system, Haldane and Priestley may have inadvertently uncovered a CO_2 sensitive ventilatory feedback loop with greater importance for ectothermic vertebrates, which experience routine limits to gas exchange compared to the mammals in which it was originally identified.

The importance of the CO_2 chemoreflex has been previously proposed to have increased in association with regulatory requirements of major transitions in vertebrate evolution (Nattie and Li, 2012). Furthermore, the dominant hypothesis suggests that CO_2 regulatory demands associated with terrestrial life are met by respiratory CO_2 chemoreceptors (Remmers et al., 2001; Smatresk, 1990, 1994). Data highlighted here show that air breathers ventilate normally, match ventilation to metabolism, and maintain P_aCO_2 despite lacking most, if not all, of their CO_2 chemoreflex. These data are not consistent with the dominant hypothesis for the evolutionary importance of the CO_2 chemoreflex. I argue that throughout the evolutionary history of tetrapods, the CO_2 chemoreflex, instead, reduced its importance with the onset and refinement of mechanisms that underlie a distinct "metabolic ventilatory drive;" specifically, sensory mechanisms that couple ventilation to organismal metabolism (Fig. 2B). This metabolic ventilatory drive may elicit appropriate ventilatory responses to maintain normal P_aCO_2/pH_a by detecting modalities associated with muscle contraction and pulmonary CO_2 excretion, but not changes in arterial CO_2/pH. Such neural mechanisms may have arisen in the ancestor of present day amniotes because most reptiles, mammals, and birds can match ventilation to metabolism over a range of metabolic rates. Future studies should use ectotherms to infer the evolutionary history of these ventilatory control mechanisms. Elucidating the sensory processes that enable ventilation to meet metabolic demands in the absence of appropriate P_aCO_2 error signals represents an exciting and crucial aspect of comparative and medically-relevant respiratory control that still lacks a mechanistic explanation.

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