

## Evolutionary trends in airway CO<sub>2</sub>/H<sup>+</sup> chemoreception

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### Abstract

In many species of air-breathing vertebrates CO<sub>2</sub>-sensitive airway receptors play an important role in ventilatory control. In ectotherms, olfactory receptors often inhibit breathing and prolong breath holding when environmental CO<sub>2</sub> levels are high. CO<sub>2</sub>/H<sup>+</sup> sensitive pulmonary receptors (intra pulmonary chemoreceptors (IPC) and pulmonary stretch receptors (PSR)) regulate breathing patterns in all vertebrates in a manner that reduces dead space ventilation and enhances the efficiency of CO<sub>2</sub> excretion under conditions of environmental hypercarbia, and/or reduces CO<sub>2</sub> loss from hyperventilation. The greater CO<sub>2</sub> sensitivity of IPC may allow them to also serve as a venous CO<sub>2</sub> receptor (at least transiently when levels of metabolically produced CO<sub>2</sub> begin to rise), prevent alkalosis during hyperpnea/polypnea, and may have contributed to the evolution of the extremely thin air/blood barrier and increased diffusion capacity associated with the rigid avian lung. The presence of all three receptor groups with different degrees of CO<sub>2</sub> sensitivity in most reptiles, however, gives rise to what appear to be anomalous responses to environmental CO<sub>2</sub>.

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### 1. Introduction

While there is no doubt that the CO<sub>2</sub>/H<sup>+</sup> sensitive receptors associated with the systemic circulation (both peripheral and central) play an essential role in ventilatory control in all air-breathing vertebrates, the role of CO<sub>2</sub>/H<sup>+</sup> sensitive receptors associated with the airways is less clear. Perhaps because the class in which

these receptors appear to play the smallest role is the mammals, they have not attracted the interest of their vascular counterparts. It has become clear, however, that in many species of air-breathing vertebrates, they play a critical role in ventilatory control. Initial interest in airway chemoreceptors was largely confined to reptiles (Boelaert, 1941; Nielsen, 1961; Templeton and Dawson, 1963; Pough, 1969). In the 1960s and early 1970s, however, there was an explosion of interest in the existence of a venous CO<sub>2</sub>/H<sup>+</sup> receptor that could help explain exercise hyperpnea (Armstrong et al., 1961; Lewis, 1975). This led to the discovery of

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intrapulmonary chemoreceptors (IPC) in birds (Burger, 1968; Peterson and Fedde, 1968), and subsequently in many (but not all) reptiles (Fedde et al., 1977; Scheid et al., 1977). It also ultimately led to a rebirth of interest in extrapulmonary, airway chemoreceptors (Ballam, 1984; Coates and Ballam, 1989). While the interest in IPCs was truly international in scope, the research group in Göttingen, led principally by Peter Scheid and Roger Fedde, focused and coordinated much of this research through a series of conferences and a host of collaborative studies (see, for instance, Fedde et al., 1977, 1982; Scheid et al., 1977). In the years that followed, there was a progression of studies of airway CO<sub>2</sub>/H<sup>+</sup> chemoreceptors that reveal a picture of growing complexity, one that we will attempt to summarize in this review.

## 2. Airway receptors and receptor responses

### 2.1. Olfactory chemoreceptors

A series of studies have identified upper airway receptors sensitive to changes in CO<sub>2</sub> in the nasal sensory epithelium, innervated by the olfactory nerve in the bullfrog (*Rana catesbeiana*, Sakakibara, 1978;

Kinkead and Milsom, 1996; but see also Smyth, 1939; Coates and Ballam, 1990), the tegu lizard (*Tupinambis nigropunctatus*, Ballam, 1984, 1985; Coates and Ballam, 1987), the garter snake (*Thamnophis sirtalis*, Coates and Ballam, 1989), rats (Coates and Silvis, 1999) and humans (Youngtob et al., 1991; Alvaro et al., 1993). These receptors are relatively rare, are stimulated by CO<sub>2</sub> levels below or near the animal's end-tidal CO<sub>2</sub> concentration, and produce a reflex inhibition of breathing (Getchell and Shepherd, 1978; Coates and Ballam, 1990; Coates et al., 1998; Coates, 2001) (Table 1). Receptors with similar properties and/or reflex effects have been reported in fish (Burlison et al., 1992); thus, at present there is no reason to believe that they are not present in all vertebrates (Fig. 1). In frogs and rats, inhibition of carbonic anhydrase (CA) attenuates the response of CO<sub>2</sub>-sensitive olfactory receptors to transient changes in CO<sub>2</sub> and it has been suggested that CA activity can serve as a marker of CO<sub>2</sub>-sensitive receptors in the olfactory epithelium (Coates, 2001).

### 2.2. Vomeronasal chemoreceptors

The vomeronasal (or Jacobson's) organ is an accessory olfactory organ found in many tetrapods but absent in most turtles, crocodylians, birds, some bats and

Table 1  
Effects of (A) increasing inspired CO<sub>2</sub> and (B) venous CO<sub>2</sub> loading or increased metabolic CO<sub>2</sub> production

Receptor	Discharge	Reflex effects	
		$\dot{V}_E$	fR/V <sub>T</sub>
(A) Effects of increasing inspired CO <sub>2</sub>			
Olfactory	Increase throughout cycle	Decrease	Decrease fR primarily
Vomeronasal	Increase	Increase	Increase fR primarily
Intrapulmonary	Decrease tonic, decrease phasic	Decrease	Decrease fR, increase V <sub>T</sub>
Pulmonary stretch	Decrease	Increase	Decrease fR, increase V <sub>T</sub>
Arterial	Increase	Increase	Increase fR, increase V <sub>T</sub>
Central	Increase	Increase	Increase fR primarily
Net effect		Increase, decrease, or no change	Increase/decrease or no change in fR, increase V <sub>T</sub>
(B) Effects of venous CO <sub>2</sub> loading or increased metabolic CO <sub>2</sub> production			
Olfactory	No effect or slight increase during expiration	No effect	
Vomeronasal	?	?	?
Intrapulmonary	No effect or slight increase phasic	No effect	
Pulmonary stretch	No effect or slight decrease during expiration	No effect or slight increase	Increase fR, increase V <sub>T</sub>
Arterial	Increase	Increase	Increase fR, increase V <sub>T</sub>
Central	Increase	Increase	Increase fR primarily
Net effect		Increase	Increase fR, increase V <sub>T</sub>

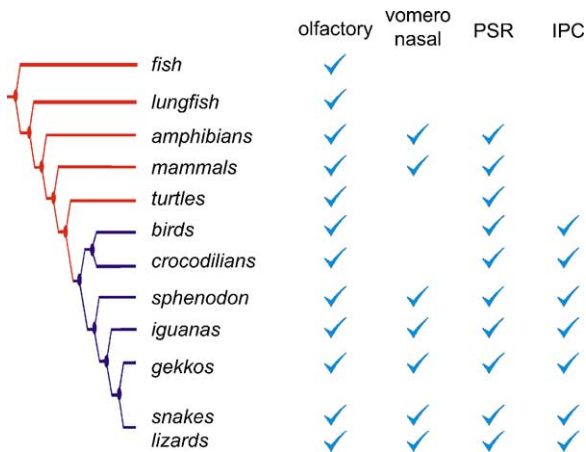


Fig. 1. Cladogram illustrating the phylogenetic relationships between various vertebrate groups (left). Symbols indicate the presence of various airway chemoreceptors in each group.

aquatic mammals (Fig. 1). Its innervation runs parallel to, but remains entirely separate from the main olfactory system. It is believed to participate primarily in sensing chemicals important to social or reproductive behaviour, as well as for feeding and prey trailing (Halpern and Kubie, 1984; Cooper and Burghardt, 1990). However, the inhibition of respiratory frequency in response to upper airway  $\text{CO}_2$  is greater after vomeronasal nerve lesions in gartersnakes, suggesting that  $\text{CO}_2$ -sensitive receptors in the vomeronasal organ of this species are excited by  $\text{CO}_2$  and elicit a reflex increase in breathing frequency (Coates and Ballam, 1989) (Table 1).

### 2.3. Intra-pulmonary chemoreceptors (IPC)

Intrapulmonary chemoreceptors (IPC) have now been described in the lungs of several species of reptiles and birds. They are located within the lung, innervated by the vagus nerve and have discharge that is inversely proportional to  $P_{\text{CO}_2}$ . The phylogenetic distribution of IPC suggests that this receptor group evolved after mammals split from the reptilian stock (Fig. 1). They have not been found in amphibians (Milsom and Jones, 1977; Kuhlmann and Fedde, 1979; Furilla and Bartlett, 1988) or mammals (Kunz et al., 1976) and their presence in turtles is questionable (Jones and Milsom, 1979; Ishii et al., 1986; Sundin et al., 2001). They are present in birds (see Scheid and Piiper, 1986 for review) and

all diapsid reptiles examined so far (lizards: Gatz et al., 1975; Fedde et al., 1977; snakes: Sundin et al., 2001; alligators: *Alligator mississippiensis*, Powell et al., 1988; Douse et al., 1989). Their  $\text{CO}_2$  sensitivity is nearly abolished by intracellular inhibitors of carbonic anhydrase and inhibitors of  $\text{Na}^+/\text{H}^+$  antiport exchange (Hempleman et al., 2000, 2003) suggesting that they respond to intracellular pH rather than  $\text{CO}_2$  per se (see Hempleman and Posner, 2004, this volume). A unique feature of these receptors is their extremely fast response and sensitivity to rate of  $\text{CO}_2$  change, resulting in a dynamic and responsive respiratory chemoreceptor (Osborne et al., 1977; Hempleman and Posner, 2004, this volume) (Table 1).

### 2.4. Pulmonary stretch receptors

Although the primary stimuli for these receptors are changes in lung volume, pressure or wall tension, increasing pulmonary  $\text{CO}_2$  inhibits their discharge by varying degrees in amphibians, reptiles, birds and mammals (see Milsom, 1995, 1998 for reviews). The effects of  $\text{CO}_2$  on receptor discharge range from insignificant to total inhibition, both between species and between individual receptors in a single individual animal in some species (Jones and Milsom, 1979; Powell et al., 1988; Sundin et al., 2001) (Table 1). The inhibition is largely (but not completely) independent of the effects of the  $\text{CO}_2$  on pulmonary smooth muscle tone, and it is unknown whether these effects result from changes in pH or  $\text{CO}_2$  (Milsom, 1995; Sundin et al., 2001).

## 3. Whole animal responses

### 3.1. Steady state responses

While the response to environmental hypercarbia of most air-breathing vertebrates is a robust increase in ventilation, this is not always the case. In many (but not all) lizards and snakes, for instance, while low levels of environmental  $\text{CO}_2$  almost always cause ventilation to increase, breathing is depressed by higher levels of environmental  $\text{CO}_2$  (>3%) (lizards, Boelaert, 1941; Nielsen, 1961; Templeton and Dawson, 1963; Pough, 1969; Ballam, 1984; Klein et al., 2002; snakes, Glass and Johansen, 1976; Gratz, 1979; Coates and

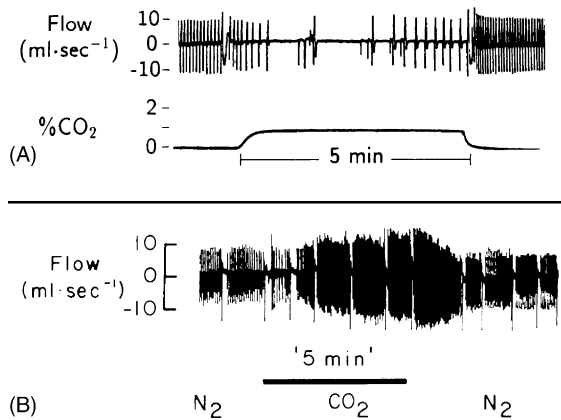


Fig. 2. (A) Ventilatory flow (upper trace) and  $\text{CO}_2$  composition of gas entering the external nares (lower trace) in the tegu lizard illustrating the effect of inspiring  $\text{CO}_2$ . (B) Ventilatory flow before, during, and after infusing  $\text{CO}_2$  into the gut of a tegu lizard illustrating the effect of increasing levels of metabolically produced  $\text{CO}_2$  (Ballam and Donaldson, 1988; Ballam and Coates, 1989).

Ballam, 1989; Andrade et al., 2004). In general,  $\text{CO}_2$  increases tidal volume, but the higher concentrations usually decrease breathing frequency and total ventilation (Fig. 2). In the tegu lizard, in which increasing levels of inspired  $\text{CO}_2$  significantly decrease ventilation, venous  $\text{CO}_2$  loading (to simulate an increase in metabolic  $\text{CO}_2$  production) dramatically increased ventilation (Ballam and Donaldson, 1988), indicating that the route of  $\text{CO}_2$  administration is important in determining the nature of the response (Fig. 2).

From these and other studies, it is clear that there are varying degrees of opposing excitatory and inhibitory influences during environmental hypercarbia, arising from different receptor groups (systemic arterial versus airway) in different species of air-breathing vertebrates. The net effect of these inputs on total ventilation results from the relative strengths of each input. Differences in the ventilatory responses to  $\text{CO}_2$  of various species are now thought to be due to differences in afferent sensitivities and/or differences in central nervous system processing of afferent information from different receptor groups (Table 1).

All air-breathing vertebrates are believed to possess peripheral arterial chemoreceptors and central  $\text{CO}_2/\text{H}^+$ -sensitive receptors. Stimulation of these receptors elevates ventilation (see Milsom, 1998 for review) (Table 1). As mentioned earlier, birds and diapsid reptiles possess intrapulmonary chemorecep-

tors that are inhibited by increasing levels of  $\text{CO}_2$ ; high levels of environmental hypercarbia act on these chemoreceptors to elevate tidal volume and reduce breathing frequency (Fedde et al., 1977; Furilla and Bartlett, 1988) (Table 1). All air-breathing vertebrates appear to possess slowly adapting pulmonary receptors. In those in which this mechano-receptor discharge is  $\text{CO}_2$ -sensitive, high levels of intrapulmonary  $\text{CO}_2$  also elevate tidal volume and reduce breathing frequency (Jones and Milsom, 1982) (Table 1). All air-breathing vertebrates also appear to possess upper airway chemoreceptors in the nasal epithelium whose discharge is stimulated by  $\text{CO}_2$  and continuous (but not phasic) hypercarbia acts on these receptors to dramatically inhibit breathing frequency with little effect on tidal volume (Fig. 3; Table 1) (Coates and Ballam, 1987, 1989; Ballam and Coates, 1989; Coates et al., 1991). Finally, snakes at least, also appear to possess a further group of vomeronasal receptors that produce an increase in breathing frequency and ventilation in response to environmental hypercarbia (Coates and Ballam, 1989) (Table 1). Depending on the balance, environmental hypercarbia may give rise to a wide spectrum of responses ranging from an increase, to no change, or even a decrease in ventilation.

### 3.2. "Post-hypercapnic hyperpnea"

In many species, return from hypercarbia to air is accompanied by a marked transient increase of ventilation relative to values during hypercarbic exposure (Fig. 4). Thus, in the South American lungfish, *Lepidosiren paradoxa* (Sanchez and Glass, 2001), the anuran amphibian, *Rana catesbeiana* (Kinkead and Milsom, 1996), the lizards, *Crotaphytus collaris*, *Lacerta viridis* and *Uromastix aegypticus* (Nielsen, 1961; Templeton and Dawson, 1963; Klein et al., 2002) and the snakes, *Acrochordus javanicus*, *Coluber constrictor* and *Crotalus durissus* (Glass and Johansen, 1976; Nolan and Frankel, 1982; Andrade et al., 2004), an immediate relative hyperpnea is seen when inspired hypercarbic gas is replaced with a normocarbic gas mixture, an effect termed the "off-response" or post-hypercapnic hyperpnea. This off-response has been interpreted to suggest that during conditions of environmental hypercarbia, the stimulating effect of systemic hypercapnia is, at least in part, masked by an inhibitory effect of tonically elevated airway

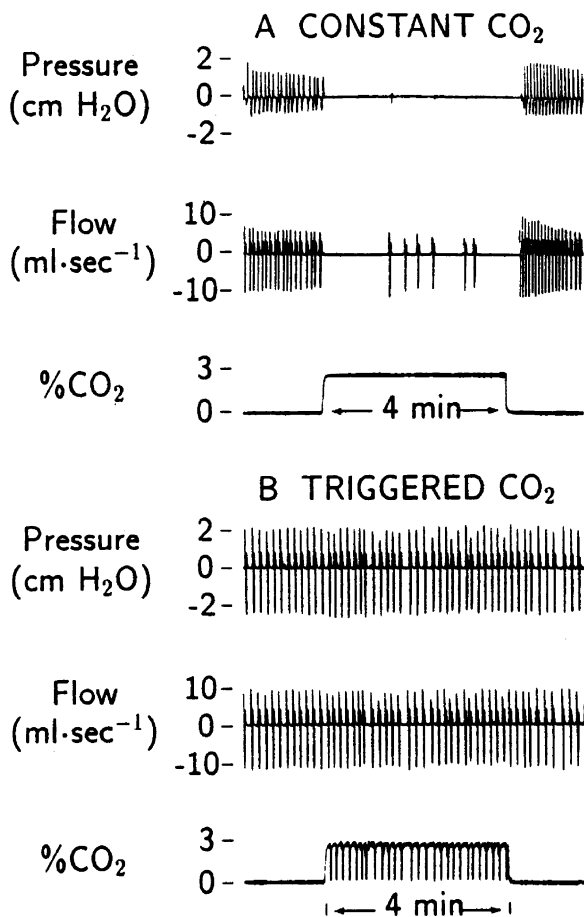


Fig. 3. Intrapulmonary pressure (upper trace) ventilatory flow (middle trace) and CO<sub>2</sub> composition of gas entering the external nares (lower trace) in the tegu lizard illustrating the effects on breathing when CO<sub>2</sub> is (A) held constant or (B) pulsed at the same mean concentration through the upper airways (Ballam and Coates, 1989).

CO<sub>2</sub> (Boelaert, 1941; Nielsen, 1961; Templeton and Dawson, 1963; Nolan and Frankel, 1982; Coates and Ballam, 1989; Kinkead and Milsom, 1996; Klein et al., 2002; Andrade et al., 2004). When animals begin to breathe normocarbic air again, arterial CO<sub>2</sub> levels will still be elevated for some time but the level of CO<sub>2</sub> in the airways will be elevated only during expiration. Arterial levels of CO<sub>2</sub> and end-expiratory levels of CO<sub>2</sub> will fall slowly as whole body CO<sub>2</sub> stores are lowered and CO<sub>2</sub> is eliminated, whereas inspired CO<sub>2</sub> levels will fall immediately. Thus, the inhibitory effect of tonically elevated airway CO<sub>2</sub> is eliminated imme-

diately, while the excitatory effect of elevated systemic CO<sub>2</sub> persists until a new steady state is attained.

If this interpretation is correct, then the post-hypercapnic hyperpnea should be greater in instances where both the excitatory effect of the systemic hypercapnia (i.e. the level of CO<sub>2</sub>) and the inhibitory effect on airway CO<sub>2</sub>-sensitive receptors (as indicated by the reduction in steady state breathing frequency during sustained hypercarbia) were greater. The literature contains reports suggesting both that there is (Templeton and Dawson, 1963; Andrade et al., 2004) (Fig. 4) and is not (Nielsen, 1961; Nolan and Frankel, 1982) a correlation between the level of environmental hypercarbia and the magnitude of the response to CO<sub>2</sub> removal. Interestingly, Klein et al. (2002) found that there was a correlation when the data were expressed in absolute terms, but not when the data were expressed in relative (proportionate) terms. Why consistent results have not been found in all studies is hard to say but this may reflect the kinetics of the receptor responses. It is possible that receptor kinetics are such that there is a maximum response that can be produced and in some species this is produced at lower levels of inspired CO<sub>2</sub> masking this correlation.

If the responses seen during the first minute of the “off-response” are an accurate indication of the “unmasked” level of excitation being provided by systemic (arterial and central) chemoreceptors, the “off-response” may be a useful tool to examine the net effect of airway CO<sub>2</sub> on ventilatory responses in all vertebrates. We have attempted to do this with data taken from the literature in Fig. 5. Here we see that in the frog, gecko and rattlesnake, total ventilation only increased modestly during hypercarbia, but there was a further increase in ventilation during the first minute of the post-hypercapnic hyperpnea, a time when arterial levels of CO<sub>2</sub> would be elevated but declining. In the gecko and rattlesnake, both breathing frequency and tidal volume were reduced during hypercarbia by the inhibitory effects of airway CO<sub>2</sub>, and the net effect of removing upper airway inhibition was extremely large. On the other hand, while the caiman responded to inhalation of CO<sub>2</sub> in a very similar fashion to the rattlesnake (a decrease in breathing frequency and a large increase in tidal volume), both variables returned towards normocarbic levels immediately when the CO<sub>2</sub> was removed; there was no “off-response”. Similar results have been recorded in the lizard, *Uromastix aegyptius* where it

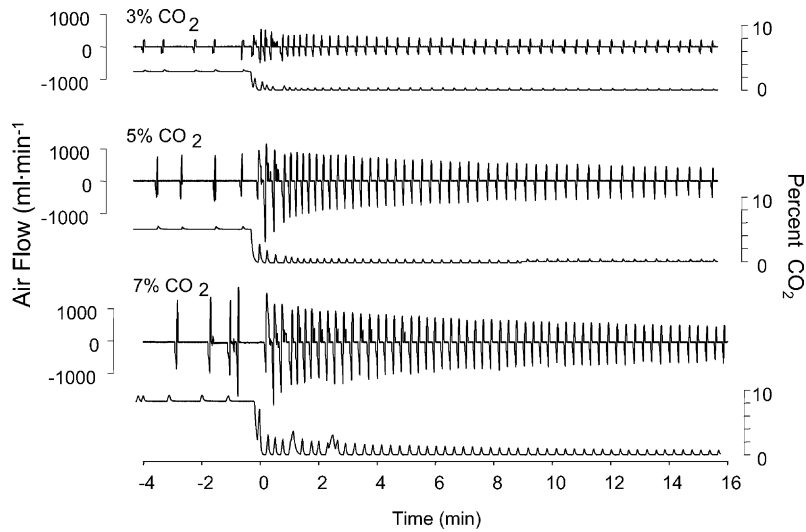


Fig. 4. Ventilatory flow (upper trace) and CO<sub>2</sub> composition of gas entering the external nares (lower trace) in a rattlesnake illustrating the post-hypercapnic hyperpnea in ventilation that occurred during the return to air after breathing various levels of CO<sub>2</sub>. The switch back to breathing air occurred at time 0 (Andrade et al., 2004).

was attributed to faster washout of CO<sub>2</sub> from blood, lungs and airways due to increased pulmonary perfusion and ventilation (Klein et al., 2002). Finally, in the turtle (as in mammals) the initial response to inhaled CO<sub>2</sub> is quite robust and ventilation immediately returns to normal on return to normocapnia.

#### 4. Relative roles of the different receptor groups

Just as the response to inhalation of CO<sub>2</sub> can be highly variable, the relative roles of different receptor groups in producing these responses are also highly variable. The data indicate that inhibition of the hypercapnic ventilatory response arises from different sites in different species (Fig. 6).

In the American bullfrog, experiments in which the olfactory nerve was transected revealed that the response to inhaled CO<sub>2</sub> increased to levels similar to, or exceeding, those seen during the “off-response” (Kinkead and Milsom, 1996). These data suggest that all of the inhibitory effect of inhaled CO<sub>2</sub> on ventilation arose from the upper airways (Fig. 6).

In the tegu lizard, inhalation of CO<sub>2</sub> decreased breathing frequency, increased tidal volume and

slightly decreased total ventilation. Introducing CO<sub>2</sub> to the lungs, bypassing the mouth, produced less of a decrease in breathing frequency but, it also produced an increase in tidal volume similar to that seen when the CO<sub>2</sub> was inspired through the nose (Fig. 6). This suggests that CO<sub>2</sub> acting on upper airway receptors may partially inhibit breathing frequency in the tegu lizard (Ballam, 1985; Coates and Ballam, 1987), but has no effect on tidal volume. Part of the reduction in breathing frequency and all of the increase in tidal volume arose from receptors within the lungs or vascular system, most likely from inhibition of intrapulmonary chemoreceptors (Coates and Ballam, 1989).

In the South American rattlesnake, neither the steady state response, nor the post-hypercapnic hyperpnea arose from receptors in the upper airways. When the upper airways were bypassed, there was a greater fall in breathing frequency and total ventilation than when they were not (Fig. 6). This suggests that, in this species, all inhibition arises from receptors in the lungs, a conclusion consistent with several other studies from other species that have shown that vagotomy, which removes feedback from lung receptors but not upper airway receptors, eliminates the post-hypercapnic hyperpnea (Boelaert, 1941; Nielsen, 1961; Templeton and

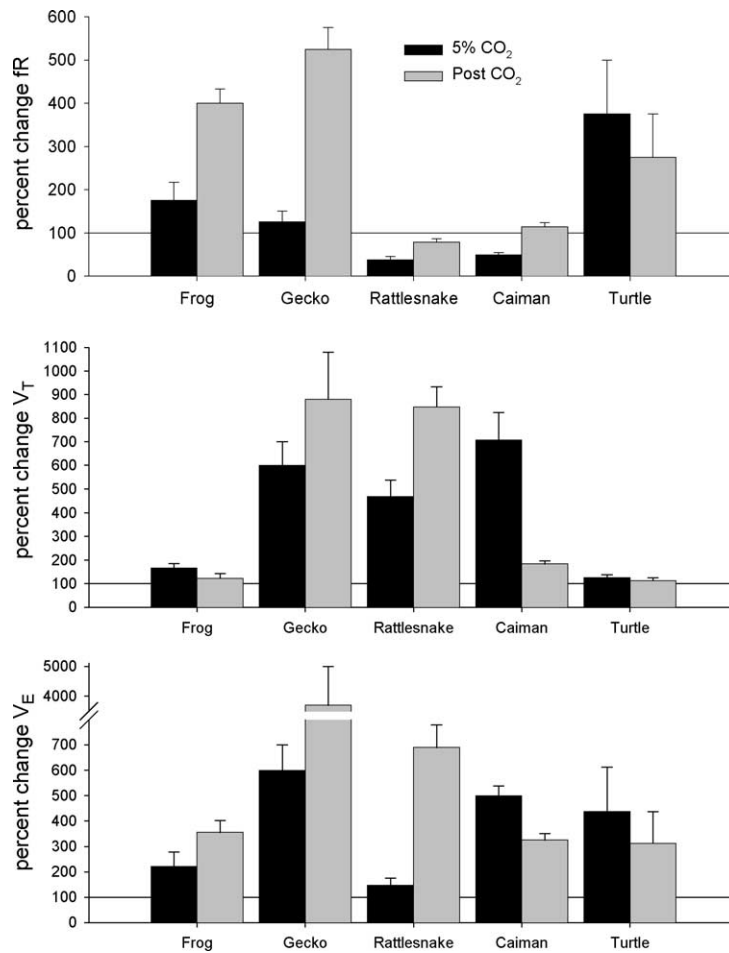


Fig. 5. Comparison of the relative changes in breathing frequency ( $f_R$ ), tidal volume ( $V_T$ ) and total ventilation ( $\dot{V}_E$ ) that occurred in various species during steady state exposure to 5%  $\text{CO}_2$  as well as during the first minute after the return to breathing air (post- $\text{CO}_2$ ). Hundred percentage represents the values for each species while animals were breathing air. Frog data is from Kinkead and Milsom (1996), gecko data from Milsom et al. (unpublished), rattlesnake data from Andrade et al. (2004), caiman data from Tattersall et al. (unpublished) and turtle data from Milsom and Jones (1979).

Dawson, 1963; Gatz et al., 1975; Glass and Johansen, 1976; Nolan and Frankel, 1982; Andrade et al., 2004). It is interesting to note that in garter snakes (Coates and Ballam, 1989), total ventilation was depressed by hypercarbia due to upper airway receptors, whereas in rattlesnakes, breathing is modestly elevated without upper airway receptor involvement.

Finally, in the turtle, where the response to inhaled  $\text{CO}_2$  is robust, bypassing the upper airways does not produce any change in the ventilatory response (Milsom and Jones, 1980).

## 5. Functional significance

These observations beg the question, “Why are there so many different  $\text{CO}_2$ -sensitive receptors in different locations with different degrees of  $\text{CO}_2$  sensitivity”? The interactions between the various receptor groups, particularly in the reptiles, appear designed to produce a robust response to metabolically produced  $\text{CO}_2$  (hypercapnia), with only a very modest or even inhibitory response to environmental  $\text{CO}_2$  (hypercarbia). Does this simply reflect the fact that hypercarbia is not commonly

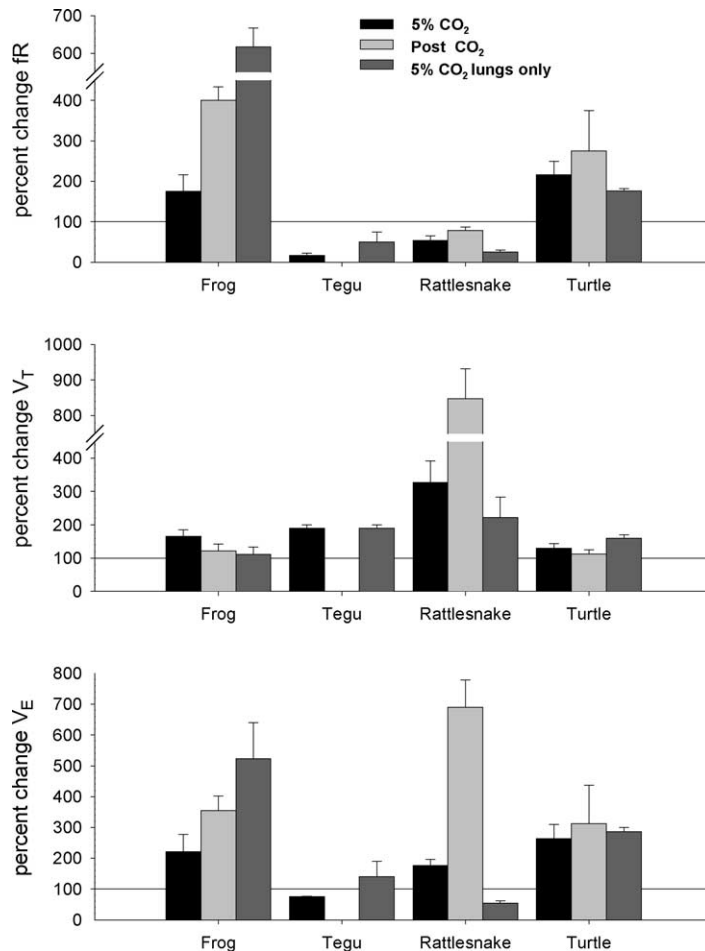


Fig. 6. Comparison of the relative changes in breathing frequency (fR), tidal volume ( $V_T$ ) and total ventilation ( $\dot{V}_E$ ) that occurred in various species during inhalation of 5% CO<sub>2</sub> via the external nares (5% CO<sub>2</sub>) as well as during the first minute after the return to breathing air (post-CO<sub>2</sub>) and when inspired either bypassing the nares or with the olfactory nerve denervated (5% CO<sub>2</sub>—lungs only). Hundred percentage represents the values for each species while animals were breathing air. Frog data is from Kinkead and Milsom (1996), tegu data from Ballam (1985), rattlesnake data from Andrade et al. (2004), and turtle data from Milsom and Jones (1979).

found in nature by these animals and, thus, the response to hypercarbia is an anomalous response of a system designed for other functions? Or, is there some biological significance to the hypercarbic response?

### 5.1. Olfactory receptors and avoidance responses

In all air-breathing vertebrates, olfactory receptors appear to have no influence on resting ventilation when environmental CO<sub>2</sub> levels are low. In species such as frogs, lizards and some snakes, these receptors inhibit breathing and prolong breath holding when environ-

mental CO<sub>2</sub> levels rise. Thus, it has been postulated that stimulation of olfactory chemoreceptors by changes in CO<sub>2</sub>/H<sup>+</sup> might function to inhibit breathing when the ambient  $P_{CO_2}$  is higher than levels in the systemic blood, reducing CO<sub>2</sub> uptake while the animals seek fresh air (Coates and Ballam, 1987). As such, CO<sub>2</sub>/H<sup>+</sup> chemosensitive olfactory receptors may serve as the afferent limb of a defensive reflex that initiates reduced ventilation or breath holding, while animals seek a better environment. Such responses have also been postulated to occur in fish as a defensive reflex (Burlison et al., 1992). Hypercarbia is not uncommon in aquatic



environments and retention of such a reflex in some terrestrial animals would not be surprising. One might predict that these receptors, and this response, would be more strongly developed in species that encounter, and can choose to avoid, hypercarbic environments. In situations where they cannot avoid the hypercarbia, however, these reflexes will be in conflict with excitatory respiratory responses.

### 5.2. Prey detection

While no role for the modulatory effects of the vomeronasal receptors on respiration has yet been proposed, it has been suggested that the receptors found in the nasal epithelium may function to detect changes in environmental CO<sub>2</sub> originating from prey or predators, especially in confined burrows (Ballam, 1985; Coates and Ballam, 1987, 1989). Why this should lead to a net inhibition of breathing and change in breathing pattern is not at all clear. While this may reduce the frequency of body wall movements, making the waiting predator, or alerted prey, more difficult to detect, the size of each breath and associated body wall expansion that occurs when they do breathe is enhanced. This would also seem to be inconsistent with the proposal that the vomeronasal chemoreceptors are involved in the chemical sensing associated with feeding and prey tracking, since stimulation of these latter receptors excites ventilation (Halpern and Kubie, 1984; Coates and Ballam, 1989; Cooper and Burghardt, 1990).

### 5.3. Control of breathing pattern

One role of CO<sub>2</sub>/H<sup>+</sup> chemosensitive pulmonary receptors (IPC and PSR) may lie in the regulation of breathing pattern to enhance the efficiency of CO<sub>2</sub> excretion under conditions of environmental hypercarbia. Both PSR and IPC contribute to inspiratory termination (Banzett and Burger, 1977; Milsom et al., 1981). CO<sub>2</sub> reduces discharge of both receptor groups and acts to prolong inspiration, increasing tidal volume and decreasing breathing frequency. This acts to reduce dead space ventilation under any condition in which pulmonary CO<sub>2</sub> is elevated. The extent to which this is of benefit to an animal will be a consequence of the magnitude of the ventilatory dead space, and the extent to which changes in pattern are offset by net changes in the level of total ventilation. The opposite will also be

true and feedback from these receptors will also act to prevent CO<sub>2</sub> loss under conditions where breathing is elevated, such as during certain behavioural displays.

### 5.4. Phylogenetic trends

In amphibians, it would appear that there are only two groups of CO<sub>2</sub>-sensitive airway receptors, the olfactory receptors and the PSR. The net effect of inspiring CO<sub>2</sub> on these two groups is a function of the net inhibition of breathing due to olfactory receptor stimulation and the change in pattern resulting from inhibition of PSR discharge. While there are too few data to draw firm conclusions, it appears that the net effect in frogs, is slower, deeper breathing (enhanced “faveolar” ventilation) during hypercarbia followed by a significant post-hypercarbic rebound that would serve to flush the lungs on return to breathing fresh air (Kinkead and Milsom, 1996). While it is tempting to ascribe this to a diving life style, where internal CO<sub>2</sub> build-up would be expected, this response is primarily due to stimulation of the olfactory receptors that would continue to be ventilated by buccal pumping even during breath-holding.

The chelonians and mammals arose from the stem reptiles at about the same time. Interestingly, olfactory receptors appear to have little effect on overall ventilation in either group, and the effect of CO<sub>2</sub> on PSR discharge does not lead to much change in net ventilation. For these groups, there is little difference between ventilatory responses to hypercapnia and hypercarbia (Ballam, 1985; Coates and Silvis, 1999). The two groups in which we see the most profound difference between ventilatory responses to hypercarbia and hypercapnia are the diapsid reptiles and the birds. These groups also both have IPC.

In the diapsid reptiles there is tremendous variation in the extent to which ventilation is affected by olfactory CO<sub>2</sub>-sensitive receptor stimulation, as well as in the magnitude to which respiratory pattern is altered by increasing levels of intrapulmonary CO<sub>2</sub>. Increasing levels of environmental CO<sub>2</sub> may inhibit total ventilation (tegu lizard) (Ballam, 1985) or lead to modest (rattlesnake) (Andrade et al., 2004) or robust changes in total ventilation (caiman) (Tattersall, G.J., Andrade, D., Abe, A.S., Brito, S., Milsom, W.K., unpublished). Compared to the pattern of ventilation seen during the “off-response”, which is believed to more closely

mimic the response to metabolically produced CO<sub>2</sub>, however, in all cases the pattern of breathing is altered in a manner that would increase relative alveolar ventilation (Figs. 5 and 6). In animals with large saccular lungs, this not only reduces dead space ventilation but also flushes the lungs and air sacs. On return to breathing air, the post-hypercarbic response (off-response) would further refresh the air in the lungs. Finally, in situations where levels of CO<sub>2</sub> fall (hypocapnia), this would act to decrease tidal volume and increase breathing frequency, concentrating air flow to the more highly developed gas exchange portions of the lung that surround the opening of the trachea and primary bronchi.

In birds, what little data there are suggest that olfactory receptors play little or no role in the control of ventilation. IPC do appear to contribute to changes in breathing pattern during hypercarbia, but given the mechanics of ventilation in birds, this would have less effect on dead space ventilation (Scheid and Piiper, 1986). In birds, however, the IPC appear to take on new roles. Birds are unique in that the portion of the lung associated with producing airflow, and the gas exchange surface, have become separated into the air sacs and lungs respectively. With this change, the lungs have become rigid and this appears to have allowed the blood gas barrier to become thinner, greatly increasing the anatomical diffusion capacity of the lung (Perry and Duncker, 1980). The IPC largely take over the role of the PSR in this rigid lung where they monitor the washout or dilution of CO<sub>2</sub> in the respiratory passages during inspiration, which is a function of the rate and depth of each inspiration (Scheid and Piiper, 1986).

It has also been suggested that IPC may contribute to the hypercapnic ventilatory response and, in particular, that these receptors might be able to monitor CO<sub>2</sub> flux from venous blood into the pulmonary space and act as a venous CO<sub>2</sub> receptor (Fedde et al., 1982). As such, they could act to minimize or even eliminate any increase in PaCO<sub>2</sub> arising from increased metabolic CO<sub>2</sub> production. This, however, might be a transient effect of changes in breathing pattern in which tidal volume increases faster than breathing frequency decreases. Presently there are data to both support (Fedde et al., 1982) and refute this hypothesis (Milsom et al., 1981). The extent to which IPC may perform a unique function may be in eliciting ventilatory responses to

hypocapnia (Osborne et al., 1977). Here the CO<sub>2</sub> sensitivity of both PSR and IPC would act to decrease tidal volume and increase breathing frequency when CO<sub>2</sub> levels are low, preventing alkalosis during the hyperpnea associated with thermal polypnea and behavioural displays.

In conclusion, it would appear that olfactory receptors have evolved to inhibit breathing and prolong breath holding as a defensive strategy when environmental CO<sub>2</sub> levels are high. They give rise to reflexes that are retained in some, but not all species of lower vertebrate. PSR evolved to terminate inspiration and promote expiration (amongst other things). CO<sub>2</sub> sensitivity allows PSR to alter breathing pattern to reduce dead space ventilation when CO<sub>2</sub> levels are high. However, the degree of CO<sub>2</sub> sensitivity of PSR is highly variable. IPC appear to have evolved to perform similar tasks, but their increased CO<sub>2</sub> sensitivity allows them to not only reduce dead space ventilation when intrapulmonary CO<sub>2</sub> levels are high, but serve as a venous CO<sub>2</sub> receptor, at least transiently when levels of metabolically produced CO<sub>2</sub> begin to rise, and prevent alkalosis during hyperpnea/polypnea, and may have contributed to the evolution of the thin air/blood barrier associated with the rigid avian lung. The presence of all three groups of receptors in most of the reptiles, however, with different degrees of CO<sub>2</sub> sensitivity, gives rise to what appear to be anomalous responses to increasing levels of inspired CO<sub>2</sub>. The biological significance of these responses is still not clear, and may not be clear until more is known about the occurrence of environmental hypercarbia for any of these species and experiments are run under more natural conditions where animals are free to choose what they breathe.

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