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ORIGINAL PAPER

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Regulation of ventilation in the caiman (*Caiman latirostris*): effects of inspired CO_2 on pulmonary and upper airway chemoreceptors

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Abstract In order to study the relative roles of receptors in the upper airways, lungs and systemic circulation in modulating the ventilatory response of caiman (*Caiman latirostris*) to inhaled CO_2, gas mixtures of varying concentrations of CO_2 were administered to animals breathing through an intact respiratory system, via a tracheal cannula by-passing the upper airways (before and after vagotomy), or via a cannula delivering gas to the upper airways alone. While increasing levels of hypercarbia led to a progressive increase in tidal volume in animals with intact respiratory systems (Series I), breathing frequency did not change until the CO_2 level reached 7%, at which time it decreased. Despite this, at the higher levels of hypercarbia, the net effect was a large and progressive increase in total ventilation. There were no associated changes in heart rate or arterial blood pressure. On return to air, there was an immediate change in breathing pattern; breathing frequency increased above air-breathing values, roughly to the same maximum level regardless of the level of CO_2 the animal had been previously breathing, and tidal volume returned rapidly toward resting (baseline) values. Total ventilation slowly returned to air breathing values. Administration of CO_2 via different routes indicated that inhaled CO_2 acted at both upper airway and pulmonary CO_2-sensitive receptors to modify breathing pattern without inhibiting breathing overall. Our data suggest that in caiman, high levels of inspired CO_2 promote slow, deep breathing. This will decrease dead-space ventilation and may reduce stratification in the saccular portions of the lung.

Keywords Reptile · *Caiman latirostris* · Caiman · Control of breathing · Breathing pattern · Hypercarbia · Hypercapnia · Upper airway receptors · Intrapulmonary chemoreceptors

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Introduction

There has been a long-standing interest in the ventilatory responses of reptiles to inspired CO_2. These responses are highly variable between reptilian groups and often at odds with expectations for air-breathing vertebrates. Thus, while low levels of environmental CO_2 often cause ventilation to increase in lizards and snakes (Boelaert 1941; Nielsen 1961; Templeton and Dawson 1963; Ballam 1984; Klein et al. 2002; Glass and Johansen 1976; Gratz 1979; Andrade et al. 2004), they decrease total ventilation in others (Pough 1969; Coates and Ballam 1989). Higher levels of environmental CO_2 ($>3\%$), on the other hand, almost always depress total ventilation. In general, CO_2 always causes tidal volume to increase but increasing concentrations usually result in a substantial fall in breathing frequency and total ventilation. This is not the case in chelonian or crocodilian reptiles where vigorous ventilatory responses are well documented (Benchetrit and Dejours 1980; Milsom and Jones 1980; Davies et al. 1982; Douse and Mitchell 1992a).

These differences in the magnitude, and direction, of the ventilatory response appear to be due to varying degrees of conflicting inputs from different receptor groups sensitive to the CO_2 in inspired air. These receptor groups include central CO_2/pH-sensitive receptors, peripheral arterial chemoreceptors, intrapulmonary (CO_2) chemoreceptors, upper airway (nasal) chemoreceptors and vomeronasal receptors in some species (see Milsom et al. 2004 for review). Differences in

the ventilatory responses to CO_2 of various species are now thought to be due to differences in the presence, distribution, afferent sensitivities and/or central nervous system processing of afferent information from these different receptor groups. Depending on the balance, a wide spectrum of responses ranging from a large increase to no change or a decrease in ventilation may ensue.

The inhibitory inputs that have been documented arise primarily from olfactory and intrapulmonary chemoreceptors. In frogs, it appears that all of the inhibitory effect of inhaled CO_2 on breathing frequency comes from the upper airways (Kinkead and Milsom 1996). In the tegu lizard, receptors in the upper airways as well as within the lungs (or vascular system) contribute to an inhibition of breathing frequency while only the intrapulmonary receptors enhance the increase in tidal volume (Ballam 1985; Coates and Ballam 1987, 1989). In the South American rattlesnake, the inhibition of breathing frequency and enhanced increase in tidal volume arise only from receptors within the lungs (Andrade et al. 2004), a finding that is consistent with suggestions from studies on several other species of snake and lizard (Boelaert 1941; Nielsen 1961; Templeton and Dawson 1963; Gatz et al. 1975; Glass and Johansen 1976; Nolan and Frankel 1982).

The physiological roles of these various CO_2-sensitive airway receptors (and the responses they produce) are still conjecture. Hypotheses based on the various ventilatory responses to inspired CO_2 include roles in avoidance (of CO_2 rich environments), prey detection and breath-by-breath control of breathing pattern (see Coates and Ballam 1987, 1989; Furilla and Bartlett 1989; Milsom et al. 2004 for reviews). Hypercarbia is not commonly found in nature; however, no hypothesis based on hypocarbia or responses to increasing metabolic CO_2 loads have yet been put forward.

While studies focused on the mechanisms underlying the effects of CO_2 on receptor discharge in the different reptile groups and the central integration of the various afferent inputs are much needed, there is also a continuing need for inter-species comparisons of the ventilatory responses and the relative roles of the different receptor groups. There is no pattern apparent in the species differences reported in the literature at present to suggest any trend associated with phylogeny or life history traits. This largely reflects a lack of data from enough species from varying habitats, modes of life and phylogenies.

In the present study, we examine the location (upper versus lower airways) and relative roles of airway receptors in the ventilatory responses to inspired CO_2 in a member (the broad-nose caiman) of another reptilian group, the crocodilia. The crocodilians are known to exhibit a vigorous response to inspired CO_2 with increases in both f_R and V_T (Douse and Mitchell 1992a) yet have intrapulmonary chemoreceptors, pulmonary stretch receptors and, presumably, upper airway (olfactory) receptors (Douse et al. 1989). Specifically, we address the questions: (1) given the robust overall response to inhaled CO_2 in crocodilians, is there any evidence of respiratory inhibition/modulation by airway CO_2 receptors and, if so, (2) what are the relative roles of receptors in the upper airways, lungs and systemic circulation in this modulation? To answer the latter question, we exploit the presence of the "post-hypercapnic hyperpnea" described in reptiles (Milsom et al. 2004), and in the process, examine the relative role of changes in resting lung volume on resting ventilation and the ventilatory response to inhaled CO_2.

Materials and methods

Experiments were performed on 13 juvenile caiman, *Caiman latirostris*, weighing 1.56 ± 0.21 kg (mean ± SE; range 0.87–3.6 kg). All caiman were bred and raised in captivity from wild-caught parental stock at the Jacarezário, UNESP-Rio Claro, Southeastern Brazil, which runs a conservation program on this species. The caimans were fasted for at least 3 days prior to experimentation and were maintained at room temperature (~25 ± 2°C) both before and during experiments. All experiments complied with guidelines established by the Canadian Council of Animal Care.

All surgical and experimental manipulations were extremely similar, if not identical to, those described by Andrade et al. (2004). They are described again only briefly here.

Surgery

For anesthesia, each caiman was placed into a plastic bag flushed with pure CO_2 until all righting and withdrawal reflexes were abolished (Mac Rae 1958; Wang et al. 1993; Kohler et al. 1999). The animals were then fixed on a surgical table, and a local anesthetic (Lidocaine® HCI 2% 1:50,000 EPI, Pearson Labs) was injected (0.2–1.0 ml) at the boundaries of the surgical site. CO_2 was then intermittently delivered to the caimans through a mask, while the right femoral artery was exposed by a short (2–3 cm) dorso-lateral incision and a catheter (PE 50) previously filled with heparinized saline (50 IU ml^{-1}) was inserted through the artery toward the dorsal aorta. The vessel was then fastened tightly around the cannula with three sutures and the incision was then closed. For Series I, all 13 caiman were fitted with a custom-made face mask using the technique described by Glass et al. (1978) and modified by Wang and Warburton (1995). A period of at least 12 h was allowed for recovery after surgery before any measurements were made. Following completion of this series of experiments, all 13 caiman were re-anesthetized, the mask removed and the caiman intubated. The tracheal cannula was sewn in place with a purse string suture sealing the glottis around the cannula and the cannula was run out through the skin behind the angle of the jaw.

A secondary, small mask was also glued over the nostrils of these animals at this time (Series II). Another period of at least 12 h (overnight) was allowed for recovery after this surgery and measurements were made the following day. Following completion of this series of experiments, eight of these same animals were re-anesthetized and cuffs were placed around their vagus nerves high in the neck through small lateral incisions (Series III). The incisions were then closed and another period of at least 12 h (again, overnight) was allowed for recovery before measurements were made the following day. Animals were housed in a large darkened chamber (60 cm×60 cm×50 cm) and care was taken to minimize disturbances in the room in which the experiments were run.

Blood pressure and blood gas measurements

The arterial catheter was connected to a Deltran pressure transducer kept level with the heart of the animal. A water column was used to calibrate the transducer before and after each experiment. The pressure signal was amplified (custom-made amplifier) and stored on computer (Sable Systems v2.0 for Datacan V sampling at a rate of one sample every 0.22 s).

For blood sampling, pressure measurements were interrupted and samples (0.6 ml) were anaerobically withdrawn into heparinized 1 ml syringes and immediately analyzed for arterial P_{O_2} ($P_{a_{O_2}}$), and pH (pH_a) using a Radiometer BMS3 system. They were also analyzed for total O_2 (CaO_2) and CO_2 content ($CaCO_2$) using a Tucker chamber and Cameron chamber, respectively (Tucker 1967; Cameron 1971). Calibrations and calculations were performed as described by Andrade et al. (2004).

Ventilation measurements

Pulmonary ventilation was measured using the pneumotachographic method in all instances. In Series I, a Fleisch tube was attached between the mask and a T-connection to a gas line through which gas flowed at 3,000 ml min^{-1}. In Series II and III, the Fleisch tube was attached between the tracheal cannula and the gas flow line (see Andrade et al. 2004 for details). The pressure difference across the grid in the Fleisch tube was monitored by a differential pressure transducer (Sable PT100) connected to a computer data acquisition system (Sable Systems v2.0 for Datacan V sampling at a rate of one sample every 0.22 s). The pneumotachographs were calibrated before and after each experiment by injecting known volumes at different flow rates through each system from a syringe inserted into the system in lieu of the caiman. In Series II and III, gas was also delivered to the nose of the animal at a rate of 500 ml min^{-1}. This gas would enter the nares whenever the nostrils opened and exit the mouth via the internal nares but would not reach the lungs since at this time the animal was breathing through the tracheal cannula.

Inspired levels of CO_2 were monitored using a gas analyzer (Sable Systems CA−1B CO_2 analyzer). In Series I, gas was sampled from just outside the facemask at 100 ml min^{-1}. In Series II and III, gas was sampled from the tracheal cannula, also at 100 ml min^{-1}. The gas analyzer was calibrated with air and CO_2 mixtures produced from bottled gases by a gas-mixing pump (GF−3MP, Cameron Instruments, Port Aransas, TX, USA).

Experimental protocol

Series I

In this series, caiman equipped with a face mask were allowed to rest breathing air overnight in an isolated chamber at room temperature (25°C). On the day of the experiment, all the equipments were calibrated and then after recording baseline values for at least 2 h while breathing air, the animals were exposed to progressive hypercarbia (3, 5 and 7% CO_2) for 1-h periods. The gases were produced with a gas-mixing pump (GF-3MP, Cameron Instruments). Animals were returned to breathing air for at least 1 h between each level of CO_2. In this series, all inspired gases would transit the entire respiratory system.

Series II and III

In these series, the caiman were equipped with a tracheal cannula that was attached, via a T-connector, to a gas line running through the chamber and exhausting outside the chamber. In this way, the composition of the gas the caiman inspired into its lungs could be controlled independently of the gas surrounding the body and upper airways. A second gas line was connected to a small mask attached over the nostrils and in this way, the composition of the gas administered to the upper airways was controlled independently of the gas going to the lungs. These animals were also allowed to rest, breathing air overnight in an isolated chamber. On the day of this experimental series, after recording baseline values for at least 2 h, the air was replaced with 5% CO_2 (balance air) provided from a gas-mixing pump (Cameron GF-3MP) and delivered to either the lungs only, the upper airways only or to both the upper airways and lungs, in random order, for 1 h each. Animals were always administered air to both lungs and upper airways for at least 1 h between the exposure to the different CO_2 treatments. In Series III (vagotomized animals), at the start of the day, local anesthetic (Xylocaine, 2%) was administered through the vagal cuffs which invariably slowed breathing, increased heart rate, and was effective for the entire experimental run. Baseline recordings were then made

for at least 2 h and the 5% CO_2 was administered to the lungs only.

Calculations and statistics

Blood gases and haematocrit were measured at the end of each initial air run as well as at the end of each CO_2 run in all series. All cardio-respiratory variables were measured continuously under steady state control (normocapnia) and hypercarbic conditions and analyzed during the last 30 min of 1-h exposures and during each minute (up to 20 min) of the recovery period following the return to inspiring air after hypercarbic exposures. For ventilation, measures were made of the tidal volume, duration of each expiration (T_E), inspiration (T_I), the pause between subsequent breaths (T_{NVP}) and the corresponding overall breathing frequency. Total ventilation was calculated by multiplying the mean tidal volume by the breathing frequency. Heart rate was counted from the systolic peaks seen on the blood pressure trace.

Data were compared using either a one-way or a two-way repeated measures analysis of variance combined with an all pairwise multiple comparison procedure using the Holm–Sidak method for differences between individual means. All values expressed as % change were arcsin transformed before the analysis was run. Significance was accepted at $P < 0.05$.

Results

Series I

Steady state responses to CO_2

This series was primarily designed to examine the effects of different levels of environmental hypercarbia on ventilation in animals breathing through both upper and lower airways with an intact glottis (i.e., wearing a mask). Table 1 lists the levels of all cardio-respiratory variables for animals breathing air in this series of experiments. Figure 1 shows breathing traces from one caiman in Series I illustrating the patterns of breathing present under steady state conditions after 1 h exposures to air and different levels of CO_2. These traces are relatively typical and they illustrate the highly variable nature of breathing in these animals and the extent to which breathing pattern could change due to disturbance (primarily, reciprocal changes in breathing frequency and tidal volume—compare top and second panel in Fig. 1).

Table 2 shows the changes in arterial blood gases and pH that occurred during exposure to each level of hypercarbia while Fig. 2 and Table 3 present the corresponding effects of each exposure on the cardiovascular and ventilatory variables. While increasing levels of hypercarbia led to a progressive increase in tidal volume, breathing frequency did not change until the highest level of CO_2 at which time it decreased (Fig. 2). The net effect was still a large and progressive increase in total ventilation (Fig. 2). The slowing of breathing and increase in tidal volume at higher levels of CO_2 was due to increases in all respiratory intervals (Table 3, Fig. 3). However, while the caiman spent longer periods inspiring, the larger tidal volumes were also due to higher mean air flow rates over these intervals (150, 246, 558 and 906 ml min^{-1} kg^{-1} while breathing 0, 3, 5 and 7% CO_2, respectively) (see Fig. 3 for peak air flow rates in one individual). It can be seen from the data (Figs. 2 , 3, Table 3) that the overall breathing frequency was not slower in animals breathing 5% CO_2 compared to animals breathing air, although the breath length was significantly longer. Thus, this level of inspired CO_2 produced breaths that were much slower and deeper but it also reduced the length of the non-ventilatory period. The net result was that there was no significant change in breathing frequency overall. There were no associated changes in heart rate and mean arterial blood pressure (Fig. 2).

Post-hypercapnic hyperpnea

Figure 4 illustrates the post-hypercapnic changes that occurred in breathing in one caiman following the return to breathing air after having been exposed to 1-h periods

Table 1 Cardio-respiratory variables of animals breathing air in the different treatment protocols: wearing a mask (Series I); with an endotracheal tube and nose mask (Series II); and with an endotracheal tube and nose mask following vagotomy (Series III)

	Series I	Series II	Series III
Arterial blood pressure (mmHg)	49.2 ± 6.4	51.2 ± 6.0	72.4 ± 11.9[a]
Heart rate (beats min^{-1})	28.3 ± 3.8	28.7 ± 2.4	35.6 ± 1.8
Breathing frequency (breaths min^{-1})	6.8 ± 1.7	0.98 ± 0.17[b]	0.29 ± 0.05[b]
Tidal volume (ml kg^{-1})	9.3 ± 2.0	23.2 ± 3.3[b]	44.3 ± 8.2[a,b]
Total ventilation (ml kg^{-1} min^{-1})	38.5 ± 3.3	17.1 ± 1.8[b]	13.4 ± 3.1[b]
Expiratory time (T_E) (s)	3.6 ± 0.8	8.0 ± 2.4	5.4 ± 0.5
Inspiratory time (T_I) (s)	3.6 ± 0.8	7.0 ± 1.7	5.8 ± 0.4
T_I/T_{TOT}	0.49 ± 0.02	0.50 ± 0.04	0.54 ± 0.04
Non-ventilatory period (s)	9.2 ± 2.6	59.0 ± 16.2	116.2 ± 48.3[b]

[a]Indicates values in Series III that are significantly different from values in Series II
[b]Denotes values that are significantly different from values in Series I

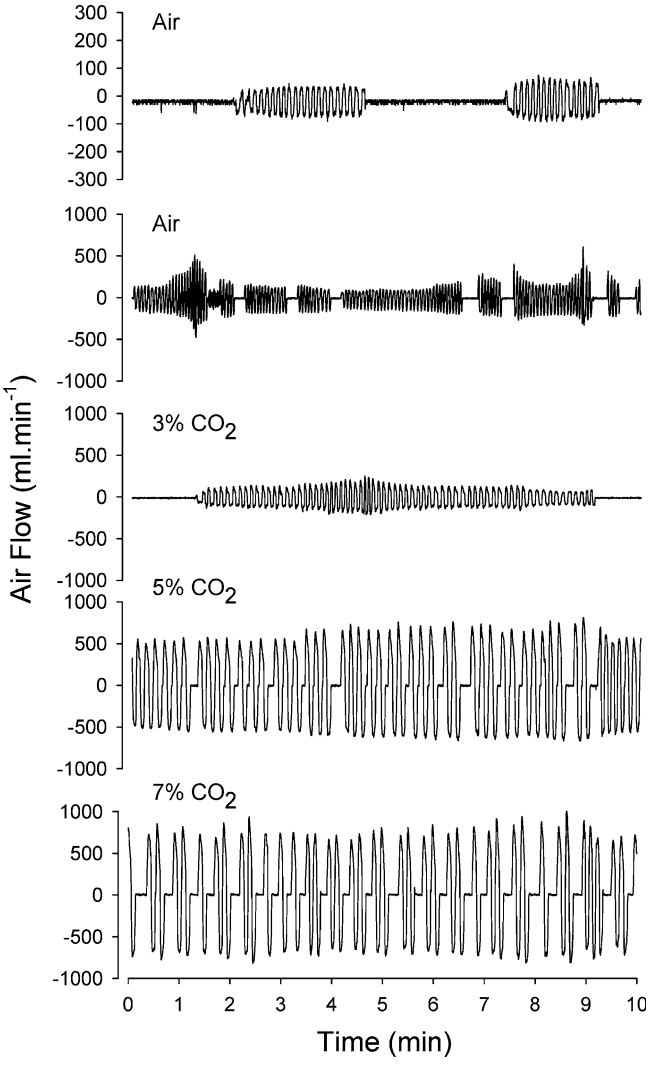

Fig. 1 Traces of respiratory air flow (inspiration is up) from one caiman (Series I) illustrating steady state responses in ventilation to various levels of hypercarbia. The second set of traces for the animal breathing air were taken after the animal had been disturbed (in this case by experimenters entering the room)

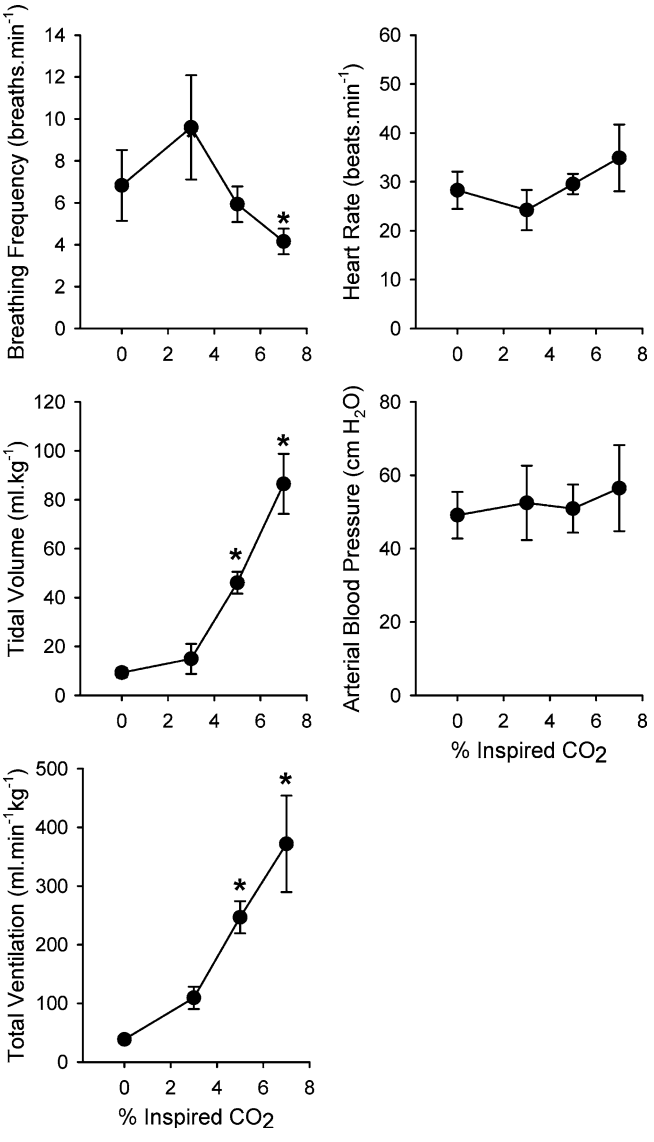

Fig. 2 Effect of 1 h exposure to different levels of hypercarbia on breathing frequency, tidal volume, total ventilation, heart rate and arterial blood pressure in caiman breathing through a mask (Series I). Values are means ± SEM ($n = 13$), * denotes significant difference from values in animals breathing air

of breathing 3, 5 and 7% CO_2 via the mask. While it appears that breathing frequency and tidal volume slowly returned to control values, when the results are viewed quantitatively (for all animals in Fig. 5, left-hand panels) it becomes apparent that breathing pattern changed dramatically immediately on the return to breathing air. Once the CO_2 was removed, breathing frequency immediately increased above air-breathing values, to roughly the same maximum level, regardless of the level of CO_2 the animal had been breathing, and remained there for over 10 min before slowly declining back to typical air breathing values (Fig. 5, left center panel). The higher the level of CO_2, the greater was the

Table 2 Values for blood gases, pH and haematocrit (Hct) of animals wearing a mask and breathing various concentrations of CO_2 ($n = 7$)

Gas	P_{O_2} (mmHg)	O_2 content (vol.%)	P_{CO_2} (mmHg)	CO_2 content (mmol l^{-1})	pH	Hct (%)
Air	69.5 ± 7.3	9.7 ± 2.0	19.6 ± 3.1	20.6 ± 3.5	7.61 ± 0.04	22.0 ± 2.30
3% CO_2	93.6 ± 5.4	10.7 ± 2.2	23.4 ± 1.7	22.9 ± 3.8	7.52 ± 0.04	23.1 ± 2.0
5% CO_2	106.5[a] ± 7.6	10.7 ± 2.2	31.5[a] ± 0.8	23.1 ± 3.2	7.39[a] ± 0.05	22.8 ± 1.8
7% CO_2	108.4[a] ± 6.5	9.1 ± 1.6	37.4[a] ± 0.9	23.2 ± 3.2	7.31[a] ± 0.07	22.9 ± 1.9

[a]Indicates values significantly different from those recorded in animals breathing air

Table 3 Respiratory intervals (s) of animals wearing a mask and breathing various concentrations of CO_2 ($n = 13$)

Gas	T_E	T_I	T_{TOT}	T_I/T_{TOT}	T_{NVP}
Air	3.6 ± 0.8	3.6 ± 0.8	6.5 ± 1.4	0.49 ± 0.02	5.5 ± 2.6
3% CO_2	3.6 ± 0.7	3.6 ± 0.6	6.3 ± 1.2	0.52 ± 0.02	2.9 ± 2.9
5% CO_2	4.6 ± 0.6	4.9[a] ± 0.5	9.0[a] ± 0.9	0.53 ± 0.01	4.0 ± 1.8
7% CO_2	5.0[a] ± 0.7	5.7[a] ± 0.3	9.6[a] ± 0.8	0.51 ± 0.05	7.4[a] ± 4.0

[a]Indicates values significantly different from those recorded in animals breathing air

tidal volume at the end of the hypercarbic episode. Within the first breath after the CO_2 was removed from the gas stream, tidal volume decreased rapidly (Fig. 5, left top panel). The net result was a small immediate drop in total ventilation followed by a slow progressive return back to air breathing values (Fig. 5, bottom left panel).

The net effect of this dramatic and immediate switch in breathing pattern can be seen in the right-hand panels of Fig. 5. While total ventilation increased in a robust fashion with increasing levels of hypercarbia, this was due exclusively to increases in tidal volume. During the first 1–2 min after the return to breathing air, there were relatively off-setting increases in breathing frequency and decreases in tidal volume. The decrease in total ventilation in the first 2 min was significant, however,

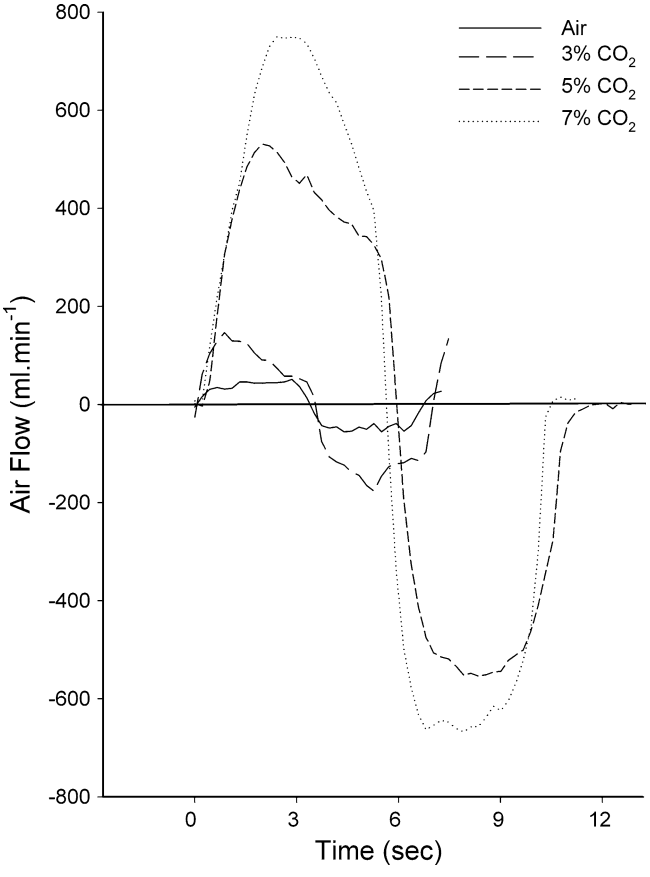

Fig. 3 Traces of respiratory air flow (inspiration is up) for single breaths from one caiman (Series I) inhaling various levels of CO_2

for animals returning to air after breathing 5 and 7% CO_2.

Series II and III

While animals in Series I wore a mask, those in Series II and III were intubated and hence their glottis was bypassed. Since reptiles normally pause between breaths at end-inspiration with a breath-hold maintained against a closed glottis, intubation leads to a reduction in resting lung volume and a change in breathing pattern. Animals now begin each breath with a small, active expiration (to below resting lung volume) followed by an active inspiration; but, instead of the inspiration being followed by a breath-hold, it is followed by a passive expiration (Fig. 6, lower right panel). The net result of intubation was a slowing of the breathing rate (primarily due to an increase in the pause between breaths), an increase in tidal volume and a fall in total ventilation (Table 1, Fig. 6). Animals in Series III were also vagotomized. Amongst other things, this will have removed all feedback from pulmonary receptors. The net result was a further slowing of breathing frequency (not significant; $P = 0.067$) and increase in tidal volume (Table 1, Fig. 6). Total ventilation, overall, was not changed (non-significant decrease; $P = 0.590$), when compared to intubated animals breathing with an intact vagus. This also removed parasympathetic vagal tone to the heart, which led to an increase in mean arterial blood pressure associated with a small, non-significant increases in heart rate ($P = 0.103$) (Table 1, Fig. 6).

Steady state responses to CO_2

In Series II and III, caiman breathing spontaneously were induced to inspire 5% CO_2 either through the upper airways and lungs, into the lungs alone or through the upper airways alone, in an attempt to resolve the location of the receptors responsible for the changes in breathing pattern seen when CO_2 was administered through the mask. The effects of each treatment on blood gases, for all series, are shown in Table 4. Note there were no changes in blood gases when the 5% CO_2 was delivered to the upper airways alone since this gas did not come in contact with the pulmonary exchange surface. On the other hand, the delivery of 5% CO_2 to

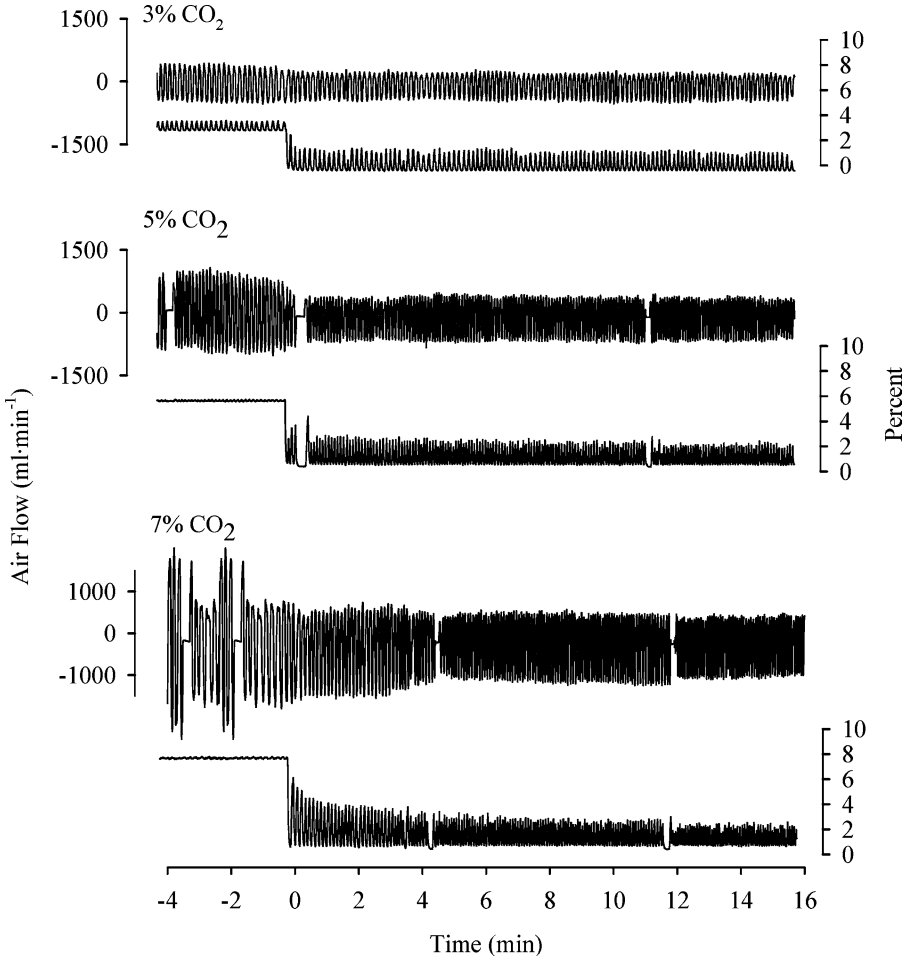

Fig. 4 Traces of respiratory air flow, and mask CO_2 levels from one caiman (Series I) illustrating the changes in ventilation that occurred during the return to air after breathing various levels of CO_2. The switch back to breathing air occurred at time 0

the lungs led to a significant rise in P_{CO_2} in all other conditions (Table 4).

When 5% CO_2 was administered to both the lungs and upper airways in these experiments, there was no change in breathing frequency but a significant increase in tidal volume, similar to what was seen when the CO_2 was administered through the mask in Series I (Fig. 7). The results were identical when CO_2 was administered to the lungs alone. Administration of CO_2 to the upper airways alone had no effect on ventilation (Fig. 7). Following vagotomy (Series III), the administration of 5% CO_2 to the lungs alone led to a significant rise in total ventilation due primarily to a significant increase in tidal volume (Fig. 7). Thus, despite the dramatic increases in tidal volume seen in animals in Series II and III while breathing air, due to the intubation or intubation plus vagotomy, tidal volume and total ventilation still increased on inspiration of 5% CO_2, although the relative increases were reduced (Fig. 7, right-hand panels).

Post-hypercapnic hyperpnea

Figure 8 illustrates the quantitative changes seen in breathing frequency on return to breathing air after 1 h of breathing 5% CO_2. A post-hypercapnic tachypnea still occurred for the animals receiving 5% CO_2 to both lungs and upper airways, to the lungs alone and to the upper airways alone. There was no effect of removing CO_2 from the air stream delivered to the lungs alone in the vagotomized animals.

Discussion

The questions addressed specifically in this paper were: (1) given the robust overall response to inhaled CO_2 previously reported in crocodilians (Douse et al. 1989), is there any evidence of respiratory inhibition/modulation by airway CO_2 receptors and, if so, (2) what are the relative roles of receptors in the upper airways, lungs and systemic circulation in this modulation? Our data suggest that in the caiman, high levels of inspired CO_2 (≥5%) promote slow, deep breathing. Once inspired CO_2 levels return to air values, but while arterial and end-expired levels of CO_2 remain transiently high, the breathing pattern changes to one of higher breathing frequency, lower tidal volume breaths with no net change in total ventilation. Thus, inhaled CO_2 (hypercarbia), acting at both upper airway and pulmonary

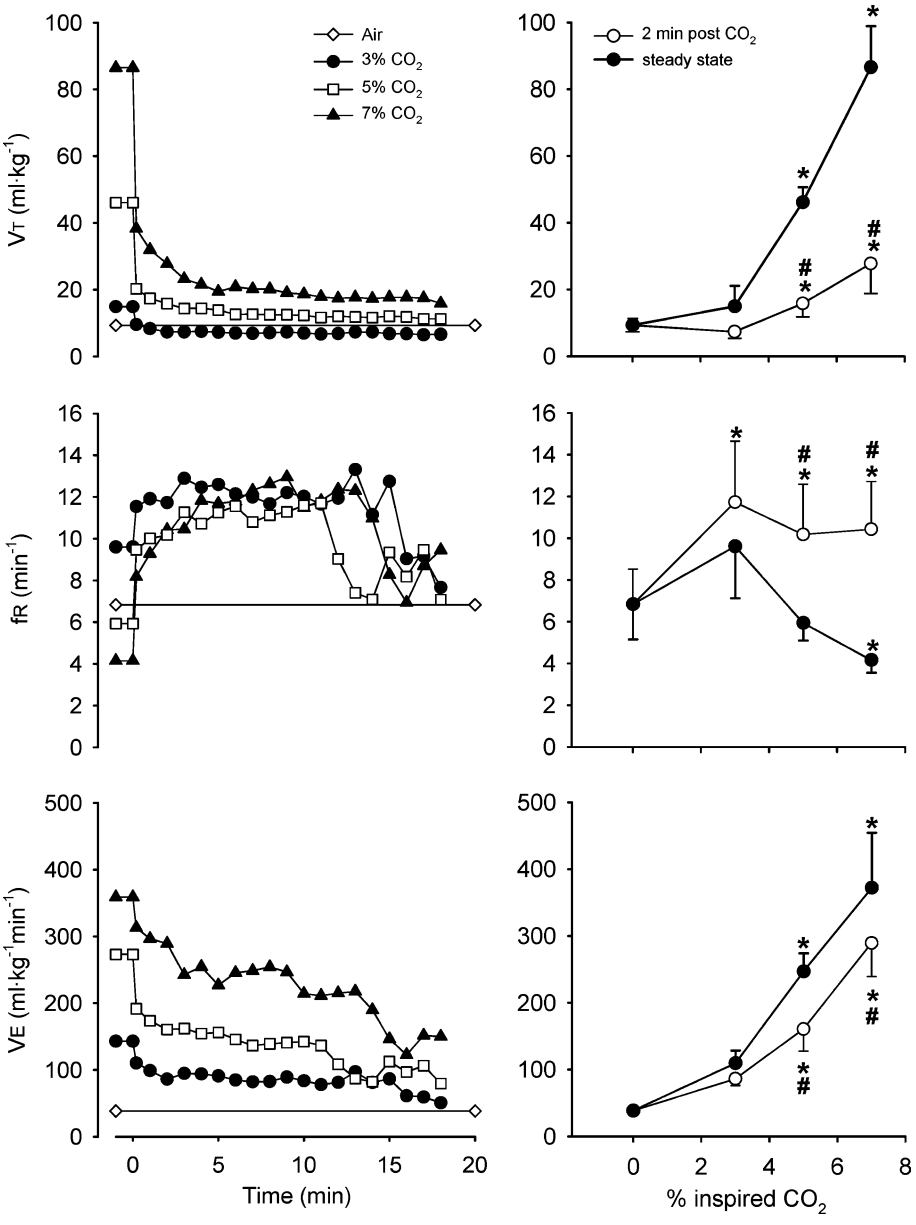

Fig. 5 *Left panel*: The changes in tidal volume, breathing frequency and total ventilation that occurred in all caiman in Series I during the return to air after breathing different levels of CO_2 for 1 h ($n = 13$). The switch back to breathing air occurred at time 0. *Right panels*: Comparison of the levels of breathing frequency, tidal volume and total ventilation in all caiman in Series I during steady state exposure to various levels of CO_2 as well as during the first 2 min after the return to breathing air from each level of CO_2 (2 min post-CO_2). Values are means ± SEM ($n = 13$), * denotes significant difference from values in animals breathing air in each treatment group and # denotes a significant difference between steady state and "2 min post-CO_2" values in animals breathing the same level of CO_2

CO_2-sensitive receptors, modifies the response to hypercapnia (increased systemic CO_2) without inhibiting breathing overall. The net effect of this modulation will be to decrease dead-space ventilation when inspired CO_2 is elevated.

Animals in Series I wore a mask while those in Series II and III were intubated and their glottis was bypassed during breathing. This led to significant changes in resting breathing pattern (reduced frequency but increased tidal volume) and total ventilation (Table 1). Reptiles normally pause between breaths at end-inspiration with a breath-hold maintained against a closed glottis. Following intubation, this was not possible since the lungs passively deflated following active inspiration with no glottal trapping. Nonetheless, intubated animals responded to inspiration of CO_2 in much the same manner as animals with an intact glottis ("both" versus "mask", Fig. 7). Both groups showed no change in breathing frequency and significant increases in tidal volume and total ventilation during hypercapnia (Fig. 7), and a significant post-hypercapnic hyperpnea (Fig. 8), and thus the data obtained using this technique were adequate for addressing our questions. Besides being intubated, animals in Series III were also vagotomized. This will have removed all feedback from pulmonary receptors and the net result was a dramatic further increase in tidal volume (Table 1). Breathing frequency and total ventilation were not statistically altered and neither were blood gases (Table 4). These animals also showed no change in breathing frequency and significant increases in tidal volume and total ventilation during hypercapnia (Fig. 7), although the post-hypercapnic hyperpnea was now gone (Fig. 8).

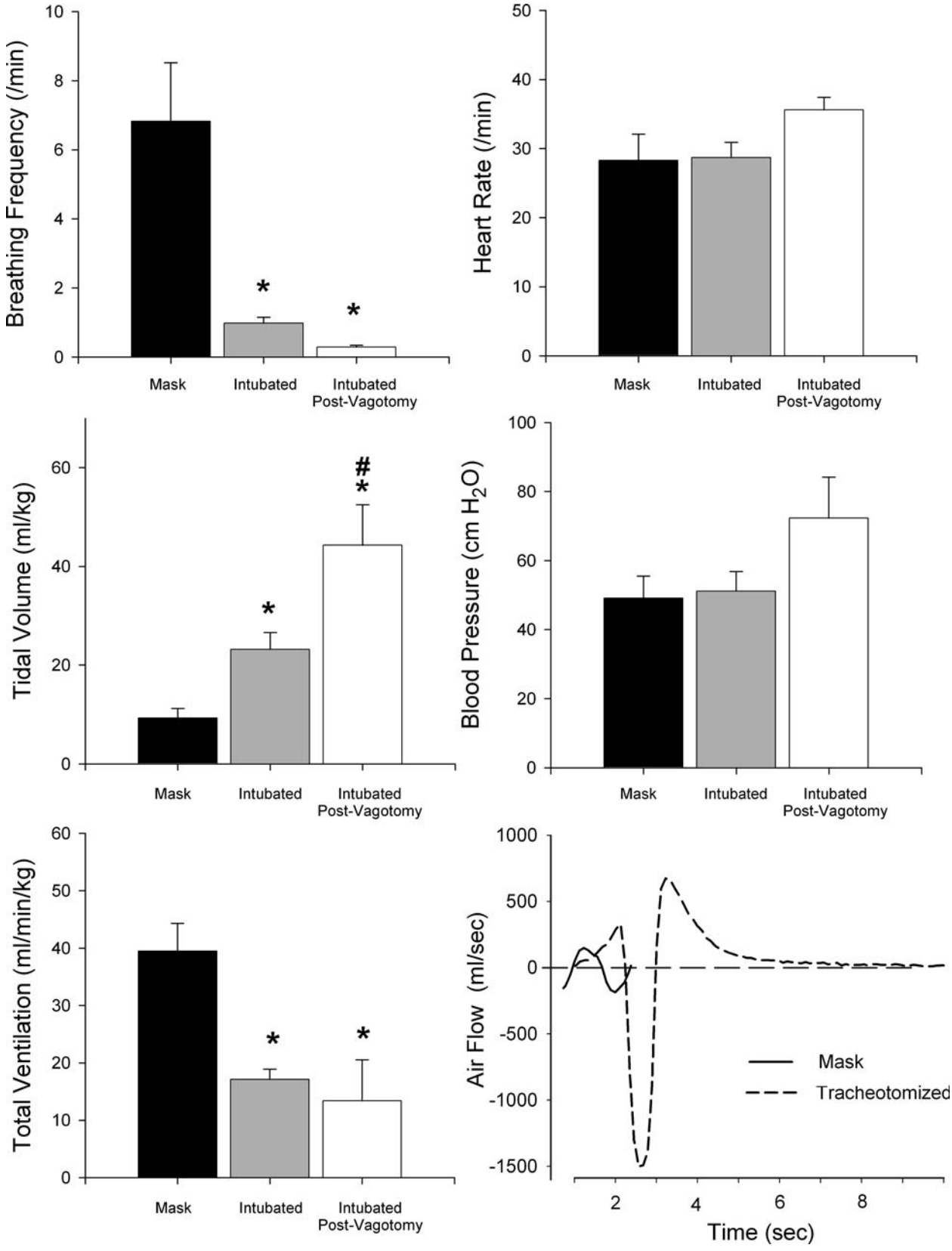

Fig. 6 Effect of intubation, and intubation plus vagotomy, on breathing frequency, tidal volume, total ventilation, heart rate and arterial blood pressure in caiman. Values are means ± SEM ($n = 13$), * denotes significant difference from values in animals breathing through a mask and # indicates a significant effect of vagotomy in animals that were intubated

Table 4 Values for blood gases, pH and haematocrit (Hct) for animals breathing air or 5% CO_2 under various conditions

Condition	Gas	P_{O_2} (mmHg)	O_2 content (vol.%)	P_{CO_2} (mmHg)	CO_2 content (mmol l^{-1})	pH	Hct (%)
Mask	Air	69.5 ± 7.3	9.7 ± 2.0	19.6 ± 3.1	20.6 ± 3.5	7.61 ± 0.05	22.0 ± 2.3
	5% CO_2	106.5 ± 7.6	10.7 ± 2.2	31.5[a] ± 0.8	23.1 ± 3.2	7.39[a] ± 0.05	22.8 ± 1.8
Both	Air	72.7 ± 7.8	10.3 ± 1.20	22.5 ± 2.2	21.9 ± 3.2	7.52 ± 0.05	25.8 ± 1.9
	5% CO_2	90.7 ± 9.5	10.4 ± 1.6	36.3[a] ± 3.0	23.8 ± 3.3	7.33[a] ± 0.06	26.6 ± 1.1
Lungs only	Air	72.7 ± 7.8	10.3 ± 1.2	22.5 ± 2.2	21.9 ± 3.2	7.52 ± 0.03	25.8 ± 1.9
	5% CO_2	94.10 ± 7.3	11.6 ± 2.1	32.9[a] ± 1.9	22.5 ± 3.0	7.35[a] ± 0.04	26.6 ± 1.6
Upper airways only	Air	72.7 ± 7.8	10.3 ± 1.2	22.5 ± 2.2	21.9 ± 3.2	7.52 ± 0.03	25.8 ± 1.9
	5% CO_2	78.8 ± 6.85	10.8 ± 1.3	21.3 ± 1.8	21.2 ± 2.9	7.53 ± 0.03	25.9 ± 1.5
Post-vagotomy	Air	64.7 ± 6.2	10.7 ± 1.8	22.8 ± 0.7	17.8 ± 0.8	7.42 ± 0.03	27.1 ± 2.3
	5% CO_2	74.7 ± 8.5	8.5 ± 1.4	39.6[a] ± 4.8	22.0 ± 3.1	7.26[a] ± 0.04	25.1 ± 1.6

$n = 13$ for all conditions except post-vagotomy where, $n = 8$
[a]Indicates values significantly different from those recorded in animals in the same condition breathing air

Steady state breathing on air and in hypercarbia

The respiratory pattern in control animals was highly variable, both within and between individuals, regardless of the gas the animals were breathing (Fig. 1). Such variability has been noted in crocodilians in the past, both in breathing pattern and in heart rate (Huggins et al. 1969; Douse and Mitchell 1992a). These animals are highly sensitive to minor disturbances even when housed in darkened chambers isolated from researchers and equipment. The slightest of disturbances has been reported to lead to large changes in breathing pattern and heart rate (Huggins et al. 1969; Douse and Mitchell 1992a). This was also true in the present study. In general, when breathing room air, caiman in the present study breathed episodically as has been described for other crocodilians (Naifeh et al. 1970, 1971; Glass and Johansen 1979; Zhao-Xian et al. 1991; Douse and Mitchell 1992a; Hicks and White 1992; Munns et al. 1998) and resting levels of ventilation (f_R, V_T and V_{TOT}), heart rate and blood pressure from undisturbed animals compared well to those reported for other crocodilians (Huggins et al. 1970, 1971; Glass and Johansen 1979; Hicks and White 1992; Branco and Wood 1993; Wang and Warburton 1995; Munns et al. 1998).

The cardio-respiratory responses to hypercarbic hypercapnia (i.e., inhalation of CO_2) also compare well with reports for other crocodilians. Cardiovascular variables remained constant while animals increased breathing exclusively through increases in tidal volume. While there was a trend in the data, overall f_R did not change until the highest level of inspired CO_2 (7%), at which point it fell significantly. Overall, there was a robust increase in total ventilation due to both longer inspiratory intervals and to higher mean air flow rates over these intervals (Fig. 3). Identical changes in breathing pattern (large increases in tidal volume with a reduction in breathing frequency at high levels of inspired CO_2) and similar robust increases in total ventilation have been reported in the American alligator (Branco and Wood 1993; Wang and Warburton 1995).

Post-hypercapnic tachypnea

It has not been uncommon to see a paradoxical increase in total ventilation in reptiles (and other air-breathing vertebrates) immediately following the return to breathing air after a period of hypercarbia (post-hypercapnic hyperpnea). This increase often occurs due to increases in both breathing frequency and tidal volume and can be large and dose dependent (Nielsen 1961; Templeton and Dawson 1963; Glass and Johansen 1976; Nolan and Frankel 1982; Coates and Ballam 1989; Klein et al. 2002; Andrade et al. 2004). In the present study, while there was no increase in tidal volume, or total ventilation during this interval, there was a large increase in breathing frequency (a post-hypercapnic tachypnea). Despite the immediate fall in inspired CO_2, breathing frequency rose, to the same maximum level independent of the level of inspired CO_2, and remained elevated for well over 10 min. Simultaneously, tidal volume dropped, rapidly at first, and then slowly returned to resting levels. As a consequence, total ventilation also returned slowly to control values (≥15 min). During the first 2 min after return to air, the breathing pattern had switched dramatically from one of low frequency, high tidal volume breaths to one of higher breathing frequency, lower tidal volume breaths (right-hand panels, Fig. 5).

The post-hypercapnic hyperpnea has been interpreted to suggest that during conditions of environmental hypercarbia, the stimulating effect of systemic hypercapnia is in part masked by an inhibitory effect of tonically elevated airway CO_2 acting at various sites (see Milsom et al. 2004 for review). When animals subsequently breathe normocarbic air again, arterial levels of CO_2 and end-expiratory levels of CO_2 fall slowly as whole body CO_2 stores are lowered gradually and CO_2 is eliminated, whereas inspired CO_2 levels fall immediately. Thus, the inhibitory effect of tonically elevated airway CO_2 is eliminated immediately, while the excitatory effect of elevated systemic CO_2 persists until a new steady state is attained. In this context, the results of the present study show that, in the caiman, while high levels of inspired

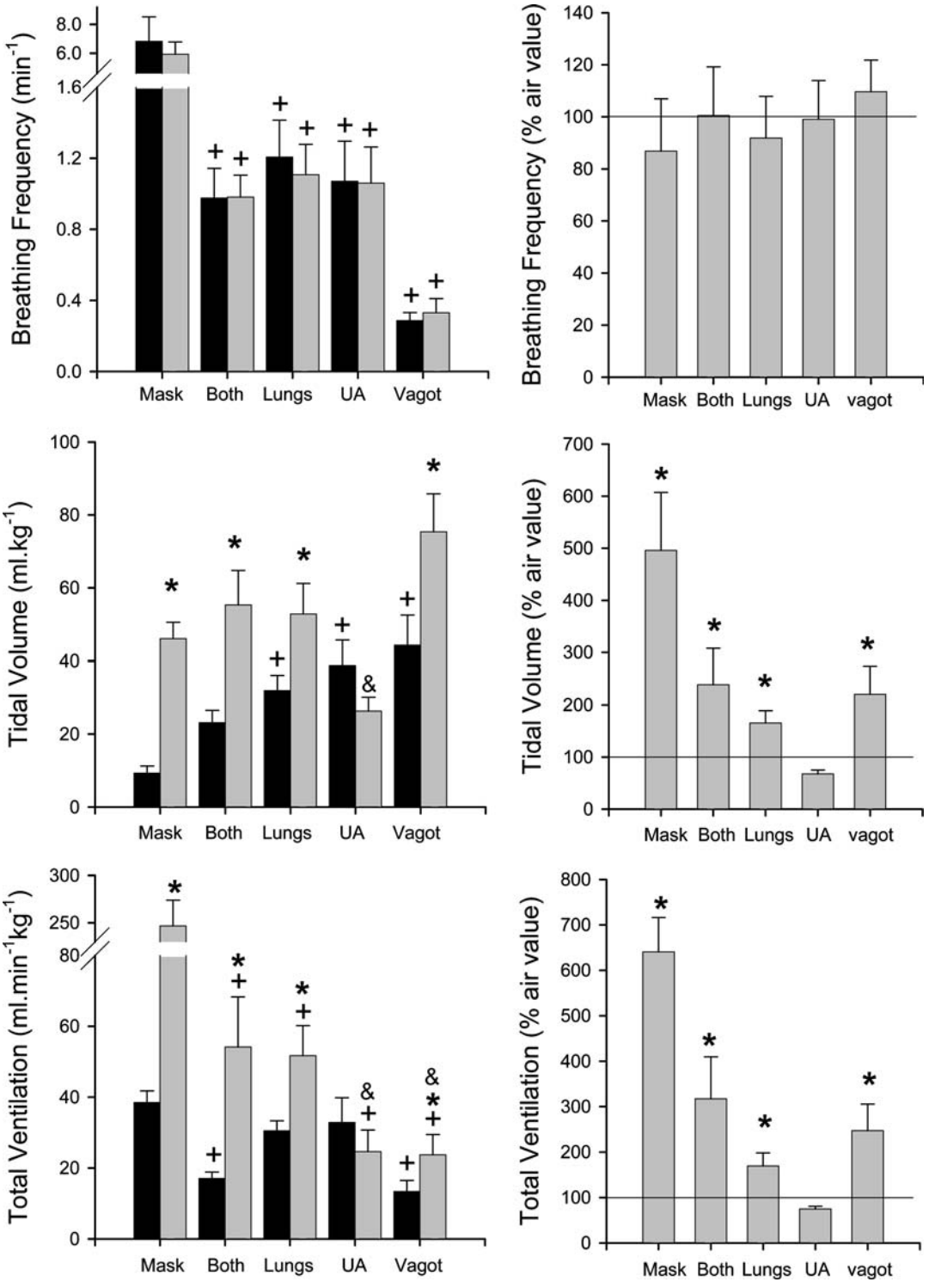

Fig. 7 Effect of 1 h exposure to 5% CO_2 on breathing frequency, tidal volume, total ventilation, heart rate and arterial blood pressure in caiman inspiring CO_2 via different routes (to both lungs and upper airways via a mask (*Mask*; $n=13$); to both lungs and upper airways simultaneously via an endotracheal tube and nose mask (*Both*, $n=13$); to the lungs alone via an endotracheal tube (*Lungs*, $n=13$), to the upper airways alone via a nose mask (*UA*, $n=13$) and to the lungs alone via an endotracheal tube following vagotomy (*Vagot*, $n=8$)). Absolute values are presented on the *left* while values expressed as a % of the values for animals breathing air before CO_2 exposure are presented on the *right*; * denotes values that are significantly different from those recorded while animals were breathing air in that treatment group; $^{+}$ denotes values that are significantly different from those recorded in animals wearing a mask breathing the same gas; $^{\&}$ denotes values that are significantly different in intubated animals breathing 5% CO_2 to either upper airways or to lungs post-vagotomy compared to animals breathing CO_2 to both upper airways and the lungs

CO_2 promote slower, deeper breathing, once inspired CO_2 levels return to air values, but while arterial and end-expired levels of CO_2 remain high, only the breathing pattern changes, not total ventilation.

Effects of altering/removing pulmonary receptor feedback

To explore the sites of the receptors involved in producing the breathing pattern we saw in the caiman during inhalation of CO_2, and the changes we saw on return to breathing air, we needed to perform more invasive experiments on intubated animals. Following intubation most of the inspired air escaped during the respiratory pause and there was little air to exhale during the active expiratory phase. Breathing became very slow and deep. Figure 6 compares the respiratory and cardiovascular variables in intubated animals as well as in intubated, vagotomized animals breathing air. It can be seen that intubation and the ensuing reduction in end-inspired lung volume had a similar effect to total lung denervation. Breathing became very slow and deep, and overall breathing was reduced. Vagotomy only led to a further significant rise in tidal volume. The additional decreases in breathing frequency and total ventilation were not significant. These changes did not alter arterial $P_{a_{O_2}}$, $P_{a_{CO_2}}$ or pH_a significantly. The differences seen in breathing pattern between the intubated animals and the intubated, vagotomized animals were presumably due to removing all remaining tonic pulmonary receptor input, as well as the phasic input, which should have now become extremely large (in proportion to the increase in tidal volume). Vagotomy will also have removed feedback from peripheral arterial chemoreceptors which may have contributed to these further changes. Although their data were not quantified, these results are very similar to those reported by Douse and Mitchell (1992a) for the American alligator. Our data point to the significance of (tonic) lung receptor feedback during the end inspiratory breath-hold for regulating breathing pattern in caiman.

Intubation had little effect on the cardiovascular variables and the increases in heart rate and mean arterial blood pressure following vagotomy were insignificant, although the power of our statistical analysis was low. While this suggests that these changes may have become significant with a larger or more complete data set, they nonetheless suggest there was not much resting vagal tone in these animals.

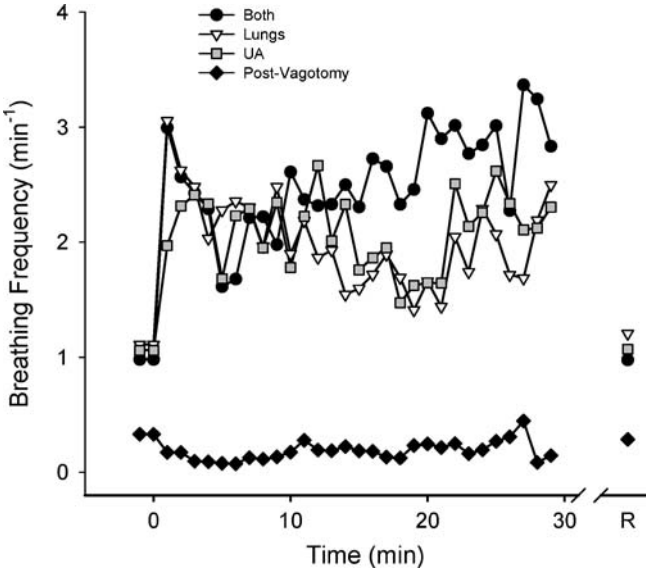

Fig. 8 The changes that occurred in breathing frequency in all caiman in Series II and III during the return to air after breathing 5% CO_2 by different routes for 1 h (to both lungs and upper airways simultaneously via an endotracheal tube and nose mask (*Both*, $n=13$); to the lungs alone via an endotracheal tube (*Lungs*, $n=13$), to the upper airways alone via a nose mask (*UA*, $n=13$) and to the lungs alone via an endotracheal tube following vagotomy (post-vagotomy, $n=8$))

Relative roles of different receptor groups

Since the studies conducted to elucidate the roles of the different receptor groups in the modulation of breathing pattern during hypercarbic exposure were performed on intubated animals, changes in breathing pattern were initiated from the slow deep breathing pattern just discussed. For these studies, 5% CO_2 was choosen as the hypercarbic stimulus because it was a level sufficient to produce a brisk increase in total ventilation when administered via the mask and a significant change in breathing pattern on immediate return to breathing air. It invariably elevated arterial CO_2 in these experiments except when administered only to the upper airways.

In these experimental runs, breathing frequency was unaffected by CO_2 regardless of the route of administration. In all cases, increases in total ventilation were due exclusively to increases in tidal volume (Fig. 7, left-hand panels). This occurred despite the fact that tidal volume was already significantly elevated although the increase in resting tidal volume probably accounted for the relative reduction in the overall increase in ventilation (Fig. 7, right-hand panels). Administration of CO_2 via the upper airways alone had no effect on ventilation. There was also no further effect of vagotomy on ventilation once the animals were breathing 5% CO_2.

Following return to air after administering 5% CO_2 via the different routes, there was a post-hypercapnic tachypnea present under all conditions except post-vagotomy. Thus, even though administering 5% CO_2 via the upper airways had no effect on ventilation, removal of the CO_2 led to an immediate increase in breathing frequency that lasted for over 30 min. It is possible that this represents a non-specific response to a sudden change in upper airway stimulation, rather than a specific respiratory response.

In the case of the rebound tachypnea seen when CO_2 was administered to the lungs alone, this was removed post-vagotomy indicating that it arose from effects on either receptors within the lungs or the peripheral systemic circulation.

We cannot distinguish between the effects of removing pulmonary receptor feedback versus arterial chemoreceptor feedback with this protocol (i.e., Series III). However, the speed of the change in breathing frequency seen in intact animals upon the return to air after having been exposed to hypercarbic hypercapnia (i.e., where pulmonary receptors are "off-loaded" immediately but arterial chemoreceptors are not), would suggest that the differences seen following vagotomy arise from the removal of the afferent information arising from pulmonary receptors. If so, these data suggest that it is the pulmonary receptors that enhance the tidal volume effect and reduce the breathing frequency response to inspired CO_2, but that both removal of CO_2 from these receptors, as well as from upper airway receptors, are involved in the post-hypercapnic tachypnea. This is very similar to what has been reported in the tegu lizard, where receptors in the upper airways, as well as within the lungs or vascular system inhibited breathing frequency while only the latter receptors contributed to the enhanced increase in tidal volume when animals inhaled CO_2 (Ballam 1985; Coates and Ballam 1987; Ballam and Coates 1989). There is one difference, however. In the caiman, there was only a change in breathing pattern, not in total ventilation.

Recordings of single fiber vagal nerve discharge arising from the lungs have shown that the alligator possesses both pulmonary stretch receptors (that are relatively CO_2-insensitive) and CO_2-sensitive intra-pulmonary chemoreceptors (IPC) (Powell et al. 1988; Douse et al. 1989). Intrapulmonary chemoreceptors have been described in reptiles, in snakes, lizards and alligators (see Milsom 1995 for review). They have discharge that is inversely proportional to CO_2 levels and it has previously been suggested that part of the inhibition of ventilation during environmental hypercarbia in many of these species arises from inhibition of this receptor group (Ballam 1985; Ballam and Coates 1989; Coates and Ballam 1989). They could well be the pulmonary receptors involved in breathing pattern modulation during inhalation of CO_2 in this study as well.

There are few other studies with comparable data. Douse and Mitchell (1992b), however, did measure the response to 5% inspired CO_2 in intubated, lightly anesthetized American alligators following vagotomy and found that the ventilatory response was totally absent. Curiously, they also found the response returned if the animals were unidirectionally ventilated. During unidirectional ventilation, vagotomy did not increase tidal volume and it was suggested that this may have reduced mechanical or reflex inhibition of the ventilatory response, allowing expression of the CO_2 response. The response to CO_2 now consisted of increases in both tidal volume and breathing frequency. The increase in tidal volume seen with vagotomy in intubated animals in that study (from 40 to 116 ml kg^{-1}) was much greater than that seen in the present study (23–44 ml kg^{-1}) and could explain why we continued to see a ventilatory response to 5% CO_2 post-vagotomy while they did not. Similar results have been obtained from chickens where bilateral vagotomy almost abolished the CO_2 response in spontaneously breathing birds (Mitchell and Osborne 1979) but not in unidirectionally ventilated animals (Peterson and Fedde 1971).

Perspective

The response to inhalation of CO_2 can be highly variable in air-breathing vertebrates and the relative roles of different receptor groups in producing these responses can also be highly variable. Inhibitory inputs have been documented arising from upper airway (olfactory) and intrapulmonary chemoreceptors. In frogs, it appears that all of the inhibitory effect of inhaled CO_2 on breathing frequency comes from the upper airways (Kinkead and Milsom 1996). In the tegu lizard, receptors in the upper airways as well as within the lungs (or vascular system) contribute to an inhibition of breathing frequency while only the intrapulmonary receptors enhance the increase in tidal volume (Ballam 1985; Coates and Ballam 1987, 1989). In the South American rattlesnake, the inhibition of breathing frequency and enhanced increase in tidal volume arise only from receptors within the lungs (Andrade et al. 2004), a finding that is consistent with suggestions from studies on several other species of snakes and lizards (Boelaert 1941; Nielsen 1961; Templeton and Dawson 1963; Gatz et al. 1975; Glass and Johansen 1976; Nolan and Frankel 1982). The situation in the caiman appears similar to that reported in the tegu lizard with the exception that the net effect is only a change in breathing pattern, not in total ventilation. The significance of these species differences in receptor roles and the physiological roles of these various CO_2-sensitive airway receptors (and the responses they produce) are still conjecture (see Milsom et al. 2004 for review). In all reptiles, however, the modulation of breathing pattern arising from the effects of inhalation of CO_2 on airway receptors will increase relative faveolar ventilation, which will in turn reduce dead-space ventilation and stratification in saccular lungs.

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