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CARDIORESPIRATORY RESPONSE TO PROGRESSIVE HYPOXIA AND HYPERCAPNIA IN THE TURTLE *TRACHEMYS SCRIPTA*

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Summary

The ventilatory responses of chelonian reptiles to hypoxic and hypercapnic stress have been fairly well described. As turtles are capable of large cardiac shunts, changes in pulmonary perfusion may be an equally viable and potent response to these stressors. To test this hypothesis, conscious unrestrained turtles were unidirectionally ventilated while blood flow in the left pulmonary artery ($\dot{Q}_{LPA}$) and left aortic arch ($\dot{Q}_{LAo}$) was monitored. Turtles were exposed to step changes (2.5 h step^{-1}) in O_2 tension (30, 15, 5, 2.5 or 0 % O_2; CO_2 inflow maintained constant) on day 1 followed by step changes in CO_2 tension (0, 2, 4, 8 % CO_2; O_2 inflow maintained constant) on day 2. Steady-state cardiorespiratory variables were recorded for the last 30 min of each step change in gas tension.

Progressive hypoxia resulted in progressive increases in ventilation, $\dot{Q}_{LPA}$ and $\dot{Q}_{LAo}$ and a small, but non-significant, increase in heart rate. Progressive hypercapnia resulted in a progressive increase in ventilation, while $\dot{Q}_{LPA}$ and $\dot{Q}_{LAo}$ did not change at any level of CO_2. These results suggest that information from the O_2-sensitive chemoreceptors appears to be stimulatory to both the cardiovascular and ventilatory control systems, while CO_2 chemoreception appears to affect primarily the ventilatory control system. These results also suggest that, in animals capable of intracardiac shunting, increasing pulmonary perfusion may be an integral component of the reflex response to hypoxia.

Key words: blood flow, ventilation, reptile, anoxia, hypoxia, hypercapnia, turtle, *Trachemys scripta.*

Introduction

Semi-aquatic turtles are diving animals that exhibit an episodic breathing pattern similar to that of many other tetrapod lower vertebrates (see Shelton and Boutilier, 1982; Milsom, 1991; Glass, 1992). This breathing pattern appears to be an inherent component of the central respiratory rhythm generators, but its output may be dynamically altered by various afferent stimuli (Douse and Mitchell, 1990). The cues that modify this respiratory pattern have intrigued comparative respiratory physiologists for many years and, as such, the ventilatory responses to altered blood gas tensions are well documented (Frankel et al., 1969; Jackson, 1973; Hitzig and Jackson, 1978; Silver and Jackson, 1985; West et al., 1989). Lowered blood oxygen tensions have been demonstrated to induce an increase in minute ventilation primarily through increasing ventilatory frequency (decreasing the non-ventilatory period), with tidal volumes being progressively increased as hypoxia becomes more severe (Jackson, 1973; Milsom and Chan, 1986). These effects are thought to be mediated through O_2-sensitive chemoreceptors located in the carotid arteries and in the pulmonary and aortic arches (Frankel et al., 1969; Benchetrit et al., 1977; Ishii et al., 1985; Kusakabe et al., 1988). Hypercapnic acidosis, in contrast, increases ventilation through changes in both frequency and tidal volume, apparently *via* stimulation of both central and peripherally located chemoreceptors (Hitzig and Jackson, 1978; Benchetrit and Dejours, 1980; Milsom and Jones, 1980; Hitzig et al., 1985).

During breathing episodes, pulmonary blood flow ($\dot{Q}_{pul}$) and heart rate (f_H) increase to match lung perfusion with ventilation (Burggren, 1975; Shelton and Burggren, 1976; Burggren et al., 1977a; Wang and Hicks, 1996a). Because turtles breath episodically and use their lungs as a potential O_2 store during non-ventilatory periods, an independent control of pulmonary perfusion would enable a finer degree of control over the rate at which O_2 is extracted from the lungs during these non-ventilatory periods. Indeed, evidence exists that supports this idea, as shown by increases in arterial P_{O_2} (and decreases in lung P_{O_2}) during dives in the absence of ventilation (Shelton and Burggren, 1976; Burggren et al., 1989). Burggren et al. (1977a), using a similar protocol to that used in the present study, found that exposure to hypoxia induced much larger changes in pulmonary perfusion than in ventilation and therefore reduced the ventilation/perfusion ($\dot{V}/\dot{Q}$) ratio, further supporting an independent control of pulmonary perfusion. In

addition, recent reports have indicated an apparent link between arterial oxygen content (Ca_{O_2}), when altered either chemically (Wang et al., 1996) or through reduced hematocrit (Wang et al., 1997), and net cardiac shunt, without concomitant changes in ventilation. However, with the apparent exception of hypoxemic hypoxia, both ventilation and pulmonary perfusion typically increase during hypoxic exposure, making the control of pulmonary perfusion difficult to resolve from the control of ventilation (Wang and Hicks, 1996a; Wang et al., 1997). Therefore, the degree to which the cardiovascular and respiratory systems are independent and capable of eliciting separate responses remains unclear. An independently controlled cardiovascular response may serve to maintain blood gas tensions during non-ventilatory periods or, alternatively, during periods of lowered O_2-carrying capacity (Wang and Hicks, 1996b; Wang et al., 1996, 1997). However, if the relationship between ventilation and pulmonary perfusion is strictly correlative, then hypercapnic stress should stimulate similar increases in both pulmonary perfusion and ventilation. The objectives of this study were therefore to examine the cardiorespiratory responses to altered steady-state arterial O_2 and CO_2 tensions.

Materials and methods

Animals

Turtles [*Trachemys scripta* (Gray), body mass 0.6–1.5 kg] were purchased from Charles Sullivan Co. (Nashville, Tennessee, USA) and housed in large aquaria with free access to water and terrestrial environments. The turtles were kept on a 14 h:10 h L:D photoperiod for at least 2 months prior to experimentation to ensure health and 'seasonal' (long-day) status. Food was given twice weekly in the form of Trout Chow (Purina) or crickets, but was withheld for 5–7 days prior to experiments. During this time and throughout the experimental protocol, the turtles were maintained at 25 °C.

Instrumentation of turtles

The turtles were anesthetized by intramuscular injections of Ketamine (140 mg kg^{-1}). Following induction of anesthesia, a 3 cm×5 cm rectangular hole was cut into the plastron above the heart using a Dremel tool. Doppler flow transducers (Triton Technology, San Diego, California, USA) were placed around the left pulmonary artery (2.0–2.8 mm i.d.), close to the bifurcation of the common pulmonary artery, and around the left aortic arch (2.4–3.0 mm i.d.). The leads from the flow transducers were passed through an incision cut into the skin at the right dorso-lateral margin of the neck and then through a hole drilled into the cranial border of the carapace. The right subclavian artery was non-occlusively cannulated (stretched PE-50 tubing) by cannulating the right ventral cervical artery and advancing the cannula into the subclavian artery. Once the cannula had been secured in place, it was flushed with heparinized Ringer's solution to ensure patency and to prevent blood clotting. The arterial cannula was led out through the hole in the plastron and taped into place. The rectangular piece of the plastron was secured back into place using 5 min epoxy resin. To cannulate the lungs, the turtle was returned to a prone position, and two holes were drilled on each midpoint of the lateral border of the third dorsal scute. The lung tissue on each side was exposed, the underlying lung chamber was cannulated (PE-205) and the cannula was secured into place using a purse-string suture. Prior to placement, the cannulae were fitted through a hole in a rubber serum cork that was then seated into the hole in the carapace and sealed with 5 min epoxy resin to ensure an air-tight seal. The two lung cannulae were then connected *via* a T-piece with a sealed end so that normal lung ventilation could occur as the animals recovered.

Experimental protocol

The turtles were allowed 48–72 h to recover after induction of anesthesia to ensure full recovery from surgery and patency of flow probes. The turtles were placed into 5 l terraria 3 h prior to the start of recording. During this time, they were unidirectionally ventilated with the appropriate initial humidified gas (350 ml min^{-1}) using a gas-mixing flow meter (Cameron Instruments). The initial air mixture was either hyperoxic (30 % O_2, 3.5 % CO_2, remainder N_2) or hypocapnic (15 % O_2, 0 % CO_2, remainder N_2), depending on the protocol. A differential pressure transducer (Validyne, Northridge, California, USA) was attached *via* the side-arm of a T-piece to the air inflow cannulae to measure ventilation frequency and changes in pulmonary pressure. Lung ventilation frequency was measured *via* changes in inflow pulmonary pressure. While tidal volumes could not be assessed directly, the peak pressure of tidal movements within a bout was used as an index of tidal effort. The position of the differential pressure transducer on the inflow cannula allowed pulmonary pressure to be monitored and ensured that the unidirectional ventilation did not induce large increases in pulmonary pressure.

To alter the alveolar P_{O_2} (P_{AO_2}) tension, the lungs were perfused for 2.5 h with 30 %, 15 % (normoxic P_{AO_2}), 5 %, 2.5 % or 0 % O_2 while holding CO_2 tension at 3.5 % (remainder N_2 in all cases). The O_2 concentrations were sequentially stepped down from 30 % to 0 % to induce progressive declines in P_{AO_2} and arterial P_{O_2} (Pa_{O_2}) during the experiments. To induce changes in CO_2 tension, the lungs were perfused for 2.5 h each at 0, 2, 4 and 8 % CO_2 while holding O_2 tension at 15 % (remainder N_2 in all cases). Measurements of responses to CO_2 were made by sequentially increasing CO_2 doses because of the long-lasting effects of acid–base change on blood ion concentrations (pH returns to the control value within 1 h of a return to normocapnia, but [HCO_3^-] remains elevated for over 48 h; Silver and Jackson, 1985). The responses of turtles to varying P_{O_2} levels were determined first, and the responses to changing P_{CO_2} levels were measured the following day. This ensured that CO_2-induced acidification did not confound the P_{O_2} response curves.

Arterial blood was drawn (0.5 ml) *via* the ventral cervical cannulae during the last 5 min of each steady-state period in each condition to measure Pa_{O_2}, arterial pH (pHa) and total CO_2 (during the CO_2 protocol). Total CO_2 (T_{CO_2}) was

measured on whole-blood samples using infrared absorption (Capnicon V, Cameron Instruments) on 20 µl samples taken from the drawn blood. The Henderson–Hasselbalch equation was used to calculate estimated values of $P_{a_{CO_2}}$ and $[HCO_3^-]$ using pK^1 and α_{CO_2} values derived in Nicol et al. (1983) and corrected to 25 °C. Arterial pH and P_{O_2} were measured using pH and O_2 microelectrodes (Radiometer, Copenhagen, Denmark). After measurements had been made, the remainder of the blood was reinjected into the turtle to prevent anemia resulting from repeated blood sampling. The cannula was then flushed with heparinized Ringer's solution to maintain patency. The P_{O_2} electrode was calibrated using a zero-oxygen solution and water equilibrated with room air using a magnetic stir bar. The pH electrode was calibrated using standard buffers (pH 7.0 and 10.0). The electrodes were maintained at the same temperature (25 °C) as the animal during the experiment.

Data analysis and statistical analysis

Blood-flow transducers were connected to a directional-pulsed Doppler flow meter (Bioengineering, University of Iowa, model 545C-4). Signals were converted to a digital signal using a DATAQ (model DI 220) A/D conversion board and recorded at 50 Hz using WINDAQ data-acquisition software (DATAQ Instruments, version 1.13 Akron, Ohio, USA). Heart rate was determined from peak-to-peak interval analysis of the left aortic blood flow. The flow probes were calibrated at five different flow rates by excising the left aorta from the animal following experiments and perfusing this vessel with blood that had been previously drawn from the animal. The pressure transducer was calibrated using a static water column prior to each experiment.

Blood flow was initially analyzed in 1 min bins for the last 30 min of each steady-state gas tension. Net cardiac shunt ($\dot{Q}_{shunt}$, ml min^{-1} kg^{-1}) was estimated as described by Wang and Hicks (1996a). In short, the total pulmonary blood flow ($\dot{Q}_{pul}$) was estimated by doubling the blood flow in the left pulmonary artery ($\dot{Q}_{LPA}$). Total systemic blood flow ($\dot{Q}_{sys}$) was estimated by multiplying the blood flow in the left aorta ($\dot{Q}_{LAo}$) by 2.8 (the combined blood flow in the right aortic arch, subclavian arteries and carotid arteries is 1.8 times the flow in the left aortic arch; Comeau and Hicks, 1994). The difference between $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ yields an estimate of the net cardiac shunt: a negative value would indicate a net right-to-left shunt (R-L shunt), whereas a positive value would indicate a net left-to-right shunt (L-R shunt). To be statistically conservative, blood flows ($\dot{Q}_{LPA}$ and $\dot{Q}_{LAo}$), f_H, the frequency of tidal movements (f_{vent}; breaths min^{-1}), the frequency of breathing bouts (f_{bout}; bouts min^{-1}), the number of tidal movements per bout (breaths bout^{-1}) and peak tidal pressure (T_P) were averaged over the last 30 min of each steady-state gas tension. While analyzing the data in this manner masked the normal cardiorespiratory synchrony that took place during active lung ventilation, it gave a better estimate of the degree to which the cardiorespiratory variables had changed during the steady-state period. A repeated-measures analysis of variance (ANOVA) was used to analyze the effects of each steady-state gas tension on blood flow, f_H and breathing variables. *Post-hoc* analysis using the Student–Newman–Keuls (SNK) method was used where significance was indicated by the ANOVA. A least-squares regression analysis was used to determine the relationship between $\dot{Q}_{LPA}$ and f_{bout}. All statistical tests were performed using Statistica (StatSoft, version 4.5). The level of significance was set at $P<0.05$, and the data are presented as means ±1 S.E.M.

Results

Cardiorespiratory responses to progressive hypoxia

Ten turtles were exposed to progressive step decreases in $P_{A_{O_2}}$ while inflow P_{CO_2} was maintained constant. Decreasing $P_{A_{O_2}}$ significantly lowered $P_{a_{O_2}}$ ($P<0.0001$), while pHa did not differ among the different conditions (Table 1).

Cardiovascular variables

Heart rate (f_H) appeared to increase slightly with decreasing $P_{a_{O_2}}$, but this increase was not significant ($P=0.07$, Fig. 1A). Blood flow in the left aortic arch increased with decreasing $P_{a_{O_2}}$ ($P<0.01$, Fig. 1B). The progressive decline in $P_{a_{O_2}}$ also induced significant increases in $\dot{Q}_{LPA}$ ($P<0.0001$, Fig. 1C). As the turtles became progressively hypoxic, the calculated net L-R shunt flow increased significantly ($P<0.05$, Fig. 1D).

Respiratory variables

Reductions in $P_{a_{O_2}}$ did not alter the tidal frequency (f_{vent}) (Fig. 2A) but did increase the frequency of breathing bouts (f_{bout}) at the lowest P_{O_2} level ($P<0.0001$, Fig. 2B). Since f_{vent} did not appear to change while f_{bout} increased, the number of breaths per bout was significantly reduced with reduced $P_{a_{O_2}}$ ($P<0.001$, Fig. 2C). Peak tidal pressure (T_P) increased significantly with decreasing $P_{a_{O_2}}$ ($P<0.001$, Fig. 2D).

Cardiorespiratory responses to progressive hypercapnia

Ten turtles were exposed to progressive step increases in

Table 1. *Arterial P_{O_2} and pHa during the last 30 min of steady-state periods of altered lung P_{O_2} tension in the turtle* Trachemys scripta

Aerated percentage O_2	$P_{a_{O_2}}$ (kPa)	pHa
30	16.1±1.1[a]	7.673±0.026
15	12.4±0.8[b]	7.686±0.015
5	7.5±0.5[c]	7.664±0.029
2.5	4.5±0.6[d]	7.677±0.022
0	2.5±0.5[e]	7.664±0.034
Recovered (15)	11.2±0.6[b]	7.661±0.033

Different letters indicate significant differences (SNK test; $P<0.05$).

Values are presented as mean ± S.E.M. ($N=10$).

$P_{a_{O_2}}$, arterial P_{O_2}; pHa, arterial pH.

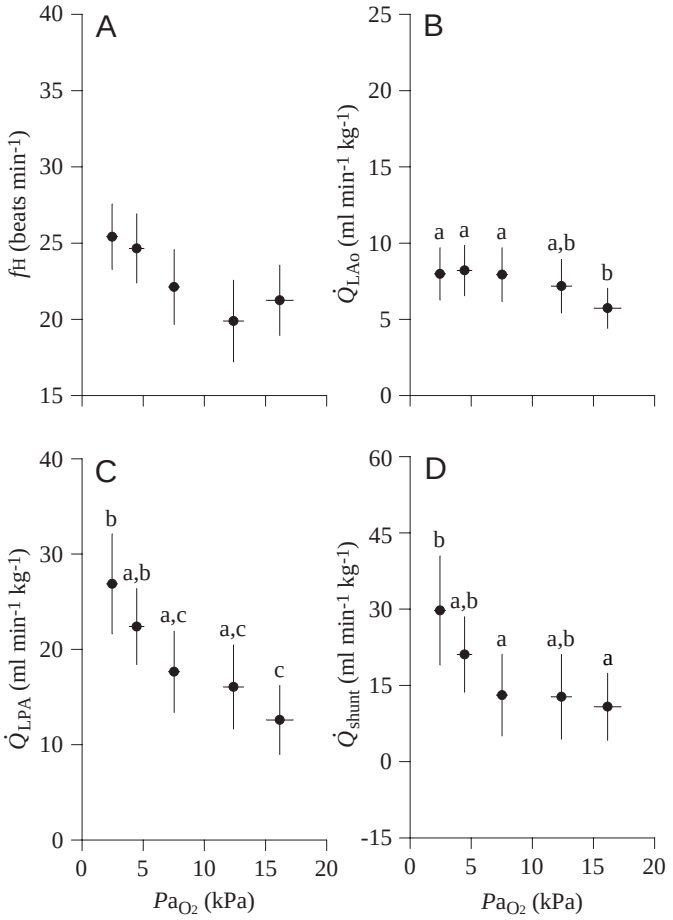

Fig. 1. Mean values of cardiovascular variables *versus* arterial P_{O_2} (P_{aO_2}) for the last 30 min of each steady-state P_{O_2} period in the turtle *Trachemys scripta* (N=10). (A) Heart rate (f_H). (B) Left aortic arch blood flow ($\dot{Q}_{LAo}$). (C) Left pulmonary artery blood flow ($\dot{Q}_{LPA}$). (D) Calculated net shunt blood flow ($\dot{Q}_{shunt}$). Different letters indicate significant differences (SNK test; P<0.05). Values are presented as means ± S.E.M.

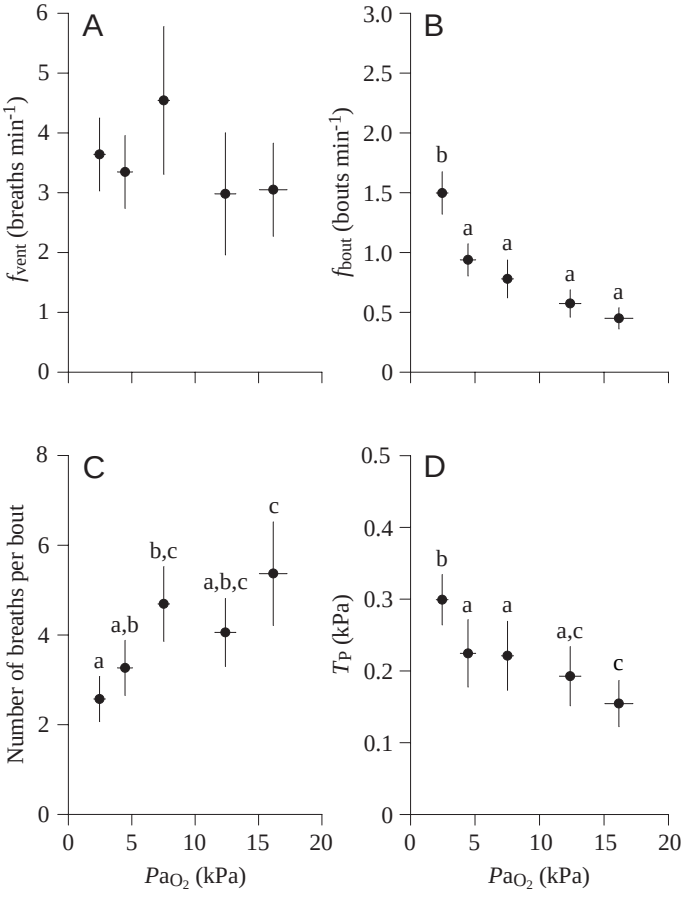

Fig. 2. Mean values of respiratory variables *versus* arterial P_{O_2} (P_{aO_2}) for the last 30 min of each steady-state P_{O_2} period in the turtle *Trachemys scripta* (N=10). (A) Breathing frequency (f_{vent}). (B) Bout frequency (f_{bout}). (C) Number of breaths per bout. (D) Peak tidal pressure (T_P). Different letters indicate significant differences (SNK test; P<0.05). Values are presented as means ± S.E.M.

P_{ACO_2} while maintaining inflow P_{O_2} constant. While there was no directional change in P_{aO_2}, there was a significant difference in P_{aO_2} between the 2% and 8% CO_2 treatments (P<0.05, Table 2). Increasing the P_{CO_2} of the unidirectional gas stream induced a significant decrease in pHa (P<0.001) and a significant elevation in T_{CO_2} (P<0.001) and in estimated P_{aCO_2} (P<0.001) and [HCO_3^-] (P<0.001, Table 2).

Cardiovascular variables

Altering the P_{ACO_2} induced significant changes in f_H (P<0.01) but not in a directional manner (Fig. 3A). $\dot{Q}_{LAo}$ did not appear to change significantly as the turtles became increasingly hypercapnic, although the ANOVA results were marginal (P=0.053, Fig. 3B). $\dot{Q}_{LPA}$ (Fig. 3C) and calculated net $\dot{Q}_{shunt}$ (Fig. 3D) also did not change with increasing P_{aCO_2}.

Respiratory variables

Increasing P_{ACO_2} had significant effects on all the respiratory variables (Fig. 4A–D). Tidal frequency (f_{vent}) increased in an almost linear fashion with progressive hypercapnia (P<0.001, Fig. 4A). Bout frequency (f_{bout}) also increased with each successive increase in CO_2 level (P<0.001, Fig. 4B). The number of breaths per bout increased initially (P<0.01) but then remained unchanged with further increases in P_{ACO_2} (Fig. 4C). Peak tidal pressure also increased in an almost linear fashion with progressive hypercapnia (P<0.001, Fig. 4D).

Correlation between respiratory events and $\dot{Q}_{LPA}$

Bout frequency and $\dot{Q}_{LPA}$ increased similarly during hypoxia and therefore their relationship was significantly correlated (r^2=0.2285, P<0.001, Fig. 5A). This correlative relationship was not maintained during hypercapnic stress (r^2=0.0439, P=0.1941, Fig. 5B).

Comparison between experimental days

The cardiorespiratory variables from the 15% O_2 level on day 1 were compared with the 2% and 4% CO_2 values (normoxic/near-normocapnic) from day 2. Heart rate was

Table 2. *Arterial* P_{O_2}, *pH,* T_{CO_2} *and* P_{CO_2} *during the last 30 min of each steady-state period of altered lung* P_{CO_2} *tension in the turtle* Trachemys scripta

Percentage CO_2	Pa_{O_2} (kPa)	pHa	T_{CO_2} (mmol l^{-1})	Pa_{CO_2} (kPa)	$[HCO_3^-]$ (mmol l^{-1})
0	10.4±0.5^{a,b}	8.423±0.061^{a}	25.12±1.12^{a}	0.25±0.02^{a}	25.04±1.12^{a}
2	9.2±0.6^{a}	7.768±0.051^{b}	29.95±1.24^{b}	1.81±0.09^{b}	29.40±1.23^{b}
4	9.7±0.5^{a,b}	7.592±0.056^{c}	31.79±0.85^{c}	2.98±0.17^{c}	30.89±0.82^{b}
8	10.9±0.7^{b}	7.405±0.049^{d}	35.25±0.41^{d}	5.43±0.53^{d}	33.62±0.55^{c}

Different letters indicate significant differences (SNK test; P<0.05). Values are presented as mean ± S.E.M.; N=10 except for T_{CO_2}, Pa_{CO_2} and $[HCO_3^-]$ where N=5).

Calculations for Pa_{CO_2} and $[HCO_3^-]$ are derived from Nicol et al. (1983).

Pa_{O_2}, arterial P_{O_2}; pHa, arterial pH; T_{CO_2}, total CO_2; Pa_{CO_2}, arterial P_{CO_2}.

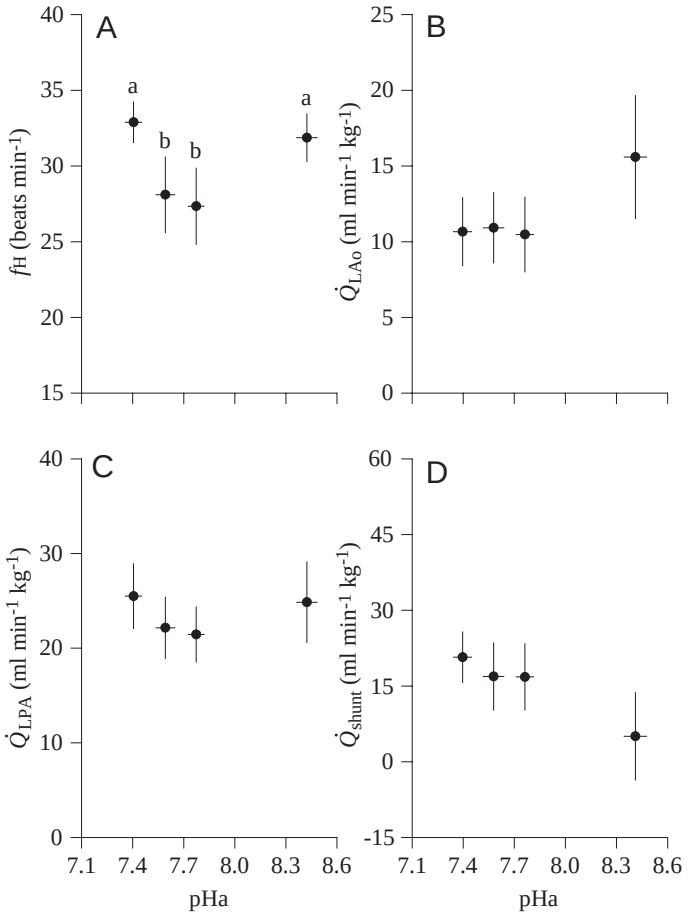

Fig. 3. Mean values of cardiovascular variables *versus* arterial pH (pHa) for the last 30 min of each steady-state P_{CO_2} period in the turtle *Trachemys scripta* (N=10). (A) Heart rate (f_H). (B) Left aortic arch blood flow ($\dot{Q}_{LAo}$). (C) Left pulmonary artery blood flow ($\dot{Q}_{LPA}$). (D) Calculated net shunt blood flow ($\dot{Q}_{shunt}$). Different letters indicate significant differences (SNK test; P<0.05). Values are presented as means ± S.E.M.

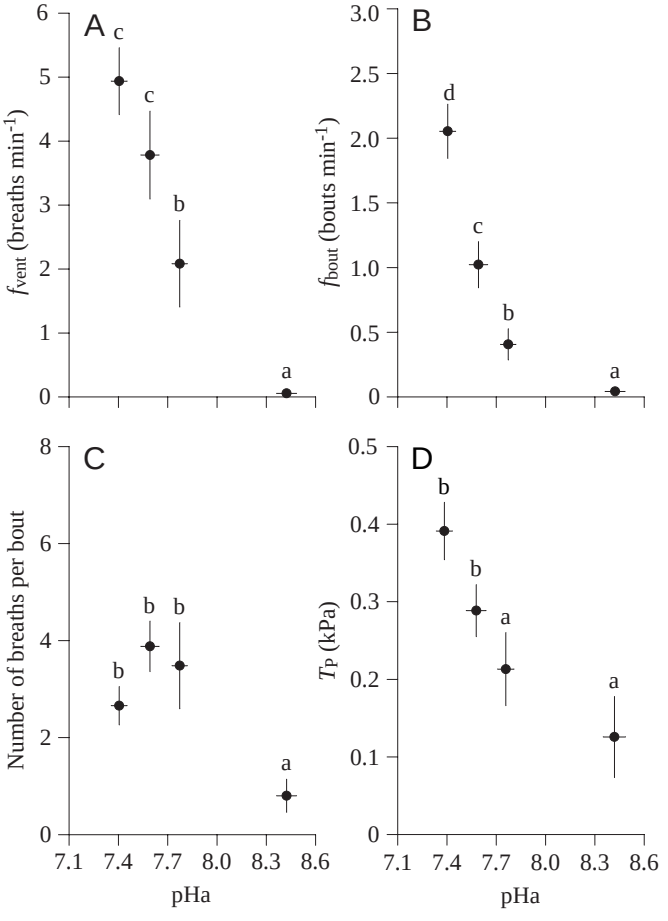

Fig. 4. Mean values of respiratory variables *versus* arterial pH (pHa) for the last 30 min of each steady-state P_{CO_2} period in the turtle *Trachemys scripta* (N=10). (A) Breathing frequency (f_{vent}). (B) Bout frequency (f_{bout}). (C) Number of breaths per bout. (D) Peak tidal pressure (T_P). Different letters indicate significant differences (SNK test; P<0.05). Values are presented as means ± S.E.M.

slightly elevated on the second day of experiments, but this did not result in significant elevations in $\dot{Q}_{LPA}$ or $\dot{Q}_{LAo}$ or in alterations in net $\dot{Q}_{shunt}$. The respiratory variables were also not affected by the day of experiments. The only respiratory variable that differed between days was f_{bout}, which was slightly lower on day 1 than at the 4% CO_2 level on day 2.

Discussion

Progressive hypoxia and progressive hypercapnia were used

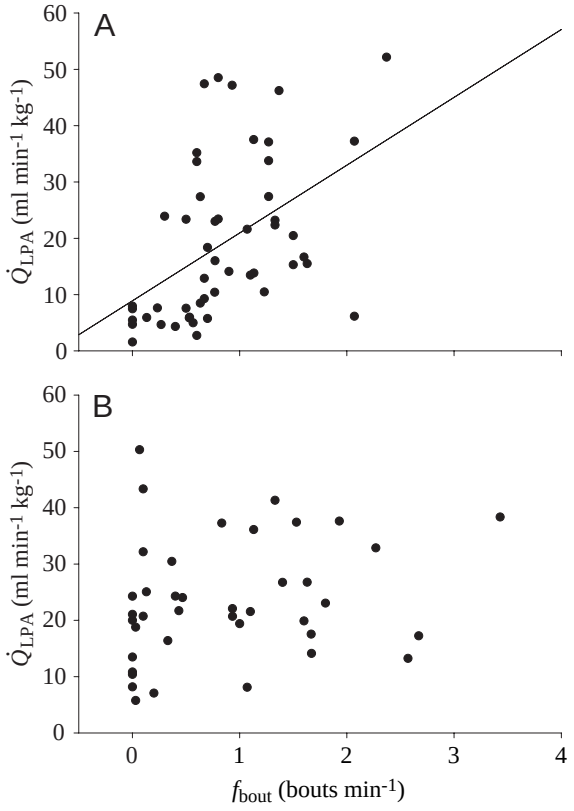

Fig. 5. Scatterplots demonstrating the relationship between left pulmonary artery blood flow ($\dot{Q}_{LPA}$) and bout frequency (f_{bout}) during (A) progressive hypoxia and (B) progressive hypercapnia. During the progressive hypoxic protocol, there is a significant correlation between $\dot{Q}_{LPA}$ and f_{bout}. During the progressive hypercapnic protocol there is no significant correlation between $\dot{Q}_{LPA}$ and f_{bout}.

separately in the present study to test the hypothesis that the control of pulmonary perfusion may be independent of the control of ventilation in the response to altered arterial blood gas tension. If the control of pulmonary perfusion is directed by output from respiratory control centers, then changes in pulmonary perfusion should match changes in respiratory output. Therefore, during both hypoxia and hypercapnia, ventilation and pulmonary perfusion should increase in a proportional manner. The hypoxic ventilatory response of turtles tends to be fairly moderate compared with their hypercapnic ventilatory response (Jackson, 1973; Burggren et al., 1977a; Hitzig and Jackson, 1978; Glass and Wood, 1983). Therefore, pulmonary perfusion should be higher during hypercapnia, reflecting the higher respiratory drive of hypercapnic stimulation. The major observation of the present study is that this is not the case and that central blood flow regulation may be uncoupled from ventilatory control during times of hypercapnic stress.

The gas concentrations used to achieve normoxic (15 % O_2) and normocapnic (3.5 % CO_2) conditions were chosen because they represent the P_{O_2} and P_{CO_2} levels that have been reported in the lungs of turtles during moderate periods of apnea (Burggren and Shelton, 1979; Shelton and Boutilier, 1982). This percentage CO_2 resulted in Pa_{CO_2} and pHa levels that were comparable with the normal range reported in other studies (Frankel et al., 1969; Jackson et al., 1974). The values of 5 % and 2.5 % O_2 were chosen in an attempt to encompass the erythrocyte P_{50} value of 3.2–3.5 kPa reported for this species (Burggren et al., 1977b). The inflow rate of the unidirectional gas stream was maintained at 350 ml min^{-1} to try to ensure a constant alveolar gas tension. Because turtles have large multicameral lungs (Perry, 1978; Jones and Milsom, 1982), this flow rate could be used without generating large intrapulmonary pressures (<0.98 kPa for all turtles). However, with this type of lung, it is possible that unidirectional ventilation is not as efficient as normal ventilatory movements at equilibrating gases within the lung. Also, because the unidirectional flows were not corrected for body mass, lung washout during unidirectional ventilation in larger animals may not have been sufficient to overcome the increased ventilatory movements observed during the severely hypoxic periods. Therefore, despite the relatively high unidirectional flow rate used, ventilatory movements by some turtles were still sufficient to elevate the Pa_{O_2} above the expected value. During the anoxic unidirectionally ventilated period, despite an inflow P_{O_2} of less than 0.4 kPa, this protocol reduced Pa_{O_2} below 1.33 kPa in only four of the 10 turtles.

Ventilatory frequency and amplitude increased with progressive hypoxia, as reported previously for other reptiles (Jackson, 1973; Benchetrit et al., 1977; Abe and Johansen, 1987). Breathing frequency increased primarily through a decrease in the non-ventilatory period, as evident from an increase in f_{bout} rather than an increased number of tidal movements (f_{vent}). In the present study, the increases in T_P preceded alterations in ventilation frequency, suggesting that tidal volume changes may be part of the initial ventilatory response to hypoxic exposure. Unlike the response reported in earlier studies (i.e. Burggren et al., 1977a; Wang et al., 1997), f_H did not appear to increase as much with hypoxic exposure as did $\dot{Q}_{LPA}$. The apparent lack of response in the present study is probably due to inter-animal variability because the ANOVA results were marginal (P=0.07). The results of the present study also differed from those of previous reports (i.e. Wang and Hicks, 1996a; Hicks and Wang, 1998) in that a net L-R shunt prevailed during normoxic/normocapnic conditions. This difference may have arisen, in part, because f_H and $\dot{Q}_{sys}$ were lower in the present study during these conditions than values reported previously. However, even when f_H was slightly elevated, as during the near-normocapnic conditions of the 2 and 4 % CO_2 protocols, the net L-R shunt flow prevailed in these turtles. Despite these differences, the relationship between $\dot{Q}_{pul}$ and Pa_{O_2} is similar to that reported by Burggren et al. (1977a) during hypoxia and to that reported by Hicks and Wang (1998) during the transition to anoxia. Furthermore, progressive hypoxia led to an increase in the net L-R shunt flow and, therefore, to a larger proportional pulmonary stroke volume, a response similar to that reported in previous studies (Burggren et al., 1977a; West et al., 1992; Hicks and Wang,

1998). During normoxic ventilation, changes in $\dot{Q}_{pul}$ appear to be better correlated to f_{bout} than to the number of breaths taken within a bout (Wang and Hicks, 1996a). In the present study, as in the study of Wang et al. (1997), because both $\dot{Q}_{pul}$ and f_{bout} increased similarly with decreasing levels of P_{aO_2}, the correlative relationship between ventilation and pulmonary perfusion was qualitatively maintained.

During anoxic unidirectional ventilation, $\dot{Q}_{LPA}$ and f_H were maximal. This differs from a recent report that pulmonary blood flow declines and f_H decreases during chronic (>2 h) anoxic exposure (Hicks and Wang, 1998). There are two possible reasons for these differences. Hicks and Wang (1998) measured these variables in submerged turtles in which breathing was measured using an inverted funnel connected to a pneumotachograph. Many amphibians (Jones and Milsom, 1982), chelonian reptiles (Wang et al., 1997) and diving mammals and birds (see Jones and Milsom, 1982; Signore and Jones, 1996) show parasympathetic bradycardia during submergence. To test this possible influence on $\dot{Q}_{LPA}$, we exposed four turtles to the step changes in P_{AO_2} while they were submerged in approximately 10 cm of water (data not shown). The blood flow responses to hypoxia in immersed and emersed turtles were not significantly different, suggesting that P_{aO_2} appears to be a more critical determinant of blood flow than does the immersion/emersion state of the animal. Recently, Crossley et al. (1998) reported that, in anesthetized turtles, pulmonary vascular resistance increases dramatically at a P_{aO_2} of approximately 1.5 kPa, a level that is lower than the mean P_{aO_2} achieved in the present study. In the study of Hicks and Wang (1998), P_{aO_2} in the anoxic turtles was reduced to below 0.3 kPa. In the four turtles from the present study in which P_{aO_2} was reduced below 1.3 kPa, $\dot{Q}_{LPA}$ was reduced below the level measured at 2.5 % P_{O_2}, but not below that recorded at the 15 % O_2 level. At this low P_{O_2} level, f_H was also irregular, which may result from anoxic impairment of cardiac function or from a vagally mediated decrease in cardiac output (Jackson, 1987). Thus, the more severe anoxic exposure in the study of Hicks and Wang (1998) probably accounts for the observed differences. Taken together, this series of findings suggests that there may be a set point at which blood flow to the lungs is no longer a viable option, so that shunting blood to the systemic circulation is enhanced. This level (<1.5 kPa P_{aO_2}) appears to be below the reported P_{50} for this species (Burggren et al., 1977b; West et al., 1989) and is nearer to the critical P_{O_2} for the maintenance of standard metabolic rate (see Ultsch and Anderson, 1988). This set point may also be an important determinant in the uncoupling of the cardiorespiratory systems, as demonstrated in Fig. 2B from the study of Hicks and Wang (1998) in which the ventilatory movements of this turtle during anoxia were not accompanied by changes in $\dot{Q}_{pul}$. We did not observe any turtles exhibiting this behavior in the present study, but this may once again reflect the P_{aO_2} differences during anoxic exposure between the present study and that of Hicks and Wang (1998).

Numerous studies have shown that hypercapnic exposure induces increases in chelonian ventilation (Frankel et al., 1969; Jackson et al., 1974; Burggren et al., 1977a; Hitzig and Jackson, 1978; Benchetrit and Dejours, 1980; Silver and Jackson, 1985). The increase in ventilation with hypercapnia is due to stimulation of both peripheral and central chemoreceptors in reptiles (Hitzig and Jackson, 1978; Hitzig et al., 1985). With the levels of CO_2 delivered to the turtles and the changes seen in the blood gas variables, there is little doubt that both sets of receptors participated in the hypercapnic responses. Assuming that pulmonary perfusion is correlated with, and possibly modified by, output from the respiratory centers in turtles, the increase in respiratory drive and respiratory output should also stimulate large increases in $\dot{Q}_{LPA}$. This was not the case in the present study. Indeed, $\dot{Q}_{LPA}$ did not differ as the animals were stepped up from severe hypocapnia (0 %) to severe hypercapnia (8 %) despite an almost linear increase in the ventilatory variables. Thus, P_{CO_2} appears to be a potent respiratory stimulant, but is unlikely to be involved in cardiovascular control. Therefore, with this stimulus modality, the correlative nature between f_{bout} and $\dot{Q}_{pul}$ was not maintained.

These findings differ from that reported by Burggren et al. (1977a). These authors, using a similar protocol to the present study, reported that in an aquatic turtle, *Pelomeduas subrufa*, and in a tortoise, *Testudo pardalis*, both hypoxia and hypercapnia increased pulmonary blood flow. The difference in the hypercapnic responses may be due to a slight difference in experimental protocol between the two studies. Burggren et al. (1977a) exposed the turtles to each new gas concentration until an apparent steady state was reached in the ventilation and blood flow traces; typically, this occurred within minutes. With this protocol, it is conceivable that blood gas tensions and acid–base balance were not yet at steady state. Studies by Silver and Jackson (1985) have shown that acid–base adjustments take over an hour to equilibrate when the turtle is exposed to 5.7 % CO_2. Thus, the differences in the pulmonary blood flow response between these two studies may have arisen, in part, from acid–base equilibration differences. However, despite these differences, the results of the study of Burggren et al. (1977a) are similar to those of the present study in that both demonstrate that hypercapnia was a much more potent ventilatory than cardiovascular stimulus. Furthermore, pulmonary blood flow in the tortoise (Burggren et al., 1977a) and in *T. scripta* (present study) increased more vigorously during hypoxia than during hypercapnic exposure. These similarities further support the hypothesis that pulmonary perfusion and ventilation may be differentially modified in the response to altered arterial blood gas tension.

The question that still needs to be addressed is why blood flow is responsive to O_2 but not to CO_2. The $\dot{V}/\dot{Q}$ ratio in turtles tends to be skewed towards ventilation when the turtles are breathing normoxic and normocapnic air (Burggren et al., 1977a; Hopkins et al., 1996). This ratio is brought closer to unity in response to hypoxia and increases in body temperature (Burggren et al., 1977a; Kinney et al., 1977). The labile nature of the $\dot{V}/\dot{Q}$ ratio is suggestive of two independent yet tightly coupled systems. The present study further demonstrates this

tight coupling in that, with respect to O_2 homeostasis, both ventilation and $\dot{Q}_{pul}$ are similarly modified. The main finding of the present study is that the correlative nature of the cardiorespiratory output is not obligatory, thereby demonstrating that the two systems are independent and may be uncoupled. Thus, the findings of the present study support the concept that oxygen homeostasis is more dependent on changes in pulmonary perfusion than is acid–base balance and provide a basis for further neurophysiological investigation of the sensory control of blood flow in turtles.

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