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HYPOXIA ELICITS AN INCREASE IN PULMONARY VASCULATURE RESISTANCE IN ANAESTHETISED TURTLES (*TRACHEMYS SCRIPTA*)

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Summary

In mammals and birds, low oxygen levels in the lungs cause a constriction of the pulmonary vasculature. This response is locally mediated and is considered to be important for local matching of perfusion and ventilation. It is not known whether reptiles respond in a similar fashion. The present study describes the effects of altering lung oxygen levels (at a constant F_{CO_2} of 0.03) on systemic and pulmonary blood flows and pressures in anaesthetised (Nembumal, 50 mg kg^{-1}) and artificially ventilated turtles *Trachemys scripta*. During severe hypoxia (1.5–3 kPa P_{O_2}), pulmonary blood flow decreased in all animals; systemic blood flow increased, resulting in an increased net right-to-left shunt blood flow. The redistribution of blood flows was associated with reciprocal changes in the vascular resistances within the pulmonary and the systemic circulations (R_{pul} and R_{sys}, respectively). At 1.5 kPa O_2, R_{pul} increased from 0.09±0.01 to 0.15±0.03 kPa ml^{-1} min kg during normoxia (means ±1 S.E.M., N=5). Concurrently, R_{sys} tended to decrease from a normoxic value of 0.12±0.01 to 0.09±0.02 kPa ml^{-1} min kg (P=0.08). The effects of hypoxia on the haemodynamic variables persisted following atropinisation (1 mg kg^{-1}) and cervical vagotomy, suggesting that the increased R_{pul} during hypoxia is locally mediated. This study therefore demonstrates that turtles exhibit hypoxic pulmonary vasoconstriction, although the threshold is low compared with that of mammals.

Key words: turtle, *Trachemys scripta*, reptile, blood flow, blood pressure, systemic circulation, pulmonary circulation, lung function, cardiovascular system, cardiac shunt, hypoxia, hypoxic pulmonary vasoconstriction, catecholamine.

Introduction

In mammals and birds, low oxygen levels in the lungs cause a constriction of the pulmonary vasculature that increases the resistance to pulmonary blood flow and elevates the pulmonary arterial blood pressure (Euler and Liljestrand, 1946; Faraci *et al.* 1984). This response is locally mediated and can be demonstrated in denervated lungs that are devoid of external neurohumoral influences (e.g. Fishman, 1976). Hypoxic pulmonary vasoconstriction (HPV) is believed to be important for the local matching of perfusion and ventilation by diverting pulmonary blood flow from poorly ventilated to well-ventilated regions of the lung (Euler and Liljestrand, 1946; Marshall *et al.* 1994*c*; Brimioulle *et al.* 1996). The effects of hypoxia on pulmonary vascular resistance have not been studied in reptiles, and it is not known whether the structurally simple lungs of these animals display HPV. In non-crocodilian reptiles, the ventricle is not fully divided and, with the notable exception of varanid lizards (e.g. Burggren and Johansen, 1982; Heisler *et al.* 1983), the distribution of blood flows between the pulmonary and systemic circulations is largely determined by differences in vascular resistances (cf. Hicks and Malvin, 1995; Hicks *et al.* 1996). Increased pulmonary vascular resistance resulting from HPV in reptiles therefore reduces pulmonary blood flow and causes a cardiac right-to-left shunt (a systemic bypass of the pulmonary circulation). Because the right-to-left shunt reduces systemic oxygen delivery (e.g. Wood, 1982; Wang and Hicks, 1996*b*), it is possible that HVP limits the extent to which lung oxygen stores can be exploited during breath-holding or during environmental hypoxia. Thus, while HPV may enhance gas exchange by reducing the ventilation–perfusion inhomogeneity in the lungs, HPV may also compromise gas exchange by inducing a cardiac right-to-left shunt.

Given these considerations, the present study investigates the effects of hypoxia on pulmonary vascular resistance in turtles. The experiments were performed on anaesthetised and artificially ventilated animals for several reasons. First, ventilation is increased in conscious turtles during hypoxia, and it can therefore be difficult to distinguish the direct effects of hypoxia on the central vascular resistances from the secondary effects associated with ventilatory responses (see

Wang *et al.* 1997). Second, anaesthesia retards cardiovascular reflexes and, therefore, reduces the possibility that the observed responses are due to stimulation of, for example, vascular chemoreceptors. Third, artificial ventilation of anaesthetised animals has the advantages that lung P_{O_2} can be effectively controlled and easily altered and that acid–base status can be maintained by keeping lung CO_2 levels constant.

Apart from the possible influence of HPV, pulmonary vascular resistance in turtles is controlled by vagal innervation of the pulmonary artery (Burggren, 1977; Milsom *et al*. 1977). To avoid vagal responses during hypoxia, the present experiments were therefore also performed following pharmacological blockade of the cholinergic receptors by atropine injection. In addition, circulating catecholamines reduce pulmonary vascular resistance (Luckhardt and Carlson, 1921; Burggren, 1977; Comeau and Hicks, 1994; cf. Milsom *et al.* 1977), and we therefore included measurements of plasma catecholamine concentrations in the present study.

Materials and methods

Experimental animals

Freshwater turtles, *Trachemys scripta* Gray (body mass ranging between 0.6 and 1.4 kg, mean 0.9 kg, N=5) were obtained from Lemberger Inc. (Oshkosh, WI, USA) and air-freighted to Aarhus University. In the animal care facility, they were housed in a large fibreglass tank containing fresh water heated at 28 °C, where they had free access to dry platforms, allowing for behavioural thermoregulation. Animals were fed on fish several times a week, but food was withheld for at least 3 days prior to experimentation.

Anaesthesia and surgery

On the day of experimentation, turtles were anaesthetised by an intramuscular injection of sodium pentobarbital (Nembumal; 50 mg kg^{-1}). Normally the pedal withdrawal response disappeared within 30–60 min, but in some cases an additional injection (25 mg kg^{-1}) was needed to abolish the withdrawal response. The trachea was then exposed by a ventral incision in the neck, and the turtle was tracheotomised for artificial ventilation. During the surgical procedure, which normally lasted for 60–90 min, the turtle was ventilated every 5 min with room air using a syringe.

To access the central vascular blood vessels, a 5 cm×5 cm portion of the plastron was removed using a bone saw. The pectoral muscles were gently loosened from the excised piece and bleeding from small superficial vessels was stopped by cauterisation (Roboz RS-232). A polyethylene catheter containing heparinized saline was occlusively inserted into the left carotid artery and pushed forward into the right aortic arch. The common pulmonary artery was non-occlusively cannulated using the Seldinger technique as described by White *et al.* (1989). Briefly, an intravenous catheter (Surflo) was inserted upstream in the artery (approximately 0.5 cm from the heart) after tapering it over a 23 gauge needle. Following insertion, the needle was withdrawn and the catheter was connected to a piece of PE-60 tubing. Finally, the catheter was secured to the artery using a small amount of tissue adhesive (cyanoacrylate). The catheters were connected to Statham pressure transducers, which were calibrated daily against a static column of water. For measurements of blood flows, 1–1.5 cm sections of the left aortic arch (LAo) and the left pulmonary artery (LPA) were freed from connective tissue for placements of 2S transit-time ultrasonic blood flow probes (Transonic System, Inc., NY, USA). To enhance the signal, acoustical gel was infused around the blood flow probes. After completion of the experimental protocol, all turtles were killed by vascular injections of KCl.

Determination of blood gas levels and plasma catecholamine concentrations

Blood samples were collected from the right aortic arch catheter. Samples were obtained during normoxia and during the most hypoxic condition (F_{O_2}=0.015; 1.5 kPa). Immediately following sampling, blood was analysed for P_{O_2}, P_{CO_2} and pH using a Radiometer BMS III system connected to a PHM 73 (Radiometer, Copenhagen, Denmark); all electrodes were maintained at the same temperature (22–23 °C) as the experimental animal. Haematocrit was determined following a 3 min centrifugation at 12,000 r.p.m. in capillary tubes.

A 0.6 ml plasma sample was obtained for subsequent determination of catecholamine levels. Before being stored at –70 °C, 10 µl of glutathione/EGTA (0.2 mol l^{-1}/0.2 mol l^{-1}) was added to prevent catecholamine oxidation. Plasma catecholamine concentrations were determined by high-performance liquid chromatography (HPLC) analysis after extraction with alumina, as described previously (Fritsche and Nilsson, 1990).

Data recording

The two Statham pressure transducers (P23G) were connected to a Beckman R511A recorder for appropriate filtering and magnification of the signal. The flow probes were connected to a Transonic dual-channel blood flow meter (T206) for measurements of instantaneous blood flow rates. Signals from the pressure transducer and the blood flow meter were recorded using an AcqKnowledge MP 100 (version 3.2.3) data-acquisition system at 50 Hz.

Calculation of blood flows, net shunt, stroke volume and resistance to blood flow in the systemic and pulmonary circulations

This study did not measure blood flows in all the systemic arteries, but several studies on anaesthetised and non-anaesthetised freshwater turtles have shown that systemic blood flow ($\dot{Q}_{sys}$) can be adequately estimated as 2.85×$\dot{Q}_{LAo}$ (Shelton and Burggren, 1976; Comeau and Hicks, 1994; Wang and Hicks, 1996*a*). Likewise, pulmonary blood flow ($\dot{Q}_{pul}$) was calculated as 2×$\dot{Q}_{LPA}$ under the assumption that blood flow in the right pulmonary artery equals that in the left. The net shunt flow ($\dot{Q}_{shunt}$) was calculated as the difference between $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ ($\dot{Q}_{pul}$–$\dot{Q}_{sys}$). Beat-to-beat heart rate (f_H) was calculated on

basis of the instantaneous blood flow profile in the left aortic arch, and total stroke volume (V_{Stot}; pulmonary + systemic) was calculated as total cardiac output ($\dot{Q}_{sys}+\dot{Q}_{pul}$) divided by f_H. The pulmonary and systemic resistances (R_{pul} and R_{sys}, respectively) were calculated as the mean blood pressure relative to blood flow ($R_{pul}=P_{pul}/\dot{Q}_{pul}$ and $R_{sys}=P_{sys}/\dot{Q}_{sys}$) under the assumption that both atrial pressures are zero.

Experimental protocol

During experiments, turtles were maintained ventral side up and artificially ventilated at a tidal volume of 20 ml and at a frequency of 24 breath min^{-1} using a Harvard Apparatus respirator (HI 665). Under these conditions, the tracheal pressure was approximately 0.8–1.0 kPa. The gas mixtures to the ventilator were delivered by a Wösthoff gas-mixing pump (Bochum, Germany). During all experiments, fractional CO_2 concentration (F_{CO_2}) was maintained at 0.03 (3 kPa) to mimic the arterial blood P_{CO_2} normally observed *in vivo* (e.g. Glass *et al.* 1983). After ensuring steady-state conditions (stable pressures and flows for 30 min) during normoxia (F_{O_2}=0.21, F_{CO_2}=0.03, balance N_2), F_{O_2} was altered in the following order: 0.10, 0.21, 0.05, 0.21, 0.03 and 0.21. The order of administration was identical for all turtles, and each hypoxic gas mixture was administered for 20 min. After completing the hypoxic exposures, atropine sulphate (1.0 mg kg^{-1}) was injected through the catheter in the right aortic arch and allowed to take effect for 30 min. Comeau and Hicks (1994) showed that a similar dose of atropine eliminated the cardiovascular changes during electrical stimulation of the efferent vagus of anaesthetised turtles. Hypoxic exposures were then repeated in the order described above. In three additional experiments, bilateral cervical vagotomy was performed instead of atropine injection; otherwise, the experimental protocol was identical to that described above. All experiments were performed at room temperature (22–23 °C).

Data analysis and statistics

All recordings of blood flows were analysed using AcqKnowledge data-analysis software (version 3.2.3; Biopac Inc.). For each oxygen exposure, mean values for $\dot{Q}_{LAo}$, $\dot{Q}_{LPA}$, P_{sys}, P_{pul}, f_H and systolic and diastolic pressures in the pulmonary and systemic circulations were determined for a 3–5 min period.

A two-way analysis of variance (ANOVA) for repeated measures was employed to determine significant effects of hypoxic gas mixtures and atropine infusion on the reported variables. Differences among means were subsequently assessed using a Student–Newman–Keuls *post-hoc* test. A fiducial limit for significance of $P<0.05$ was applied, and the data are presented as means ±1 S.E.M.

Results

Fig. 1 shows an example of blood flows and pressures in the systemic and pulmonary circulations in a turtle where P_{O_2} was reduced from 21 to 1.5 kPa. Shortly after the initiation of hypoxia, $\dot{Q}_{LPA}$ increased transiently for a few minutes, followed by a progressive decline until $\dot{Q}_{LPA}$ stabilised at a reduced level compared with that observed during normoxia. $\dot{Q}_{LAo}$ changed in a reciprocal manner. The changes in blood flows were associated with an initial increase in blood pressures in both circulations, followed by a decrease that was most pronounced in the systemic circulation. Similar patterns of blood flow and blood pressure changes were observed in all animals whenever P_{O_2} was reduced from 21 kPa to either 3 or 1.5 kPa.

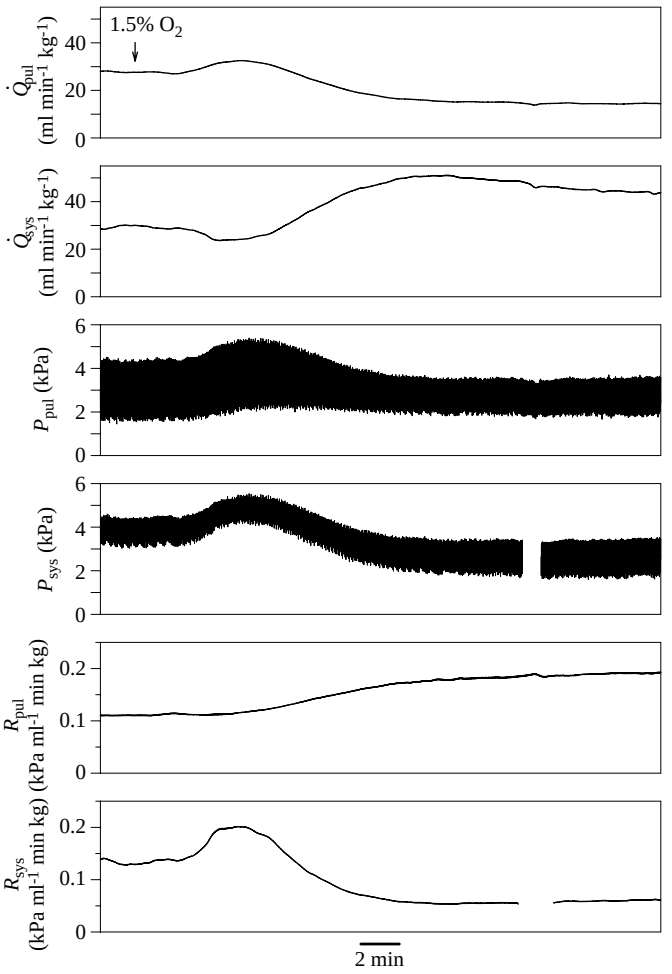

Fig. 1. An original recording of pulmonary and systemic blood flow ($\dot{Q}_{pul}$ and $\dot{Q}_{sys}$, respectively), pulmonary and systemic pressure (P_{pul} and P_{sys}, respectively) and pulmonary and systemic vascular resistance (R_{pul} and R_{sys}, respectively) immediately before and following hypoxia (F_{O_2}=0.015; 1.5 kPa O_2) in an anaesthetised and artificially ventilated turtle.

The effects of hypoxia on blood flows, blood pressures and the calculated resistances in turtles before and after atropinisation are presented in Fig. 2. In the pulmonary circulation (Fig. 2A–C), hypoxia caused a significant (P=0.0004) reduction in blood flow from a control value of 35.1±8.0 to 23.3±8.3 ml min^{-1} kg^{-1} at a P_{O_2} of 1.5 kPa in the inspired air. P_{pul} remained virtually constant within the

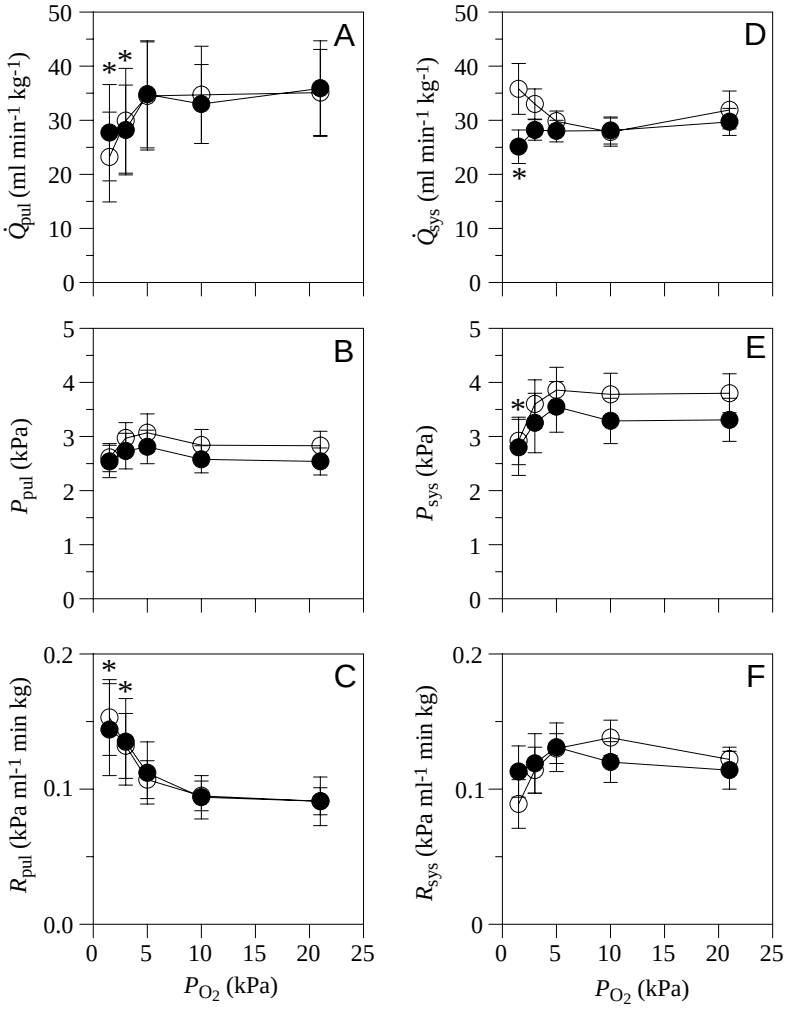

Fig. 2. Pulmonary (A) and systemic (D) blood flow ($\dot{Q}_{pul}$ and $\dot{Q}_{sys}$, respectively), pulmonary (B) and systemic (E) pressure (P_{pul} and P_{sys}, respectively) and pulmonary (C) and systemic (F) vascular resistance (R_{pul} and R_{sys}, respectively) during hypoxia in anaesthetised and artificially ventilated turtles. Open symbols denote the responses of untreated turtles and filled symbols denote the responses following atropine injection (1 mg kg^{-1}). Values are mean ±1 S.E.M. (N=5). Mean values that are significantly different (P<0.05) from the mean during normoxia are marked with an asterisk.

range 2.6–3.0 kPa, but there was a significant (P=0.009) reduction in pulmonary systolic blood pressure at a P_{O_2} of 1.5 kPa (Table 1). Hypoxia elicited a significant (P=0.0021) increase in R_{pul} from 0.09±0.01 to 0.15±0.03 kPa ml^{-1} min kg at P_{O_2} values of 21 and 1.5 kPa, respectively. Atropinisation had no significant effect on the responses of $\dot{Q}_{pul}$, P_{pul} or R_{pul} to hypoxia (Fig. 2). In the systemic circulation (Fig. 2D–F), there was a slight increase (but not statistically significant) in blood flow from 31.9±3.5 to 35.8±4.7 ml min^{-1} kg^{-1} as P_{O_2} was reduced from 21 to 1.5 kPa, and this was associated with a significant (P=0.007) decrease in P_{sys} from 3.80±0.36 to 2.92±0.44 kPa. The decrease in P_{sys} was due to a reduction in both systolic and diastolic pressures (Table 1). Concomitantly, R_{sys} decreased from 0.12±0.01 to 0.09±0.02 kPa ml^{-1} min kg (but not statistically significant; P=0.08). Atropine did not affect P_{sys}

Table 1. *The effects of hypoxia on diastolic and systolic blood pressures in anaesthetised turtles (*Trachemys scripta*) before and after injection of atropine (1 mg kg^{-1})*

Inspired P_{O_2} (kPa)	Pressure in pulmonary circulation (kPa)				Pressure in systemic circulation (kPa)			
	Control		Atropinised		Control		Atropinised	
	Diastolic	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic	Systolic
21	1.33±0.08	4.02±0.35	1.03±0.10	3.87±0.42	3.21±0.29	4.29±0.38	2.74±0.43	3.91±0.40
10	1.36±0.21	3.98±0.31	1.02±0.10	4.05±0.49	3.22±0.35	4.31±0.43	2.92±0.54	3.98±0.47
5	1.63±0.39	4.04±0.33	1.18±0.12	4.15±0.49	3.28±0.38	4.35±0.45	3.01±0.58	4.15±0.42
3	1.71±0.13	4.08±0.43	1.20±0.12	3.93±0.52	2.95±0.47	4.18±0.46	2.45±0.56	3.93±0.57
1.5	1.58±0.12	3.55±0.39*	1.13±0.11	3.52±0.35*	2.27±0.51*	3.54±0.37*	1.91±0.48*	3.63±0.41*

Values are means ± S.E.M., N=5.
*Significantly different from the value during normoxia (F_{O_2}=0.21) (P<0.05).

or R_{sys}, but caused a significant (P=0.02) reduction in $\dot{Q}_{sys}$ during hypoxia.

Total cardiac output ($\dot{Q}_{pul}+\dot{Q}_{sys}$) decreased significantly from 67.0±10.7 to 59.1±8.3 ml min^{-1} kg^{-1} when P_{O_2} was reduced to 1.5 kPa (Fig. 3A; P=0.02). This reduction was ascribed to a significant (P=0.005) reduction in V_{Stot} (from 1.73±0.25 to 1.50±0.16 ml kg^{-1}; Fig. 3C), whereas f_H was not affected and remained within the range 38–40 beats min^{-1} (Fig. 3B). Atropine did not alter the effects of hypoxia on $\dot{Q}_{tot}$, f_H or V_{Stot} (Fig. 3). Owing to the reciprocal changes in $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ (Fig. 2A,D), hypoxia caused a significant reduction and in the net $\dot{Q}_{shunt}$ (P=0.02) and $\dot{Q}_{pul}/\dot{Q}_{sys}$ (P=0.05) (Fig. 4A,B). Thus, while a small left-to-right shunt prevailed during normoxia, hypoxia induced a right-to-left shunt. Although injection of atropine reduced the right-to-left shunt during hypoxia, this effect was not statistically significant.

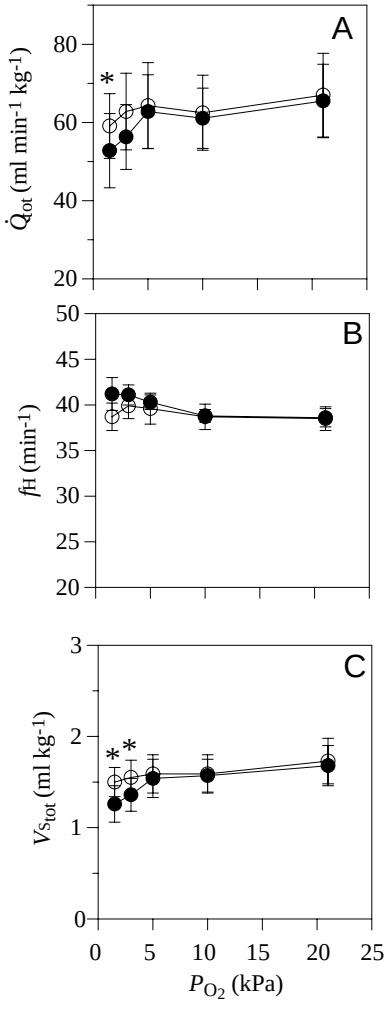

Fig. 3. (A) Total cardiac output ($\dot{Q}_{tot}=\dot{Q}_{pul}+\dot{Q}_{sys}$), (B) heart rate ($f_H$) and (C) total stroke volume (V_{Stot}) during hypoxia in anaesthetised and artificially ventilated turtles. Open symbols denote the responses of untreated turtles and filled symbols denote the responses following atropine injection (1 mg kg^{-1}). Values are mean ±1 S.E.M. (N=5). Mean values that are significantly different (P<0.05) from the mean during normoxia are marked with an asterisk.

In three specimens, bilateral cervical vagotomy had no effects on the haemodynamic variables during normoxia. Thus, f_H was 42.4±2.7 ml min^{-1} before vagotomy and 40.7±3.2 ml min^{-1} after vagotomy; $\dot{Q}_{pul}$ was 26.2±5.0 ml min^{-1} kg^{-1} before vagotomy and 26.3±1.8 ml min^{-1} kg^{-1} after vagotomy. Furthermore, vagotomy did not alter the cardiovascular responses to hypoxia. For example, hypoxia (1.5 kPa inspired P_{O_2}) elicited a 68 % increase in R_{pul} in the intact animals and a 60 % increase in R_{pul} in the vagotomised animals. These findings are, therefore, consistent with the data obtained following atropinisation.

The arterial blood gas levels during normoxia and severe hypoxia are summarised in Table 2. Arterial P_{O_2} (Pa_{O_2}) was reduced as expected from the hypoxic treatment, while acid–base status (Pa_{CO_2} and pH) and haematocrit were not affected. Plasma noradrenaline levels increased markedly during hypoxia, and this effect was more pronounced following atropinisation (Fig. 5). Plasma adrenaline levels did not increase during hypoxia, but there was a significant elevation in plasma adrenaline levels during hypoxia following atropinisation (Fig. 5).

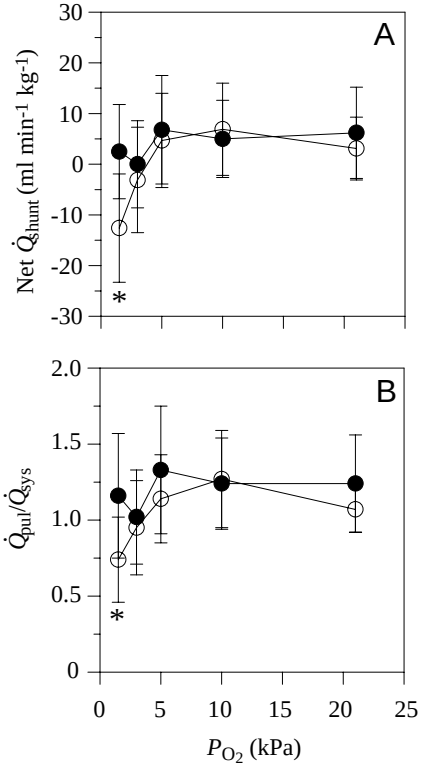

Fig. 4. (A) Net cardiac shunt flow ($\dot{Q}_{shunt}=\dot{Q}_{pul}-\dot{Q}_{sys}$) and (B) $\dot{Q}_{pul}/\dot{Q}_{sys}$ during hypoxia in anaesthetised and artificially ventilated turtles. Open symbols denote the responses of untreated turtles and filled symbols denote the responses following atropine injection (1 mg kg^{-1}). Values are mean ±1 S.E.M. (N=5). Mean values that are significantly different (P<0.05) from the mean during normoxia are marked with an asterisk.

Table 2. *The effects of hypoxia and atropine on blood gas levels in anaesthetised turtles (*Trachemys scripta*)*

Inspired P_{O_2} (kPa)	Pa_{O_2} (kPa)		Pa_{CO_2} (kPa)		pH		Haematocrit (%)	
	Control	Atropinised	Control	Atropinised	Control	Atropinised	Control	Atropinised
21	15.5±0.3	16.7±0.4	2.6±0.1	2.8±0.1	7.71±0.03	7.62±0.03	27±2	29±1
1.5	1.5±0.2*	1.5±0.1*	2.8±0.1	2.8±0.1	7.63±0.03	7.60±0.03	30±2	29±2

Values are means ± S.E.M., N=5.
*Significantly different from the value during normoxia (21 kPa) (P<0.05).

Discussion

The present study describes blood flows and vascular resistances in the pulmonary and systemic circulations during hypoxia in anaesthetised turtles (*Trachemys scripta*). As a main finding, we report that severe hypoxia almost doubled R_{pul} in anaesthetised turtles, whereas R_{sys} decreased by approximately 25 %, although this reduction was not statistically significant. These effects are probably locally mediated and show that turtles exhibit hypoxic pulmonary vasoconstriction. This finding is consistent with the situation in mammals and birds, although the sensitivity appears to be lower in turtles.

Comparison with previous studies

The haemodynamic variables measured in the present study compare favourably with previous studies on anaesthetised turtles (Table 3), but show marked differences compared with values for conscious and fully recovered animals (e.g. Shelton and Burggren, 1976; Wang and Hicks, 1996*a*). Thus, V_{Stot} in conscious turtles is almost twice the value for anaesthetised animals (3.1 *versus* 1.4–1.7 ml kg^{-1}; Shelton and Burggren, 1976; Wang and Hicks, 1996*a*; Table 3). Furthermore, while conscious turtles exhibit a right-to-left shunt during breath-holding and pronounced elevations of $\dot{Q}_{pul}$ and f_H during ventilation (e.g. Shelton and Burggren, 1976; Wang and Hicks, 1996*a*), f_H and blood flows are high and uniform during anaesthesia and a net left-to-right shunt dominates (see Table 3). These differences are probably caused by the withdrawal of vagal tone on the heart and pulmonary artery during anaesthesia since atropinisation and vagal sectioning had no effects in the present study (Figs 2, 3). In contrast, atropine injection elicits large increases in f_H, $\dot{Q}_{pul}$ and a net left-to-right shunts in conscious turtles (Burggren, 1975; Hicks and Wang, 1998).

Critique of the present study

As in previous studies on turtles (Comeau and Hicks, 1994; Hicks and Comeau, 1994; Hicks *et al.* 1996), we calculated R_{pul} as $P_{pul}/\dot{Q}_{pul}$ under the assumption that left atrial pressure (P_{LAt}) is negligible. Badeer and Hicks (1994) warned that such an assumption is potentially dubious in a low-pressure system, and our approach could, consequently, overestimate R_{pul}; the

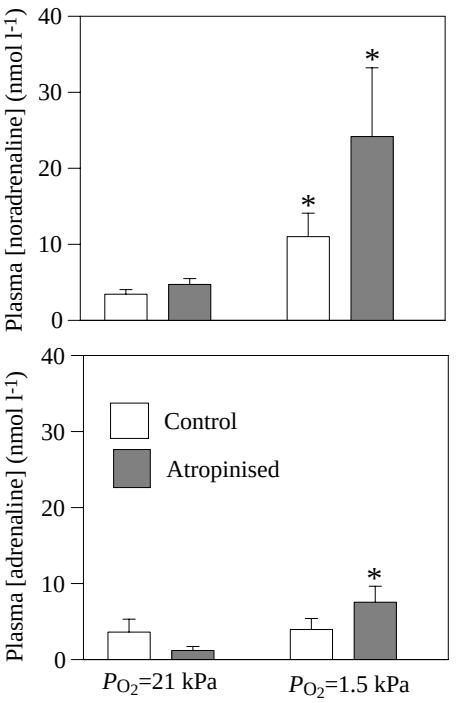

Fig. 5. Plasma concentrations of catecholamines (noradrenaline and adrenaline) during normoxia and severe hypoxia in anaesthetised and artificially ventilated turtles. Values are mean +1 S.E.M. (N=5). Mean values that are significantly different (P<0.05) from the mean during normoxia are marked with an asterisk.

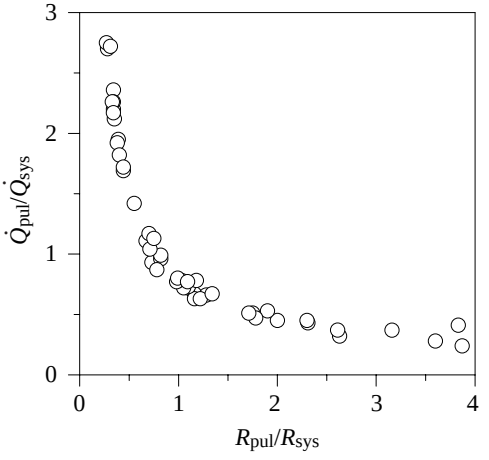

Fig. 6. The relationship between the ratio of vascular resistances (R_{pul}/R_{sys}) and the ratio of blood flows ($\dot{Q}_{pul}/\dot{Q}_{sys}$) in the pulmonary and systemic circulations. Each data point represent the blood flows and resistances obtained from an individual turtle at each of the hypoxic exposures.

Table 3. *Values of haemodynamic variables in anaesthetised turtles (*Trachemys scripta*) determined previously*

$\dot{Q}_{pul}$ (ml min^{-1} kg^{-1})	$\dot{Q}_{sys}$ (ml min^{-1} kg^{-1})	f_H (min^{-1})	V_{Stot} (ml kg^{-1})	R_{sys} (kPa ml^{-1} min kg)	R_{pul} (kPa ml^{-1} min kg)	Reference
42.3	19.5	43.2	1.37	0.15	0.05	Hicks *et al.* (1996)
57.4	–	44.0	–	–	0.06	Hicks and Comeau (1994)
33.8	29.2	38.5	1.67	0.13	0.06	Comeau and Hicks (1994)
35.1	31.9	38.5	1.73	0.12	0.09	Present study

$\dot{Q}_{pul}$, pulmonary blood flow; $\dot{Q}_{sys}$, systemic blood flow; f_H, heart rate; V_{Stot}, total stroke volume; R_{sys}, systemic resistance to blood flow; R_{pul}, pulmonary resistance to blood flow.

higher is P_{LAt}, the lower is R_{pul}. However, P_{LAt} is 0–0.5 kPa during normoxia (J. W. Hicks and S. G. Comeau, unpublished observations) and would, therefore, need to increase to 1.0–1.3 kPa during hypoxia if R_{pul} proper remained constant. P_{LAt} could increase during hypoxia as result of impairment of left atrial and/or ventricular contractility, but since $\dot{Q}_{pul}$ decreased and $\dot{Q}_{sys}$ tended to increase during hypoxia, this possibility seems unlikely. Furthermore, an increased P_{LAt} would be expected to be transmitted to the arterial side and to be associated with clearly visible left atrial distension. Thus, it is unlikely that changes in P_{LAt} would alter the conclusion that hypoxia elicits an increase in R_{pul}.

This study was performed on anaesthetised animals to allow for precise control of lung gas composition, to reduce cardiovascular reflexes involving the central nervous system and to preclude the cardiovascular changes associated with spontaneous breathing. However, pentobarbital markedly attenuates HPV in excised and *in-situ* perfused lungs from young sheep (Wetzel and Martin, 1989), and it is therefore possible that the Nembumal used in the present study reduced HPV compared with values in conscious turtles.

Release of catecholamines

The normoxic plasma noradrenaline level in the present study is similar to those reported previously in anaesthetised and in chronically cannulated resting turtles, while the adrenaline level is slightly lower (Cipolle *et al.* 1986; Wasser and Jackson, 1991). Hypoxia elicited a rise in noradrenaline level, which was more pronounced after atropinisation, whereas the adrenaline level was unchanged. A qualitatively similar response has been observed in mammals (Favier *et al.* 1985; Koller *et al.* 1983; Maher *et al.* 1975). The catecholamine levels during severe hypoxia in the present study are similar to those in anoxic turtles, where acidosis has been shown to augment the release of both adrenaline and noradrenaline (Wasser and Jackson, 1991). Noradrenaline appears to be the predominant sympathetic neurotransmitter in reptiles (Cooper *et al.* 1965), and the elevated catecholamine levels during hypoxia in the present study may, therefore, have resulted from both sympathetic nerve terminal spill-over and release from the adrenal medulla.

Hypoxic pulmonary vasoconstriction in turtles

In these experiments, $\dot{Q}_{pul}$ and blood pressures increased transiently within 3–10 min after application of the most hypoxic gas mixtures (Fig. 1). Initially, these changes were associated with an increase in R_{sys}, whereas R_{pul} increased gradually after 5–10 min. After approximately 15 min, R_{sys} decreased below normoxic levels and R_{pul} reached a steady level above the normoxic value. The initial changes in vascular resistances may, at least in part, be due to the release of catecholamines (Fig. 5), which cause a vasoconstriction in the systemic circulation and a vasodilatation in the pulmonary circulation (Luckhardt and Carlson, 1921; Burggren, 1977; Comeau and Hicks, 1994; cf. Milsom *et al.* 1977). While the effects of catecholamines are expected to persist, the increased R_{pul} and the decreased R_{sys} are probably due to locally mediated effects that develop over a slower time course. Thus, the time course for HPV in turtles is slower than in mammals, where HPV is manifested within minutes after hypoxia (e.g. Morrell *et al.* 1995). During more severe hypoxia, HPV in mammals is biphasic because the initial rapid constriction is followed by a subsequent dilatation (de Canniere *et al.* 1992; cf. Marshall and Marshall, 1983*b*). A similar response was not observed in the present study. The P_{O_2} threshold to elicit HPV in turtles is between 3 and 5 kPa, and is therefore substantially lower than that in most mammals and birds, in which HPV is often elicited at a P_{O_2} above 7–8 kPa, although the exact threshold does vary (Peake *et al.* 1981; Faraci *et al.* 1984). The lower sensitivity for HPV in turtles is consistent with the general observation that ectotherms are more tolerant to hypoxia than are endotherms.

Because anaesthesia reduces vagal tone on the cardiovascular system and impairs cardiovascular reflexes, the hypoxic response in the present experiments differs from that of conscious animals. In conscious turtles, $\dot{Q}_{pul}$ and f_H increase during hypoxia, but these changes are primarily associated with the increased ventilation (Burggren *et al.* 1977; West *et al.* 1992; Wang *et al.* 1996) and, at a fractional inspired O_2 concentration (F_{IO_2}) of 0.05, $\dot{Q}_{pul}$ remains high (Wang *et al.* 1997). These observations, therefore, are consistent with the present study showing that HPV only develops during very severe hypoxia. Turtles can tolerate complete anoxia (e.g. Jackson, 1987), and Hicks and Wang (1998) have recently shown that $\dot{Q}_{pul}$ is drastically reduced within an hour of anoxia in conscious turtles. Furthermore, although f_H and $\dot{Q}_{sys}$ were increased following atropinisation during anoxia in conscious turtles, there was no effect on $\dot{Q}_{pul}$ (Hicks and Wang, 1998).

Thus, in the light of the present experiments, it seems that HVP accounts for the large reduction in $\dot{Q}_{pul}$ during anoxia.

The present study does not reveal the location of the vasoconstriction within the pulmonary circulation. However, because the hypoxia-induced constriction persisted following vagotomy or atropine injection, it is reasonable to conclude that the constriction is not caused by a vagally mediated constriction of the pulmonary artery (see Burggren, 1977; Milsom *et al.* 1977). In mammals, the site of HPV is the subject of debate. Nevertheless, although the HPV appears to take place throughout the pulmonary vasculature, the precapillary pulmonary arterioles are the most important and major locus (Fishman, 1976; Voelkel, 1986; Weir and Archer, 1995). Furthermore, in mammals, HPV is independently augmented by reductions in both lung P_{O_2} and O_2 levels in the blood perfusing the lungs (Marshall and Marshall, 1988; Marshall *et al.* 1994*a*), suggesting the presence of a sensor in the precapillary region (Marshall and Marshall, 1983*a*). In the present study, hypoxia was applied simultaneously in the lungs and blood and it is, therefore, not possible to determine whether blood P_{O_2} also elicits HPV in turtles. Finally, in mammals, HPV depends on acid–base status, and metabolic acidosis augments and alkalosis reverses HPV in dogs (Brimioulle *et al.* 1990). In the present experiments, F_{CO_2} and acid–base status did not vary and they cannot, therefore, reveal whether acid–base status affects HPV in turtles.

Implications of HVP for in vivo *blood flows and an evolutionary speculation*

Because of the undivided ventricle in turtles, blood flows between the systemic and pulmonary circulation are determined by the resistances in these two circuits (Hicks *et al.* 1996). For the data obtained in the present experiment, this relationship is depicted in Fig. 6, which shows a decreased $\dot{Q}_{pul}/\dot{Q}_{sys}$ (indicative of an increased right-to-left shunt) as R_{pul}/R_{sys} increases. Thus, as HPV develops during hypoxia, the right-to-left shunt will increase and systemic oxygen delivery will be reduced (Wang and Hicks, 1996*b*). As an interesting analogy, HPV is viewed as detrimental for gas exchange in neonates with tetralogy of Fallot (Marshall *et al.* 1994*b*). If hypoxaemia arises as a result of hypoventilation or coughing in neonates with this vascular malformation, the ensuing HVP elevates R_{pul} and increases the right-to-left shunt, which worsens the hypoxaemia. Similarly, HVP may be deleterious in diseased humans with large pulmonary shunts because HVP causes a diversion of blood flows to shunt vessels within the lung.

The breathing pattern of many reptiles, particularly aquatic species, is characterised by breathing episodes consisting of one or several breaths interspersed between breath-holding periods of varying duration. During these breath-holding periods, lung and blood P_{O_2} decrease as the oxygen stores are exhausted (e.g. Burggren and Shelton, 1979) and a blunted HVP may, therefore, ensure that pulmonary blood flow can be increased during, for example, periods of submerged activity (Shelton and Burggren, 1976; West *et al.* 1992; Wang *et al.* 1997). Thus, from an evolutionary point of view, it may be speculated that, as progressively more complex lungs developed, HPV evolved to improve ventilation–perfusion homogeneity. However, in species with a poorly divided ventricle, this benefit is counterbalanced by the fact that HPV impairs the ability to increase pulmonary blood flow during breath-holding periods. As a testable hypothesis for this scenario, it is expected that the potency of HPV within amphibians and reptiles will be greater in species with a high degree of ventricular separation and with increased lung complexity and in animals that do not normally exhibit prolonged periods of apnoea. Future studies are planned to investigate this hypothesis.

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