J. exp. Biol. **186**, 109–126 (1994)
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VAGAL REGULATION OF INTRACARDIAC SHUNTING IN THE TURTLE *PSEUDEMYS SCRIPTA*

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Accepted 6 September 1993

Summary

Two principal hypotheses account for intracardiac shunting in reptiles. The 'pressure shunting' hypothesis proposes that there is no fuctional separation between the ventricular cava during systole. The 'washout shunting' hypothesis suggests that the cavum pulmonale is functionally separated from the rest of the ventricle during systole. The purpose of this study was to test the two principal hypotheses in a turtle, *Pseudemys scripta,* after inducing a right-to-left shunt by electrical stimulation of the vagus nerve. Animals were anaesthetized with sodium pentobarbital (30–40 mg kg^{-1}), tracheotomized and mechanically ventilated. Two experimental groups were used. Both groups had the right and left cervical vagi exposed and sectioned and silver bipolar electrodes were attached for electrical stimulation. In addition, cardiac function was evaluated by determining the pulmonary blood flow, pulmonary arterial pressure, peak systolic pressure in the cavum pulmonale, central arterial pressure, pulmonary vascular resistance and heart rate. In group I, hydrogen electrodes were inserted into the right aorta, the left aorta and the pulmonary artery. Hydrogen, dissolved in saline, was infused into the left atrium, jugular vein and cavum pulmonale. Blood flow from these sites was deduced from detection of a H_2 signal in the right and left aortae and the pulmonary artery. In group II, catheters were inserted in the left and right atria and aortae for the measurement of blood gases. For both groups, the protocol consisted of control periods and periods of electrical stimulation of the efferent and afferent ends of the vagus nerve. During the control periods, infusion of a H_2 solution into either the left atrium or the jugular vein resulted in the detection of H_2 in the right and left aortae and the pulmonary artery. This suggested that both right-to-left and left-to-right intracardiac shunts were present. H_2 infused into the cavum pulmonale was always detected in the pulmonary artery but never in the left or right aortae. During stimulation of the right vagal efferents, a bradycardia developed (heart rate declined by 65 %), pulmonary blood flow was reduced by 73 % and pulmonary vascular resistance increased by 158 %. This was accompanied by a reduction in the P_{O_2} of both the right and left aortae, although the P_{O_2} of the left and right atria

Key words: reptiles, cardiovascular, washout shunting, pressure shunting, cholinergic regulation, *Pseudemys scripta.*

remained constant. Under these conditions, H_2 infused into the jugular vein and the left atrium was detected in the right and left aortae and the pulmonary artery of all animals studied. Infusion of H_2 into the cavum pulmonale was detected in the right and left aortae in only two animals. The results supported the washout mechanism for right-to-left intracardiac shunting.

Introduction

In the chelonians (turtles) and squamates (lizards and snakes), the heart consists of two atrial chambers and a single ventricle. The ventricle is subdivided into three anatomically interconnected chambers. These chambers are the cavum pulmonale (CP), the cavum venosum (CV) and the cavum arteriosum (CA). A distinctive feature of the ventricular anatomy is a septum-like structure called the muscular ridge. The muscular ridge originates from the ventral ventricular wall and divides the CP from the CV and CA (Van Mierop and Kutsche, 1981). The dorsolateral border of the muscular ridge is unattached, resulting in potential communication among the three ventricular chambers. In all reptiles, three great vessels arise from the ventricle. The pulmonary artery (PA) originates from the CP, whereas the right and left aortic arches (RAo, LAo) emerge from the CV (see Fig. 1). During diastole, deoxygenated blood from the right atrium (RAt) fills the CV and CP, while the CA receives oxygenated blood from the lungs by way of the left atrium (LAt). During systole, mixtures of oxygenated and deoxygenated blood flow into the RAo, LAo and PA. The mechanism that causes RAt blood to bypass the pulmonary circulation (right-to-left; R–L shunt) is controversial (Heisler and Glass, 1985; Hicks and Malvin, 1992).

There are two principal hypotheses termed *pressure shunting* and *washout shunting* that address the mechanism of intracardiac shunts (Heisler and Glass, 1985). The pressure shunting hypothesis states that the muscular ridge does not separate the CP from the CV or CA during systole. This allows nearly unimpeded blood flow between the ventricular chambers. Consequently, when the pulmonary vascular resistance increases relative to the systemic vascular resistance, some blood within the CP will be ejected into the systemic circuit (i.e. a R–L shunt) (White and Ross, 1966; Shelton and Burggren, 1976). In contrast, the washout hypothesis states that the muscular ridge separates the CP from the CV and CA during systole. This will prevent blood within the CP from being ejected into the systemic circuit. A R–L shunt results from the deoxygenated blood being washed from the CV into the systemic arteries by blood being ejected from the CA during systole. Accordingly, variations in the magnitude of the R–L shunt result from any factors that affect diastolic filling and/or systolic ejection from the CV and CA (Heisler *et al.* 1983).

A recent study (Hicks and Malvin, 1992) attempted to resolve the mechanism of intracardiac shunting. Helium (He) dissolved in saline was injected into the cardiac chambers and was detected in the systemic arteries with a mass spectrometer. This procedure allowed us to determine directly the source of blood in the systemic circuit. A R–L intracardiac shunt was induced by infusion of acetylcholine (ACh) into the venous circulation. We concluded, from the detection of He in the systemic circuit, that the muscular ridge functioned as a complete septum during systole, effectively separating the pulmonary and systemic circulations. However, we did not measure any cardiovascular

variables other than the heart rate. This made it impossible to assess the haemodynamic conditions under which these results were obtained.

The purpose of the present study was to test the two principal hypotheses in a turtle, *Pseudemys scripta,* after inducing a R–L shunt by electrical stimulation of the vagus nerve. The cardiac function was evaluated by determining the pulmonary blood flow ($\dot{Q}_{pul}$), pulmonary arterial pressure (P_{PA}), peak systolic pressure in the CP ($P_{CP,sys}$), right aortic pressure (P_{RAo}), pulmonary vascular resistance (R_{pul}) and heart rate (f_H). Intracardiac shunting was assessed by analysis of blood P_{O_2} sampled from the four central vascular sites, LAt, RAt, RAo and LAo. In addition, blood flow patterns were deduced by injecting hydrogen (H_2) into the cardiac chambers and detecting its presence in the systemic and pulmonary arteries with H_2 electrodes. An abstract of this study has been published previously (Hicks and Comeau, 1992).

Materials and methods

Animals

Studies were conducted on turtles, *Pseudemys scripta* Gray, ranging in mass from 1.3 to 2.3 kg (mean ± S.D. 1.74±0.31 kg; *N*=14). Animals were obtained from a commercial supplier (Lemberger Co., Oskosh, WI), housed in a large aquarium at room temperature and were not fed for at least 1 week before the study.

Surgical preparation

Experiments were conducted on two groups of animals. In group I (*N*=9; mass 1.9±0.2 kg), vagal effects on blood flow patterns were studied using H_2 electrodes. In group II (*N*=5; mass 1.6±0.33 kg), vagal effects on central P_{O_2} levels were studied. In both groups, animals were prepared for application of flow probes and insertion of H_2 electrodes as previously described (Hicks and Malvin, 1992). Animals were supine, anaesthetized (sodium pentabarbital, 30–40 mg kg^{-1}), tracheotomized and mechanically ventilated (12 min^{-1}). The humidified gas mixture inspired by the animals contained 21 % O_2, 4 % CO_2 and 75 % N_2, prepared by a gas-mixing pump (GF3, Cameron Instruments). To expose the heart and great vessels, a rectangular opening (approximately 3 cm×4 cm) was made in the plastron over the heart with a bone saw. The piece of plastron was removed from the underlying musculature and any bleeding was stopped by cauterization. The LAt was catheterized by a previously described method (Heisler *et al.* 1983). In addition, a catheter (PE 50) was inserted into the left carotid artery and advanced 2–3 cm towards the heart. This catheter was connected to a strain-gauge pressure transducer (Spectromedic, PX23L) for the measurement of P_{RAo}. f_H was determined by inserting needle electrodes into the right and left forelegs and the left hindfoot. The electrodes were connected to a cardiotachometer (type 9857; Sensormedics, Yorba Linda). In both groups, the right and left cervical vagi were exposed, carefully dissected away from surrounding tissue and bilaterally sectioned (Comeau, 1992). A small loop of silk suture (4-0) was tied onto both afferent and efferent ends of the cut nerve so that they could be independently manipulated onto the stimulating electrode during the experiment.

Group I (hydrogen electrodes)

Besides the above procedures, group I had three H_2 electrodes inserted into the great vessels for detection of a H_2 tracer. The H_2 electrodes were fashioned after the electrodes described by Clark *et al.* (1960). Each electrode consisted of a 32 g platinum wire (diameter 0.2 mm), insulated by PE 50 tubing. Approximately 1 cm of platinum wire was left exposed and its tip was sharpened. The sensitivity of the electrode to flow and concentration was determined before the experiments in the following way. The electrode was inserted into a piece of Tygon tubing (diameter 3 mm). The tubing was connected to a peristaltic pump (Masterflex; model 7524-000) which withdrew saline from a large glass beaker and perfused the Tygon tubing. A silver reference electrode was placed inside the beaker. The reference electrode and the H_2 electrode were connected directly to a d.c. amplifier on a Beckman R610 polygraph system. Amplification was set at 100 mV full scale. The flow of saline through the Tygon tubing was varied from 1 to 30 ml min^{-1}. An additional volume of saline was continuously tonometered at 25 ˚C with pure H_2. At each flow rate, 300 μl of the H_2 solution was injected upstream from the electrode. The amount of dissolved H_2 injected into the perfusion apparatus was varied by diluting the stock solution. The amount of H_2 injected ranged from 0.19 to 0.76 μmol. The peak signal from the electrode was determined at each flow rate and for each amount of H_2 injected. Injections of the H_2 solution were in triplicate.

In group I, the H_2 electrodes were inserted into the RAo, LAo and PA by puncturing the vessel wall. The silver reference electrode was brought into contact with the exposed muscle tissue. The silver reference electrode and the H_2 electrodes were connected directly to d.c. amplifiers on a Beckman R610 polygraph system. Amplification was set at 100 mV full scale. A catheter for infusion of H_2 or pressure measurements was inserted into the CP by way of the PA. A small section (3–5 mm) of the PA was exposed and a 20 g intravenous catheter was gently inserted upstream. The catheter was connected to a strain-gauge pressure transducer (P23XL; Spectramed Inc., Oxnard) by an additional piece of catheter tubing (PE 50) and advanced until it entered the CP. This was determined by the characteristic ventricular waveform. The position of the CP catheter was confirmed during *post-mortem* examination. An additional infusion catheter (PE 50) was inserted into the right jugular vein (JV) and advanced 2–3 cm towards the heart. A transit-time ultrasonic flow probe (2R; Transonic Inc., Ithaca) was applied to the left pulmonary artery (LPA) for the measurement of blood flow. Total pulmonary blood flow ($\dot{Q}_{pul}$) was determined by multiplying the flow of the LPA by 2. The $\dot{Q}_{pul}$ measured in group II (see below) and in recent studies (Comeau, 1992) indicated that the LPA and the right pulmonary artery (RPA) blood flows were not significantly different. Recent studies suggest that the flow relationship between LPA and RPA was not altered during vagal nerve stimulation (Comeau, 1992).

Group II (blood gases)

In group II, a catheter was inserted into the RAt by the method described above. The LAo was non-occlusively cannulated using a 22 g intravenous catheter. The catheter was connected to an additional piece of PE tubing and advanced 2–3 cm. The main branch of the

RAo was non-occlusively cannulated by way of a small branch of the left or right subclavian artery. $\dot{Q}_{pul}$ was determined by application of a transit-time ultrasonic flow probe (2R; Transonic Inc.) to both the LPA and RPA (Comeau, 1992). Flow probes had been calibrated at the factory at 25 ˚C. Calibration of the flow probes was checked at the end of the study by removing the LAo from an animal, applying a 2R flow probe and generating known flows through the vessel from a peristaltic pump (Masterflex, model 7524-00). Pulmonary vascular resistance was calculated from the standard equation, assuming that LAt pressure was approximately atmospheric and was not significantly affected by vagal efferent or afferent stimulation. Recent studies (Comeau, 1992) support this assumption.

Protocol

Experiments for group I and group II included electrical stimulation of the right vagal efferent (RVEF), the left vagal efferent (LVEF) and the right vagal afferent (RVAF) nerves. Each stimulation period was bracketed by control periods. Stimulation of the nerve was accomplished by attaching silver bipolar electrodes to either the cut distal (efferent) or central (afferent) end. Stimulation was provided by an A310 Accupulser pulse generator coupled to an A360 D/R constant-current stimulus isolator (World Precision Instruments, Inc., New Haven). Stimulation was at 2–4 Hz, 8 V, 20–40 μA and 200 ms pulse duration. Electrical stimulation of the RVEF was adjusted to produce an approximately 60–70 % reduction in f_H. This level was maintained for a sufficient time to inject the indicator or to withdraw blood samples. The same level of stimulation was then used for the LVEF. The level of stimulation for the RVAF was adjusted to produce an approximate doubling of pulmonary blood flow. The order of stimulation for LVEF and RVAF was randomly determined. Stimulation periods were always bracketed by control periods. In group I, saline was continuously tonometered at 25 ˚C with H_2. During each control and stimulation period, 300 μl of the H_2 solution was sequentially infused into the LAt, JV and CP for up to 20 s each. The rate of infusion was varied to account for differences in blood flow during the experiment. The lowest infusion rates were during RVEF and LVEF stimulation, when blood flow was the lowest. Fig. 1 is a diagram of the infusion and recording sites. The analysis of the signal was not complicated by recirculation through the pulmonary circulation. This was probably because of the low solubility of H_2 and the continuous ventilation of the animal during the experiment. In addition, recirculation from the systemic circuit was never observed. In group II, during each control and stimulation period, blood samples (0.25–0.3 ml) were simultaneously drawn from the LAt, RAt, LAo and RAo into 1 ml all-glass tuberculin syringes after first clearing the dead space (0.2 ml) within the catheters. Following each withdrawal, blood samples were stored in an ice slurry and sequentially analyzed for P_{O_2}, P_{CO_2} and pH using a Radiometer BMS3 MK2 blood gas analyzer maintained at the animal’s body temperature. Haematocrit was determined periodically throughout each experiment. Unused blood was reinfused into the animal.

Data analysis and presentation

The sensitivity of the H_2 electrodes was analyzed by a multiple regression analysis. The concentrations of H_2 and the flow of saline were the independent variables and the

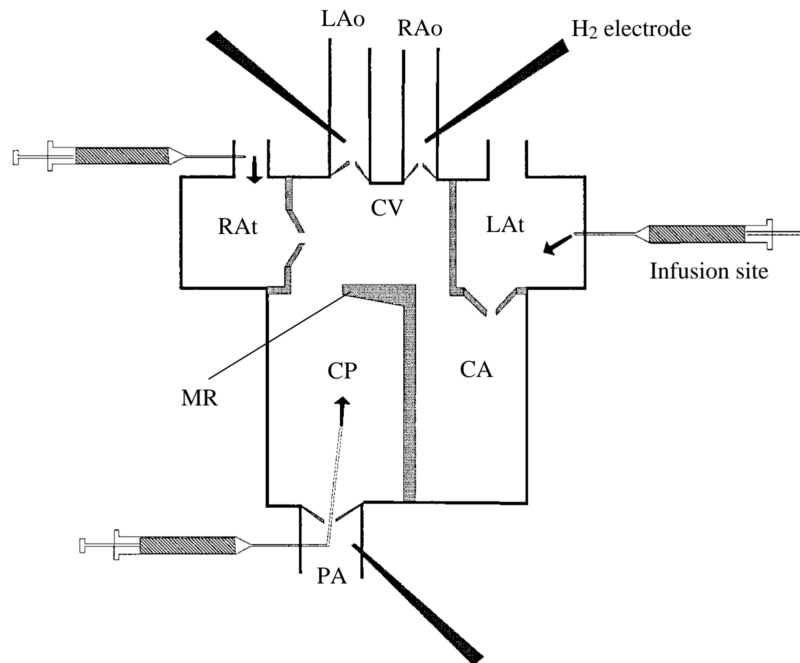

Fig. 1. Diagram of the turtle heart, showing the infusion sites (syringe symbols) and the recording sites for the H_2 electrodes. CP, cavum pulmonale; CV, cavum venosum; CA, cavum arteriosum; MR, muscular ridge; RAt, right atrium; LAt, left atrium; RAo, right aortic arch; LAo, left aortic arch; PA, pulmonary artery. The small black arrows represent H_2 injection.

peak H_2 signal was the dependent variable. Intracardiac shunts were assessed by two methods. In group I, the detection of H_2 in the RAo and LAo following infusion into the JV and CP indicated a R–L shunt. In contrast, detection of H_2 in the PA following infusion in the LAt indicated a L–R shunt. The criterion for the presence of a H_2 signal was an increase in the potential above the average baseline (see Fig. 5). The patterns of detection of H_2 in the RAo, LAo and PA at each infusion site and during each experimental condition were analyzed by a χ^2 analysis. Blood P_{O_2} values were analyzed in group II. If the RAt P_{O_2} remained constant, then a significant difference between the LAt and RAo P_{O_2} values or the LAt and LAo P_{O_2} values suggested the presence of a R–L shunt. The differences between controls and the effects of efferent and afferent vagal stimulation on both blood gases and haemodynamic variables were determined by analysis of variance (ANOVA). All values are shown as the mean ± standard deviation. Significance was set at the P=0.05 level.

Results

H_2 electrodes

At each flow rate, the H_2 electrodes exhibited a linear response to an increase in the amount of H_2 injected (Fig. 2). In addition, for each amount of H_2 injected, the peak

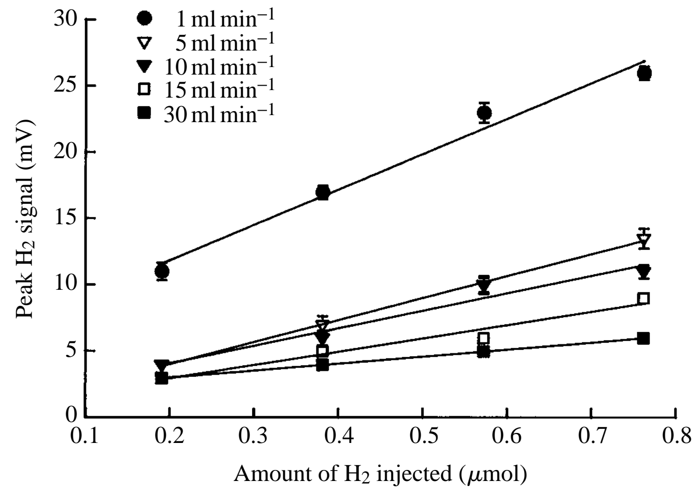

Fig. 2. The effects of saline flow rate and the amount of H_2 injected on the peak signal recorded by the H_2 electrodes. Each point represents mean ± S.D. (N=3).

signal recorded from the electrodes decreased as flow rate increased (Fig. 2). The relationship between flow rate and the amount of H_2 injected was:

$$\text{peak } H_2 \text{ signal (mV)} = 7.12 + 14.32[H_2] - 0.39\dot{Q}\,.$$

This relationship had an overall r value of 0.63 and was statistically significant (P<0.001).

Controls

Cardiovascular variables

Each experiment included four control periods. The haemodynamic variables common to groups I and II ($\dot{Q}_{pul}$, P_{RAo}, f_H) were compared between groups and during all control periods. These variables were not significantly different between groups I and II and were not different throughout the control periods. The peak systolic pressure in the CP ($P_{CP,sys}$), the mean pulmonary artery pressure (P_{PA}) and pulmonary vascular resistance (R_{pul}) in group I were not significantly different during the controls. For presentation purposes, all control values were combined. Control values for the cardiovascular variables are summarized in Table 1.

Blood gases

The P_{O_2} values from LAt, RAt, RAo and LAo during the control periods are summarized in Table 2. The P_{O_2} of the LAt was not different from those of the RAo or LAo. The RAt P_{O_2} was significantly less than the corresponding LAt, RAo and LAo values.

H_2 indicator patterns

Infusion of H_2 into the LAt was detected in both aortic arches (LAo and RAo) and the

Table 1. *Haemodynamic variables during control periods in* Pseudemys scripta

Mass (kg)	$\dot{Q}_{pul}$ (ml min^{-1} kg^{-1})	P_{PA} (kPa)	R_{pul} (kPa ml^{-1} min^{-1} kg^{-1})	$P_{CP,sys}$ (kPa)	P_{RAo} (kPa)	f_H (beats min^{-1})
1.7±0.3	57.4±11.6	3.6±0.7*	0.06±0.01*	3.9±0.8†	4.2±1.1	44±4

Values represent means ± S.D. Values marked by asterisks were obtained only for group II (N=5). Values marked by daggers were obtained only for group I (N=9). All other values represent a combination of both groups I and II.

$\dot{Q}_{pul}$, total pulmonary blood flow; P_{PA}, mean pulmonary arterial pressure; R_{pul}, pulmonary vascular resistance; $P_{CP,sys}$, peak systolic pressure in the cavum pulmonale; P_{RAo}, mean central arterial pressure; f_H, heart rate.

Table 2. P_{O_2}, P_{CO_2} *and pH measured in* Pseudemys scripta *from four central vascular sites during control periods*

	P_{O_2} (kPa)	P_{CO_2} (kPa)	pH
LAt	17.4±1.5	3.2±0.3	7.69±0.06
RAo	16.8±0.2	3.1±0.1	7.71±0.004
LAo	17.0±1.9	3.0±1.0	7.71±0.06
RAt	7.5±0.9	3.3±0.4	7.70±0.06

Values represent mean ± S.D. (N=5).

LAt, left atrium; RAo, right aortic arch; LAo, left aortic arch; RAt, right atrium.

PA for almost all experimental animals. In one animal, infusion of H_2 into the LAt was not detected in the LAo. Infusion of H_2 into the CP was detected only in the PA. Infusion of H_2 into the JV was detected in both the RAo and LAo in five of eight animals. A summary of the H_2 infusion results is given in Table 3. Experimental records of H_2 detection under control conditions are shown in Fig. 5.

Right vagal efferent stimulation

Cardiovascular variables

During right vagal efferent (RVEF) stimulation, f_H decreased from 44±4 beats min^{-1} to 15±3 beats min^{-1} (Fig. 3). $\dot{Q}_{pul}$ was reduced by approximately 73 %, decreasing to 15.4±6.8 ml min^{-1} kg^{-1} (Fig. 3). P_{PA} was reduced by approximately 30 %, decreasing to 2.5±0.8 kPa. During RVEF stimulation, there was a 158 % increase in R_{pul} to a value of 0.16±0.03 kPa ml^{-1} min^{-1} kg^{-1} (Fig. 3). RVEF stimulation also reduced P_{RAo} by 30 % to 2.9±1.1 kPa. In contrast, RVEF stimulation did not affect $P_{CP,sys}$, which was 3.2±0.8 kPa.

Blood gases

The LAt and RAt P_{O_2} values were not affected by RVEF stimulation. In contrast, RVEF stimulation produced a significant reduction in P_{O_2} in both the RAo and LAo. The

Table 3. *Detection of the H_2 signal in the aortic arches and pulmonary artery following injection of H_2 saline into left atrium, jugular vein and cavum pulmonale*

	Experimental condition and detection site											
	Control			RVEF			LVEF			RVAF		
Injection site	RAo +/–	LAo +/–	PA +/–	RAo +/–	LAo +/–	PA +/–	RAo +/–	LAo +/–	PA +/–	RAo +/–	LAo +/–	PA +/–
LAt	9/0	7/1	6/0	9/0	7/1	6/0	7/0	7/0	4/0	5/0	5/0	3/0
JV	5/3	5/3	5/0	7/1	8/0	5/0	4/3	5/2	4/0	0/6	0/6	3/0
CP	0/9	0/8	6/0	1/8	2/6	6/0	1/7	2/5	5/0	0/6	0/5	3/0

(+) H_2 detected, (−) H_2 not detected.

The sum of the +/– values is equal to the total number of animals and infusions at that site.

RVEF, right vagal efferent stimulation; LVEF, left vagal efferent stimulation; RVAF, right vagal afferent stimulation; LAt, left atrium; JV, jugular vein; CP, cavum pulmonale; other abbreviations are defined in Table 2.

RAo P_{O_2} was reduced to 14.9±1.1 kPa and LAo P_{O_2} decreased to 11.7±2.3 kPa. These changes in central vascular P_{O_2} values resulted in a significant increase in the P_{O_2} gradient between the LAt and the systemic arteries (RAo and LAo) (Fig. 4).

H_2 indicator patterns

Infusion of H_2 tracer into the LAt was detected in both aortic arches and the PA for almost all experimental animals. In one animal, infusion of H_2 into the LAt was not detected in the LAo. Infusion of H_2 into the JV was detected in the LAo of all animals and in the RAo of seven animals. Infusion of H_2 into the CP was always detected in the PA. Finally, infusion of H_2 into the CP was detected in the RAo in one animal and in the LAo of two animals. The occurrence of the H_2 signal in the systemic arches, following infusion into the CP, was not significantly different from the control pattern. These experiments are summarized in Table 3. An experimental record of the H_2 detection during RVEF stimulation is shown in Fig. 5.

Left vagal efferent stimulation

Cardiovascular variables

The effects of left vagal efferent (LVEF) stimulation on f_H, $\dot{Q}_{pul}$ and R_{pul} are summarized in Fig. 3. Stimulation of the LVEF resulted in an 11 % reduction in f_H. The reduction in f_H was less than the decrease resulting from RVEF stimulation (39 beats min^{-1} *versus* 15 beats min^{-1}). $\dot{Q}_{pul}$ was reduced by approximately 50 % from control values, decreasing to 29.8±8.2 ml min^{-1} kg^{-1}. This reduction in $\dot{Q}_{pul}$ was less than the reduction in $\dot{Q}_{pul}$ resulting from RVEF stimulation. P_{PA} was also reduced by 30 % (P_{PA}=2.5±0.5 kPa). LVEF stimulation resulted in a 36 % increase in R_{pul}, which increased to 0.08±0.02 kPa ml^{-1} min^{-1} kg^{-1}. LVEF stimulation reduced P_{RAo} by 30 % to 2.9±1.1 kPa. There was no significant difference between LVEF and RVEF stimulation on P_{RAo}, P_{PA} or $P_{CP,sys}$.

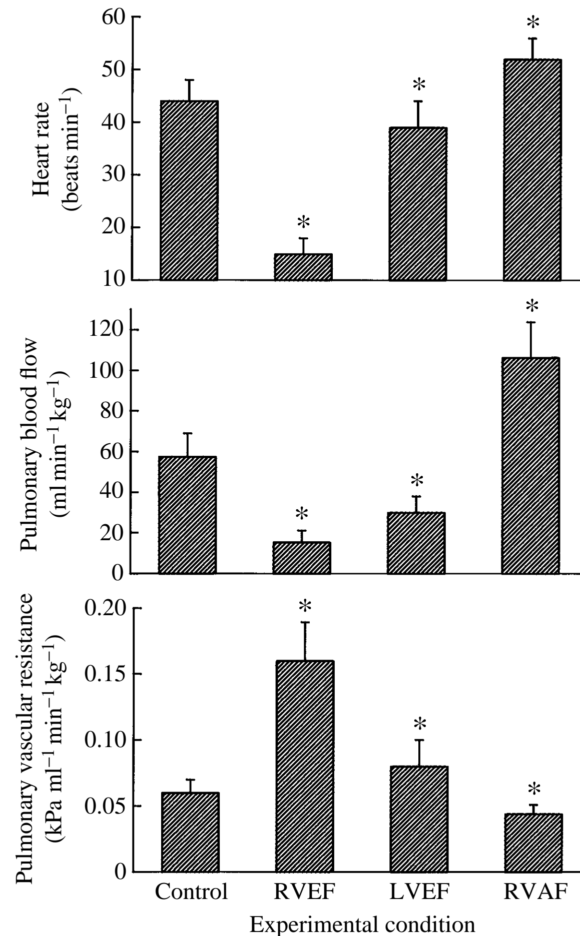

Fig. 3. Summary of the effects of vagal nerve stimulation on heart rate (f_H), pulmonary blood flow ($\dot{Q}_{pul}$) and pulmonary vascular resistance (R_{pul}). All values represent mean + S.D., N=5 for R_{pul} and N=14 for $\dot{Q}_{pul}$ and f_H. Asterisks denote values that are significantly different (P<0.05) from control. Abbreviations for experimental conditions are explained in the text.

Blood gases

LVEF stimulation did not affect central vascular P_{O_2} levels. Neither the LAt nor the RAt P_{O_2} values were affected by LVEF stimulation. There was no significant decrease in the RAo or LAo P_{O_2} (Fig. 4). Analysis of central vascular P_{O_2} values suggested that a R–L shunt was not present during this period.

H_2 indicator patterns

During LVEF stimulation, the patterns of detection of H_2 were not significantly different from control patterns (Table 3). Infusion of H_2 into the LAt was detected in both aortic arches (LAo and RAo) and the PA for all experimental animals. The pattern of detection of H_2 tracer following infusion into the JV was not significantly different from

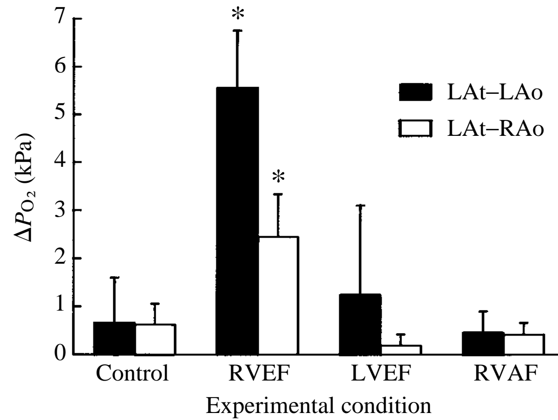

Fig. 4. The effect of vagal nerve stimulation on the P_{O_2} gradient between the left atrium (LAt) and the left aorta (LAo) and right aorta (RAo). The values represent the mean + S.D., N=5. Asterisks indicate values that are significantly different from control.

control values. Infusion of H_2 into the CP was always detected in the PA and was detected in the RAo of one animal and in the LAo of two animals.

Right vagal afferent stimulation

Cardiovascular variables

The effects of right vagal afferent (RVAF) stimulation on f_H, $\dot{Q}_{pul}$ and R_{pul} are shown in Fig. 3. Stimulation of the RVAF produced a significant tachycardia. f_H increased by approximately 20 % to 52±4 beats min^{-1}. $\dot{Q}_{pul}$ approximately doubled from control values, increasing to 106.4±17.5 ml min^{-1} kg^{-1}. P_{PA} increased by 30 % to 4.7±1.2 kPa, and R_{pul} decreased by approximately 30 % to 0.044±0.007 kPa ml^{-1} min^{-1} kg^{-1}. P_{RAo} increased by approximately 50 % during RVAF stimulation to a value of 6.4±1.4 kPa. Finally, RVAF stimulation increased $P_{CP,sys}$ to 6.2±0.8 kPa. All values of f_H, $\dot{Q}_{pul}$, P_{PA}, P_{RAo}, R_{pul} and $P_{CP,sys}$ during RVAF stimulation were significantly different from the RVEF and LVEF values.

Blood gases

RVAF stimulation did not affect central vascular P_{O_2} levels. The LAt and RAt P_{O_2} values were unaffected by RVAF stimulation. Analysis of central vascular P_{O_2} values suggested that a R–L shunt was not present during this period (Fig. 4).

H_2 indicator patterns

H_2 infused into the LAt was detected in both aortic arches (LAo and RAo) and in the PA in all experimental animals. During RVAF stimulation, infusion of H_2 into the JV was never detected in either the RAo or LAo. This was significantly different from the patterns recorded during control, RVEF and LVEF stimulation. Infusion of H_2 tracer into

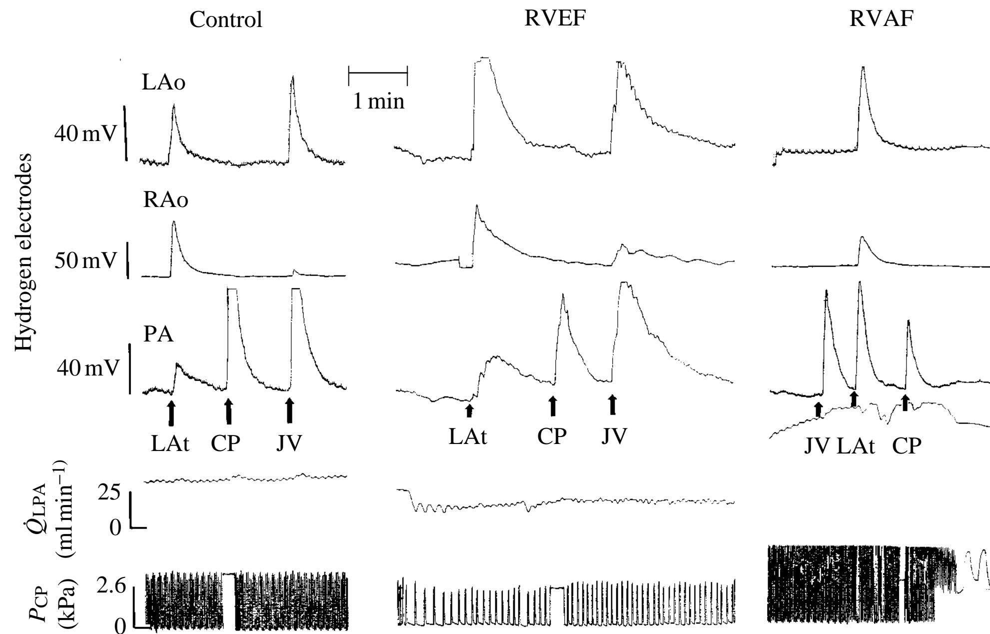

Fig. 5. The H_2 signals (mV) in the left aortic arch (LAo), right aortic arch (RAo) and pulmonary artery (PA). In addition, the mean blood flow through the left pulmonary artery ($\dot{Q}_{LPA}$) and pressure within the cavum pulmonale (P_{CP}) are measured. Recordings are from a 1.8 kg turtle under control conditions and during right vagal efferent stimulation (RVEF) and right vagal afferent stimulation (RVAF). Infusion of H_2 into the left atrium (LAt), cavum pulmonale (CP) and jugular vein (JV) is shown by the arrows. Note the change in the order of injection during RVAF. For all injections into the CP, note the disruption of the pressure signal as the catheter is switched from a pressure port to an infusion port. Also note that during the RVAF stimulation period the CP catheter is drawn back into the PA to indicate the PA pressure. Finally, during RVEF, note the reduction in $\dot{Q}_{LPA}$ and the reduction in f_H. In contrast, note the increase in $\dot{Q}_{LPA}$ and the increase in f_H during RVAF.

the CP was always detected in the PA. An experimental record of H_2 detection during RVAF stimulation is included in Fig. 5.

Discussion

This study showed that, in the turtle *Pseudemys scripta,* blood from the CP was not the primary source of deoxygenated blood ejected into the RAo or LAo during R–L intracardiac shunting. This conclusion was supported by our inability to detect a significant increase in the appearance of H_2 in the LAo or RAo following injection into the CP during RVEF stimulation (Table 3). These results confirm the conclusions of a previous study (Hicks and Malvin, 1992), which suggested that the muscular ridge effectively separated the CP from the CV and CA during systole. The results strongly support the washout hypothesis for R–L intracardiac shunting.

We used an invasive surgical preparation that may have altered cardiac function.

Specifically, the placement of flow probes and the insertion of catheters and H_2 electrodes within the heart chambers and great vessels may have adversely affected the cardiovascular dynamics. However, the range of values measured for P_{RAo}, P_{PA}, $P_{CP,sys}$ and $\dot{Q}_{pul}$ were similar to the values reported for other chelonians. In the turtles *Chrysemys scripta*, *Chelydra serpentina, Testudo gracea* and *Testudo pardalis*, the mean P_{RAo} and P_{PA} ranged from 1.7 to 3.8 kPa (Steggarda and Essex, 1957; White and Ross, 1966; Shelton and Burggren, 1976). $P_{CP,sys}$ in turtles ranged from 3.3 to 4.8 kPa (Shelton and Burggren, 1976) and $\dot{Q}_{pul}$ varied from 14 ml min^{-1} kg^{-1} during diving to more than 35 ml min^{-1} kg^{-1} during ventilation (Shelton and Burggren, 1976). The similarity between the cardiovascular variables measured in our study and the values reported in previous studies suggests that the invasive nature of our procedures did not seriously depress cardiac function.

The use of H_2 electrodes for the assessment of intracardiac shunting has been well characterized (Clark and Bargeron, 1959; Clark *et al.* 1960). The electrical potential that is obtained from such electrodes depends on the partial pressure of H_2 near the electrode and on the surface area of the platinum wire. In our study, the electrical potential obtained from the electrodes was proportional to the amount of H_2 injected and inversely proportional to the flow rate. The electrodes were very sensitive and could detect the smallest amount of H_2 (0.19 μmol) at the highest flow rate (30 ml min^{-1}). This made it unlikely that during RVEF and LVEF stimulation, when systemic blood flows would be at their lowest (Comeau, 1992), H_2 flowing from the CP into the systemic arteries would go undetected.

During control conditions, five of the animals exhibited a H_2 signal in the systemic arteries (RAo and LAo) following infusion into the JV. In addition, H_2 infused into the CP was never detected in the RAo or LAo during controls. These results suggested that a R–L shunt was present and that the source of systemic venous blood in the RAo and LAo was the RAt. This supported the washout hypothesis. In contrast, in group II, the analysis of blood P_{O_2} suggested that the values for RAo and LAo were not significantly different from the LAt values. This suggested that a R–L shunt was not present during control conditions. There was, therefore, a discrepancy between the results of the two methods used to analyze intracardiac shunts. In summary, the H_2 method detected a R–L shunt under control, RVEF- and LVEF-stimulated conditions, but not during RVAF stimulation. The O_2 method detected a R–L shunt only during RVEF stimulation. It is unlikely that the H_2 electrodes would have provided a signal without being exposed to H_2. Consequently, the H_2 method would not have falsely identified a R–L shunt. The discrepancy between the results for groups I and II may have revealed a difference in the ability of the H_2 and O_2 methods to detect small R–L shunts. To enhance the sensitivity of the O_2 method, the animals were pump-ventilated with an inspired gas mixture that ensured a high lung P_{O_2}. At these P_{O_2} values, the blood leaving the lungs would be almost fully saturated and the blood P_{O_2} would be on the plateau portion of the oxygen dissociation curve (Maginiss *et al.* 1980). In our study, this is demonstrated by the LAt P_{O_2}, which was much higher than one might expect in conscious animals. As a result, any mixing of systemic venous blood with the pulmonary venous blood should have produced a large decrease in the P_{O_2} of the RAo and LAo. The discrepancy between groups I and II

suggested that, even under these conditions, the O_2 method was not capable of detecting small R–L shunts. However, it is possible that if simultaneous measurements of H_2 and O_2 were made there would have been better agreement between the two methods. A recent study (Hicks and Malvin, 1992) reported that five out of twelve turtles exhibited a R–L shunt during similar control conditions. In that study, the use of a mass spectrometer permitted the simultaneous measurements of blood P_{O_2} and a tracer gas (He) in the LAt, RAt, RAo and LAo. In all five cases in which the tracer gas that was infused into the RAt was detected in the systemic arteries, the blood P_{O_2} measurements also indicated a R–L shunt.

Many intermittent lung breathers exhibit a cardiorespiratory synchrony. In *Pseudemys scripta*, f_H and $\dot{Q}_{pul}$ measured during ventilation are 2–3 times higher than during apnoea (Burggren, 1975; Shelton and Burggren, 1976; White *et al.* 1989). In addition, changes in R_{pul} are coupled with ventilatory state, with R_{pul} increasing during apnoea (Shelton and Burggren, 1976). These cardiovascular changes and the spontaneous development of a R–L intracardiac shunt are thought to be under cholinergic control (White, 1976; Milsom *et al.* 1977). In our preparation, f_H, $\dot{Q}_{pul}$ and R_{pul} were not coupled with the respiratory state. The absence of cardiorespiratory synchrony probably indicated a reduction in vagal tone resulting from the anaesthesia. Nembutal is known to have vagolytic properties (Rall, 1990). In our study, however, electrical stimulation of the vagus nerves produced changes in f_h, R_{pul} and $\dot{Q}_{pul}$. This suggested that the cholinergic modulation of these variables was still intact. The reductions in f_H, P_{PA}, $\dot{Q}_{pul}$ and the increase in R_{pul} during RVEF stimulation were similar to the values recorded in turtles during diving. In diving turtles, *Pseudemys scripta*, f_H was 11 beats min^{-1}, P_{PA} was 1.5 kPA, $\dot{Q}_{pul}$ was 11 ml min^{-1} kg^{-1} and R_{pul} was 0.14 kPa ml^{-1} min^{-1} kg^{-1} (Shelton and Burggren, 1976).

Stimulation of the RVEF resulted in a significant reduction in the P_{O_2} of both the LAo and RAo, whereas the P_{O_2} values of the LAt and RAt remained constant. Interestingly, the reduction in LAo P_{O_2} was greater than the reduction in RAo P_{O_2}. This suggested that the systemic venous blood was differentially distributed between the systemic arches. A similar distribution of deoxygenated blood into the systemic arches has been shown to occur in the lizard *Varanus niloticus* (Ishimatsu *et al.* 1988).

The reductions in arterial P_{O_2} during RVEF stimulation suggested the development of a R–L shunt. In the turtle, electrical stimulation of the vagus nerve resulted in a reduction in the LAo P_{O_2} (Burggren, 1978). A recent study of *Pseudemys scripta* showed that an intravenous infusion of acetylcholine (ACh) resulted in a reduction of both the RAo and LAo P_{O_2} values, although the LAt and RAt P_{O_2} values remained constant (Hicks and Malvin, 1992). The reductions in arterial P_{O_2} during vagal stimulation or following infusion of ACh support the suggestion that the development of a R–L shunt is under cholinergic control. Reductions in arterial P_{O_2} values have also been reported during voluntary diving in the turtle, *Pseudemys scripta* (White *et al.* 1989). The reductions in arterial P_{O_2} occurred within minutes following the onset of a dive and continued throughout submergence, although the LAt and RAt P_{O_2} values remained at pre-dive levels. It was concluded that the changes in arterial P_{O_2} resulted from an increased level of R–L shunting during the dive (White *et al.* 1989).

During RVEF stimulation, the infusion of H_2 into the JV resulted in the detection of a

H_2 signal in either the RAo or the LAo of all animals studied. In contrast, a signal was detected in the systemic arteries in only two of eight animals following infusion of H_2 into the CP. This showed that the primary source of systemic venous blood shunted into the arterial circulation was the RAt and not the CP. These results supported the washout hypothesis. H_2 infused into the LAt was always detected in the PA, suggesting a L–R shunt. Although the mechanism is unknown, bidirectional intracardiac shunting during both ventilation and apnoea has been reported in lizards (Heisler *et al.* 1983; Ishimatsu *et al.* 1988) and turtles (Heisler and Glass, 1985; White *et al.* 1989).

LVEF and RVEF stimulation produced similar cardiovascular changes. However, LVEF-induced changes were of smaller magnitude than those produced by RVEF stimulation. In several preliminary experiments, doubling the LVEF stimulus intensity did not produce cardiovascular changes similar to those during RVEF stimulation. The differences between RVEF and LVEF stimulation on the f_H response may reflect the differential distribution of vagal efferent fibres to the heart. It has been suggested that in chelonians the sinus venosus, RAt and LAt are primarily innervated by the right vagus. In contrast, the ventricle is primarily innervated by the left vagus (see Burggren, 1985).

During LVEF stimulation, the detection of H_2 in the systemic arches following its infusion into the JV was not significantly different from the control condition. In addition, there was a discrepancy between the H_2 and O_2 methods. Analysis of the H_2 method indicated a R–L shunt, with the primary source of blood being the RAt. This supported the washout hypothesis. In contrast, analysis of the LAt, RAo, LAo and RAt P_{O_2} values suggested that a R–L shunt did not develop. As discussed above, simultaneous measurements of both H_2 and O_2 could have clarified the discrepancy between these two methods.

During RVAF stimulation, f_H, P_{PA}, $P_{CP,sys}$, P_{RAo} and $\dot{Q}_{pul}$ increased and R_{pul} was decreased. In turtles, sympathetic fibres join the vagus nerve and run towards the heart, forming the vagosympathetic trunk (Burnstock, 1969). Stimulation of the intact cervical vagus nerve could therefore produce an adrenergic response. In our study, however, the cervical vagus was bilaterally sectioned. Therefore, the exact mechanism or neural pathway that produced these cardiovascular responses is unknown. A previous study of turtles has suggested that stimulation of pulmonary vagal afferents induces changes in f_H and $\dot{Q}_{pul}$ (Johansen *et al.* 1977). A reduction in the lung volume resulted in a bradycardia and a reduction in $\dot{Q}_{pul}$. In contrast, reinflation of the lung resulted in an increase in f_H and $\dot{Q}_{pul}$. A recent study has shown that stimulation of the vagus nerve produces a pulmonary vasoconstriction in the rat snake *Elaphe obseleta* (Donald *et al.* 1990). This was followed by a pulmonary vasodilation during the post-stimulatory period, which was unaffected by the administration of atropine but was eliminated following administration of propranolol. It was concluded that the changes in R_{pul} resulted from the reciprocal interplay of cholinergic vasoconstriction and adrenergic vasodilation (Donald *et al.* 1990). In our study, the changes in f_H, $\dot{Q}_{pul}$ and R_{pul} may have resulted from an adrenergic mechanism. Using a similar animal preparation and experimental protocol, Comeau (1992) reported that the infusion of adrenaline ($0.1\,\mu g\,kg^{-1}$) resulted in a 30 % increase in f_H, a doubling of $\dot{Q}_{pul}$ and a 30 % decrease in R_{pul}. Preliminary evidence has suggested that the cardiovascular changes during RVAF stimulation were prevented by propranolol

(J. W. Hicks, in preparation). Further studies are required to describe fully the mechanisms and neural pathways responsible for the cardiovascular changes observed during RVAF stimulation.

H_2 infused into the JV was never detected in the RAo or LAo during RVAF stimulation, suggesting that the R–L shunt was abolished under these conditions. A similar response has been reported in the turtle *Pseudemys scripta* following administration of adrenaline (Hicks and Malvin, 1992). The elimination of R–L shunting under these conditions would have two important functional consequences. First, the aortic P_{O_2} and oxygen content would increase during this period when O_2 demands may be increased. Second, the elimination of the R–L shunt would ensure that all of the venous return was directed into the pulmonary circulation. These effects could have important consequences for pulmonary O_2 and CO_2 exchange (J. W. Hicks, in preparation).

The mechanism responsible for the disappearance of the H_2 signal from the systemic circulation during RVAF stimulation is unknown, but there are four distinct possibilities. First, the amount of H_2 ejected from the CV into the systemic arteries may have been too small to detect. In our study, the H_2 electrodes gave a smaller response as the amount of H_2 decreased and flow increased (Fig. 2). During RVAF stimulation, the end-diastolic volume of the CV may have been reduced and blood flows elevated. Therefore, a H_2 signal may not have been detectable. Second, the disappearance of the H_2 signal may have resulted from an alteration in the diastolic filling pattern of the CV and CP during RVAF stimulation. During control conditions, some RAt blood normally filled the CV. However, during RVAF stimulation, the RAt blood may have been passed directly into the CP, essentially bypassing the CV. An analysis of the ventricular anatomy has previously suggested the potential for this type of diastolic filling pattern (Van Mierop and Kutsche, 1985). Third, the diastolic filling of the CA and CV may have been altered by RVAF stimulation when $\dot{Q}_{pul}$ was very high. Under these conditions, the high pulmonary venous return could have filled both the CA and CV during diastole and displaced RAt blood from the CV into the CP. Finally, the disappearance of the H_2 signal may have resulted from changes in the systolic ejection pattern. In the turtle heart, ejection of blood from the CP into the pulmonary artery precedes the ejection of blood into systemic arteries. The timing difference can be as much as 150 ms (Shelton and Burggren, 1976). A reduction in R_{pul}, during RVAF stimulation, may have increased the timing differential. This could have caused a significant volume of the CV and CA blood to be translocated around the muscular ridge into the CP and ejected into the PA. It was not possible to determine which of these mechanisms was responsible for the elimination of the R–L shunt. Additional studies are required to examine the temporal relationship of ventricular ejection of blood into the systemic and pulmonary circulations.

H_2 infused into the LAt was always detected in the PA, under all experimental conditions. This provided direct evidence for a L–R shunt. Systemic blood flow ($\dot{Q}_{sys}$) and PA blood oxygen content were not measured, so the magnitude of the L–R shunt could not be measured. The mechanism for L–R shunting could not be determined from this study. The washout hypothesis predicts that the L–R shunt results from the volume of blood in the CV at the end of systole, which fills the CP during the subsequent diastole.

An increase in the L–R shunt would be associated with an increase in the end-systolic volume of the CV. In contrast, the pressure hypothesis predicts that during the early phase of systole some blood in the CV and CA is translocated around the muscular ridge into the CP and pulmonary circulation. In our study, $\dot{Q}_{pul}$ during RVAF stimulation almost doubled. Recent studies in this turtle have suggested that, during RVAF stimulation, a large L–R shunt developed (Comeau, 1992). It seems unlikely that all of the increased $\dot{Q}_{pul}$ and the large L–R shunt were caused by a washout mechanism. Under certain conditions, therefore, both pressure and washout shunting may contribute to cardiac function in reptiles. Further experiments are needed to resolve this question.

This study was supported by the National Science Foundation Grant, DCB-9004570, to J.W.H. The authors express their gratitude to Ms Elizabeth Heinz for assisting in these experiments.

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