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THE MECHANISM OF CARDIAC SHUNTING IN REPTILES: A NEW SYNTHESIS

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Summary

The mechanism of cardiac shunting in reptiles is controversial. Recent evidence suggests that a right-to-left shunt in turtles results primarily from a washout mechanism. The mechanism that accounts for left-to-right (L–R) shunting is unresolved. This study used haemodynamic analysis and digital subtraction angiography to determine the mechanism of L–R cardiac shunting in the turtle *Trachemys* (*Pseudemys*) *scripta*. Animals were instrumented with ultrasonic blood flow probes (Transonic Systems, Inc.) for the measurement of total pulmonary blood flow and total systemic blood flow. In addition, catheters were inserted into the common pulmonary artery (PA), the systemic arteries, the left atrium and right atrium. These catheters were used for the measurement of blood pressure or for the infusion of radio-opaque material. Haemodynamic conditions were altered by electrical stimulation of the afferent (VAF) or efferent vagal nerves or by infusion of vasoactive drugs. Under control conditions, the peak systolic pressure in the systemic arteries was slightly higher than that in the PA (30.6 *versus* 28.3 mmHg; 4.08 *versus* 3.77 kPa), whereas diastolic pressure in the PA was significantly less than that in the systemic arteries (9.8 *versus* 24.4 mmHg; 1.31 *versus* 3.25 kPa). During VAF stimulation, the peak systolic pressures in the PA and aortae almost doubled. Diastolic pressure in the systemic arteries also doubled, but it increased by only 45 % in the PA. Ejection of blood into the PA preceded that into the left aorta by 53 ms under control conditions. This difference increased (by as much as 200 ms) as the difference in the diastolic pressures between the two circulations increased during VAF stimulation. This resulted in the development of a large net L–R shunt. Under these conditions, digital subtraction angiography showed that the L–R shunt resulted from a combination of both washout and pressure mechanisms.

Key words: turtle, intracardiac shunting, *Trachemys* (*Pseudemys*) *scripta*, pulmonary circulation, systemic circulation, digital subtraction angiography.

Introduction

In the chelonians (turtles) and squamates (lizards and snakes), the heart consists of two atria and three ventricular chambers. A distinctive anatomical feature of the ventricle is the presence of an incomplete septum called the muscular ridge (Van Mierop and Kutsche, 1981, 1985). This septum separates the ventricle into a large cavum dorsale and a smaller cavum pulmonale (CP). The cavum dorsale is further subdivided into the cavum venosum (CV) and cavum arteriosum (CA) by the presence of another septum, the vertical septum. However, a distinct vertical septum is not found in all reptiles (Van Mierop and Kutsche, 1985). Three outflow vessels emerge from the ventricle. The pulmonary artery originates from the CP and the two aortic arches, the right and left aortae (RAo, LAo), emerge from the CV.

The anatomy of the reptilian ventricle results in the potential for intracardiac shunting. Cardiac shunts are defined as right-to-left (R–L) or left-to-right (L–R). A R–L shunt represents a fraction of systemic venous blood that bypasses the pulmonary circulation and re-enters the systemic circulation. A L–R shunt represents a fraction of pulmonary venous blood recirculated

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This paper is dedicated to Mrs Sylvia Glage, who is fondly remembered by all who have worked in Göttingen.

into the pulmonary circulation. The mechanisms that cause intracardiac shunting in reptiles are controversial (Heisler *et al.* 1983; Heisler and Glass, 1985; Hicks and Malvin, 1992; Hicks and Comeau, 1994; Hicks, 1994), but two principal hypotheses termed pressure shunting and washout shunting have been put forward (Heisler and Glass, 1985).

The pressure shunting hypothesis states that the muscular ridge does not separate the CP from the CV or CA. Consequently, under the appropriate haemodynamic conditions, blood can flow between the ventricular chambers. For example, when the pulmonary vascular resistance increases relative to the systemic vascular resistance, some blood within the CP will be ejected around the muscular ridge, into the systemic circuit during systole (i.e. R–L shunt) (White and Ross, 1966; Shelton and Burggren, 1976). In contrast, if the pulmonary vascular resistance decreases relative to the systemic vascular resistance, then a fraction of blood within the CV and CA will be ejected, around the muscular ridge, into the CP and pulmonary artery (i.e. L–R shunt).

A central feature of the washout mechanism is that blood cannot flow between the CP and CV/CA during the systolic phase of the cardiac cycle. Cardiac shunting results instead from the volume of blood within the CV at the end of diastole or at the end of systole being transposed into the alternative circulation. During diastole, blood from the right atrium fills both the CP and CV. During ventricular systole, blood within the CA is ejected through the CV into the aortic arches. Consequently, the R–L shunt is dependent on the volume of blood remaining in the CV at the end of diastole and the volume of blood ejected from the CA during systole (Heisler *et al.* 1983). The ratio of these volumes determines the O_2 level of systemic arterial blood. At the end of systole, the CV is filled with blood from the CA. This end-systolic blood, which is of pulmonary venous origin, is washed from the CV into the CP during the subsequent diastole. This increases the O_2 level of pulmonary arterial blood and accounts for the L–R shunt. The volume of blood remaining in the CV at the end of systole and thus the size of the L–R shunt will change with the end-systolic volume and the systemic venous return. Variability in intracardiac shunting between individuals and during different physiological states can be accounted for by the ratio of stroke volume to the volume of the CV during the cardiac cycle (Heisler *et al.* 1983). These volumes may be subject to modulation by changes in preload, afterload or contractility of the heart (Heisler and Glass, 1985).

In the turtle *Trachemys* (*Pseudemys*) *scripta*, R–L shunting is accounted for by the washout mechanism (Hicks and Malvin, 1992; Hicks and Comeau, 1994). In these animals, a R–L shunt is induced by intravenous injection of acetylcholine or by electrical stimulation of the vagus nerves. During these conditions, an indicator substance (He or H_2), injected into the CP, is not detected in the aorta. Therefore, the muscular ridge effectively separates the pulmonary and systemic circulations (Hicks and Malvin, 1992; Hicks and Comeau, 1994).

A large L–R shunt in *Trachemys* (*Pseudemys*) *scripta* results from electrical stimulation of vagal afferent nerves and from intravenous infusion of epinephrine (Comeau and Hicks, 1994), but the mechanism responsible is unresolved. The purpose of the present study was to determine the mechanism of L–R intracardiac shunting in the turtle, utilizing a combination of digital subtraction angiography and measurement of blood flow in the pulmonary artery and the aortae.

Materials and methods

Animals

Studies were performed on commercially obtained (Lemberger, Oshkosh, WI, USA) red-eared turtles *Trachemys* (*Pseudemys*) *scripta* Gray, ranging in body mass from 1.41 to 2.1 kg (mean ± S.D.=1.65±0.23 kg, N=7) airfreighted to the laboratories in Germany and California. They were kept in large aquaria equipped with dry basking areas and infrared lamps to allow behavioural regulation of body temperature. Water temperature in the aquaria was set at 25 °C. Animals were regularly fed chopped beef liver and chicken meat. Food was withheld for 3 days before experimentation.

Haemodynamic studies

Surgery

Anaesthesia was induced as previously described by Hicks and Malvin (1992). Animals were then tracheotomized and mechanically ventilated (12–20 min^{-1}) with a humidified gas mixture of 17 % O_2, 3 % CO_2 and 80 % N_2 prepared using a gas-mixing pump (Wösthoff, Bochum, Germany). A rectangular opening (approximately 3 cm×4 cm) was cut in the plastron using a mechanical bone saw to expose the heart and central blood vessels. The plastron piece was carefully removed from the underlying musculature. Bleeding from the musculature was stopped by cauterization. Short sections of the central vessels were freed from the surrounding connective tissue and blood flow probes (2R, Transonic Systems, Inc., Ithaca, NY, USA) were placed around the vessels. In all experiments, the blood flow probes were placed on the left pulmonary artery (LPA), left aorta (LAo), the subclavian and common carotid arteries of one side (RSC) and the main trunk of the right aorta (RAo). The blood flow probes were connected to two dual-channel blood flow meters (T201, Transonic Systems, Inc., USA). Instantaneous blood flow signals from two sites (the LPA and one of the systemic arches) were displayed on an oscilloscope (Gould 20 MHz digital storage type 1421) to check the signals during experimentation. Five central vascular sites (PA, LAo, RAo, LAt and RAt) were non-occlusively catheterized in a manner similar to that described by White *et al.* (1989). The PA and LAo catheters were connected to pressure transducers (Statham P23), with the transducer maintained at the level of the heart. The analogue outputs from pressure transducers and flow meters were recorded on a chart recorder (Gould). Core body temperature was monitored by a small thermistor probe inserted into the cloaca of the animal. Finally, the right and left cervical vagus nerves were exposed and prepared for stimulation as described

by Hicks and Comeau (1994). Some animals showed vasoconstriction following probe or catheter placement and these animals were excluded from data analysis.

Protocols

During the experiment, animals were placed supine and were mechanically ventilated as described above. Five different conditions for vagal stimulation were tested, including measurement following vagus sectioning without any electrical stimulation (control), efferent stimulation of either the right (RVEF) or left (LVEF) vagus, and afferent stimulation of either side (RVAF and LVAF). Vagal stimulation was conducted at frequencies between 1 and 3 Hz, applying voltage of 4–8 V for a duration of 200 ms. Animals were allowed to stabilize between stimulation periods. In some animals, epinephrine (0.24 μg) or sodium nitroprusside (32 μg) was intravenously injected to evoke large changes in cardiovascular variables. Experiments were performed at room temperature maintained at 25±1 °C.

Statistical analyses

Analysis of variance with a randomized block design was used to detect differences in the measured haemodynamic variables (heart rate, blood flow, blood pressure and vascular resistance) during the experimental conditions. Dunnett and Bonferroni tests were used for pairwise comparison as appropriate. Several additional relationships between the following physiological variables were analyzed. The ejection timing difference between LPA and LAo, or the net cardiac shunt, was correlated with the diastolic pressure difference between the PA and LAo. In addition, the diastolic pressure difference between the PA and LAo, or the net cardiac shunt, was correlated with the ratio of pulmonary to systemic vascular resistance (R_{pul}/R_{sys}). For each animal, the physiological variable of interest was regressed either on the diastolic pressure difference or on R_{pul}/R_{sys} and the functions were compared by analysis of covariance. Significance was at the P=0.05 level. Data are expressed as mean ± S.D.

Digital subtraction angiography

Surgery

Animals were prepared for surgery as described above. Transient-time ultrasonic blood flow probes (2R, Transonic Systems, Inc., Ithaca, NY, USA) were placed on the LPA and LAo. Catheters (PE50) for the injection of radio-opaque material were inserted into the left atrium (LAt), right atrium (RAt), cavum arteriosum (CA) or cavum pulmonale (CP). The catheters were positioned under fluoroscopy and their position was confirmed by *post-mortem* examination. The animals were heparinized (0.2 ml, 1000 i.u. ml^{-1} infused intravenously) every 30 min to prevent blood clotting during experimentation.

Image acquisition and processing

All images were acquired using a conventional X-ray tube (Dynamax 79-45/120, Machlett Laboratories, Stamford, CT, USA), a constant potential X-ray generator (Optimus M200, Philips Medical Systems, Shelton, CT, USA), a 23/15 cm CsI image intensifier (II), a focused grid (8:1 grid ratio, 36 lines cm^{-1}) and a CCD (charge coupled device) camera (Multicam MC-1134GN, Texas Instruments Inc., Dallas, TX, USA). Light intensity in front of the camera was controlled by an adjustable aperture. The video signal was linearly digitized to 512×512×8-bit precision and the image processing was performed using a Fischer DA-100 image processor (Fischer Imaging, Denver, CO, USA). Images were acquired using a 60 kVp X-ray beam, the 15 cm II mode, the small X-ray tube focal spot (0.6 mm nominal) and a magnification of 2.0.

A four-channel digitizer (LeCroy 8013A, LeCroy, Pleasonton, CA, USA) was employed to record the instantaneous and mean volumetric flow signals, the kVp waveform and the injector signal simultaneously. The signals were digitized at 100 Hz and stored using a microcomputer (Data386, Data World, Los Angeles, CA, USA). Approximately 100 images were acquired at 30 frames s^{-1}. Temporal subtraction images were formed after logarithmic transformation.

Protocol

Contrast material (Hexabric, Mallinckrodt Medical Inc., St Louis, MO, USA; 320 mg I ml^{-1}, organically bound iodine) was infused (0.4 ml at 1 ml s^{-1}) into the cardiac sites under control conditions and during electrical stimulation of the vagal afferent nerves.

Results

Blood flow, heart rate and vascular resistances

Haemodynamic variables prior to nerve stimulation or other experimental manipulations are summarized in Table 1. The effects of vagal nerve stimulation on $\dot{Q}_p$, $\dot{Q}_s$, heart rate (f_H), R_{pul} and R_{sys} are shown in Fig. 1. The results were qualitatively similar to those previously described in detail (Comeau and Hicks, 1994; Hicks and Comeau, 1994). RVEF stimulation resulted in a significant bradycardia (P<0.0001). In contrast, RVEF stimulation caused a 164 % increase in R_{pul}, an increase that was significantly different from the control measurement (P=0.02). There was no significant effect of RVEF stimulation on R_{sys} (P=0.072). The combined effects of reduced f_H and changes in vascular resistances resulted in significant reductions in $\dot{Q}_p$ (P=0.003) and $\dot{Q}_s$ (P=0.0027) during RVEF stimulation (Fig. 1A). The haemodynamic changes during LVEF stimulation were qualitatively similar, although f_H was reduced by only 15 %. This small reduction in f_H was significant (P=0.020). Electrical stimulation of the vagal afferents resulted in changes in the systemic and pulmonary circulation that were different from those produced by efferent stimulation. RVAF and LVAF stimulation resulted in a small tachycardia (P=0.0446; Fig. 1B), a doubling of R_{sys} (P<0.0001), but no significant change in R_{pul} (P=0.167). During RVAF and LVAF stimulation, there was no change in $\dot{Q}_s$, but there was a twofold increase in $\dot{Q}_p$ (P=0.0046; Fig. 1A).

Table 1. *Haemodynamic variables for the turtle* Trachemys (Pseudemys) scripta

Haemodynamic variable	Value	Units
Blood flow		
Systemic, $\dot{Q}_s$	19.5±6.4	ml min^{-1} kg^{-1}
Pulmonary, $\dot{Q}_p$	42.3±14.4	ml min^{-1} kg^{-1}
Blood pressure		
Systemic, P_{sys}		
Systolic	30.6 (4.08)±10.6 (1.42)	mmHg (kPa)
Diastolic	24.4 (3.25)±9.8 (1.31)	mmHg (kPa)
Pulmonary, P_{pul}		
Systolic	28.3 (3.77)±10.5 (1.40)	mmHg (kPa)
Diastolic	9.8 (1.31)±3.9 (0.52)	mmHg (kPa)
Vascular resistance		
Systemic, R_{sys}	1.12±0.3	mmHg ml^{-1} min^{-1} kg^{-1}
	(0.15±0.04)	(kPa ml^{-1} min^{-1} kg^{-1})
Pulmonary, R_{pul}	0.37±0.06	mmHg ml^{-1} min^{-1} kg^{-1}
	(0.05±0.008)	(kPa ml^{-1} min^{-1} kg^{-1})
Heart rate	43.2±3.9	min^{-1}
Stroke volume		
Systemic	0.44±0.11	ml kg^{-1}
Pulmonary	0.93±0.29	ml kg^{-1}
Ejection timing,		
LPA–LAo	53±19	ms

Values are means ± S.D., N=7.

Systolic and diastolic blood pressures

Systolic pressure in the aorta was slightly higher than in the pulmonary artery under control conditions (Table 1). This difference was significant (P<0.01). Electrical stimulation of the vagal afferent nerves or infusion of epinephrine increased the systolic pressures in these two vessels by approximately 75 %. In contrast, electrical stimulation of the vagal efferent nerves or infusion of sodium nitroprusside reduced the systolic pressures in these two vessels by as much as 75 %. The systolic pressures, over these ranges, were different (Fig. 2), with the systolic pressures in the aorta being higher than those in the pulmonary artery (P=0.0019).

Under control conditions, diastolic pressure in the pulmonary artery was lower than that in the aorta (Table 1). Electrical stimulation of the vagal afferents and epinephrine infusion increased diastolic pressure in the aorta by as much as 100 %, whereas diastolic pressure in the pulmonary artery increased by only 45 % (Fig. 2). Under these conditions, the diastolic pressure in the pulmonary artery was significantly lower than that in the aorta (P<0.0001). In contrast, electrical stimulation of vagal efferent nerves or infusion of nitroprusside lowered blood pressure in the pulmonary artery and aortae, but diastolic pressures in the aorta remained, on average, higher than those in the pulmonary artery (P<0.001) (Fig. 2).

Instantaneous blood flow in the pulmonary artery and aorta

Ejection of blood into the pulmonary artery preceded ejection into the aorta during control conditions (52.8±19.1 ms; N=7). This interval was correlated with differences in diastolic pressures between the PA and the LAo (Fig. 3). The slopes for the individual animals did not differ (P=0.8162) and the average slope was significantly different from zero (P<0.0001). This relationship indicated that, as the diastolic pressure in the LAo became greater than the diastolic pressure in the PA, the interval between the ejection of blood into the PA and LAo increased (Fig. 3). It is of interest to note that in an extreme case the diastolic pressure in the LAo was less than that in the PA (following nitroprusside injection into the systemic circulation). Under these conditions, the ejection of

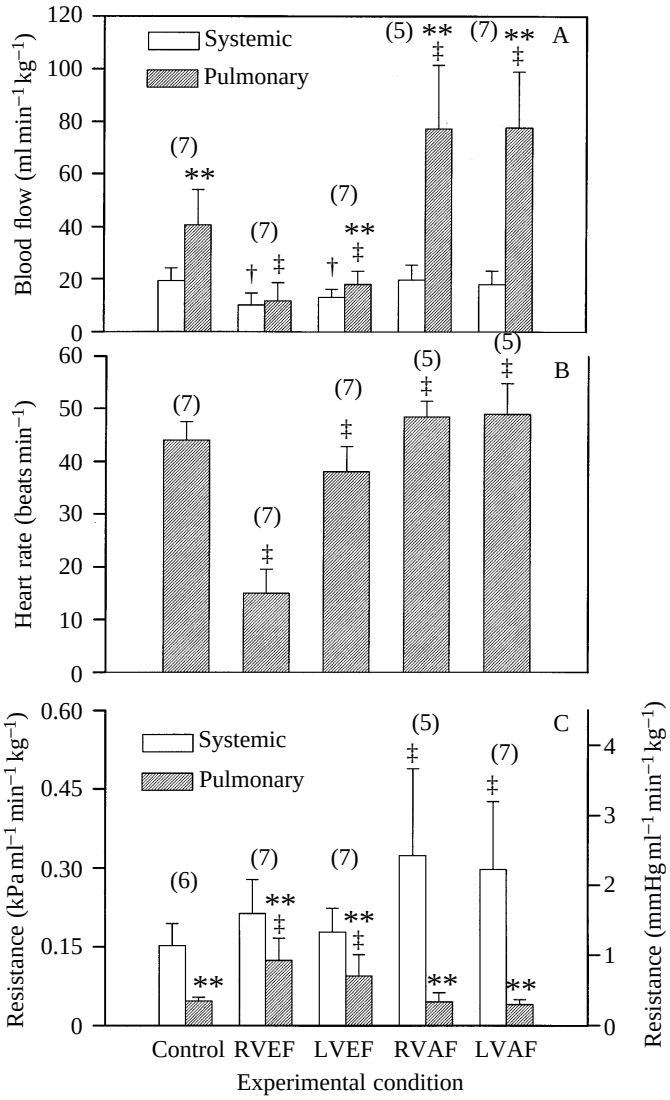

Fig. 1. The effects of electrical stimulation of vagal efferents (RVEF and LVEF) and vagal afferents (RVAF and LVAF) on selected cardiovascular parameters in the turtle *Trachemys* (*Pseudemys*) *scripta.* (A) Effects of vagal stimulation on pulmonary and systemic blood flow. (B) Effects of vagal stimulation on heart rate (f_H). (C) Effects of vagal stimulation on pulmonary and systemic vascular resistance (R_{pul} and R_{sys}). **Significantly different from systemic value; †,‡significantly different from control value. See text for P values. All values represent means + S.D.; values of N are given in parentheses.

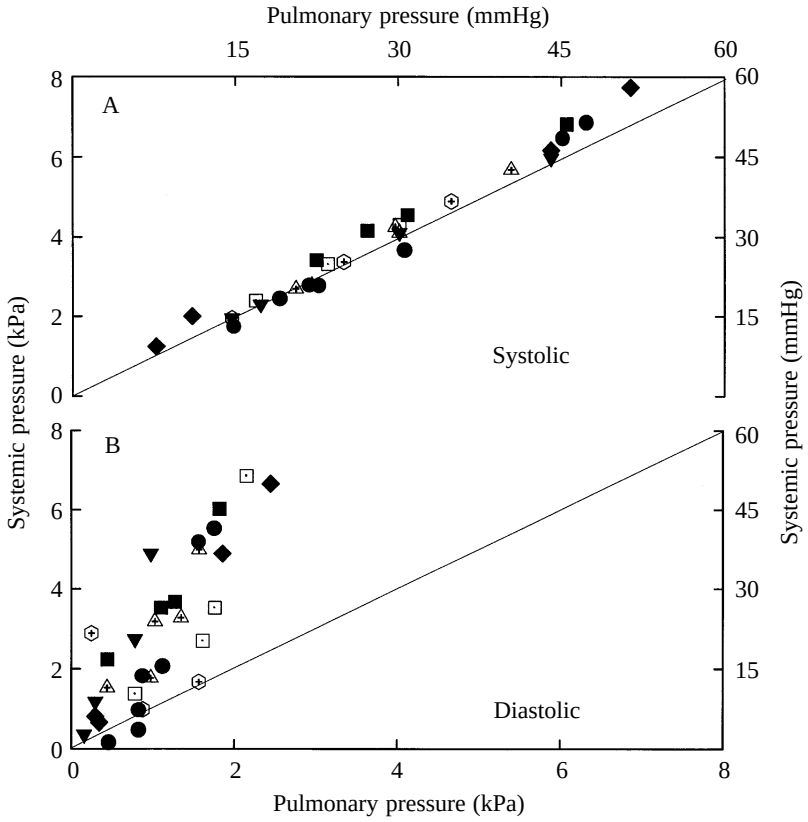

Fig. 2. (A) The relationship between peak systolic blood pressure in the pulmonary artery (abscissa) and peak systolic pressure in the left aortic arch (ordinate) in the turtle *Trachemys* (*Pseudemys*) *scripta*, treated with vagal stimulation, epinephrine or nitroprusside infusion. The solid line is the line of identity. (B) The relationship between minimum diastolic pressure in the pulmonary artery (abscissa) and minimum diastolic pressure in the left aortic arch (ordinate). Same treatments as for A. The solid line is the line of identity. Different symbols represent different animals (N=7). See text for description of correlation analysis.

blood into the LAo preceded ejection into the PA by approximately 40 ms (Fig. 3) and a small net R–L shunt developed.

In general, the difference in diastolic pressure was influenced by the ratio of pulmonary vascular resistance to systemic vascular resistance (R_{pul}/R_{sys}) (Fig. 4). The slopes for the individual animals were not different (P=0.7134) and the average slope was significantly different from zero (P<0.001). This correlation indicated that when the pulmonary vascular resistance (R_{pul}) decreased relative to the systemic vascular resistance (R_{sys}), i.e. R_{pul}/R_{sys} was reduced, the difference in diastolic pressure between PA and LAo became larger (Fig. 4).

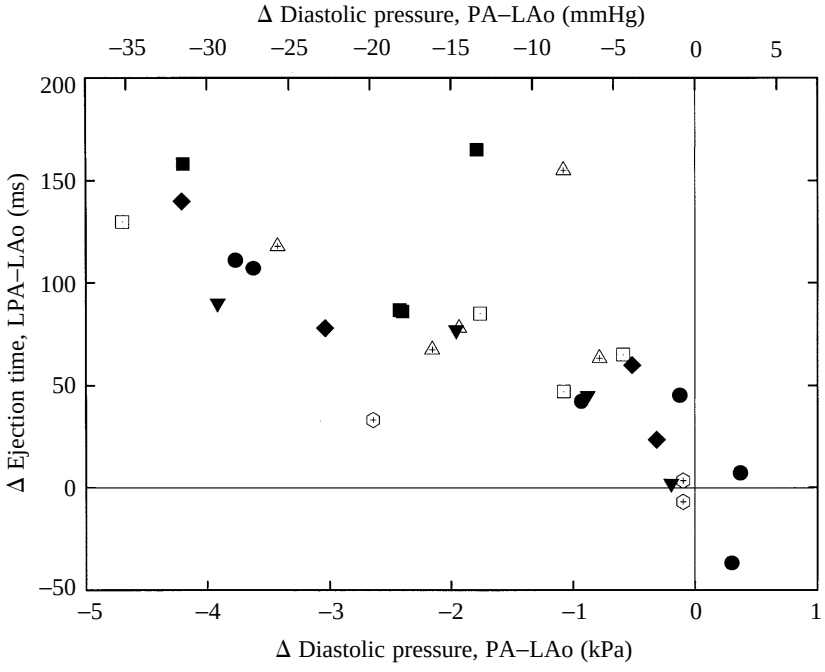

Fig. 3. Relationship between the difference in diastolic pressure in the pulmonary artery (PA) and the left aortic arch (LAo) and the interval between ejection of blood into the left pulmonary artery (LPA) and into LAo. Different symbols represent different animals (N=7). See text for description of correlation analysis.

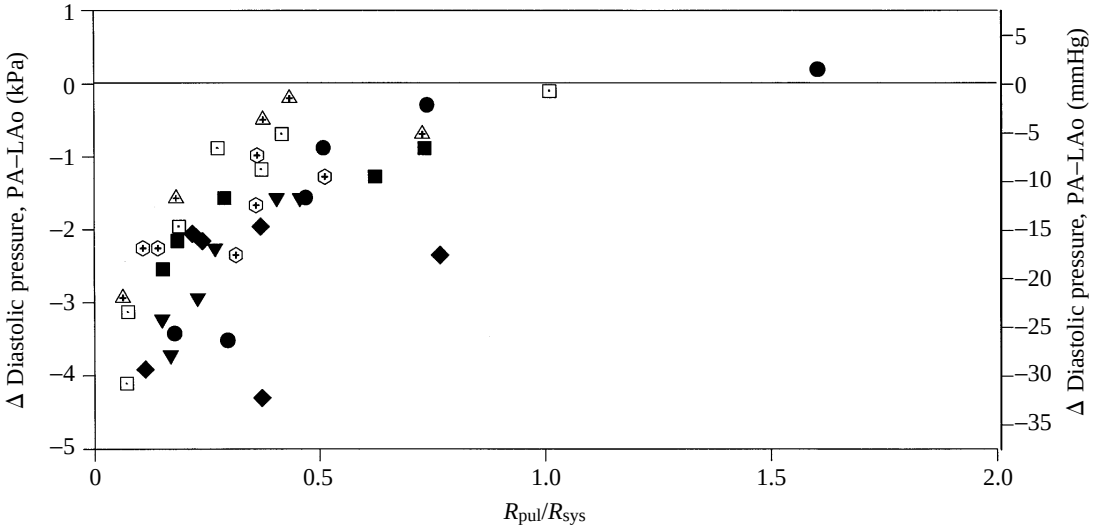

Fig. 4. The effects of changing the ratio of pulmonary vascular resistance to systemic vascular resistance (R_{pul}/R_{sys}) on the diastolic pressure gradient between the pulmonary artery (PA) and the left aortic arch (LAo). Different symbols represent different animals (N=7). See text for description of correlation analysis.

The volume of blood ejected into the LPA prior to ejection of blood into the LAo was defined as Q_{pre}. The volume of blood ejected into the LPA after ejection had started into the LAo was defined as Q_{post} (Fig. 5). The fractional contribution of Q_{pre} to the total blood volume flowing through the LPA (Q_{pre}+Q_{post}) increased as the timing difference between the pulmonary artery and aorta increased (Fig. 5). At very large timing differences, as much as 20 % of the blood flowing into the LPA was ejected prior to ejection of blood into the LAo (Fig. 5).

Net cardiac shunts

Total cardiac output ($\dot{Q}_{tot}$=$\dot{Q}_p$+$\dot{Q}_s$) was varied by vagal nerve stimulation or infusion of epinephrine. As $\dot{Q}_{tot}$ increased, a net L–R shunt accounted for approximately 80 % of $\dot{Q}_p$ (Fig. 6). At very low $\dot{Q}_{tot}$, induced by electrical stimulation of vagal efferent nerves, $\dot{Q}_p$ and $\dot{Q}_s$ were equal or a small net R–L shunt developed (Fig. 6).

The level of net cardiac shunting was related to the timing difference between ejection of blood into the LPA and the LAo (Fig. 7). The slopes for the individual animals did not differ (P=0.0759) and the average slope was significantly different from zero (P<0.001). This relationship indicated that, as timing difference increased, the size of the net L–R shunt increased (Fig. 7). In a few cases, for example during RVEF stimulation or following nitroprusside injection, the timing difference was reversed (ejection of blood into the LAo before the LPA) and a small net R–L shunt developed (Fig. 7). Finally, the size of the net cardiac shunt was correlated with the ratio, R_{pul}/R_{sys}. As with the other relationships, the slopes for the individual animals were not different (P=0.8345) and the average slope was significantly different from zero (P<0.001). This relationship indicated that as R_{pul}/R_{sys} decreased a net L–R shunt developed (Fig. 8).

Digital subtraction angiography

Injection of radio-opaque substance into the RAt preferentially filled the CP during diastole (Fig. 9A). During the subsequent systole, the marker was observed to flow into

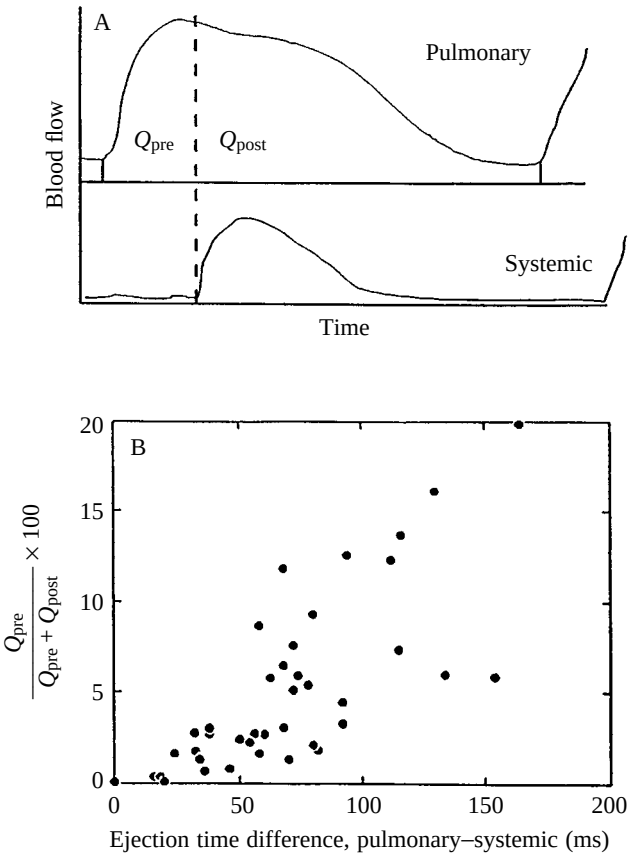

Fig. 5. (A) Typical instantaneous flow traces for the pulmonary artery and left aortic arch. Q_{pre} is defined as the volume of blood (area under the curve) ejected into the pulmonary artery prior to the ejection of blood into the left aortic arch. Q_{post} is defined as the volume of blood ejected into the pulmonary artery after blood has started to flow into the aortic arch. (B) The percentage contribution of Q_{pre} to the total volume of pulmonary blood ejected as a function of ejection time difference between the pulmonary artery and the left aortic arch.

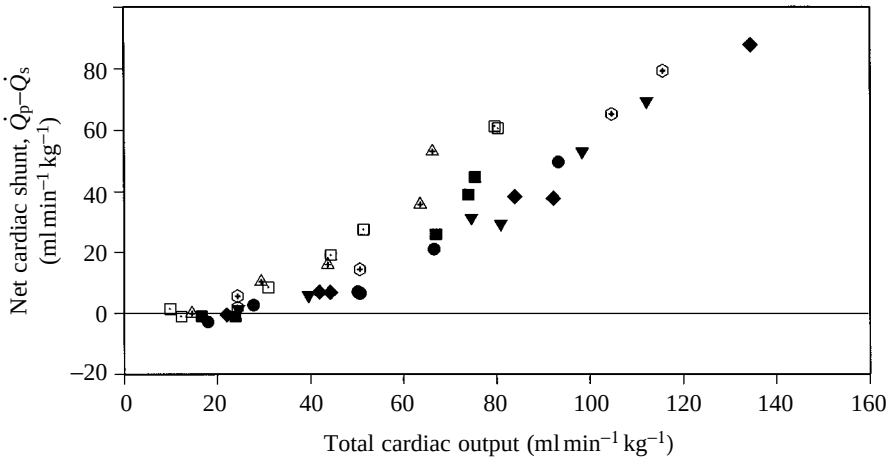

Fig. 6. The relationship between total cardiac output and the net cardiac shunt ($\dot{Q}_p-\dot{Q}_s$). If $\dot{Q}_p-\dot{Q}_s$ is greater than zero, then there is a net L–R shunt. Values below zero represent a net R–L shunt. Note that the majority of the increase in total cardiac output is accounted for by an increase in net L–R shunt. Different symbols represent different animals (N=7).

the pulmonary artery (Fig. 9B). In our preparation, no contrast material was seen to enter the aortic arches from the CP. Under control conditions, contrast material in the LAt filled the CA during diastole (Fig. 9C). During the subsequent systole, contrast material was ejected through the interventricular canal and filled the systemic arterial tree, which included both the RAo and the LAo (Fig. 9D). During the next diastole, contrast material in the CV and CA flowed into the CP.

Demonstrating the relative contribution of the CA and CV to the PA blood flow during conditions that promote L–R shunting was technically difficult. This required that radio-opaque material be injected into the ventricle just prior to the beginning of the systolic phase. Normally, the systolic phase of the cardiac cycle represents approximately 20–30 % of the total cycle. If contrast material is injected into the CA and CV during late systole, the flow of blood is directed towards the aortic arches only. During the next cardiac cycle, the radio-opaque material remaining at the end of systole is washed into the CP. Additionally, if the contrast material is injected during early to mid diastole, it is translocated into the CP. While these results demonstrate the contribution of the washout mechanism, they provide little insight into the contribution of the CA and CV to pulmonary blood flow. Our experience indicated that it was critical to inject contrast material into the CA and CV at the end of diastole, just prior to systole. The short time available for injection of the material made successful injection technically difficult to achieve.

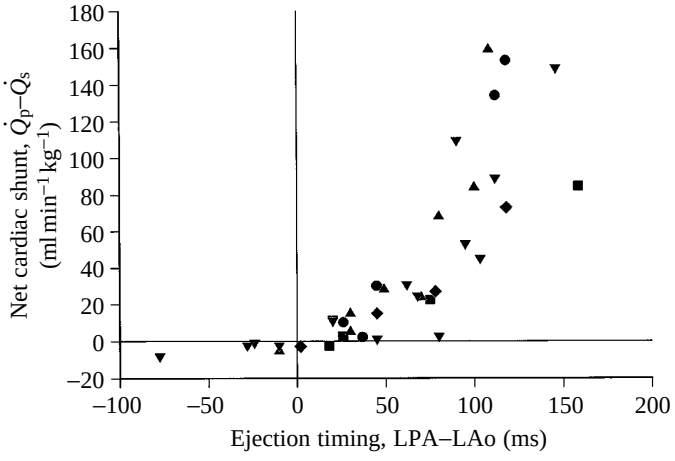

Fig. 7. The effects of changing the timing of ejection into the LPA and LAo on the level of net cardiac shunting ($\dot{Q}_p-\dot{Q}_s$). A $\dot{Q}_p-\dot{Q}_s$ value greater than zero represents a net L–R shunt and a value below zero represents a net R–L shunt. Note that, as the ejection interval becomes greater, the level of net L–R shunting is increased. Also note that when ejection of blood into the LAo preceded ejection into the LPA (ejection timing values are negative) there was a net R–L shunt. Different symbols represent different animals (N=6). See text for description of correlation analysis.

An example of a successful injection during vagal afferent stimulation is shown in Fig. 10, with a sequence of X-ray images corresponding to the instantaneous flow traces in the same animal during injection of contrast material. The first image shows contrast in the CA and CV just prior to systolic ejection. The next image, representing the upstroke of the instantaneous flow trace in the LPA, shows contrast material beginning to move into the PA. The third and fourth images, corresponding to the peak instantaneous flow signal, clearly show that radio-opaque material is now in the PA. At this point, flow is just beginning in the LAo. The last image corresponds to the diastolic filling period and shows contrast material entering the CP.

Discussion

This study has indicated that under certain conditions diastolic pressure differences between the pulmonary and systemic circulations may have important functional consequences for ventricular flow patterns in the turtle. These pressure differences are correlated with the vascular resistances in the pulmonary and systemic circulations and affect the timing of blood ejection into the pulmonary artery and the aortic arches. The time interval between blood ejection into these two circulations influences the size and direction of net cardiac shunting. During haemodynamic conditions that

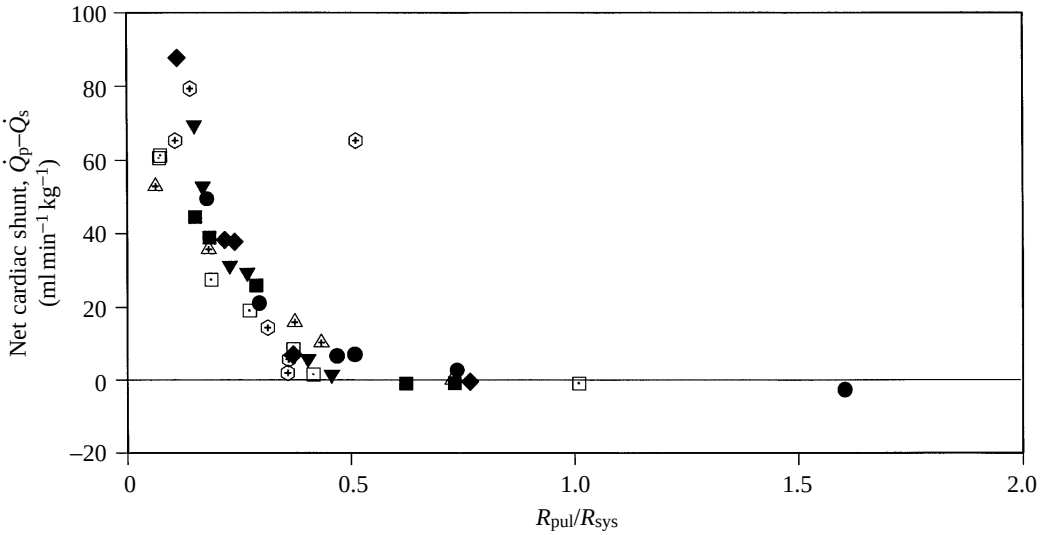

Fig. 8. The effects of changing the vascular resistance in the pulmonary and sytemic circulations (R_{pul}/R_{sys}) on the level of net cardiac shunting ($\dot{Q}_p-\dot{Q}_s$). A $\dot{Q}_p-\dot{Q}_s$ value greater than zero represents a net L–R shunt and a value less than zero represents a net R–L shunt. Different symbols represent different animals (N=7).

promote net a L–R shunt, both washout and pressure difference mechanisms contribute to intracardiac shunting.

The cardiovascular responses that we observed are similar to those previously measured in a similar preparation. The mean $\dot{Q}_p$ measured under control conditions (42.3±14.4 ml min^{-1} kg^{-1}) was slightly larger than the $\dot{Q}_p$ (33.8±6.7 ml min^{-1} kg^{-1}) reported by Comeau and Hicks (1994). In contrast, the mean $\dot{Q}_p$ in our study was lower than the $\dot{Q}_p$ (57.4±11.6 ml min^{-1} kg^{-1}) measured by Hicks and Comeau (1994). R_{pul}, f_H and total cardiac output measured in the present study were close to those of previous studies. In all cases, cardiovascular variables measured in our acute preparation were in good agreement with data from animals fitted with devices for long-term measurement. In studies on *Chrysemys scripta*, *Chelydra serpentina*, *Testudo graeca* and *Testudo pardalis*, the mean values of P_{sys} and P_{pul} were found to range from 12.7 to 28.5 mmHg (1.7–3.8 kPa; Steggerda and Essex, 1957; White and Ross, 1966; Shelton and Burggren, 1976). In these studies, $\dot{Q}_p$ varied from 14 ml min^{-1} kg^{-1} during diving to more than 35 ml min^{-1} kg^{-1} during periods of ventilation (Shelton and Burggren, 1976). The close similarity in cardiovascular variables reported in this paper and previous studies suggests that the invasive nature of the present preparations does not seriously depress cardiac function.

The effects of vagal nerve stimulation on cardiovascular dynamics in *Trachemys* (*Pseudemys*) *scripta* have been described in detail (Hicks and Comeau, 1994; Comeau and Hicks, 1994; Hicks, 1994) and are qualitatively confirmed by our study. Electrical stimulation of the vagal efferent nerves resulted in cholinergically mediated effects (Comeau and Hicks, 1994; Hicks, 1994): a pronounced bradycardia, reductions of $\dot{Q}_s$ and $\dot{Q}_p$, increases in R_{pul} and R_{sys} and the development of a small net R–L shunt. In contrast, vagal afferent nerve stimulation resulted in tachycardia, a twofold increase in R_{sys}, a reduction in R_{pul} and, while $\dot{Q}_s$ was unchanged, $\dot{Q}_p$ doubled. The neural pathways responsible for these changes are unknown, but the response is adrenergically mediated, as indicated by the lack of response following treatment with bretylium (Comeau and Hicks, 1994).

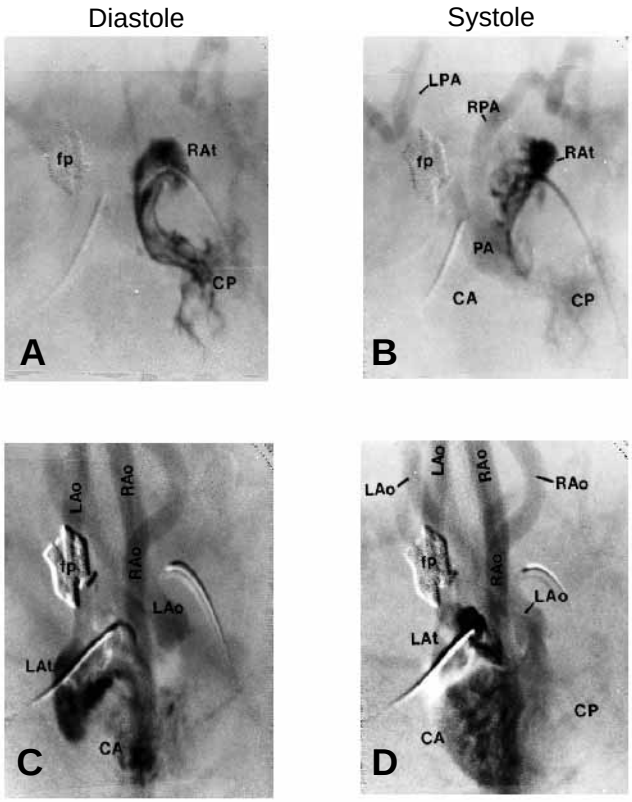

Fig. 9. (A) Injection of radio-opaque material into the right atrium (RAt) during diastole. Note the flow of opaque material from the RAt into the cavum pulmonale (CP). (B) During systole, note the flow of opaque material from the CP into the pulmonary artery (PA). (C) Injection of contrast material during diastole into the left atrium (LAt). Note the flow of radio-opaque material into the cavum arteriosum (CA). (D) During the subsequent systole, note the increase in contrast material in the right aortic arch (RAo) and left aortic arch (LAo). LPA, left pulmonary artery; RPA, right pulmonary artery; RAt, right atrium; fp, ultrasonic flow probe.

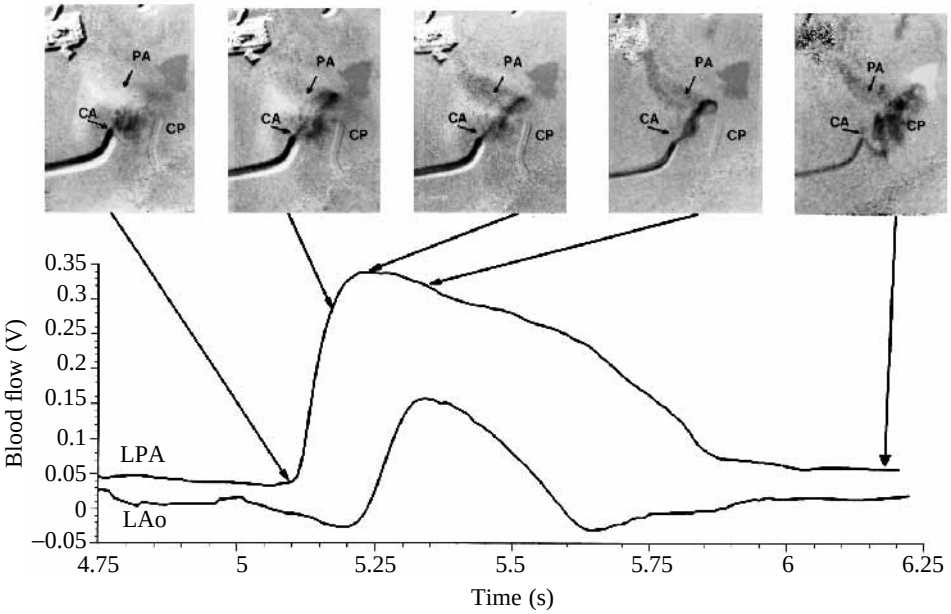

Fig. 10. Injection of radio-opaque material into the cavum arteriosum (CA) during vagal afferent nerve stimulation. The lower traces represent the instantaneous blood flows into the left pulmonary artery (LPA) and left aortic arch (LAo). The upper panels represent sequential X-ray images, with arrows connecting them to the corresponding stage of the instantaneous flow cycle. Note the catheter in the CA, filled with radio-opaque material. Also note that the ordinate is given as a voltage. CP, cavum pulmonale; PA, pulmonary artery.

In Chelonia, the rate of ventricular pressure development is the same in all ventricular subchambers (Steggerda and Essex, 1957; Shelton and Burggen, 1976; Burggren *et al.* 1977; Burggren, 1977*a*). Slight differences have been reported in previous studies of turtles, with the pulmonary pressure being 0.7–5.6 mmHg (0.09–0.75 kPa) lower than the systemic pressure (Steggerda and Essex, 1957; White and Ross, 1966; Johansen *et al.* 1970; Shelton and Burggren, 1976). Our study confirms these earlier measurements. The slight depression in pulmonary artery pressure is attributable to a small band of cardiac muscle wrapped around the base of the pulmonary artery, called the bulbus cordis (Johansen and Burggren, 1980). Contraction of this small band, during late ventricular systole, may significantly narrow the pulmonary outflow tract and accordingly increase the resistance from the CP to the pulmonary artery. The effects of the bulbus cordis on pulmonary pressure have been well documented *in vitro* (Burggren, 1977*a*). In addition to the slight systolic pressure difference during control conditions, in the present study the peak systolic pressure in the aorta remained slightly higher even when pressure was varied over a fourfold range.

In the present study, diastolic pressure in the aorta under control conditions was 2.5 times larger than that in the pulmonary artery, an observation well supported by other studies in reptiles, including chelonians (Steggerda and Essex, 1957; Johansen *et al.* 1970; Shelton and Burggren, 1976; White and Ross, 1966), snakes (Johansen, 1959; Burggren, 1977*b*) and lizards (White, 1968; Johansen and Burggren, 1984; Ishimatsu *et al.* 1988). Although their central circulatory anatomy differs, several amphibians show a similarly lower diastolic pressure in the pulmocutaneous compared with the systemic arteries (Shelton and Jones, 1965, 1968).

At any given blood flow and f_H, diastolic pressure is a function of the *in vivo* compliance of the blood vessels and the vascular resistance. The present study shows a positive correlation of diastolic pressure with relative resistances between the pulmonary and systemic circulations (R_{pul}/R_{sys}). When R_{pul}/R_{sys} was reduced, either by increasing R_{sys} or by reducing R_{pul}, the difference in diastolic pressure gradient between the PA and LAo increased. In contrast, as R_{pul}/R_{sys} was increased, either by reducing R_{sys} or by increasing R_{pul}, the diastolic pressure difference decreased. Interestingly, intravenous infusion of nitroprusside resulted in the development of a large R_{pul}/R_{sys} (>3) in one case, causing a reversal of the diastolic pressure difference.

Different diastolic pressures between the pulmonary artery and the aorta have important haemodynamic consequences, in particular during ventricular systole. As systole proceeds, the lower diastolic pressure in the pulmonary artery results in the opening of the pulmonary valve before the aortic valves, leading to ejection of blood into the pulmonary artery before ejection into the aortae. Such a dichotomy has been reported for snakes, in which the systolic pulmonary pressure starts to rise 40–100 ms before the pressure rise in the aorta (*Tripodonotus natrix*, 40–50 ms, Johansen, 1959; *Thamnophis* sp., 100 ms, Burggren, 1977*b*), and in *Trachemys* (*Pseudemys*) *scripta* fitted with long-term recording devices blood flow in the pulmonary artery achieves maximal velocity almost 100 ms prior to the ejection of blood into the aorta (Shelton and Burggren, 1976). The present study confirms this, with ejection of blood into the pulmonary artery beginning 53 ms prior to aortic ejection under control conditions. The magnitude of the aortic ejection delay is positively correlated with the difference in diastolic pressures between pulmonary and systemic vessels, with increasing diastolic pressure differences leading to a progressively longer delay before the start of aortic ejection. Reversal of the diastolic pressure difference reverses the ejection interval, so that blood is expelled into the aorta before expulsion into the pulmonary artery.

The anatomy of the reptilian heart, coupled with the observed differences in ejection timing, results in the development of cardiac shunts. In reptiles, $\dot{Q}_p$ does not always equal $\dot{Q}_s$, with the difference being termed the net cardiac shunt (Hicks, 1994). If $\dot{Q}_p$ is larger than $\dot{Q}_s$, then a net L–R shunt occurs, while the reverse applies for net R–L shunts. After the suggestion of Johansen (1959) that the ejection of blood into the PA prior to ejection into the aortic arches favours the development of a L–R shunt, the present study provides the first quantitative description of this relationship. The level of intracardiac L–R shunting increased in parallel with the ejection interval (i.e. the time by which pulmonary ejection preceded systemic ejection), with negative values for the ejection interval eventually resulting in a small net R–L shunt. A similar relationship has been reported in amphibians (Jones and Shelton, 1972).

The present results have clarified the haemodynamic conditions promoting cardiac shunting. An important factor is the timing of ejection into the pulmonary and systemic arteries, which is a function of the diastolic pressures in these two circuits. These diastolic pressure differences are, in turn, determined by the relative resistances (R_{pul}/R_{sys}) between the two circuits. Size and direction of net cardiac shunting is correlated with the timing of ejection into the pulmonary and systemic arteries. Specifically, if the ejection of blood into the pulmonary circuit precedes the ejection of blood into the systemic circuit, the L–R shunt will increase in size. In contrast, if the systemic ejection precedes the pulmonary ejection, net R–L shunting will occur.

The mechanism of the left-to-right shunt

Our study has described the haemodynamic conditions that are correlated with cardiac shunting in turtle ventricles. There are two principal hypotheses that specifically address the mechanisms of intracardiac shunting: the washout mechanism and the pressure difference mechanism (Heisler and Glass, 1985), which have previously been discussed in detail (Heisler *et al.* 1983; Heisler and Glass, 1985; Hicks and Malvin, 1992; Hicks and Comeau, 1994). The essential difference between these two mechanisms is the functional separation of the CP from the CV and CA during systole by the muscular ridge.

Direct tests of the washout mechanism have been limited to a few species. In the tegu lizard *Tupinambis teguixin*, angiography indicates that a radio-opaque substance injected into the CP during apnoea can flow into both the PA and LAo (Johansen *et al.* 1987), apparently supporting the pressure difference mechanism for R–L shunting. However, in that study, the injection catheter was advanced into the CP *via* the right atrium, which could have affected the functional separation of the CP from the CV by the muscular ridge. In contrast, recent studies have indicated that the R–L shunt developing during vagal nerve stimulation or following acetylcholine injection results from washout of the cavum venosum (Hicks and Malvin, 1992; Hicks and Comeau, 1994). Indicator substances (H_2 or He) injected into the CP during periods of R–L shunting could not be detected in the aortic arches, suggesting that blood from the CP did not contribute to the reduction in arterial oxygen saturation during R–L shunting. In the present study, a net R–L shunt developed when the diastolic pressure in the pulmonary artery exceeded that in the aortic arch and ejection of blood into the aorta preceded ejection of blood into the pulmonary artery. Under these specific haemodynamic conditions, it is possible that blood from within the CP is translocated around the muscular ridge and ejected into the aortic arches. Further experiments are required to test this hypothesis directly.

During stimulation of vagal afferent fibres or following injection of epinephrine, a large net L–R cardiac shunt developed. Tracer substance (H_2) injected into the left atrium (LAt) during vagal afferent nerve stimulation is detected in the PA (Hicks and Comeau, 1994, using a preparation similar to that described in this study). This indicates that blood from the LAt is contributing to the observed L–R shunt. However, the indicator technique cannot distinguish between the washout mechanism and the pressure mechanism. In the present study, we used digital subtraction angiography to demonstrate that, under haemodynamic conditions promoting L–R shunting, blood from the CA and CV is ejected into the PA during the early phase of systole. In addition, during diastole, the tracer was shown to flow from the CA and CV into the CP. This clearly supports the hypothesis that L–R shunting results from a combination of the two mechanisms.

The mechanism of cardiac shunting: a new synthesis

Our results led us to propose a new mechanism for cardiac shunting in reptiles. This mechanism amalgamates important aspects of both the washout and pressure difference hypotheses and asserts that they are not mutually exclusive. It also emphasizes the influence of peripheral vascular factors on the dynamic flow events that occur in the ventricle during the cardiac cycle.

During diastole, the CA is filled from the LAt with blood returning from the pulmonary circulation. Concurrently, blood from the RAt flows into the CV, around the muscular ridge and fills the CP. As blood flows from the atria into the ventricular chambers, mixing may be reduced by the atrio-ventricular valves. At the end of diastole, the CV is filled with systemic venous (oxygen-poor) blood and the CA is filled with pulmonary venous (oxygen-rich) blood. As systole begins, the flow of blood within the heart will be determined by the myocardial contractility and peripheral vascular factors. An increase in R_{sys} and/or a reduction in R_{pul} will result in the pulmonary diastolic pressure being lower than the diastolic pressure in the aortic arches. Under these conditions, the pulmonary valves will open before the aortic valves during ventricular systole. Consequently, the ejection of blood into the pulmonary artery starts before the ejection of blood into the aortic arches. This may promote the flow of blood from the CV around the muscular ridge into the CP and the PA. A moderate translocation of blood in this direction will reduce the R–L shunt by reducing the amount of poorly oxygenated blood in the CV, which will be expelled with the main flow of highly

oxygenated blood from the CA as soon as the aortic valves open. However, if as a result of large diastolic pressure differences the flow of blood flowing around the muscular ridge is large enough, then even highly oxygenated blood from the CA may be translocated into the pulmonary circulation, creating a larger L–R shunt.

As systole continues, the muscular ridge will press against the dorsal ventricular wall, occluding the transfer path for pressure difference shunting. The volume of blood that is translocated from the CV and CA into the CP and PA will be determined by (1) the timing difference for ejection between the PA and the aortic arches, which in turn is determined by the diastolic pressure difference between the two circuits, (2) the rate of ventricular pressure development (dP/dt), and (3) the resistance to flow offered by the pulmonary circuit.

In our study, digital subtraction angiography has indicated that there is a flow of blood from the CV into the pulmonary circulation during L–R shunting induced by vagal afferent nerve stimulation. Further support for the existence of this flow path comes from the data of Ishimatsu *et al.* (1996), showing a significant rise in P_{O_2} in both the LAo and RAo under the same conditions. Furthermore, a recent study showed that, during vagal afferent stimulation, an indicator substance (H_2) injected into the RAt is not transmitted into the aortic arches (Hicks and Comeau, 1994). This result suggests that, under haemodynamic conditions promoting net L–R shunting, blood remaining in the CV from diastole is not ejected into the systemic circulation, thus reducing the R–L shunt and improving the efficiency of respiratory gas exchange with the systemic tissues.

However, this advantageous effect will only occur when there is a difference between the pulmonary and the systemic diastolic pressures. The pressure difference can be reduced by a rise in R_{pul} or a fall in R_{sys}, while in the present study reduced diastolic pressure differences were produced by vagal efferent nerve stimulation. Under these conditions, the pulmonary and aortic valves will open almost simultaneously and ejection of blood into these two vessels occurs concurrently. This will prevent blood flowing from the CV and CA into the CP, thus allowing the end-diastolic volume of poorly oxygenated blood in the CV to be expelled into the systemic circulation. The resulting shunt is completely attributable to the washout mechanism.

Under extreme conditions, vascular resistances may be altered sufficiently to reverse the diastolic pressure difference so that pressure is lower in the aortae than in the PA. In the present study, this situation was provoked by injection of nitroprusside. When blood enters the aorta before the pulmonary artery, a reflux of blood from the CP around the muscular ridge and into the aortic arches may be possible. The volume of blood thus relocated from the CP will be a function of (1) rates of ventricular pressure development, (2) the diastolic pressure difference and (3) the resistance to blood flow in the aorta.

The mechanisms of cardiac shunting have been studied in only a few representatives of extant non-crocodilian reptiles. Given the degree of variability of the ventricular anatomy (Van Mierop and Kutsche, 1985), the degree of development of the muscular ridge (Van Mierop and Kutsche, 1981), and the varying physiological requirements of reptiles, it is not surprising to discover that, at least in the Chelonia, the effects of pressure differences and of washout may both contribute to the overall intracardiac shunting. In varanid lizards, the functional separation of the pulmonary and systemic circuits during systole by the well-developed muscular ridge allows wide differences in the rate and magnitude of ventricular pressure development in the CP and the CV and CA, providing a system that is probably independent of peripheral resistance and the timing of pulmonary and aortic valve opening. In these animals, the washout of blood remaining in the CV at the end of each heart half-cycle into the inappropriate circulatory system is the primary mechanism responsible for cardiac shunting (Heisler *et al.* 1983; Heisler and Glass, 1985). In varanids, in contrast to turtles, the additional effect of pressure difference shunting cannot ameliorate the less variable shunt provided by the washout mechanism (Heisler *et al.* 1983; Heisler and Glass, 1985), but this arrangement also limits the level of shunting to relatively low values. It thus provides a system much less subject to inefficiency under extreme conditions and having the additional advantage of pulmonary and systemic pressures being adjusted to the specific requirements of the respective circulation.

This study has described the correlation between haemodynamic changes and cardiac shunting patterns in the turtle heart. Relationships between the diastolic pressure gradients in the pulmonary and systemic circulation, the timing of ejection into these circulations and the net level of cardiac shunting have been presented. Future studies should test these pressure and flow relationships in chronically instrumented animals.

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