Adrenergic and Cholinergic Regulation of Intracardiac Shunting

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Abstract

Reciprocal variations in the heart rate (HR), pulmonary vascular resistance (R_{pul}) and pulmonary blood flow ($\dot{Q}_{pul}$) are associated with intermittent lung breathing in reptiles. During ventilation R_{pul} decreases and the HR and the $\dot{Q}_{pul}$ increase. In contrast, R_{pul} increases and the HR and $\dot{Q}_{pul}$ decrease during apnea. Besides these changes, intermittent ventilation is associated with changes in the distribution of blood flow between the pulmonary and systemic circulations. A right-to-left (R-L) intracardiac shunt predominates during apnea, while during ventilation a left-to-right intracardiac (L-R) shunt predominates. These changes in the HR, $\dot{Q}_{pul}$, R_{pul}, and intracardiac shunting may be under adrenergic and cholinergic control. Recent experiments in the turtle Pseudemys scripta *support this hypothesis. In this species, cholinergic stimulation resulting from electrical stimulation of vagal efferent nerves or infusion of acetylcholine resulted in a bradycardia, an increased R_{pul}, a reduced $\dot{Q}_{pul}$, and the development of a net R-L intracardiac shunt. The net R-L shunt flow was 6 mL/min/kg and represented approximately 40% of the systemic blood flow. The changes in HR, R_{pul}, and $\dot{Q}_{pul}$ were eliminated by atropine. The R-L shunt was also eliminated by atropine. In contrast, adrenergic stimulation, resulting from electrical stimulation of vagal afferent nerves or infusion of epinephrine resulted in a tachycardia, a decrease in R_{pul}, an increased $\dot{Q}_{pul}$ and the development of a net L-R shunt. The net L-R shunt flow was 28 mL/min/kg, representing 58% of the $\dot{Q}_{pul}$. Preliminary evidence suggested that the changes in the HR, $\dot{Q}_{pul}$, and R_{pul} during vagal afferent stimulation were reduced by propranolol. The results of this study support the hypothesis that the size and direction of the intracardiac shunt is determined by the relative resistances offered by the pulmonary and systemic circulations.*

Introduction

Intracardiac shunting normally occurs in chelonians (turtles) and squamates (lizards and snakes). The complex cardiac anatomy of these animals allows

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the systemic venous blood to bypass the lungs and pulmonary venous blood to bypass the systemic circulation. The magnitude of cardiac shunting may be affected by the physiological state of the animal. In particular, cardiac shunting may vary as a function of ventilatory state. Many reptiles are intermittent lung breathers. Systemic venous blood bypassing the lungs may predominate during apnea, while recirculation of pulmonary venous blood through the lungs may occur during the brief ventilatory periods. The ability to regulate intracardiac shunting may provide several adaptive functions for intermittently breathing reptiles (table 1; Burggren 1987).

The mechanisms controlling the direction and size of intracardiac shunting are not well understood. Factors that affect cardiac function (heart rate [HR] and contractility) and the vascular resistances of various vascular beds can potentially regulate intracardiac shunting. These factors may include adrenergic and cholinergic mechanisms as well as nonadrenergic noncholinergic (NANC) systems. The purpose of this article is to review the cardiac anatomy of noncrocodilian reptiles and to describe the possible role of cholinergic and adrenergic regulation in cardiac shunting. Experimental evidence supporting cholinergic and adrenergic control of intracardiac shunting in turtles will be presented.

Cardiac Anatomy

The hearts of noncrocodilian reptiles are made up of two separate atrial chambers and a single ventricle. The ventricle is subdivided into three anatomically interconnected chambers or cava (fig. 1). A distinctive feature of the ventricular anatomy is the presence of a septum-like structure called the muscular ridge or *Muskelleiste.* The muscular ridge originates from the ventral ventricular wall, running from apex to base, and divides the ventricle into a smaller cavum pulmonale (CP) and larger cavum dorsale (Van Mierop and Kutsche 1981). The dorsolateral border of the muscular ridge is free, allowing potential communication between the CP and cavum dorsale (Van Mierop and Kutsche 1981). A second incomplete vertical septum, which originates from the dorsal aspects of the muscular ridge to the dorsal wall of the cavum dorsale, further subdivides the cavum dorsale into the cavum arteriosum (CA) and the cavum venosum (CV).

In all reptiles, three great vessels arise from the ventricle: the pulmonary artery, the right aortic arch (RAo), and the left aortic arch (LAo). The pulmonary artery emerges to the left of the two aortic arches and originates from the CP. The RAo and LAo arise from the CV. In addition, the RAo divides into subclavian and carotid arteries, and a third branch of the RAo

TABLE 1

Functional significance of intracardiac shunting during intermittent ventilation in reptiles

Functional Significance	Shunt Direction	Reference
Apnea:		
Saves cardiac energy[a]	R-L	Burggren 1987
"Metering" lung oxygen stores[a]	R-L	Burggren and Shelton 1979; Burggren, Smits, and Evans 1989
Reduces CO_2 flux into the lungs[a]	R-L	White 1985
Reduces plasma filtration into the lungs[a]	R-L	Burggren 1982
Ventilation:		
Facilitates CO_2 elimination into the lung[a]	L-R	Ackerman and White 1979; White 1985
Minimizes physiological shunt in the lung due to $\dot{V}/\dot{Q}$ mismatching	L-R	Wood 1984

[a] Summarized from Burggren (1987).

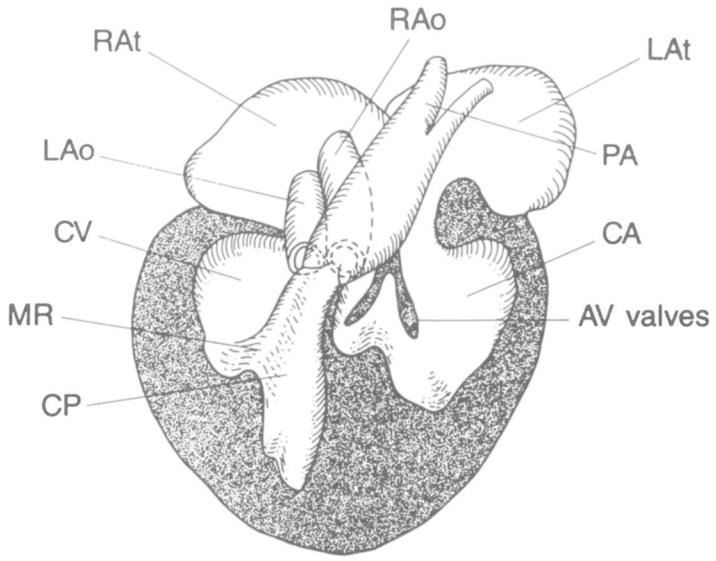

Fig. 1. Schematic representation of the heart of noncrocodilian reptiles. PA, pulmonary artery; RAt, right atrium; LAt, left atrium. Drawing is modified from original line drawing by F. N. White (unpublished).

unites with the LAo mediocaudally to form the dorsal aorta (Van Mierop and Kutsche 1985).

Intracardiac Shunts

These anatomical features of the reptilian heart result in the potential for intracardiac shunting. Cardiac shunts are defined as right-to-left (R-L) or left-to-right (L-R). An R-L shunt represents systemic venous blood bypassing the pulmonary circulation and reentering the systemic circulation. In contrast, recirculation of pulmonary venous blood into the pulmonary arterial circulation is called an L-R shunt. If the systemic blood flow ($\dot{Q}_{sys}$) and pulmonary blood flow ($\dot{Q}_{pul}$) are measured, then intracardiac shunting can be represented as the difference between these two flow rates ($\dot{Q}_{pul} - \dot{Q}_{sys}$). This difference is referred to as the net shunt flow.

In reptiles, the net shunt flow does not provide a complete description of intracardiac shunting. The anatomical arrangement of the ventricular cava (see fig. 1) allows for bidirectional shunting, in which an L-R and R-L shunt both occur during the cardiac cycle (Heisler and Glass 1985; Ishimatsu, Hicks, and Heisler 1988; Hicks and Comeau 1994). In hearts with bidirectional shunting, the net shunt flow may underestimate the actual shunt flows. A simple model illustrates this point (fig. 2). In this model, the $\dot{Q}_{pul}$ is equal to

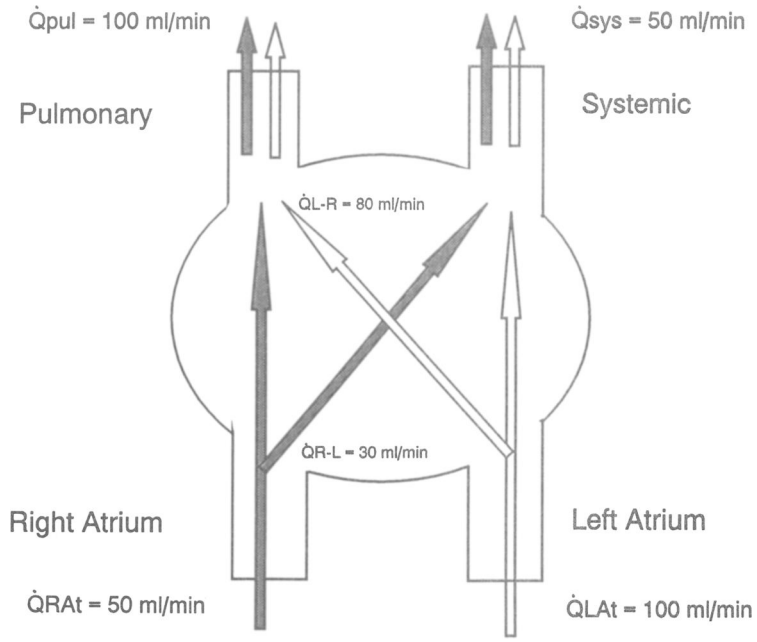

Fig. 2. Model of bidirectional intracardiac shunting in reptiles. Open arrows represent oxygenated blood; filled arrows represent deoxygenated blood. See text for detailed description.

the systemic venous return ($\dot{Q}_{RAt}$) plus the actual L-R shunt flow ($\dot{Q}_{L\text{-}R}$) minus the actual R-L shunt flow ($\dot{Q}_{R\text{-}L}$). Accordingly, the $\dot{Q}_{sys}$ is the sum of the pulmonary venous return ($\dot{Q}_{LAt}$) and the $\dot{Q}_{R\text{-}L}$ minus the $\dot{Q}_{L\text{-}R}$. In this example, the net L-R shunt flow is 50 mL/min. However the $\dot{Q}_{L\text{-}R}$ is 80 mL/min, which represents 80% of the $\dot{Q}_{pul}$. This contribution of the $\dot{Q}_{L\text{-}R}$ to the pulmonary circulation is called the shunt fraction ($\dot{Q}_{L\text{-}R}/\dot{Q}_{pul}$). In this model, the $\dot{Q}_{sys}$ results from a $\dot{Q}_{R\text{-}L}$ of 30 mL/min and the R-L shunt fraction ($\dot{Q}_{R\text{-}L}/\dot{Q}_{sys}$) is 60%. Under the flow conditions provided in this example, the R-L shunt flow cannot be detected from measurements of net shunt flow. Bidirectional shunting can be quantified by measurements of blood oxygen content from arterial and cardiac sites (Ishimatsu et al. 1988) or by the simultaneous injection of radioactively labeled microspheres into the left and right atrium (Heisler, Neumann, and Maloiy 1983). The measurement of net shunts, either L-R or R-L, require careful interpretation if bidirectional shunting is present.

Intracardiac Shunting Patterns and Ventilatory State

Intermittent ventilatory patterns are a characteristic feature of many chelonians and squamates. Such patterns consist of brief periods of ventilation

interspersed among apneas of variable duration (Shelton, Jones, and Milsom 1986). The ventilatory state (ventilation or apnea) is often associated with changes in the size of the R-L and L-R intracardiac shunt. However, the precise flow ratios and absolute magnitudes of these intracardiac shunts are virtually unknown. Regardless, for a few species of chelonians and squamates, the cardiovascular events that occur during intermittent ventilation have been described.

Apnea

During apnea, associated with quiet breathing or diving, there is a bradycardia coupled with an increase in the pulmonary vascular resistance (R_{pul}), which leads to a reduction in the $\dot{Q}_{pul}$. For example, in the freely diving turtle *Pseudemys scripta,* the HR decreases by 80% and the R_{pul} increases by 150%. These changes result in an 80% reduction in the $\dot{Q}_{pul}$ (Shelton and Burggren 1976). Blood flow measurements suggest that a net R-L shunt develops during apnea (White and Ross 1966; Shelton and Burggren 1976). The net R-L shunt flow is estimated to be 5–10 mL/min/kg. In the turtle, analysis of measurements of the partial pressure of oxygen (P_{O_2}) of blood from arterial and cardiac sites confirms an R-L intracardiac shunt during apnea (Burggren and Shelton 1979; White, Hicks, and Ishimatsu 1989).

The actual shunt flows during apnea have been estimated in a varanid lizard and in turtles (Heisler and Glass 1985; White et al. 1989). In the lizard *Varanus exanthematicus,* at a body temperature of 30°C, the R-L shunt flow is 11 mL/min/kg, or approximately 31% of the $\dot{Q}_{sys}$ (Heisler and Glass 1985). In the turtle *Chrysemys picta* at 30°C, the R-L shunt is 18 mL/min/kg, or approximately 61% of the $\dot{Q}_{sys}$ (Heisler and Glass 1985). In this turtle, the R-L shunt flow decreases to 8 mL/min/kg at a body temperature of 15°C. The R-L shunt flow at this temperature is 60% of the $\dot{Q}_{sys}$. In these reptiles, the fractional R-L shunt did not change during ventilation (Heisler and Glass 1985). In contrast, a microsphere study of the turtle *P. scripta* shows that the fractional R-L shunt is greater during apnea than during ventilation (White et al. 1989).

Several studies have shown that an L-R shunt also occurs during apnea. In the lizard *V. exanthematicus* and in the turtle *C. picta* a fractional L-R shunt of 11%–20% occurs during apnea (Heisler and Glass 1985). Measurements of blood P_{O_2} from the pulmonary artery and the left and right atrium confirms an L-R intracardiac shunt during apnea (White et al. 1989). The mechanism responsible for this L-R shunt has been discussed in detail (Heisler and Glass 1985; Hicks and Comeau 1994).

Ventilation

During ventilatory periods, the cardiovascular changes are the reciprocals of those occurring during apnea. For example, in the turtle *P. scripta* the HR doubles and the R_{pul} decreases by more than 50%, which leads to a threefold increase in the $\dot{Q}_{pul}$ (Shelton and Burggren 1976). Blood flow measurements from selected arteries estimate that a net L-R shunt develops during ventilation (White and Ross 1966; Shelton and Burggren 1976). The net L-R shunt is 30–50 mL/min/kg. Recent studies of the snake *Acrochordus granulatus* show that $\dot{Q}_{pul}$ is 10-fold higher than $\dot{Q}_{sys}$ during ventilation (Lillywhite and Donald 1989). This may be the largest net L-R shunt measured for an intermittently breathing reptile. In the turtle *P. scripta* analysis of the blood P_{O_2} from the left atrium, right atrium, and pulmonary artery confirms an L-R intracardiac shunt during ventilation (White et al. 1989).

The magnitude of the L-R shunt flow during ventilation has been estimated in the lizard *V. exanthematicus* and the turtle *C. picta* at 30°C. In the lizard, the $\dot{Q}_{L\text{-}R}$ is approximately 3 mL/min/kg and is 11% of the $\dot{Q}_{pul}$. In the turtle, the $\dot{Q}_{L\text{-}R}$ shunt is 5.5 mL/min/kg, which represents 18% of the $\dot{Q}_{pul}$ (Heisler and Glass 1985).

An R-L intracardiac shunt also occurs during ventilation. In the lizard *V. exanthematicus* and the turtle *C. picta,* the fractional R-L shunt is 10%–25% during ventilation (Heisler and Glass 1985). In the turtle *P. scripta,* measurements of blood P_{O_2} from cardiac and arterial sites indicate a 20%–30% fractional R-L shunt during ventilation (White et al. 1989). The mechanism responsible for this R-L intracardiac shunt has been discussed in detail (Heisler and Glass 1985; Hicks and Comeau 1994).

Adrenergic and Cholinergic Regulation of the Cardiovascular System

The size and direction of intracardiac shunting will be determined by factors that control cardiac function (HR and myocardial contractility) and the vascular resistance of the pulmonary and systemic circulations. These factors certainly include adrenergic and cholinergic mechanisms (Berger and Burnstock 1979; Nilsson 1983). In addition, NANC systems may play an important role in controlling cardiovascular function in reptiles (Lillywhite and Donald 1989; Conlon, Hicks, and Smith 1990).

Cholinergic Regulation

The vagus may be the primary regulator of the HR during ventilation and apnea (Burggren 1975). A great deal of evidence shows that both the atria

and the ventricle are innervated by the vagus nerve (see Berger and Burnstock [1979] for review). In the turtle *P. scripta* the bradycardia that occurs during apnea is eliminated by an intravenous infusion of atropine (Burggren 1975). In contrast, intravenous administration of propranolol has no effect on the bradycardia that occurs during apnea (Burggren 1975). In *P. scripta,* electrical stimulation of the vagus nerve results in a bradycardia that is abolished by atropine (Comeau and Hicks 1994).

The pulmonary vasculature of chelonians and squamates exhibits a cholinergic vasoconstrictor innervation. Electrical stimulation of the vagus nerve results in an increase in the R_{pul} in turtles, lizards, and snakes (Luckhardt and Carlson 1921; Berger 1972, 1973; Burggren 1977; Milsom, Langille, and Jones 1977; Smith and MacIntyre 1979; Donald, O'Shea, and Lillywhite 1990; Hicks and Comeau 1994; Comeau and Hicks 1994). This increase is abolished by atropine. The development of an R-L shunt may be under cholinergic control (White 1976). In the turtle *P. scripta* electrical stimulation of the vagus nerves results in a reduction in the P_{O_2} of both the LAo and RAo (Burggren 1978; Hicks and Comeau 1994). This reduction in the arterial P_{O_2} occurs even though the pulmonary venous and systemic venous P_{O_2} remain unchanged. The systemic arterial P_{O_2} is also decreased by the intravenous infusion of acetylcholine (ACh) (Hicks and Malvin 1992). The effects of cholinergic stimulation on the $\dot{Q}_{R\text{-}L}$ or the net R-L shunt have not been determined.

Adrenergic Regulation

In turtles cardiac sympathetic nerves leave the spinal cord at the level of the tenth spinal nerve, run forward through three ganglia of the sympathetic chain, and extend toward the heart (Gaskell and Gadow 1884). Sympathetic fibers innervate the atrium and ultrastructural observations confirm that fibers containing vesicles typical of adrenergic nerves occur in the ventricular myocardium of turtles (Yamauchi and Chiba 1973). Stimulation of these fibers result in an acceleration of the HR. This effect is blocked by bretylium (an adrenergic neurone-blocking agent) (see Berger and Burnstock [1979] for review). Increases in the HR occur after intravenous administration of epinephrine and norepinephrine (Berger and Burnstock 1979; Comeau and Hicks 1994).

Histochemical studies show adrenergic nerves in the pulmonary vasculature of the lacertilian *Trachysaurus rugosus* (McLean and Burnstock 1967; Furness and Moore 1970) and the file snake, *A. granulatus* (Lillywhite and Donald 1989). In the rat snake *Elaphe obsoleta,* adrenergic innervation is present on the pulmonary artery, the smaller pulmonary

arteries and veins, and the main pulmonary vein (Donald et al. 1990). The functional role of adrenergic nerves in the pulmonary vasculature is contradictory. Luckhardt and Carlson (1921) reported a differential effect of epinephrine on the pulmonary vasculature. In the turtles *Chrysemys* and *Malacoclemys* small doses of epinephrine produce a vasodilation, whereas larger doses produce a vasoconstriction. In contrast, the adrenergic agents norepinephrine, isoprenaline, or phenylephrine have no effect on the pulmonary arterial tone in the tortoise (Berger 1972). In the turtle *C. scripta* electrical stimulation of the cervical sympathetic nerves produces no change in the pulmonary perfusion pressure (Milsom et al. 1977). In addition, infusion of epinephrine, in a dose range from 1 to 100 μg, does not affect the pulmonary artery pressure (Milsom et al. 1977). In contrast, a marked pulmonary vasodilation results from the injection of epinephrine into the pulmonary artery of the rat snake (Donald et al. 1990). Administration of propranolol eliminates this response. In the rat snake, electrical stimulation of the vagus nerve produces a biphasic response in R_{pul}. During the stimulation there is a pulmonary vasoconstriction, followed by a poststimulatory pulmonary vasodilation (Donald et al. 1990). The administration of bretylium or propranolol eliminates this poststimulatory vasodilation. This later observation suggests the $\dot{Q}_{pul}$ is regulated by the reciprocal interaction of adrenergic and cholinergic nerves (Donald et al. 1990).

Recent evidence from the turtle *P. scripta* shows that electrical stimulation of vagal afferent fibers results in an increase in HR, a reduction in R_{pul}, and an increase in systemic vascular resistance (R_{sys}). These changes result in an increase in $\dot{Q}_{pul}$ and a decrease in $\dot{Q}_{sys}$ (Comeau and Hicks 1994). These cardiovascular changes are reduced following administration of bretylium. Finally, an intravenous infusion of epinephrine (0.1 μg/kg) produces cardiovascular changes similar to those measured during vagal afferent stimulation (Comeau and Hicks 1994).

Adrenergic control of intracardiac shunting is not well studied. Analysis of blood P_{O_2} from the systemic arteries and from cardiac sites indicates that adrenergic stimulation may abolish the R-L shunt. An intravenous infusion of epinephrine eliminates the systemic venous admixture in the aortic arches of the turtle *P. scripta* (Hicks and Malvin 1992). A similar result is obtained during electrical stimulation of vagal afferent nerves in this same species (Hicks and Comeau 1994). Intravenous infusion of epinephrine may produce a large net L-R shunt in turtles (Comeau and Hicks 1994).

Material and Methods

In our laboratory we have focused on the role of the vagus nerve on control of central vascular blood flow in the turtle *Pseudemys scripta.* Recently, we have addressed the hypothesis that the size and direction of intracardiac shunting results from the reciprocal interplay of the adrenergic and cholinergic mechanisms (Comeau 1992; Hicks and Malvin 1992; Comeau and Hicks 1994; Hicks and Comeau 1994). The purpose of this study was to quantify the effects of vagal nerve stimulation on the size of the net R-L or net L-R shunt flow. Studies were conducted in five animals ($\bar{X} = 1.65 \pm 0.34$ kg, mean ± SD), anesthetized (Nembutal, 30 mg/kg), in the supine position and ventilated (tidal volume = 25 mL/kg and breathing frequency = 7–10 beat/min). The following cardiovascular variables were measured or calculated: HR, $\dot{Q}_{pul}$, $\dot{Q}_{sys}$, pulmonary arterial pressure (P_{pa}), pulmonary venous pressure (P_{pv}), central venous pressure, arterial pressure (P_{sys}), R_{pul}, R_{sys}, net L-R shunt flow, and net R-L shunt flow. The $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ were determined with ultrasonic transit-time flow probes (2R; Transonic). The $\dot{Q}_{pul}$ was measured by placing a 2R flow probe on the left and right pulmonary arteries. The $\dot{Q}_{sys}$ was calculated by measuring the LAo flow (2R flow probe) and multiplying by a factor of 2.8. This flow factor was previously determined in this preparation (Comeau 1992) and was in good agreement with a previous study (Shelton and Burggren 1976). Central vascular pressures were measured in four sites. A small section (3–5 mm) of the common pulmonary artery was exposed and a 20-gauge i.v. catheter was gently inserted downstream for the measurement of P_{pa}. The P_{pv} was measured by inserting a catheter into the left atrium by a method previously described (Heisler et al. 1983). The central venous pressure was measured by inserting a catheter (PE 50) into the right jugular vein and advancing 2–3 cm toward the heart. Finally, the P_{sys} was measured by inserting a catheter into the right common carotid artery. The pressure catheters were connected to four strain gauge pressure transducers (P23XL; Spectramed, Oxnard, Calif.). The HR was measured by inserting needle electrodes into the right and left foreleg and the left hindlimb and connecting to a cardiotachometer (Type 9857; Sensormedics, Yorba Linda, Calif.). The R_{pul} and R_{sys} were calculated from the Poiseuille equation. The net intracardiac shunt flow was calculated by the difference between $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$. Analog outputs from the flowmeters, pressure transducers, and cardiotachometer were connected to a data acquisition system (Series 500; Keithley Metrabyte). All cardiovascular variables were sampled at 1 Hz and stored onto disk for later off-line analysis. Experiments were conducted at room temperature, 21°–23°C.

In this preparation, the right and left cervical vagus nerves were isolated and bilateral sectioned (Comeau 1992). Bipolar, silver, stimulating electrodes were placed on either the efferent or afferent end of the sectioned vagus nerve. Stimulation was provided by an A310 Accupulser pulse generator coupled with an A360 D/R constant current stimulus isolator (World Precision Instruments, New Haven, Conn.). All experiments were conducted at room temperature (21°–23°C). The protocol for these experiments consisted of control periods followed by periods of electrical stimulation of either the vagal afferents or vagal efferents. Stimulation levels were at 10–40 μA; 2–5 V, 200 ms duration, 1–3 Hz, and lasted up to 1 min. In addition, intravenous infusion of epinephrine, ACh, propranolol, or atropine was periodically conducted.

Results

Vagal Efferent Stimulation and ACh Infusion

The cardiovascular variables during control conditions are shown in table 2. Electrical stimulation of the right VEF resulted in a large increase in R_{pul}, a reduction in HR and a reduction in $\dot{Q}_{pul}$ (fig. 3). Similar changes in $\dot{Q}_{pul}$, HR, and R_{pul} were observed following a bolus injection of ACh (200 nmol/kg; fig. 3). These cardiovascular changes were completely blocked after intravenous administration of 6 μmol/kg atropine (fig. 3).

Magnitude of R-L Intracardiac Shunting

A net R-L shunt developed during vagal efferent stimulation (fig. 4). The size of the net shunt was a function of the ratio R_{pul}/R_{sys} (fig. 5). As the R_{pul}/R_{sys} ratio increased, the net R-L shunt increased. Individual measurements of net R-L shunt ranged up to 25 mL/min/kg. The average net R-L shunt was 6 ± 5 mL/min/kg ($\bar{X}$ + SD, n = 5). The net R-L represented 40% ± 7% of the $\dot{Q}_{sys}$. A net R-L shunt did not develop after administration of atropine.

Vagal Afferent Stimulation and Epinephrine Infusion

Electrical stimulation of vagal afferents resulted in an increase in HR, a reduction in R_{pul}, and an increase in $\dot{Q}_{pul}$ (fig. 6). An intravenous infusion of epinephrine (4–5 nmol/kg/min) resulted in similar changes in these cardiovascular variables (fig. 6). Preliminary evidence showed that these changes were abrogated by administration of propranolol (10 μmol/kg; fig. 6).

TABLE 2

Cardiovascular variables during control conditions in the turtle Pseudemys scripta *at a body temperature of 22° C*

Value (n = 5)	Mass (kg)	$\dot{Q}_{pul}$ (mL/min/kg)	P_{pul} (mmHg)	R_{pul} (mmHg/mL/min/kg)	$\dot{Q}_{sys}$ (mL/min/kg)	P_{sys} (mmHg)	R_{sys} (mmHg/mL/min/kg)	HR (beat/min)
Mean	1.65	30	15	.40	24	19	.75	43
SD	.34	6	3	.13	6	3	.20	4

Note. P_{pul} represents mean blood pressure in common pulmonary artery.

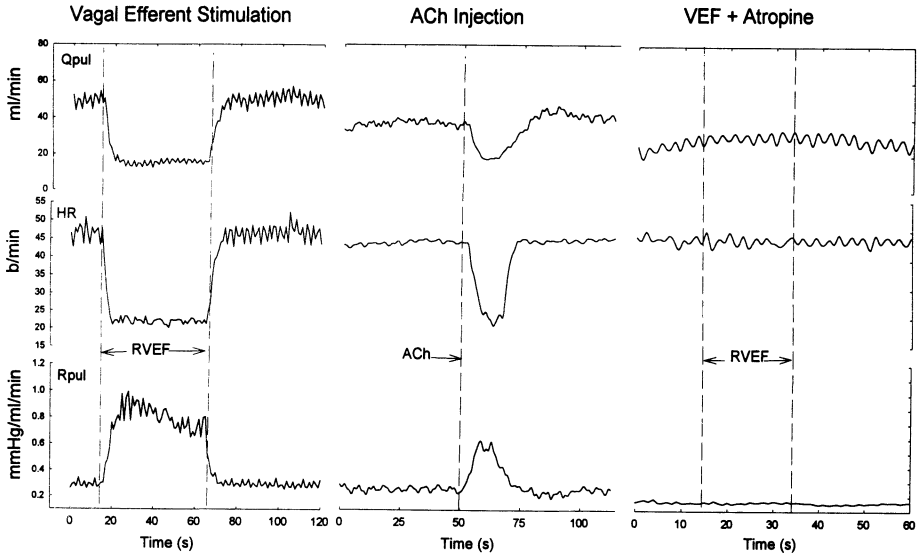

Fig. 3. Changes in $\dot{Q}_{pul}$, *HR, and* R_{pul} *in a single turtle,* Pseudemys scripta, *during electrical stimulation of the right vagal efferent nerve (RVEF), after ACh injection and during RVEF after administration of atropine. In this series of experiments the level of nerve stimulation was 20 μA, 2.5 V, 200 ms duration, 1.5 Hz. The injection of ACh was intravenous at a dose of 200 nmol/kg and the dose of atropine was 6 μmol/kg. The animal was 1.73 kg.*

Magnitude of L-R Intracardiac Shunting

A net L-R shunt developed during vagal afferent stimulation (fig. 4). The size of the net L-R shunt flow was a function of the ratio R_{pul}/R_{sys} (fig. 5). As the R_{pul}/R_{sys} ratio decreased, the net L-R shunt flow increased. Individual measurements of net L-R shunt ranged up to 55 mL/min/kg. The overall magnitude of the net L-R shunt was 28 ± 7 mL/min/kg ($\bar{X}$ ± SD, n = 5). This represented 58% ± 8% of the $\dot{Q}_{pul}$. The size of the net L-R shunt was reduced after administration of propranolol (10 μmol/kg)(L-R shunt = 15 ± 10 mL/min/kg; $\bar{X}$ ± SD, n = 3).

$\dot{Q}_{pul}$ *versus* $\dot{Q}_{sys}$

Figure 7 shows a summary of all flow measurements made in this study. The increase in the total cardiac output resulted primarily from an increase in the $\dot{Q}_{pul}$.

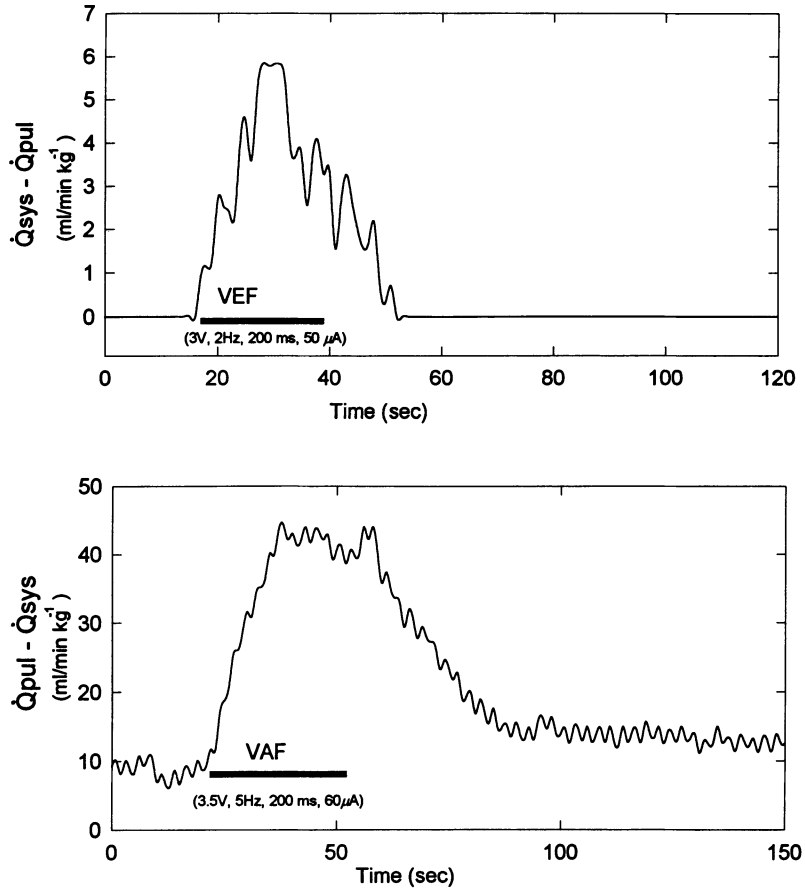

Fig. 4. Upper panel, the development of a net R-L shunt during electrical stimulation of vagal efferent nerves in the turtle Pseudemys scripta. *Electrical stimulation was 3 V, 2 Hz, 200 ms, 50 μA. Bottom panel, the development of a net L-R shunt during electrical stimulation of vagal afferent nerves in the turtle. Electrical stimulation was 3.5 V, 5 Hz, 200 ms, 60 μA. The animal was 1.8 kg.*

Discussion

These experiments support the hypothesis that a net R-L shunt is of cholinergic origin and that a net L-R shunt is of adrenergic origin. In addition, these results support the hypothesis that the size and direction of intracardiac shunts result from the reciprocal interplay of cholinergic and adrenergic mechanisms.

Regulation of Intracardiac Shunt

The results of this study strongly support the hypotheses that the direction and size of intracardiac shunts are dictated by the relative resistances offered

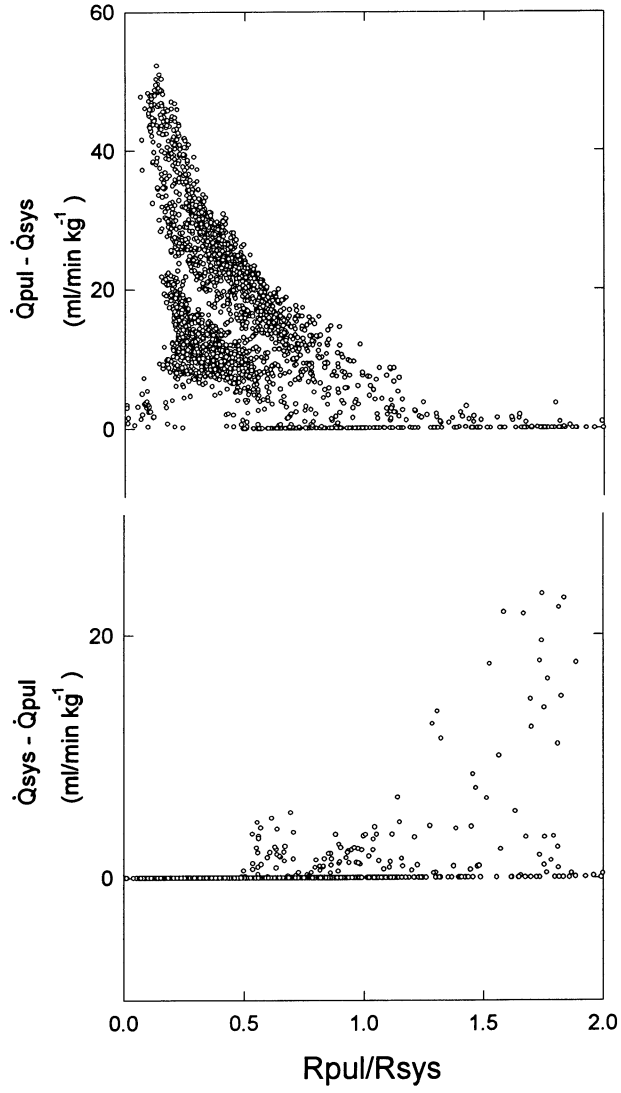

Fig. 5. The effects of the ratio R_{pul}/R_{sys}, *on the net L-R shunt (upper panel) and net R-L shunt (bottom panel).*

by the pulmonary and systemic circulations (White and Ross 1966; White 1976; Burggren 1985). The precise mechanism for the effects of vascular resistance on intracardiac shunting is controversial. Currently, there are two hypotheses addressing the mechanism of intracardiac shunt: "pressure shunting" and "washout shunting." The pressure shunting hypothesis states that low-resistance connections exist between the ventricular cava during systole that permit nearly unimpeded blood flow through the ventricle. Consequently, when the R_{pul} increases relative to the R_{sys}, a portion of blood

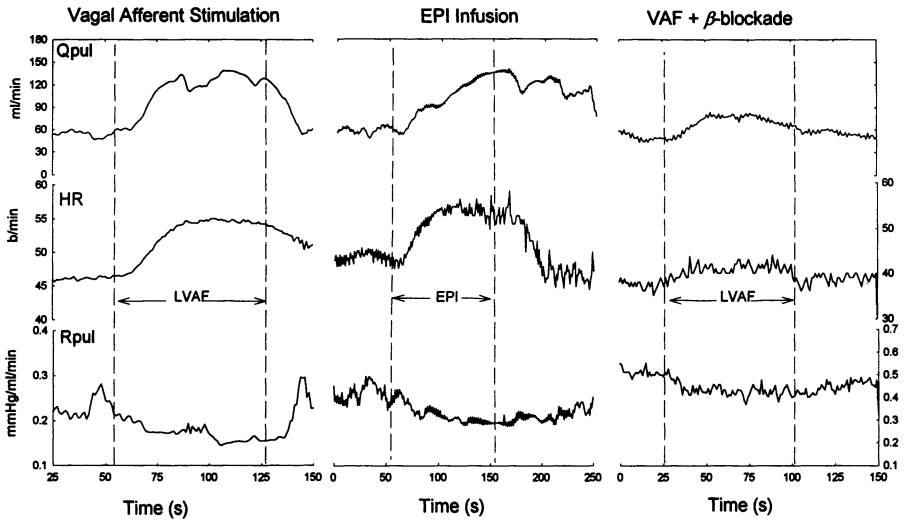

Fig. 6. Changes in $\dot{Q}_{pul}$, *HR, and* R_{pul} *in a single turtle,* Pseudemys scripta, *during electrical stimulation of the left vagal afferent nerve (LVAF), following epinephrine (EPI) infusion and during (LVAF) after administration of propranolol. In this series of experiments the level of nerve stimulation was 20* μ*A, 3 V, 100 ms duration, 5 Hz. The infusion of EPI was intravenous at a dose of 4.5 nmol/kg/min and the dose of propranolol was 10* μ*mol/kg. The animal was 1.6 kg.*

within the CP (see fig. 1) will be ejected around the muscular ridge into the systemic circulation. Conversely, when R_{pul} decreases relative to R_{sys}, a portion of blood in the CV and CA will be ejected around the muscular ridge into the pulmonary circulation. Evidence supporting the pressure hypothesis has been reported (Shelton and Burggren 1976). In contrast, the washout hypothesis states that during systole, the muscular ridge forms a functional separation of the CP from the CV and CA, and thus blood cannot flow from the CP into the systemic circulation during systole. In addition, the functional separation resulting from the muscular ridge prevents blood within the CV and CA from flowing into the pulmonary circulation during systole. Therefore, the size of the R-L shunt results from the end-diastolic volume of blood in the CV that is "washed" into the systemic circulation during systole. Conversely, the size of the L-R shunt is determined by the end-systolic volume of blood in the CV that is washed into the CP during the subsequent diastole. Variations in the size of the intracardiac shunt result from factors affecting diastolic filling and/or systolic ejection from the CV and CA (Heisler and Glass 1985). Such factors would include the resistance offered by the pulmonary and systemic circulations (Heisler and Glass 1985).

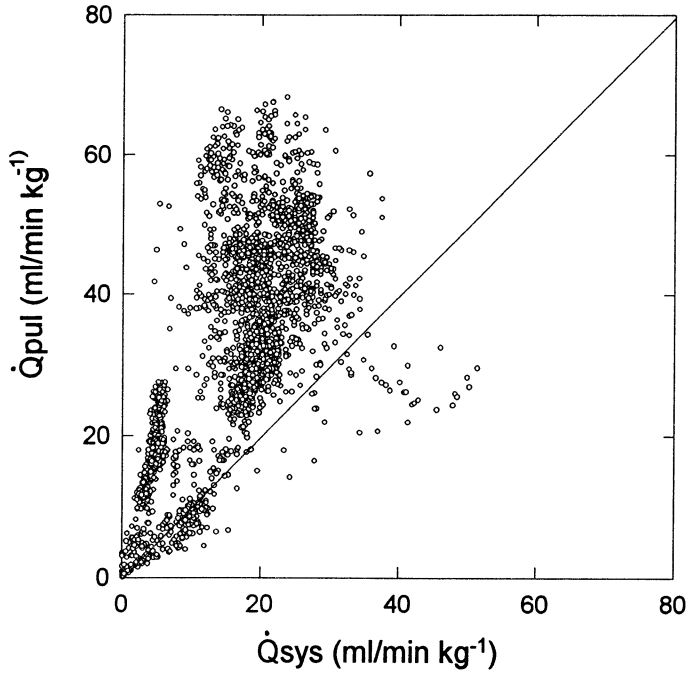

Fig. 7. The relationship between total $\dot{Q}_{pul}$ and total $\dot{Q}_{sys}$ in the turtle Pseudemys scripta *during electrical stimulation of the vagus nerve. Individual data points are from five animals and represent both afferent and efferent nerve stimulation.*

Evidence supporting the washout hypothesis has been recently reported (Hicks and Malvin 1992; Hicks and Comeau 1994).

Regardless of the precise mechanism for intracardiac shunting, it is clear from this study that factors that affect the relative vascular resistances in the pulmonary and systemic circulations will influence the size and direction of the net intracardiac shunt. Therefore, it is not surprising that the administration of atropine, which blocks cholinergic vasoconstriction of the pulmonary circulation, eliminated the net R-L shunt that developed during vagal efferent stimulation.

The effects of adrenergic blockade are more difficult to interpret. The net L-R shunt results primarily from the large increase in $\dot{Q}_{pul}$ (fig. 7). It is possible that β-adrenergic blockade prevented the development of a pulmonary vasodilation and the subsequent increase in $\dot{Q}_{pul}$. Thus the net L-R shunt was reduced. Alternatively, β-adrenergic blockade may have reduced the effects of adrenergic stimulation on myocardial contractility. This study could not differentiate between these factors. In addition, this study did not

address the effects of α-adrenergic blockade. The α-adrenergic receptors play an important role in the regulation of the R_{sys} (Nilsson 1983). It is likely that α-adrenergic blockade will also reduce the size of the net L-R shunt by preventing an increase in R_{sys} during adrenergic stimulation.

Finally, this study did not address the role of NANC mechanisms in controlling intracardiac shunt. Various peptides may play a role in cardiovascular function in reptiles. In the snake *Acrochordus granulatus* there is the presence of vasoactive intestinal peptide immunoreactivity in the pulmonary vasculature (Donald and Lillywhite 1989). In the turtle *Pseudemys scripta* intravenous administration of Thr^6-bradykinin results in a systemic vasodilation and a increase in the LAo blood flow. Clearly any NANC factor that effects the R_{pul}/R_{sys} ratio will influence the direction and size of the net intracardiac shunt. The NANC control of cardiovascular function in reptiles remains a topic for research.

Intracardiac Shunts and Other Physiological States

The number of studies that have simultaneously measured the $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ in chronically instrumented animals is limited. Therefore, the direction and size of intracardiac shunting during other physiological states, such as activity, changes in body temperature, feeding, and digestion, is virtually unknown. Undoubtedly, these physiological states may alter the R_{pul}/R_{sys} ratio and therefore affect the size and direction of the net shunt. Recently, West, Butler, and Bevan (1992), measured left pulmonary artery blood flow ($\dot{Q}_{Lpul}$) and left aortic arch blood flow ($\dot{Q}_{LAo}$) during rest and swimming in the green sea turtle, *Chelonia mydas.* This study revealed that during exercise the HR increased, the R_{pul} decreased, and the $\dot{Q}_{pul}$ increased. The total increase in cardiac output was accounted for primarily by an increase in $\dot{Q}_{Lpul}$; that is, $\dot{Q}_{Lpul}$ increased more than $\dot{Q}_{LAo}$ during exercise. Qualitatively, the relationship between $\dot{Q}_{Lpul}$ and $\dot{Q}_{LAo}$ during exercise in *C. mydas* is very similar to the relationship between $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ in *P. scripta* measured in this study (fig. 7).

Functional Significance of L-R Intracardiac Shunting

Several hypotheses have addressed the adaptive functions of intracardiac shunting (table 1). Many of these hypotheses have emphasized the R-L shunt that develops during apnea. Of the current set of hypotheses, none appear to satisfactorily explain the functional advantage of a large net L-R shunt. In terms of oxygen transport efficiency, a large L-R shunt associated with an increase in $\dot{Q}_{pul}$ would minimize physiological shunting due to the

ventilation-perfusion ratio ($\dot{V}/\dot{Q}$) mismatching in the lung (Wood 1984; West et al. 1992). This would improve the level of oxygenation of pulmonary venous blood. The $\dot{V}/\dot{Q}$ heterogeneity within the reptilian lung has been described only for the alligator *Alligator mississipiensis* (Powell and Gray 1989) and the teju lizard *Tupinambis nigropunctatus* (Hlastala et al. 1985). The effects of increasing $\dot{Q}_{pul}$ or increasing the size of the L-R shunt on $\dot{V}/\dot{Q}$ heterogeneity have not been determined. The effects of an increase in $\dot{Q}_{pul}$ or L-R shunt on the expired P_{O_2} to pulmonary venous P_{O_2} gradient has not been reported. Recent evidence suggests that the increase in $\dot{Q}_{pul}$ during adrenergic stimulation does not significantly improve the P_{O_2} of pulmonary venous blood (Hicks and Malvin 1992; Hicks and Comeau 1993). In contrast, an increase in the level of the L-R shunt may adversely affect oxygen exchange. The mixing of pulmonary venous and systemic venous blood would increase the pulmonary arterial P_{O_2}. This increase would reduce the P_{O_2} gradient from lung gas to blood and offset any benefit that would result from the increase in $\dot{Q}_{pul}$.

In terms of CO_2 exchange, the L-R shunts may be beneficial (Ackerman and White 1979; White 1985). The mixing of pulmonary venous blood with systemic venous blood increases the oxygen saturation of the pulmonary arterial blood. This mixing diminishes the capacity of blood to carry CO_2 (the Haldane effect). These changes promote CO_2 elimination from the lung (White 1985). Theoretically, the amount of CO_2 eliminated depends on the minute ventilation, the $\dot{Q}_{pul}$, the size of the L-R shunt, the slope of the CO_2 dissociation curve, and the magnitude of the Haldane effect (White 1985). The effects of an L-R shunt on CO_2 elimination have not been experimentally verified.

An additional consequence of an L-R shunt, not previously appreciated, may be an improvement in systemic oxygen transport. The systemic oxygen transport is the product of mean arterial oxygen content (CaO_2) and $\dot{Q}_{sys}$. During periods of increased oxygen demand, the systemic oxygen transport can be improved by increasing CaO_2, $\dot{Q}_{sys}$, or both. In turtles, the capacity to increase $\dot{Q}_{sys}$ may be constrained (West et al. 1992; fig. 7). This probably results from the adrenergic vasodilation of the pulmonary circulation, making the pulmonary circuit the favored route for blood flow. Recent studies have shown that an adrenergically induced L-R shunt eliminates the R-L shunt and subsequently raises the systemic arterial oxygen saturation to levels that are equal to pulmonary venous values (Hicks and Malvin 1992; Hicks and Comeau 1994). Thus, total systemic oxygen transport is improved. These observations suggest that the elimination of the R-L shunt and the subsequent increase in the CaO_2 may be important during activity or when there is an increased demand for oxygen.

This article has shown that the direction and size of intracardiac shunting may result from the reciprocal interplay of adrenergic and cholinergic factors. Specifically, these factors may alter the relative resistances offered by the pulmonary and systemic circulations. The development of an R-L shunt is of vagal origin and is under cholinergic control. In contrast, the development of an L-R shunt is under adrenergic control. The manipulation of intracardiac shunting, either by nerve stimulation or the action of specific agonist and antagonist, can be used in both acute and chronic animal preparations. Future studies will use these techniques to experimentally test the hypotheses that address the functional role of intracardiac shunting.

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