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The unequal influences of the left and right vagi on the control of the heart and pulmonary artery in the rattlesnake, *Crotalus durissus*

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SUMMARY

Autonomic control of the cardiovascular system in reptiles includes sympathetic components but heart rate (f_H), pulmonary blood flow ($\dot{Q}_{pul}$) and cardiac shunt patterns are primarily controlled by the parasympathetic nervous system. The vagus innervates both the heart and a sphincter on the pulmonary artery. The present study reveals that whereas both the left and right vagi influence f_H, it is only the left vagus that influences pulmonary vascular resistance. This is associated with the fact that rattlesnakes, in common with some other species of snakes, have a single functional lung, as the other lung regresses during development. Stimulation of the left cervical vagus in anaesthetised snakes slowed the heart and markedly reduced blood flow in the pulmonary artery whereas stimulation of the right cervical vagus slowed the heart and caused a small increase in stroke volume (V_S) in both the systemic and pulmonary circulations. Central stimulation of either vagus caused small (5–10%) reductions in systemic blood pressure but did not affect blood flows or f_H. A bilateral differentiation between the vagi was confirmed by progressive vagotomy in recovered snakes. Transection of the left vagus caused a slight increase in f_H (10%) but a 70% increase in $\dot{Q}_{pul}$, largely due to an increase in pulmonary stroke volume ($V_{S,pul}$). Subsequent complete vagotomy caused a 60% increase in f_H accompanied by a slight rise in $\dot{Q}_{pul}$, with no further change in $V_{S,pul}$. By contrast, transection of the right vagus elicited a slight tachycardia but no change in $V_{S,pul}$. Subsequent complete vagotomy was accompanied by marked increases in f_H, $\dot{Q}_{pul}$ and $V_{S,pul}$. These data show that although the heart receives bilateral vagal innervation, the sphincter on the pulmonary artery is innervated solely by the left vagus. This paves the way for an investigation of the role of the cardiac shunt in regulating metabolic rate, as chronic left vagotomy will cause a pronounced left–right shunt in recovered animals, whilst leaving intact control of the heart, *via* the right vagus.

Key words: cardiovascular, nervous control, reptile.

INTRODUCTION

Autonomic control of the cardiovascular system in reptiles has both sympathetic and parasympathetic components (Morris and Nilsson, 1994). Although there was clear evidence of chronotropic adrenergic control of the heart in rattlesnakes, inhibitory vagal control was predominant (Wang et al., 2001b). The vagus nerve in vertebrates, including reptiles, runs to the heart, trachea, lungs, pulmonary and coronary vasculature as well as other visceral organs such as the digestive tract. In reptiles, peripheral electrical stimulation of the vagus or intravenous injection of acetylcholine results both in bradycardia and an increase in pulmonary vascular resistance, which reduces pulmonary blood flow ($\dot{Q}_{pul}$) (Burggren, 1977a; Burggren, 1977b; Milsom et al., 1977; Hicks, 1998). This is because the heart and a sphincter on the pulmonary artery are innervated by the vagus, which terminates on muscarinic cholinoceptors (Luckhardt and Carlson, 1921). Efferent activity in the vagus slows the heart and causes constriction of the sphincter on the pulmonary artery, which shunts blood away from the lungs [a right-to-left (R–L) cardiac shunt]. Thus, the net direction and magnitude of shunt flow is largely determined by changes in resistance in the pulmonary circuit, relative to the systemic circuit, due to active vagal, cholinergic regulation of pulmonary arterial resistance (Hicks, 1994). Reptiles are typically periodic breathers. During apnoea, resistance in the pulmonary circuit is increased, resulting in a marked R–L cardiac shunt whereas during bouts of breathing, a reduced resistance in the pulmonary circuit can increase blood flow to the lungs [a left-to-right (L–R) cardiac shunt] (see Wang et al., 2001b). Thus, vasomotor control is important in diverting blood between the pulmonary and systemic systems (Woodbury and Robertson, 1942) and may even enable an increase in oxygen demand to be satisfied in the absence of increased lung ventilation (Wang and Hicks, 1996).

Complete vagotomy in the rattlesnake caused heart rate (f_H) to rise and become unvarying, and the breathing rhythm to become very slow, with very large tidal volumes (Wang et al., 2001a). These data were interpreted as loss of vagal tone on the heart, which is known to be responsible for f_H variability as well as setting f_H plus denervation of lung stretch receptors, with the resultant loss of the Hering–Breuer reflex (Sundin et al., 2001; Wang et al., 2001b; Campbell et al., 2006), In a series of exploratory experiments it became apparent that the left and right vagi were not equal in their mediation of these classic responses. This may relate to the fact that the rattlesnake, in common with some other species of snakes, only possesses a single functional lung. Thus, the vagal afferent innervation of lung receptors and efferent innervation of the sphincter on the pulmonary artery may be similarly unilateral. The present study sought to quantify these differences.

MATERIALS AND METHODS

Animals

We used South American rattlesnakes (*Crotalus durissus* Linnaeus 1758) with body mass ranging from 260 to 850 g, in three experimental series. The snakes were obtained from the Butantan Institute in São Paulo and transported to Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil, where they were fed on rodents and chickens and maintained at 28±5°C under a natural photoperiod. All animals used in the experiments appeared healthy. Prior to the experiments, the snakes were fasted for more than one week.

Experimental protocols

Vagal stimulation in anaesthetised snakes

These experiments were designed to investigate putative differences between the left and right vagi with respect to their cardiovascular control. All procedures were in accordance with the ethical requirements imposed on animal experimentation in Brazil and were based on the standards applied in Denmark and the UK. Eight snakes were terminally anaesthetised with the pentobarbiturate, Mebumal (20 mg kg^{-1}), injected into the caudal vein. The snakes normally lost all behavioural reflexes within 15 min and were then placed in a supine position. A ventrolateral incision was made immediately above the heart to expose the central blood vessels. A cannula (PE 60 containing heparinised saline) was inserted into the right aortic arch (RA_O) *via* the occluded vertebral artery, for measurements of blood pressure. We placed transonic blood flow probes (2R or 2S depending on the size of the snake) around the main branch of the pulmonary artery and around the left aortic arch (LA_O) to measure pulmonary blood flow ($\dot{Q}_{pul}$) and left aortic blood flow ($\dot{Q}LA_O$). All cardiovascular variables stabilised quickly after completion of the surgery and base-line data were obtained for up to 30 min before proceeding to stimulate the vagus nerves.

The cervical vagus was exposed on either side *via* another ventral midline incision in the neck, and both vagi were dissected free from connective tissue, so that they could be lifted onto platinum hooks for electrical stimulation (Physiological Stimulator, Farnell Instruments, Wetherby, UK). Peripheral stimulation consisted of positive-going 2 ms stimuli delivered at frequencies between 0.2 and 70 Hz at 2–10 V, depending on the measured responses obtained from each preparation. The appropriate stimulation voltage was determined by progressively increasing voltage until a maximal response was obtained at a frequency eliciting changes in f_H and/or changes in $\dot{Q}_{pul}$. A range of frequency–response determinations was then made for each nerve. Each stimulation was followed by a period of recovery that constituted the control conditions for the subsequent stimulation. The same stimulation parameters were then retained for subsequent central stimulation of the same nerve. In four animals, atropine (3 mg kg^{-1}) was injected and allowed to take effect for 15 min before peripheral vagal stimulation was repeated to investigate whether the haemodynamic responses had been blocked.

Effects of vagotomy on cardiovascular variables in fully recovered snakes

Given that stimulation of the right and left vagi in anaesthetised snakes revealed pronounced differences in the effects on $\dot{Q}_{pul}$, we aimed to characterise the effects of sectioning the left or right vagus on central vascular blood flows and f_H in fully recovered snakes. To implant blood flow probes and snares around the vagi, ten snakes were anaesthetised by inhalation of CO_2. This method of anaesthesia has been used many times on reptiles and induces lack of movement and insensitivity to physical stimulation such as handling for 4–10 min in rattlesnakes (Wang et al., 1993). During anaesthesia the snakes were hypoxaemic and acidotic but plasma acid–base status and oxygen levels returned to normal values within 2 to 6 h (Wang et al., 1993). Furthermore, rattlesnakes subject to operative procedures under CO_2 anaesthesia, have survived for several weeks, feeding and acting normally. One ventrolateral incision, 5–7 cm long, was made cranial to the heart, so that one Transonic blood flow probe (2R, 2S or 1.5S depending on the size of the animal) could be placed around the left pulmonary artery. The site of each incision was injected with xylocaine as an analgesic. While the left lung of *Crotalus* is completely reduced, the right lung is perfused by the left pulmonary arch (Van Bourgondien and Bothner, 1969). Consequently, $\dot{Q}_{pul}$ can be readily assessed by placement of a single blood flow probe around the left pulmonary artery. Another probe was then placed around the LA_O. The leads of each probe were secured to the skin with a single suture and exteriorised from the animal through the incision, which was closed with intermittent sutures. Another ventral mid-line incision was made in the neck and snares, made of 3–0 silk suture (Kruuse A/S, Marslev, Denmark), were placed around the left and right vagi and exteriorised on the back of the snake. All snakes resumed spontaneous ventilation within 30 min of completion of the operative procedures and appeared to regain normal behavioural patterns within a few hours. All snakes were held in 60 cm×30 cm×15 cm plastic boxes at 24–28°C and allowed to recover overnight.

On the following day, blood flows and f_H were allowed to stabilise for one to two hours after connecting the probes to the flow meter. The snare surrounding one of the vagi was then pulled, with minimum disturbance to the unanaesthetised snake. After this unilateral vagotomy, blood flows were allowed to stabilise over the next 30–60 min, while measurements were taken. Complete vagotomy was then achieved by pulling the remaining snare. In five snakes, the left side was vagotomised before the right side whereas an opposite order of vagotomy was performed in the other five snakes.

Measurements of blood pressure, f_H and blood flows

Systemic arterial blood pressure (P_{sys}) was measured by connecting the systemic arterial catheter to a Baxter Edward (model PX600, Irvine, CA, USA) disposable pressure transducer. The signals from the pressure transducers were amplified using an in-house built pre-amplifier and were calibrated daily against a static column of water. The blood flow probes were connected to a Transonic dual channel blood flow meter (T206) for measurements of instantaneous blood flow rates. Data were collected electronically using a Biopac 100 acquisition package and analysed using AcqKnowledge data analysis software (v. 3.7.1; Biopac, Goleta, CA, USA).

Statistical analysis

The effects of left or right vagal stimulation on the relative changes in haemodynamic variables were analysed with a one-way analysis of variance (ANOVA) for repeated measures. A one-way ANOVA for repeated measures was also applied to assess the effects of unilateral and subsequent complete vagotomy on haemodynamic variables. Effects were considered significant whenever $P<0.05$.

RESULTS

Means of all cardiovascular variables measured in the anaesthetised and the fully recovered snakes at the start of the experiments are presented in Table 1. Heart rate was markedly higher in the anaesthetised snakes, compared with those that were fully recovered. Stroke volume was only slightly lower and total cardiac output was, therefore, higher than the recovered animals. Systemic blood flow was estimated as $3.3\times\dot{Q}LA_O$ (see Galli et al., 2005b) that allows

Table 1. Cardiovascular variables of anaesthetised and fully recovered rattlesnakes

	f_H (min^{-1})	$\dot{Q}_{pul}$ (ml kg^{-1} min^{-1})	$\dot{Q}LA_o$ (ml kg^{-1} min^{-1})	$V_{s,pul}$ (ml kg^{-1})	$V_{s,sys}$ (ml kg^{-1})	P_{sys} (cm H_2O)	$\dot{Q}_{pul}/\dot{Q}_{sys}$
Anaesthetised	40.6±3.2	36.7±7.2	14.1±4.7	0.91±0.11	1.12±0.15	36.5±5.2	0.79±0.12
Recovered	28.2±2.4*	33.6±4.2	9.3±1.1	1.14±0.17	1.21±0.13	–	1.20±0.14

The data for the two groups of snakes used for the studies involving vagotomy on the left or the right side did not differ and have been pooled in this table. f_H, heart rate; $\dot{Q}_{pul}$, pulmonary blood flow; $\dot{Q}LA_o$, blood flow in the left aortic arch; $V_{s,pul}$, stroke volume in the pulmonary circulation, $V_{s,sys}$, stroke volume in the systemic circulation (calculated as $\dot{Q}_{sys} = 3.3 \times \dot{Q}LA_o / f_H$); P_{sys}, systemic arterial blood pressure; $\dot{Q}_{sys}$, systemic blood flow (calculated as $\dot{Q}_{sys} = 3.3 \times \dot{Q}LA_o$). Statistical differences between anaesthetised and recovered animals are marked with an asterisk (Student's *t*-test).

for an estimation of cardiac shunt patterns, which is expressed as $\dot{Q}_{pul}/\dot{Q}_{sys}$. In the fully recovered snakes, a small net L–R cardiac shunt prevailed whereas the anaesthetised snakes were characterised by a small net R–L cardiac shunt.

Effects of vagal stimulation in anaesthetised snakes

The cardiovascular changes elicited by electrical stimulation of the efferent vagus differed substantially between the right and left vagi. Thus, although stimulation of either side led to pronounced reductions in f_H, stimulation of the left vagus also led to large reductions in $\dot{Q}_{pul}$. An experimental recording from one experiment, shown in Fig. 1, highlights these differences. The records depict similar reductions in f_H following stimulation of the right or left vagi. The bradycardia elicited by electrical stimulation of the right vagus was associated with an increase in V_s in both the systemic and pulmonary circulations. By contrast, a similar bradycardia during left vagal stimulation was associated with a marked and progressive reduction in the V_S to the pulmonary circulation whereas the increased V_S to the systemic circulation resembled the response to right vagal stimulation.

A similar response was observed in all nine animals and the means of the combined data are illustrated in Fig. 2. In this figure, we present the changes in systemic and pulmonary stroke volumes, as well as P_{sys}, during moderate reductions (to approximately 60%) and maximal reductions (to approximately 20%) in f_H, arising from different rates of stimulation of the left or the right vagi. Regardless of which of the vagi was stimulated, P_{sys} decreased and $V_{S,sys}$ increased as a proportion of the decrease in f_H. Although $V_{S,pul}$ increased slightly on stimulation of the right vagus, it decreased significantly on stimulation of the left vagus, independently of the reduction in f_H, implying pulmonary vasoconstriction.

All f_H and blood flow responses arising from electrical stimulation of the right or the left vagus were abolished upon infusion of atropine (3 mg kg^{-1}).

Afferent central stimulation of either side of the vagus was performed in five snakes. In none of the animals did we observe

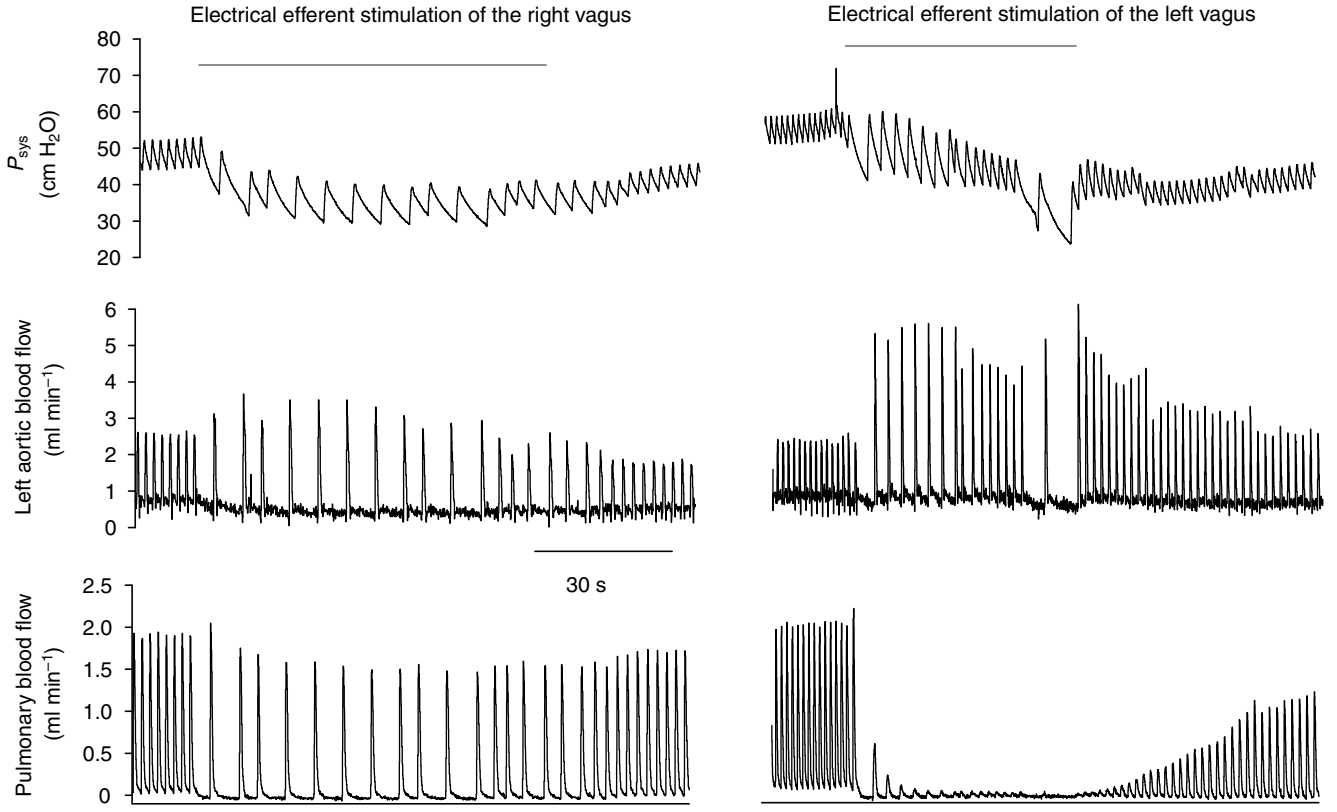

Fig. 1. A recording from an anaesthetised rattlesnake (*Crotalus durissus*, 754 g) of systemic arterial blood pressure (P_{sys}) as well as blood flows in the pulmonary artery ($\dot{Q}_{PA}$) and the left aortic arch ($\dot{Q}LA_O$) during a period of peripheral electrical stimulation of the left or right cervical vagus. The periods of electrical stimulation are shown by the right bars.

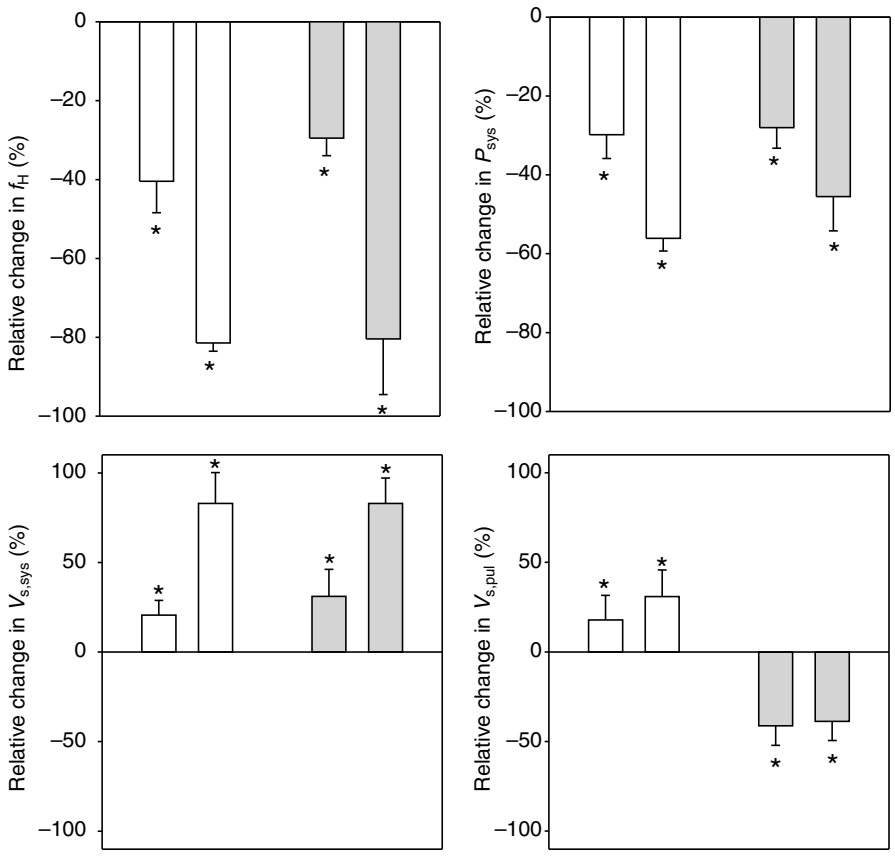

Fig. 2. Cardiovascular changes upon electrical stimulation of the right (open bars) or the left (closed bars) vagus in the rattlesnake (*Crotalus durissus*). Data are presented as the relative changes in heart rate (f_H), systemic blood pressure (P_{sys}), systemic stroke volume ($V_{s,sys}$) and stroke flow in the pulmonary artery ($V_{s,pul}$), caused by peripheral electrical simulation of the left or right vagus at intensities causing approximately 20 or 60% reductions in f_H, respectively. Data are presented as means ± s.e.m (N=9). Values that are significantly different from the resting undisturbed condition are marked with an asterisk.

changes in f_H or blood flow but small 5–10% reductions of systemic blood pressure were observed in some animals.

Effects of vagotomy on haemodynamic variables in fully recovered snakes

The differential actions of the left and right vagi were also evident from the cardiovascular changes that followed from selective vagotomy of either the right or left side in recovered snakes (Fig. 3). Transection of the left vagus, while leaving the right vagus intact, elicited a small but significant increase in f_H of approximately 10% (Fig. 3C) but was associated with a 70% increase in $\dot{Q}_{pul}$, while $\dot{Q}LA_O$ was not affected (Fig. 3A,B). The increased $\dot{Q}_{pul}$ could largely be ascribed to an elevated $V_{S,pul}$ (Fig. 4A). Subsequent complete vagotomy caused a 60% increase in f_H accompanied by a slight rise in $\dot{Q}_{pul}$, with no further change in $V_{S,pul}$ (Fig. 4A). By contrast, transection of the right vagus, leaving the left vagus intact, elicited a small increase in f_H and $\dot{Q}_{pul}$ but no change in $V_{S,pul}$. Subsequent complete vagotomy, following transection of the left vagus, was accompanied by marked increases in f_H, $\dot{Q}_{pul}$ and $V_{S,pul}$ (Fig. 3A,D; Fig. 4C), indicating that the left vagus plays a predominant role in controlling pulmonary blood flow.

DISCUSSION

In a study of lung morphology in snakes Cope labelled the lung in *Crotalus* as the left (Cope, 1894). This accords with our finding that stimulation of the left vagus reduced $\dot{Q}_{pul}$. However, Wallach, (Wallach, 1998), quoting other historical sources (e.g. Butler, 1895), stated emphatically that Cope was mistaken and that snakes typically retain the right lung. This apparent paradox was solved by Van Bourgondien and Bothner (Van Bourgondien and Bothner, 1969). Their careful study of the arterial systems of crotalid snakes showed that the 'right' (*sic*) lung was effectively perfused by the left pulmonary artery. Their detailed description of the topography of the major arteries surrounding the heart match our observations during the placement of flow probes around the pulmonary artery and the LA_O.

Blood flows, P_{sys} and f_H of the anaesthetised snakes are similar to previous studies on this species using a similar preparation (Galli et al., 2005a; Galli et al., 2005b; Galli et al., 2007; Hagensen et al., 2008). However, f_H and $\dot{Q}_{pul}$ of fully recovered snakes were lower than snakes anaesthetised with pentobarbital (Table 1). A similar effect has been observed in turtles and rattlesnakes (Crossley et al., 1998; Skals et al., 2005). Rattlesnakes equipped with data loggers for ECG that had recovered from anaesthesia for 120 h, carrying ECG electrodes attached to a data logger so that they were unrestrained, showed progressive recovery of f_H to nocturnal lows around 12 beats min^{-1} (Campbell et al., 2006). Thus, in the present study the combined implantation of flow probes and vagal snares seems to have reduced the extent of postoperative recovery of cardiac vagal tone. However, partial recovery was clearly achieved as pulling the snares increased f_H and had differential left/right effects on $\dot{Q}_{pul}$.

The vagus nerve exerts several important functions in the regulation of cardio–respiratory function in vertebrates and our study confirms this role in a reptile. On the efferent side, the vagus innervates the heart, smooth muscle surrounding the pulmonary artery as well as smooth muscle in the lungs. In *Crotalus*, the action of the vagus on the heart was confirmed by the observation that peripheral electrical stimulation of the vagus caused a pronounced bradycardia in anaesthetised snakes and that vagotomy led to a marked tachycardia in fully recovered snakes, indicating that the heart operates under a degree of vagal tone. Similarly, stimulation of the vagus as well as vagotomy elicited large changes in $\dot{Q}_{pul}$.

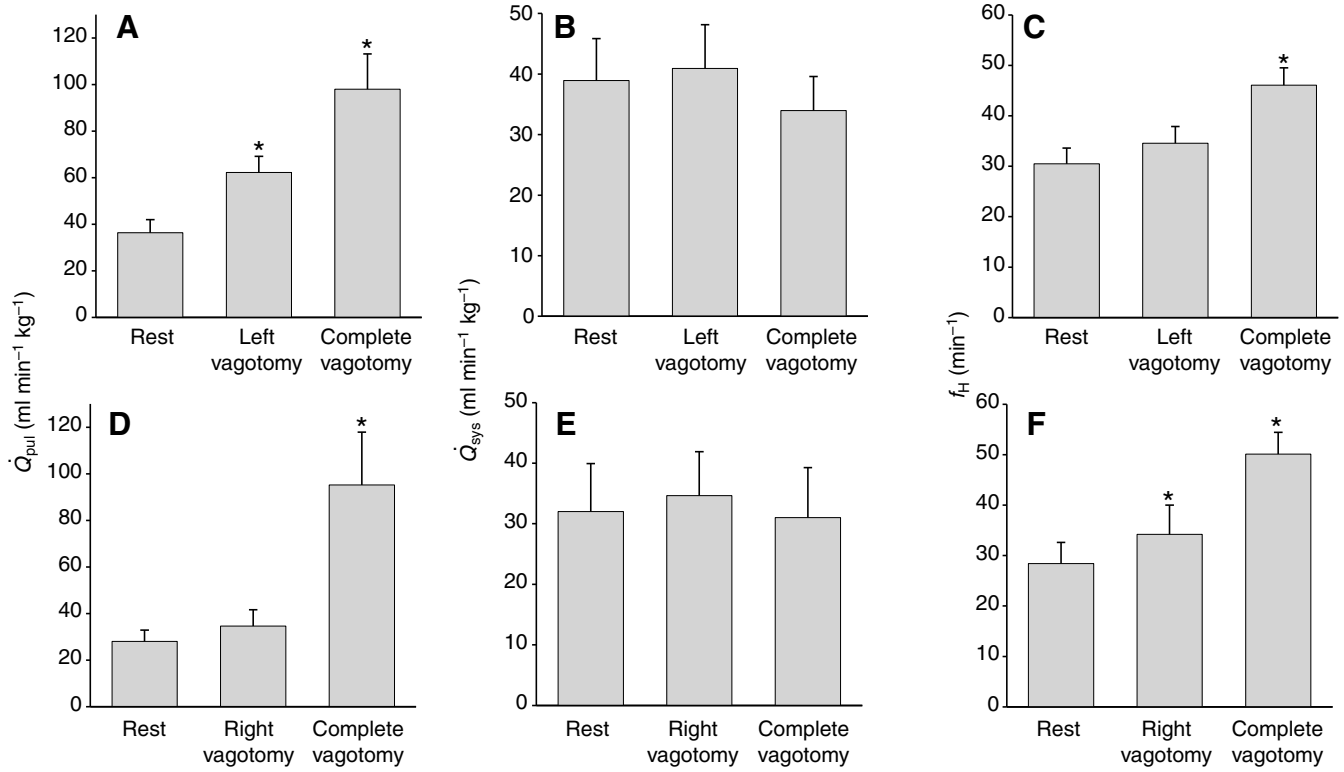

Fig. 3. The effects of progressive transection of the vagi on stroke volume in the pulmonary and systemic blood flows ($\dot{Q}_{pul}$ and $\dot{Q}_{sys}$, respectively) as well as heart rate (f_H) in unanaesthetised rattlesnakes (*Crotalus durissus*). In the first series of experiments (A–C), the left vagus was transected before the right whereas this order was reversed in the second series, i.e. right vagus was transacted before the left vagus (D–F). Either route terminated in complete vagotomy. $\dot{Q}_{sys}$ was calculated as 3.3 × blood flow in the left aortic arch. N=6 in each of the two experimental protocols and data are presented as means ± s.e.m. Values that are significantly different from the resting undisturbed condition are marked with an asterisk.

However, our study documents a pronounced unequal influence of the vagi on either side of the animal on pulmonary blood flow, whilst the vagi on both sides have similar influences on f_H. This observation is novel.

Bilateral differences in vagal efferent control on the heart have been reported in turtles (Gaskell, 1882; Mills, 1885; Garrey, 1911) and in isolated Langendorff perfused rabbit hearts, where stimulation of the right vagus causes larger reductions in f_H than the left vagus (Ng et al., 2001). Hicks and Comeau found that electrical stimulation of the right vagus of a turtle induced greater reductions in f_H and $\dot{Q}_{pul}$ than stimulation of the left vagus (Hicks and Comeau, 1994). However, the responses of the heart and the pulmonary circulation were qualitatively similar (Hicks and Comeau, 1994). In anaesthetised *Crotalus*, stimulation of either vagus slowed the heart but $\dot{Q}_{pul}$ was controlled solely by the left vagus. These observation are supported by vagotomy in recovered snakes where denervation of the left vagus caused large increases in $\dot{Q}_{pul}$ and $V_{S,pul}$ whereas $\dot{Q}_{pul}$ was unaffected by vagotomy on the right side. When the right side was denervated after the left side, there was nevertheless a further increase of $\dot{Q}_{pul}$. Although this could indicate a release of remaining tone on the pulmonary artery, it seems more likely that the rise in $\dot{Q}_{pul}$ is caused by the elevated f_H, where the increased cardiac output is directed into the low resistance circuit of the pulmonary circulation.

The vagal influence on the heart in *Crotalus* is consistent with numerous studies on different reptiles and other vertebrates, showing cardiac slowing upon vagal stimulation or infusion of acetylcholine (e.g. Gaskell, 1882; De la Lande et al., 1962; Kirby and Burnstock, 1969; Berger, 1971; Hedberg and Nilsson, 1975; Burggren, 1977a; Burggren, 1977b; Milsom et al., 1977; Wojtaszek, 1979; Donald et al., 1990b; Comeau and Hicks, 1994; Hicks and Comeau, 1994). As the vagus releases acetylcholine onto muscarinic cholinoceptors on the heart, its influence is antagonised by atropine (e.g. Burnstock, 1969; Hedberg and Nilsson, 1975; Berger and Burnstock, 1979; Morris and Nilsson, 1994). Although other neurotransmitters have been implicated in control of the heart in reptiles (Donald et al., 1990a; Lillywhite and Donald, 1994; Wang et al., 2001a), in *Crotalus*, atropine completely blocked the reduction in f_H during vagal stimulation. Furthermore, f_H after complete vagotomy (47.8±2.6 min^{-1} for all 12 snakes) was almost identical to the value of 48.3±1.1 min^{-1} that was observed in *Crotalus* after infusion with atropine (T.W., A.S.A. and E.W.T., unpublished observations). Thus, it seems that efferent control of f_H by the vagus in *Crotalus* can be attributed solely to the release of acetylcholine.

In reptiles, the vagus normally runs together with sympathetic nerves, in a mixed vagosympathetic nerve, so that vagotomy by nerve transection will result in removal of sympathetic tone as well as vagal tone on the heart. In some reptiles, f_H increases during electrical stimulation of the vagus following treatment with atropine, which has been interpreted as release of an adrenergic neurotransmitter from sympathetic nerves (De la Lande et al., 1962; Hedberg and Nilsson, 1975). This was not observed in *Crotalus*, although this species exhibits clear chronotropic responses to β-adrenergic stimulation (Skals et al., 2005). The heart in *Crotalus* operates under a degree of inhibitory vagal tone that overrides a smaller sympathetic tonus (Wang et al., 2001a; Wang et al., 2001b).

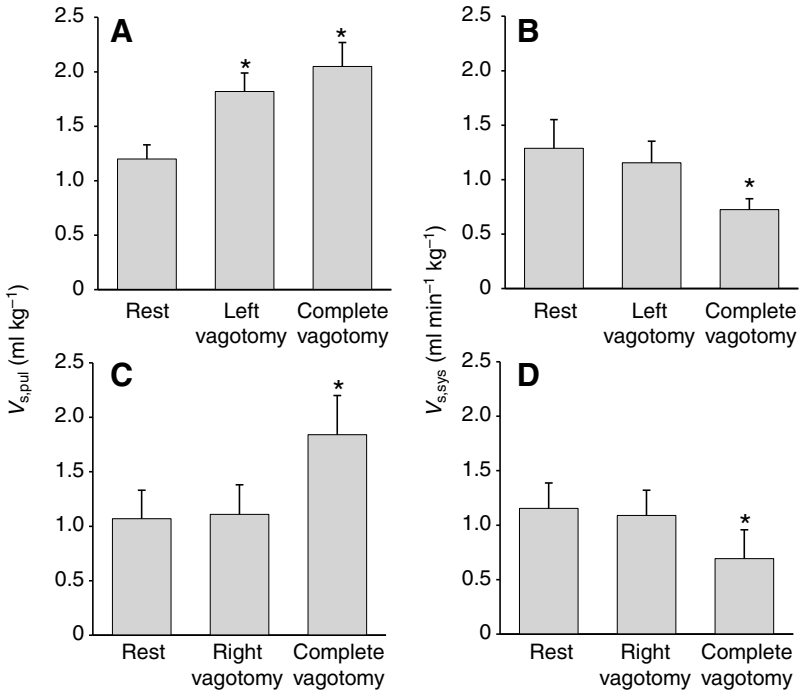

Fig. 4. The effects of progressive transection of the vagi on stroke volume in the pulmonary and systemic circulations ($V_{s,pul}$ and $V_{s,sys}$, respectively) in unanaesthetised rattlesnakes (*Crotalus durissus*). In the first series of experiments (A,B), the left vagus was transected before the right whereas this order was reversed in the second series (C,D). Either route terminated in complete vagotomy. $V_{s,sys}$ is calculated as 3.3 × stroke volume in the left aortic arch. N=6 in each of the two experimental protocols and data are presented as mean ± s.e.m. Values that are significantly different from the resting undisturbed condition are marked with an asterisk.

Furthermore, Galli and colleagues showed that the degree of adrenergic regulation of the pulmonary circulation is relatively small in *Crotalus* (Galli et al., 2007).

The absence of a pronounced effect of central stimulation of the vagus nerve is surprising as the vagus carries information from oxygen sensitive chemoreceptors on the aortic arches as well as stretch and CO_2 sensitive receptors within the lungs. Sundin and colleagues recorded lung stretch receptor responses from the peripheral cut end of the vagus in snakes (Sundin et al., 2001). Vagal innervation of pulmonary stretch receptors (PSRs) was also revealed by a pronounced increase in tidal volume following complete vagotomy (Wang et al., 2001a; Wang et al., 2001b). Stimulation of these and other vagal inputs, such as those from baroreceptors, would be expected to cause changes in cardiovascular variables (Hicks and Comeau, 1994). At present, we cannot offer a convincing explanation for why this was not observed in the rattlesnake, although changes would be mediated in part *via* the vagus nerve and the centrally stimulated branch was transected in this preparation.

The inhibitory role of vagal activity on $\dot{Q}_{pul}$ in *Crotalus* is consistent with previous studies on a range of reptiles (Burggren, 1977a; Burggren, 1977b; Milsom et al., 1977; Donald et al., 1990a; Donald et al., 1990b; Hicks and Comeau, 1994). Although the present study cannot reveal the precise location of the vagal regulation of pulmonary vascular resistance, it shows clearly that it is on a portion of the pulmonary artery that is innervated solely by the left vagus. In turtles, the vagus innervates smooth muscle on the main pulmonary artery (Burggren, 1977a; Milsom et al., 1977) and in the garter snake, *Thamnophis*, the innervation appears to be very close to the heart (Burggren, 1977b). Acetylcholine is recognised as the major neurotransmitter for the vagal innervation of the pulmonary circulation in a number of reptiles (Donald et al., 1990a; Donald et al., 1990b; Hicks, 1998) but non-adrenergic-non-cholinergic (NANC) regulation has been reported in the garter snake (Smith and Mcintyre, 1979).

Very little is known about the normal patterns of cardiac shunting in snakes and although some species possess prominent ventricular pressure separation (Wang et al., 2003), others can exhibit sizeable cardiac shunts (Lillywhite and Donald, 1989). *Crotalus* does not possess pressure separation between the systemic and pulmonary arteries (Galli et al., 2005a; Galli et al., 2005b) and large R–L cardiac shunts at low temperatures have been inferred from arterial oxygen levels (Wang et al., 1998). The very large increase in $\dot{Q}_{pul}$ with no attendent changes in $\dot{Q}_{sys}$ certainly indicates that L–R cardiac shunts can be very large in *Crotalus*. We did not measure blood flows in systemic arteries other than the LA_O but we are able to estimate $\dot{Q}_{sys}$ using the flow distribution among the systemic arches of anaesthetised *Crotalus* measured in a previous study (Galli et al., 2005b). On this basis, we estimate that the net cardiac shunt ($\dot{Q}_{pul}$–$\dot{Q}_{sys}$) of resting undisturbed *Crotalus* is rather small but that a net L–R shunt dominates. In turtles and toads, a net R–L shunt normally prevails in resting undisturbed animals and the development of a net L–R shunt is normally associated with exercise or hypoxia (West et al., 1992; Wang and Hicks, 1996; Wang et al., 1997; Wang et al., 1998; Gamperl et al., 1999; Wang and Hicks, 2002).

The physiological consequences of cardiac shunts in reptiles have been discussed for more than a century but it remains uncertain whether the ability to mix systemic and venous blood within the ventricle confers functional advantages (see Burggren, 1987; Hicks and Wang, 1996; Wang and Hicks, 2008). The many unresolved issues can, at least partially, be ascribed to the lack of good animal candidates on which we could manipulate cardiac shunts, without incurring unwanted side effects, and then measure long-term effects in performance (e.g. exercise, growth, digestion, etc.). The differential regulation of $\dot{Q}_{pul}$ by the left and right vagi in *Crotalus* raises an interesting possibility of using this species to study some functional consequences of cardiac shunts. Unilateral transection of the vagus did not cause major changes in f_H (Fig. 3C,F), which suggest that the other side remained functional and capable of maintaining an adequate tone on the heart. Thus, it is likely that unilaterally vagotomised *Crotalus* would retain the ability to exhibit normal f_H responses to altered metabolic demands. However, upon left vagal denervation, the remaining right vagus would not exert

neural control of pulmonary vascular resistance. Under such conditions, $\dot{Q}_{pul}$ would be determined exclusively by f_H as well as humoral and local influences on vascular resistances, which appear to be rather small in the pulmonary circulation of *Crotalus* (Galli et al., 2005a; Galli et al., 2005b; Galli et al., 2007). As a result, denervation of the left vagus is expected to chronically abolish the ability of *Crotalus* to reduce $\dot{Q}_{pul}$. It would be interesting to investigate whether this inability affected measurable physiological parameters such as minimal and maximal metabolic rate, temperature selection and lung fluid homeostasis, and whether in the long run appetite and growth rate were affected.

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REFERENCES

Berger, P. J. (1971). The vagal and sympathetic innervation of the heart of the lizard *Tiliqua rugosa*. *Aust. J. Exp. Biol. Med. Sci.* **49**, 297-304.

Berger, P. J. and Burnstock, G. (1979). Autonomic nervous system. In *Biology of the Reptilia. Neurology*, vol 10B (ed. C. Gans, R. G. Northcutt and P. Ulinski), pp. 1-57. New York: Academic Press.

Burggren, W. W. (1977a). The pulmonary circulation of the chelonian reptile: morphology, haemodynamics and pharmacology. *J. Comp. Physiol.* **116**, 303-323.

Burggren, W. W. (1977b). Circulation during intermittent lung ventilation in the garter snake *Thamnophis*. *Can. J. Zool.* **55**, 1720-1725.

Burggren, W. W. (1987). Form and function in reptilian circulations. *Am. Zool.* **27**, 5-19.

Burnstock, G. (1969). Evolution of the autonomic innervation of the visceral and cardiovascular systems in vertebrates. *Pharmacol. Rev.* **21**, 247-324.

Butler, G. W. (1895). On the complete or partial suppression of the right lung in the Amphisbaenidae and of the left lung in snakes and snake-like lizards and amphibians. *Proc. Zool. Soc. Lond.* **1895**, 691-712.

Campbell, H. A., Leite, C. A. C., Wang, T., Skals, M., Abe, A. S., Egginton, S., Rantin, F. T., Bishop, C. M. and Taylor, E. W. (2006). Evidence for a respiratory component, similar to mammalian respiratory sinus arrhythmia, in the heart rate variability signal from the rattlesnake, *Crotalus durissus terrificus*. *J. Exp. Biol.* **209**, 2628-2636.

Comeau, S. G. and Hicks, J. W. (1994). Regulation of central vascular blood flow in turtle. *Am. J. Physiol.* **36**, R569-R578.

Cope, E. D. (1894). On the lungs of the ophidia. *Proc. Am. Philos. Soc.* **33**, 217-224.

Crossley, D., Altimiras, J. and Wang, T. (1998). Hypoxia elicits an increase in pulmonary vasculature resistance in anaesthetised turtles (*Trachemys scripta*). *J. Exp. Biol.* **201**, 3367-3375.

De la Lande, I. S., Tyler, M. J. and Pridmore, B. R. (1962). Pharmacology of the heart of *Tiliqua* [*Trachysaurus*] *rugosa* (the sleepy lizard). *Aus. J. Exp. Biol.* **40**, 129-138.

Donald, J. A., O'Shea, J. E. and Lillywhite, H. B. (1990a). Somatostatin and innervation of the heart of the snake *Elaphe obsoleta*. *Am. J. Physiol.* **27**, R1001-R1007.

Donald, J. A., O'Shea, J. E. and Lillywhite, H. B. (1990b). Neural regulation of the pulmonary vasculature in a semi-arboreal snake, *Elaphe obsoleta*. *J. Comp. Physiol.* **159B**, 677-685.

Galli, G., Skovgaard, N., Taylor, E. W., Abe, A. S., Conlon, J. M. and Wang, T. (2005a). Purification and characterization of rattlesnake bradykinin and its cardiovascular actions in the South American rattlesnake *Crotalus durissus terrificus*. *Am. J. Physiol.* **288**, R456-R465.

Galli, G., Skovgaard, N., Abe, A. S., Taylor, E. W. and Wang, T. (2005b). The role of nitric oxide in the regulation of the systemic and pulmonary vasculature of the South American rattlesnake *Crotalus durissus terrificus*. *J. Comp. Physiol.* **175**, 201-208.

Galli, G. L. J., Skovgaard, N., Abe, A. S., Taylor, E. W. and Wang, T. (2007). The adrenergic regulation of the cardiovascular system in the South American rattlesnake *Crotalus durissus*. *Comp. Biochem. Physiol.* **148**, 510-520.

Gamperl, A. K., Milsom, W. K., Farrell, A. P. and Wang, T. (1999). The cardiovascular and ventilatory responses to hypoxia at two different temperatures in toads (*Bufo marinus*). *J. Exp. Biol.* **202**, 3647-3658.

Garrey, W. E. (1911). Rhythmicity in the turtle's heart and comparison of action of the two vagus nerves. *Am. J. Physiol.* **28**, 330-351.

Gaskell, W. H. (1882). On the innervation of the heart with especial reference to the heart of the tortoise. *J. Physiol.* **6**, 43-127.

Hagensen, M. K., Abe, A. S., Falk, E. and Wang, T. (2008). Physiological importance of the coronary arterial blood supply to the rattlesnake heart. *J. Exp. Biol.* **211**, 3588-3593.

Hedberg, A. and Nilsson, S. (1975). Vago-sympathetic innervation of the heart of the puff-adder, *Bitis arietans*. *Comp. Biochem. Physiol.* **53C**, 3-8.

Hicks, J. W. (1994). Adrenergic and cholinergic regulation of intracardiac shunting. *Physiol. Zool.* **67**, 1325-1346.

Hicks, J. W. (1998). Cardiac shunting in reptiles: mechanisms, regulation and physiological functions. In *Biology of Reptilia, Morphology G: Visceral Organs* (ed. C. Gans and A. S. Gaunt), pp. 425-483. New York: SSAR Press.

Hicks, J. W. and Comeau, S. G. (1994). Vagal regulation of intracardiac shunting in the turtle *Pseudemys scripta*. *J. Exp. Biol.* **186**, 109-126.

Hicks, J. W. and Wang, T. (1996). Functional role of cardiac shunts in reptiles. *J. Exp. Zool.* **275**, 204-216.

Kirby, S. and Burnstock, G. (1969). Comparative pharmacological studies of isolated spiral strips of large arteries from lower vertebrates. *Comp. Biochem. Physiol.* **28**, 307-319.

Lillywhite, H. B. and Donald, J. A. (1989). Pulmonary blood flow regulation in an aquatic snake. *Science* **245**, 293-295.

Lillywhite, H. B. and Donald, J. A. (1994). Neural regulation of arterial blood pressure in snakes. *Physiol. Zool.* **67**, 1260-1283.

Luckhardt, A. B. and Carlson, A. J. (1921). Studies on the visceral sensory nervous system VIII. On the presence of vasomotor fibers in the vagus nerve to the pulmonary vessels of the amphibian and the reptilian lung. *Am. J. Physiol.* **56**, 72-112.

Mills, T. W. (1885). The innervation of the heart of the slider terrapin (*Pseudemys rugosa*). *J. Physiol.* **6**, 246-286.

Milsom, W. K., Langille, B. L. and Jones, D. R. (1977). Vagal control of vascular resistance in the turtle, *Chrysemys scripta*. *Can. J. Zool.* **55**, 359-367.

Morris, J. L. and Nilsson, S. (1994). The circulatory system. In *Comparative Physiology and Evolution of the Autonomic Nervous System* (ed. S. Nilsson and S. Holmgren), pp.193-246. Newark, NJ: Harwood Academic Publishers.

Ng, G. A., Brack, K. E. and Coote, J. H. (2001). Effects of direct sympathetic and vagus nerve stimulation on the physiology of the whole heart-a novel model of isolated Langendorff perfused rabbit heart with intact dual autonomic innervation. *Exp. Physiol.* **86**, 319-329.

Skals, M. G., Skovgaard, N., Abe, A. S. and Wang, T. (2005). Venous tone and cardiac function in the South American rattlesnake (*Crotalus durissus*): mean circulatory filling pressure during adrenergic stimulation in anaesthetised and fully recovered animals. *J. Exp. Biol.* **208**, 3747-3759.

Smith, D. G. and MacIntyre, D. H. (1979). Autonomic innervation of the visceral and vascular smooth muscle of a snake lung (Ophidia: Colubridae). *Comp. Biochem. Physiol.* **62C**, 187-191.

Sundin, L., Burleson, M., Wang, T., Reid, S., Salgado, H., Abe, A., Glass, M. and Milsom, W. (2001). Pulmonary receptors in reptiles: discharge patterns of receptor populations in snakes *versus* turtles. *J. Comp. Physiol.* **171**, 103-111.

Van Bourgondien, T. and Bothner, R. C. (1969). A comparative study of the arterial systems of some new world crotalinae (Reptilia: Ophidia) *Am. Midl. Nat.* **81**, 107-147.

Wallach, V. (1998). The lungs of snakes. In *Biology of Reptilia, Morphology G: Visceral Organs* (ed. C. Gans and A. S. Gaunt), pp. 93-295. New York: SSAR Press.

Wang, T. and Hicks, J. W. (1996). The interaction of pulmonary ventilation and the right–left shunt on arterial oxygen levels. *J. Exp. Biol.* **199**, 2121-2129.

Wang, T. and Hicks, J. W. (2002). An integrative model to predict maximum oxygen uptake of animals with central vascular shunts. *Zoology* **105**, 45-53.

Wang, T. and Hicks, J. W. (2008). Changes in pulmonary blood flow do not affect gas exchange during intermittent ventilation in resting turtles. *J. Exp. Biol.* **211**, 3759-3763.

Wang, T., Fernandes, W. and Abe, A. S. (1993). Blood pH and O_2 homeostasis upon CO_2 anaesthesia in the rattlesnake (*Crotalus durissus*). *The Snake* **25**, 21-26.

Wang, T., Krosniunas, E. and Hicks, J. W. (1997). The role of cardiac shunts in the regulation of arterial blood gases. *Am. Zool.* **37**, 12-22.

Wang, T., Abe, A. S. and Glass, M. L. (1998). Effects of temperature on lung and blood gases in the South American rattlesnake, *Crotalus durissus terrificus*. *Comp. Biochem. Physiol.* **121A**, 7-11.

Wang, T., Taylor, E. W., Andrade, D. and Abe, A. S. (2001a). Autonomic control of heart rate during forced activity and digestion in the snake *Boa constrictor*. *J. Exp. Biol.* **204**, 3553-3560.

Wang, T., Warburton, S. J., Abe, A. S. and Taylor, E. W. (2001b). Vagal control of heart rate and cardiac shunts in reptiles: relation to metabolic state. *Exp. Physiol.* **86**, 777-786.

Wang, T., Altimiras, J., Klein, W. and Axelsson, M. (2003). Ventricular haemodynamics in *Python molurus*: separation of pulmonary and systemic pressures. *J. Exp. Biol.* **206**, 4241-4245.

West, N. H., Butler, P. J. and Bevan, R. M. (1992). Pulmonary blood flow at rest and during swimming in the green turtle, *Chelonia mydas*. *Physiol. Zool.* **65**, 287-310.

Wojtaszek, J. (1979). Motor reactivity of isolated heart of the grass-snake (*Natrix natrix*). Effect of vagal stimulation and selected cholinergic and adrenergic agents. *Acta Physiol. Pol.* **30**, 607-615.

Woodbury, R. A. and Robertson, G. G. (1942). The one ventricle pump and the pulmonary arterial pressure of the turtle: the influence of artificial acceleration of the heart, changes in temperature, hemmorrhage and epinephrine. *Am. J. Physiol.* **137**, 628-636.