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# The adrenergic regulation of the cardiovascular system in the South American rattlesnake, *Crotalus durissus*

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**Abstract**

The present study investigates adrenergic regulation of the systemic and pulmonary circulations of the anaesthetised South American rattlesnake, *Crotalus durissus*. Haemodynamic measurements were made following bolus injections of adrenaline and adrenergic antagonists administered through a systemic arterial catheter. Adrenaline caused a marked systemic vasoconstriction that was abolished by phentolamine, indicating this response was mediated through α-adrenergic receptors. Injection of phentolamine gave rise to a pronounced vasodilatation (systemic conductance ($G_{sys}$) more than doubled), while injection of propranolol caused a systemic vasoconstriction, pointing to a potent α-adrenergic, and a weaker β-adrenergic tone in the systemic vasculature of *Crotalus*. Overall, the pulmonary vasculature was far less responsive to adrenergic stimulation than the systemic circulation. Adrenaline caused a small but non-significant pulmonary vasodilatation and there was tendency of reducing this dilatation after either phentolamine or propranolol. Injection of phentolamine increased pulmonary conductance ($G_{pul}$), while injection of propranolol produced a small pulmonary constriction, indicating that α-adrenergic and β-adrenergic receptors contribute to a basal regulation of the pulmonary vasculature. Our results suggest adrenergic regulation of the systemic vasculature, rather than the pulmonary, may be an important factor in the development of intracardiac shunts.


*Keywords:* Reptile; Snake; Cardiovascular regulation; Adrenaline; Adrenergic stimulation; Pulmonary circulation; Systemic circulation

## 1. Introduction

Similar to mammals, the circulatory system of reptiles is under continuous autonomic nervous regulation by excitatory adrenergic sympathetic fibres as well as inhibitory cholinergic parasympathetic fibres (Morris and Nilsson, 1994). The overall regulation of systemic and pulmonary vascular tone has important implications for most reptiles, because their undivided hearts permit intracardiac shunting (Hicks, 1998). The degree and direction of the cardiac shunt flow depends on the relative vascular conductances of the systemic and pulmonary circulations (Hicks, 1994). Thus, an increase in systemic conductance ($G_{sys}$), or a reduction in pulmonary conductance ($G_{pul}$), will promote a right to left (R→L) cardiac shunt, so that blood by-passes the pulmonary circulation and is re-circulated within the systemic vasculature. Alternatively, if $G_{sys}$ is reduced or $G_{pul}$ increased, a net left to right (L→R) shunt will develop, with blood flow being directed towards the pulmonary circulation.

It is well established that regulation of reptilian cardiac shunts are primarily achieved via cholinergic vagal innervation of smooth muscle surrounding the pulmonary artery, where increased vagal activity causes pulmonary constriction and a subsequent decrease in $G_{pul}$ (Berger, 1972; Burggren, 1977; Smith and Macintyre, 1979; Lillywhite and Donald, 1994). However, histochemical studies have also identified

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adrenergic nerves innervating the pulmonary vasculature of reptiles (Smith and Macintyre, 1979; Donald and Lillywhite, 1988; Donald et al., 1990a). In particular, the rat snake, *Elaphe obsoleta,* has extensive innervation throughout the entire pulmonary circulation, including the lung parenchyma, indicating sympathetic regulation of pulmonary blood flow in the lung itself (Donald et al., 1990a,b). Nevertheless, the extent to which the sympathetic nervous system affects pulmonary vascular conductance in the different species of reptiles is not clear (for a review see Hicks, 1994). Although it is well established that cholinergic vagal control of the pulmonary artery determines shunt patterns, altering vascular resistance through adrenergic stimulation of either the systemic or pulmonary circulations may also contribute to control of intracardiac shunting (Lillywhite and Donald, 1989). Increased adrenergic tone is mainly associated with exercise and the classic "flight or flight" response and is often associated with reduced vagal tone (*e.g.* Wang et al., 2001a for a study on snakes). These reciprocal responses reduce the R→L shunt, and may even cause the development of a L→R shunt. A reduction in the R→L shunt increases arterial oxygen levels, which increases systemic oxygen delivery as metabolic demands are elevated (Wang and Hicks, 1996, 2002; Wang et al., 2001b).

In the present study, we seek to determine the extent to which adrenergic stimulation affects systemic and pulmonary conductance, and how adrenergic stimulation affects intracardiac shunt patterns in the South American rattlesnake (*Crotalus durissus*). *C. durissus* is common in arid areas throughout South America and we have recently documented a strong vagal influence on heart rate and pulmonary blood flow in this species, and have previously shown that they exhibit a large R→L cardiac shunt

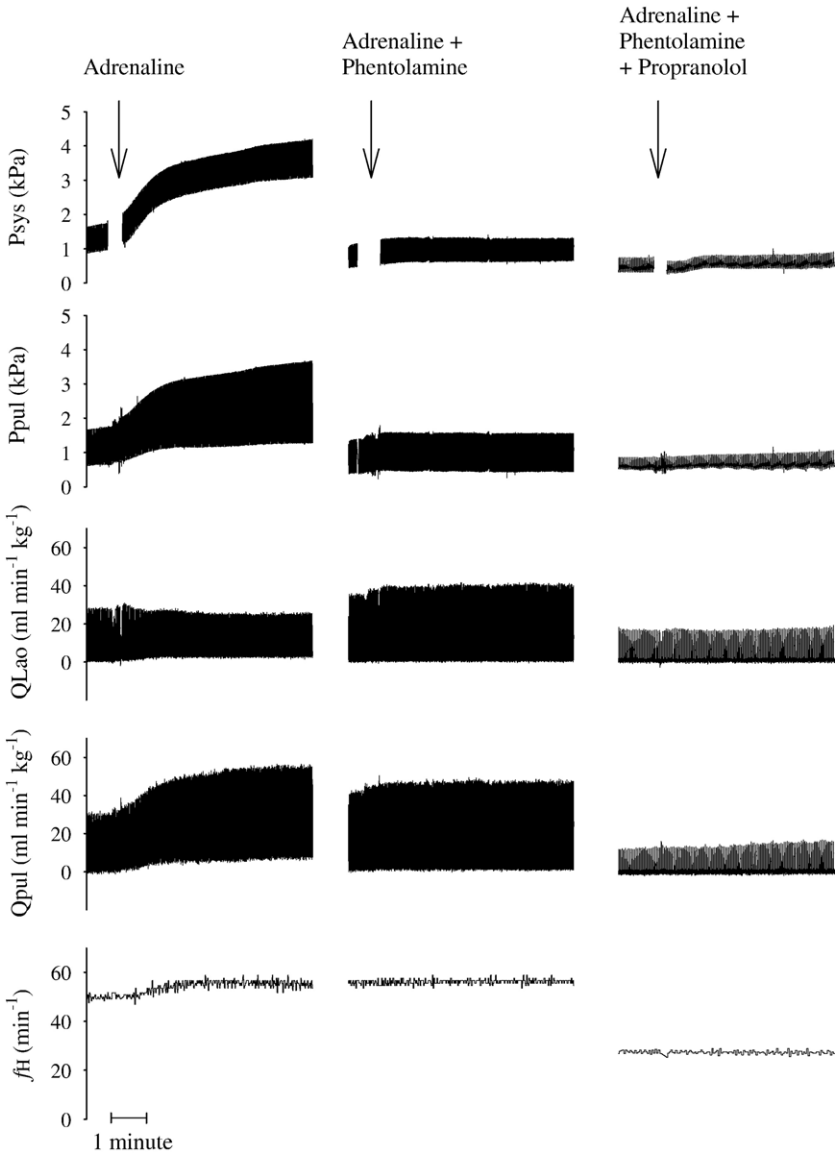


Fig. 1. Original traces obtained from a 550 g *Crotalus* showing haemodynamic variables during protocol 1, where adrenaline (2 μg $kg^{-1}$) was infused before and after phentolamine (2 mg $kg^{-1}$) and after complete adrenergic blockade following injection of propranolol (2 mg $kg^{-1}$).

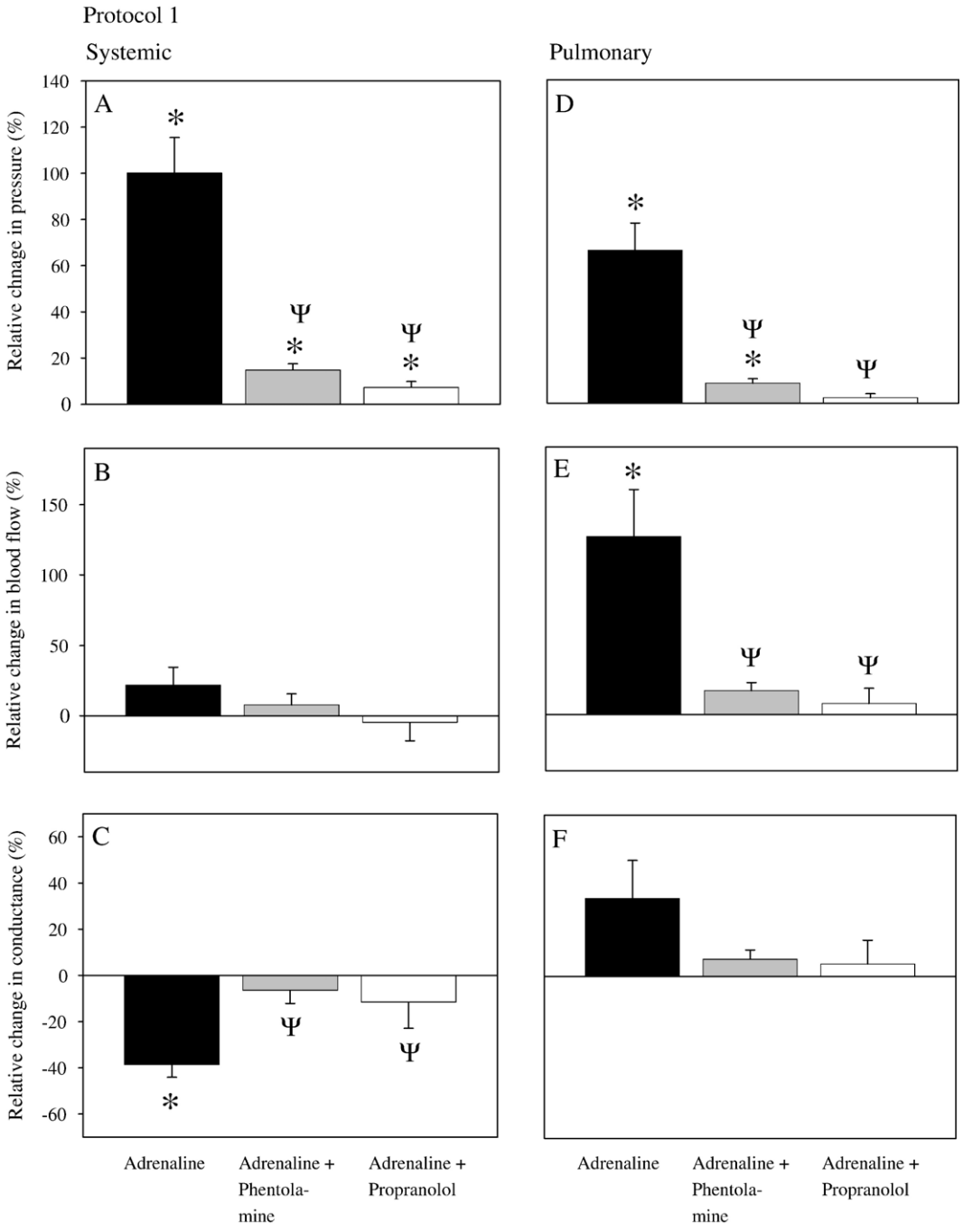


Fig. 2. Relative change in mean haemodynamic values in protocol 1 following a bolus injection of adrenaline (2 μg $kg^{-1}$); adrenaline after phentolamine (2 mg $kg^{-1}$); adrenaline after phentolamine and propranolol, (2 mg $kg^{-1}$). Systemic (A) and pulmonary (D) arterial blood pressure; systemic (B) and pulmonary blood flow (E); systemic (C) and pulmonary (F) vascular conductance. Values are mean with S.E.M. $N=8$, * indicates significant difference of mean from pre-injection value, $\psi$ indicates significant difference of mean from control adrenaline injection.

at low temperatures when metabolic demands are reduced (Wang et al., 1998).

## 2. Materials and methods

### 2.1. Experimental animals

Studies were performed on sixteen South American rattlesnakes, *C. durissus*, of both sexes and body masses ranging between 0.20 to 1.5 kg (0.64±0.06 kg; mean±S.E.M). Snakes were obtained from the Butantan Institute in São Paulo and transported to UNESP, Rio Claro, SP (Brazil) where they were housed in 0.5×0.5 m vivaria at a natural photoperiod and a temperature of 25–30 °C. The snakes had free access to water, but food was withheld one week prior to experimentation. All animals appeared healthy. All experiments were performed in accordance with guidelines for animal experiments under Universidade Estadual Paulista, Rio Claro (Brazil).

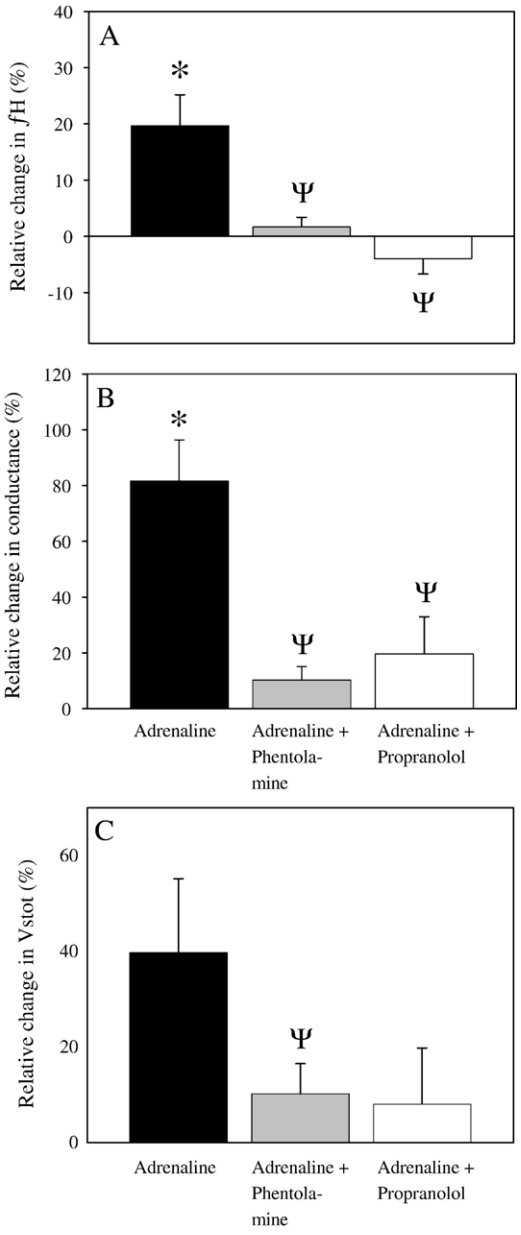


Fig. 3. Relative change in mean haemodynamic values in protocol 1 following a bolus injection of adrenaline (2 μg $kg^{-1}$); adrenaline after phentolamine (2 mg $kg^{-1}$); adrenaline after phentolamine and propranolol, (2 mg $kg^{-1}$). Heart rate, $f_H$ (A); shunt fraction, $Q_{pul}/Q_{sys}$ (B); total stroke volume, $Vs_{tot}$ (C). Values are mean with S.E.M. $N=8$, an asterisk indicates significant difference of mean from pre-injection value, $\psi$ indicates significant difference of mean from control adrenaline injection.

### 2.2. Surgery and instrumentation

Snakes were anesthetised by an injection of 30 mg $kg^{-1}$ pentobarbital into the tail muscle (Mebumal, Sygehusapotekerne, Denmark). All reflexes disappeared within 20 min and the animals were placed in a prone position, so that they could be tracheotomised for artificial ventilation at 4 breaths $min^{-1}$ and a tidal volume of 25 mL $kg^{-1}$ using a Harvard Apparatus mechanical ventilator. A 5 cm ventral incision was made cranial to the heart, and a PE50 catheter containing heparinised saline (50 IU/mL) was advanced into the right aortic arch through the vertebral artery. An additional incision was made immediately above the heart to allow for occlusive cannulation of a small branch of the pulmonary artery supplying the dorsal part of the lung with PE50 catheter containing heparinised saline. Both catheters were connected to Baxter Edward (model PX600, Irvine, CA, USA) disposable pressure transducers and the signals were amplified using an in-house built preamplifier. The pressure transducers were positioned at the level of the heart of the snake and were calibrated daily against a static water column.

For measurements of blood flows, 2S or 2R transit-time ultrasonic blood flow probes (Transonic System, Inc., NY) were placed around the left aortic arch (LAo) and the pulmonary artery. These flow probes were calibrated at the manufacturers at 25 °C and we periodically verified the calibration by generating known flows through either excised vessels or polyurethane tubing. Acoustical gel was infused around the blood flow probes to enhance the signal. Both flow probes were connected to a Transonic dual-channel blood flow meter (T206). Signals from the pressure transducers and the blood flow meter were recorded with a Biopac MP100 data acquisition system (Biopac Systems, Inc., Goleta, CA, USA) at 50 Hz.

### 2.3. Experimental protocol

Once all haemodynamic parameters had stabilised following surgery (approximately 30 min), the snakes were randomly divided into two experimental groups in which drugs were administered according to the following protocols:

Protocol 1: adrenaline (2 μg $kg^{-1}$), phentolamine (2 mg $kg^{-1}$), adrenaline (2 μg $kg^{-1}$), propranolol (2 mg $kg^{-1}$), adrenaline (2 μg $kg^{-1}$).

Protocol 2: animals in this protocol were subjected to the same protocol as the previous protocol, except that the injections of phentolamine and propranolol were reversed.

The dose of adrenaline used in these protocols was the dose found to produce maximal adrenergic stimulation in rattlesnakes, as assessed by maximal increase in pressure and flow (data not shown). All injections were given through the systemic arterial catheter in 1 mL $kg^{-1}$ aliquots. The catheter was flushed with 0.3 mL saline immediately following injection, and all haemodynamic variables were allowed to return to baseline levels before subsequent injections. There were no effects of sham injections of saline. The experimental protocol lasted for approximately 1.5 h. All experimental animals were sacrificed once the protocols were completed.

### 2.4. Calculation of blood flows, stroke volume and vascular conductances

Because rattlesnakes only have one lung and a single pulmonary artery, pulmonary blood flow ($Q_{pul}$) can be measured with a single flow probe. Total systemic blood flow

Table 1
Effects of adrenaline and adrenergic antagonists on haemodynamic variables in the anaesthetised rattlesnake, *Crotalus durissus*

| Protocol 1 | $P_{sys}$ (kPa) | $Q_{sys}$ (mL kg$^{-1}$ min$^{-1}$) | $G_{sys}$ (mL kPa$^{-1}$ min$^{-1}$ kg$^{-1}$) | $P_{pul}$ (kPa) | $Q_{pul}$ (mL kg$^{-1}$ min$^{-1}$) | $G_{pul}$ (mL kPa$^{-1}$ min$^{-1}$ kg$^{-1}$) | $f_H$ (min$^{-1}$) | $Vs_{tot}$ (mL kg$^{-1}$) | $Q_{pul}/Q_{sys}$ |
|---|---|---|---|---|---|---|---|---|---|
| Resting | 2.7±0.3 | 46.1±6.9 | 18.4±3.7 | 2.4±0.2 | 24.6±6.1 | 11.2±3.0 | 45.1±3.0 | 1.4±0.3 | 0.6±0.1 |
| Adrenaline | 5.2±0.6* | 54.3±8.6 | 10.3±1.5* | 3.9±0.5* | 45.4±6.4* | 12.5±2.2 | 52.9±1.5* | 1.8±0.3 | 1.0±0.2* |
| Pre-injection | 3.6±0.2 | 47.6±8.8 | 12.6±2.6 | 3.0±0.2 | 37.9±10.7 | 12.9±3.6 | 45.2±2.7 | 1.6±0.4 | 1.0±0.2 |
| Phentolamine | 2.1±0.3* | 54.9±11.2 | 28.9±8.3* | 2.0±0.2* | 28.7±10.1* | 12.9±3.6 | 51.2±2.2* | 1.5±0.4 | 0.6±0.2* |
| Pre-injection | 2.1±0.3 | 55.8±10.7 | 30.2±8.8 | 2.0±0.2 | 31.5±12.9 | 15.4±4.9 | 51.2±2.2 | 1.5±0.4 | 0.7±0.2 |
| Adrenaline | 2.4±0.4* | 61.7±13.1 | 29.0±8.7 | 2.2±0.2* | 34.0±11.7 | 15.8±4.6 | 52.0±1.9 | 1.6±0.4 | 0.7±0.2 |
| Pre-injection | 2.4±0.5 | 61.0±10.9 | 32.8±13.4 | 2.2±0.3 | 39.5±16.7 | 18.4±6.1 | 52.7±1.9 | 1.6±0.5 | 0.7±0.2 |
| Propranolol | 1.4±0.3* | 18.8±4.1* | 18.5±7.7* | 1.4±0.2* | 10.8±4.3* | 7.5±2.8 | 27.7±1.0* | 0.9±0.3* | 0.9±0.3 |
| Pre-injection | 1.5±0.3 | 19.8±4.5 | 19.3±8.0 | 1.4±0.2 | 11.7±4.9 | 8.1±3.3 | 27.8±1.0 | 0.9±0.3 | 0.9±0.3 |
| Adrenaline | 1.6±0.3* | 16.6±3.4 | 15.9±7.0 | 1.4±0.2 | 10.3±3.4 | 7.2±2.0 | 26.8±1.6 | 0.8±0.2 | 0.9±0.3 |

Values are mean±S.E.M. ($N$=8). An asterisk indicates a significant effect relative to the pre-injection value. $P_{pul}$, mean pulmonary blood pressure; $P_{sys}$, mean systemic blood pressure; $Q_{pul}$, pulmonary blood flow; $Q_{sys}$, systemic blood flow; $Q_{pul}/Q_{sys}$, shunt fraction; $G_{pul}$, pulmonary conductance; $G_{sys}$, systemic conductance; $f_H$, heart rate; $Vs_{tot}$, total stroke volume.

($Q_{sys}$) can be estimated as 3.3 times blood flow in left aortic arch ($Q_{LAo}$) (Galli et al., 2005b). Heart rate ($f_H$) was derived from the instantaneous blood flow trace from the left aortic arch and total stroke volume ($Vs_{tot}$; pulmonary+systemic) was calculated as $Q_{pul}+Q_{sys}/f_H$. When baseline blood flow changes more than baseline blood pressure, which is the case in most *in vivo* situations, conductance provide a better index for comparing vascular tone than resistance (Lautt, 1989; O'Leary, 1991). Pulmonary and systemic conductance ($G_{pul}$ and $G_{sys}$, respectively) were calculated from mean blood flow and mean blood pressure ($G_{pul}=Q_{pul}/P_{pul}$ and $G_{sys}=Q_{sys}/P_{sys}$) assuming that central venous blood pressures are negligible.

### 2.5. Data analysis and statistics

All data are presented as mean±S.E.M. Recordings of blood flows and pressures were analyzed using AcqKnowledge data analysis software (version 3.7.1.; Biopac, Goleta, CA). Effects on haemodynamic variables following injection were assessed by a paired *t*-test. To test whether the effects of adrenaline before and after pharmacological blockade were significantly different, a one-way repeated measures ANOVA on ranks was performed, followed by a Dunnett *post hoc* test to identify values that were significantly different from control values. A limit for significance of $P<0.05$ was applied.

## 3. Results

Fig. 1 shows an example of the changes in haemodynamic variables in protocol 1 following injection of adrenaline before and after injection of phentolamine, and before and after a subsequent injection of propranolol. The relative changes for these parameters are depicted in Figs. 2 and 3, while the absolute values are listed in Table 1. Table 2 and Figs. 4–6 display data from protocol 2 in the same format as the figures from protocol 1.

### 3.1. Resting haemodynamic values

The haemodynamic parameters of untreated animals did not differ between the two protocols (Tables 1 and 2). Systolic blood pressures were always similar between the systemic and

Table 2
Effects of adrenaline and adrenergic antagonists on haemodynamic variables in the anaesthetised rattlesnake, *Crotalus durissus*

| Protocol 2 | $P_{sys}$ (kPa) | $Q_{sys}$ (mL kg$^{-1}$ min$^{-1}$) | $G_{sys}$ (mL kPa$^{-1}$ min$^{-1}$ kg$^{-1}$) | $P_{pul}$ (kPa) | $Q_{pul}$ (mL kg$^{-1}$ min$^{-1}$) | $G_{pul}$ (mL kPa$^{-1}$ min$^{-1}$ kg$^{-1}$) | $f_H$ (min$^{-1}$) | $Vs_{tot}$ (mL kg$^{-1}$) | $Q_{pul}/Q_{sys}$ |
|---|---|---|---|---|---|---|---|---|---|
| Resting | 3.3±0.6 | 76.5±13.5 | 29.2±7.9 | 2.7±0.3 | 36.7±5.1 | 14.5±1.0 | 52.3±3.7 | 2.1±0.2 | 0.6±0.1 |
| Adrenaline | 5.4±0.7* | 101.5±17.0 | 21.6±4.4* | 3.9±0.3* | 51.3±6.3* | 15.1±1.1 | 58.3±2.1* | 2.6±0.3 | 0.6±0.1 |
| Pre-injection | 4.3±0.5 | 91.2±14.9 | 24.0±6.2 | 3.4±0.3 | 43.9±4.4 | 13.4±1.3 | 54.5±2.9 | 2.4±0.3 | 0.6±0.1 |
| Propranolol | 2.7±0.3* | 60.1±8.1* | 21.6±4.5 | 2.5±0.1* | 22.1±3.8* | 9.3±1.3 | 30.6±0.7* | 2.7±0.3 | 0.4±0.1* |
| Pre-injection | 2.7±0.3 | 59.9±8.1 | 25.4±5.5 | 2.6±0.1 | 21.6±3.8 | 9.1±1.4 | 30.1±0.7 | 2.7±0.3 | 0.4±0.1 |
| Adrenaline | 4.2±0.5* | 50.7±9.6 | 15.3±4.6* | 3.4±0.3* | 23.3±2.5 | 7.3±1.1 | 29.6±0.8 | 2.5±0.3 | 0.6±0.1* |
| Pre-injection | 3.5±0.4 | 52.0±9.4 | 18.0±5.0 | 2.9±0.2 | 21.5±2.1 | 7.9±0.7 | 28.2±0.7 | 2.6±0.3 | 0.6±0.2 |
| Phentolamine | 1.3±0.1* | 53.9±11.6 | 43.3±10.2* | 1.6±0.1* | 14.7±1.8* | 11.9±2.3* | 27.7±1.1 | 2.4±0.3 | 0.4±0.1 |
| Pre-injection | 1.3±0.1 | 53.9±11.6 | 43.3±10.2 | 1.6±0.1 | 14.7±1.8 | 11.9±2.3 | 27.7±1.1 | 2.4±0.3 | 0.4±0.1 |
| Adrenaline | 1.6±0.1* | 64.1±14.4 | 42.6±11.1 | 1.9±0.1* | 17.5±2.7* | 11.4±1.9 | 29.7±1.2* | 2.7±0.4 | 0.4±0.1 |

Values are mean±S.E.M. ($N$=8). An asterisk indicates a significant effect relative to the pre-injection value. $P_{pul}$, mean pulmonary blood pressure; $P_{sys}$, mean systemic blood pressure; $Q_{pul}$, pulmonary blood flow; $Q_{sys}$, systemic blood flow; $Q_{pul}/Q_{sys}$, shunt fraction; $G_{pul}$, pulmonary conductance; $G_{sys}$, systemic conductance; $f_H$, heart rate; $Vs_{tot}$, total stroke volume.

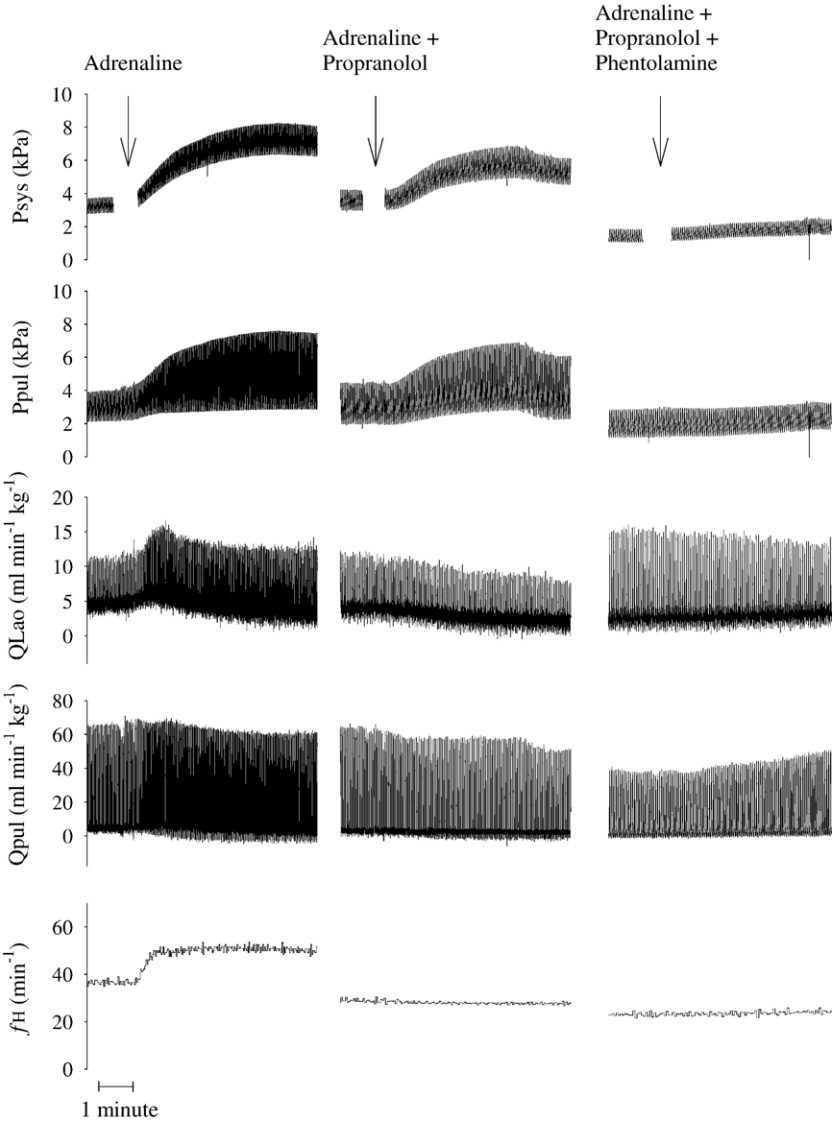


Fig. 4. Original traces of a 600 g *Crotalus* showing haemodynamic variables during protocol 2, where adrenaline (2 μg $kg^{-1}$) was infused before and after propranolol (2 mg $kg^{-1}$) and after complete adrenergic blockade following injection of phentolamine (2 mg $kg^{-1}$).

pulmonary circulations, but mean $P_{pul}$ was lower than mean $P_{sys}$ due to a lower diastolic pressure in the pulmonary circulation. In both protocols, a net R→L shunt was observed in untreated snakes where $Q_{sys}$ exceeded $Q_{pul}$.

### 3.2. Adrenergic control of the systemic circulation

Adrenaline caused a marked systemic vasoconstriction manifested as a large increase in $P_{sys}$ and decrease in $G_{sys}$ (Figs. 2A–C and 5A–C; Tables 1 and 2). Injection of phentolamine caused a systemic vasodilatation, where the approximate doubling of $G_{sys}$ was accompanied by a decrease in $P_{sys}$, whilst $Q_{sys}$ remained unchanged (Tables 1 and 2). Following treatment with phentolamine, the effect of adrenaline on $P_{sys}$ and $G_{sys}$ was virtually abolished (Fig. 2A-C). β-adrenergic blockade with propranolol caused a systemic vasoconstriction, which was accompanied by reductions in $P_{sys}$, $Q_{sys}$ and $G_{sys}$ (Tables 1 and 2). In protocol 2, treatment with propranolol increased the systemic vasoconstriction elicited by adrenaline, resulting in a diminished rise in $P_{sys}$ and a reduction in $Q_{sys}$ (Fig. 5A–C). Following double adrenergic blockade, adrenaline failed to elicit significant changes in $Q_{sys}$ or $G_{sys}$. However, $P_{sys}$ increased, indicating that adrenaline may stimulate additional pathways, which could not be inhibited by pharmacological blockade (Figs. 2A–C and 5A–C).

### 3.3. The pulmonary circulation

The pulmonary circulation was much less responsive to adrenergic stimulation than the systemic vasculature. Adrenaline had no effect on $G_{pul}$ whereas both $P_{pul}$ and $Q_{pul}$ increased (Figs. 2D–F, 5D–F; Tables 1 and 2). In protocol 1, phentolamine had no effect on $G_{pul}$, whilst $P_{pul}$ and $Q_{pul}$ decreased (Table 1). There were also no effects of adrenaline on $G_{pul}$ following treatment with phentolamine and the rise in $P_{pul}$ and $Q_{pul}$ were attenuated (Fig. 2D–F). Subsequent treatment with propranolol caused a non-significant pulmonary constriction decreasing

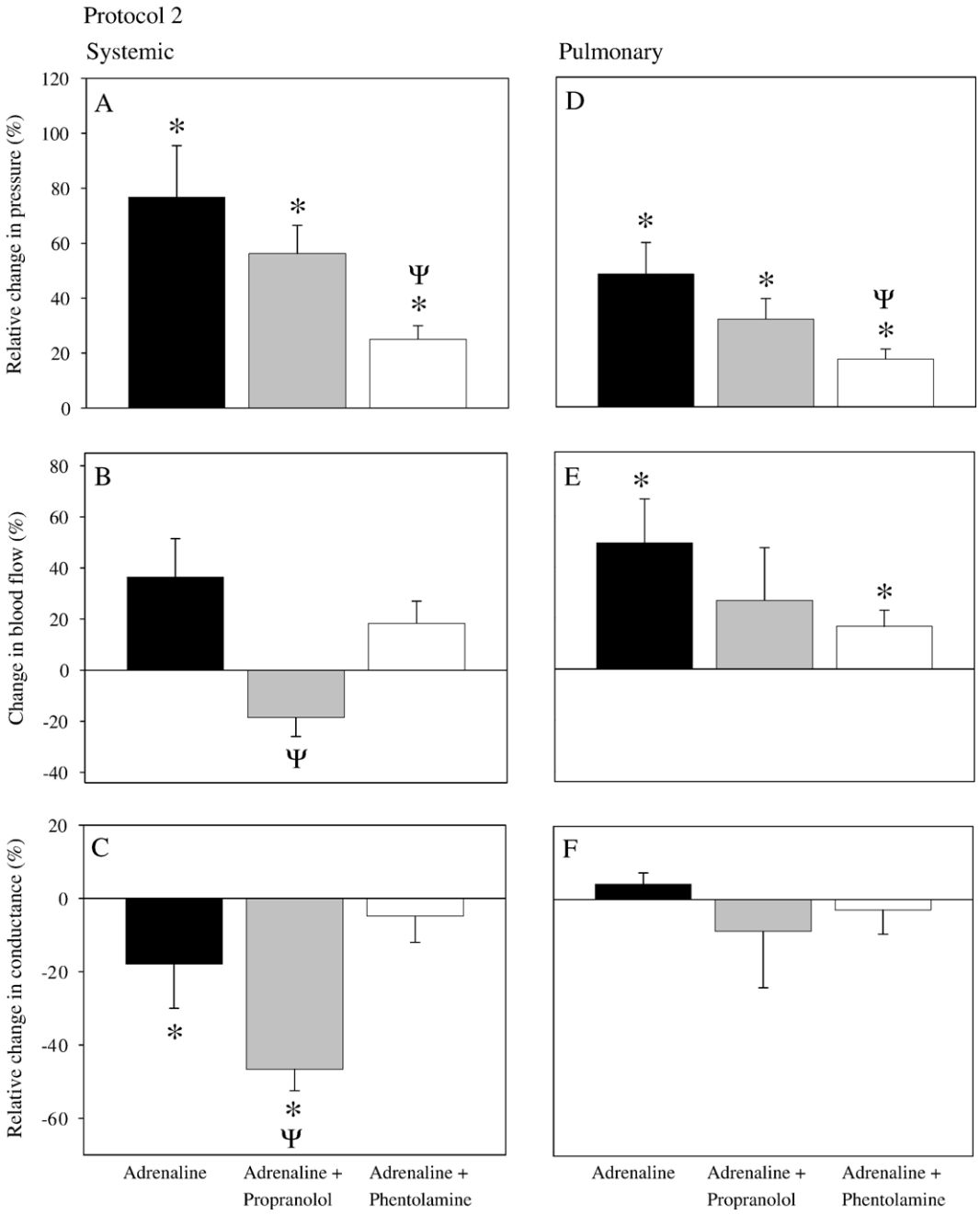


Fig. 5. Relative change in mean haemodynamic values in protocol 2 following a bolus injection of adrenaline (2 μg $kg^{-1}$); adrenaline after propranolol (2 mg $kg^{-1}$); adrenaline after propranolol and phentolamine (2 mg $kg^{-1}$). Systemic (A) and pulmonary (D) arterial blood pressure; systemic (B) and pulmonary blood flow (E); systemic (C) and pulmonary (F) vascular conductance. Values are mean with S.E.M. $N=8$, * indicates significant difference of mean from pre-injection value, $\psi$ indicates significant difference of mean from control adrenaline injection.

$P_{pul}$, $Q_{pul}$, and $G_{pul}$ ($P=0.06$). In protocol 1, there were no effects of adrenaline on the pulmonary circulation following total adrenergic blockade (Fig. 2D–F).

In protocol 2, injection of propranolol caused a small, but non-significant, pulmonary vasoconstriction accompanied by significant reductions in $P_{pul}$ and $Q_{pul}$ (Table 2). There were no changes in $G_{pul}$ when adrenaline was infused after propranolol, and the effects on $Q_{pul}$ and $P_{pul}$ were reduced (Fig. 5D–F). Phentolamine produced a significant vasodilatation in the pulmonary circulation after β-adrenergic blockade, accompanied by a decrease in $P_{pul}$ and $Q_{pul}$ (Table 2). Significant effects of adrenaline on $P_{pul}$ and $Q_{pul}$ persisted after total adrenergic blockade, indicating that adrenaline may have stimulated additional pathways which could not be inhibited by pharmacological blockade (Fig. 5D–F).

### 3.4. Heart rate and stroke volume

Injection of adrenaline caused a marked tachycardia (Figs. 3A and 6A; Tables 1 and 2). In protocol 1, injection of phentolamine caused an increase in $f_H$, and following this treatment, the

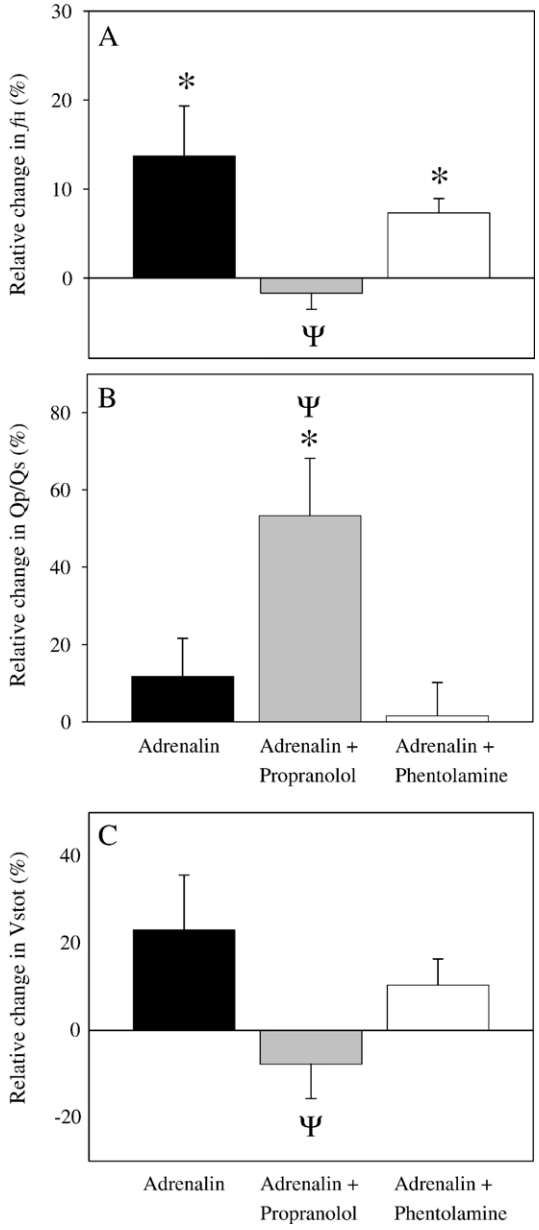


Fig. 6. Relative change in mean haemodynamic values in protocol 2 following a bolus injection of adrenaline (2 μg $kg^{-1}$); adrenaline after propranolol (2 mg $kg^{-1}$); adrenaline after propranolol and phentolamine (2 mg $kg^{-1}$). Heart rate, $f_H$ (A); shunt fraction, $Q_{pul}/Q_{sys}$ (B); total stroke volume, $Vs_{tot}$ (C). Values are mean with S.E.M. $N=8$, * indicates significant difference of mean from pre-injection value, $\psi$ indicates significant difference of mean from control adrenaline injection.

tachycardia associated with adrenaline was abolished (Table 1 and Fig. 3A). A reduction in $f_H$ was observed following injection of propranolol in protocol 1 (Table 1). Similarly, propranolol caused a decline in $f_H$ during protocol 2 and abolished the tachycardia associated with adrenaline injection (Table 2; Fig. 6A). In protocol 2, $f_H$ remained unchanged after injection of phentolamine. Following total adrenergic blockade, the $f_H$ response to adrenaline was abolished in protocol 1 but not in protocol 2 (Tables 1 and 2). Total stroke volume remained virtually unchanged throughout all treatments, except for injection of propranolol in protocol 1 where it decreased (Tables 1 and 2).

### 3.5. Cardiac shunt pattern

In protocol 1, adrenaline caused a reduction in the R→L shunt (increase in $Q_{pul}/Q_{sys}$), (Fig. 3B; Table 1). Injection of phentolamine caused an increase in the R→L shunt (Table 1). Following treatment with phentolamine, adrenaline and propranolol had no effect on $Q_{pul}/Q_{sys}$ (Fig. 3B; Table 1). In protocol 2, injection of propranolol produced an increase in the R→L shunt (Table 2). In the presence of propranolol, adrenaline reduced the R→L shunt (Fig. 6B). No significant effects of phentolamine on cardiac shunt patterns were observed, and there was no effect of adrenaline on $Q_{pul}/Q_{sys}$ after total adrenergic blockade (Fig. 6B).

## 4. Discussion

### 4.1. Limitations of the study

Heart rate of the anaesthetised rattlesnake studied here, and in our previous studies (Galli et al., 2005a,b), was considerably higher than fully recovered animals at similar temperatures, where $f_H$ is around 25 $min^{-1}$ (Wang et al., 2001b; Skals et al., 2005). A tachycardia during anaesthesia with barbiturates has previously been reported for *Crotalus* and turtles (Crossley et al., 1998; Skals et al., 2005) and seems to be caused by a rise in adrenergic tone and decreased parasympathetic activity. In fully recovered *Crotalus* and *Boa*, adrenergic tone on the heart is normally low, whilst parasympathetic tone is high (Wang et al., 2001a,b). It is likely that the higher adrenergic tone in anaesthetised animals also applies to the vascular tone and the effects of infusing adrenergic antagonists may therefore be larger than they would be in fully recovered animals. Studies on anaesthetised animals, however, have the advantage that autonomic and barostatic responses are muted, making it easier to identify the direct effects of pharmacological manipulations. A second limitation of our study is that phentolamine does not allow for the discrimination between hormonal and neural adrenergic regulation of the cardiovascular system. Nevertheless, our approach of having used both adrenaline and adrenergic antagonists allows for an assessment of the respective roles of both α- and β-adrenergic receptors on the systemic and pulmonary vasculatures as well as the heart. Within reptiles, a similar quantitative assessment only exists for freshwater turtles.

### 4.2. Adrenergic control of the systemic circulation

Adrenaline caused a systemic vasoconstriction in *Crotalus* that was abolished by phentolamine, indicating that it is mediated by α-adrenergic receptors. Moreover, phentolamine caused a considerable vasodilatation ($G_{sys}$ more than doubled, Table 1), pointing to a potent α-adrenergic tone in the systemic vasculature. Histochemical studies confirm a dense adrenergic innervation of most arteries in several species of reptiles (Donald and Lillywhite, 1988; Lillywhite and Donald, 1994; Morris and Nilsson, 1994) and many pharmacological studies on reptiles have documented that the systemic vasculature constricts in response to α-adrenergic

stimulation, whilst β-adrenergic stimulation leads to a small dilatation (*e.g.* Wang et al., 2000; Hicks and Farrell, 2000; Overgaard et al., 2002). In *Crotalus*, injection of propranolol caused a reduction in $G_{sys}$, implying a basal β-adrenergic tone in the systemic vasculature. The dilatory role of β-adrenergic stimulation was also revealed as a rise in $G_{sys}$ following injection of adrenaline in snakes treated with phentolamine.

### 4.3. Adrenergic control of the pulmonary circulation

While adrenergic stimulation consistently yields similar results in the systemic circulation of reptiles, conflicting data has been obtained in the pulmonary vasculature. Adrenaline and the adrenergic antagonists had relatively small effects on $G_{pul}$ in *Crotalus*, and it is clear that the pulmonary vasculature is less responsive than the systemic circulation. This is also the case in turtles (Overgaard et al., 2002). In both protocols, adrenaline caused a small, but non-significant, vasodilatation which was slightly reduced following treatment with either phentolamine or propranolol. In both protocols, there was a tendency for phentolamine to increase $G_{pul}$, and for propranolol to cause a vasoconstriction. This suggests that α-adrenergic receptors contribute to a basal constriction of the pulmonary vasculature, while β-adrenergic receptors contribute to a tonic dilatation. Our findings are, therefore, consistent with those in the rat snake, *E. obsoleta* (Donald et al., 1990a,b). In this species, adrenaline causes a marked vasodilatation of the pulmonary vasculature, which is reversed to a constriction following treatment with propranolol, and this constriction can be abolished by phentolamine. Thus, similar to *Crotalus*, it seems that stimulation of β-adrenergic receptors cause a vasodilatation, whilst α-adrenergic stimulation causes vasoconstriction in the lungs of rat snake (Donald et al., 1990a). In the turtles *Chrysemys* and *Malacoclemys*, the pulmonary response to catecholamine injections seem to be dose dependant; while small doses of epinephrine produce a vasodilatation, larger doses elicit a vasoconstriction (Luckhardt and Carlson, 1921). However, in *Chrysemys scripta*, stimulation of cervical sympathetic nerves, or injection of epinephrine failed to invoke a response in the pulmonary circulation (Milsom et al., 1977). This is also the case with the tortoise, *Chelodina longicollis* (Berger, 1972).

The rather small responses of the pulmonary circulation in reptiles seems somewhat surprising in light of the numerous histochemical studies that have revealed extensive adrenergic innervation of the pulmonary vasculature in lizards (McLean and Burnstock, 1967; Furness and Moore, 1970) and snakes (Lillywhite and Donald, 1989; Donald et al., 1990a,b). The rat snake *E. obsoleta*, for example, has extensive innervations throughout the entire pulmonary circulation, including the lung parenchyma, indicating sympathetic regulation of local pulmonary blood flow within the lung itself (Donald et al., 1990a). The low response of the rattlesnake pulmonary circulation to adrenergic stimulation is consistent with recent studies on various regulatory peptides and NO in different species of reptiles. Thus, in turtles, *Crotalus* and *Python regius*, there are marked effects of neuropeptide gamma, bradykinin, NO, and inhibition of nitric oxide synthase in the systemic circulation, but corresponding responses in the lungs are small, or even completely absent (Crossley et al., 2000; Galli et al., 2005a,b; Skovgaard et al., 2005a,b). These small responses may reflect the relatively simple lung structure of reptiles where the need for local regulation to secure ventilation-perfusion matching may be less important than in more complex lungs. Whatever the explanation, it is clear that parasympathetic control via the vagally innervated smooth muscle surrounding the pulmonary artery is the main source of pulmonary regulation (see Hicks, 1998; Wang et al., 2001b).

### 4.4. Adrenergic tone on the heart and the effects of adrenergic stimulation on stroke volume

The adrenergic fibres that innervate the reptilian heart exert positive ionotropic and chronotropic effects that are mediated through β-adrenergic receptors located in the pace-maker region, atria and ventricle (Van Harn et al., 1973; Morris and Nilsson, 1994). As in other snakes (Hedberg and Nilsson, 1975; Wojtaszek, 1979; Wang et al., 2000, 2001a,b; Zaar et al., 2007), the chronotropic effect of adrenaline was abolished by propranolol in *Crotalus*. In protocol 1, phentolamine also appeared to abolish the tachycardia caused by adrenaline, but, rather than representing the presence of α-adrenergic receptors on the heart, it is more likely that $f_H$ had already reached the maximal rate in response to the low blood pressure that followed α-adrenergic blockade. Chiu and Sham (1985), however, characterised the responses to adrenergic agonists and antagonists contractions of isolated atria from two species of snakes (*Naja naja* and *Ptyas korros*) and provided some evidence for an α-adrenergic chronotropic effect. In their preparation, the rate of beating of the isolated atria increased during exposure to phenylephrine and this response was abolished by phentolamine (Chiu and Sham, 1985). Nevertheless, β-adrenergic stimulation had considerably larger effects, and it was proposed that part of the response could be ascribed to pre-synaptic release of catecholamines in response to α-adrenergic stimulation (Sham et al., 1987).

Total stroke volume ($Vs_{tot}$) remained relatively constant in response to adrenergic stimulation even though $f_H$ rose, so it seems that the decrease in filling time did not compromise cardiac filling. The maintenance of $Vs_{tot}$ is likely to result from increased contractility, which would explain the observation that propranolol decreased $Vs_{tot}$ (Table 2). However, increased venous tone and elevated venous return is also likely to contribute to a rise in cardiac filling and an associated elevation of $Vs_{tot}$ through the Frank-Starling mechanism (*e.g.* Frank, 1895). Indeed, catecholamines increase central venous pressure in the snake, *E. obsoleta* (Lillywhite, 1987) and Skals et al. (2005) recently showed that venous tone and central venous pressure increases in response to α-adrenergic stimulation in *Crotalus*. This could explain that adrenaline failed to increase $Vs_{tot}$ after treatment with phentolamine (Table 1).

### 4.5. Adrenergic regulation of cardiac shunts — implications

High adrenergic tone is of paramount importance to the fight-or-flight response, where stimulation of metabolic pathways and

the cardiovascular system enable higher performance. Systemic oxygen delivery can be enhanced by a rise in $Q_{sys}$, but in reptiles arterial oxygen content may also be increased through a reduction in the cardiac R→L shunt (see Wang and Hicks, 1996, 2002). In *Crotalus*, adrenergic stimulation via adrenaline caused an increase in $Q_{pul}/Q_{sys}$, directing blood to the pulmonary circulation (reduced R→L shunt). Since $G_{pul}$ remained relatively constant, the pulmonary circulation is not actively contributing to this response, it is purely the result of the adrenergically induced systemic vasoconstriction, shunting blood into the pulmonary circuit. Indeed, all of the changes in $Q_{pul}/Q_{sys}$ observed in this study can be attributed to alterations in systemic conductance, rather than pulmonary. Thus, it is possible adrenergic regulation of systemic conductance may contribute to the development of intracardiac shunts. In contrast, although the pulmonary vasculature may be tonically adrenergically mediated, changes in conductance, and therefore shunt patterns, are primarily bought about through the well-established vagal control of the pulmonary artery.

Periods of elevated metabolic state in snakes, such as exercise or digestion, are associated with both an increase in adrenergic stimulation and a release of vagal tone (Wang et al., 2001a,b), inducing a decrease in systemic conductance, and an increase in pulmonary conductance respectively. These two responses will work oppositely but synergistically to shunt blood towards the pulmonary circulation (reduce the R→L shunt), thereby increasing arterial oxygen content. Thus, it seems that both adrenergic and parasympathetic regulation of the cardiovascular system in *Crotalus* may act together to facilitate intracardiac shunting.

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