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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

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Summary

The effects of adrenergic stimulation on mean circulatory filling pressure (MCFP), central venous pressure (P_{CV}) and stroke volume (V_S), as well as the effects of altered MCFP through changes of blood volume were investigated in rattlesnakes (*Crotalus durissus*). MCFP is an estimate of the upstream pressure driving blood towards the heart and is determined by blood volume and the activity of the smooth muscle cells in the veins (venous tone). MCFP can be determined as the plateau in P_{CV} during a total occlusion of blood flow from the heart.

V_S decreased significantly when MCFP was lowered by reducing blood volume in anaesthetised snakes, whereas increased MCFP through infusion of blood (up to 3 ml kg^{-1}) only led to a small rise in V_S. Thus, it seems that end-diastolic volume is not affected by an elevated MCFP in rattlesnakes. To investigate adrenergic regulation on venous tone, adrenaline as well as phenylephrine and isoproterenol (α- and β-adrenergic agonists, respectively) were infused as bolus injections (2 and 10 μg kg^{-1}). Adrenaline and phenylephrine caused large increases in MCFP and P_{CV}, whereas isoproterenol decreased both parameters. This was also the case in fully recovered snakes. Therefore, adrenaline affects venous tone through both α- and β-adrenergic receptors, but the α-adrenergic receptor dominates at the dosages used in the present study. Injection of the nitric oxide donor SNP caused a significant decrease in P_{CV} and MCFP. Thus, nitric oxide seems to affect venous tone.

Key words: reptile, *Crotalus durissus*, cardiovascular control, adrenergic regulation, venous tone, mean circulatory filling pressure, venous return.

Introduction

A primary function of the cardiovascular system is to provide adequate blood flow to secure nutrient and oxygen transport during different behaviours and environmental conditions. It is imperative, therefore, that cardiac output, the product of heart and stroke volume, can be altered accordingly. Increased heart rate and stroke volume during exercise or digestion have been documented in a number of reptiles (e.g. Gleeson et al., 1980; Stinner, 1987; Wang et al., 1997, 2001; Secor et al., 2000; Hicks et al., 2000; Farmer and Hicks, 2000; Munns et al., 2004; Clark et al., 2005). Stroke volume is the difference between end-diastolic and end-systolic volumes and is determined by myocardial contractility and cardiac filling. Increased venous return raises end-diastolic volume, which increases contractility and stroke volume *via* the Frank–Starling relationship (Guyton, 1955; Guyton et al., 1955, 1957; Bishop et al., 1964), and it is well established that the venous system plays an important role in determining the rate at which venous blood returns to the heart in mammals (Guyton, 1955; Guyton et al., 1957; Rothe, 1986; Tabrizchi and Pang, 1992; Pang, 2000).

Venous tone, which reflects the tonic contraction of the venous blood vessels, is a determining parameter for venous return and cardiac filling and can be assessed as the central venous pressure (P_{CV}) during a brief cessation of blood flow from the heart (Guyton, 1955; Pang, 2001). When cardiac output has stopped, blood will be redistributed between the arterial and venous system and pressures within the entire systemic circulation equalise (Rothe, 1993). This pressure, defined as mean circulatory filling pressure (MCFP) by Guyton et al. (1954), is determined by blood volume and compliance of the entire circulatory system. Importantly, MCFP represents the pressure in the small venules and is the best available estimate of the upstream pressure driving blood towards the heart (Guyton, 1955, 1963; Rothe, 1993). Thus, at a given right atrial pressure, venous return is proportional to MCFP (Tabrizchi and Pang, 1992). An increase in MCFP can be induced by increasing sympathetic tone or by increasing total blood volume. Conversely, a decrease in MCFP can be induced by decreasing sympathetic tone or decreasing total blood

volume (Pang, 2000). By altering blood volume and, thereby, changing MCFP, it is possible to evaluate the effect of a changed venous tone on stroke volume.

Changes in venous return through blood volume alterations markedly affect stroke volume in anaesthetised turtles and that venous return was significantly affected by adrenergic stimulation (S. Warburton, D. C. Jackson, V. T. Bobb and T. Wang, unpublished data). However, it is not known whether this response is mediated by α- or β-adrenergic receptors. Therefore, the present study was undertaken to investigate the role of these receptors on venous tone in anaesthetised rattlesnakes. Also, the effect of changed venous tone on cardiac filling was investigated. This was accomplished by the use of specific adrenergic agonists and antagonists and by blood infusions and withdrawals. Because anaesthetics may have depressive effects on the cardiovascular system, we also measured P_{CV} and MCFP during adrenergic stimulation in fully recovered snakes.

Materials and methods

Experimental animals

Twenty South American rattlesnakes *Crotalus durissus* L., of undetermined sex and a body mass ranging between 170 and 1850 g (644±83 g; mean ± S.E.M.) were obtained from the Butantan Institute in Sâo Paulo and maintained at UNESP, Rio Claro, Sâo Paulo state, Brazil for several weeks before experimentation. The animals had free access to water, whereas food was withheld for at least one week prior to experimentation. All experiments were performed at 25–28°C. The experiments were performed in accordance with guidelines for animal experiments under Universidade Estadual Paulista, Rio Claro (Brazil).

Surgery and instrumentation

Anaesthetised snakes

Twelve snakes were anaesthetised by an injection of 30 mg kg^{-1} pentobarbital (Mebumal, Sygehusapotekerne, Denmark) into the red muscle in the tail. When all reflexes had disappeared, the animals were tracheostemised and artificially ventilated at 4 breaths min^{-1} and a tidal volume of 35 ml kg^{-1} using a Harvard Apparatus mechanical ventilator (Cambridge, MA, USA).

A 5 cm ventral incision was made to expose the heart and major vessels. To measure systemic arterial blood pressure (P_{sys}), the vertebral artery was occlusively cannulated with a PE50 catheter containing heparinised saline (50 IU ml^{-1}). To measure central venous blood pressure (P_{CV}) a small vein running to the jugular vein was occlusively cannulated with a PE50 or PE90 catheter containing heparinised saline, and the tip of the catether was advanced into the sinus venosus. Both catethers were connected to Baxter Edward disposable pressure transducers (model PX600; Irvine, CA, USA) and the signals were amplified using an in-house-built preamplifier. Both transducers were positioned at the level of the heart of the animal and calibrated daily against a static water column.

For measurements of systemic and pulmonary blood flows, transit-time ultrasonic blood flow probes (2R or 2S; Transonic System, Inc., Ithaca, NY, USA) were placed around the left aortic arch (LAo) and the pulmonary artery. Flow probes were connected to a Transonic dual-channel blood flow meter (T206). Acoustic gel was infused around the flow probes to enhance the signal.

For measurements of MCFP, a 0.5–1 cm incision was made in the pericardium and a suture (1-0 silk) was placed around the common outflow tract of the heart, which included both aortic arches and the common pulmonary artery. The pericardium was subsequently closed with one or two sutures (4-0 silk).

Signals from the pressure transducers and flow meters were recorded with a Biopac MP100 data acquisition system (Biopac System, Inc., Goleta, CA, USA).

Recovered snakes

Eight snakes were anaesthetised with CO_2 (Wang et al., 1993) and given a local injection of lidocain ventrally above the heart. In all animals, a 5 cm incision was made ventrally to expose the heart and major vessels, and catheters were inserted to measure P_{sys} and P_{CV} as described for the anaesthetised snakes. In four snakes, a 0.5–1 cm incision was made in the pericardium, and a vascular occluder (In Vivo Metric, Healdsburg, CA, USA) was placed around the common outflow tract of the heart. The pericardium was subsequently closed with one or two sutures (4-0 silk). The animals were allowed to recover for 24 h after surgery before measurements started.

Experimental protocols

Anaesthetised snakes

The 12 anaesthetised animals were divided into two groups with two different experimental protocols. In protocol 1, the adrenergic control of the venous system was investigated by injection of adrenaline and specific α- and β-agonists (phenylephrine and isoproterenol, respectively). In this series of experiments, blood volume alterations were also made to investigate the effect on P_{CV}, MCFP and blood flows. The second protocol was designed to characterise the effect of blood volume alterations after α-adrenergic receptor blockade by injection of phentolamine, an α-antagonist. The effect of NO on the venous system was also investigated in the second series by injection of the NO donor sodium nitroprusside (SNP).

When all haemodynamic variables had stabilised following surgery, baseline values were recorded. Then, arterial outflows from the heart were occluded by tightening the suture around the common outflow tract, until both P_{CV} and P_{sys} had stabilised. This normally occurred within 35 s, and the stable and elevated P_{CV} during the occlusion was taken to indicate MCFP. Blood pressures and flows returned to baseline values within minutes after releasing the occlusion, and drugs were now administered in the manner described below for the two protocols. In all cases, MCFP was measured when the effect

of the drug on P_{sys} was maximal. All haemodynamic variables were allowed to return to baseline values, and MCFP was measured before each subsequent injection. Repeated measurements of MCFP were performed at the beginning of each experiment to show that repeated occlusions of blood flow did not affect haemodynamic variables.

When haemodynamic variables had returned to baseline values after the last injection, MCFP was recorded and blood was infused or withdrawn, as described for the two protocols below, and MCFP was measured immediately after each volume change. Each infusion or withdrawal was completed as quickly as possible. Infused blood came from a donor snake and the order of infusion or withdrawal was randomised. All injections were given through the systemic catheter, and the catheter was flushed with heparinised saline immediately following all injections.

Protocol 1. Two dosages of adrenaline (2 μg kg^{-1} and 10 μg kg^{-1}), two dosages of phenylephrine (2 μg kg^{-1} and 10 μg kg^{-1}) and two dosages of isoproterenol (2 μg kg^{-1} and 10 μg kg^{-1}) were injected. Blood was infused in steps of 9±0.5, 16.8±0.9 and 30.5±1.3 ml kg^{-1}. Blood was withdrawn in steps of 8.6±0.5, 14.8±0.5 and 23.1±1.1 ml kg^{-1}.

Protocol 2. Adrenaline (10 μg kg^{-1}), two dosages of SNP (2.5 μg kg^{-1} and 25 μg kg^{-1}), phentolamine (2 mg kg^{-1}) and adrenaline (2 μl kg^{-1}) were injected. Blood was infused in steps of 8±0.4, 15.7±0.7 and 29.6±0.4 ml kg^{-1}. Blood was withdrawn in steps of 8.4±0.8, 16.2±1.1 and 23.2±0.3 ml kg^{-1}. Each drug was dissolved in saline (0.9% w/v) and was administered in 1 ml kg^{-1} aliquots.

Recovered snakes

When the snakes had remained undisturbed for 2 h and exhibited stable haemodynamic variables, baseline values were recorded and adrenergic agonists were administered as described for protocol 1 for anaesthetised snakes. In the four animals with vascular occluders, MCFP was measured during rest and when the effects of the various drugs on P_{sys} were maximal by inflating the occluder. Haemodynamic variables normally returned to control values within 60 s after releasing the occlusion. Manipulation of blood volume and administration of SNP were not performed in the recovered snakes.

Calculation of cardiac output, heart rate, stroke volume and vascular resistance

Systemic cardiac output ($\dot{Q}_{sys}$) can be calculated as 3.3 times the flow in the LAo (Galli et al., 2005a,b). Since rattlesnakes only have a single pulmonary artery, pulmonary cardiac output ($\dot{Q}_{pul}$) can be measured using a single flowprobe. Total cardiac output ($\dot{Q}_{tot}$) was calculated as $\dot{Q}_{sys}+\dot{Q}_{pul}$. Heart rate ($f_H$) was derived from the flow trace of the LAo, and total stroke volume (V_{Stot}; systemic and pulmonary) was calculated as $\dot{Q}_{tot}/f_H$. Systemic vascular resistance (R_{sys}) was calculated from the difference between arterial and central venous blood pressures divided by the systemic cardiac output [$R_{sys}=(P_{sys}-P_{CV})/\dot{Q}_{sys}$]. Venous resistance ($R_{ven}$) was calculated from the difference between MCFP and P_{CV} divided by systemic cardiac output [$R_{ven}=(MCFP-P_{CV})/\dot{Q}_{sys}$; for details on venous resistance see Guyton et al., 1952; Pang, 2000, 2001].

Data analysis and statistics

All data are presented as means ± S.E.M. Blood pressure and flow recordings were analysed using AcqKnowledge data analysis software (version 3.7.1; Biopac, Goleta, CA, USA). Effects on haemodynamic variables after injections of the various drugs were tested using a paired *t*-test. Differences between the anaesthetised and recovered snakes were tested using a *t*-test. Effects on haemodynamic variables after infusion and withdrawal of blood within each protocol were tested using a one-way analysis of variance (ANOVA) for repeated measurements, followed by a Dunnet's *post hoc* test to identify values that were significantly different from control values. Differences in MCFP during blood volume alterations between the two protocols were tested using a two-way ANOVA. A limit for significance of $P<0.05$ was applied.

Results

Anaesthetised snakes

Examples of the measurements of MCFP before and immediately after injection of the high dosages of the adrenergic agonists are shown in Fig. 1. Occlusion of the common outflow tract from the heart, shown by the grey bars, caused a progressive decline of P_{sys} and a rise in P_{CV}. The stable P_{CV} during occlusion was taken as MCFP. Adrenaline delayed the rate at which P_{sys} declined during occlusion, but P_{CV} stabilised within 30 s in all anaesthetised snakes.

Effects of adrenergic agonists

Mean haemodynamic parameters before and after adrenergic stimulation are shown in Figs 2, 3, where black bars represent control values, and grey bars represent values after injection of the agonists. Both dosages of adrenaline caused a marked increase in P_{sys}, P_{CV} and MCFP, reflecting a constriction of the systemic vasculature. This constriction was manifested as a rise in R_{sys}, but adrenaline also increased R_{ven}, which indicates a marked constriction of the venous system. Heart rate tended to increase, although not significantly, after infusion of adrenaline. Adrenaline also caused an increase in $\dot{Q}_{pul}$ and a decrease in $\dot{Q}_{sys}$, causing a rise of $\dot{Q}_{pul}/\dot{Q}_{sys}$, which reflects an increased L–R cardiac shunt (recirculation of blood within the pulmonary circulation). All changes in haemodynamic variables due to adrenaline were dose dependent, being more pronounced at the highest dose.

The α-agonist phenylephrine generally elicited similar responses on the systemic vasculature to those of adrenaline but did not affect f_H and $\dot{Q}_{pul}$. Thus, phenylephrine elicited a significant rise in P_{CV} and MCFP, reflecting an increased venous tone. The high dosages of phenylephrine caused a significant increase in P_{sys}, R_{sys} and R_{ven} and a significant decrease in $\dot{Q}_{sys}$ as the systemic vasculature constricted.

Injection of the β-agonist isoproterenol caused significant decreases in P_{sys}, P_{CV}, MCFP, R_{sys} and R_{ven}. Thus,

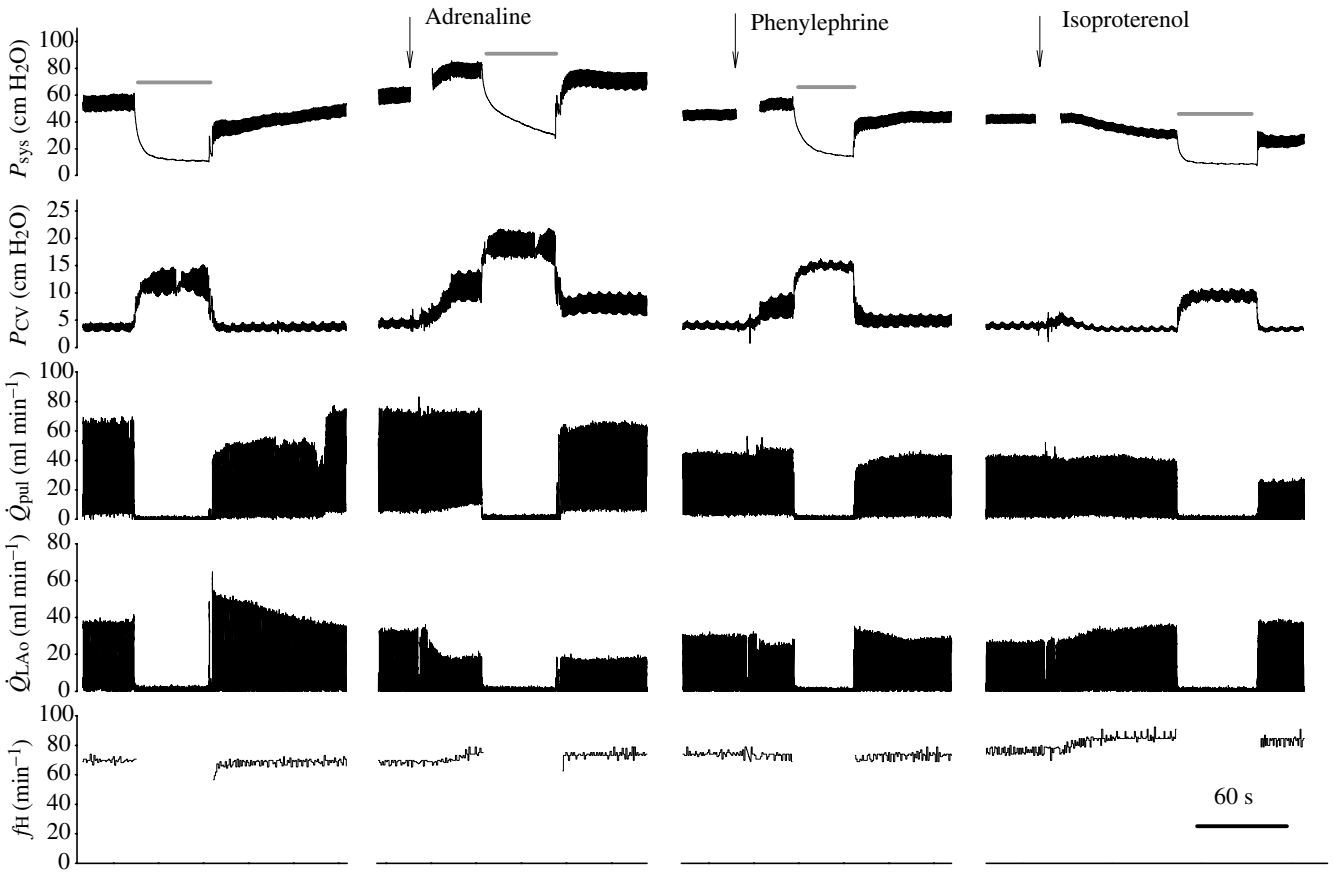

Fig. 1. Haemodynamic parameters in an anaesthetised South American rattlesnake *Crotalus durissus* (525 g), before and after injection of adrenaline (10 μg kg^{-1}), phenylephrine (10 μg kg^{-1}) and isoproterenol (10 μg kg^{-1}). Arrows indicate injection of the various drugs. Grey bars indicate occlusion of blood flow from the heart to measure mean circulatory filling pressure (MCFP). P_{sys}, systemic blood pressure; P_{CV}, central venous pressure; $\dot{Q}_{pul}$, pulmonary cardiac output; $\dot{Q}_{LAo}$, left aortic cardiac output; f_H, heart rate. (1 cm H_2O=0.098 kPa.)

Table 1. *The effects of injected SNP in anaesthetised South American rattlesnakes* Crotalus durissus

	P_{CV} (cm H_2O)	P_{sys} (cm H_2O)	f_H (min^{-1})	MCFP (cm H_2O)	$\dot{Q}_{LAo}$ (ml kg^{-1} min^{-1})	$\dot{Q}_{pul}$ (ml kg^{-1} min^{-1})	$\dot{Q}_{sys}$ (ml kg^{-1} min^{-1})
Baseline	3.1±0.22	40.8±3.49	49.2±1.86	8.4±1.15	32.3±10.5	57.1±22.1	106.5±34.8
SNP[1]	2.8±0.18*	20.3±3.20*	51.7±4.35	6.6±0.94	33.1±9.24	23.1±5.95	109.3±30.5
Baseline	3.2±0.28	41.6±4.84	50.3±3.49	8.64±1.08	30.2±13.9	61.3±28.6	99.6±46.2
SNP[2]	2.7±0.21*	19.9±1.96*	55.4±5.37	5.5±0.85*	30.7±6.97	24.9±8.1	101.4±23.0

	$\dot{Q}_{tot}$ (ml kg^{-1} min^{-1})	V_{Ssys} (ml kg^{-1})	V_{Spul} (ml kg^{-1})	V_{Stot} (ml kg^{-1})	R_{sys} (cm H_2O ml^{-1} min^{-1} kg^{-1})	R_{ven} (cm H_2O ml^{-1} min^{-1} kg^{-1})	$\dot{Q}_{pul}/\dot{Q}_{sys}$
Baseline	159.3±35.4	2.16±0.71	1.06±0.36	3.20±0.70	0.58±0.19	0.07±0.02	0.77±0.23
SNP[1]	134.9±35.2	2.02±0.58	0.49±0.18	2.52±0.71*	0.25±0.09*	0.04±0.01*	0.29±0.08
Baseline	145.3±40.9	1.97±0.83	0.92±0.3	2.88±0.77	1.03±0.54	0.13±0.06	1.31±0.72
SNP[2]	122.9±25.0	1.89±0.43	0.42±0.13	2.29±0.51	0.25±0.08*	0.04±0.01*	0.28±0.07

Values are means ± S.E.M. (N=5). Asterisks denote a significant difference relative to the baseline value. SNP[1] (2.5 μg kg^{-1}), SNP[2] (25 μg kg^{-1}).

P_{CV}, central venous blood pressure; P_{sys}, mean systemic blood pressure; f_H, heart rate; MCFP, mean circulatory filling pressure; $\dot{Q}_{LAo}$, left aortic arch cardiac output; $\dot{Q}_{pul}$, pulmonary cardiac output; $\dot{Q}_{sys}$, systemic cardiac output; $\dot{Q}_{tot}$, total cardiac output; V_{Ssys}, systemic stroke volume; V_{Spul}, pulmonary stroke volume; V_{Stot}, total stroke volume; R_{sys}, systemic resistance; R_{ven}, venous resistance; $\dot{Q}_{pul}/\dot{Q}_{sys}$, ratio of pulmonary cardiac output to systemic cardiac output. (1 cm H_2O=0.098 kPa.)

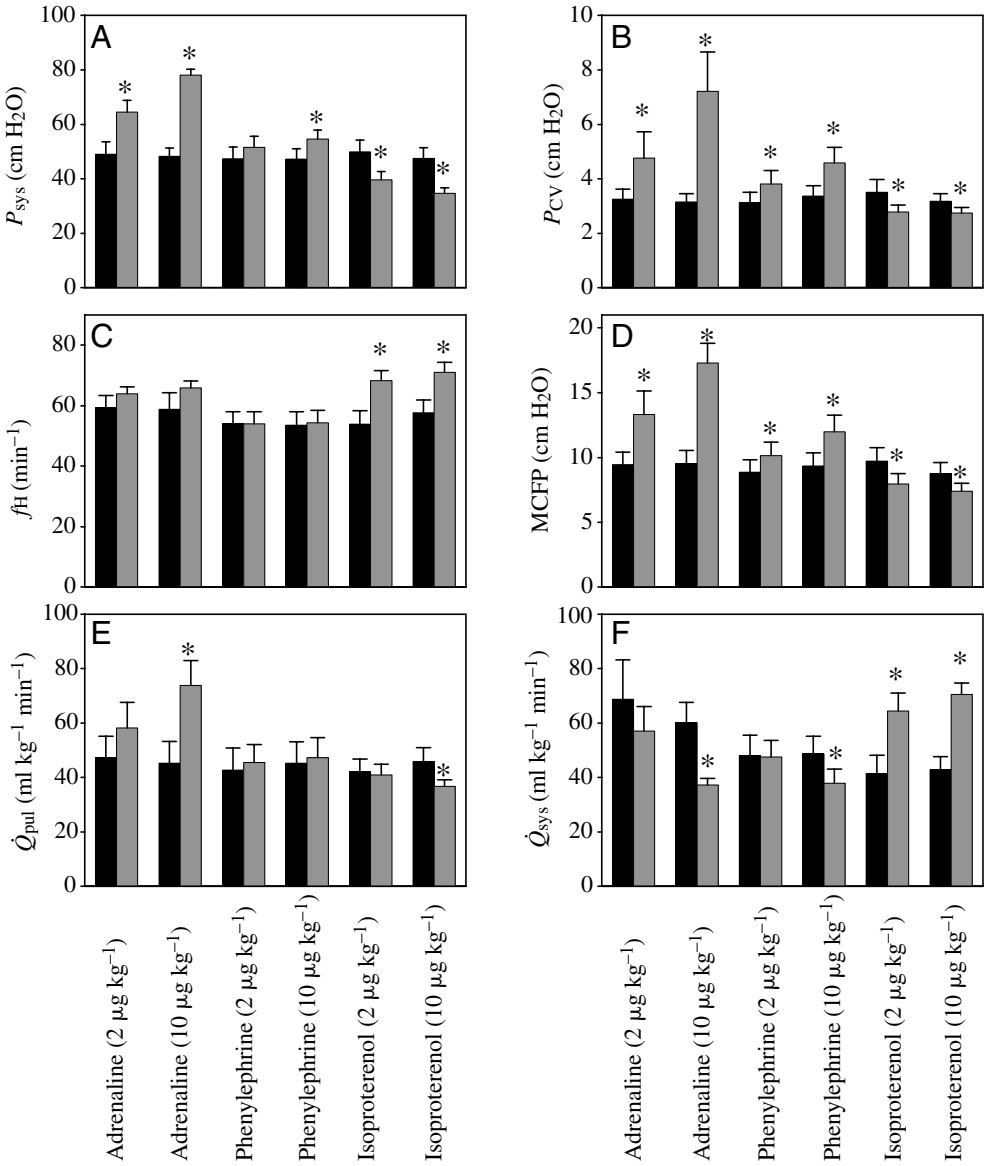

Fig. 2. Effects of bolus injections (2 and 10 μg kg^{-1}) of adrenaline, phenylephrine and isoproterenol on haemodynamic variables in anaesthetised South American rattlesnakes *Crotalus durissus*: (A) P_{sys}, mean systemic blood pressure; (B) P_{CV}, central venous blood pressure; (C) f_H, heart rate; (D) MCFP, mean circulatory filling pressure; (E) $\dot{Q}_{pul}$, pulmonary cardiac output; (F) $\dot{Q}_{sys}$, systemic cardiac output. Black bars represent control values. Grey bars represent values after injection of the various adrenergic agonists. Values are means ± S.E.M. (N=6). Asterisks indicate a significant difference relative to control values. (1 cm H_2O=0.098 kPa.)

isoproterenol seemed to cause an overall relaxation of both the arterial system and the venous system. Furthermore, isoproterenol caused significant increases in f_H and systemic blood flow and a decrease in pulmonary blood flow. As a consequence, $\dot{Q}_{pul}/\dot{Q}_{sys}$ decreased.

Effects of nitric oxide

The effects of injecting the NO donor SNP are shown in Table 1. SNP caused a significant decrease in P_{sys}, P_{CV}, MCFP and a decrease in both R_{sys} and R_{ven}, reflecting an overall relaxation of the vasculature. Regarding blood flows, SNP caused no changes in $\dot{Q}_{sys}$ but did affect $\dot{Q}_{pul}$, resulting in a decrease in $\dot{Q}_{tot}$ and V_{Stot}.

MCFP at blood volume alterations

The effects of manipulating blood volume by withdrawal and infusion of blood are shown in Figs 4–6 for untreated animals (black symbols) and the group of snakes where the α-adrenergic receptors had been blocked by phentolamine (grey symbols). Before treatment with phentolamine, both groups responded similarly to adrenaline (Table 2; Figs 2, 3), and the efficacy of the α-adrenergic blockade was evident from the lack of vasoconstriction following infusion of adrenaline (Table 2).

As shown in Fig. 4, MCFP increased significantly for both the untreated and the α-blocked snakes when blood volume was elevated by blood infusion and tended to decrease when blood was withdrawn. This presentation of MCFP as a function of blood volume represents 'capacitance curves', and the inverse slope of the capacitance curve represents the overall compliance (C) of the vasculature, under the assumption that there is no transcapillary fluid movement during manipulation of blood volume (Samar and Coleman, 1978). In the present study, compliance was estimated to be 3.3±0.3 and

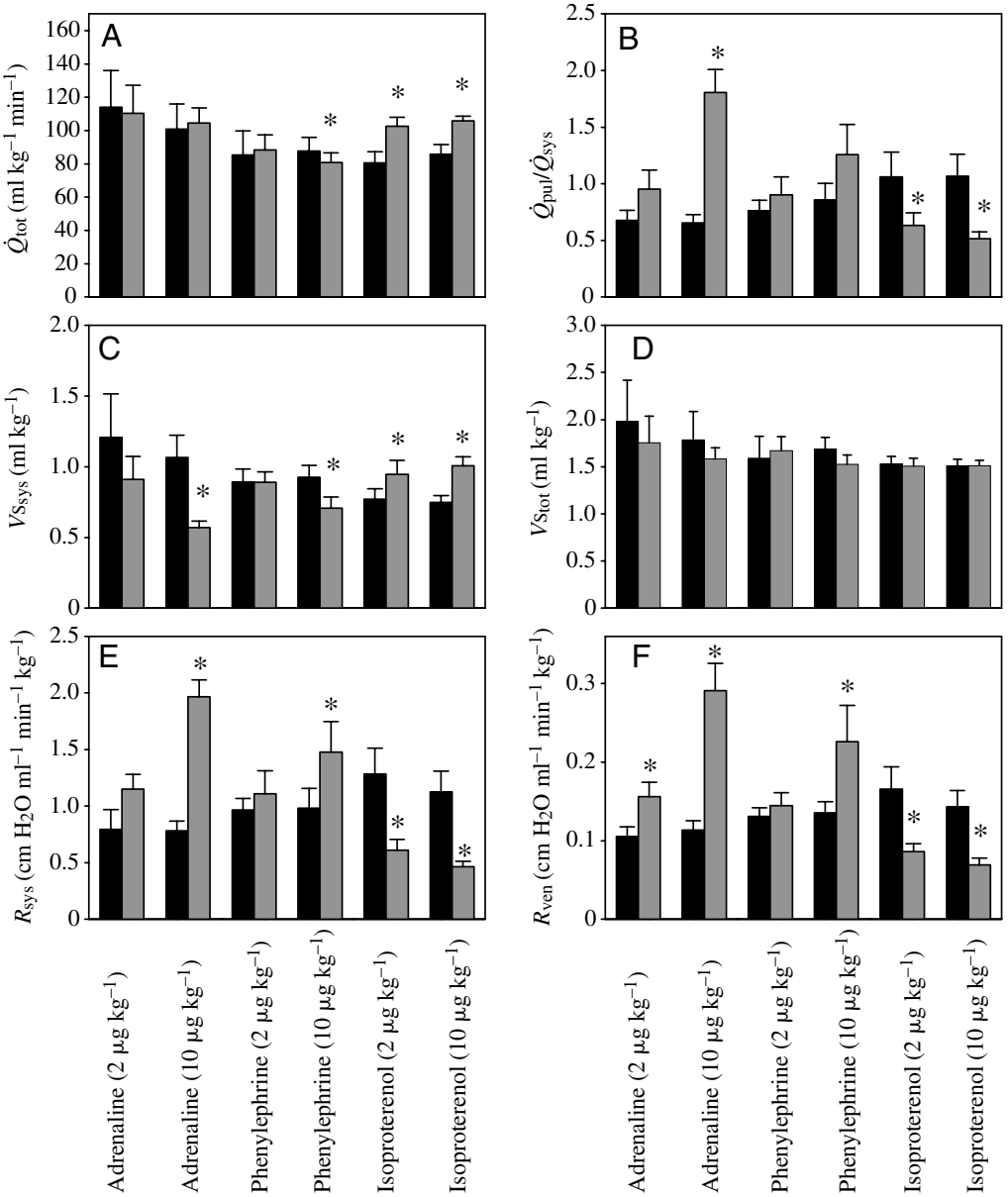

Fig. 3. Effects of bolus injections (2 and 10 μg kg^{-1}) of adrenaline, phenylephrine and isoproterenol on haemodynamic variables in anaesthetised South American rattlesnakes *Crotalus durissus*: (A) $\dot{Q}_{tot}$, total cardiac output; (B) $\dot{Q}_{pul}/\dot{Q}_{sys}$, ratio of pulmonary cardiac output to systemic cardiac output; (C) Vs_{sys}, systemic stroke volume; (D) Vs_{tot}, total stroke volume; (E) R_{sys}, systemic resistance; (F) R_{ven}, venous resistance. Black bars represent control values. Grey bars represent values after injection of the various adrenergic agonists. Values are means ± S.E.M. (N=6). Asterisks indicate a significant difference relative to control values. (1 cm H_2O=0.098 kPa.)

3.9±0.7 ml kg^{-1} cmH_2O^{-1} for untreated and α-blocked snakes, respectively. Unstressed blood volume (USBV), the blood volume at zero distending pressure, can also be estimated from the capacitance curves by extrapolating the curve to a MCFP value of zero (Samar and Coleman, 1978; Rothe, 1993; Fig. 4). We did not measure total blood volume, but using the value of 54 ml kg^{-1} measured on the closely related *Crotalus viridis* (Smits and Lillywhite, 1984), we estimate USBV to be 20.7±1.8 and 18.3±7.1 ml kg^{-1} for the untreated and α-blocked snakes, respectively (Fig. 4). There were no significant differences between C and USBV between the two groups; i.e. untreated and α-blocked snakes.

Figs 5, 6 show the effect of changing blood volume on the various haemodynamic parameters in untreated snakes (black symbols), where the haemodynamic variables are presented as a function of MCFP at each blood volume. In these snakes, P_{sys}, P_{CV}, $\dot{Q}_{pul}$, $\dot{Q}_{sys}$, $\dot{Q}_{tot}$, Vs_{pul}, Vs_{sys} and Vs_{tot} were significantly affected by MCFP. Increased MCFP by volume loading did not lead to a significant rise in $\dot{Q}$ or Vs, but a lowering of MCFP by blood withdrawal generally resulted in a decline of $\dot{Q}$ and Vs. Blood volume did not affect f_H and R_{ven}, but there was tendency of R_{sys} to increase in response to low blood volume.

The effects of phentolamine at normal blood volume are listed in Table 2, and the relationships between haemodynamic variables and MCFP, obtained after manipulation of blood volume, are also included in Figs 5, 6 (grey symbols). The group of snakes that received α-blockade had higher $\dot{Q}_{sys}$ and Vs_{sys} than the group of untreated snakes; as a consequence, these snakes had significantly higher $\dot{Q}_{pul}/\dot{Q}_{sys}$ than untreated snakes. At normal blood volume, the snakes responded to phentolamine by reductions in P_{sys} and R_{sys}, but these changes were not statistically significant (Table 2). During the

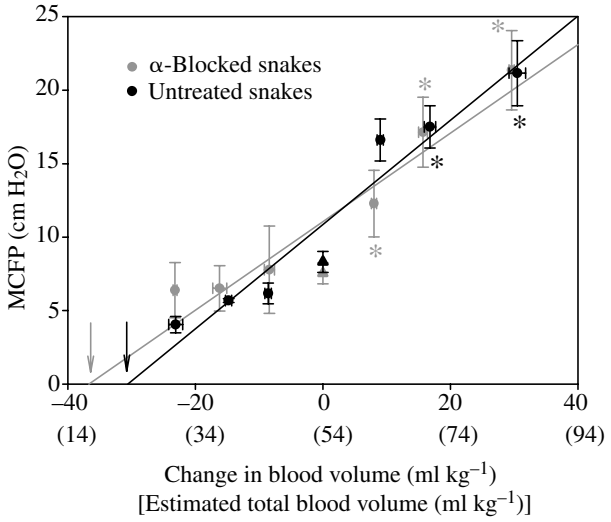

Fig. 4. Capacitance curves. Mean circulatory filling pressure (MCFP) as a function of a change in blood volume (ml kg^{-1}) in anaesthetised South American rattlesnakes *Crotalus durissus.* 0 ml kg^{-1} represents normal blood volume and the control value. Values in parentheses represent blood volume, assuming this species to have a blood volume of 54 ml kg^{-1}. Black symbols represent the untreated snakes and grey symbols represent the α-blocked snakes. Triangles indicate control values at normal blood volume. A weighted linear regression curve has been fitted to each group of snakes, where the black curve (y=0.306x+10.17) and the grey curve (y=0.256x+9.14) represent the untreated and the α-blocked animals, respectively. Arrows indicate unstressed blood volumes. Values are means ± S.E.M. (N=6). Asterisks indicate a significant difference relative to control values. (1 cm H_2O=0.098 kPa.)

subsequent manipulation of blood volume, the phentolamine-treated snakes responded qualitatively similarly to the untreated snakes, but $\dot{Q}_{sys}$ and $\dot{Q}_{tot}$ as well as Vs_{sys} and Vs_{tot} remained elevated at all MCFPs. As a major difference, the phentolamine-treated snakes had significantly lower R_{sys} and R_{ven}, and there was no compensatory increase in R_{sys} during volume depletion of the α-blocked snakes.

Recovered snakes

Measurements of MCFP before and after injections of adrenergic agonists from one animal are shown in Fig. 7. The three other animals with vascular occluders responded similarly. Blood pressures and f_H for the two groups of snakes with and without vascular occluders are illustrated in Fig. 8, where the left hand-panel (Fig. 8A–D) shows data from snakes with a vascular occluder, whereas results from the animals without an occluder are shown in the right-hand panel (Fig. 8E–G). Baseline values for heart rates and blood pressures were similar in the two groups, and they responded similarly to adrenergic agonists, although the group without a vascular occluder generally showed more pronounced responses. As in the anaesthetised snakes, adrenaline caused a marked rise in P_{sys}, P_{CV} and MCFP, which was mirrored after injection of phenylephrine, while isoproterenol caused a decrease in P_{sys} and a rise in f_H.

Discussion

The present study is the first to report on MCFP in fully recovered reptiles and the first study to discern the roles of α-

Table 2. *The effects of injected adrenaline and phentolamine in anaesthetised South American rattlesnakes,* Crotalus durissus

	P_{CV} (cm H_2O)	P_{sys} (cm H_2O)	f_H (min^{-1})	MCFP (cm H_2O)	$\dot{Q}_{LAo}$ (ml kg^{-1} min^{-1})	$\dot{Q}_{pul}$ (ml kg^{-1} min^{-1})	$\dot{Q}_{sys}$ (ml kg^{-1} min^{-1})
Baseline	3.0±0.4	44.9±4.8	50.9±2.8	7.8±1.7	38.8±12.5	63.8±20.1	128.2±41.2
Adrenaline[1]	9.4±1.3*	80.7±4.9*	66.0±3.8*	16.9±1.3*	28.6±9.9	89.0±33.6	94.4±32.8
Baseline	3.1±0.2	36.5±4.1	48.4±5.7	7.7±1.2	56.3±25.0	26.7±11.4	185.6±82.5
Phentolamine	3.1±0.4	27.5±3.3	63.8±2.8		42.0±11.8	38.3±9.4	138.7±39.0
Baseline	3.1±0.4	27.5±3.3	63.8±2.8		42.0±11.8	38.3±9.4	138.7±39.0
Adrenaline[2]	3.7±0.4	29.4±3.5	66.1±3.3		62.7±12.1	48.4±9.8	207.1±39.8
Baseline	3.3±0.3	25.6±3.5	64.5±2.0	7.1±1.0	48.0±14.1	33.8±10.7	158.3±46.4

	$\dot{Q}_{tot}$ (ml kg^{-1} min^{-1})	Vs_{sys} (ml kg^{-1})	Vs_{pul} (ml kg^{-1})	Vs_{tot} (ml kg^{-1})	R_{sys} (cm H_2O ml^{-1} min^{-1} kg^{-1})	R_{ven} (cm H_2O ml^{-1} min^{-1} kg^{-1})	$\dot{Q}_{pul}/\dot{Q}_{sys}$
Baseline	192.0±38.9	2.4±0.7	1.3±0.4	3.7±0.7	0.53±0.18	0.06±0.02	2.6±0.8
Adrenaline[1]	183.0±32.5	1.5±0.5	1.4±0.5	2.8±0.6	1.08±0.26*	0.15±0.05*	5.1±1.7*
Baseline	212.4±74.8	3.5±1.3	0.6±0.3	4.1±1.1	0.45±0.19	0.05±0.02	1.6±0.8
Phentolamine	176.9±45.5	2.1±0.6	0.6±0.1	2.7±0.7	0.17±0.06	0.08±0.03	1.2±0.3
Baseline	176.9±45.5	2.1±0.6	0.6±0.1	2.7±0.7	0.17±0.06	0.08±0.03	1.2±0.3
Adrenaline[2]	255.5±40.6	3.1±0.6	0.7±0.2	3.9±0.6	0.15±0.04		0.9±0.4
Baseline	192.1±53.0	2.4±0.7	0.5±0.2	3.0±0.8	0.25±0.10	0.03±0.01	0.9±0.3

Values are means ± S.E.M. (N=5). Asterisks denote a significant difference relative to the baseline value. Adrenaline[1], 10 μg kg^{-1}; adrenaline[2], 2 μg kg^{-1}; phentolamine, 2 mg kg^{-1}.

See Table 1 for definitions of abbreviations.

or β-receptors on venous tone in reptiles. In addition, MCFP was manipulated by changing blood volume to assess how venous return affects stroke volume.

The haemodynamic variables of the anaesthetised rattlesnakes in our study were similar to those reported previously on the same species using pentobarbital as anaesthetic (Galli et al., 2005a,b). Anaesthetised snakes had higher P_{sys} and f_H than fully recovered snakes, and it is likely that anaesthesia lowers parasympathetic tone and increases sympathetic tone as in turtles (Crossley et al., 1998). However, P_{CV} and MCFP were not significantly affected by anaesthesia in rattlesnakes.

Measurements of MCFP

In rattlesnakes, P_{CV} and P_{sys} usually stabilised at equal values within 35 s after occlusion, and P_{CV} at that time was taken to indicate MCFP. It is possible that vascular tone changed shortly upon occlusion in response to the lowered arterial blood pressure and ischemia of vascular beds, and such compensatory mechanisms could affect our estimation of MCFP (Guyton, 1963; Pang, 2001). Compensatory mechanisms are rapidly activated in mammals, which is evident from a rise in P_{CV} after approximately 10 s of occlusion (Guyton, 1963; Rothe and Dress, 1976; Hainsworth, 1986), and trout also appear to have fast compensatory responses (Sandblom and Axelson, 2005). In our study, however, there was no rise in P_{CV} within 35 s of occlusion, indicating that compensatory reflexes were not yet activated and that our assessment of MCFP is valid.

After infusion of adrenaline and phenylephrine, the marked

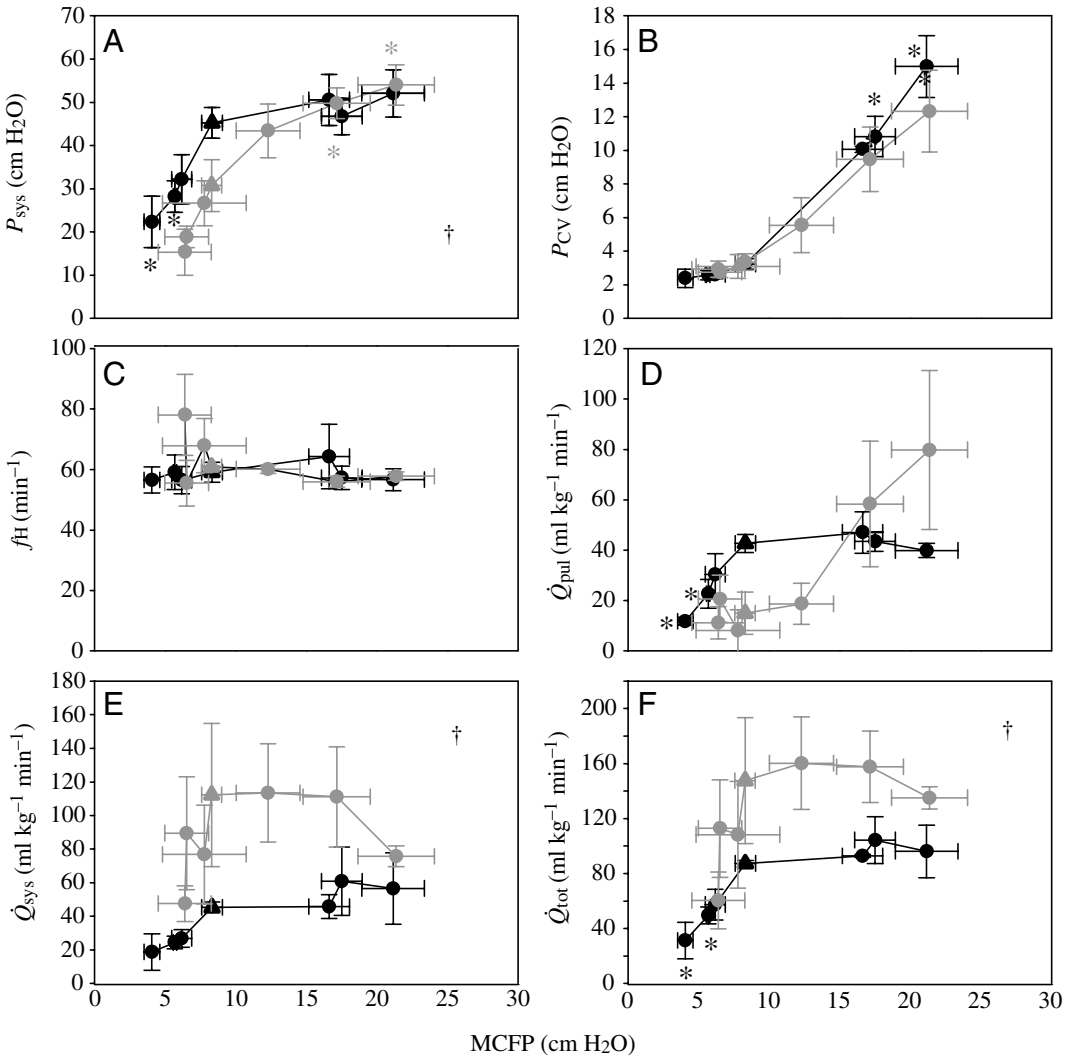

Fig. 5. Haemodynamic effects of blood volume alterations in South American rattlesnakes *Crotalus durissus*, as a function of mean circulatory filling pressure (MCFP) measured at each blood volume change. (A) P_{sys}, mean systemic blood pressure; (B) P_{CV}, central venous pressure; (C) f_H, heart rate; (D) $\dot{Q}_{pul}$, pulmonary cardiac output; (E) $\dot{Q}_{sys}$, systemic cardiac output; (F) $\dot{Q}_{tot}$, total cardiac output. Black symbols represent the untreated group and grey symbols represent the α-blocked group. Triangles indicate control values at normal blood volume. Values are means ± S.E.M. (N=6 for each group). * indicates a significant difference relative to control values. † indicates a significant difference between the two groups of snakes. (1 cm H_2O=0.098 kPa.)

rise in resistance often delayed the equilibration between systemic and venous pressures. In these instances, P_{CV} after no more than 40 s of occlusion was taken to indicate MCFP, although P_{CV} and P_{sys} were not equal. However, given that the venous compliance is approximately six times higher than the arterial compliance in this species (estimated from the decline in P_{sys} relative to the rise in P_{CV} during occlusion; P_{sys} declined from 49.0±4.6 to 11.0±1.1 cm H_2O (1 cm H_2O=0.098 kPa), while P_{CV} increased from 3.3±0.4 to 9.5±1.0 cm H_2O), the lack of complete equilibration would only have minor effects on the estimated MCFP.

It was necessary to open the pericardium to place the occluder for measurement of MCFP. This could influence haemodynamic variables, so we determined P_{sys}, P_{CV} and f_H in a group of recovered snakes without a vascular occluder and an intact pericardium. At rest and when undisturbed, both groups had similar blood pressures and heart rates, but, after injection of the various adrenergic drugs, the group without a vascular occluder exhibited larger blood pressure responses.

Adrenergic effects on blood pressures

Adrenaline increased P_{sys}, P_{CV} and MCFP in both anaesthetised and recovered snakes, reflecting a marked rise in overall vascular tone, and elicited a small tachycardia. Similar responses have been reported in recovered rats and fish (Trippodo, 1981; Zhang et al., 1998). The α-agonist phenylephrine elicited similar, albeit less pronounced, responses without affecting f_H. In recovered dogs,

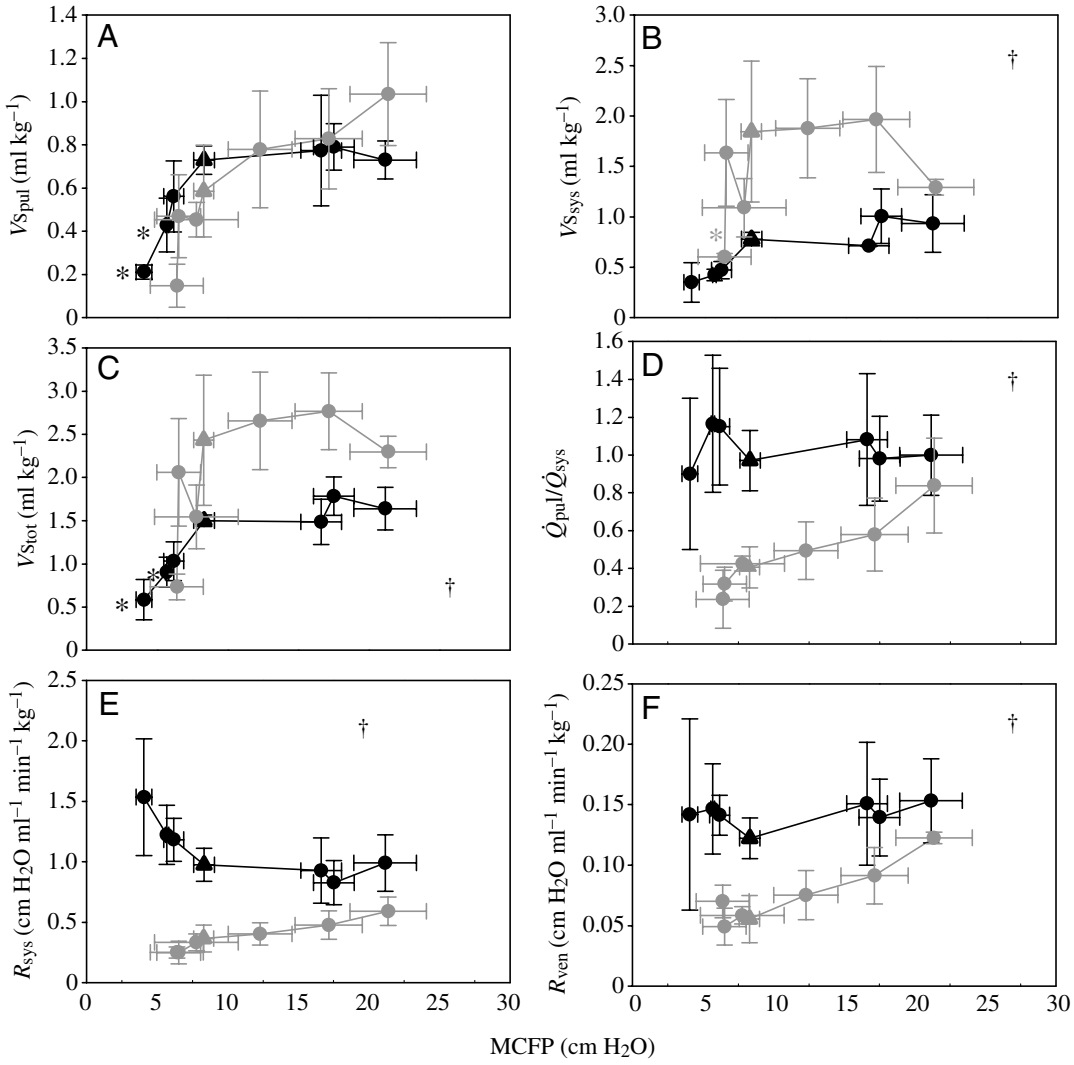

Fig. 6. Haemodynamic effects of blood volume alterations in South American rattlesnakes *Crotalus durissus*, as a function of mean circulatory filling pressure (MCFP) measured at each blood volume change. (A) Vs_{pul}, pulmonary stroke volume; (B) Vs_{sys}, systemic stroke volume; (C) Vs_{tot}, total stroke volume; (D) $\dot{Q}_{pul}/\dot{Q}_{sys}$, ratio of pulmonary cardiac output to systemic cardiac output; (E) R_{sys}, systemic resistance; (F) R_{ven}, venous resistance. Black symbols represent the untreated group and grey symbols represent the α-blocked group. Triangles indicate control values at normal blood volume. Values are means ± S.E.M. (N=6 for each group). * indicates a significant difference relative to control value. † indicates a significant difference between the two groups of snakes. (1 cm H_2O=0.098 kPa.)

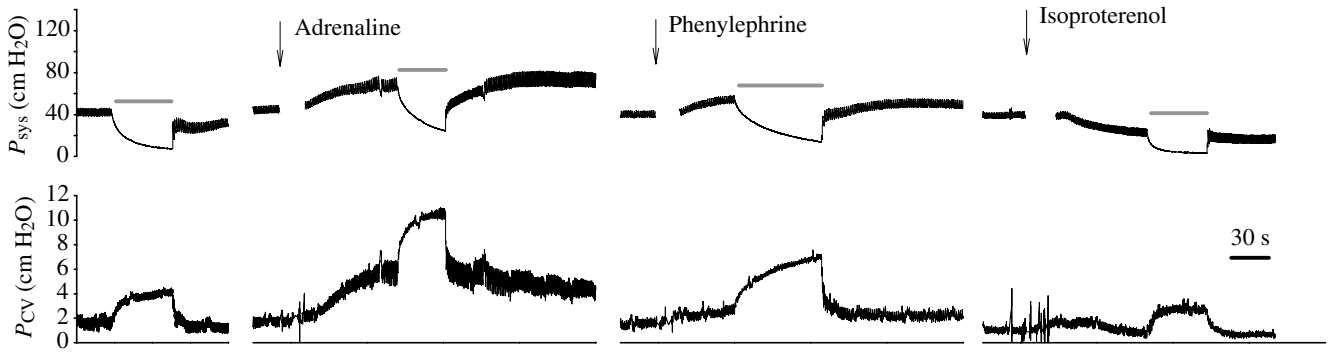

Fig. 7. Haemodynamic parameters in a recovered South American rattlesnake *Crotalus durissus* (550 g), before and after injection of adrenaline (10 μg kg^{-1}), phenylephrine (10 μg kg^{-1}) and isoproterenol (10 μg kg^{-1}). Arrows indicate injection of the various drugs. Grey bars indicate occlusion of blood flow from the heart to measure MCFP. P_{sys}, mean systemic blood pressure; P_{CV}, central venous pressure. (1 cm H_2O=0.098 kPa.)

phenylephrine caused large changes in P_{sys} but had little effect on P_{CV} (Bennett et al., 1984). The smaller response to phenylephrine compared with adrenaline in rattlesnakes could be due to a lower α-receptor affinity of phenylephrine relative to adrenaline. Generally, isoproterenol elicited opposite responses and was associated with a fall in P_{sys}, P_{CV} and MCFP. Therefore, the constriction of the arterial and venous vasculature in response to adrenaline is primarily mediated by α-adrenergic receptors, which is consistent with previous studies on the arterial vasculature of other reptiles (Nilsson, 1983; Overgaard et al., 2002). Our results clearly show that activation of β-receptors can decrease P_{CV} and MCFP through dilatation of the venous system, and β-receptors in the veins may contribute to regulating the venous system in rattlesnakes. Isoproterenol, however, does not significantly affect P_{CV} or MCFP in dogs (Rothe et al., 1989).

The pressure gradient for venous return and its effect on stroke volume during adrenergic stimulation

In untreated anaesthetised snakes, the pressure gradient of venous flow (MCFP–P_{CV}) was 6.2 cm H_2O and increased to 10.1 cm H_2O after the high dose of adrenaline. Surprisingly, V_{Stot} did not increase in response to the higher pressure gradient for venous return and the higher filling pressure (P_{CV}) but actually tended to decrease from 2.0±0.4 to 1.6±0.1 ml kg^{-1}. V_{Stot}, the difference between end-diastolic and end-systolic volumes, is determined by cardiac filling, contractility and afterload (P_{sys}). Adrenaline is expected to increase contractility, but also increased afterload and could have increased end-systolic volume. The effects of adrenaline on V_{Stot} in rattlesnakes differ from those seen in frogs and turtles, where V_{Stot} increases (S. Warburton, D. C. Jackson, V. T. Bobb and T. Wang, unpublished data). This rise in V_{Stot} in frogs and turtles was ascribed to an increased cardiac filling as MCFP rose after adrenaline. In fish, administration of adrenaline did not significantly affect stroke volume (Zhang et al., 1998).

V_{Stot} did not change after phenylephrine or isoproterenol despite an increased and decreased pressure gradient, respectively (Figs 2, 3). Contractility is unlikely to have been affected by phenylephrine, but, as afterload and R_{ven} increased, it seems that the unchanged V_{Stot} after α-adrenergic stimulation results from a balance between venous constriction and higher afterload. An increase in R_{ven} in response to α-adrenergic stimulation has also been reported for anaesthetised dogs (Imai et al., 1978). In our study, isoproterenol reduced afterload and venous resistance, which would be expected to decrease end-systolic volume and to increase end-diastolic volume, respectively. These effects would be enhanced by β-adrenergic stimulation of contractility. However, V_{Stot} was not affected by isoproterenol in rattlesnakes, which may be due to shorter filling time as f_H increased. In anaesthetised dogs, administration of isoproterenol and noradrenaline increased venous return by decreasing venous resistance through stimulation of β-receptors (Imai et al., 1978).

The marked effects of the various adrenergic drugs on MCFP and R_{ven} in rattlesnakes show that this species has a strong adrenergic regulation of the venous system. This is consistent with previous studies on isolated central veins from the ratsnake, *Elaphe obsolete*, which contract in response to adrenaline (Conklin et al., 1996). Also, Donald and Lillywhite (1988) showed dense innervation of the central venous vasculature in *Elaphe*. Although an increased venous tone did not increase V_S in untreated conditions in this species, it is likely that an increased sympathetic activity could exert strong influence on venous return and, therefore, may mediate an increased cardiac output under conditions such as exercise, digestion or increased temperature where metabolism is elevated.

MCFP and blood volume changes in anaesthetised snakes

Neither V_S nor $\dot{Q}$ increased when blood volume was raised, which indicates that end-diastolic volume is maximal at normal blood volume. Furthermore, V_S may not have been affected because P_{CV} increased as much as MCFP during volume loading, so that the pressure gradient for venous flow was virtually unchanged. Decreasing blood volume, on the other hand, markedly reduced V_S, which correlated with a substantial decrease in MCFP and a much lower pressure gradient for venous flow. Thus, as shown in frogs, turtles and mammals

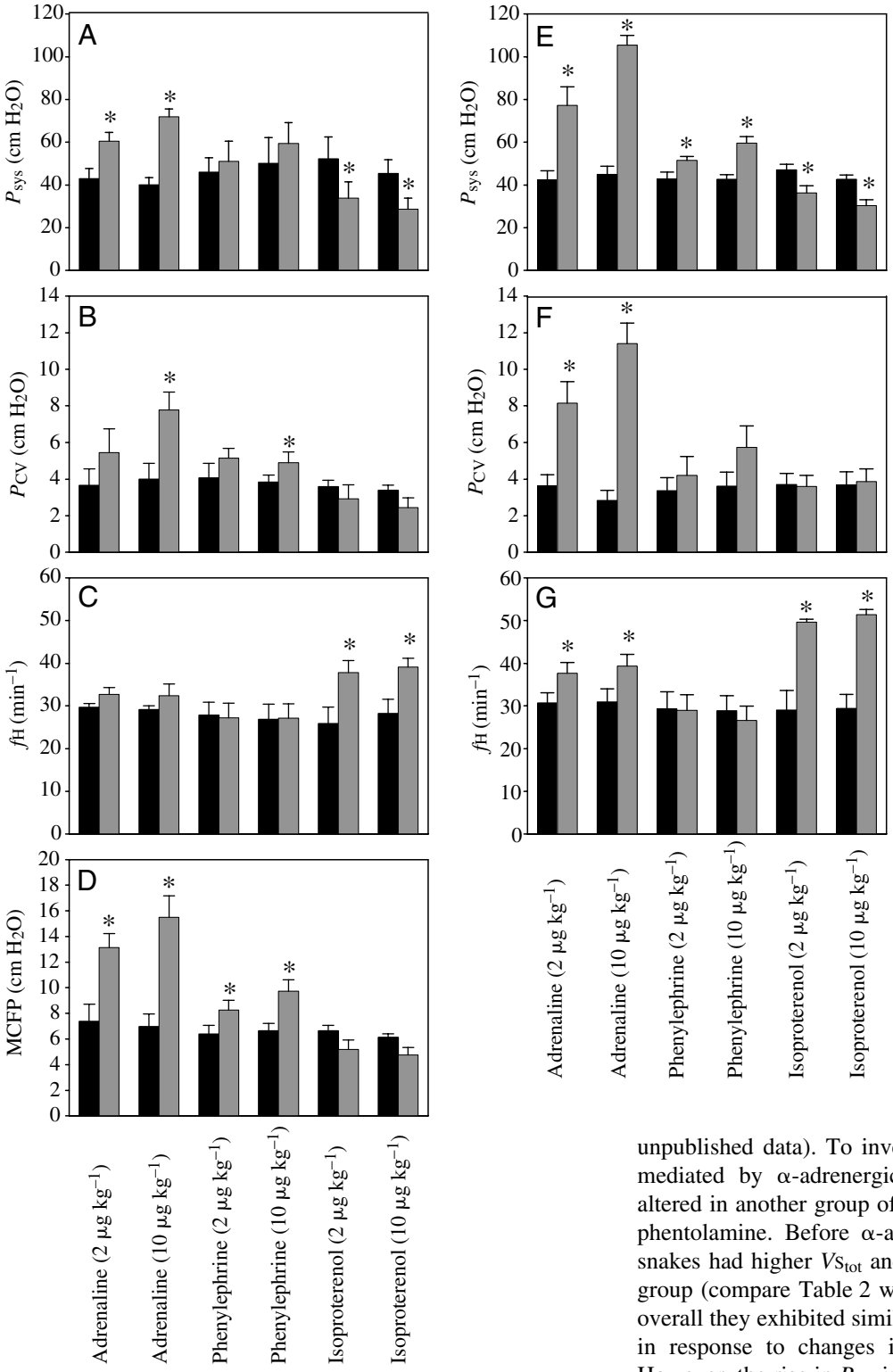

Fig. 8. Effects of bolus injections of adrenaline, phenylephrine and isoproterenol (2 and 10 μg kg^{-1}) on haemodynamic variables in the recovered South American rattlesnakes *Crotalus durissus*. A–D represent the group with a vascular occluder, and E–G represent the group without a vascular occluder. P_{sys}, mean systemic blood pressure; P_{CV}, central venous blood pressure; f_H, heart rate; MCFP, mean circulatory filling pressure. Black bars represent control values. Grey bars represent values after injection of the various adrenergic agonists. Values are means ± S.E.M. (N=4) for each group. * indicates a significant difference relative to control values. (1 cm H_2O=0.098 kPa.)

(Guyton, 1955; S. Warburton, D. C. Jackson, V. T. Bobb and T. Wang, unpublished data), there is a clear relationship between MCFP and cardiac filling and Vs in rattlesnakes.

In rattlesnakes, blood withdrawal was accompanied by a rise in R_{sys}, as previously observed in turtles and frogs (S. Warburton, D. C. Jackson, V. T. Bobb and T. Wang, unpublished data). To investigate whether this response was mediated by α-adrenergic stimulation, blood volume was altered in another group of snakes following α-blockade with phentolamine. Before α-adrenergic blockade, this group of snakes had higher V_{Stot} and $\dot{Q}_{tot}$ compared with the untreated group (compare Table 2 with values given in Fig. 3A,D), but overall they exhibited similar changes in P_{CV}, MCFP and V_{Stot} in response to changes in blood volume (Figs 4, 5B, 6C). However, the rise in R_{sys} in response to volume depletion was fully abolished after α-adrenergic blockade, suggesting an increased sympathetic tone in response to lowered blood pressure and volume.

Compliance and unstressed blood volume

The measurements of MCFP at the various blood volumes also allow for an estimation of unstressed blood volume

(USBV), vascular compliance (*C*) and stressed blood volume (SBV) as the difference between total blood volume and USBV. In doing so, we assumed that there was no net fluid movement across the capillary wall during blood volume changes and we used the total blood volume of 54 ml kg^{-1}, which has been determined in *Crotalus viridis* (Smits and Lillywhite, 1984). Blood was infused and withdrawn as fast as possible, leaving the animals hypo- and hypervolemic for as short a time as possible, and MCFP was always measured immediately after blood volume alteration to avoid compensatory responses.

USBV is the volume required to fill the circulatory system until a transmural pressure of zero and is accordingly considered to be haemodynamically inactive, whereas SBV is haemodynamically active (Pang, 2000, 2001). USBV is determined by the capacity and activity of the smooth muscle cells surrounding the blood vessels, and an increased vascular tone translocates blood from USBV to SBV (Pang, 2001). We estimated USBV to be 20.7±1.8 ml kg^{-1} and 18.3±7.1 ml kg^{-1} for the untreated and α-blocked snakes, respectively. Compliance is a measure of vascular elasticity and is defined as a change in volume relative to a change in transmural pressure (Rothe, 1993; Pang, 2001). In rattlesnakes, *C* was estimated to be 3.3±0.3 ml kg^{-1} cmH_2O^{-1} for the untreated snakes and 3.9±0.7 ml kg^{-1} cmH_2O^{-1} for the α-blocked snakes. These estimates are similar to those determined in trout and two species of anurans (*Bufo marinus* and *Rana catesbeiana*). In recovered trout, USBV and *C* are 18.3±0.7 ml kg^{-1} and 3.0±0.2 ml kg^{-1} $mmHg^{-1}$, respectively (Zhang et al., 1998), and another study determined *C* in trout to be 2.8±0.3 ml kg^{-1} $mmHg^{-1}$ (Minerick et al., 2003). In recovered *Bufo*, USBV and *C* are 2.5 ml kg^{-1} and 3.7 ml kg^{-1} $mmHg^{-1}$, and in recovered *Rana*, USBV and *C* are 14.2 ml kg^{-1} and 2.2 ml kg^{-1} $mmHg^{-1}$, respectively (Hoagland, 1997).

Neither compliance nor unstressed volume were significantly affected by α-blockade in rattlesnakes. Similarly, compliance and unstressed volume were not affected by phentolamine in trout, but there were small rises in both parameters after infusion of another α-antagonist, prasozin (Zhang et al., 1998).

The effect of NO on the venous system

To investigate the role of nitric oxide (NO) on the venous circulation, the NO donor SNP was injected. It is well established that SNP reduces systemic resistance in reptiles (Crossley et al., 2000; Galli et al., 2005a; Skovgaard et al., in press) and dilates central veins from *Elaphe in vitro* (Conklin et al., 1996). The effect of NO on the venous circulation of reptiles, however, has not been investigated *in vivo*. In rattlesnakes, SNP decreased P_{sys}, R_{sys} and Vs_{tot} as previously shown in this species (Galli et al., 2005a) and caused marked decreases in P_{CV} and MCFP, which indicate that NO has a marked effect on the veins in this species. In trout, injection of SNP had no effect on either P_{CV} or *V*s and only slightly decreased MCFP (Olson et al., 1997), whereas in toads, SNP caused dilatation of the central veins (Broughton and Donald, 2005). In rattlesnakes, MCFP decreased proportionally more than P_{CV}, which led to a decreased venous return, reflected in a significant decreased total *V*s when the low dose of SNP was injected.

Summary

Adrenaline, phenylephrine and isoproterenol significantly affected venous tone, reflected as a change in MCFP, in both anaesthetised and fully recovered rattlesnakes. Since phenylephrine showed similar responses as adrenaline, we conclude that sympathetic responses are mediated though α-receptors in rattlesnakes. Stimulation of β-receptors with isoproterenol decreased venous tone. Since SNP decreased P_{CV} and MCFP, nitric oxide seems to regulate venous tone in anaesthetised snakes. When venous return was increased by elevating blood volume, there was only a small rise in *V*s, which indicates that end-diastolic volume is maximal during normal conditions. However, venous return clearly affected *V*s when MCFP was reduced through volume depletion. Since blocking α-receptors with phentolamine did not markedly affect MCFP, USBV or *C*, rattlesnake did not appear to have a significant α-adrenergic tone during resting conditions.

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