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Adrenergic control of the cardiovascular system in the turtle *Trachemys scripta*

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Summary

Freshwater turtles, *Trachemys scripta*, like all non-crocodilian reptiles, are able to shunt blood between the pulmonary and systemic circulations owing to their undivided ventricle. The prevailing hypothesis is that the ratio of pulmonary and systemic resistances is the primary determinant of cardiac shunting in turtles. In the present study, we have examined the adrenergic influences on vascular resistances in the pulmonary and systemic circulations and the associated effects on cardiac shunts in turtles. To achieve this objective, systemic blood flow and pressures and pulmonary blood flow and pressures were measured simultaneously in anaesthetised turtles during bolus injections of α- and β-adrenergic agonists and antagonists. Total cardiac output, systemic vascular resistance, pulmonary vascular resistance, heart rate and cardiac stroke volume were derived from these measurements. Anaesthetised turtles showed cardiovascular characteristics that were similar to those of non-apnoeic non-anaesthetised turtles, because anaesthesia blocked the cholinergically mediated constriction of the pulmonary artery that is normally associated with apnoea. As a result, the anaesthetised turtles exhibited a large net left-to-right shunt, and the adrenergic responses could be observed without confounding changes resulting from apnoea. Potent α-adrenergic vasoconstriction and weaker β-adrenergic vasodilation were discovered in the systemic circulation. Modest β-adrenergic vasodilation and possible weak α-adrenergic vasodilation were discovered in the pulmonary circulation. This adrenergically mediated vasoactivity produced the largest range of cardiac shunts observed so far in turtles. Regression analysis revealed that 97% of the variability in the cardiac shunts could be accounted for by the ratio of the pulmonary and systemic resistances. Thus, we conclude that, independent of whether the pulmonary vascular resistance is modulated (as during apnoea) or the systemic resistance is modulated with adrenergic mechanisms (as shown here), the consequences on the cardiac shunt patterns are the same because they are determined primarily by the ratios of the pulmonary and systemic resistance.

Key words: turtle, *Trachemys scripta*, adrenergic response, cardiac shunting, cardiovascular, pulmonary resistance, systemic resistance.

Introduction

The circulatory system of vertebrates is primarily regulated by the autonomic nervous system, as well as by hormonal and local factors. The autonomic nervous system consists of a parasympathetic vagal inhibitory component and a sympathetic adrenergic excitatory component. The autonomic nervous system is of paramount importance for regulation of blood pressures, cardiac contractility and distribution of blood flows between various vascular beds (Nilsson, 1983; Morris and Nilsson, 1994). In turtles, the ventricle is undivided, and blood-flow distribution between the systemic and pulmonary circulations and the intracardiac shunt patterns are largely determined by the vascular resistances of these two circulations (e.g. Shelton and Burggren, 1976; Hicks et al., 1996). It is well established that cholinergic control of pulmonary vascular resistance exerts an important role in the distribution of blood flow (e.g. Johansen and Burggren, 1980; Hicks, 1998), but an adrenergic component also seems to be involved in the regulation of vascular resistances of the pulmonary and systemic circulations (Burggren, 1977; Hicks, 1994). Increased sympathetic tone is often associated with exercise and stressful conditions, where oxygen delivery needs to be augmented through increased systemic blood flow through increased heart rate (f_H) and/or stroke volume. A sympathetically mediated reduction in the re-circulation of systemic venous blood within the systemic circulation (i.e. a reduced right-to-left shunt) will improve systemic oxygen transport through increased oxygen saturation of systemic arterial blood (Wang and Hicks, 1996b). Despite this benefit, a clear picture of the role of adrenergic stimulation in the regulation of pulmonary vascular resistance (R_{pul}) in turtles has yet to emerge because contradictory results appear in the literature (Hicks, 1994); some studies show no effects of catecholamine injections, while others report reductions in R_{pul} (Luckhardt and Carlson, 1921; Berger, 1972; Milsom et al., 1977; Burggren, 1977; Comeau and Hicks, 1994).

While the overall haemodynamic effects of adrenergic stimulation have been characterised to some degree in turtles, it remains unknown as to what extent the changes in cardiac shunt pattern are caused by increased systemic resistance (R_{sys}) or decreased R_{pul}. Furthermore, the specific role of α-adrenergic and β-adrenergic receptors underlying the regulation in the pulmonary circulation is largely unknown. Non-anaesthetised turtles primarily regulate the ratio of the vascular resistances (R_{pul}/R_{sys}), and thereby cardiac shunt pattern, through a cholinergically mediated constriction of the pulmonary circulation. However, owing to the lack of vagal tone in anaesthetised turtles, the present study allowed us to examine how the ratios of vascular resistances affects shunt patterns when the resistances are manipulated through adrenergic stimulus or blockade. We measured haemodynamic responses following bolus injections of norepinephrine and the specific α- and β-adrenergic agonists phenylephrine and isoproteronol. Furthermore, we investigated the effects of pharmacological blockade using the α-adrenergic antagonist phentolamine and the β-adrenergic antagonist propranolol. Here, we aim to characterise the adrenergic regulation of the cardiovascular system in turtles and its possible functional implications.

Materials and methods

Experimental animals

Freshwater turtles *Trachemys scripta* (Gray) with a body mass ranging between 0.6 kg and 1.5 kg (mean ± S.D., 0.85±0.07 kg) were obtained from Lemberger Inc. (Oshkosh, WI, USA) and air-freighted to Aarhus University, where they were housed in a large fibreglass tank containing fresh water heated to 25±2°C and had free access to dry platforms, allowing for behavioural thermoregulation. All procedures were in accordance with the laws of animal care and experimentation in Denmark.

Anaesthesia and surgery

Turtles were anaesthetised by an intramuscular injection of sodium pentobarbital (Nembumal, Sygehusapotekerne, Denmark; 50 mg kg^{-1}) and normally ceased to exhibit responses to pinching of the legs or tail within 60 min. The trachea was then exposed by a ventral incision in the neck, and the turtle was tracheotomised for artificial ventilation. During surgery and the entire experimental protocol, turtles were maintained ventral side up and received continuous artificial ventilation with a tidal volume of 20–30 ml kg^{-1} at a frequency of 20–30 breaths min^{-1} using an HI 665 Harvard Apparatus Respirator (Cambridge, MA, USA). Because of the high ventilation rate, we used a gas mixture of 97% room air and 3% CO_2 delivered by a Wösthoff gas-mixing pump (Bochum, Germany) to mimic the arterial blood PCO_2 *in vivo* (e.g. Glass et al., 1983; see Crossley et al., 1999 for arterial blood gas composition of anaesthetised turtles using a similar experimental design).

To access the central vascular blood vessels, a 5 cm × 5 cm portion of the plastron was removed using a bone saw, and the pectoral muscles were gently loosened from the excised piece. A polyethylene catheter, containing heparinised saline, was occlusively inserted into the left carotid artery and advanced into the right aortic arch. The common pulmonary artery was non-occlusively cannulated using an intravenous catheter (Surflo, Terumo Medical Cooporation, Elkton, MD, USA) inserted upstream into the common pulmonary artery to within 0.5 cm of the heart. This required that the pericardium was opened by a 1 cm incision. After insertion, the intravenous catheter was connected to PE-60 tubing, which, in turn, was connected to a Baxter Edward disposable pressure transducer (model PX600, Irvine, CA, USA). The signals from the pressure transducers were amplified using an in-house-built preamplifier and were calibrated daily against a static column of water. For measurements of systemic and pulmonary blood flows, a 1–1.5 cm section of the left aortic arch (LAo) and the left pulmonary artery (LPA) were freed from connective tissue for placements of transit-time ultrasonic blood-flow probes (2R or 2S probes; Transonic System, Inc., NY, USA). To improve the signal, acoustical gel was infused between the blood vessel and flow probe. The flow probes were connected to a Transonic dual-channel blood-flow meter (T206) for measurements of instantaneous blood-flow rates. Signals from the pressure transducer and the blood-flow meter were recorded with a Biopac MP100 data acquisition system (Biopac Systems, Inc., Goleta, CA, USA) at a sampling frequency of 50 Hz.

Calculation of blood flows, net shunt, stroke volume and resistance to blood flow in the systemic and pulmonary circulations

This study did not measure blood flow in all systemic and pulmonary arteries. Several studies on anaesthetised and non-anaesthetised freshwater turtles have shown that systemic blood flow ($\dot{Q}_{sys}$) can be adequately estimated as $2.85\dot{Q}_{LAo}$ (Shelton and Burggren, 1976; Comeau and Hicks, 1994; Wang and Hicks, 1996a; this relationship also persists after injection of adrenergic antagonists and agonists in fully recovered turtles; J. A. W. Stecyk, J. Overgaard, A. P. Farrell and T. Wang, unpublished data). Likewise, pulmonary blood flow ($\dot{Q}_{pul}$) can be calculated as $2\dot{Q}_{LPA}$ under the assumption that blood flow in the right pulmonary artery equals that in the left. f_H was calculated from the instantaneous blood-flow trace from the LAo. Total cardiac output ($\dot{Q}_{tot}$) was calculated as $\dot{Q}_{sys}+\dot{Q}_{pul}$, and total stroke volume (V_{Stot}; pulmonary + systemic) was calculated as $\dot{Q}_{tot}$ divided by f_H. Pulmonary and systemic resistances (R_{pul} and R_{sys}, respectively) were calculated from mean blood pressure (P) and mean blood flow ($R_{pul}=P_{pul}/\dot{Q}_{pul}$ and $R_{sys}=P_{sys}/\dot{Q}_{sys}$) using the assumption that central venous blood pressures are zero. This assumption may lead to a small overestimation of vascular resistances, as pointed out by Badeer and Hicks (1994).

Experimental protocol

After ensuring steady-state conditions (stable pressures and flows for a minimum of 30 min), the turtles were sequentially

injected with adrenergic receptor antagonists and agonists using the protocol described below. The injections were administered through the systemic catheter, which was subsequently flushed with saline. Although not studied in detail, injections of adrenergic agonists in the pulmonary artery yielded similar responses to those following systemic injections. The total volume injected never surpassed 2 ml, and control injections of 2 ml saline did not cause haemodynamic changes. Following injection of each drug, we recorded haemodynamic parameters at a maximal response. This usually occurred after 1–2 min with the agonists and after 30 min with the antagonists. After injections of agonists, the haemodynamic variables were allowed to return to baseline values before continuing the protocol. Following injection of antagonists, we waited for at least 30 min until a new steady-state was established.

The animals were randomly divided into two groups (body mass of 0.78±0.03 kg and 0.92±0.12 kg for group 1 and 2, respectively) and presented with the following pharmacological protocols (all chemicals were purchased from Sigma-Aldrich, Vallensbæk Strand, Denmark).

Group 1: norepinephrine 5 μg kg^{-1}, phenylephrine 5 μg kg^{-1}, propranolol 3 mg kg^{-1}, norepinephrine 5 μg kg^{-1}, phenylephrine 5 μg kg^{-1}, phentolamine 3 mg kg^{-1}. Group 2: these animals were exposed to the same protocol as group 1 with the exception that the injections of phentolamine and propranolol were reversed. In addition, eight of the twelve animals (four from each group) were injected with a lower dose of norepinephrine (1 μg kg^{-1}), and four animals (two from each group) were injected with isoproteronol (1 μg kg^{-1}). These injections were performed before administration of the first antagonist. After completion of the experimental protocol, all turtles were euthanized by a lethal injection of saturated KCl.

Data analysis and statistics

Recordings of blood flows and pressures were analysed using AcqKnowledge data analysis software (Biopac Systems, Inc., Goleta, CA, USA, version 3.7.0) where the mean values for f_H, $\dot{Q}_{LAo}$, $\dot{Q}_{LPA}$, P_{sys}, P_{pul}, R_{sys} and R_{pul} were determined over a 1–2 min period following each injection. Differences in control values between groups 1 and 2 were tested using a *t*-test. In each group, the results obtained following injections of agonists and antagonists were tested for significant differences from untreated baseline values using a one-way analysis of variance (ANOVA) for repeated measures. Significant differences among mean values were identified using a *post hoc* Student–Newman–Keuls (SNK) test. Effects of agonists (phenylephrine and norepinephrine) after injection of antagonists (propranolol or phentolamine) were compared with the baseline value that prevailed 30 min after injection of the antagonist. Similarly, the eight turtles that received two doses of norepinephrine were tested using a one-way ANOVA for repeated measures and a *post hoc* SNK test. A paired *t*-test was used to assess for significant differences from control values in the four animals injected with isoproteronol. In all tests, a limit of $P<0.05$ was applied to identify significant differences. Owing to low *N* values for some comparisons, type 1 errors are possible, and lack of significant differences should be interpreted cautiously. The data are presented as means ± S.E.M. and normalised to a 1 kg turtle.

Results

The cardiovascular status for each of the two experimental groups during the control condition is shown in Table 1. f_H, P_{pul} and P_{sys} were similar between the two groups. Although blood flows seemed higher in group 1 compared with group 2, this difference was not significant. Similarly, there were no significant differences between the two groups in any of the other reported parameters, and the ratios of $\dot{Q}_{pul}/\dot{Q}_{sys}$ and R_{pul}/R_{sys} were very similar in both groups. In both groups, $\dot{Q}_{pul}$ greatly exceeded $\dot{Q}_{sys}$, P_{sys} was considerably higher than P_{pul}, and R_{sys} was approximately threefold greater than R_{pul}.

Effect of norepinephrine

The temporal changes in heart rate, blood flows and pressures following injection of norepinephrine are illustrated in Fig. 1A, 2A for two turtles from each experimental group. The group data are presented in Table 1. Data from the eight turtles (four from each group) that received two doses of norepinephrine were pooled and are presented in Fig. 3. Both concentrations of norepinephrine had significant chronotropic and inotropic effects. $\dot{Q}_{tot}$, $\dot{Q}_{pul}$, f_H and Vs_{tot} all increased significantly following injection of 1 μg kg^{-1} norepinephrine, and f_H also increased further following injection of 5 μg kg^{-1} norepinephrine (Fig. 3A). $\dot{Q}_{sys}$ was unchanged following injection of 1 μg kg^{-1} norepinephrine but decreased significantly after injection of 5 μg kg^{-1} norepinephrine. Norepinephrine caused significant dose-dependent increases in P_{pul} and P_{sys} but did not affect R_{pul}, while R_{sys} increased significantly from baseline values following injection of 5 μg kg^{-1} norepinephrine (Fig. 3D).

Effect of α-adrenergic stimulation and blockade

Injection of the α-agonist phenylephrine led to an increase in $\dot{Q}_{tot}$, although the effect was only significant in group 2 (Table 1). The increase in $\dot{Q}_{pul}$ and $\dot{Q}_{tot}$ following injection of phenylephrine was slightly lower than that following norepinephrine, and this correlated with the lack of a chronotropic effect of phenylephrine, but there were similar increases in Vs_{tot} after injection of either phenylephrine or norepinephrine (Table 1). As with norepinephrine, phenylephrine elicited a significant increase in $\dot{Q}_{pul}$, while R_{pul} was not affected. The change in $\dot{Q}_{sys}$ following phenylephrine was not statistically significant, although R_{sys} almost doubled (Table 1; Figs 1A, 2A).

Blockade of the α-receptors by phentolamine induced significant reductions in R_{sys}, P_{sys}, P_{pul} and $\dot{Q}_{pul}$ without altering f_H, $\dot{Q}_{sys}$ and R_{pul} (Table 1). The pronounced α-adrenergic systemic vasoconstriction was abolished by

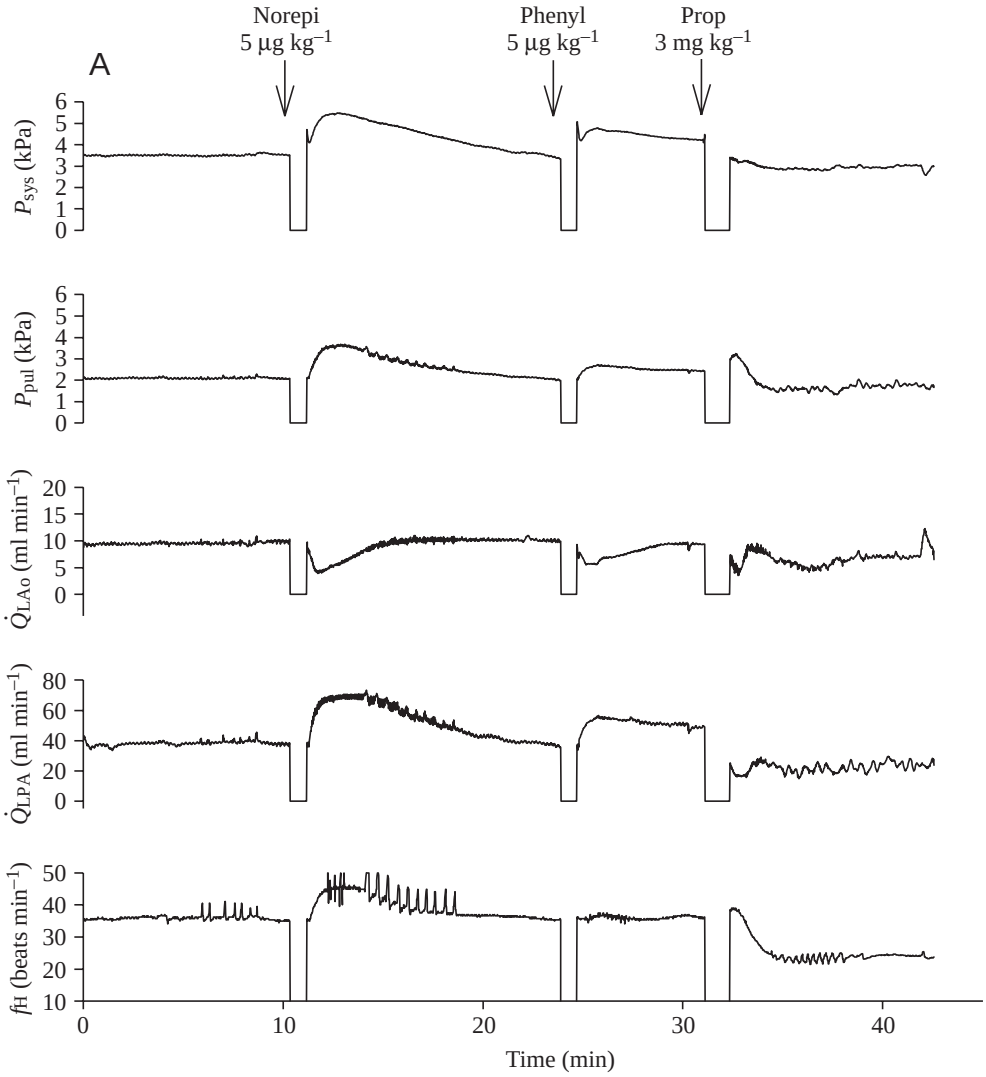
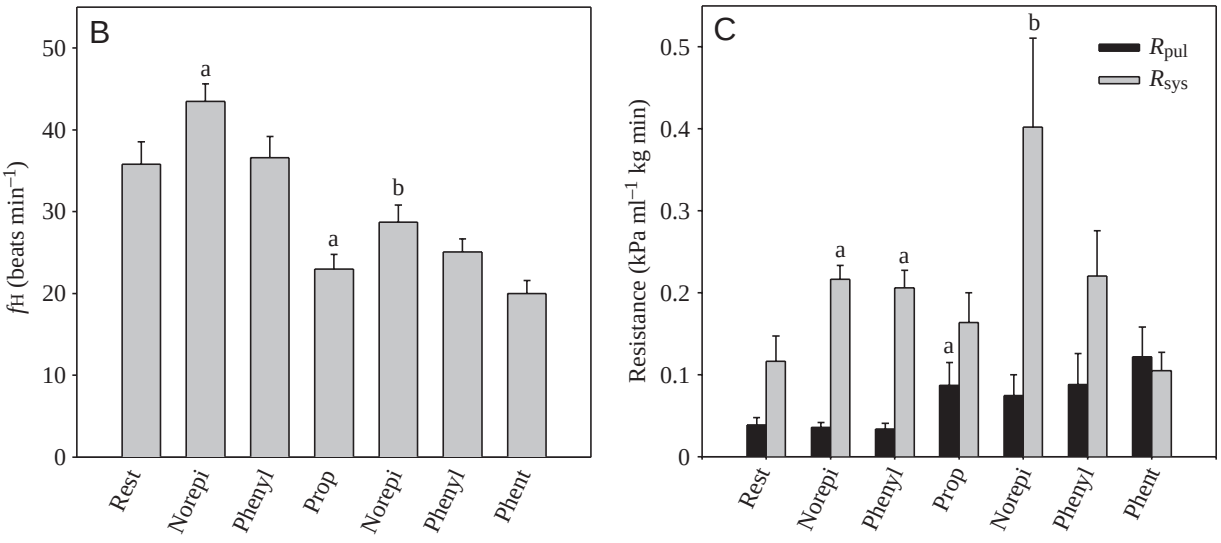


Fig. 1. (A) Effects of adrenergic agonists and antagonists on haemodynamic variables in the turtle *Trachemys scripta*. Recordings from one individual (#15/group 1) of mean arterial blood pressures and mean blood flows in the pulmonary and systemic circuits, as well as heart rate (f_H). P_{pul} and P_{sys} are the arterial pressures in the pulmonary and systemic circulation, respectively. $\dot{Q}_{LAo}$ is blood flow in the left aortic arch, and $\dot{Q}_{LPA}$ is the blood flow in the left pulmonary artery. The trace depicts resting conditions and measurements following injections of norepinephrine (norepi), phenylephrine (phenyl) and propranolol (prop). (B) Heart rate and (C) pulmonary and systemic resistances in group 1 turtles (N=6) following injection of agonists and antagonists. [a] indicates a significant difference (P<0.05) compared with resting conditions, and [b] indicates a significant difference (P<0.05) compared with the variables obtained following propranolol. Phent, phentolamine.



phentolamine. Thus, when phenylephrine or norepinephrine were injected after phentolamine, there were no significant cardiovascular changes except for an increase in P_{sys} and $\dot{Q}_{tot}$ following phenylephrine.

Effect of β-adrenergic stimulation and blockade

The haemodynamic changes caused by β-adrenergic receptor stimulation using isoproteronol are presented in Fig. 4. Isoproteronol produced significant positive

chronotropic effects and significantly reduced $V_{S_{tot}}$, while there were no significant changes in $\dot{Q}_{pul}$ or $\dot{Q}_{sys}$. Although there were no significant changes in R_{sys} and R_{pul}, isoproteronol caused significant hypotension in both the pulmonary and systemic circuits.

Abolishment of β-adrenergic tone, using propranolol, significantly reduced f_H and $\dot{Q}_{tot}$ but did not affect $V_{S_{tot}}$. A significant increase in R_{pul} resulted in a decrease in $\dot{Q}_{pul}$ and no change in P_{pul}. The changes in R_{sys} and $\dot{Q}_{sys}$ were not statistically significant. Similarly, when propranolol was applied after phentolamine (group 2), f_H decreased significantly, without any further change in $\dot{Q}_{tot}$ and $\dot{Q}_{pul}$ (which had already been reduced significantly by phentolamine). The increases in R_{sys} and R_{pul} following propranolol injection did not reach statistical significance. Compared with norepinephrine injection before β-adrenergic blockade, norepinephrine injection following propranolol (group 1) caused an even larger increase in R_{sys}, while the systemic hypertension was of similar magnitude. Phentolamine injection following β-adrenergic blockade (group 1) caused cardiac collapse, with $\dot{Q}_{tot}$ decreasing to 25% of the initial control values, and systemic and pulmonary blood pressures decreasing to 50% of their initial control values (Table 1).

Table 1. *Cardiovascular parameters following injections of adrenergic agonist and antagonist in the turtle* Trachemys scripta

	N	f_H (beats min^{-1})	$\dot{Q}_{pul}$ (ml kg^{-1} min^{-1})	$\dot{Q}_{sys}$ (ml kg^{-1} min^{-1})	$\dot{Q}_{tot}$ (ml kg^{-1} min^{-1})	$V_{S_{tot}}$ (ml kg^{-1})	P_{pul} (kPa)	P_{sys} (kPa)	R_{pul} (kPa ml^{-1} kg min)	R_{sys} (kPa ml^{-1} kg min)	$\dot{Q}_{pul}/\dot{Q}_{sys}$
Group 1											
Control	6	35.8±2.7	74.1±13.8	33.9±5.4	107.9±16.9	3.1±0.5	2.29±0.18	3.23±0.26	0.04±0.01	0.12±0.03	2.38±0.41
Norepinephrine	6	43.5±2.2[a]	124.6±18.7[a]	25.1±2.3	149.7±19.6[a]	3.5±0.5	3.93±0.26[a]	5.27±0.24[a]	0.04±0.01	0.22±0.02[a]	5.05±0.83[a]
Phenylephrine	6	36.6±2.6	107.7±16.6[a]	22.7±2.0	130.5±16.5	3.7±0.6	3.06±0.17[a]	4.49±0.21[a]	0.03±0.01	0.21±0.02[a]	4.94±0.86[a]
Propanolol	6	23.0±1.8[a]	40.5±11.6[a]	23.8±5.4	64.3±10.6[a]	2.9±0.6	2.09±0.26	3.07±0.32	0.09±0.03[a]	0.16±0.04	2.14±0.73
Norepinephrine	6	28.7±2.1[b]	64.3±12.7	15.6±3.6	80.0±12.3	2.9±0.6	3.52±0.28[b]	4.60±0.26[b]	0.08±0.02	0.40±0.11[b]	5.60±1.49[b]
Phenylephrine	6	25.1±1.6	52.0±10.9	20.3±3.3	72.3±12.2	2.9±0.6	2.82±0.38	3.73±0.42	0.09±0.04	0.22±0.05	2.84±0.52
Phentolamine	6	20.0±1.6	11.6±1.8[b]	14.9±2.8	26.5±3.7[b]	1.3±0.1[b]	1.22±0.28[b]	1.38±0.32[b]	0.12±0.04	0.11±0.02	1.00±0.32
Group 2											
Control	6	35.4±3.4	46.6±8.0	22.0±2.8	68.7±9.1	2.0±0.3	2.26±0.21	3.13±0.26	0.05±0.01	0.16±0.03	2.30±0.43
Norepinephrine	6	42.8±2.4[a]	102.3±15.1[a]	16.0±2.3	118.3±13.6[a]	2.9±0.4[a]	4.43±0.49[a]	5.82±0.58[a]	0.05±0.01	0.41±0.08[a]	7.81±2.29[b]
Phenylephrine	6	35.6±3.6	80.9±10.7[a]	17.5±2.9	98.4±9.3[a]	2.9±0.4[a]	3.08±0.26[a]	4.47±0.36[a]	0.04±0.01	0.30±0.06[a]	5.62±1.47[b]
Phentolamine	6	35.4±3.1	26.4±5.2[a]	24.6±3.8	51.0±7.5	1.4±0.2	1.39±0.11[a]	1.62±0.05[a]	0.08±0.03	0.08±0.02[a]	1.11±0.20
Norepinephrine	6	38.0±2.4	40.3±12.1	27.8±4.0	68.0±13.2	1.8±0.4	1.76±0.09	2.30±0.15[b]	0.08±0.02	0.10±0.02	1.51±0.37
Phenylephrine	5	36.3±3.4	47.4±14.2	32.4±5.6	79.7±18.4[b]	2.3±0.6	1.77±0.14	2.32±0.13[b]	0.05±0.02	0.09±0.02	1.20±0.33
Propanolol	6	23.0±3.0[b]	23.3±7.2	26.8±7.8	50.1±14.0	2.1±0.6	1.64±0.17	2.35±0.21[b]	0.13±0.05	0.16±0.07	0.98±0.22

Values are means ± S.E.M.

Norepinephrine and phenylephrine, 5 μg kg^{-1}; propanolol and phentolamine, 3 mg kg^{-1}.

f_H, heart rate; $\dot{Q}_{pul}$, pulmonary bloodflow; $\dot{Q}_{sys}$, systemic bloodflow; $\dot{Q}_{tot}$, total bloodflow; $V_{S_{tot}}$, stroke volume; P_{pul}, mean pulmonary blood pressure; P_{sys}, mean systemic blood pressure; R_{pul}, pulmonary resistance; R_{sys}, systemic resistance; $\dot{Q}_{pul}/\dot{Q}_{sys}$; ratio of pulmonary and systemic blood flow.

[a] signifies a significant difference from the control and [b] signifies a significant difference following injection of the first antagonist. Statistical significance is taken at the level of $P<0.05$.

Discussion

The haemodynamic variables reported during the untreated control condition of our study are in general agreement with previous studies on anaesthetised *Trachemys scripta* (see table 3 in Crossley et al., 1998). Nevertheless, $\dot{Q}_{pul}$ in group 1 of our study was somewhat higher, and f_H was slightly lower, than previous reports (Hicks et al., 1996; Hicks and Comeau, 1994; Comeau and Hicks, 1994; Crossley et al., 1998, 2000). As in previous studies on anaesthetised turtles, $\dot{Q}_{pul}$ always exceeded $\dot{Q}_{sys}$, resulting in a net left-to-right cardiac shunt. Furthermore, our results clearly show that an increase in R_{sys} increased this left-to-right shunt by increasing $\dot{Q}_{pul}$, often without compromising $\dot{Q}_{sys}$.

Comparison of haemodynamic variables between anaesthetised and fully recovered animals

We studied anaesthetised turtles to isolate the adrenergic control of the cardiovascular system without the confounding effects of the often-profound cardiovascular changes associated with ventilation in non-anaesthetised animals. The haemodynamic variables of anaesthetised turtles

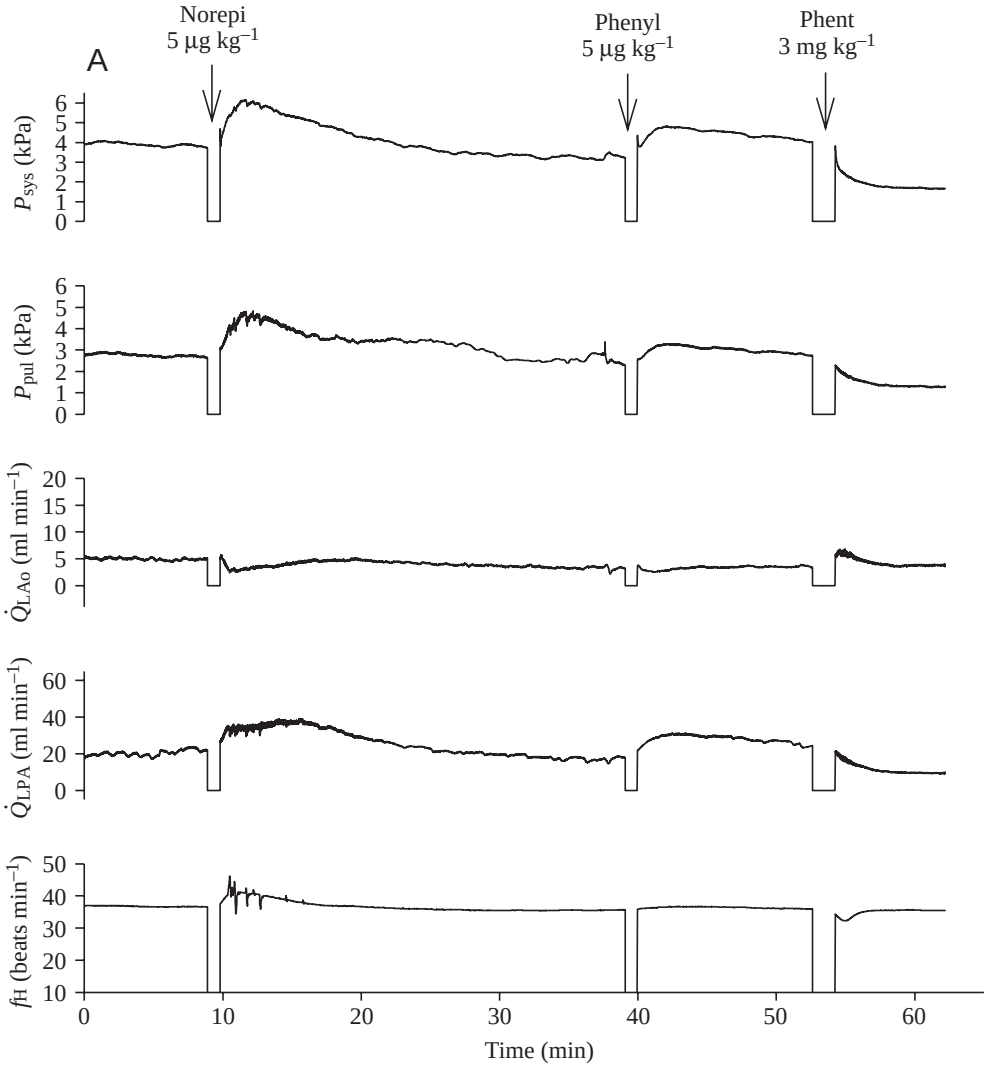
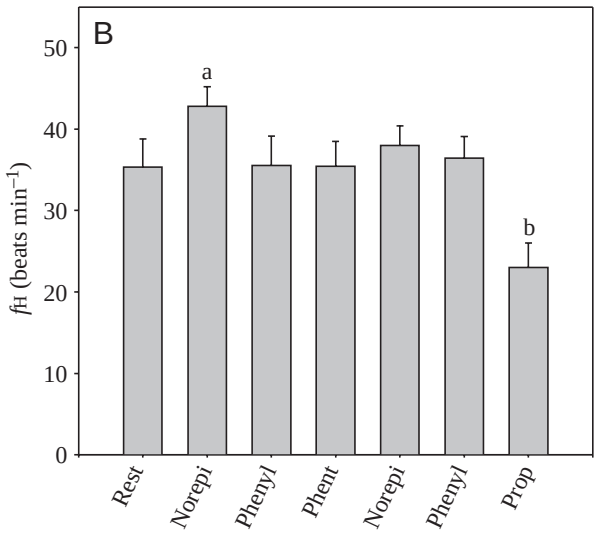
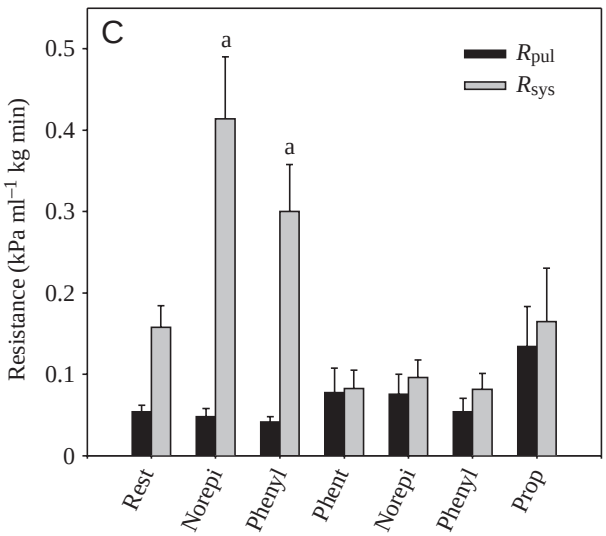


Fig. 2. (A) Effects of adrenergic agonists and antagonists on haemodynamic variables in the turtle *Trachemys scripta*. Recordings from one individual (#11/group 2) of mean arterial blood pressures and mean blood flows in the pulmonary and systemic circuits, as well as heart rate (f_H). P_{pul} and P_{sys} are the arterial pressures in the pulmonary and systemic circulation, respectively. $\dot{Q}_{LAo}$ is blood flow in the left aortic arch, and $\dot{Q}_{LPA}$ is the blood flow in the left pulmonary artery. The trace depicts resting conditions and measurements following injections of norepinephrine (norepi), phenylephrine (phenyl) and phentolamine (phent). (B) Heart rate and (C) pulmonary and systemic resistances in group 2 turtles (N=6) following injection of agonists and antagonists. [a] indicates a significant difference (P<0.05) compared with resting conditions, and [b] indicates significant difference (P<0.05) compared with the variables obtained following phentolamine. Prop, propranolol.





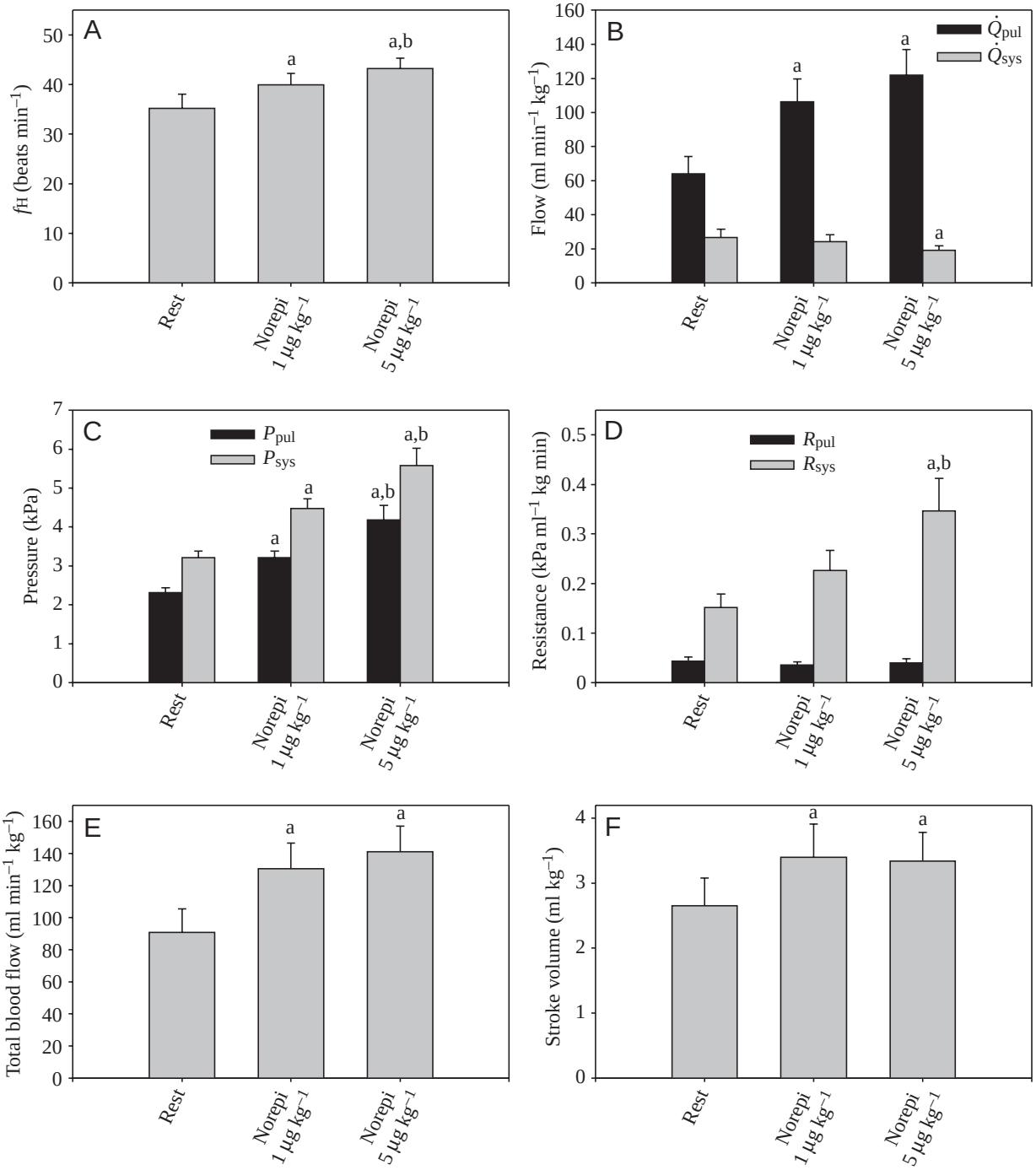


Fig. 3. Effect of norepinephrine dose (1 μg kg^{-1} or 5 μg kg^{-1}) on (A) heart rate (f_H), (B) pulmonary ($\dot{Q}_{pul}$) and systemic ($\dot{Q}_{sys}$) blood flow, (C) pulmonary (P_{pul}) and systemic (P_{sys}) pressure, (D) pulmonary (R_{pul}) and systemic (R_{sys}) resistance, (E) total blood flow ($\dot{Q}_{tot}$) and (F) stroke volume (Vs_{tot}). [a] indicates a significant difference (P<0.05) compared with resting conditions, and [b] indicates a significant difference (P<0.05) compared with the conditions following injection of 1 μg kg^{-1} norepinephrine. N=8.

differ markedly from that of non-anaesthetised and recovered animals. For example, during non-ventilatory periods, recovered animals are characterised by a low f_H and a large right-to-left shunt, whereas lung ventilation produces increases in f_H, $\dot{Q}_{pul}$ and $\dot{Q}_{pul}/\dot{Q}_{sys}$ (White and Ross, 1966; Shelton and Burggren, 1976; West et al., 1992; White et al., 1989; Wang and Hicks, 1996a). The low f_H and $\dot{Q}_{pul}$ during apnoea appear to stem from a large vagal tone, as injection of atropine increases both f_H and $\dot{Q}_{pul}$ and abolishes their reduction during breath holding (Berger, 1972; Burggren, 1975; Hicks and Wang, 1998; Hicks, 1998; Hicks and Farrell, 2000). Injection of a beta-blocker did not affect the changes in f_H associated

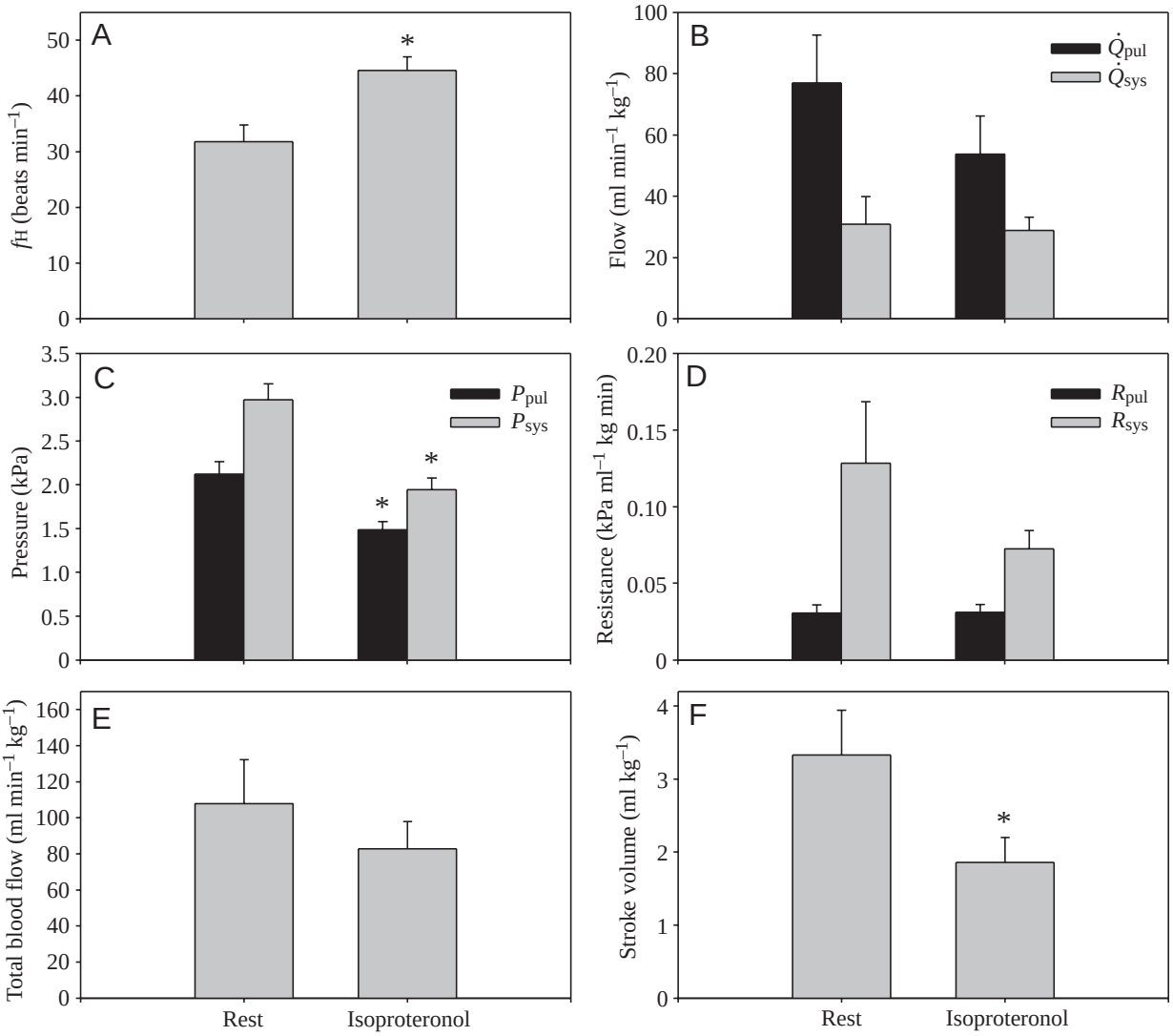


Fig. 4. Effect of isoproteronol (1 μg kg^{-1}) on (A) heart rate (f_H), (B) pulmonary ($\dot{Q}_{pul}$) and systemic ($\dot{Q}_{sys}$) blood flow, (C) pulmonary (P_{pul}) and systemic (P_{sys}) pressure, (D) pulmonary (R_{pul}) and systemic (R_{sys}) resistance, (E) total blood flow ($\dot{Q}_{tot}$) and (F) stroke volume (V_{Stot}). * indicates a significant difference compared with resting conditions. N=4.

with intermittent ventilation (Burggren, 1975), although nadolol reduced mid-apnoeic f_H (Hicks and Farrell, 2000). It is unknown whether the decreased f_H results from blocking the effects of circulating catecholamines or the adrenergic innervation of the heart. Hicks (1994) suggested that part of the increased $\dot{Q}_{pul}$ and f_H during ventilation can be attributed to increased adrenergic tone. Indeed, in anaesthetised turtles, afferent stimulation of the vagus causes tachycardia and increased $\dot{Q}_{pul}$, which is abolished by inhibiting adrenaline release from nerve endings with bretylium (Comeau and Hicks, 1994). Hence, it seems that the reciprocal changes in f_H and blood flows associated with breathing are predominantly caused by alterations in vagal tone, but that changes in adrenergic tone contribute to cardiac rhythm in non-anaesthetised turtles.

Blood flow and f_H of anaesthetised turtles are higher than in non-anaesthetised, apnoeic turtles because vagal tone on the heart and pulmonary artery is lost in anaesthetised and ventilated animals (Crossley et al., 1998). As a result, blood flow and f_H in anaesthetised turtles are quantitatively more similar to those measured in non-anaesthetised turtles during ventilation, when vagal tone is reduced. Nevertheless, anaesthetised turtles maintain an adrenergic tone similar to recovered animals, as the 35% reduction in f_H occurring with propranolol injection in the present study (Table 1) agrees well with the 16–35% reduction in f_H after nadolol injection in non-anaesthetised turtles (Hicks and Farrell, 2000).

α- and β-adrenergic responses on the heart and vasculature

This is the first study to simultaneously examine α- and β-adrenergic regulation of the heart as well as the pulmonary and systemic circulations in turtles. Previous studies have shown that systemic injection of catecholamines leads to an increased heart rate that can be blocked or greatly attenuated by injection

of β-receptor antagonists (Burggren, 1975; Hicks and Farrell, 2000). In our study, propranolol caused a large reduction in f_H but did not completely abolish the chronotropic effect of norepinephrine (Fig. 1; Table 1). This is likely to result from competitive binding between propranolol and norepinephrine on the cardiac β-adrenergic receptors, as phentolamine and phenylephrine did not affect f_H, whereas specific stimulation of β-adrenergic receptors using isoproteronol elicited marked tachycardia (Fig. 4). These observations are consistent with studies on isolated ventricular strips from *Trachemys*, where no chronotropic effects could be demonstrated following α-adrenergic stimulation (Van Harn et al., 1973).

In this study, we used bolus injections of adrenergic agonists and antagonists to evaluate the adrenergic regulation of the heart and vascular tones. This experimental design does not allow for a differentiation between the effects of circulating catecholamines relative to the effects of catecholamine release from nerve endings.

In our study, high doses of norepinephrine caused a marked increase in R_{sys} (Table 1). This response was mimicked by phenylephrine and could be blocked by phentolamine, demonstrating that the systemic vascular constriction following norepinephrine injection is caused by α-adrenergic receptors (Table 1; Fig. 2). Phentolamine halved R_{sys}, pointing to a substantial α-adrenergic tone on the systemic circulation in anaesthetised turtles (Table 1). This is also the case in fully recovered turtles, where phentolamine reduced R_{sys} from 0.08 kPa ml^{-1} min kg to 0.06 kPa ml^{-1} min kg (A. W. Stecyk, J. Overgaard, T. Wang and A. P. Farrell, unpublished data). Thus, as in virtually all other vertebrates examined so far, stimulation of the α-adrenergic receptors in the systemic vascular beds is associated with constriction (Nilsson, 1983). Conversely, stimulation of β-adrenergic receptors with isoproteronol was associated with relaxation of the systemic vascular beds (Fig. 4), and blockade of β-adrenergic receptors increased R_{sys} by a factor of 1.4 (Table 1; Fig. 1). Similar changes have been observed in fully recovered turtles, where β-adrenergic blockade using nadolol led to a threefold increase in R_{sys} from 0.05 kPa ml^{-1} min kg to 0.15 kPa ml^{-1} min kg (Hicks and Farrell, 2000).

Comeau and Hicks (1994) reported a small decrease in R_{pul} of anaesthetised turtles following catecholamine injection, whereas other studies were unable to detect reductions in R_{pul} (Luckhardt and Carlson, 1921; Berger, 1972; Milsom et al., 1977). In our study, both norepinephrine and phenylephrine elicited a small, statistically non-significant reduction in R_{pul} (Table 1; Fig. 1). A potentially minor dilatory role for α-adrenergic receptors was further suggested by the increase in R_{pul} (albeit not statistically significant) following phentolamine injection (Table 1). Thus, the pulmonary circulation is much less responsive to α-adrenergic stimulation than is the systemic circulation, where R_{sys} doubled after injection of phenylephrine. Burggren (1977) earlier reported a 15–20% reduction in the resistance of the isolated distal pulmonary artery upon injection of epinephrine. In the lizard *Trachydosaurus rugosus*, stimulation of β-receptors dilates the pulmonary vasculature, while stimulation of α-receptors appears to cause a constriction (Berger, 1973). In the snake *Elaphe obsoleta*, there is also evidence for β-adrenergic relaxation of the pulmonary artery (Donald et al., 1990). The role of β-receptors in the pulmonary circulation is less clear in turtles. Thus, while propranolol caused a significant increase in R_{pul}, suggesting a tonically active β-adrenergic mediated relaxation (Table 1; Fig. 1), injection of isoproteronol did not reduce R_{pul} (Fig. 4), perhaps because this vasoactive mechanism was already fully activated.

Our study demonstrates marked differences in the vascular response to adrenergic stimulation between the pulmonary and systemic circulations. The pulmonary circulation is much less responsive to catecholamines, and α-adrenergic receptors could even mediate opposite changes in vascular tone. Other studies also show that vasoactivity in the systemic and pulmonary circulations of turtles differs in other respects. Thus, hypoxia causes vasodilation in the systemic circulation as opposed to vasoconstriction in the pulmonary circulation (Crossley et al., 1998). NO exerts a substantial role in maintaining systemic vascular tone but does not seem important in the pulmonary circulation (Crossley et al., 2000). Conversely, pulmonary resistance is under strong cholinergic control (Berger, 1972; Burggren, 1977; Milsom et al., 1977; Hicks and Comeau, 1994), whereas the cholinergic tone on the systemic circulation is either low or absent (Kirby and

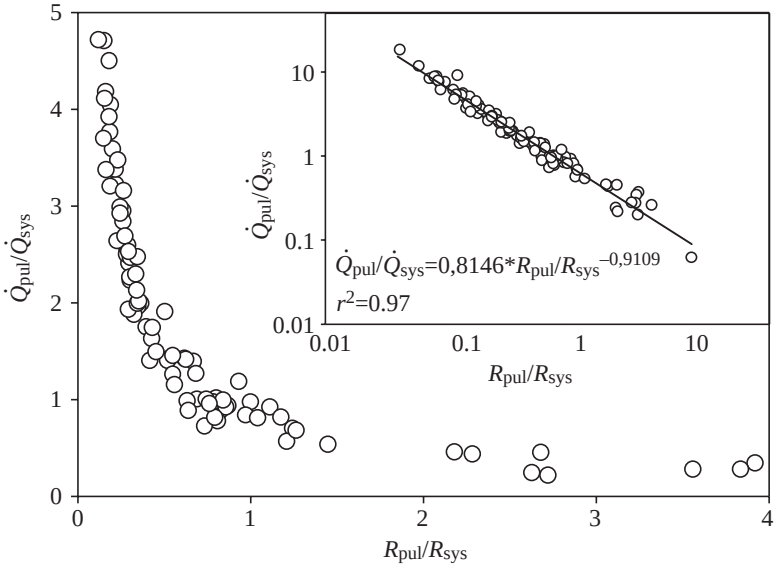


Fig. 5. Relationship between the ratio of vascular resistance (R_{pul}/R_{sys}) *versus* the ratio of blood flows ($\dot{Q}_{pul}/\dot{Q}_{sys}$) in the pulmonary and systemic circuits. The box insert shows the same data points on a double-log scale fitted to a power function. All data points are from individual measurements following the protocol of groups 1 and 2. For clarity, individual values with $\dot{Q}_{pul}/\dot{Q}_{sys}$ of >5 and R_{pul}/R_{sys} of >4 were not included in the main figure but are included in the double-log plot.

Burnstock, 1969; Berger and Burnstock, 1979; Comeau and Hicks, 1994). Indeed, more studies are needed to better define the basis of these differences in vasoactivity.

The present study is revealing in terms of a potential role of venous venoconstriction on cardiac function. For example, despite the slight decrease in $\dot{Q}_{sys}$ and the marked increase in R_{sys} with both phenylephrine and norepinephrine, there was an increase in $\dot{Q}_{tot}$ and Vs_{tot}. The increase in Vs_{tot} could be a result of an α-adrenergically mediated venoconstriction. Conversely, the apparent negative inotropic effect of isoproteronol on the heart (i.e. the decrease in Vs_{tot}) might be best explained by a β-adrenergically mediated venodilation reducing venous return to the heart. These venous effects of adrenergic stimulation need not be manifest in changes in overall vascular resistance but warrant further attention because of their potential effects on Vs_{tot} and $\dot{Q}_{tot}$. Thus, although it seems that arterial vascular resistances determine the relationship between pulmonary and systemic blood flows, it is likely that the magnitude of changes in total blood flow is determined, in part, by the degree of venous vasoactivity.

Functional implications of adrenergic regulation of cardiac shunts

Our study is the first to comprehensively evaluate effects on adrenergic stimulation on cardiac shunt patterns. Fig. 5 depicts the relationship between blood-flow distribution, expressed as $\dot{Q}_{pul}/\dot{Q}_{sys}$, and the ratio of the vascular resistances in the pulmonary and systemic circulations (R_{pul}/R_{sys}). A similar relationship illustrated that the distribution of blood flows between the systemic and pulmonary circuits in turtles correlates closely with the ratio of pulmonary and systemic vascular resistances (Hicks et al., 1996; Crossley et al., 1998). We were able to show that an even more profound range of cardiac shunting can be produced with adrenergic drug injections that act primarily on the systemic circulation. Consequently, anaesthetised turtles exhibit large net left-to-right shunts ($\dot{Q}_{pul}/\dot{Q}_{sys}$ of >1) when R_{pul} is lower than R_{sys} (R_{pul}/R_{sys} of <1), while a net right-to-left shunt ($\dot{Q}_{pul}/\dot{Q}_{sys}$ of <1) exists when R_{pul} is higher than R_{sys} (R_{pul}/R_{sys} of >1). This relationship implies that systemic outflow resistance, regulated through α-adrenergic systemic vasoconstriction at the arterial level (as shown by the fourfold changes in R_{sys} between α-adrenergic stimulation and blockade), is the primary determinant of distribution of $\dot{Q}_{tot}$ to the blood flows between the two circuits in these anaesthetised turtles. In non-anaesthetised turtles, changes in the shunt pattern are effected primarily by cholinergically mediated constrictions of the pulmonary circulation. Thus, by focusing on adrenergic control, our work has provided evidence that modulation of the systemic vascular resistance produces the same types of cardiac shunts in turtles as does modulation of pulmonary resistance. Consequently, for turtles, it appears that up to 97% of the observed variability in the cardiac shunting (Fig. 5) can be explained by the ratio of the resistances in the two circulations.

It remains unanswered whether the profound changes in cardiac shunting produced here by adrenergic mechanisms play a role in non-anaesthetised turtles. Increased adrenergic tone is often associated with exercise and stressful conditions such as hypoxia, where sympathetic stimulation safeguards systemic oxygen delivery through increased f_H and cardiac output. In addition, reduced cardiac right-to-left shunting through increased adrenergic tone and decreased vagal tone increases oxygen delivery (Wang and Hicks, 1996b, 2002). Our study is consistent with this view. However, because anaesthetised turtles have no vagal tone, adrenergic stimulation serves to increase the net left-to-right shunt rather than abolishing the net right-to-left shunt that would occur *in vivo*. Furthermore, the changes in shunt pattern are primarily accomplished through increased R_{sys} and are associated with reductions in $\dot{Q}_{sys}$. Therefore, adrenergic stimulation of anaesthetised animals is unlikely to improve systemic oxygen delivery. It is probable, however, that oxygen delivery in fully recovered animals would benefit from increased sympathetic tone, as resting and undisturbed animals are characterised by net right-to-left shunts and low arterial oxygen levels (see tables 1, 2 in Wang and Hicks, 1996a).

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